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- To serve and promote the scientific cause and to advance academic interest in the field of plant genetic resources.
- To disseminate knowledge relating to various aspects on plant genetic resources.
- To provide a forum for organizing symposia/conferences with a view to develop close relationship among the scientists engaged and interested in plant genetic resources activities.

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**EDITORS:** Sunil Archak  
Anuradha Agrawal  
Anjula Pandey  
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## Managing Agrobiodiversity through Use: Changing Paradigms

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### Preamble

Anthropogenic activities have profoundly re-shaped the earth's land, oceans, air and biodiversity to such an extent that geologists have proposed a new epoch called the 'Anthropocene', marking the end of 'Holocene' (since 12,000 years). This new epoch is being said to begin from 1950, when radioactive elements from nuclear testing were likely spread all over the globe, and characterised by mass extinctions, plastic pollution, and spike in carbon emissions in the atmosphere (Waters *et al.*, 2016). Consequently, biological diversity got reduced, the earth became warmer with greater incidence of natural catastrophic events. Whilst all biodiversity is critical for life on earth, the genetic diversity of agriculturally important species have a direct bearing on our food and nutritional security globally. A recent study shows that about 58% of the world's land surface, and 9 out of 14 of the world's terrestrial biomes, have fallen below 'safe threshold' of biodiversity, impacting a wide range of services provided by biodiversity, including crop pollination, waste decomposition, regulation of the global carbon cycle, and cultural services that are critical to human well-being (Newbold *et al.*, 2016). Added to this is the concern about projections that global food production will need to be doubled by 2050 to feed the 9 billion plus population, by either intensification of existing agricultural systems or by expansion into new lands, scope for which is very limited.

This current paradox concerning the cause of decline of earth's life-sustaining elements and also having the potential to keep hunger and malnutrition at bay, would require adoption of a new paradigm as to how we manage our natural resources whilst fulfilling the commitments to meet the new sustainable development goals (SDGs). United Nations Conference on Environment and Development was one such major shift in the management of biodiversity subject to the rights of individual nations, which required to be

protected with proper legal/*sui generis* instruments. Further, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilisation of genetic resources, including appropriate access to genetic resources and by using most relevant technologies, also got enshrined in the Convention on Biological Diversity (CBD). The CBD thus envisioned a new paradigm that available genetic resources were to be conserved for posterity. Subsequently it was realized that conservation is not only for 'posterity', but for 'use' for the overall benefit to the society. Hence, 'conservation through use' emerged to be a new paradigm, also known as 'New Mantra'. What we now know is that there is less use of genetic diversity today than what we had previously which led to Green Revolution. The Food and Agriculture Organization (FAO) of the United Nations has, therefore, initiated with the support of Bill and Melinda Gates Foundation, a project to strengthen plant breeding capacity and research on global scale, so that use of genetic resources is enhanced globally. This project, known as the Global Partnership Initiative for Plant Breeding Capacity Building (GIPB), is a multi-partner platform with an aim of improving institutional capacity for effective crop variety development and their distribution through seed systems (<http://www.fao.org/in-action/plant-breeding/en/>).

In the past, national agricultural research systems, including that of India, had strong national breeding programmes for developing improved varieties. However, there is now greater dependence on pre-breeding materials being provided by many of the international centres, especially those of the CGIAR. For another paradigm shift in agriculture from sustained food security to that of household nutritional security, we now need higher research investments as well as intensified scientific understanding of agriculturally important species (be those of crop, animal, aquatic and microbial).

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## Managing Agrobiodiversity in the Geopolitical Landscape

Changes in the perception on genetic resources (GR) during the last three decades of the twentieth century has dramatically transformed the way they are now being managed. Member countries, including India, have either legislated or developed policy frameworks under the obligations of the CBD. In the pre-CBD era, all biodiversity was considered, managed and used as global public goods, with easy access, relatively free exchange and absence of ownership issues. It is now almost difficult to imagine how Nikolai Ivanovich Vavilov could have carried out his historical collection expeditions of genetic resources and identified centres of origin of crop plants in the post-CBD scenario which now demands Prior Informed Consent, and the Access and Benefit Sharing (ABS) mechanisms. However, this paradigm shift is a reality in today's context of biodiversity management. The reality is that all biodiversity is now classified as 'sovereign rights of nations'. This and the concurrent rapid loss of genetic diversity in existing agro-ecosystems are the reasons for initiating short-, medium- and long-term genetic resources conservation programmes at the national, regional and global levels.

Moreover, the conservation and use of genetic resources have many facets including research and development, intellectual property rights, food security and health related issues, which are governed by different institutions and agreements. India was among the first few countries to ratify the International Treaty for Genetic Resources for Food and Agriculture (ITPGRFA) in 2002. The Treaty came into force in 2004. In 2006, the Governing Body of the Treaty also adopted the Standard Material Transfer Agreement (SMTA) as an instrument for carrying out germplasm exchange. Under the CBD, it was envisioned that there would be both multilateral and bilateral systems of germplasm exchange, whereas multilateral exchange would exclusively be the domain under ITPGRFA. Obviously, these processes have not been easy though India tried to move forward by enacting the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act in 2001, the Biological Diversity Act (BDA) in 2002. As a consequence, the process of germplasm exchange got significantly reduced globally, while ensuring a new paradigm shift from no regimentation to that of a new international regulatory regime. Earlier, the genetic resources were being exchanged freely for the welfare of

humankind, which helped significantly in expanding our food basket globally. Imagine, what would have been the food options for us had these regulations were in place prior to CBD asking for protection of all available biodiversity.

As a matter of principle, rules and procedures enshrined in ITPGRFA must now be followed by the global community. Also there had been significant debate as to why soybean should not be included in Annex 1 of 64 crops despite being an important food crop in the entire south east Asia. To my utter disappointment, being present in the FAO deliberations then, soybean was not included mainly because of other considerations than the scientific and food security ones. Similarly, a few other crops were also discussed but not agreed upon. Finally, to overcome the stalemate, a decision on the list of crops under Annex 1 was taken almost by four o'clock in the morning on the last day of Treaty negotiations. Also there was an understanding that countries would revisit the list to expand it further, which somehow has not happened over the last 15 years since then. Another unfortunate part is that those countries that were most vocal in getting ITPGRFA adopted have yet not enacted the Treaty and no one knows why? At the same time, even countries like India having ratified the Treaty is not very open to sharing genetic resources. Obviously, therefore, there is an urgent need to review the process of ABS for improved germplasm exchange and management in the overall interest of humanity.

The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (2010) had reviewed and assessed the developments concerning ITPGRFA. A perusal of the report illustrates that we need build capacity, partnerships, fulfil our legal obligations and need to refrain from putting hurdles in the implementation of the Treaty. What is of greater concern today, particularly for the developing countries, is to ensure that the policy framework regulating access and use of genetic resources, while keeping pace with technological developments, also addresses the livelihood security issues of the poorest of the poor. Another point of concern is that agrobiodiversity policy and legislation are not clearly demarcated and mostly treated under the similar rules and regulations as biodiversity. The fundamental difference between management of biodiversity as compared to agrobiodiversity needs to be understood to deal with them differently. Interestingly, another paradigm change has taken place in the process

of exchange of genetic resources, which used to be the domain of scientists earlier. Instead, it is now dealt with by the bureaucrats and legal experts with little appreciation of science that has human face.

### Addressing Exchange and ABS Regimes

Studies have clearly shown how nations have historically been dependant on each other for their needs of genetic resources for increased agricultural productivity. This dependence is predicted to increase more in future, given the current trends of climate change, emerging needs for expanding food basket and changing consumer preferences for more healthy foods (Galluzzi *et al.*, 2016). Related to this is the existence of international and national legal obligations and Treaties under which exchange of are governed, which dictate how access is to be provided and what benefit sharing mechanisms will be agreed upon. As already stated, administrative, structural, and political compulsions have made the exchange of biodiversity much more complex. Instead of easing the process, the Treaties such as the Nagoya Protocol of the CBD and ITPGRFA have indirectly led to reduced exchange of germplasm between nations, despite clear recognition of multilateral system (MLS) for exchange. Experience tells that neither the MLS has functioned at the anticipated level, nor it has helped in generating financial benefits through the proposed international Benefit-Sharing Fund (BSF). In India, there is still unsettled debate concerning exchange of germplasm even with the local private seed sector organizations engaged in plant breeding. Even SMTA has not yet been put into practice for want of procedural clearances and lack of understanding. During mid-eighties, ICAR, as a policy, allowed free access to the parental lines of hybrids bred by the public system recognising well that seeds of these hybrids would otherwise not reach the end users i.e., the smallholder farmers. This policy decision then not only accelerated the coverage under hybrid seeds resulting in increased crop productivity but on the contrary strengthened existing private seed sector in India. On the contrary, with the advent of Plant Breeders' Rights and the adoption of IPR regime, there is an obvious hesitation to share the germplasm, either for the fear of loss of ownership or for biopiracy. Hence, there is an obvious concern for much needed trust-building and partnership. This would demand an enabling policy environment for sharing the germplasm as well as information between public and private sectors.

In many cases, the farmers are the custodian of traditional varieties and their rights are now being protected through PPV&FRA. The Authority needs to be congratulated for recognising these Rights. The Authority has also been assured of Government support to build initially an Indian Gene Fund of Rs. 50 crores (around \$7.5 million) in order to recognise, reward and give incentives to farming communities engaged in conserving valuable genetic resources. It is also expected that benefit-sharing mechanism in future will help in building the proposed Gene Fund. Simultaneously, it calls for developing a clear mechanism to benefit directly the farmers for their invaluable service to the society. It is expected that this fund will soon be around \$20 million with the provision of ABS and expected funding support from the seed sector.

### Conservation Continuum

The genetic diversity in crops and animals are manifestation of diverse agricultural systems evolved over many centuries by the farmers and breeders. On the contrary, the modern agriculture tends to use mainstream varieties/breeds, resulting in monoculture practice that prevails in modern agricultural landscapes. In this context, preserving available diversity, as a safety-net, is emerging as a big threat to be addressed. Hence, sustainable use of genetic resources would demand both strengthening and expanding of conservation approaches, be those *ex situ* or *in situ*. In fact, complimentary and rather holistic conservation practices that are low cost and sustainable are the needs of the hour. Also there is an urgency to develop a 'conservation continuum', encompassing *in situ*, on-farm, *ex situ*, permafrost and other methods with adequate funding support. Also it is of prime importance today that the farmers, livestock keepers, aquaculture practitioners or foresters engaged in conserving the target varieties, breeds and species derive direct (financial) or indirect (livelihood security) benefits by engaging in such activities. Despite sporadic efforts in this regard, much is still to be done to research and conserve the wild relatives and underutilised species as crops for the future. Programmes and policies are thus warranted both at the national and global levels to move forward rather aggressively in this regard.

### Mainstreaming Agrobiodiversity to Meet SDGs

Plant and animal breeding provides an important foundation to address effectively the sustainable development goals (SDGs), and contributes to raising

yields, increasing resource use efficiency and reducing the negative environmental impacts on food production. In this context, enhanced and integrated use of genetic resources would be instrumental, not only through breeding better varieties and breeds, but also through smarter deployment of genetic diversity to combat biotic (pests and diseases) and abiotic (drought, heat, cold) stresses, derive greater ecosystem services (pollinators) and reduce the use of costly inputs (e.g. fertilizers, pesticides). It would also demand for exploring new sources for food, nutrition and good healthy foods for ever increasing global population. Sound management of genetic resources will thus be the key to achieve increased food production, without negatively impacting available agrobiodiversity. We now need to ensure up-scaling and out-scaling of innovations to achieve dietary diversity and improved nutrition at household levels. Better information management and accessibility to databases/informed knowledge for policy makers and stakeholders would help in strengthening on-going efforts to use agrobiodiversity for food and nutrition being important SDGs. There is also an urgent need to promote use of more nutritious species such as millets, indigenous fruits, vegetables, roots and tubers, as compared to major emphasis that we gave in the past to only few selected staples.

### **Harnessing New Science**

Scientific advances like CRISPR-Cas would help breeders/researchers to tap new genetic resources more at ease for both food and nutritional security. New technologies pervading agriculture in terms of smart-phones, satellite imaging, automated farm practices, even use of drones, is allowing farmers to grow more food on their land while reducing their water, fertilizer, and pest-control needs. However, the availability of appropriate planting material/breeds remains the most critical factor for productivity, adaptability and resilience of agro-ecosystems. Developments in science and technology in the areas of genetic engineering, genomics, biotechnology, nanotechnology, bioinformatics, synthetic biology etc. have increased the speed, scale and efficiency in research outputs. These technologies are the game changers that will dictate how genetic resources are researched in future and used. Nonetheless, existing agrobiodiversity would remain the “hardware and software codes of nature” requiring systematic deciphering for designing agricultural crops and breeds for their use through new science. An important aspect

with application of new technologies for agricultural production would be to generate awareness and dispel fears in the minds of general public about use of new products (e.g. golden rice) that are outcomes of cutting-edge technologies as international public goods.

### **Role of Farmers and Civil Society**

To ensure synergy among agrobiodiversity management and agricultural development, the role of stakeholders such as: decision-makers, public administration, civil society, local communities (including farmers, non-government organisations) and even media is crucial, especially in developing countries that are gene-rich. Given the multitude of stakeholders involved in operating/ utilising the genetic resources as well as the traditional knowledge associated with genetic resources, capacity building through awareness-raising campaigns would be necessary. Also greater partnership among the stakeholders, including public and private sectors, NGOs and farmers is required. Many farmers do conserve useful collections of different varieties and crops by sheer self motivation and with hardly any funding support. The question, therefore, emerges as to when there is no support, why then the tribal communities protect these resources for others while living at sub-subsistence level? In this context, enhanced efforts are obviously needed to develop community level gene banks and link them with national/international gene bank networks. Also the traditional agricultural systems are linked with traditional knowledge related to agrobiodiversity. Such knowledge systems need to be documented before these are lost forever. Scientific validation of such traditional knowledge is also essential for improved understanding of the ecological functions of agrobiodiversity especially in the context of physical environment and the socio-economic factors. A delicate balance is obviously needed for maintaining agrobiodiversity and ecological processes while optimizing societal benefits, as we move forward.

### **Conclusion**

Managing agrobiodiversity in the present context requires paradigm shifts in our understanding the role and implications of legal instruments for protection and sharing of genetic resources for betterment of humankind. Agrobiodiversity in its own right must now be managed more scientifically through involvement and partnership of all stakeholders at the national, regional and global levels. For meeting the SDGs, efforts to diversify

existing food basket through better understanding and use of available genetic resources, using new science, will have to be intensified and supported well. This paper addresses some of the emerging paradigms for effective and efficient management of agrobiodiversity for human welfare.

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## Indian Agrobiodiversity System

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Agrobiodiversity occupies a distinctive place within biodiversity. It recognises that agriculture evolved from bioprospecting, selection and development of a few species from plant and animal kingdoms, to meet human needs of food, fibre and fuel. All biotic factors related to agriculture, such as, plants, animals, fish, reptiles, insects, birds and microbes are components of agrobiodiversity. The conservation, management and sustainable use of these organisms (and their wild progenitors/relatives) require specific attention. The Indian Council of Agricultural Research (ICAR) addresses the issue of management of genetic resources through its five Bureaux, namely, National Bureau of Plant Genetic Resources (ICAR-NBPGR), National Bureau of Animal Genetic Resources (ICAR-NBAGR), National Bureau of Fish Genetic Resources (ICAR-NBFGR), National Bureau of Agriculturally Important Microbes (NBAIIM) and the National Bureau of Agricultural Insect Resources (ICAR-NBAIR) to address the management of their respective genetic resource. The respective Bureaux coordinate the management involving various stakeholders in their functional areas.

Effective conservation and use of agrobiodiversity is of immense importance in the present scenario. There is an urgent need for managing genetic resources for food and agriculture in ways that promote the evolution and conservation of agrobiodiversity, and to ensure that they are available to all stakeholders for use to adapt to changing environment by developing new climate resilient varieties/breeds to ensure the food and nutritional security. Climate change will also increase the importance of minor or under-utilized crops and plant species. Therefore, it is essential that all forms of agrobiodiversity are collected through regular explorations, conserved through suitable means, characterised and evaluated using modern technologies to ensure their availability for improvement programmes of food and agriculture. Resources and activities of each of the five bureaux is presented briefly:

National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi was established in 1976 as a nodal institute for assembly of global diversity of plant genetic resources (PGR) that are of direct or indirect value to humans. The component activities include PGR collection through exploration, characterisation, evaluation, safe conservation using both conventional storage and biotechnological approaches for *in vitro* conservation and cryopreservation; generation and conservation of genomic resources. So far over 2,600 explorations have assembled over 2.66 lakh crop species and their wild relatives. The national Genebank houses ~0.43 million accessions of over 1800 species in seed genebank, 11,000 accessions in cryo genebank and 1,900 accessions in *in vitro* genebank. Exchange of germplasm is carried out with >100 countries and CGIAR institutes under bi- or multilateral agreements. Besides, it is a nodal institute authorized by Government of India to facilitate international exchange of germplasm for research purposes and to undertake quarantine which has resulted in the interception of over 50 pests of high economic significance. Till date, over 2.35 lakh accessions of different agri-horticultural crops have been characterised and evaluated. A mega evaluation executed in collaboration with SAUs for 21,822 accessions of wheat and 18,775 of chickpea earned its place in Limca Book of Records. DNA fingerprinting of agri-horticultural crops, conservation and use of genomic resources and GM detection are also important activities at ICAR-NBPGR. About 2300 varieties of >35 crops have been fingerprinted to safeguard against biopiracy. It is mandatory for the notification of any new variety to be registered with ICAR-NBPGR. A PGR portal, a gateway to information on PGR conserved, is hosted on ICAR-NBPGR website. The PGR information has been documented in user friendly databases. HRD including PGR Education, awarding M.Sc. and Ph.D. degrees in PGR discipline in collaboration with ICAR-IARI, are also integral components of its functioning.

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National Bureau of Animal Genetic Resources (ICAR-NBAGR) was established in 1984 at Bangalore in order to safeguard the existence of precious livestock of the country. The institute was later shifted to Karnal in 1985. It is working for the enhancement of indigenous livestock and sustainable utilisation of animal genetic resources. It is divided into three divisions: Animal Genetics, Animal Biotechnology and Animal Genetic Resources, and also work with various agencies involved in livestock improvement through a Network Programme on Animal Genetic Resources. ICAR-NBAGR has a mandate for identification, evaluation, characterisation, conservation and utilisation of livestock and poultry genetic resources and conducts systematic surveys to characterise, evaluate and catalogue farm livestock and poultry genetic resources and to establish their National database; designs methodologies for *ex situ* conservation and *in situ* management and optimal utilisation of farm animal genetic resources and undertakes studies on genetic characterisation using modern molecular biological techniques. About 1,1,30,000 cryo-preserved semen doses representing important and endangered breeds of seven species (Cattle, Buffalo, Goat, Sheep, Camel, Equine and Yak) are being maintained in the National Gene Bank. Animal Genomic Resource Bank is established which has collection of DNA samples of more than 90% of the Indian breeds of various livestock species besides some embryos.

National Bureau of Fish Genetic Resources (ICAR-NBFGR), was established in 1983 at Lucknow with the mandate of collection, classification and cataloguing of fish genetic resources of the country, maintenance and preservation of fish genetic material for conservation of endangered fish species and evaluation and valuation of indigenous and exotic fish species. The Bureau has created excellent infrastructure and expertise in several research areas including development of fish databases, genetic characterisation, gene banks, fish germplasm and habitat inventory, risks analysis of exotic species, diagnostics for OIE notified pathogens, aquatic microbes and other areas of germplasm conservation with special focus on threatened, prioritized and exotic fish species. A database on Fish Diversity of India, containing information on about 2936 indigenous fish species (936 freshwater, 113 brackish water and 1887 marine species) and 462 exotic species, has been developed. The Bureau has prepared fish diversity checklists for eight states, viz. Uttar Pradesh, Chhattisgarh, Madhya Pradesh, Rajasthan,

Kerala, Karnataka, Tamil Nadu and Maharashtra and three ecosystems such as Western Ghats, Gulf of Mannar and Vembanad lake. The Bureau has discovered forty new species during explorations from North-eastern States and Western Ghats in collaboration with other partner organizations. The cytogenetic profiling of over 70 fish species mainly from freshwater and marine systems, collected from various part of the country including biodiversity hot-spots, like North-Eastern part and Western Ghats, Kerala, has been accomplished. Using the molecular markers, especially microsatellite DNA, concerted efforts have provided description of genetic variation and population structure for 24 prioritized fish species from their major range of natural distributions. At ICAR-NBFGR, a National Repository of Fish Cell lines (NRFC) has been established where at present 50 cell lines are being maintained. ICAR-NBFGR is also coordinating the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) which is being implemented in 15 States and 2 Union Territories through 24 collaborating partners.

National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM), was established in the year 2001 at Maunath Bhanjan for cataloguing and preservation of agriculturally important microflora, which is a necessity of the present time for judicious microbial resource management, bio-prospecting and fundamental scientific research. The Bureau started functioning at ICAR-NBPGR, New Delhi and was later shifted to Maunath Bhanjan, Uttar Pradesh in 2004. ICAR-NBAIM was designated as a Recognized Repository under the National Biodiversity Act (2002) for storing microbial wealth in India. It is a premier organisation of agricultural and microbial biotechnology prioritizing its responsibilities in the area of collection, isolation, conservation, management and utilisation of agriculturally important microorganisms (AIMs) in the country. The Bureau is engaged in the multifarious activities in the area of microbial diversity, biological control, microbial genomics, preservation and maintenance of microbial cultures. Diversity analysis of northern Indo-Gangetic plains undertaken indicated that the soils do have a population of *Bacillus* isolates and fluorescent *Pseudomonas* in high numbers but the isolates/strains have lost the ability to express plant growth promoting attributes. Diversity analysis of various extreme environments has led to the development of huge database of microorganisms from extreme habitats. The Diversity

of *Bacillus* and predominant genera has been mapped and novel *cry* genes with insecticidal properties have been deciphered. The Bureau has also made significant progress in nutrient management and biocontrol and has developed bioformulations to be used as biofertilizers, biocontrol agents, abiotic stress alleviators, agricultural residue decomposers etc. DNA based diagnostic kits were developed for the identification and ecological monitoring of *Bacillus*, *Pseudomonas*, *Alternaria*, *Colletotrichum*, *Fusarium udum* and *Macrophomina phaseolina*. The Bureau established the Microbial Genomic Resource Repository (MGRR) where over 10,000 accessions of genomic DNA, clones, plasmids, vectors and gene constructs from various sources are being maintained. The Bureau is also engaged in supply of pure cultures to various research organizations and provides microbial identification services.

National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru is involved in the study of invertebrates particularly insects, spiders, mites and their associated organisms (including microbes). A radical shift in perspective in 2009 saw the institution transform itself from one devoted solely to the use of insects as biological control agents for the management of insect pests in agricultural situations to one which acknowledged the multifarious roles of insects in agroecosystems in particular and all of nature in general. While research in biocontrol would continue to be conducted at ICAR-NBAIR, it would henceforth have the responsibility of not only documenting and studying the entire agricultural insect diversity of the country but also that of the fauna of associated organisms, viz., insect pathogenic bacteria, fungi, viruses and nematodes as well as endosymbionts of insect pests and their natural enemies. ICAR-NBAIR has been undertaking surveys to document agricultural insect diversity from all agroecological regions of the country with special emphasis on the collection and documentation of insects in the biodiversity hotspots of the country. Many new taxa have been discovered and described from various parts of the country. Over 200 types in the collection of the ICAR-NBAIR have been digitized and are hosted on the ICAR-NBAIR website which is available for free access to taxonomists all over the world. ICAR-NBAIR leads other organizations in the country in the generation of barcodes of Indian insects. Over 600 barcodes have been generated for insect pests, their natural enemies, mites and nematodes of agricultural importance. Identification services for

insects of agricultural importance, including both insect pests and their natural enemies are being provided free. The insect collection in the ICAR-NBAIR museum is home to over 100,000 dry land wet specimens. In recognition of the expertise in insect taxonomy available at ICAR-NBAIR, the National Biodiversity Authority of the Government of India has designated this bureau as a National Repository for agriculturally important insects, spiders and mites.

The main threats to biodiversity as perceived and submitted to the Convention on Biological Diversity (CBD) to which India is a signatory include habitat fragmentation, degradation and loss, over-exploitation of resources, shrinking genetic diversity, invasive alien species; declining forest resource base, climate change and desertification, impact of development projects, and impact of pollution. In the backdrop of the varying socio-cultural milieu and often conflicting demands of various stakeholders, there is an urgent need for augmenting and accelerating the efforts for conservation and sustainable use of biodiversity, and for the fair and equitable sharing of benefits arising from the utilisation of genetic resources.

India has ratified all the three treaties-CBD, International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA) and Nagoya Protocol (NP) and also enacted its own Biological Diversity Act (BDA) in 2002 that governs the access and benefit sharing (ABS) mechanisms of genetic resources held within its political boundaries. Following the ratification of CBD, India notified the Biological Diversity Rules in 2004 to give effect to the provisions of the CBD, including those related to its ABS. India was one of the first few countries to enact such a legislation. The Act is to be implemented through a three-tiered institutional structure: National Biodiversity Authority (NBA), State Biodiversity Boards (SBBs), Biodiversity Management Committees (BMCs) at the local level, in line with the provisions for decentralized governance contained in the Constitution. The Biological Diversity Act is a path-breaking and progressive legislation which has the potential to positively impact biodiversity conservation in the country.

At a policy level, the National Policy and Macro level Action Strategy (1999) was developed that called for consolidating existing biodiversity conservation programmes and initiating new steps in conformity with the spirit of the Convention. This was followed

by implementation of a UNDP/GEF-sponsored NBSAP Project (2000-2004) that yielded micro-level action plans adequately integrating cross-cutting issues and livelihood security concerns. Besides, subsequent to the approval of the National Environment Policy (NEP) in 2006, preparation of the National Biodiversity Action Plan was taken up by revising the 1999 document in consonance with the NEP, using the NBSAP project report as one of the inputs. The National Biodiversity Action Plan (2008) defines targets, activities and associated agencies for achieving the goals, drawing upon the main principle in the NEP that human beings are at the centre of concerns of sustainable development and they are entitled to a healthy and productive life in harmony with nature.

The ICAR envisaged the Agrobiodiversity Platform to address management of genetic resources of plants, animals, fish, microbes and insects. The respective Bureaux coordinate these management functions involving other stakeholders in their functional areas from National Agricultural Research System (NARS) consisting of ICAR Institutes, All India Coordinated Research Project (AICRP) centres and State Agricultural Universities (SAUs). The objective is to suitably characterise, evaluate and conserve the genetic resources to ensure the availability of desirable trait specific

germplasm of food and agriculture for improvement programmes. In order to ensure achievement of this objective, the CRP on Agrobiodiversity of ICAR has eight sub-projects addressing management of genetic resources of plants, animals, fish, microbes, insects, veterinary type culture collections and documentation of fungal biodiversity through fungal barcoding and on island ecosystems. The project will provide long-term strategies for management and sustainable use of agro- biodiversity. Through this project there would be enhanced conservation, management and sustainable use of these genetic resources (and their wild progenitors/relatives) in the improvement programmes of different components of agrobiodiversity, namely, plants, animals, fish, microbes, insects and documentation of fungal biodiversity through fungal barcoding. During past three years, a total of 40 thousand germplasm accessions of agri-horticultural crops were multiplied, characterised and evaluated. The project would establish and strengthen the different Institutions governing genetic resources in ways that would promote the evolution and conservation of agrobiodiversity, and to ensure that they are available to be used by all regions to adapt better changing environment, as climate change is also expected to change interdependence patterns and levels.



## Reducing Mineral and Vitamin Deficiencies through Biofortification: Progress under HarvestPlus

**Howarth Bouis**

*Founding Director, HarvestPlus*

When HarvestPlus first started in 2003, there was much doubt among a range of stakeholders, that biofortification would work. Over the last 15 years, biofortification research has demonstrated broadly that (Saltzman *et al.*, 2013):

- Conventional breeding can add extra nutrients in the crops without reducing yields.
- When consumed, the increase in nutrient levels can make a measurable and significant impact on human nutrition.
- Farmers are willing to grow biofortified crops and consumers to eat them.

The final task is to mainstream biofortification as central activities of a number of organizations.

### Crop Development and Release

First, we had to prove to the plant science community that higher target levels iron, zinc, and provitamin A could be bred into high-yielding, profitable varieties. The crop development work focused on breeding crops with the desired nutrient levels, in collaboration with international research institutes and national research partners in target countries, to submit the best-performing varieties to national governments for release. Crop development research demonstrated that increased nutrient levels could be bred into crops without compromising yield, or other farmer-desired traits.

Biofortification research has greatly expanded the field of knowledge with regard to vitamin and mineral heritability and mechanisms of mineral loading in rice and wheat grain, in particular. Advances in genomic research, such as the identification of the alleles for lycopene epsilon cyclase (*lycE*) and beta-carotene hydroxylase 1 (*crtrB1*), which substantially affect the accumulation of beta-carotene in grain, have allowed plant breeders to use marker-assisted selection to more efficiently breed for the nutrient traits (Hares *et al.*, 2008). Coupled with

breakthroughs in high throughput screening technologies, like XRF and NIRS, these advances reduce the time to market for current and future biofortified varieties. Breeding pipelines at CGIAR centers and NARS are filled, with next wave varieties to be released in the near future and even better varieties in development.

Presently, over 150 varieties across twelve biofortified crops have passed the agronomic tests of varietal release committees in 30 developing countries. They are in multi-location testing in an additional 25 countries.

### Human Nutrition

Second, the nutrition community questioned the efficacy of biofortified crops – would the levels of retained nutrients and absorption be high enough? HarvestPlus has commissioned fourteen efficacy trials, all undertaken in developing countries. While four of these studies are still in process, there is already sufficient positive published evidence for iron and provitamin A, that the World Health Organization is now undertaking a systematic review of the evidence. This review will be completed and findings published by the WHO in 2017.

Bioavailability and efficacy studies have tested the effects of biofortified crops when consumed. Vitamin A nutrition research found efficient conversions from provitamin A to retinol, in most cases, more efficient than originally estimated. Efficacy studies have demonstrated that increasing provitamin A intake through consuming vitamin A-biofortified crops results in increased circulating beta-carotene, and has a moderate effect on vitamin A status, as measured by serum retinol. Consumption of orange maize specifically has been demonstrated to improve total body vitamin A stores as effectively as supplementation (Gannon *et al.*, 2012). Nutritionists agree that vitamin A biofortified crops have the potential to improve vitamin A status in deficient populations, but additional research is needed, using

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other, more sensitive biochemical indicators, as well as functional indicators, to fully understand the health impact of consuming biofortified foods.

Iron nutrition research demonstrated the efficacy of biofortified iron bean and iron pearl millet in improving the nutritional status of target populations (Finkelstein *et al.*, 2015). Iron studies also suggest that reducing absorption inhibitors, such as polyphenols and phytic acid, may further improve the efficacy of iron beans, in particular, and research in this area continues. Zinc studies have demonstrated that biofortified zinc wheat is bioavailable, and initial results of zinc wheat efficacy studies are promising. Because plasma zinc concentration, the biomarker widely used to estimate zinc status, has limitations in measuring changes in dietary zinc, foundational research to identify and test more sensitive biomarkers is underway. These biomarkers will be tested in the zinc rice efficacy trial scheduled for late 2016.

### Scaling Up

Third, could the adoption of biofortified crops by farmers be scaled up, and a public health impact demonstrated? We are not there yet on the measurement of a public health impact at a national scale, but for example, introduction of orange sweetpotato in Mozambique showed substantial reductions in diarrhea among children (Jones *et al.* 2015). Just four years after release of high-yielding, iron bean varieties in Rwanda, we now have rigorous evidence that 30% of farmers in Rwanda are growing biofortified beans on a regular basis. We estimate that 20 million farmers and consumers presently grow and consume biofortified crops.

HarvestPlus and its partners believe that biofortified crops can and must be delivered through the same seed system through which farmers typically obtain seed. Each country and seed system presents its own challenges for integrating biofortified crops. Many lessons, however, apply across the spectrum of seed and market systems. These include: multiplication of a sufficient amount of planting material is a crucial first step; integrating biofortified crops into sustainable value chains, as well as creating knowledge and demand, are essential to scaling; and partnerships are the future.

Without planting material, whether seed, vines, or stem cuttings, there are no biofortified crops. In these early years of implementation, HarvestPlus and its partners have focused on strengthening capacity and reducing risk to ensure that planting material is available

for farmers. In most countries, HarvestPlus has worked closely with NARS to ensure that sufficient breeder and foundation seed is available for the production of certified seed by cooperatives or the private sector. In countries with robust private seed systems that reach smallholder farmers, private seed companies are a natural partner, which is particularly advantageous in crops where hybrid seeds predominate (e.g. Seed Co. in Zambia (hybrid maize) and Nirmal Seeds in India (hybrid pearl millet) and where seed companies operate regionally).

Demonstrations and trials have been key demand drivers at the farm level. Seed delivery, in most cases, has led with small promotional seed packs, which allow interested farmers to try the new product without taking on a great deal of risk in cultivating a crop for which the market has not yet been tested.

Particularly for vitamin A crops, which differ in colour from their non-biofortified counterparts, farmers need to see and taste the yellow or orange product to believe. To improve acceptability of the colour of vitamin A crops, HarvestPlus and its partners have used community opinion leaders and tasting fairs to pique interest. Nutrition messaging aimed at both men and women has also been key, and in general, involving women farmers has been key to increasing demand for biofortified crops. While many biofortified crops are acceptable to farmers and consumers without further information about their nutrition traits, nutrition information helps ensure that the biofortified foods are integrated into child diets.

### Mainstreaming

In short, we have proven that biofortification works.

To reach its full potential, biofortification must be integrated as a core activity within a range of global institutions. This will require three critical elements.

*Demand:* Both rural and urban consumers come to see the value of, and demand, high mineral and vitamin content in their staple foods.

*Policy:* A wide range of national and international public officials come to recognise the significant impact of biofortification for improving and sustaining public health, as well as the high economic return to investments in biofortification and the legitimacy conferred by international recognition (especially by standards bodies).

*Supply:* Agricultural research entities, both public and private, come to recognise high mineral and vitamin content as core plant breeding objectives; varietal release committees make minimum levels of minerals and vitamins a requirement for approval for release (in addition to the standard agronomic traits, such as high yield).

### Vision

Our vision is that the quality of food staples globally will be continuously improved as a matter of routine, not just with iron, zinc, and provitamin A, but eventually with additional minerals, vitamins, and compounds as well. Not only do crop yields need to be increased to feed a growing population under serious land and water constraints in the context of climate change, but to nourish these populations properly, the density of minerals, vitamins, and other compounds in crops needs to be increased. The science is there to accomplish all this quite cost-effectively, if the investments are made now.

HarvestPlus has set an ambitious target of having one billion people benefit from biofortified crops by the

year 2030. HarvestPlus intends to provide leadership in this effort over the next decade in one of the largest nutrition interventions ever undertaken.

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## From Industrial Agriculture to Diversified Agroecological Systems

**Emile Frison**

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Today's food and farming systems have succeeded in supplying large volumes of foods to global markets, but are now generating negative outcomes on multiple fronts. Many of these problems can be linked specifically to 'industrial agriculture', i.e. the industrial-scale feedlots and uniform crop monocultures that dominate agricultural landscapes, and rely on chemical fertilizers and pesticides as a means of managing agro-ecosystems. This form of agriculture is associated with widespread degradation of land, water and ecosystems; high greenhouse gas (GHG) emissions; biodiversity losses; persistent hunger and micro-nutrient deficiencies alongside the rapid rise of obesity and diet-related diseases; and livelihood stresses for farmers around the world.

### What is Keeping Industrial Agriculture in Place

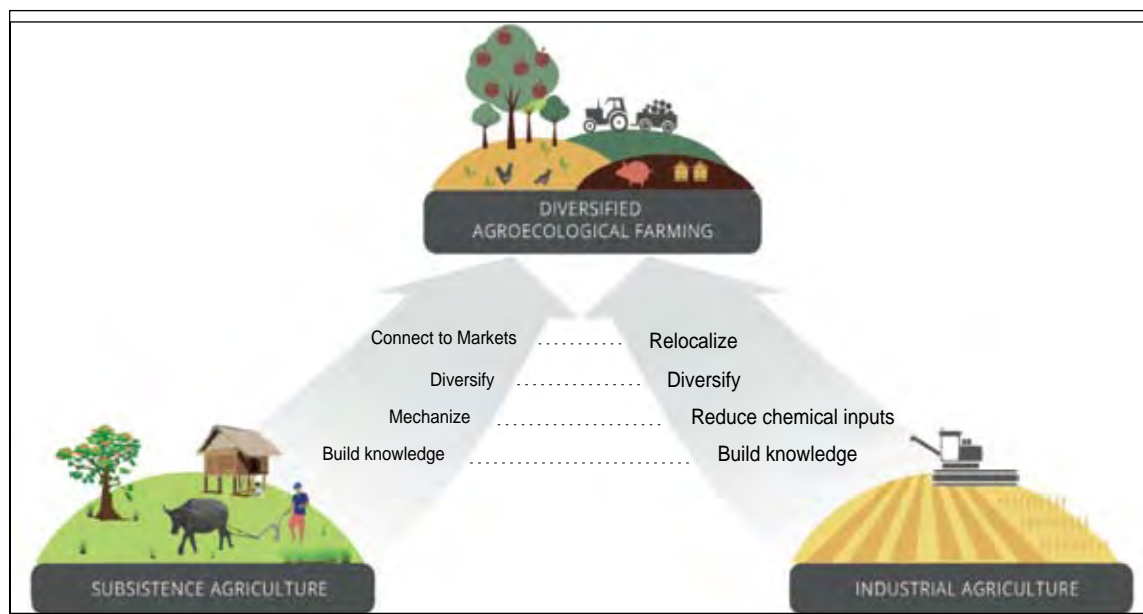
Eight 'lock-ins' can be identified, referring to the key feedback loops that characterise modern food systems and keep industrial agriculture in place:

#### *Lock-in 1: Path Dependency*

Industrial agriculture requires significant up-front investments, in terms of equipment, training, networks and retail relationships, and often requires farmers to scale up. Once these investments and structural shifts have been made, it is increasingly difficult for farmers to change course ('path dependency').

#### *Lock-in 2: Export Orientation*

As industrial agriculture has spread, generating abundant supplies of uniform, tradable crop commodities, trade has taken on disproportionate political importance. Specific supply chains (e.g. for animal feed, for processed food ingredients) have become increasingly export-oriented and export-dependent. Supporting these chains has often been prioritized over other interests (e.g. ensuring resources for local food production) and in spite of the risks and problems associated with export orientation and regional monocultures (e.g. price volatility,



*This paper is based on a report by the International Panel of Experts on Sustainable Food Systems (IPES-Food) published in June 2016: from uniformity to diversity: a paradigm shift from industrial agriculture to diversified agroecological systems (<http://www.ipes-food.org/>).*

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environmental degradation, competition for land) various policy measures have incentivized export orientation.

### ***Lock-in 3: The Expectation of Cheap Food***

Industrial agriculture and shifting consumer habits have helped to facilitate the emergence of mass food retailing, characterised by the abundance of relatively cheap highly-processed foods, and the year-round availability of a wide variety of foods. In many countries, consumers have become accustomed to spending less on food. In this context, farmers have received clear signals to industrialize their production in order to respond to the increasing demand for large volumes of undifferentiated commodities.

### ***Lock-in 4: Compartmentalized Thinking***

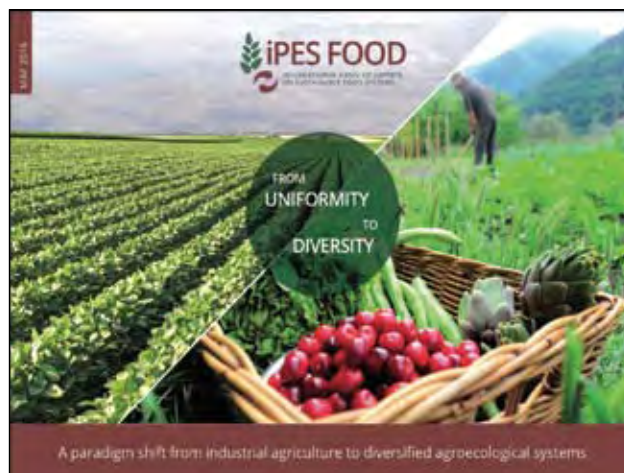
Highly compartmentalized structures continue to govern the setting of priorities in politics, education, research and business, allowing the solutions offered by industrial agriculture to remain at centre stage. Agricultural ministries, committees and lobbies retain a privileged position relative to other constituencies (e.g. environment, health) in setting priorities and allocating budgets for food systems. Increasingly privatized agricultural R&D programmes remain focused on the handful of commodities for which there is a large enough market to secure significant returns. Educational silos remain in place, and sectoral ‘value chain’ organizations share knowledge vertically (by product) rather than encouraging food systems approaches.

### ***Lock-in 5: Short-term Thinking***

Diversified agroecological systems offer major benefits for farmers and for society, however, the advantages will not be immediately visible, given the time needed to rebuild soil health and fertility, to increase biodiversity in production systems, and to reap the benefits of enhanced resilience. Unfortunately, key players in food systems are often required to deliver short-term results. Politicians are locked into short-term electoral cycles that encourage and reward policies that deliver immediate returns and publicly-traded agribusiness firms are required to deliver rapid returns to shareholders.

### ***Lock-in 6: ‘Feed the World’ Narratives***

Despite the fact that food security is recognized primarily as a distributional question tied to poverty and access to food, achieving food security continues to be framed



by many prominent actors as a question of how to ‘feed the world’, or in other words, how to produce sufficient calories at the global level. These narratives and approaches have been particularly prominent in the wake of the 2007-2008 food price spikes.

### ***Lock-in 7: Measures of Success***

The criteria against which farming is typically measured – e.g. yields of specific crops, productivity per worker – tend to favour large-scale industrial monocultures. Evidence in recent long-duration studies, suggest that diversified agroecological systems can compete well on productivity grounds. However, they are still disadvantaged by such comparisons. Diversified systems are by definition geared towards producing diverse outputs, while delivering a range of environmental and social benefits on and off the farm. Narrowly-defined indicators of agricultural performance fail to capture many of these benefits. Current systems will be held in place insofar as they continue to be measured in terms of what industrial agriculture is designed to deliver, at the expense of the many other outcomes that really matter to society.

### ***Lock-in 8: Concentration of Power***

The way food systems are currently structured allows value to accrue mainly to a limited number of actors, reinforcing their economic and political dominance, and thus their ability to influence the governance of those systems and the interests of these powerful actors converge around supporting industrial agriculture.

## **The Potential of Diversified Agroecological Systems**

In contrast to industrial agriculture, diversified agroecological farming can deliver simultaneous and mutually-reinforcing benefits for productivity, the environment and society. These alternative systems deliver strong and stable yields over time by building healthy ecosystems where different species interact in ways that improve soil fertility and water retention. They perform particularly strongly under environmental stress and deliver production increases in the places where additional food is most needed. These systems have major potential to keep carbon in the ground, increase resource efficiency and restore degraded land, turning agriculture from a major contributor to climate change to one of the key solutions. Diversified agriculture also holds to key to increasing dietary diversity at the local level, as well as reducing the multiple health risks from industrial agriculture (e.g. pesticide exposure, antibiotic resistance).

### **Recommendations: How to Shift the Centre of Gravity in Food Systems**

The IPES-Food report identifies a set of coherent steps that strengthen the emerging opportunities while simultaneously breaking the vicious cycles that keep industrial agriculture in place. Together, these steps must shift the centre of gravity in food systems, allowing harmful dependencies to be cut, the agents of change to be empowered, and alliances to be forged in favour of change.

#### ***Recommendation 1: Develop New Indicators for Sustainable Food Systems***

It is essential to adopt a broader range of indicators, covering long-term ecosystem health; total resource flows; sustainable interactions between agriculture and the wider economy; the sustainability of outputs; nutrition and health outcomes; livelihood resilience; and the economic viability of farms with respect to debt, climate shocks, etc.

#### ***Recommendation 2: Shift Public Support Towards Diversified Agroecological Production Systems***

Governments must shift public support away from industrial production systems, while rewarding the array of positive outcomes in diversified agroecological systems. Governments must implement measures that allow farms to diversify and transition towards

agroecology. In particular, they must support young people to enter agriculture and adopt agroecological farming – before they are locked into the cycles of industrial agriculture.

#### ***Recommendation 3: Support Short Supply Chains and Alternative Retail Infrastructures***

Governments should support and promote short circuits in order to make them a viable, accessible and affordable alternative to mass retail outlets, e.g. by repurposing infrastructure in cities to favour farmers' markets. More attention should also be paid to the role of informal markets and policy measures must be put in place that empower emerging initiatives linking farmers to consumers.

#### ***Recommendation 4: Use Public Procurement to Support Local Agroecological Produce***

Public procurement should be used with increasing ambition in order to ensure sales outlets for diversified agroecological farms, while providing fresh, nutritious food and diversified diets for the users of public canteens, particularly schoolchildren.

#### ***Recommendation 5: Strengthen Movements that Unify Diverse Constituencies Around Agroecology***

Governments can support farmers' groups, community-based organizations and social movements which encourage the spread of agroecological practices and advocate for sustainable food systems, and ensure the participation of diverse civil society groups from the global North and South in global governance processes and forums.

#### ***Recommendation 6: Mainstream Agroecology and Holistic Food Systems Approaches into Education and Research Agendas***

Public research agendas must be redefined around different priorities. Investments must be redirected towards equipping farmers to shift their production. The mission of university research should be redefined around the delivery of public goods. FAO and other international agencies should mainstream agroecology into all of their work, in order to spread existing knowledge and plug the remaining gaps in our understandings. Research conducted by the CGIAR Centres should be refocused around diversified agroecological systems and farmer participatory research.

***Recommendation 7: Develop Food Planning Processes and ‘Joined-Up Food Policies’ at Multiple Levels***

It is crucial to implement joined-up policymaking for food systems. Long-term, inter-ministerial planning – reaching across political boundaries and transcending electoral cycles – should be supported, building on landscape management and territorial planning initiatives, where food security can be meaningfully targeted and understood in terms other than ‘feeding the world’.

Crucially, food systems planning must be based on broad participation of various constituencies and groups with a stake in food systems reform. At the global level, the Committee on World Food Security (CFS) should advocate for coherent food policies and contribute to strengthening diversified agroecological food systems.

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[http://www.ipes-food.org/images/Reports/UniformityToDiversity\\_FullReport.pdf](http://www.ipes-food.org/images/Reports/UniformityToDiversity_FullReport.pdf)

## Animal Genetic Resources (AnGR) for Food Security

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Demand for livestock products is increasing against a background of rapid change in the agricultural sector, genetic erosion of indigenous livestock diversity and increasing impact of climate change.

In developed countries, improved livestock genetics associated with better husbandry—better feeds, health care and housing—have resulted in significant and sustained increases in livestock productivity. New technologies, including better recording of production traits, statistical models to predict genetic merit, genetic marker information in selection programmes (Goddard *et al.*, 2007; Clark *et al.*, 2013) and new reproductive technologies that speed up genetic gains (Kasinathan *et al.*, 2015) are leading to further productivity gains in the commercial sectors. This is not yet happening in developing countries (Marshall, 2014). The reasons are multiple, they include but are not limited to, a lack of understanding of the potential of indigenous livestock genetic resources for productivity improvement; investment prioritized other avenues of livestock development; heterogeneity of livestock systems and preferences of livestock keepers; lack of supportive policies, institutional arrangements and capacity to undertake genetic improvement (Kosgey and Okeyo, 2007; Rege *et al.*, 2011).

Indigenous livestock breeds have sustained human livelihoods for centuries (FAO, 2015). Today, they represent a treasure trove of diversity and adaptation that remains largely under-exploited (Okeyo *et al.*, 2015). The world faces the challenge to respond quickly yet sustainably to a rapid increase in demand for livestock products, while addressing the poverty and poor nutrition typically found in livestock-producing households. This will only be achieved rapidly through the understanding of the diversity and unique traits of the indigenous animal genetic resources and their utilisation in within breeds and crossbreeding improvement programmes as well as in breed replacements strategies.



**Indigenous village chicken diversity**

In this presentation, I will first put into context the diversity and adaptation of animal genetic resources (AnGR) in relation to its history of domestication and dispersion. I will present then the today on-going and expected future trajectories of the livestock sector (Smith *et al.*, 2013). In particular, I will present the three main trajectories as detailed in Smith *et al.* (2013) and their link with AnGR: (i) The “*rapid inclusive growth systems*”, which address the need to develop sustainable food systems that deliver key animal-source nutrients to the poor while facilitating a structural transition in the livestock sector of developing countries. In this trajectory the productivity as well as the adaptive traits of animal genetic resources are important. (ii) The “*high growth systems with externalities*”, a system which rely mainly on the development and the use of new technologies and high inputs. The diversity and adaptive traits of AnGR are playing here a minor role. (iii) The “*fragile growth systems*” which recognizes that rapid, market-focused growth will not be the trajectory for all livestock keepers. Indeed, in areas where productivity is severely limited by remoteness, harsh climates or environments, and/or by poor institutions, infrastructure and market access,

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Coat colour variation in African sheep (Ethiopia) (Photo credits: ILRI – Camille Hanotte)



Women and indigenous chicken, Oromia, Ethiopia

the emphasis needs to be to enhance the important roles of AnGR diversity and adaptation in the resilience of people and communities to environmental variability.

Examples of ongoing genome wide livestock characterisation project led by ILRI and its partners will be presented, including example of diversity and/or adaptive traits of AnGR that may be utilised for the benefit of livestock productivity and food security (e.g. cattle dairy sector, small ruminant, poultry). I will end by examining the possibilities of new breeding approaches (e.g. genomics selection), new technologies (e.g. gene editing) as shortcuts to exploit the diversity and adaptive traits of AnGR for immediate improvement of productivity.

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## Aquatic Genetic Resources and Technologies for Food and Environmental Security

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### Introduction

Aquatic genetic resources (AqGR) of farmed aquatic species and their wild relatives have been used and exchanged for millennia. In China evidence of fish farming is found over two thousand years ago; in ancient Rome marine species were held in special coastal enclosures for consumption and as an indication of wealth and status; European monks in the middle-ages farmed and transferred the common carp from its native range in Asia and the Danube River to many parts of Europe (Nash, 2011).

Today aquatic genetic resources continue to provide the world with food and recreation: the world's fisheries harvest over 2000 species including fish, crustaceans, molluscs, coelenterates, echinoderms and aquatic plants (FAO, 2014). The number of farmed aquatic species is smaller, but still extremely diverse (Table 1).

As capture fisheries reach the limit of their productive capacity, aquaculture will be called on to provide a greater share of the world's aquatic food. Aquaculture production is growing (Figure 1); it is the fastest growing food production sector globally and more aquatic species are being farmed today than ever before. However, for aquaculture to continue to provide food for a growing population, better use of and information on aquatic genetic resources will be needed.

The member countries (Members) of the Food and Agriculture Organization (FAO) realized these needs and tasked FAO, through the FAO Commission on Genetic Resources for Food and Agriculture (Commission) and the Fisheries and Aquaculture Department, with overseeing the production of the first *Report on the State of the World's Aquatic Genetic Resources for Food and Agriculture* (Report). This Report will complement the ongoing process of reporting on fisheries and aquaculture at the species level that is routinely done by Members

Table 1. Diversity of aquatic species (FAO FishStatJ, 2016)

Taxon	Wild species	Number of farmed species (number of families)
Finfish	31,000	359 (>90)
Molluscs	85,000	103 (27)
Crustaceans	47,000	61 (>13)
Other aquatic animals	**	15 (>8)
Aquatic plants	13,000	~37 (>22)
Total	180 000	575

\*\*These include echinoderms, coelenterates and tunicates too numerous to list, many of which have no potential as food and are all marine species, as well as a few amphibian and reptiles.

and summarized in FAO's flagship publication, the *State of World Fisheries and Aquaculture* (see for example FAO, 2014). Members are currently preparing country reports<sup>1</sup> on *inter alia*, the status of their AqGR, and the drivers, policies, threats and opportunities related to them. A draft Report has been made publicly available<sup>2</sup> and this present document describes early results of the process as countries continue to submit their country reports to FAO. It is hoped that the process and the country reports will help countries use and conserve AqGR.

### Methodology

Information was collected from the FAO fishery statistics database (FishStatJ<sup>3</sup>) and from the country reports submitted by Members. A total of 47 country reports were analysed for this preliminary assessment.

<sup>1</sup>Country reports are based on an on-line questionnaire available in multiple languages at <http://www.fao.org/fishery/AquaticGeneticResources/en>

<sup>2</sup><http://www.fao.org/fi/static-media/MeetingDocuments/AqGenRes2016/Inf2e.pdf>

<sup>3</sup><http://www.fao.org/fishery/statistics/software/fishstatj/en>

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## Results and Discussion

Unlike terrestrial agriculture in which farmers have been using hundreds of useful breeds and varieties for thousands of years, the domestication of aquatic species in aquaculture became more widely practiced only during the last century (Nash, 2011). The common carp is a notable exception to this as it was domesticated centuries ago (Balon, 1995). None the less, aquaculture is the fastest growing food production sector and is expected to play a major role in providing seafood in the future as production from capture fisheries has plateaued (FAO 2014; Fig. 1). Currently about 50% of the seafood we eat comes from aquaculture.

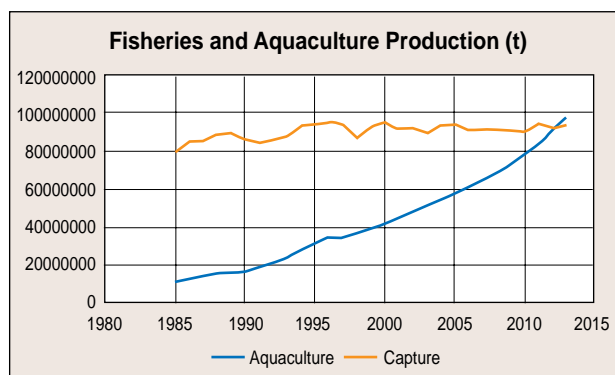
The FAO database<sup>4</sup> contains over 500 species items from inland, marine and coastal waters (Table 2) derived from two kingdoms and over four phyla (chordata, mollusca, arthropoda and echinodermata).

However, information in the FAO database is largely at the species level. The FAO standard for reporting statistics, the Aquatic Sciences and Fisheries Information System (ASFIS), does not have the capacity to register information on stocks, strains, polyploids, or other genetically altered farmed types of aquatic species. ASFIS does contain certain hybrids however.

Analysis of the country reports revealed that more species and more farmed types, e.g. hybrids, are being farmed than have been previously reported to FAO. For example, the ASFIS currently includes 11 hybrid species items and additional hybrids reported in country reports included:

- *Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans*, (and reciprocal crosses) and *Pseudoplatystoma reticulatum* × *Phractocephalus hemiliopterus* from Brazil;
- *Oreochromis mossambicus* × *O. niloticus* from the

<sup>4</sup> <http://www.fao.org/fishery/collection/asfis/en>



**Fig. 1. Fisheries and aquaculture production as reported to FAO**

Philippines;

- *Epinephelus lanceolatus* × *E. coides*, *E. coides* × *E. fuscoguttatus*, *E. lanceolatus* × *E. fuscoguttatus* from Viet Nam and Malaysia;
- *Onchorhynchus mykiss* × *O. masou* from Japan;
- *Barbonymus gonionotus* × *B. schwanefeld* and *Clarias batrachus* × *C. microcephalus* from Thailand;
- *Channa micropeltes* × *C. striata* from Lao PDR;
- *Patinopecten caurinus* × *P. yessoensis* from Canada.

Further analysis revealed that introduced species play a large role in aquatic food production from aquaculture. Aquaculture is the main reason for the deliberate introduction of aquatic species (Bartley and Casal, 1998). From the country reports about 200 species items were reported cultured in countries where they are non-native and there were over 300 reports of countries farming non-native species. In fact, the most commonly farmed species are farmed in more non-native areas than where they are native (Table 3).

Although the country reports listed several types of genetic improvement being applied to farmed aquatic species, the wild type was the most common farmed type reported. Additionally, the collection of seed, early

**Table 2. Number of farmed aquatic species**

Taxa/Region	Africa	Americas	Asia	Europe	Oceania
Finfish	81	119	194	122	30
Molluscs	16	41	31	35	21
Crustaceans	14	19	39	20	17
Other animals	3	4	11	7	1
Plants	8	11	23	14	3
Total – all aquaculture taxa	122	194	298	198	72

**Table 3. For the most commonly farmed species reported, the number of countries where the farmed species is native and introduced**

Species	Native	Introduced
<i>Oncorhynchus mykiss</i>	2	16
<i>Cyprinus carpio</i>	4	4
<i>Macrobrachium rosenbergii</i>	5	8
<i>Penaeus vannamei</i>	8	9
<i>Oreochromis niloticus/spp</i>	5	23
<i>Clarias gariepinus</i>	5	6
<i>Kappaphycus alvarezii</i>	3	7
<i>Crassostrea gigas</i>	1	9

life history stages and broodstock from the wild was reported in 85% of the responses. Thus, AqGR in the wild, e.g. lakes, rivers, rice fields, estuaries, seas and oceans contribute greatly to aquaculture production.

The wild relatives of farmed aquatic species contribute to capture fisheries as well as providing resources for aquaculture, thus demonstrating the close relationship between farming and fishing aquatic genetic resources. However, about 1/3 of the capture fisheries that are based on wild relatives were reported to be declining. Loss of habitat, pollution and climate were the three top reasons cited for the decline in capture fisheries. As the sector advances and technologies for genetic improvement become easier and less costly, genetically improved farmed types will become more common, but the wild AqGR and their habitat must still be protected and conserved.

Genetic information and technologies are useful, but are not commonly being used in fisheries and aquaculture. Although there are examples of genetic information being used for the development and management of some important species, these examples are usually of commercially important species in developed countries. Countries reported that genetic information is used not at all or to only a minor extent in 75% of the species being farmed; about 2/3 of the capture

fisheries reported on used no genetic information in fishery management. Tremendous potential exists to use genetic information and technologies for increased food production, livelihoods and poverty alleviation. The need for seafood is expected to increase by about 2%/year; genetic improvement through selective breeding can provide increases of 5-12%/year. However, there will be challenges in implementing genetic improvement and management in many areas.

Aquaculture is already one of the most efficient and cost effective means to produce high quality protein. With the diversity of species and habitats available to grow aquatic species, new strains and species can be farmed to increase the global food supply in a responsible and sustainable manner. For aquaculture to reach its full potential consumers, industry and governments must be aware of the opportunities and risks associated with genetic technologies. Policies and risk/benefit analysis must be put in place to ensure that aquaculture provides high quality food, efficiently produced in an environmentally and socially acceptable manner now and in the future.

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## Microbial Diversity of Extreme Regions: An Unseen Heritage and Wealth

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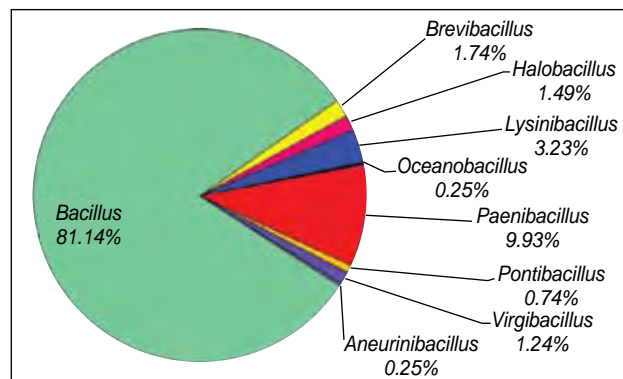
Extreme environments represent a unique ecosystem which harbour novel microbial flora. India is one among 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world. Thermophiles from hot springs can be a source for enzymes that are active at high temperatures. They can also be used for decomposition process. Psychrophiles can be a source of anti freezing compounds. Halophiles and osmophiles can be a source of genes coding for osmolytes and can be used for the development of transgenic plants tolerant to salt and drought stress.

Different surveys were carried out to collect soil, plant and water samples for analysis of microbial diversity from the cold desert of Leh and Rohtang, from mangroves of Sunderbans, West Bengal and Bhitarkanika, Orissa; thermal springs of Rajgir, Manikaran, Bakreshwar, Balrampur and Vashisht, hypersaline soils of Rann of Kutch, Pulicat lake (Tamil Nadu), Sambhar salt lake and Chilka lake; acidic soils of Manipur, Kerala, Meghalaya and Mizoram for isolation of microbes growing under extreme conditions. All the collected isolates were characterised through biochemical analysis for PGP traits, and identified on the basis of 16s rRNA gene sequencing. Many novel genera and species not reported from country were identified. A database of predominant genera was developed and base line information generated for extreme environments in the country.

During these surveys a total of 1,540 bacteria, 157 archaea, 260 fungi and 200 actinomycetes were isolated and the occurrence of *Bacillus*, *Bacillus* derived genera and other predominant genera are depicted in Fig 1.2 (Yadav *et al.*, 2015). The archaeal and bacterial isolates were further screened for molecular and functional diversity. A huge database comprising microbes tolerant to high salinity, low and high temperature, drought and low pH was generated.

Microorganisms isolated from extreme environments can be developed as inoculants for crops grown in these

stressed environments. Eubacteria and archaea isolated from saline habitats were used as inoculants to alleviate salt stress and to influence the growth and yield of crop plants. Bacterial isolates obtained from Sambhar salt lake and capable of exhibiting plant growth promoting traits at high salt concentration improved the growth of wheat in saline soils. Inoculation induced significantly higher proline, total soluble sugar accumulation in plants and reduced Na:K ratio (Upadhaya *et al.*, 2012). Likewise, a preliminary investigation on archaea isolated from hypersaline environment of Rann of Kutch, Gujarat, India suggests its role in supporting the growth of plants in saline soils. Among 157 archaeal isolates, twenty exhibited phosphate solubilization both in plates and broth. The ability of archaeal isolates to solubilise tricalcium phosphate ranged from  $51.92 \pm 0.8$  to  $1986.86 \pm 1.8 \mu\text{g mg}^{-1}$  protein, the maximum being produced by *Halococcus hamelinensis* strain IARI-SNS2 ( $1986.9 \text{ mg l}^{-1}$ ) followed by *Natrinema* sp. strain IARI- WRAB2 ( $1341.5 \text{ mg l}^{-1}$ ) (Yadav *et al.*, 2015). In addition archaeal isolates solubilizing potassium, capable of producing IAA, Zeatin, gibberellic acid and siderophore at high salt concentration were reported for the first time. Acid tolerant bacteria isolated from soils of Kerala were used as inoculant to improve the growth of horticultural crops like coconut, areca nut, cocoa and vanilla. Psychrophilic bacteria isolated from Leh and Rohtang Pass significantly



**Fig. 1. Abundance of different *Bacillus* and *Bacillus* derived genera in the samples surveyed (Source: Yadav *et al.*, 2015)**

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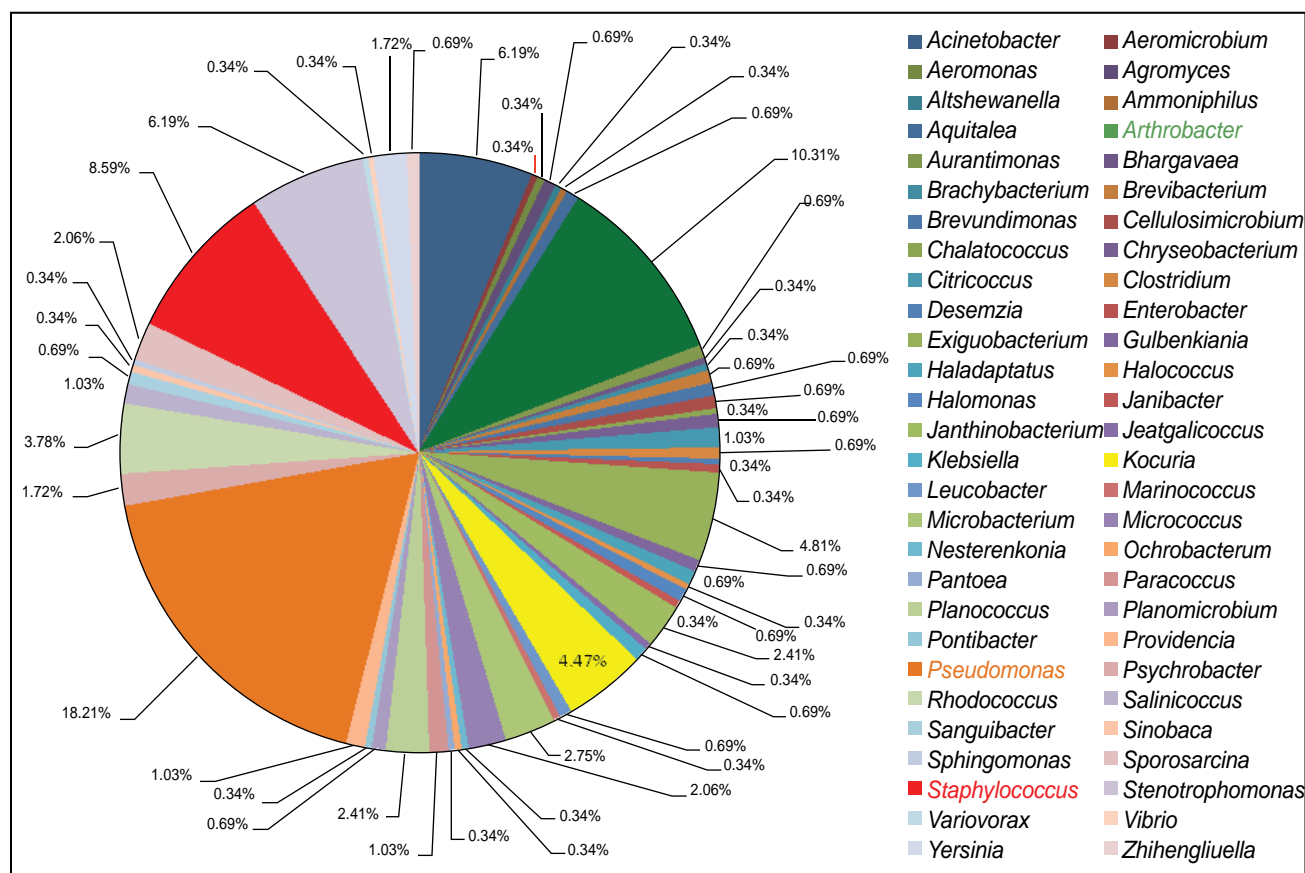


Fig. 2. Abundance of other predominant bacterial genera in the samples surveyed (Source: Yadav *et al.*, 2015)

influence the growth of wheat on inoculation (Verma *et al.*, 2015).

Low temperature tolerant microbial strains were screened for production of lignocellulolytic enzymes active at low temperature and a consortia consisting of two bacteria (*Bacillus atropheus* and *Bacillus* spp) and two fungi (*Eupenicillium crustaceum* and *Penicillium citrinum*) was developed for the preparation of compost at low temperature (4 to 10°C) (Shukla *et al.*, 2016). Psychrophilic bacteria are a source of cold active enzymes. Psychrotrophic microbes isolated from samples collected from high mountain pass (Khardungla and Rohtang), rivers (Indus, Zaskar, I-Z confluence and Beas) and sub-glacial Lakes (Pangong, Dashair and Chandratat) of Indian Himalayas were found to produce amylase,  $\beta$ -glucosidase, pectinase, protease, cellulase, xylanase,  $\beta$ -galactosidase, laccase, chitinase and lipase enzymes active at 4°C (Yadav *et al.*, 2016). These cold active enzymes have importance for both industrial and agricultural application. Psychrophilic bacteria capable of producing anti freeze proteins could be used to

counter ice nucleation protein produced by bacteria like *Pseudomonas syringae* and *Erwinia herbicola* and thereby protect the plants from frost damage. Within the last two decades, antifreeze proteins have been found in variety of bacteria from cold habitats (Wilson and Walker 2010; Kawahara *et al.*, 2007).

The microbes obtained from extreme environments are a good source of enzymes to be used in agriculture and industry. Many of the isolates were found to produce, cellulases, amylases and proteases (Sahay *et al.*, 2012). Candidate genes for abiotic stress tolerance were amplified from many extremophiles and can be used for the development of transgenic plants tolerant to salt, drought or temperature stress (Kumar *et al.*, 2013). The gene *dnaK* from *Bacillus pumilus* strain B3; superoxide dismutase (*sodD*) gene from *Halobacillus* sp. and cyanobacteria *Lyngbya* sp.; pyrroline-5-carboxylate reductase gene from *Bacillus pumilus*; genes for glutamate dehydrogenase, ectoine biosynthesis and sorbitol dehydrogenase from *Oceanobacillus iheyensis*; mannitol-1-phosphate-5-dehydrogenase gene from

*Bacillus licheniformis*; genes for trehalose-6-phosphate synthase (TPS), 2-Cys peroxiredoxin (Prx2), hydroxy methyl butenyl diphosphate reductase (HDR), chloroplast lycopene beta-cyclase (LCYB), duplicated carbonic anhydrase (DCA1) from *Dunaliella viridis* were successfully amplified, cloned, sequenced and expressed in heterologous strain.

The database of microbes from extreme environments is a resource that can be tapped as and when required for screening of different molecules, enzymes, genes and alleles.

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## Agrobiodiversity and Achieving the Zero Hunger Challenge

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Agrobiodiversity provides the basic building blocks for food, livelihood and health security. The Svalbard Gene Vault provides an opportunity for preserving, a representative sample of existing agrobiodiversity for posterity. We need more initiatives of this kind so that agrobiodiversity will be available at all times and for all.

The most urgent task today is the conservation of diversity and pluralism in all living organisms ranging from microbes to man. In particular, we need to promote gene banks for a warming planet. In this connection, the following three kinds of community gene banks developed by MS Swaminathan Research Foundation (MSSRF) are worthy of replication:

1. *Genetic garden of halophytes* – this is to preserve gene pools for breeding crop varieties tolerant to sea water in coastal areas, as a part of anticipatory research to face the challenge of sea level rise.
2. *Genetic garden of biofortified plants* – species like *Moringa*, sweet potato, etc. This is for helping to find agricultural remedies for prevailing nutritional maladies, in particular micronutrient deficiencies like vitamin A, vitamin B<sub>12</sub>, zinc, iron, etc.
3. *Farm gene bank for the in situ conservation* – these will help agrobiodiversity relevant to promoting a climate smart agriculture.

The above three types of community gene banks may serve to illustrate the need and opportunities for conserving agrobiodiversity for launching an era of biohappiness. At the same time, there is need for greater emphasis on developing sustainable water security systems.

### Malnutrition Burden

According to available data the malnutrition burden tends to be high in tribal areas. This is an enigma since such areas are rich in agrobiodiversity. An example is Attappadi, Kerala, where tribals are suffering from several nutritional deficiencies. An opportunity is now

available for converting this malnutrition hot-spot into a leader of the nutri-farm movement. The Government of Kerala has decided to distribute 1800 acres of fertile land to 1000 landless tribal families in Attappadi. Land confers multiple benefits such as food, work and income. The challenge now is to integrate nutrition with land use. These landless tribal families to whom land is being given can be trained to include in the farming system crops which will help to address specific nutritional disorders. The former Finance Minister Mr P Chidambaram had provided 200 crore in 2013-14 budget for establishing nutri-farms. Unfortunately this programme was not implemented. The Attappadi opportunity therefore should be taken to introduce specific agricultural remedies to overcome the nutritional maladies of the area. For example, *Moringa*, jackfruit, orange flesh sweet potato, amla, quality protein maize and many such crops can help to improve the nutritional status of the malnourished families. I therefore suggest that the distribution of land to the tribal families may be accompanied with assistance for converting a part of the land into a nutri-farm which will help to banish in a short time both protein and hidden hunger. Thus, Attappadi can become the flagship of the nutri-farm movement and erase its current image as a malnutrition hot-spot. I hope this unique opportunity will not be missed.

### Culinary Diversity and Agrobiodiversity

Among the methods of protecting community knowledge and technologies under World Trade Organisation (WTO), Geographic Indication (GI) is an important one. UNESCO's World Heritage Site and FAO's Globally Important Agricultural Heritage System (GIAHS) are other forms of recognition of the conservation ethos of local communities. In this context, the Government of Paschim Bangla has done well in seeking GI protection for *Rasagulla* (Indian sweet). Odisha feels that it has also a claim on *Rasagulla* from the point of view of GI. There is need for according recognition to communities which have conserved agrobiodiversity, i.e., biodiversity

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used for various human needs. Such diversity can take the form of cultural, culinary and curative (i.e. medicinal plants) diversity. Thanks to the work of MSSRF, the agrobiodiversity based farming systems in the Koraput region of Odisha and the below sea level rice cultivation of Kuttanad in Kerala have both received GIAHS recognition from FAO. We should make similar efforts to get global recognition for the work of rural and tribal communities in areas of significance to human quality of life.

### **Nobel Prize in Medicine 2015: Importance of Conservation of Curative Agrobiodiversity**

Dr Tu Youyou, China along with Dr Satoshi Omura and Dr William Campbell are the three medical professionals who have been chosen for this year's Nobel Prize in Medicine for discovering "therapies that have revolutionised the treatment of some of the

most devastating parasitic diseases". Dr Tu developed Artemisinin a drug that reduces the death rates from malaria. The interesting feature of this drug is its herbal origin. The sweet wormwood plant *Artemisia annua* was its main source. Dr Tu has been working on herbal medicines and I am happy that her work has led to a significant medical contribution. This emphasises the need for the conservation and study of medicinal plants belonging to the category of curative agrobiodiversity. AYUSH (Ayurveda, Yoga and Naturapathy, Unani, Siddha and Homoeopathy) in our country should help to intensify work in this field. I know several other drugs of herbal origin which are under advanced stages of testing including a drug for HIV/AIDS. More support for the conservation and scientific analyses of medicinal plants will be of great value in preparing drugs which are not only effective, but also economical and safe.

## Biodiversity Use for Food Security

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The origin and survival of our civilization depends upon agricultural biodiversity. We are the guests of green plant on this planet. I fondly remember a sticker on the bumper of a car that I once read, "Have you thanked the green plants today?" Approximately 250,000 plant species have been identified and described to date. More than 30,000 are edible and 7000 have been used as food. More than 300 are cultivated for food and feed at present.

The grass family, Poaceae is the most important source of food for feeding the world. Its three species, namely rice, wheat and maize provide 50% of the calories consumed by world population. Rice is the staple food of half of the world population. Genus *Oryza* to which cultivated rice belongs is distributed in four continents, e.g. Asia, Africa, Australia and Latin America. It has two cultivated and 20 wild species. Asian cultivated rice, *O. sativa* is grown on all continents. African cultivated rice, *O. glaberrima* is grown on limited areas in the West Africa.

Genetic relationships between *O. sativa* and wild species have been investigated thoroughly and various genomes have been designated based on crossability and chromosome pairing in  $F_1$  hybrids. Thus, both cultivated and six wild species have AA genomes. Wild species with AA genomes are easy to cross with cultivated rice and useful genes for disease and insect resistance have been transferred to cultivated rice. Species with BB, CC, BBCC, CCDD, EE and FF genomes can be crossed with cultivated rice and  $F_1$  hybrids can only be obtained through embryo rescue. Very useful genes for disease and insect resistance have been transferred to cultivated rice from these species. These include resistance to brown plant hopper, white backed plant hopper, blast and bacterial blight. Some of the progenies from the cross of *O. sativa* and *O. officinalis*, with resistance to brown planthopper were released as varieties in Vietnam. Monosomic alien addition lines (MAALs) having full chromosome complement of cultivated rice and one chromosome of a wild species have been selected from

these interspecific crosses. These MAALs are very useful source of variation for rice improvement.

Asian cultivated rice *O. sativa* is very diverse species. It is grown from below sea level in Kerala to seven thousand feet above sea level in Nepal and between 47° north latitude in China and 35° south latitude in Australia and Chile. Moreover, it is adapted to growing under diverse ecologies, from deep-water to uplands. Perhaps more than 250,000 rice varieties exist that have been preserved in the world's gene banks. Three of the largest collections are in genes banks of IRRI Philippines, India and China. Almost all other rice-growing countries have rice gene banks.

Kato *et al.* (1928) recognised two varietal groups of rice i.e. indica and japonica. This classification agreed with empirical distinction Chinese people had recognized since ancient times between two types of rices called 'Hsien' and 'Keng' that correspond to indica and japonica respectively. Matsuo (1952) classified rice into three groups later referred to as indica, japonica and javanica. Oka (1958) suggested that japonica and javanica should be considered as temperate and tropical ecotypes of single group. Glaszman (1987) studied allelic variation at 15 polymorphic loci coding for eight enzymes in 1,688 traditional rice cultivars from Asia. He divided the varieties into 6 groups on the basis of multivariate analysis of data. Group 1 varieties found throughout tropical Asia are typical indica types. Group II includes short duration varieties of Eastern India and Bangladesh locally called aus. Groups III and IV include photoperiod-insensitive and photoperiod-sensitive deep-water rices of Bangladesh respectively. Aromatic rices of Indian subcontinent belong to group V. Group VI has temperate japonicas of Northern China, Japan, Korea and tropical japonicas distributed along with indicas in Thailand, Vietnam, Philippines and Indonesia (Table 1).

During green revolution, there has been massive exchange of germplasm internationally. Rice varieties

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**Table 1. Distribution of rice varietal groups**

Varietal group	Distribution
Group I Indica	Tropical Asia
Group II Aus	Eastern India, Bangladesh
Group III Photoperiod insensitive	DW Bangladesh
Group IV Photoperiod sensitive	DW Bangladesh
Group V Aromatic	Indian subcontinent
Group VI Temperate Japonica	North China, Japan, Korea
Tropical Japonica	Thailand, Philippines, Indonesia

belonging to different groups have been used in varietal improvement programmes. As an example IR 36 has 10 parents; eight indica, one tropical japonica and one wild species. Similarly IR 64 has 20 parents, 16 indica, one temperate japonica, two tropical japonica and one wild species. Two widely used genes for bacterial blight resistant–*Xa5* and *Xa21* were derived from an aus variety DZ192 and wild species *O. longistaminata*, respectively. Thus traits from different varietal groups and wild species have been combined to develop high yielding varieties with disease and insect resistance, improved grain quality and adapted to diverse growing conditions. Improved varieties have also been developed

from crosses of two cultivated species of *Oryza*, e.g. NERICA rices in Africa. These modern varieties are now grown on 90% of world's riceland. Wide scale adoption of these varieties has led to unprecedented increases in rice production and world food security. For example, world rice production increased from 200 million tons in 1960 to 720 million tons in 2015 and price of rice is 50% lower than what it was during 1960s. Poor people who spend 50-60% of their income on food have greatly benefited from low price of rice. Thus, genus *Oryza* is an excellent example of use of biodiversity for the world food security.

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## Exploring the Benefits of a Systems-based Approach to Plant Genetic Resources Conservation

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Plant genetic resources make essential contributions to increasing food security and nutrition, improving rural livelihoods, supporting the maintenance of ecosystem services (Frison *et al.*, 2011; FAO/PAR, 2011) and to meeting the challenges of adapting to climate change (Hodgkin and Bordon, 2012; Jarvis *et al.*, 2015). Ensuring that plant genetic resources can make these contributions requires that they are conserved for present and future generations in the face of continuing genetic erosion in many production systems, as a result of the expansion of more uniform cultivars and the spread of intensive agricultural practices (IPES Food, 2016).

Over the past sixty years a variety of ways of ensuring the long-term conservation of plant genetic resources have been developed. These include: *ex situ* and *in situ* conservation programmes and a range of national and international policies and collaboration. These programmes have been supported by a growing body of knowledge of the many different aspects of conservation of different types of plant genetic resources, and an increasing capacity to undertake conservation activities. The reports by FAO of the State of the World's Plant Genetic Resources for Food and Agriculture (FAO, 1996; 2010) document the developments that have occurred in conservation and use of plant genetic resources while the different Global Plans of Action set out perceived needs and priorities for future conservation efforts (FAO, 2012). The global commitment to, and recognition of the importance of, plant genetic resources conservation is reflected in the entry into force in 2004 of the International Treaty on Plant Genetic Resources for Food and Agriculture, which currently has 140 contracting parties ([http://www.planttreaty.org/list\\_of\\_countries](http://www.planttreaty.org/list_of_countries)).

Despite considerable efforts by international and national organizations, there are still considered to be substantial failings in our efforts to effectively conserve and make available plant genetic resources and the

genetic diversity they contain. It has been reported that the genetic diversity present in many species and crops is not adequately conserved (Padulosi *et al.*, 2002; Ford Lloyd *et al.*, 2011), that the continuing contributions by farmers growing traditional varieties of crops are not recognized and understood (e.g. Chapter 12 in Jarvis *et al.*, 2016; Zimmerer, 2003), that the links between *ex situ* and *in situ* conservation programmes are poor (Brush, 1995) and that, overall, the resources for conservation remain inadequate (Gepts, 2006).

The conservation of plant genetic resources involves a number of different activities undertaken by a wide variety of actors with often divergent interests, concerns and objectives (Baker *et al.*, 2013). The many different elements and activities that contribute to the conservation and use of plant genetic resources can be thought of as constituting a more or less integrated and dynamic system (Hodgkin *et al.*, 2013). Systems have been described as sets of interconnected elements and processes that result in a particular function or set of functions. Systems change over time in response to developments and changes in external forces, and themselves influence their external environment.

Exploring the systems features of plant genetic resources conservation involves investigating: (a) the connections between the elements, activities and processes (either distinct or overlapping) that support or influence conservation and use; (b) the different institutions and actors involved; and (c) the ways in which the operations of the system contribute to the maintenance and flows of materials and knowledge. A systems approach can help evaluate the extent to which a system is achieving its objectives. One systems approach, dynamic systems modelling, has been used to explore the food system and the ways in which diversity can contribute to desired food security and nutrition outcomes (Allen *et al.*, 2014; Allen and Prosperi, 2016). Systems

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approaches have also been used to explore aspects of biodiversity conservation.

In this paper we use systems perspectives to explore the features of plant genetic resources conservation efforts with a primary focus on the national or country level. We consider the processes, linkages and relationships that operate in the conservation system and the different actors involved. Our objective is to explore alternative ways of investigating the strengths and weaknesses of current conservation efforts with a view to identifying possible improvements.

### Elements of Systems Approaches

Systems approaches usually include a consideration of:

- The boundaries of the system – what is considered to be inside or outside the system of interest (which may vary depending on the perspectives of the observer);
- Inter-relationships or connections between the processes involved rather than just the different elements themselves; and
- The different actors, their interests and concerns – farmers still using traditional varieties may have very different roles and concerns from those who fund *ex situ* conservation activities;

They will often consider also:

- Hierarchies and the relationships between different subsystems – in the case of conservation systems, from local to international;
- The ways in which knowledge is used, shared and maintained; and
- The ways in which decisions are made and the relative power of different actors within the system.

### The Plant Genetic Resources Conservation System

One way of identifying what might constitute a plant genetic resources conservation system and its boundaries is to identify the activities that contribute to conserving plant genetic resources and to making them available to current and future generations (Hodgkin *et al.*, 2012) *viz*:

- Locating diversity, planning conservation and collecting;
- Management and maintenance of materials;

- Diversity analysis, characterisation and evaluation;
- Managing information and knowledge and making this available to users;
- Distribution and exchange of materials and the information associated with these;
- Selection, breeding, and variety development or improvement.

These processes are connected in a more or less linear manner. The final step, selection and variety development, leads to a range of new plant genetic resources in the production system which may themselves be the target of conservation activities thus forming a continuing cycle. The connections include internal smaller feedback loops or cycles so that, for example, diversity analysis provides information to support conservation planning and use of genetic resources.

Five identifiable areas of operation or domains can be identified in which these activities occur:

- *Ex situ* programmes, usually carried out as part of national or international programmes;
- Deliberate *in situ* and *on-farm* conservation actions for crop wild relatives and traditional varieties, undertaken or supported by national programmes, civil society groups and farmers themselves;
- Research and breeding efforts that support use of plant genetic resources;
- The agricultural production system itself and the plant genetic resources and genetic diversity found in it; and,
- The market operations involved in making products available and providing seed and planting materials.

While only the first two domains involve deliberate conservation activities carried out by identified organizations, all the domains contribute to the resources conserved for present and future generations.

As noted above, an important part of the analysis of the system is identification of the different actors involved in the various activities and in describing their interests and concerns (Table 1). The listing of actors in Table 1 is preliminary and subject to discussion but allows identification of some relevant questions and areas for further investigation. Thus, both conservation professionals and farmers undertake similar activities but with quite different objectives

**Table 1. Direct contributions of selected actors to conservation related activities (X, XX – estimate of extent to which the actor undertakes specific activities)**

Actor	Processes					
	Locating and planning	Maintenance of materials	Characterisation and evaluation	Information management	Distribution of materials	Selection and breeding
Farmers and rural communities	XX	XX	XX	XX	XX	XX
Farmer organizations and civil society groups	X	X	X	X	X	X
Extension officers				X	XX	
National agricultural research and development workers		X	XX	XX	X	
Biodiversity conservation organizations and environment agencies	XX	XX	X	XX		
PGR conservation professionals	XX	XX	XX	XX	XX	
Private and public sector breeders (including breeding research)		X	XX	XX	XX	XX
Seed distributors and sellers				X	XX	
National and international agribusiness			X	X	XX	XX
National policy makers				X		

and to different extents. Seed distributors and sellers are only likely to contribute to seed distribution and exchange although they may contribute to knowledge management and sharing information, and again have different objectives (maximizing sales and turnover) than those of conservation professionals (long-term maintenance of diversity) or farmers (production to meet livelihood needs). Policy makers are crucially important in setting the rules for different operations, especially seed distribution, but play no direct part in undertaking any of the specific activities apart from information management.

Actors influence both the links and the flows within the system in different ways. For example, national and private plant breeders and breeding organisations provide the main pathway for movement of *ex situ* conserved genetic diversity back to the production system. But, they also act as a substantial genetic bottleneck, supporting the return of very much less diversity than was obtained from a genebank's location and collecting activities. Analysis of flows between genebanks and breeding indicates that research, breeders' demands, crop identity and the biological nature of the materials are all important in determining the movement of materials into breeding programmes (Dulloo *et al.*, 2013). Exploring flows of resources between all the different processes and the roles of different actors is a further area where a systems approach would be rewarding.

### Application to Key Issues

An important test of the value of systems approaches to plant genetic resources conservation is the extent to which it contributes to identifying ways in which some of the key problems identified above might be overcome. In this section we highlight how a systems approach can help identify insights to strengthen conservation activities. We use two examples: (1) limited conservation of key genetic resources and (2) the links between *in situ* and *ex situ* conservation.

#### (1) Limited Conservation of Key Genetic Resources

Neglected and underutilized crop species (NUS) have been reported to be under represented in conservation efforts with an average of possibly 8 accessions or less conserved *ex situ* for many of these species (Padulosi *et al.*, 2002). Exploring the processes and actors entailed in conserving genetic resources, it is apparent that it is farmers who are the main actors involved in locating and planting many NUS, maintaining materials, characterizing and evaluating them, managing information about them, distributing them, and selecting and breeding new varieties. Systems approaches can explore how the contributions of other actors might be enhanced (e.g. increasing *ex situ* conservation in genebanks, using agricultural extension services to share materials and information, expanding support for

research and breeding programmes on NUS) or how to improve recognition of the contribution farmers make to conserving NUS (e.g. direct financial support, *on-farm* conservation programmes). Alternatively, such approaches might analyse the dynamics behind the replacement of NUS to identify intervention points to stem or reverse replacement. Consideration of a hierarchy of sub-systems might yield insights into missing processes or conflicting purposes at different levels from the individual farmer to global level.

## (2) *The Links between In situ and Ex situ Conservation*

Ideally *ex situ* and *in situ* conservation efforts would be closely linked but, in practice, the links are weak. Systems approaches can be used to analyse boundaries to explore what is inside different actors' systems of interest that contribute or could contribute to conservation. For example, *in situ* conservation of CWR often depends on management of protected areas, but CWR are rarely within the boundaries of a protected area (PA) manager's system of interest. In the case of traditional varieties, the role of farming communities who *de facto* maintain crop genetic diversity *on-farm* is often ignored by many PGRFA conservation professionals. Systems approaches can contribute to bringing together the different stakeholders – conservation professionals, government workers in forestry and environment and biodiversity departments as well as men and women farmers from local communities – to explore their perspectives on the processes under consideration and to create new, meaningful linkages between the actors.

## Conclusion

Although this paper provides only a brief overview, the preliminary results suggest that systems approaches may shed light on some key issues that confront those involved in the conservation and use of plant genetic resources. More work is required to explore the full complexity of the system as a whole and of its constituent parts. A next step could be to explore the conservation system for a range of individual crops and undertake a comprehensive stakeholder analysis to determine present and potential roles of all actors. This exploration would allow for a more complete and less general description of the processes involved, the relative contributions of the different areas of operation and the roles of the different actors. It would also make clear the benefits of a systems approach in increasing the efficiency of

conservation and use of plant genetic resources for food and agriculture.

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## Why Conserve Crop Diversity?

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Crop diversity is the foundation of our food. For millennia, food plants have been domesticated, selected, exchanged, and improved by farmers in traditional ways, within traditional production systems (Plucknett *et al.*, 2014). In the 20th century, this process was hugely accelerated and focused by scientific crop improvement, leading to historic achievements such as the Green Revolution and the steady rise in yields since then. Half of the increases in food production globally can be attributed to genetic improvement, whose benefits also include reduced reliance on environmentally harmful inputs, more stable yields and higher nutritional value.

At the same time, the world still has two billion people who are malnourished, and of these about 749 million do not get enough calories (IFPRI, 2015). Meanwhile, the rate of yield gains is decreasing for some major food crops and climate change has introduced additional, urgent challenges. Adaptation of agriculture will be crucial to ensure food security for a global population of nine billion people in 2050. Today, modern tools allow researchers to be more accurate and efficient in managing and using genetic diversity. However, for plant breeders to continue delivering benefits, they require continued access to plant genetic diversity from around the world.

Recognition of the significance of crop diversity is epitomized by the agreement of a global treaty addressing the issue, the International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty), which came into force in 2004. Now ratified by more than 135 countries, it provides a legal framework for how crop diversity is conserved and made available for nutritional security. More recently, the UN Sustainable Development Goals (SDGs) have challenged the global community to eradicate hunger. The SDGs recognise the important role crop diversity plays in helping us achieve this goal, and explicitly call for its protection by the year 2020 (in Targets 2.5 and 2.a).

## Role of Plant Genebanks

Much of the world's genetic diversity of crops was lost as agriculture developed. The establishment of many of the world's genebanks took place during the 1970s and 1980s in an atmosphere of crisis. Every day, traditional crop varieties and their wild relatives were disappearing from farmers' fields and from natural ecosystems, cast aside in favour of genetically uniform, potentially high-yielding types or victim to changing cropping patterns and habitats. This led to the mobilization of a worldwide effort to collect imperilled crop genetic resources for safeguarding in genebanks.

This eleventh hour rescue effort would eventually lead to the establishment of about 1,700 genebanks around the world, holding more than 7 million accessions. These include the genebanks of the CGIAR centres, which collectively host the foremost international effort to conserve and manage crop, forage and agroforestry genetic resources. One of these genebanks, that of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is located in India, in Hyderabad.

Despite their prescience in setting up crop diversity collections, however, a large number of genebanks established during the crisis years did not make sufficient provision for on-going financial support. Many of the collections maintained in developing countries are in a precarious state. And even the best of them struggle for funding. Reliable long-term conservation requires stable, long-term financial support.

Crop diversity in farmers' fields is diminishing too, and the wild relatives of our food crops continue to disappear in their natural habitats – be it by clearing of forests or urban sprawl. In short, agrobiodiversity today is threatened on three fronts: in the wild, in farmer's fields, and even in the crop genebanks that are intended to be safe houses for the future. We must secure safe havens from the threats posed in all of these places.

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### Role of the Crop Trust

The reliability of genebank funding is absolutely crucial given that a shortfall in financial resources can lead to the permanent loss of unique varieties. The Crop Trust's objective is to ensure stable, predictable funding for a global system of crop collections, in perpetuity, from an endowment fund.

This global system of crop diversity is based on three pillars: international crop collections (as shown in Table 1); national and regional collections of the highest importance for food security; and the Svalbard Global Seed Vault, the final back-up of the world's crop collections.

#### International crop collections

The Crop Trust has signed agreements to provide 'in perpetuity' funding to some of the world's most important collections of 17 crops (Table 2), all recognized under Article 15 of the Plant Treaty. These are among the most comprehensive and widely used collections of crop diversity. A partnership between the CGIAR and the Crop Trust has ensured stable funding for the genebanks for the past 5 years, and a proposal is in place to extend this guarantee for another six years. Over time, the Crop Trust will be able to secure more and more crops through fully-funded long-term grants

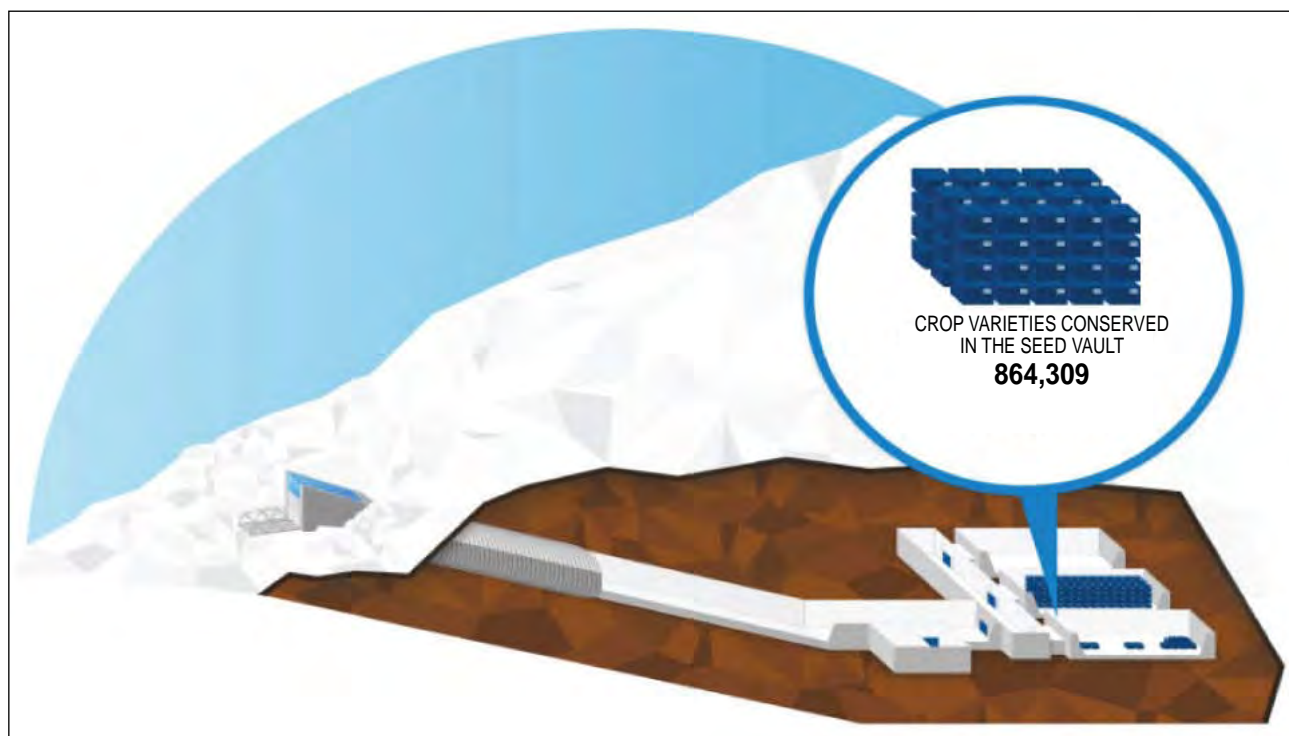
from its endowment. Genebanks provide technical and financial reports to the Crop Trust through an Online Reporting Tool, and 19 agreed Performance Indicators are monitored for each of the crop collections involved. The goal is to make this diversity available to breeders and researchers in a manner that meets international scientific standards, and which is cost efficient, secure, reliable, sustainable over the long-term and supportive of the Plant Treaty.

#### National Crop Collections

Beyond the Article 15 collections, the Crop Trust is also planning to help conserve and make available other key collections of the 25 Annex 1 crops which are most important to agriculture in Least Developed Countries (LDCs), as reflected by production statistics. Global crop strategies have been developed, and will be revised, to help identify priority collections for both short- and long-term support.

#### The Svalbard Global Seed Vault

Deep inside a mountain on a remote island in the Svalbard archipelago, halfway between the northernmost tip of mainland Norway and the North Pole, lies the Svalbard Global Seed Vault. This is a fail-safe, last-chance backup facility for the world's crop diversity. It currently holds more than 860,000 samples of crop



**Table 1. Genebanks supported by the Crop Trust through Long-Term Grants from the Endowment Fund and the Genebanks CRP**

CGIAR Center	Country	Crops	# of Crop Accessions
Africa Rice	Benin	Rice	19,983
International Institute for Tropical Agriculture	Nigeria	Cowpea, yam and cassava	30,388
Bioversity International	Belgium	Banana	1,455
International Maize and Wheat Improvement Center	Mexico	Maize and wheat	175,526
International Center for Agricultural Research in the Dry Areas	Syria	Barley, chickpea, faba bean, forages, lentil and wheat	136,350
International Center for Tropical Agriculture	Colombia	Bean, cassava and forages	67,574
International Crops Research Institute for the Semi-Arid Tropics	India	Chickpea, groundnut, minor millet, pearl millet and sorghum	129,081
International Livestock Research Institute	Kenya	Forages	17,716
International Potato Center	Peru	Andean roots and tubers, potato and sweet potato	15,756
International Rice Research Institute	Philippines	Rice	121,595
World Agroforestry Center	Kenya	Agroforestry trees	5,490
Centre for Pacific Crops and Trees	Fiji	Aroids and yams	1,467

**Table 2. Long-Term Grants provided by the Crop Trust**

- Edible Aroids – SPC
- Banana and Plantain – Bioversity International
- Barley – ICARDA
- Bean – CIAT
- Cassava – CIAT
- Cassava – IITA
- Chickpea – ICRISAT
- Faba bean – ICARDA
- Forages – ICARDA
- Forages – ILRI
- Grass pea – ICARDA
- Lentil – ICARDA
- Maize – CIMMYT
- Pearl millet – ICRISAT
- Rice – IRRI
- Sorghum – ICRISAT
- Sweet Potato – CIP
- Wheat – CIMMYT
- Yam – SPC
- Yam – IITA

diversity from more than 60 genebanks, and nearly every country in the world. The Crop Trust maintains the Vault in partnership with the Norwegian government and the Nordic Genetic Resources Center, which is responsible for its management and operation.

Last October, seeds were withdrawn from the Global Seed Vault for the first time when ICARDA, the international agricultural research centre formerly based in Aleppo, Syria withdrew 40,000 of its crop accessions in order to re-establish collections in facilities in Lebanon and Morocco. This demonstrates that the Vault can help overcome the kinds of crises that may threaten genebanks in these turbulent times.

The Crop Trust's work is not restricted to long-term funding for plant genebanks. It also implements short-term, strategic projects that underpin the global system and strengthen crop diversity conservation worldwide. This includes efforts to collect, conserve and use the wild relatives of 29 crops of global importance to food security, adding them to the pool of resources available under the Plant Treaty. The Crop Trust is also building an information system to ensure ready access not just to the diversity itself, but also to any information that exists about it: [Genesys](#), an online portal bringing together information from genebanks worldwide. The Crop Trust has also supported, in close partnership with the United States Department of Agriculture, the development of the GRIN-Global genebank data management software.

Securing the world's food supply is going to require support and work beyond crop diversity conservation. Yet nothing in agriculture can be effective if the genetic base of our food supply is lost. Genebanks are vital to a sustainable and healthy food system for our children's children.

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## Germplasm Management for Enhanced Genetic Gains

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### Challenges to Global Agriculture

Feeding 9.3 billion population by middle of 21<sup>st</sup> century the safe and nutritious food is the greatest challenge to the humanity. Agriculture is vulnerable to global warming and depletion of natural resources. Biodiversity loss, risk of change in pest dynamics, declining food quality and greater risk to food contamination (mycotoxins) are other adverse impact of global warming (Dwivedi *et al.*, 2013). South Asia and sub-Saharan Africa are predicted to be worst affected due to climate change and variability effects and it is in these regions that ICRISAT mandate crops (chickpea, pigeonpea, groundnut, pearl millet, sorghum, and finger millet) are largely grown and consumed (FAO, 2014). More importantly, the risk absorbing capacity of the farmers in these regions is low and therefore, developing climate-resilient technologies including improved and nutritious seeds together with judicious management of natural resources is the way forward to address food and nutritional security.

### Yield Increase and Genetic Gains

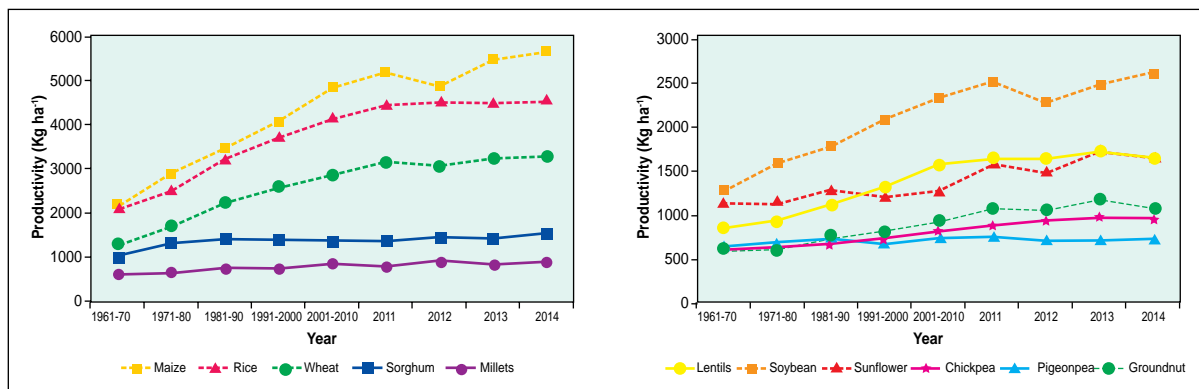
Globally, following adoption of improved technologies including seed, productivity of maize, rice and wheat has been drastically increased, while such a dramatic increase was not observed in coarse grains and legumes (Fig. 1) (FAO, 2014). Of recent, the yield in many crops either stagnated, declined or showed only marginal increase

(Fig. 1). Genetic gain is defined as the annual increase in yield realized through crop breeding. A review of literature reveals  $<1.0 \text{ year}^{-1}$  genetic gains through breeding in many crops, for example, from  $0.65\% \text{ year}^{-1}$  to  $0.74\% \text{ year}^{-1}$  in wheat (Zhou *et al.*, 2007; Sharma *et al.*, 2012),  $0.85\% \text{ year}^{-1}$  in sorghum (Woldesemayat *et al.*, 2015), and from  $0.43\% \text{ year}^{-1}$  to  $1.89\% \text{ year}^{-1}$  in groundnut (Hagos *et al.*, 2012; Haro *et al.*, 2013). There is therefore a need to double the genetic gains in most crops to increase food and nutritional security to growing world population.

### Plant Genetic Resources (PGR) for Enhanced Genetic Gains

PGR are the basic raw materials to power current and future progress in crop improvement programmes. Globally, 7.4 million accessions are conserved in more than 1,750 genebanks (FAO, 2010). RS Paroda genebank at ICRISAT, Patancheru, India has the largest collection (1,24,305 accessions) of its mandate crops from 144 countries ([www.icrisat.org](http://www.icrisat.org)), conserved as active ( $4^{\circ}\text{C}$  temperature and 30% relative humidity in medium-term storage) and (116,491 accessions) in base ( $-20^{\circ}\text{C}$  temperature in long-term storage) collections.

Low use of germplasm is a major concern as only less than 1% of assembled germplasm has been used in breeding programmes globally (Upadhyaya *et*



**Fig. 1. Trends in productivity of important cereals and legume crops during 1961-2014.**

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*al.*, 2006) leading to a narrow genetic base in most crop cultivars. For example, 50% of wheat, 75% of potato, 50% of soybean cultivars grown in USA trace back to 9, 4 and 6 genotypes in their pedigrees, respectively (World Conservation Monitoring Centre, 1992). Enhanced use of germplasm can be achieved through developing representative core (10% of entire collection) and mini-core (10% of core or 1% of entire collection) collections facilitating extensive evaluation of germplasm and identification of genetically diverse accessions with agronomically beneficial traits for use in breeding programmes.

### Forming Core and Mini-core Collections

Large size of collections and lack of reliable data on traits of economic importance, which show high genotype  $\times$  environment interaction are considered as the main reasons for low use of genetic resources. To overcome this core and mini-core (Upadhyaya and Ortiz, 2001) collections, based on passport, characterisation and evaluation data and reference set based on genotyping information are now available in ICRISAT mandate crops and small millets (Upadhyaya, 2015, [www.generationcp.org](http://www.generationcp.org)).

Due to its reduced size and representativeness of species diversity, the mini-core collections are an ideal genetic resources for an in depth characterisation of its biological diversity and use in crop improvement programmes (Fig. 2). So far, the Genebank has provided 274 sets of mini-core collections of different crops to scientists in 36 countries and 114 sets to scientists at ICRISAT.

### Identifying New Sources of Variations using Mini-core Collections

Systematic evaluation of core/mini-core collections resulted in identification of a number of germplasm lines with agronomically and nutritionally beneficial traits in addition to resistance/tolerance to abiotic and biotic stresses. For example, multiple stress resistance in chickpea (Upadhyaya *et al.*, 2013); multiple stress resistance and nutrient dense (oil, protein, Fe, Zn, oleic acid) in groundnut (Upadhyaya *et al.*, 2014a); high sugar stalk sorghum germplasm (Upadhyaya *et al.*, 2014b); downy mildew resistant pearl millet (Sharma *et al.*, 2015) or nutrient dense finger and foxtail millets (Upadhyaya *et al.*, 2011 a, b).

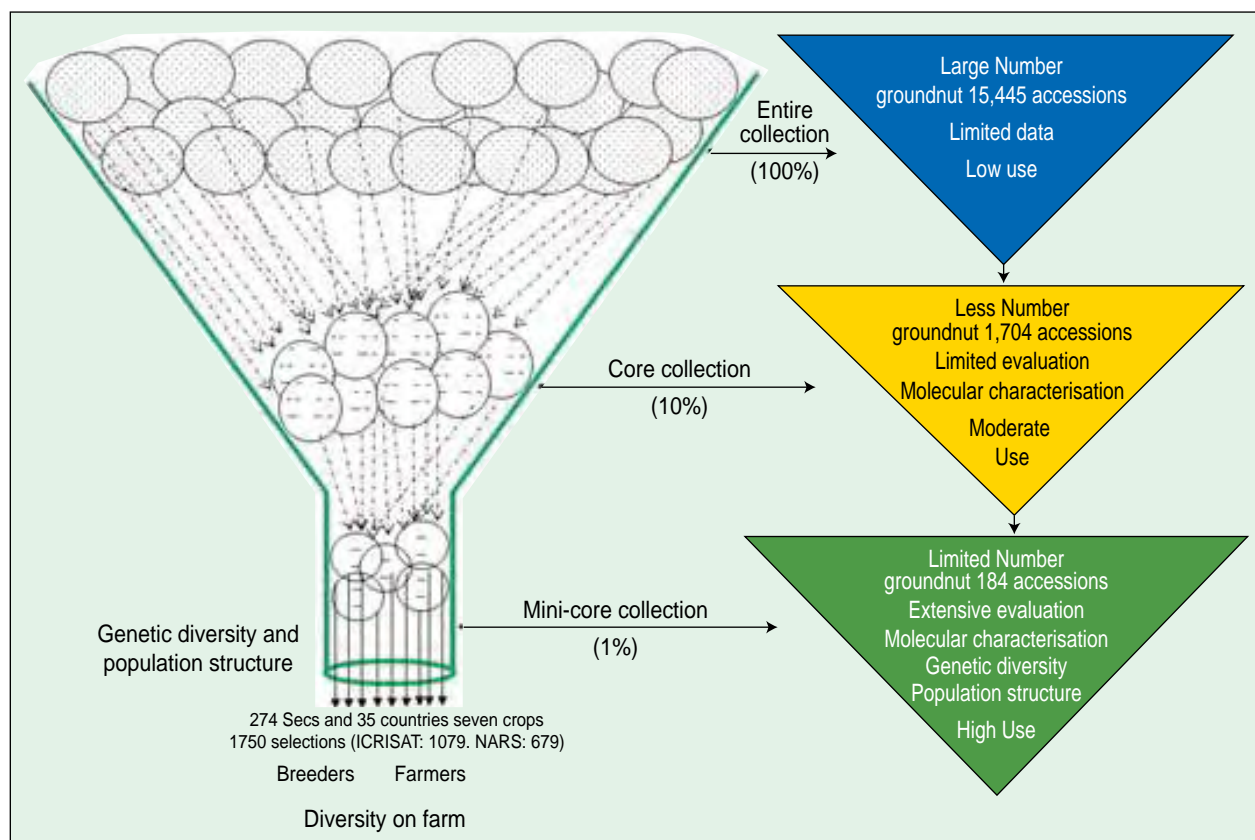


Fig. 2. Mini-core collections for enhanced use of germplasm

### ***Enhanced Uptake of Germplasm in Crop Breeding***

An analysis of the uptake of germplasm in crop improvement programmes at ICRISAT showed that germplasm use has increased since the formation of the mini-core collections in some crops. For example, 30% stresses tolerant accessions used in breeding during 2005-2009 were from mini-core, while in 2010-2014, more emphasis (17%) was given to those germplasm possessing yield and seed nutritional traits. The emphasis in groundnut during 2005-2009 was on stress tolerance (37% from mini-core), which during 2010-2014 changed to involving both stress tolerance (54%) and yield and quality (52%) traits in breeding.

### ***Germplasm Released as Cultivars***

To date, 109 germplasm lines supplied by RS Paroda genebank were directly released as 146 cultivars in 51 countries, few lines in more than three countries. For example, a chickpea landrace ICC 11879 was released in eight Mediterranean countries; a vegetable type pigeonpea landrace ICP 7035 in Fiji, India, China, Nepal and Philippines or a groundnut accession ICG 7827 in nine countries. Over 800 cultivars were released in 79 countries utilising germplasm and breeding lines from ICRISAT.

### ***Economic Impact of PGR Use in Cultivar Development***

The PGR contribute to development of breeding populations or direct release of germplasm as cultivars. PGR has been particularly useful in adding disease resistance through hybridization or when a resistant PGR has been released as cultivar. This has significant economic impact on farmers. For example, a wilt resistant pigeonpea landrace ICP 8863 released as 'Maruti' in India in 1986 benefited US\$75 million by 1996. Similarly, in case of groundnut a single germplasm line PI 203396, resistant to tomato spotted wilt virus has contributed >200 million US \$ annually in the USA.

### ***Crop Wild Relatives (CWR) for Enhancing Cultigen Genepools***

CWR harbour genes for stress tolerance, seed yield and nutritional traits (Rao *et al.*, 2003; Upadhyaya, 2008). RS Paroda genebank conserves a total of 2,876 accessions of wild relatives. Several promising sources were identified for agronomic and nutritional traits and abiotic and

biotic stress resistances in all mandate crops. ICRISAT made systematic efforts to infuse diversity from wild relatives to enhance resistance to pod borer in chickpea and pigeonpea, and to rust and leaf spots in groundnut (Upadhyaya, 2015). In groundnut wild species through amphidiploid has been successfully used to enhance 100-seed weight, pod yield (Upadhyaya, 2008) and traits related to drought tolerance such as specific leaf area and SPAD value using chlorophyll meter.

### ***Accelerating Genetic Gain through Use of Genetic and Genomic Resources***

ICRISAT mandate crops are no more orphan but genetic (germplasm representative subsets, stress tolerant and nutritionally dense seed, genetic mapping populations) and genomics (DNA markers, high density genetic maps, reference genomes) resources rich crops. Researchers are now using high throughput phenotyping and genotyping platforms (including genotyping by sequencing) to dissect the genetic and physiological basis of trait expression and deployment to accelerate genetic gains in crop breeding. In addition, researchers are now sequencing genomes of diversity panels (for example, 300-chickpea or groundnut genomes) and its comparison with reference genome is expected to associate sequence difference with agronomically beneficial traits.

The judicious use of these genetic and genomic resources have enabled researchers identify significant marker-trait associations or candidate genes associated with agronomic traits, for example, stress tolerance and agronomic traits in sorghum, chickpea and groundnut. In addition, the use of groundnut germplasm lines identified from mini-core collection have resulted in developing exceptionally high oil (up to 63%, compared to ~48% in control cultivar) and high-yielding breeding lines, indicating that new germplasm sources contribute to enhancing genetic gains.

In conclusion, plant genetic resources play an important role in developing cultivars with high genetic gains to meet the adverse effects of climate change on global agriculture. Use of representative subsets such as mini-core collections serves as an ideal diversity panels for trait discovery. The availability of high throughput genotyping and phenotyping facility at ICRISAT has opened the gateway to accelerate understanding of the genetic control of the trait expression and subsequently to rapid genetic gains in crop breeding.

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## ICARDA Efforts to Promote *In Situ/On-farm* Conservation of Dryland Agrobiodiversity

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### The Importance of Dryland Agrobiodiversity

Dryland biodiversity has only recently got due attention because of its potential to contribute in overcoming the effects of global challenges caused by land degradation and climate change (FAO, 1989). More particularly, the non-tropical dryland agrobiodiversity is of special significance since it encompasses the four major Vavilovian centers of diversity (Mediterranean, West Asia, Central Asia and Abyssinian) where most of the world today's major food and feed species have evolved. The Fertile Crescent (including Jordan, Lebanon, the Palestinian Authority, Syria, southeast Turkey and southern Iran) is considered an area of megadiversity where wheat, barley, lentil, pea, vetch, alfalfa and others have their centers of origin, diversity and domestication and where their landraces and wild relatives are still prevailing within traditional farming systems (Harlan, 1975). This agrobiodiversity, in addition to its role in sustaining the livelihoods of poor communities living under harsh conditions, are a rich reservoir for supplying genetic resources to ensure continuous genetic gains in breeding programmes and for use in the rehabilitation/restoration of degraded farming and eco-systems or their diversification. Despite limited information available, all indications show that this agrobiodiversity is subject to a rapid and alarming loss calling for more concerted efforts at local, national, regional and global levels for its conservation and sustainable use. The *ex situ* conservation in genebanks should be complemented with *in situ/farm* management of agrobiodiversity to ensure dynamic conservation of larger diversity including that of recalcitrant species and their associated local knowledge Jarvis *et al.*, 2001.

ICARDA through its coordination of the Global Environment Facility (GEF)-funded project on "conservation and sustainable use of dryland agrobiodiversity in Jordan, Lebanon, Palestine and

Syria" implemented during 1999-2005 has developed a holistic community based *in situ* conservation approach which is adopted in several initiatives promoting on-farm conservation of landraces in many countries (Amri *et al.*, 2005).

### Community-based *in situ* Conservation of Agrobiodiversity

*In situ* conservation requires a holistic approach based on the full involvement farmers and herders who are the main custodians of agrobiodiversity, the use of the integrated management of ecosystems and farming systems, the development of sound scientific basis and the collaboration at the national, regional and international levels. The strategy is based on agreeing with key stakeholders on technological, add-value, alternative sources of income, institutional and policy options which could increase the productivity and profitability of landraces and natural habitats targeted including:

- Assessment of status and threats to agrobiodiversity and undertaking gap analysis to identify biodiversity hotspots for protection;
- Development dissemination of low-cost agricultural packages;
- Increasing the demand for products of local biodiversity through add-value technologies and promoting alternative sources of income;
- Reforms of the existing policies and legislation (property rights of farmers, government incentives, research/extension/education programmes on agrobiodiversity, empowerment of local communities over natural resources use, benefit sharing mechanisms, etc.);
- Increasing public awareness at all levels using all available means (media, school curricula and extra-curricula, etc.).

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## Major Achievements and Impacts

### 1. *Better Understanding of the Status of Agrobiodiversity and of its Major Factors of Degradation*

- The results of the farming surveys showed that 100 % of the farmers in all drylands and mountainous sites surveyed in Jordan, Lebanon, Morocco, Palestine, Syria, Tunisia and Yemen are still predominantly using landraces of barley, lentil, faba bean, figs and olives. In case of wheat, almonds, apricots, plums and prunes, 50 % of the farmers are using improved varieties.
- The eco-geographic and botanic surveys conducted showed that the natural habitats are highly affected by overgrazing, land reclamation and fragmentation, and successive droughts. Remote sensing has allowed to show the negative trend of climate change on vegetation cover. These surveys along with gap analysis using DIVA-GIS programme have allowed to determine biodiversity hotspots for establishment of natural reserves;

### 2. *Development and Dissemination of Low-cost Technologies*

- **Technological options for landraces:** For cereals, food and feed legumes, seed cleaning and treatment against seed born diseases showed an increase in grain yield of up to 50 % for some farmers. Similarly, participatory improvement of landraces showed also yield gains. The development of seed producers groups and associations helped in establishing an efficient informal seed production and delivery system for promoting the use of landraces. For landraces of fruit trees, water harvesting, pruning, integrated pest management and use of fertilizer and establishment of nurseries were rewarding.
- **Natural habitats management options:** reseeding/replanting with native species, application of phosphorous, rest grazing, along with the use of water harvesting techniques, plantation of shrubs, introduction of feed block technology, animal culling and of Para-Vet services are among the recommendations for rangeland and livestock management options.
- **Demonstration of add-value technologies:** Most of the products of landraces have a price premium attributes and are more suited for traditional dishes.

Training of local communities and mainly women on food processing for the production of jams, compotes, syrups and dried fruits, along with better packaging and libeling allowed better marketing of products.

### 3. *Investigation of Alternative Sources of Income*

Agriculture is the major activity of the custodians of dryland agrobiodiversity, but its contribution to the income of families is diminishing continuously, which is threatening the remaining agrobiodiversity. Additional alternative sources of income (dairy production, honey production, mushroom production, eco-tourism, etc.) were initiated and showed potential for adoption.

### 4. *Reforms of National Biodiversity Policies and Legislations*

The project was able to develop a framework which includes all the steps to follow in the development of national biodiversity policies and legislation. This includes institutional and policy options to be undertaken at international, regional, national, community, farm and plot levels to promote the conservation of biodiversity in general and agrobiodiversity in particular. Among actions initiated in some countries, the shift to use native species in reforestation/afforestation, introduction of biodiversity in education system, establishment of agrobiodiversity units within the Ministries of Agriculture.

## Conclusion

The dryland agrobiodiversity in CWANA region is key to sustain the livelihoods of poor communities and food security and is important for the resilience of dryland farming systems and for ensuring other ecosystems benefits. The success of *in situ* conservation will require more research and more concerted efforts at all levels to empower the custodians of the agrobiodiversity and allow economic returns for their efforts. Management plans including technological, socio-economic, institutional and policy options need to be developed for each situation with full involvement of key stakeholders. However, collecting and conserving in genebanks should be continued and enforced mainly for threatened and endangered populations. ICARDA plays a crucial role in promoting the conservation and sustainable use of dryland agrobiodiversity through *ex situ* conservation of more than 154,000 accessions held in-trust in its genebank and through providing training and technical backstopping to national genebank in CWANA region.

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## Gene Banks: Management of Genetic Erosion in *Ex Situ* Collections

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### Introduction

Plant genetic resources are the fundamental source for crop improvement which is being conserved *in situ* and *ex situ*. While *ex situ* is static form of conservation of plant resources, *in situ* is dynamic; representing diverse populations (including both alleles and genotypes) and aids the evolutionary processes of gene flow between different populations and natural and artificial selection/interaction in heterogeneous environments. Apropos to conservation of biological diversity *ex situ* in post CBD scenario, but with special reference to genetic erosion there in, also gained immense significance in the wake of IP regimes among all the CBD member countries. There exists imminent danger to the very existence of rich diversity in nature in the wake of raising global temperatures and climate change. In the backdrop of several species becoming rare and endangered in their natural habitats, *ex situ* conservation of large chunk of diversity is though safeguarded to certain extent; the threat of erosion still haunts us as the genetic erosion in *ex situ* collections is looming large.

### Historic Plant Explorers/Collectors

For many thousands of years, plants have travelled around the world along with people journeying to different countries for e.g. tomato, maize, pepper (chilli) (South America), banana (South East Asia), carrots (Afghanistan), potato (Andean region, South America), onion (Central Asia), wheat (Near East) and pumpkins (tropical America). Contributions of the following are worth mentioning.

- Queen Hatsheput (Egypt, 3500 years ago) – collected resin of myrrh plant and frankincense trees near Somalia.
- Christopher Columbus (Italy – in 1492) – Potatoes, sweet potatoes, maize, tomatoes, peanut, cassava, cacao, peppers, tobacco, beans and squashes.

- Thomas Jefferson (3<sup>rd</sup> President of USA) – Vanilla, tea, olives.
- Nicolai Vavilov (Russia) – Centres of origin.
- Carlos Ochoa (Peru) – Wild and endangered species of Potato.
- John George Jack (Canada) – Tree genetic resources.
- Jack Harlan (USA) – Centres of diversity.
- Gregory WC – Peanut germplasm
- Krapovickas A – Peanut germplasm
- Brown WL – Maize germplasm
- Hawkes JG (UK) – PGR science.
- Otto Frankel (Australia) – PGR science.
- Zeven AC (The Netherlands) and PM Zhukovsky (Russia) – Centres of origin.

In the Indian context, Emperor Akbar (1542-1605) established a mango orchard (*Lakhi Bagh*) in Darbhanga, Bihar during his regime. Emperor Ashoka (304-232 BC) patronized the establishment of fruit and shade trees in his kingdom.

### Status of World PGR in Genebanks

Over 1750 gene banks and 2,500 botanical gardens conserve a total of 7.4 million germplasm accessions and 80,000 species respectively around the world. Currently, 7,74,601 samples are deposited at Svalbard, Norway by 53 genebanks. It is estimated that more than one third of the globally *distinct* accessions of 156 crop genera stored in genebanks as orthodox seeds are conserved in the global seed vault. Global holdings of commodity groups viz., cereals (31,57,578); food legumes (10,69,897); roots and tubers (2,04,408); vegetables (5,02,889); nuts, fruits and berries (4,23,401); Oil crops (181,752); Forages (6,51,024); sugar crops (63,474), fibre crops (1,69,969),

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medicinal, aromatic and spice crops (1,60,050); industrial and ornamental plants (1,52,325) and others (2,62,993) reported by FAO (2014).

### Importance of Conservation

*Ex situ* conservation is designed to maintain genetic diversity available in and genetic integrity of the collected material, to avoid loss or degeneration. Use of conserved plant genetic resources have been successful in mitigating major challenges of abiotic stresses [e.g. chromosomal translocation from rye to wheat has conferred adaptation potential to wheat in marginal environments, Borner *et al.* (2000)], biotic stresses [e.g. rust resistance in wheat varieties by incorporation of single major gene that conferred resistance to specific races of rust pathogens (Borlaug, 1953)] and climatic changes [e.g. drought tolerant maize/stress resilient maize in Africa, (Chebotar *et al.*, 2003)] and facilitated in increased crop production and productivity. Following appropriate protocols of regeneration of the *ex situ* conserved material is the critical step in maintaining genetic diversity and genetic integrity.

*Ex situ* collections are vulnerable to genetic erosion resulting in the loss of amount of genetic diversity and loss in genetic integrity, in terms of presence and absence of genes (alleles). Frankel (1975) reported in a survey of genetic resources that many collections had suffered genetic erosion due to hybridization, selection, genetic drift, unsuitable growing conditions, or human error during propagation. Quantification of genetic erosion requires time series analysis and using morphometric traits has always proven to be problematic (Thormann and Engels 2015). These changes were detected both by SNP and SNP haplotype analysis during regeneration in maize and significant differences in allelic frequencies were also reported in barley, rye, and *Brassica* (Parzies *et al.*, 2000; Soengas *et al.*, 2009), although in using limited number of accessions.

### Challenges in *ex situ* Conservation

The conservation of the diverse plant genetic resources, as a possible source of wide gene/allelic diversity, under *ex situ* genebanks faces immense challenges and issues some of which are not entirely within managers' control. In *ex situ* crop wild relatives are threatened by changed habitat (soil, water and soil microbial populations etc.) agricultural/silvicultural practices, human selection etc., that impact on its fertility and seed production. The challenges are broadly defined by environmental factors

for *in situ*/on-farm conservation. The extent of the impact of environmental factors varies from species to species; for example, whereas the cultivated plant species are faced with the challenges of habitat destruction, fragmentation, climate change and restoration efforts while the crop wild relatives of the plant species could be facing the challenge of climate change on a much bigger scale than the habitat destruction and fragmentation *in situ* (Guarino *et al.*, 2011). These challenges precipitate into genetically less diverse populations of the plant species. Jump *et al.* (2009) stated similarly that, genetic erosion can check the resilience, evolutionary potential for adaptation in the short term and survival of any plant species in the long-term, in the face of rapid environmental change.

Reducing and managing the loss of genetic integrity and genetic variation of the conserved germplasm during regeneration is an important objective of genetic resource conservation programmes, i.e. reduction in genetic drift and genetic shift. Genetic integrity may be lost due to inadvertent selection and reduction in genetic variation due to cumulative bottleneck effects that would have started at the time collecting through small seed samples used for subsequent regeneration/multiplication. The management of seed accessions in different genebanks can lead to differential loss of genetic integrity. Identification and rationalization of duplicate accessions in genebanks requires information on the genetic integrity of the accessions. In addition, different genebanks may use different methods of identification of duplicate samples and rationalization of collections which can lead to further genetic erosion. To recommend better practices for maintaining panmictic populations of germplasm accessions, studies on genetic integrity during seed multiplication and regeneration using molecular markers from other seed or clonally propagated crops can be useful.

### Assessing Genetic Erosion in Genebanks

#### Factors Causing Genetic Erosion in Genebanks

Some of the factors causing erosion in *ex situ* collections are physiological changes in seeds, inappropriate storage conditions and management procedures, accidental errors/mixing of seed samples before regeneration, lack of adequate financial resources for maintaining collections, human behaviour (accidental destruction, fire), damage of field collection (animals, meteorological anomalies including natural disasters), armed conflicts, war, regeneration backlogs, economic instability, pest

and disease outbreaks, abiotic stresses (heat, drought), lack of resources and skills, and loss of samples during regeneration etc. Genetic erosion in gene bank collections depends on the quality and quantity of the original material stored, and on the conditions under which the germplasm is maintained, multiplied and regenerated.

### **Population Size at the Time of Collecting and at the Time of Regeneration**

Number of accessions and their isolation during collection and at the time of regeneration are also indicators for genetic erosion. Small Populations are at risk of loss of alleles, increased inbreeding and extinction due to random environmental events. Secondly, sample size analyzed—small sample size may result in missing the allele detection, especially for rare alleles. Direct comparison of samples collected at different times in the *ex situ* collections are warranted to assess the genetic erosion.

### **Avoiding Genetic Drift**

Genetic drift describes random fluctuations in the numbers of gene variants in a population. Genetic drift takes place when the occurrence of variant forms of a gene, called alleles, increases and decreases by chance over time. These variations in the presence of alleles are measured as changes in allele frequencies. Hence, the genetic purity of the conserved sample by avoiding genetic drift and inbreeding; the population size/population genetics theory of sampling at the time of regeneration to maintain the genetic integrity of the accessions in the genebank. Typically, genetic drift occurs in small populations, where infrequently occurring alleles face a greater chance of being lost.. Genetic drift can result in the loss of rare alleles and decrease the genepool. Genetic drift can cause a new population to be genetically distinct from its original population, which has led to the hypothesis that genetic drift plays a role in the evolution of new species.

### **Morphological and Molecular Characterisation**

Stable and unique morphological traits should be effectively used for assessing the degree of genetic variation in the initial as well as regenerated *ex situ* conserved samples. Morphological characterisation of *ex situ* collections should be based on standardized format and the data follow internationally agreed descriptors list (Breese, 1989; Engels and Rao, 1995). Molecular characterisation of germplasm accessions is a

useful tool for better management and to study genetic diversity and integrity of conserved germplasm. Previous studies using molecular tools have been performed on the genetic integrity of genebank accessions of some crop species during regeneration. The genetic integrity of the wheat accessions (Borner *et al.*, 2000) and rye accessions (Chebotar *et al.*, 2003) conserved in the gene bank were studied using microsatellite markers. Chebotar *et al.* (2003) found that there were 4 accessions had significantly different allele frequencies and nearly 50% of alleles identified in the original samples were lost in the regenerated samples. Also, interestingly, some alleles detected in the most recently propagated sub-populations were not observed in the investigated plants of the original seed stocks. Soengas *et al.* (2009) investigated the effect of regeneration on the genetic integrity of *Brassica oleracea* accessions based on simple sequence repeats (SSRs) and found that there were significant changes in the population structure and the allelic frequency at individual loci due to the action of genetic drift, directional selection, and possibly assortative mating. The new molecular marker system, known as single nucleotide polymorphism (SNP), is widely used in different crops *viz.*, barley (Rostoks *et al.*, 2006), maize (Yan *et al.*, 2009) etc. Fingerprinting of genebank accessions can help manage genetic integrity of the germplasm accessions as well as the molecular diversity.

### **Recommendation**

- Base collections, associated with information on traits of importance should be used as a reference point to estimate the extent of genetic erosion. Sample size at the time of collecting should be as large as possible.
- Sub sample (sufficiently large to avoid bottleneck effects) of the base collection to be used for evaluation and rejuvenation/multiplication.
- Define the genetic integrity at the time of collection and the method of maintenance (size of sample for regeneration, period of regeneration cycle, exposure to current stresses) for maintaining original genetic composition.
- Revisiting the original locations of collection to assess the extent of genetic erosion.
- Research on protocols and scales and indicators (individual crop-wise) to maximize the genetic integrity.

- Follow proper regeneration protocols (Germplasm adaptation to environment, taxonomy, reproductive biology and genetic diversity studies are to be taken up).
- Modern molecular tools may be used to estimate the genetic erosion and the resultant diversity created during conservation should also be utilized for crop improvement.

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## Conservation of PGR for Effective Utilisation: New Initiatives at NBPGR

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The Indian National Genebank (NGB) comprising four kinds of conservation facilities, namely, Seed Genebank ( $-18^{\circ}\text{C}$ ), Cryogenebank ( $-170$  to  $-196^{\circ}\text{C}$ ), *In vitro* Genebank ( $25^{\circ}\text{C}$ ) and Field Genebank, was established at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) to cater the requirement of long-term as well as short-term conservation of plant genetic resources (PGR) of local as well as global importance. Whilst the seed, *in vitro* and cryogenebanks are located in NBPGR, New Delhi, the field genebanks are spread across the 10 regional stations of NBPGR in various agro-climatic zones of India. The NGB is also supported by the active partnership of other crop-based institutions designated as the National Active Germplasm Sites (NAGS), responsible for maintaining, evaluating and distributing germplasm from their active collections to NGB and other user scientists. Till date about 0.48 million accessions belonging to nearly 2,000 species are conserved in the NGB comprising 0.42 million in the seed genebank, 11,200 in cryogenebank, 1,902 in the *in vitro* genebank and 51,000 in the field genebanks of NBPGR.

Genebanks today are not only viewed as instruments important for safeguarding plant genetic diversity for future availability, but also as an active source in providing genetic resources to the research and plant breeding community for immediate and short-term use. Rapid strides in molecular biology (sequencing technologies, synthetic biology and gene editing) and computational science (informatics), is directly impacting operational management and agendas of genebanks. For instance, genebanks need to provide novel services, such as germplasm linked with trait-specific data, PGR portals with multiple query systems to select specific germplasm, with data on varied parameters such as agronomy, yield, quality, stress and gene sequences. Accordingly, with the objective to be a source of authentic, quality germplasm to users, several initiatives related to genebanking procedures, protocols and standards have been taken in the recent past at NBPGR, and described hereunder.

### Characterisation of Germplasm and Development of Core Collections—Morphological and Molecular Approaches

Characterisation and evaluation of germplasm held in the NGB was undertaken in the past by screening small holdings of each crop, at a time (200-500 accessions). This approach was modified during 'National Initiative on Climate Resilient Agriculture' (NICRA), a network project of the Indian Council of Agricultural Research (ICAR) launched in February, 2011. Under a sub-project entitled 'Acquisition, Evaluation and Identification of Climate Resilient Wheat and Rice Genetic Resources for Tolerance to Heat, Drought and Salt Stresses', a mega characterisation and evaluation approach was adopted, to screen the entire cultivated gene pool of wheat (22,469 accessions) and chickpea (14,651 accessions) held in the NGB. In the case of wheat, characterisation for 22 qualitative, and 12 quantitative parameters was undertaken after which a core set (of 2,208 accessions) was developed using PowerCore Software with step-wise approach and grouping method and validated using Shannon-Diversity Index and summary statistics. The core set comprised accessions of *T. aestivum* (1,770), *T. durum* (386), and *T. dicoccum* (52) as a representative of the total diversity recorded in the wheat germplasm (Dutta *et al.*, 2013; Bansal *et al.*, 2013).

Similarly, for chickpea germplasm held in NGB (14,651 accessions), eight quantitative and 12 qualitative agro-morphological traits were characterised in a single large-scale experiment (Fig.1). Allelic richness procedure was employed to assemble a core set comprising 1,103 accessions, 70% of which were of Indian origin. Comparable values of total variation explained by the first three principal components in the entire collection (51.1%) and the core (52.4%) together with conservation of nine pairwise *r* values among quantitative traits in the core collection and a coincidence rate around 99.7% indicated that the chickpea core was indeed an excellent representation of the entire chickpea collection in the NGB (Archak *et al.*, 2016).

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**Fig. 1. Characterisation of chickpea germplasm conserved in National Genebank at ICAR-NBPGR, New Delhi (inset: an accession with upright peduncle suitable for mechanical harvesting)**

From the 4,274 accessions of french bean germplasm originating from 58 countries and held in the NGB, large scale characterisation was carried out for 22 phenotypic traits. Based on multivariate analysis, the entire collection could be grouped into 10 genetically diverse clusters irrespective of the origin or place of collection of accessions. First three components obtained through principal component analysis explained 80.44 % of the total variance and it was contributed mainly by pod length, pod width, seed length, seed width, pods/plant and seed weight (Rana *et al.*, 2015).

A systematic characterisation of entire genebank collections have been initiated in 2014 to develop the cores and minicores under Consortium Research Platform (CRP) on Agrobiodiversity, with dedicated funding from ICAR. So far more than 50,000 accessions of various crops for 25-32 descriptors have been characterised and data are being analyzed for further use.

Besides agro-morphological data, molecular markers (SNP) are also being used to identify core collections in the NGB. Simple sequence repeat (SSR) and Single Nucleotide Polymorphic (SNP) were assessed for genetic diversity and population structure in rice (375 varieties) conserved in the NGB, using 36 genetic markers. Analysis of molecular variance (AMOVA) indicated that maximum diversity was partitioned between and within individual level but not between populations. The resolution of population was higher with SNP

markers, but SSR were more efficient for diversity analysis (Singh *et al.*, 2013a). Based on these results, genetic diversity and associated population structure of 6,984 rice accessions, originating from the North-East Region (NER) of India, were assessed using 36 genome wide unlinked SNP markers distributed across the 12 chromosomes. AMOVA analysis showed that maximum diversity was partitioned at the individual accession level (73% for Nagaland, 58% for Arunachal Pradesh and 57% for Tripura). Using POWERCORE software, a core set of 701 accessions was obtained, which accounted for ~10% of the total NER collections, representing 99.9% of the allelic diversity (Choudhary *et al.*, 2014)

It is widely accepted that development of core and mini-core collection is an efficient way for effective utilisation genetic resources in crop improvement programmes. The above and other core sets of germplasm identified in the NGB, with well-recorded morphological, agronomical and genetic traits are proving to be valuable resources for genomic studies and crop improvement strategies to utilise further in developing the climate-resilient varieties. Their information can be accessed at <http://www.nbpgr.ernet.in/pgrportal>.

### Identification of Trait-Specific Germplasm

Another recent thrust being accorded to collections held in NGB is the identification of accession(s) with specific trait(s). For instance, wheat germplasm was



Fig. 2. Evaluation of wheat germplasm against rust disease under NICRA project



Fig. 3. Evaluation of wheat germplasm against spot blotch. Inset showing resistant (R) and susceptible (S) lines

screened against major diseases (the three rusts, foliar blight and Karnal bunt) under hot-spot and artificially epiphytotic conditions (Sharma *et al.*, 2012). Out of the evaluation of 2,200 selected bread wheat germplasm screened under field condition against leaf rust (*Puccinia triticina*), 1,526 exhibited symptom of leaf tip necrosis (LTN). Ninety-eight per cent of accessions showing LTN revealed reduced leaf rust severities from traces to 40 compared to 80-100 in susceptible infector rows. LTN reduced leaf rust severities independent of its low, medium and high expressions across the accessions. The data indicated presence of durable resistance gene *Lr34* associated with the trait of LTN, either singly or in complementation with other genes (Kumar *et al.*, 2014). In another set of experiments, evaluation of wheat germplasm at rust hot-spots in field yielded identification of 689 accessions being non-race-specific resistant/moderately resistant to different rusts diseases. Out of these, under artificial inoculation conditions, 19 multiple rust resistant to dominant races; 101 accessions resistant to leaf and stem rust; 37 accessions to stem and yellow rust; 23 accessions to leaf and stripe rusts; 50 accessions to leaf rust, were identified (Sharma *et al.*, 2012). Wheat accessions IC5361402 and EC573562 have been registered as genetic stocks for rust resistance, possessing 3 minor/APR genes for rust resistance (*Lr34/Sr57/Yr18/Pm38; Lr46/Sr?/Yr29/Pm39; Lr67/Sr55/Ys46/Pm46* (Sivasamy *et al.*, 2014).

Entire collection of wheat (19,460) of *Triticum aestivum*, *T. durum* and *T. dicoccum* available in NGB was evaluated to identify sources of rust and spot blotch resistance during the 2011-14 (Fig. 2). In first round, 4925 accessions were found to be resistant for stripe rust and spot blotch at respective hot-spots. During second round of evaluation, 498 accessions potentially resistant to multiple rusts and 868 accessions resistant to spot blotch were identified (Fig. 3). Evaluation of rust resistant accessions for seedling resistance against seven virulent pathotypes of three rusts under artificial epiphytotic conditions identified 137 accessions with multiple disease resistance. Molecular analysis was done to identify different combinations of genetic loci imparting resistance to three rusts and spot blotch using linked molecular markers; consequently 45 wheat accessions containing resistance genes against all three rusts as well as a QTL for spot blotch resistance were identified. The resistant germplasm accessions can be employed to transfer multiple disease resistance into the background of high yielding wheat cultivars.

Biochemical analysis is also an important tool for identifying important germplasm for nutritional traits. In one project undertaken for evaluating traditional varieties (100) of rice from tribal regions of eastern India, nutritional traits (protein, amino acid, starch, amylose and amylopectin) was assessed to identify superior cultivars. Seeds of tested cultivars exhibited wide variation for nutritional traits. Higher amylose content (>20%) were recorded in 16% cultivars, and accessions GC/JH/2007/187 and GC/OR/2007/168 were identified with very high values for protein and amylose contents. Several superior cultivars were identified with better combination of two or more nutritional traits which can be recommended for cultivation by the farmers and tribal communities for their livelihood (Radhamani *et al.*, 2014).

In chickpea accession IC436088 has been identified with a unique trait of upright peduncle and podding behaviour. This genetic stock has special significance in breeding varieties amenable to mechanical harvesting of chickpea (Singh *et al.*, 2013b). In french bean, screening for bean anthracnose (*Colletotrichum lindemutianum*) disease led to identification of 600 accessions with resistance under field conditions. When these were subjected to screening under artificial conditions against four most prevalent races of *C. lindemutianum* (03, 515, 598 and 529), 16 accessions were identified which have complete resistance and good agronomic superiority (Rana *et al.*, 2015).

Another approach for identification trait-specific germplasm in the NGB has been the use of informatics (Agrawal *et al.*, 2011). Based on analysis of passport information, crop catalogues and other publications, abiotic and biotic trait-specific germplasm has been identified for collections of rapeseed-mustard (Radhamani *et al.*, 2013), pigeonpea (Singh *et al.*, 2013c) and cotton (Kak *et al.*, 2016). Similar efforts are underway in other important crops. Germplasm identified is available for use by the breeders.

### Unlocking the Potential of Crop Wild Relatives

Crop wild relatives (CWR), which are potentially valuable sources of important biotic/abiotic traits, play a very prominent role as donors of genes are important resources for crop improvement for the unpredictable future. Thus, their conservation in the genebanks is of paramount importance for their availability in breeding programmes. It is now well acknowledged that most of the

world's genebank collections of the CWR are inadequate or under-represented. Gap analysis is an important tool to assess the genetic as well as geographic diversity of the crop and its wild relatives, providing a more defined line of action with respect to collection and conservation strategies. A gap analysis for collected and conserved CWRs in NGB revealed that out of a total of ~2,000 taxa (Anon and Nayar, 1982; Pradeep *et al.*, 2014) CWRs reported from India only 94 species are conserved in NGB (Gupta *et al.*, 2016). Based on the analysis, areas have been identified for future collection to enrich the NGB with CWRs. Thus, priorities and strategies for collection followed by their conservation will be defined based on the economic value of cultivated species, distribution of wild species and its potential use in crop improvement programme for enhancing the food and nutritional security.

Whilst conservation of seeds of crops is now a routine practice to the extent that international guidelines are followed world-wide (Tyagi and Agrawal, 2015), CWR still pose challenge for genebank curators. For instance, *Aegilops* species (wild wheat) exhibit inherent problems caused by erratic and staggered germination due to dormancy imposed by presence of tenacious glumes. At NGB, we devised a method to overcome this impediment by removal of tenacious glume in eight wild species of wheat, before their long-term storage in genebank. Germination in seeds with glume was recorded as 20-70% and in seeds without glume was 72-100%. The tenacious glumes contribute to the physical dormancy and removal of glumes increased the germination and seedling vigour in all eight species namely *Ae. bicornis*, *Ae. geniculata*, *Ae. kotschyi*, *Ae. longissima*, *Ae. triuncialis*, *Ae. umbellulata*, *Ae. vavilovii*, and *Ae. ventricosa* (Srinivasan *et al.*, 2013). Incidentally, from 335 accessions of *Aegilops* spp. evaluated at hot-spots for rusts, 229 accessions with D genome of wheat were found resistant to leaf rust (Vikas *et al.*, 2014).

### Why Genebanks Will Continue to Remain in Demand

That genetic resources are important and required for survival of humans is an unquestionable fact. Also what is well-known is the genetic erosion and loss of diversity is a reality of the modern agriculture. And that is why genebanks exist. To safeguard the rapid loss of diversity. In a very recent study, we have used genebank material to demonstrate loss in genetic diversity, using Indian rice varieties, as a model. Trends of genetic diversity

and genetic relationship in 729 Indian rice varieties (released between 1940–2013), conserved in the NGB were assessed using 36 HvSSR markers. A total of 112 alleles was amplified with an average of 3.11 alleles per locus with mean Polymorphic Information Content (PIC) value of 0.29. Cluster analysis grouped these varieties into two clusters whereas the model based population structure divided them into three populations. AMOVA study based on hierarchical cluster and model based approach showed 3% and 11% variation between the populations, respectively. Decadal analysis for gene diversity and PIC showed increasing trend from 1940 to 2005, thereafter, values for both the parameters showed decreasing trend between years 2006–2013. In contrast to this, allele number demonstrated increasing trend in these varieties released and notified between 1940 to 1985, it remained nearly constant during 1986 to 2005 and again showed an increasing trend. Results demonstrated that the Indian rice varieties harbor huge amount of genetic diversity. However, the trait based improvement programme in the last decades forced breeders to rely on few parents, which resulted in loss of gene diversity during 2006 to 2013. The present study indicates the need for broadening the genetic base of Indian rice varieties through the use of diverse parents in the current breeding programme (Singh *et al.*, 2016).

### Epilogue

Although many basic conservation principles, organizations and initiatives have persisted almost unchanged for decades, the framing and purpose of conservation have shifted recently. These shifts primarily relate to viewing conservation as the beginning point for utilisation rather than an end in itself. This is to adopt to the ever-changing agro-climatic requirements, development of novel crop varieties in response to new biotic and abiotic stresses and changing consumer preferences. The NGB has been re-orienting its priorities and programmes accordingly. The thrust has been to increase the efficiency in the terms of ensuring PGR security of wider amplitude of species, while not compromising on the genetic diversity captured. Systematic characterisation, regeneration and multiplication of *ex situ* accessions are being undertaken to satisfy needs of efficient conservation, and making diverse genetic material and associated information available to researchers and breeders. We are aiming to provide trait-specific germplasm for breeding more productive and resilient varieties, with emphasis on enhancing nutritional quality, stress tolerance, and resource use efficiency.

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## Agrobiodiversity: Prospects for a Genetic Approach to *In situ* Conservation of Crop Landraces

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Agrobiodiversity refers to all crops and livestock, their wild relatives, and the species that interact with and support these species, including pollinators, symbionts, pests, parasites, predators and competitors (Qualset *et al.*, 1995) that provide the fundamental basis for agricultural productivity. Crop landraces (CLR) are a fundamental form of agrobiodiversity and in many instances have been replaced by improved varieties or by different crops. CLRs are generally at risk. Hence, there has been great erosion in the frequency of CLRs in almost all crops because of the dynamic nature of agricultural production practices. This includes the rise and fall of particular crop species in the farming systems, but more often because of replacement of CLRs by 'improved' varieties that meet the farmers' desires for greater yields and financial profits. These shifts in the farming systems have been the bases for improving the global food status (the Green Revolution), but have also brought along some environmentally distressing changes, in soil as pesticide and fertilizer contamination of ground water, and, in the view of some consumers, a decline in crop quality.

On-farm (*in situ*) conservation is generally not the result of a planned programme, but based on farmers' preferences. Farmers are free to discard CLRs in favour of improved varieties or other crops. *In situ* conservation is therefore a 'happening' without policy, laws, or other means of regulating the conservation of CLRs. Some farmers will sustain the use of CLRs because of their family history or because alternatives are not acceptable.

Incentives for *in situ* conservation are needed and several means have been proposed to sustain *on-farm* use of CLRs. These include payments to farmers from government programmes, public education to encourage farmers to keep their landraces, niche markets

whereby farmers can sell their CLR products at a profit, and deliberate activities by farm families and local communities to preserve the legacy of many centuries of their agriculture. Finally, an incentive using genetic means is the main focus of this presentation (Qualset *et al.*, 1997). Unfortunately, none of these incentives is sustainable, largely because government payments for growing CLRs will have short political life spans, markets for CLRs may be small, ephemeral, and locally driven, and finally because farmers have the ultimate decision power in choosing their crop species and varieties.

The genetics-based incentive for CLR conservation may be closely linked to the mainstream breeding programmes or adapted to on-farm breeding (Qualset *et al.*, 1997). The main premise of genetic improvement of CLRs is that the basic characteristics of the CLR must be retained while genetic changes are introduced. The most obvious target for CLR improvement is disease and pest resistance, but other traits, such as improvement of nutritional quality of the edible crop products may be modified. The genetic improvements to be made can be done by traditional plant breeding techniques, but great cost and time efficiencies can be made by modern methods, such as marker-assisted selection, when the appropriate molecular markers are available.

The obvious disadvantage of the genetic approach is that many CLRs exist in a single crop and the gene transfer activity is prohibitive in a large number of CLRs. An exception, will be discussed in this presentation, is that mass selection in maize or other outcrossing (allogamous) crops can be made on farm-by-farm and CLR-by-CLR bases.

The strategies for genetic enhancement of CLRs follow basic plant breeding principles (Smith *et al.*, 2001) with attention given to the recovery of the basic

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CLR phenotype and genotype during the gene transfer process. The following strategies illustrate the process, but other strategies are appropriate.

- **Defect Elimination:** Single progenies are selected from a CLR and grown separately and evaluated for the trait(s) of interest. The plants having undesired traits are eliminated. The surviving plants are harvested *en masse* and the new population may be evaluated on farmers' fields or planted again one or more times for selection.
- **New Trait Introduction:** If a CLR lacks a desired trait, then the trait must be introduced from an external genetic resource. For example, disease resistance genes may be introduced by hybridization (crossing) of the resistance source to a sample of plants from a CLR population. The hybrid ( $F_1$ ) plants are hybridized to a sample of the CLR plants. This is a backcross hybridization procedure. The resulting progenies should be evaluated and resistant plants are selected and hybridized to a sample of the CLR plants. This process is repeated 4 to-6 times and the result is disease-resistant plants that have all of the characteristics of the CLR. This population is then taken to the farmers' fields for the farmers' evaluation. Farmer participation in this phase is essential to learn if the new version is acceptable.
- **Mass Selection:** This strategy is appropriate for outcrossing crop species. CLRs are planted in the farmers' field in the usual way. A portion of the field is marked in a grid fashion to produce 50 or more subplots in the field. Observations are taken in each of the subplots and undesirable plants may be removed before pollination. At harvest time seeds from the best plants in each subplot are selected (5 to 10% of the total). The harvest from each plant in each subplot is maintained separately and combined with the plants from all other subplots. Each of the plants is evaluated and the best 20 to 30% are retained and the seeds are bulked. This is the seed stock for the next planting. This process is repeated for several generations. To judge progress, a sample of seeds from each generation is saved for demonstration plantings in the farmers' fields.

### Case Study: On-Farm Mass Selection in Maize in Mexico

Maize, an outcrossing crop, is a very important food crop in Mexico. About 8 million ha are planted annually.

Of this large area about 1.5 million ha are planted with improved open-pollinated or hybrid varieties. The remaining 6.5 million ha (about 80% of the total area) are represented by some 60-80 CLRs. Much of this production is planted in very harsh environments at high elevation with steep slopes with thin soil profile. In these environments maize is the dominant component of a mixed cropping system called the milpa. Squash and beans are the additional crops grown in the mixture. In addition, more than 50 weedy species are harvested as food plants. These are called quelite species. The CLRs are highly diverse in seed morphology and end-uses. Improved varieties or hybrids have not been bred for the areas where CLRs are presently grown and therefore do not perform better than CLRs (Perales *et al.*, 1998). Professional breeding programmes do not address the needs of these CLRs. Conservation of maize landraces in this center of domestication of the crop is essential and presently many of them are at risk of loss (Perales *et al.*, 2003). Some are vulnerable to replacement by hybrids. Genetic improvement provides an incentive for farmers to retain the CLRs. A farmer-based participatory mass selection breeding programme was initiated in 1995 with the 'Chalqueno' landrace. The activity was guided by Fernando Castillo-G, a professional maize researcher. The grid system, described above, was used. The farmers participated in the selection of plants (ears) from the subplots. The farmers then bulked all of the selected ears followed by selection of ears from this bulk population to produce the seed for the next season's planting. This process has been repeated for 12 seasons. Seed from each harvest was retained and comparisons were made in replicated yield trials to document the effects of the selection method.

Grain yield gains of 2.2% per generation in 9 Chalqueno populations have been sustained through 12 generations. In addition, reduction in ear rot (a fungal infection) steadily declined in frequency by 4.8% per generation. Likewise, ear height decreased 0.4% per generation. These results validated the methodology which was easily applied by the farmers. Individual farmers have adopted and applied the on-farm improvement protocol as an alternative to their centuries-old seed selection methods. This genetic improvement methodology has provided incentive for farmers to retain and improve the production of their CLRs.

### Case Study: CLRs of Wheat in Turkey

Wheat is a self-pollinated crop, grown in its region

of domestication on more than 8 mil ha in Turkey. In mountainous areas small-holding farmers grow CLRs (Kan *et al.*, 2015, 2016). Some of those farmers grow both improved varieties and CLRs. A rough estimate of the total area in Turkey planted with CLRs annually is less than 1 mil ha (Morgounov *et al.*, 2016). Farmers who continue to grow CLRs prefer the grain for home food use and the straw for animal feeding. The farmers would like to have better disease and pest resistances and higher yield potential. CLRs grown under conditions typical of small-farm mountainous areas were competitive with improved varieties (Zanatta *et al.*, 1998), but improved varieties utilise added fertilizers more efficiently resulting in generally higher yields.

A high loss of CLRs was judged by comparison of estimates made about 90 years earlier (Kan *et al.*, 2015). Clearly, the CLRs are at risk and incentives are needed if farmers are to retain their CLRs. The first criterion for maintaining these CLRs is to retain traditional on-farm end-uses. As outlined above, both defect elimination and new trait introduction are appropriate. Neither method has been used to improve CLRs of wheat. A current project is assessing the feasibility of these methods. A large number of CLRs were found in a national survey (Kan *et al.*, 2015) and a small number have been selected for field evaluation of single progenies at a research station. After elimination of undesired types a new composite of a CLR will be produced for farmer participatory evaluation. Specific genes for disease resistance will be introduced by the backcross method. For example, covered smut is a serious disease of wheat and requires chemical seed treatment to prevent infection in the resulting grain. Genes for resistance are known in Turkish CRLs and resistance genes can be introduced by backcrossing. The important result is that the chemical pesticide will no longer be needed, thereby improving human safety and decreasing production costs. Molecular markers are available to facilitate the gene transfers. This will be done by researchers at breeding centers. The resulting resistant lines will be evaluated by farmers for acceptability.

## Conclusions

Incentives to farmers for maintaining crop landraces may be introduced, but are generally unsustainable. The genetic approach discussed here may increase

the profitability of CLR production and provide better attributes without sacrificing traditional CLR traits. The genetic approach is highly practical for an outcrossing crop such as maize, but for self-pollinated crops, such as wheat, the gene transfer activity is labor intense and limited in scope. The defect-elimination option is practical and can be accomplished more readily to meet local needs. Care must be taken to retain the traditional end-use traits of the CLR. On-farm participatory selection is essential.

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## Integrated Seed Sector Development (ISSD) – How Informal and Formal Seed Systems Can Work Together for the Conservation and Use of Agricultural Biodiversity

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### Abstract

Agricultural biodiversity conservation and its availability for use are central to robust seed systems that support food security, poverty eradication and resilience to climate change. Recognising that smallholder farmers source seed of different qualities from a diversity of seed systems has resulted in the Integrated Seed Sector Development (ISSD) approach that supports the development of a pluralistic seed sector, which provides smallholder farmers with quality seed of the varieties they desire. The focus of the ISSD approach on the importance of different seed sources to cater for the diversity in demand by farmers offers also opportunities for better and more balanced support for both *in situ* and *ex situ* conservation of plant genetic resources, and strengthened use of agricultural biodiversity on farmers' fields and in both community and commercial seed development programmes.

### The Background and Principles of Integrated Seed Sector Development (ISSD)

Farmers' access to seed is through a diversity of channels. A proportion of seed of especially maize and vegetable hybrids reaches farmers through formal and regulated channels. Informal, usually non-regulated seed systems do however continue to play an important role for seed supply to farmers, in particular for smallholder and family farmers in low and middle income countries. Estimates of the proportion of seed originating from informal sources range from on average 80% in Africa (Louwaars and de Boef, 2012) to more than 95% in six countries around the world (McGuire and Sperling, 2016). Farming households are likely to use a diversity of seed sources at the same time.

The individual farmers themselves use different seed systems for different crops, such as (international) commercial seed for exotic vegetables; seed from

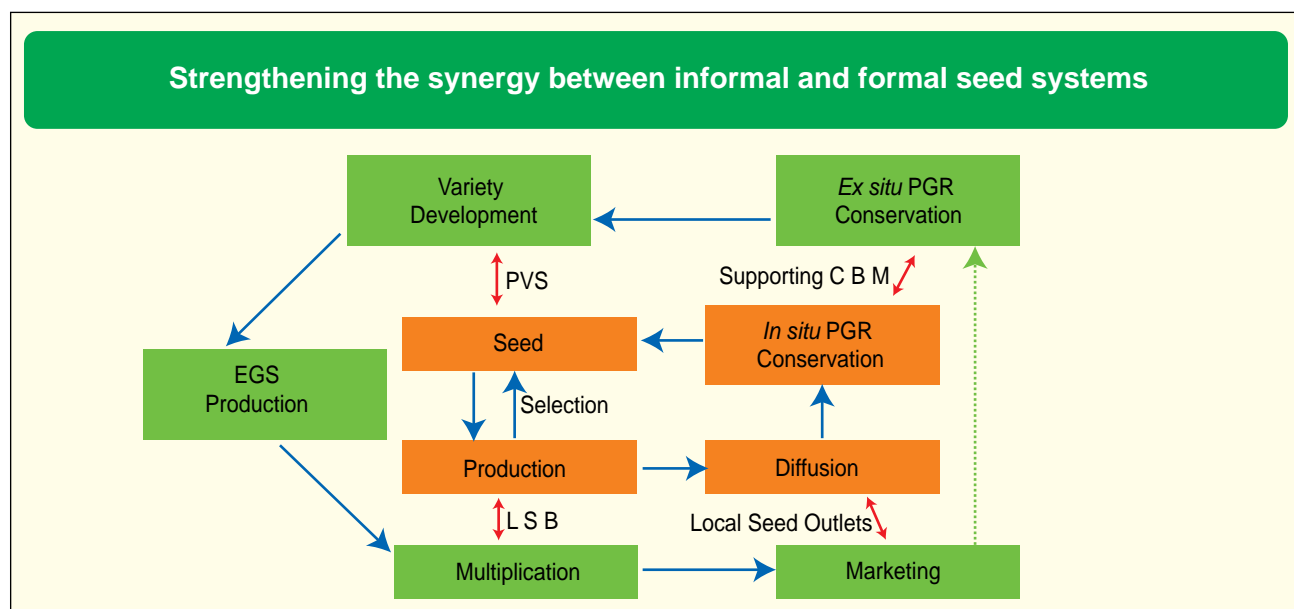
national commercial chains based on international or public research for maize; local semi-commercial sources for groundnut seed produced for the city market; and farm-saved seed for mainly home-consumed crops like sorghum, finger millet, and beans (Louwaars and de Boef, 2012).

Based on these insights the Integrated Seed Sector Development (ISSD) approach supports the development of a vibrant, pluralistic, and market-oriented seed sector. The ISSD approach strives to intervene in different seed systems simultaneously, and aims to bridge emerging gaps and create synergies between the formal (public and private) seed systems and the informal seed systems. ISSD is based on the following eight principles (ISSD, 2014):

1. Foster pluralism and build programmes on diversity of seed systems
2. Work according to the structure of the seed value chain
3. Promote entrepreneurship and market orientation
4. Recognise the relevance of informal seed systems
5. Facilitate interactions between informal and formal seed systems
6. Recognise complementary roles of the public and private sector
7. Support enabling and evolving policies for a dynamic sector
8. Promote evidence based seed sector innovation

Louwaars and de Boef (2012) have visualised how the formal and informal seed systems operate, the latter usually in a more narrow and local environment than the former (Fig. 1). The figure also highlights the role and importance of agrobiodiversity conservation and use, with the formal sector being more linked to *ex situ*

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**Fig. 1. Overview of ways to strengthen the synergy between formal and informal seed systems (red arrows), including agricultural biodiversity conservation and use. Orange – informal seed system; green – formal seed system. PGR – plant genetic resources; EGS – early generation seed; CBM – community biodiversity management; PVS – participatory variety selection; LSB – local seed businesses [adapted from Louwaars and de Boef (2012)]**

and the informal sector to *in situ* conservation. Since in particular for *in situ* situations conservation and local use of agricultural biodiversity are strongly interwoven, the importance of Community Biodiversity Management (CBM) has been recognized (de Boef *et al.*, 2013). CBM contributes to the empowerment of farming communities to manage their biological resources and make informed decisions on the conservation and use of agricultural biodiversity.

The ISSD approach<sup>1</sup> has been embraced by the African Union Commission and several large programmes have been started. Both ISSD Ethiopia and ISSD Uganda started in 2012, and are entering their 2nd phase. ISSD Burundi started in 2014 for four years, and in Mozambique an education support programme introducing ISSD thinking was launched in 2015 for five years. The cross-cutting ISSD Africa programme was launched in 2014. It provides for a continental learning platform on Integrated Seed Sector Development and aims to provide African policy makers and seed sector decision makers with background studies to achieve not only country level but also continent-wide implementation and synergies.

<sup>1</sup> <http://www.issdseed.org/> (accessed on 19 September 2016)

### Specific Effects of ISSD on Improved Agrobiodiversity Conservation and Use

Figure 1 indicates where ISSD in its quest to develop the seed sector by integration of pluralistic seed systems can contribute to interactions and synergy between Community Biodiversity Management and *ex situ* plant genetic resources conservation and use. Some examples are:

#### Improved Exchanges of Materials between CBM and Genebanks

Commonly the formal seed system is more linked with *ex situ* conservation of agricultural biodiversity in genebanks, and the informal seed system with *in situ* conservation as part of CBM, through the continued use of local varieties and community seed banks. By acknowledging the fact that genebanks need to make informed choices to limit themselves to high value and unique genotypes while *in situ* conservation is more adapted to genetically diverse populations, it is clear that creating room for both systems and coordination between them can optimize costly investments in conservation. It also can facilitate studies of change, for example between *ex situ* stored samples of local varieties and their *in situ* and in-use versions in the informal system,

informing both the conservation experts and breeders about the genetic stability and flexibility of agricultural biodiversity *in situ*. Bioversity International, one of the partners in ISSD Africa, uses the crowd sourcing approach to get feedback from smallholder farmers on genebank material distributed in tiny packets containing three to four accessions (Bioversity International, 2015; Kiambi, 2016). The Seeds for Needs initiative is showing that re-introducing selected varieties conserved in the national genebank of Ethiopia and Kenya to farming communities, can provide concrete options to increase productivity and satisfy multiple farmers' needs.

More dynamic exchange of material between seed user communities and *ex situ* conservation is becoming more and more relevant and important. As a result of climate change rain-fed agriculture is becoming less predictable, and failed harvests as a result of drought or flooding are becoming more frequent, with higher needs to call on genetic resources with potentially appropriate resilience. These could be old local varieties as well as germplasm from regions with climatic conditions resembling the changed environment of the farmer. Active exchange between *ex situ* conservation and local user communities can assure fast (re-)introduction of lost and new diversity. Studies undertaken as part of the ISSD Africa Theme 3 'Matching Global Commitments with National Realities' in Rwanda, Uganda, Zambia and Zimbabwe, showed that relevant germplasm is indeed available in genebanks, but the systems and the legal procedures [such as the Standard Material Transfer Agreement (SMTA) under the ITPGRFA] were found not to be ready (yet) to facilitate the introduction and use of these materials in CBM (Halewood *et al.*, 2016).

### **Seed Health Improvement in Community-based Seed Systems based on Improved Knowledge of Local Seed Businesses**

Seed quality is not only dependent on genetics but also on phytosanitary health. The support by ISSD programmes for the development of local seed businesses will increase local knowledge about seed physiology, seed pests and diseases, and the effect of storage methods on seed health. Such knowledge when applied to community seedbanks may support more efficient storage of local varieties and other materials, not only of the more major crops that are likely to be part of the business of the local seed businesses, but also for the other less commercial local crops that are also part of the rotation

of smallholder farmers, and are mainly conserved *in situ* but not *ex situ*.

### **Improved Use of Agricultural Biodiversity in Breeding Programmes at Community, Public and Private Level**

Figure 1 shows many points of entry for collaboration and/or synergy between the formal and informal sector, including participatory plant breeding. Increased mutual understanding of materials and selection approaches will on the one hand enable breeders to better target new varieties to the demands of smallholder farmers, while smallholder farmers will be able to start looking beyond their local diversity and explore the potential of a wider pool of more exotic and released varieties that may enable them to market more of their produce.

### **Developments and Trends in the Formal Sector**

It is clear that agricultural biodiversity in farming systems, and hence a diverse portfolio of modern (hybrid) varieties as well as traditional local varieties, will depend on the use of the various crops by the farmer for food, fodder, market and/or crop-rotation practices, and that this diversity needs to be protected and further optimized within frameworks such as sustainable intensification. The importance of quality seed and improved varieties for meeting global food security and poverty reduction goals is widely acknowledged. Providing more of the world's smallholder farmers with quality seed is most frequently seen as a task of the formal sector, and increasingly so of the private sector actors in these. At the same time formal (*ex situ*) conservation of agricultural biodiversity continues to be considered a responsibility of the public sector.

It is noteworthy that more and more commercial seed companies recognise smallholder and family farms as an important client segment and use different strategies to enable access to their quality seed of improved varieties to such farmers (Access to Seeds Index 2016<sup>2</sup>). Very few commercial companies, however, were found to engage in the improvement of minor crops and/or in participatory plant breeding and variety selection activities. Regarding conservation global seed companies mainly collaborate with international genebanks and those in developed countries, while very few were found to have links with *ex situ* collections in low and middle

2 [www.accessstoseeds.org](http://www.accessstoseeds.org) (accessed 19 September 2016)

income countries. Although seed companies active in and originating from Eastern African countries were found to collaborate mainly with *ex situ* collections in their countries of origin, there is a danger that with the growing importance of global seed companies in low and middle income countries there will be a shift to global level *ex situ* conservation, resulting in an even weaker link between *ex situ* conservation and CBM at the local farm level.

## Conclusions

Importance of crops in smallholder agriculture are sometimes part of a largely informal and unregulated seed system (beans, finger millet), and in other crops (maize, vegetables) a more formal and regulated one. The ISSD approach aims to bridge gaps between breeders, seed producers and smallholder farmers in this formal-informal reality, and to mobilise the synergy between actors, including for better agricultural biodiversity management both from a conservation and use perspective. Agricultural biodiversity is a cornerstone for the development of the innovative sustainable agricultural systems the world needs, on smallholder farms as well as large estates. Therefore better interactions between the formal and informal seed sector in the area of agricultural biodiversity conservation and use deserve high priority.

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## On-farm/*In Situ* Conservation of Tropical Fruit Tree Diversity: Emerging Concepts and Practices<sup>1</sup>

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The Convention on Biological Diversity (CBD) defined *in situ* conservation as “the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated and cultivated species, in the surroundings where they have developed their distinctive properties” (CBD, 1992). Defining *in situ* and on-farm conservation of agricultural biodiversity was an important step; the main dilemma over the past two decades has been its practical implementation in conservation practices that fit into the context of the sustainable livelihoods of smallholder and poor farmers (Jarvis *et al.*, 2011; Sthapit *et al.*, 2012; Bellon *et al.*, 2014). While *ex situ* conservation poses largely technical challenges, *in situ* and on-farm conservation needs additionally to consider several social parameters involving farming communities and the knowledge they hold. It is impossible to undertake on-farm conservation of ‘nature’ without considering people, their rights, their needs, their values and their relationships. Despite the threat of rapidly shrinking biodiversity in farmers’ fields with associated loss of evolutionary options for the future, appropriate community-driven methods for the on-farm conservation of agricultural biodiversity continue to be meagre.

Research carried out to date has remained quite academic and descriptive, so government organizations have difficulty in translating the theory into practice on

the ground and garnering support from policy makers and communities. Many theories of on-farm conservation of agricultural biodiversity have been put forward in literature (Bellon *et al.*, 1997; Brush, 2000; Bellon, 2004; De Boef *et al.*, 2013) but few studies have been conducted long-term research to assess the impact of on-farm conservation projects in developing countries (Bellon *et al.*, 2014). We asked basic research questions to assess whether: i) empowering communities improve use and safe-guard traditional genetic resources for improved livelihood strategies and people well-being, and ii) supporting roles of custodian farmers and their local organisations support the evolutionary process of on-farm conservation of biodiversity. We aim to deepen our understanding of farmer management of diversity to determine when, where and how such strategies can be further developed to improve the resilience of such systems and farmer well-being.

### Methodology

We employed Community Biodiversity Management (CBM) method (Sthapit *et al.*, 2016). The CBM approach originally emerged from the experience of on-farm conservation projects carried out in Nepal from 1998 to 2004 during a period of civil conflict (Sthapit *et al.*, 2012a; Subedi *et al.*, 2013) and further evolved by diverse actors in several other countries where biodiversity assets are high but other resources are low. CBM is a

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community-driven participatory approach that empowers farmers and communities to organise themselves and develop strategies that support the on-farm management of agricultural biodiversity for the improvement of their livelihoods. The CBM approach integrates knowledge and practices with social systems, institutions and regulations that support conservation and development goals set by participating communities.

We tested this approach in 36 communities of GEF project, “Conservation and sustainable use of wild and tropical fruit tree diversity in India, Indonesia, Malaysia and Thailand” to: i) understand characteristics of farmers that are maintaining fruit tree diversity despite pressure of globalization, and ii) assess whether empowering diversity-rich farmers and community and local institutions helps to realise the dual goals of on-farm conservation and development, and (iii) discuss key principles and practices that empower community.

## Discussions

### Conceptual Framework

The goal of the CBM approach is to realize conservation and sustainable use of tropical fruit tree diversity for present and future generation in farmers’ fields, home gardens and orchards, and also in the wild. If crop genetic diversity is going to be conserved on-farm, it must happen as an integral part of farmers’ production and livelihood strategies. This means conservation efforts must be carried out within the framework of farmers’ livelihood and income-generating systems (Sthapit *et al.*, 2012a; Bellon *et al.*, 2014).

The CBM concept aims to that three specific outcomes to achieve the overarching on-farm conservation goal: i) community empowerment ii) livelihood development and iii) biodiversity conservation. These outcomes can be achieved if an enabling environment is created for the men and women of the community to enhance their: i) knowledge, ii) practices and iii) institutions. Communities, after initial community awareness, usually easily agree that the three outcomes are mutually supportive and needed for sustainable agriculture. However, they often lack the confidence, skills and strategies to achieve these objectives in a synergistic manner. The success of the CBM approach hinges on its ability to build the community’s commitment, confidence and problem-solving skills to achieve these widely accepted outcomes.

### CBM Process for Strengthening Farmer and CBOs Capacity

There are eight generic steps (Fig. 1) in the CBM process that enrich farmers’ knowledge with scientific knowledge. It is assumed that the process will facilitate local innovation at each step and thereby current farmer practice will evolve and improve with changing challenges and contexts. Each of the steps is guided by the principles of CBM approach, as described above. A range of methods and tools are available for each step (Sthapit *et al.*, 2016) and can be customized in the CBM process to suit practitioners’ preferences and specific purposes. Complex GEF project activities were implemented using the same steps and allowing community to develop own community action plans. An empowered community tends to demonstrate good capability in: i) situation analysis, ii) critical thinking about what is good for the environment rather than focusing on short-term gains or external resources, and iii) appropriate decision making that considers community well-being. This is only possible if local government creates an enabling environment and institutional platforms for social learning and promotes community level action plans.

### Custodian Farmers and Local Innovations

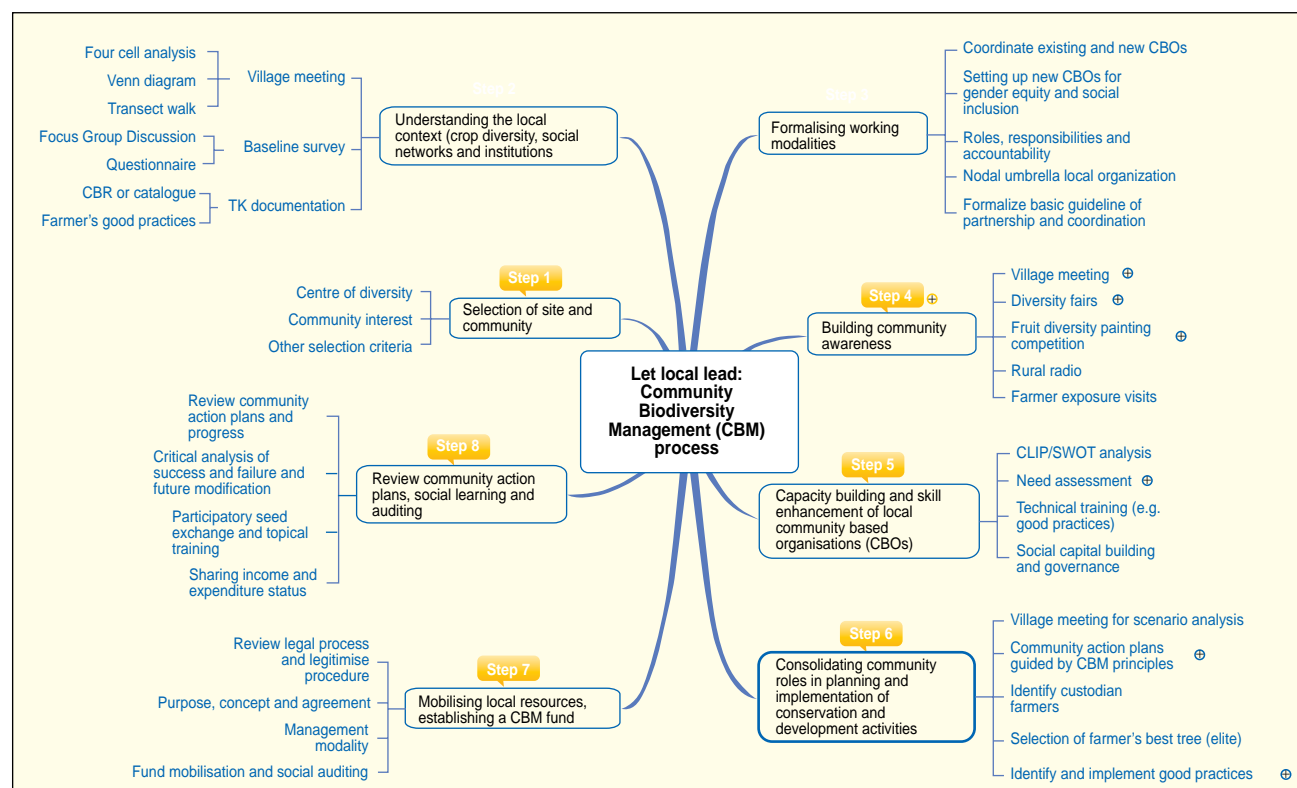
Whilst searching for good practices of tropical fruit management, researchers identified what they have called ‘custodian farmers’ (Sthapit *et al.*, 2013; 2016). These are farmers who maintain portfolios of diverse crop species and varietal diversity of agricultural biodiversity. Three features characterise these farmers: i) they maintain high richness of agricultural biodiversity, ii) they adapt or select available diversity, and iii) they disseminate materials and knowledge on a wider scale in the community. These farmers select varieties adapted to local conditions and promote the use and conservation of local diversity among their friends and neighbours, even in the absence of any extrinsic incentives. Despite commercialisation and global biodiversity loss, a few such farmers can be found in every country. These individuals can be an entry point for any community biodiversity management initiative as they are often knowledge and material holders without whom knowledge is lost. In five years, 95 elite varieties of *Mangifera*, 32 of *Citrus*, 5 of *Garcinia* and 2 of *Nephelium* were identified, characterised and documented using farmer fruit tree catalogues from four countries. Of these, a total of 75

farmer varieties of *Mangifera*, 16 of *Citrus*, 5 varieties of *Garcinia*, and 2 of *Nephelium* were registered by the respective competent authority of the government. These elite farmer materials are potentially valuable natural assets developed by farmer innovation that help income and livelihoods of farmers. In this way selected unique elite material in the name of the farmer or community can be registered with the appropriate Government Authority, according Farmers' Rights (<http://www.planttreaty.org/content/farmers-rights>) and making the information available for public and private nurseries. This is important strategy in neglected and under-utilized crops where plant breeding is long-term investment and international and national research investment is also meagre compared to arable crops and commercial vegetables. Consolidating roles of custodian farmers in the identification, piloting and mainstreaming of GPDs within a community biodiversity management approach might be one way forward.

One low-cost, efficient strategy to strengthen community biodiversity management is to work with custodian farmers (Sthapit *et al.*, 2013) to identify elite materials, which are the best trees ('plus trees') available in the community, characterise and evaluate them and further multiply them for community benefits.

## Conclusion

On-farm conservation efforts are sustainable only when local efforts are embedded within a wider context of government policies and programmes at national and local level. Mainstreaming on-farm/*in situ* conservation into the agroecosystem is grounded in a vision that firmly links research on the species and genetic diversity assessment, access to and value of crop genetic diversity to farmers with the benefits obtained by the farming community from the sustainable use of this diversity. Considerable gaps in knowledge exist as to how to consolidate local and individual efforts on the ground and with regard to local and wider contexts and requisite supportive policies and institutions. We found that community empowerment is the key driver to achieving both conservation and development. We have shown that this can be achieved by the process of community-based biodiversity management (CBM) approach—a set of principles and practices—by which communities enhance knowledge of local inter- and intraspecific diversity and improve traditional practices through continued engagement in platforms of social learning led by community organizations. These platforms may use a set of good practices (adapted to local context), tools and methods that engage both men and women, poor and rich



in collective planning and learning and practising. Here we illustrate some of those good practices that support participatory fruit tree improvement (though simple selection and propagation) for improving livelihoods, promote *in situ/ex situ* linkage and safeguarding fruit tree genetic resources. Roles of farmers, especially those of custodian farmers, as user, conservator, innovator and promoter are considered important for supporting on-farm management of tropical fruit tree diversity. We suggest that tree selection from farmers' orchards is the best approach for under-researched fruit crops. We put forward CBM as a key strategy to promote community resilience contributing to the on-farm/*in situ* conservation of plant genetic resources in general and tropical fruit genetic resources in particular.

### Acknowledgements

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## Conservation Dynamics of Roots and Tuber Crops under On-Farm Management

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Understanding the conservation dynamics of smallholder managed landrace populations requires understanding their human ecology and the selective pressures that farmers exert when they manipulate diversity. Ongoing evolution of crop genetic resources in their center of origin and diversity, where they are exposed to a dynamic state of management, environmental stress, proximity to crop wild relatives among other forces of natural and human selection, is commonly considered an essential contribution of farmer-driven conservation. It is hypothesised to lead to adaptable evolution that reconfigures diversity to contemporary cropping environments and societal needs. In this process some diversity may get lost, while new diversity may be created.

At least four pathways contribute to conservation dynamics. First, geneflow among landraces and/or compatible wild relatives and the eventual incorporation of new genotypes into farmer landrace stocks (Bonnave *et al.*, 2015). Second, the collection of selected semi-wild genotypes that are brought into cultivation. Third, mutations leading to intra-clonal variation. Fourth, Darwinist selection based on exposure of landrace pools to stressors and other selection pressures resulting in a 'survival of the fittest' of best-adapted genotypes, changes in phenology, and/or shifts in agro-ecologies (Vigouroux *et al.*, 2011). Vegetatively propagated roots and tuber crop landraces are genetically fixed once

created. This facilitated the study of their conservation dynamics on-farm (McKey *et al.*, 2012), even though the lack of time series data, geographical benchmarking, and other factors still pose challenges for field-based crop evolutionary studies.

### Methodology

In this paper we explore the four mechanisms outlined above for potato and cassava based on our own research findings and comparisons with the global literature have been discussed. We make use of several multi-year studies that were conducted by the International Potato Center (CIP), International Center for Tropical Agriculture (CIAT) and partners, involving additionally multiple (inter) national research institutes and farmer communities. Some of the studies contributing to the "big picture" are specified in Table 1.

### Discussion

*Geneflow and feral capacity:* geneflow between wild and cultivated potatoes readily occurs (Scurrah *et al.*, 2008). The subsequent steps of successful (A) germination of botanical seed, (B) seedling establishment, (C) naturalisation though advantageous feral capacity, and (D) subsequent farmer "discovery" and incorporation into landrace stocks are essential. Our finding, involving 55 hybrids exposed to natural conditions (3 environments), showed that steps A to C do occur at low frequencies

**Table 1. Summary description of selected studies conducted**

Study	Period	Country	Methodologies included
a. Geneflow in landraces and wild relatives (potato)	2002-2007	Peru	Pollinator identification, ALFP and SSR markers
b. Feral capacity of interspecific hybrids (potato)	2011-2014	Peru	Population ecology, GxE interaction trials
c. Population genetics and phenology of Araque (potato)	2011-2015	Peru	Phenological characterisation, SSR markers
d. Diversity monitoring in selected hotspots (potato)	2012-2016	Bolivia, Peru	Morphological and SSR marker characterisation, pGIS
e. Impact study of varietal releases (cassava)	2014-2015	Colombia	SNP markers, extensive varietal sampling
f. pGIS and spatial diversity transitioning (potato)	2012-2016	Bolivia, Peru	Extensive varietal sampling, cartography, pGIS

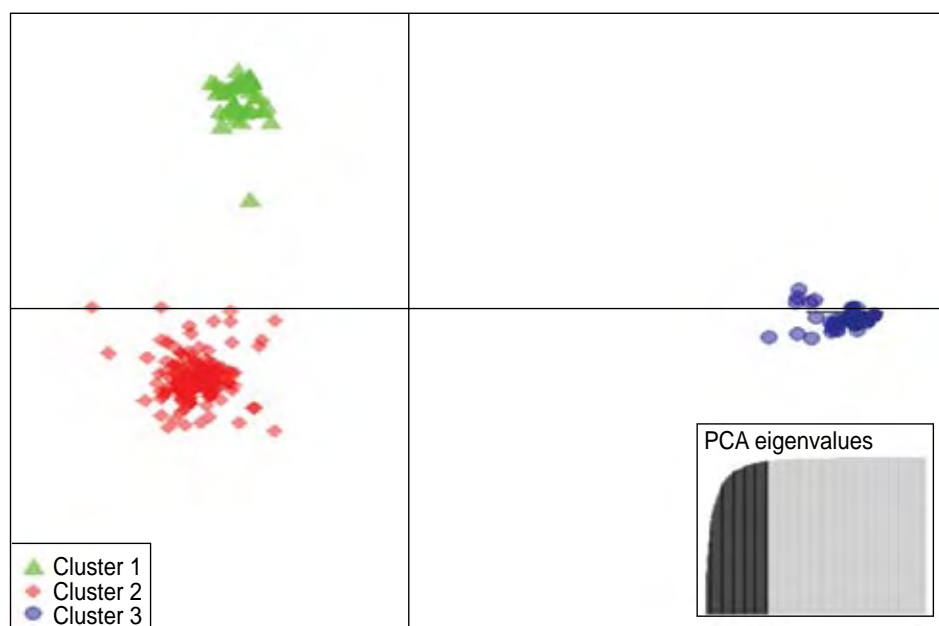
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till at least 3 years after establishment. Naturalisation rates were genotype and environmentally determined, but particularly hybrids between *Solanum tuberosum* landraces (tetraploid Andigenum group) and interspecific hybrids of *S. tuberosum* (diploid Andigenum group) with wild *S. chiquidenum* showed high survival rates. Similarly, gene flow and pathways leading to the use of new cassava hybrids have been shown in the literature (Deputié, 2008).

**Use of semi-wild genotypes:** Araq potatoes are a folk taxon with considerable cultivar diversity that is commonly found as a weed in Andean maize fields. These semi-wild potatoes are altitudinally separated from cultivated landraces. A characterisation experiment aimed at comparing the phenology of 6 Araq cultivars, 11 cultivated Andigenum landraces and 1 outgroup (*S. Juzepczukii*) showed that tuber skin thickness and vegetative reproduction through few and large tubers were significantly different. These characters in combination with prolonged dormancy and early bulking provide incentives for farmers to collect and consume Araq in times of food scarcity. Incorporation of selected cultivars into cultivated landrace stocks is uncommon, but has been observed by the authors. A SSR-marker based

population genetic study involving 668 Araq cultivars from Peru's Huanuco region and 23 primers shows that considerable allelic and clonal diversity exists, with some sub-populations (Fig. 1) being genetically very distinct from the cultivated Andigenum landraces present in CIP's ex situ reference collection. Even low-level frequencies of smallholder incorporation of Araq cultivars can have a significant effect on the overall novel diversity influx into *on-farm* managed landrace stocks.

**Mutations:** The presence of numerous potato landraces that are morphologically alike (except for tuber skin and/or bud colour), showed close similarity based on SSR-markers and belong to consistent farmer recognized cultivars groups are numerous. The occurrence of so-called "sports", or skin/bud colour variants, of well-known bred potato varieties such as Yungay (Peru) provides evidence that even recent cultivars undergo simple point mutations. Such event, which are difficult to track without detailed knowledge of time-tagged landraces diversity, are numerous for original domesticates that have been continually exposed for centuries to intense UV radiation at high altitude. The role of simple mutations in cassava and resulting chimerical



**Fig. 1. Discriminant analysis of principal components (DAPC\*) showing three discriminant clusters of semi-wild Araq potatoes (n=668), providing infrequent influxes into cultivated landrace stocks**

\* DAPC (Jombart *et al.*, 2010) evidenced 3 discriminant clusters. Cluster 1 included 117 samples (85% from Huamalíes province, 15% from Yarowilca province), cluster 2 included 357 samples (62% from Yarowilca province, 38% from Huamalíes province), and cluster 3 included 214 samples (all of them from Ambo province). Bayesian inference confirmed there are at least 3 different (sub) populations

variants is less documented compared to other root and tuber crops.

*Temporal–spatial shifts:* geographically delimited comparisons of landrace diversity in key hotspots with sizeable landrace diversity frequently show an influx and outflow of cultivar and allelic diversity. This dynamic state may be the result of a classic Darwinist selection process in which individual landraces need to comply with (changing) environmental conditions and anthropogenic use rationales. Shifts in the agroecological distribution patterns of landrace diversity are concurrent. For example, potato landrace production zones throughout the Andes have gone up 300 m in altitude during the last 50 years. Recent studies by CIAT and CIP have shown that novel landrace diversity, frequently uncovered in genebanks, is abundantly found in close proximity to the research stations where collection efforts and breeding were most intense.

## Conclusions

We show that farmer managed diversity is actively changing and continues to produce novel and unique diversity. An important implication is that the global community should more systematically monitor farmer-managed landrace populations in centers of crop genetic diversity to be able to track their conservation status and eventually increase ex situ coverage based on quantitative gap analysis. Essentially *on-farm* “conservation” projects should ideally not only elucidate temporal and spatial

change patterns of the diversity itself (landraces, alleles), but also the processes that contribute to adaptive evolution in times of accelerated global change.

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## Priority Science for the Preservation of Priority Crops

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The International Treaty on Plant Genetic Resources for Food and Agriculture identifies 64 crops or crop complexes for conservation and sustainable use. Although many can be banked conventionally as seed, lifespans will vary. For seeds that are short-lived, and the 10 species requiring explant recovery *in vitro*, cryobiotechnology is required for their preservation.

This contains what is known about the long-term *ex situ* preservation potential of the world's major crops as delineated by Annex I of the ITPGRFA. Whilst *in vitro* storage is not considered here, recovery growth *in vitro* is an important step in cryopreservation protocols for explants of those species that do not produce seeds or for which the retention of genetic homogeneity is essential.

**Seed Viability Constants:** quantify the effects of moisture content and temperature on orthodox seed lifespan (for reviews, see Roberts and Ellis, 1989; Pritchard and Dickie, 2003) and are available for 19 species from 17 genera out of a total of 76 genera (Table 1). The genera are: *Beta*, *Brassica*, *Cicer*, *Eleusine*, *Helianthus*, *Hordeum*, *Malus*, *Oryza*, *Pennisetum*, *Phaseolus*, *Phleum*, *Pisum*, *Sorghum*, *Trifolium*, *Triticum*, *Vigna* and *Zea*. As this coverage of genera represents only 22% of the total (i.e., 17 / 76) in Annex I, it is recommended that more, detailed studies of seed longevity sensitivity to water and temperature are undertaken on a wider range of agrobiodiversity, as the viability constants form the basis of predictions of longevity in agricultural seed (gene) banks across the world.

**Longevity Estimates:** of these 'easy-to-store' seeds, either based on the viability constants or on half-lives (P50s), indicates that not all seeds are long-lived (Table 1). Apart from *Raphanus* and *Sinapis*, with P50s around the 100 y mark, some other genera in the *Brassica* complex have estimated half-lives about 25 y. Interestingly, when using non-conventional banking conditions (-5 to -10°C, and ultra-dried with silica gel)

seeds of many genera in this complex, e.g. *Barbarea*, *Brassica* and *Sinapis*, survive well for 40 y. Only eight of 45 species for which long-term P50 estimates of lifespan in cold storage are available have values ~ 100 years or more: *Agrostis stolonifera*, *Avena sativa*, *Lens culinaris*, *Lolium temulentum*, *Medicago sativa*, *Pisum sativum*, *Vigna radiata* and *Raphanus sativus*. This represents only 17% of the sampled species. As shorter seed lifespans may be a feature of accessions in the world's gene banks, cryopreservation needs further exploration as a means of improving longevity, even for orthodox seeds (Li and Pritchard, 2009). There can be considerable variation in longevity performance for the same species in different genebanks. This probably relates to seed lot differences and to the use of slightly different methodology. A review of this variability would be helpful in redefining gene bank standards.

**Cryobiotechnology:** It is the combination of ultra-low temperature storage and explant recovery *in vitro*, is required for 10 of the 64 crop and crop complexes: breadfruit (*Artocarpus*), citrus (*Citrus*), coconut (*Cocos*), major aroids (*Colocasia*, *Xanthostoma*), yams (*Dioscorea*), sweet potato (*Ipomoea*), apple (*Malus*), cassava (*Manihot*), banana and plantain (*Musa*) and potato (some *Solanum*) (Table 1). For breadfruit and coconut the challenge is that the seeds produced are recalcitrant (desiccation sensitive), and across the genus *Citrus* seed desiccation tolerance is variable. Nonetheless, some progress has been made on the cryopreservation of embryos and embryonic axes of these species. Some of the other species in this cluster may need to be maintained clonally and shoot tip cryopreservation has often been used successfully, particularly employing vitrification or droplet vitrification. Post-cryo survival of shoot tips can be relatively low (<40%) in *Cocos* (coconut) and *Ipomoea* (sweet potato), indicating the need for further protocol development. Some of these species also produce 'true' seed (e.g. cassava, apple) that are orthodox and could be cryopreserved. For

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**Table 1. *Ex situ* preservation of priority crops (Annex I, ITPGRFA). Examples from the literature are given as a starting point to other reading. When material for storage is highlighted in bold, there is a need for recovery growth after cryopreservation *in vitro***

Crop	Genus	Material for storage	Example of preservation*
<b>Foods</b>			
Breadfruit	<i>Artocarpus</i>	<b>embryo axis</b>	Vitrification/cryopreservation of embryonic axes of <i>Artocarpus heterophyllus</i> with 50% developing into plants (Thammasiri, 1999). No reliable method apparent for <i>Artocarpus altilis</i> embryonic axis cryopreservation.
Asparagus	<i>Asparagus</i>	seed	Survival of <i>A. officinalis</i> seed known over c. 5 y storage under room or cool conditions (RBG Kew, 2016)
Oat	<i>Avena</i>	seed	P50: <i>A. sativa</i> = 117 y (Walters <i>et al.</i> , 2005).
Beet	<i>Beta</i>	seed	Seed viability constants: <i>B. vulgaris</i> : Ke 8.943; Cw 4.723; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016)
Brassica complex	<i>Brassica, etc</i>	seed	[Includes species in: <i>Brassica</i> , <i>Armoracia</i> , <i>Barbarea</i> , <i>Camelina</i> , <i>Crambe</i> , <i>Diplotaxis</i> , <i>Eruca</i> , <i>Isatis</i> , <i>Lepidium</i> , <i>Raphanobrassica</i> , <i>Raphanus</i> , <i>Rorippa</i> , <i>Sinapis</i> ]. P50s: <i>Brassica juncea</i> , <i>B. napus</i> , <i>B. oleracea</i> = 23-59 y; <i>Crambe abyssinica</i> = 21 y; <i>Isatis tinctoria</i> = 27 y; <i>Lepidium sativum</i> = 26 y; <i>Raphanus sativus</i> = 120 y; <i>Sinapis alba</i> P50 = 76 y (Walters <i>et al.</i> , 2005) Seed viability constants: <i>Brassica juncea</i> , Ke 7.768; Cw 4.56; Ch 0.0329; Cq 0.000478. <i>Brassica napus</i> , Ke 7.718; Cw 4.54; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016). Ultra-dry seeds of many species of Brassicaceae survived after almost 40 years of storage at -5 to -10°C, for example in the genera <i>Barbarea</i> , <i>Brassica</i> , <i>Sinapis</i> (Pérez-García <i>et al.</i> , 2007).
Pigeon pea	<i>Cajanus</i>	seed	No problem for <i>C. cajan</i> seed storage under international standard seed bank conditions (RBG Kew, 2016).
Chickpea	<i>Cicer</i>	seed	P50: <i>C. arietinum</i> = 70 y (Walters <i>et al.</i> , 2005) Seed viability constants: <i>C. arietinum</i> , Ke 8.502; Cw 4.602; Ch 0.295; Cq 0.000491 (RBG Kew, 2016)
Citrus	<i>Citrus</i>	<b>embryo, embryo axis (+ decoated seed)</b>	Partially dried explants (seeds, zygotic embryos, embryonic axes) have been cryopreserved for at least 17 species (See Malik <i>et al.</i> , 2012). Citrus shoot apices, cell suspensions and somatic embryos have also been successfully cryopreserved.
Coconut	<i>Cocos</i>	<b>embryo, shoot tip</b>	Zygotic embryos from different cultivars were cryopreserved without inducing gross morphological, genetic or epigenetic changes in the recovered plants (Susunandar <i>et al.</i> , 2010). Shoot tips cryopreserved by encapsulation-dehydration had 20% regrowth into leafy shoots 8 months after cryopreservation (N'Nan <i>et al.</i> , 2008).
Major aroids	<i>Colocasia, Xanthosoma</i>	<b>shoot tip</b>	[Includes taro, cocoyam, dasheen, tannia]. Droplet vitrification cryopreservation of <i>in vitro</i> shoot tips of taro ( <i>Colocasia</i> ), with post-thaw regeneration of 73-100% (Sant <i>et al.</i> , 2008). Cocoyam is more difficult to cryopreserve. Few studies on <i>Xanthosoma</i> .
Carrot	<i>Daucus</i>	seed	P50: <i>D. carota</i> = 30 y (Walters <i>et al.</i> , 2005)
Yams	<i>Dioscorea</i>	<b>shoot tip</b>	Following cryopreservation by a modified droplet vitrification technique, 52% of surviving explants developed further within 1 month (Leunufna and Keller, 2003).
Finger millet	<i>Eleusine</i>	seed	Seed viability constants: <i>E. coracana</i> , Ke 9.508; Cw 5.08; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016).
Strawberry	<i>Fragaria</i>	seed	<i>F. virginiana</i> seed had 75 % viability after pre-drying to 15 % RH and storage for 107 days at -20°C (RBG Kew, 2016).
Sunflower	<i>Helianthus</i>	seed	P50: <i>H. annuus</i> = 50 y (Walters <i>et al.</i> , 2005) Seed viability constants: <i>H. annuus</i> , Ke 6.74; Cw 4.16; Ch 0.039; Cq 0.000478 (RBG Kew, 2016).
Sweet potato	<i>Ipomoea</i>	<b>shoot tip</b>	Shoot tips of 24 sweet potato accessions had shoot formation levels of 2 – 66% after droplet vitrification cryopreservation (Vollmer <i>et al.</i> , 2014).
Barley	<i>Hordeum</i>	seed	Seed viability constants: <i>H. vulgare</i> , Ke 9.144; Cw 5.342; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016).
Grass pea	<i>Lathyrus</i>	seed	P50: <i>L. odoratus</i> = 40 y (Walters <i>et al.</i> , 2005)
Lentil	<i>Lens</i>	seed	P50: <i>L. culinaris</i> = 365 y (Walters <i>et al.</i> , 2005)
Apple	<i>Malus</i>	<b>dormant bud, shoot tip (+ seed)</b>	Of 77 accessions (31 species and hybrids) in the <i>Malus</i> collection, 37 had recovery >40 % (69%) when winter vegetative-buds were cryopreserved (Höfer, 2015). <i>In vitro</i> shoot tips from four <i>Malus</i> species had 57% recovery after encapsulation-dehydration cryopreservation (Li <i>et al.</i> , 2014). Seed viability constants: <i>M. pumila</i> , Ke 7.316; Cw 4.119; Ch 0.04; Cq 0.000428 (RBG Kew, 2016)

Contd.

Crop	Genus	Material for storage	Example of preservation*
Cassava	<i>Manihot</i>	<b>shoot tip</b> (+ seed)	<i>In vitro</i> shoot tips of cassava had 79% average recovery after cryopreservation via droplet vitrification (Dumet <i>et al.</i> , 2013). Seed had no loss in viability after 14 y dry, hermetic storage at -20°C (RBG Kew, 2016)
Banana / plantain	<i>Musa</i>	<b>shoot tip</b>	[Except: <i>M. textilis</i> ] <i>In vitro</i> shoot tips of 56 accessions (8 genomic groups of <i>Musa</i> spp. and one <i>Ensete</i> spp.) had 53% average post-thaw regeneration after droplet vitrification cryopreservation (Panis <i>et al.</i> , 2005).
Rice	<i>Oryza</i>	seed	P50: <i>O. sativa</i> = 34 y (Walters <i>et al.</i> , 2005). Seed viability constants: <i>O. sativa</i> , Ke 8.668; Cw 5.03; Ch0.0329; Cq 0.000478 (RBG Kew, 2016).
Pearl millet	<i>Pennisetum</i>	seed	Seed viability constants: <i>P. glaucum</i> , Ke 8.728; Cw 4.86; Ch0.0329; Cq 0.000478 (RBG Kew, 2016). At least another 19 species with orthodox seed storage behaviour (RBG Kew, 2016).
Beans	<i>Phaseolus</i>	seed	[Except: <i>P. polyanthus</i> ]. P50: <i>P. vulgaris</i> = 31 y (Walters <i>et al.</i> , 2005) Seed viability constants: <i>P. vulgaris</i> , Ke 9.09; Cw 4.761; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016). <i>P. lunatus</i> seed held under international standard seed bank conditions (RBG Kew, 2016). <i>P. maculatus</i> seed had 100 % viability after 15 % RH and 11 weeks at -20°C (RBG Kew, 2016). <i>P. microcarpus</i> seed had 100 % viability after 15 % RH and 46 days at -20°C (RBG Kew, 2016).
Pea	<i>Pisum</i>	seed	P50: <i>P. sativum</i> = 97 y (Walters <i>et al.</i> , 2005) Seed viability constants: <i>P. sativum</i> , Ke 9.858; Cw 5.39; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016).
Rye	<i>Secale</i>	seed	P50: <i>S. cereale</i> = 36 y (Walters <i>et al.</i> , 2005).
Potato	<i>Solanum</i>	<b>shoot tip</b>	[Except: <i>S. phureja</i> ]. Full plant recovery after cryopreservation of 1028 accession of nine <i>Solanum</i> species / subspecies ranged from 34 to 59% for PVS2 treated <i>in vitro</i> shoot tips (Vollmer <i>et al.</i> , 2016)
Eggplant	<i>Solanum</i>	seed	P50: <i>S. melongena</i> = 46 y (Walters <i>et al.</i> , 2005)
Sorghum	<i>Sorghum</i>	seed	Seed viability constants: <i>S. bicolor</i> , Ke 10.588; Cw 6.305; Ch 0.041; Cq 0.000349 (RBG Kew, 2016).
Triticale	<i>Triticosecale</i>	seed	No problem for triticale seed storage under international standard seed bank conditions (RBG Kew, 2016).
Wheat	<i>Triticum</i>	seed	[Includes: <i>Agropyron</i> sp. (see Forage – grass below)]; <i>Elymus</i> sp. (P50s: <i>E. agropyroides</i> = 19 y; <i>E. antarcticus</i> = 64 y; <i>E. batalinii</i> = 26y; <i>E. canadensis</i> = 25 y; <i>E. caninus</i> = 18 y; <i>E. ciliaris</i> = 14 y; <i>E. dahuricus</i> = 22 y; <i>E. drobovii</i> = 16 y; <i>E. fibrosus</i> = 21 y; <i>E. hystrix</i> = 76 y; <i>E. lanceolatus</i> = 26 y; <i>E. mutabilis</i> = 20 y; <i>E. nutans</i> = 35 y; <i>E. patagonicus</i> = 73 y; <i>E. semicostatus</i> = 19 y; <i>E. sibericus</i> = 80 y; <i>E. trachycaulus</i> = 14 y; <i>E. transhyrcanus</i> = 178 y; <i>E. tsukushiensis</i> = 38 y; <i>E. villosus</i> = 56 y; <i>E. virginicus</i> = 109 y (Walters <i>et al.</i> , 2005); <i>Secale</i> sp. (P50: <i>S. cereale</i> = 36 y; Walters <i>et al.</i> , 2005). Seed viability constants: <i>T. aestivum</i> , Ke 9.42 ; Cw 5.859 ; Ch 0.0329 Cq 0.000478 (RBG Kew, 2016)
Faba bean / vetch	<i>Vicia</i>	seed	P50: <i>Vicia</i> sp. = 71 y (Walters <i>et al.</i> , 2005)
Cowpea <i>et al.</i>	<i>Vigna</i>	seed	P50: <i>V. radiata</i> = 457 y (Walters <i>et al.</i> , 2005) Seed viability constants: <i>V. radiata</i> , Ke 10.858; Cw 6.27; Ch 0.0329; Cq 0.000478. <i>V. unguiculata</i> Ke 9.401; Cw 5.118; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016)
Maize	<i>Zea</i>	seed	[Excludes: <i>Z. perennis</i> , <i>Z. diploperrenis</i> , <i>Z. luxurians</i> ]. Seed viability constants: <i>Zea mays</i> , Ke 8.579; Cw 4.91; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016)
<b>Forage – legume</b>			
Astragalus	3 species	seed	[Includes: <i>A. arenarius</i> , <i>A. chinensis</i> ] <i>A. cicer</i> seed germination changed from 95 to 100% after 13 y storage under international standard seed bank conditions (RBG Kew, 2016).
<i>Canavalia</i>	<i>ensiformis</i>	seed	[NB Six species in the genus are thought to be orthodox in storage response, and one species ‘uncertain’ (RBG Kew. 2016)]. Information needed on <i>C. ensiformis</i> .

Contd.

Crop	Genus	Material for storage	Example of preservation*
<i>Coronilla</i>	<i>varia</i>	seed	[NB Five in the genus are thought to be orthodox in storage response (RBG Kew, 2016)]. Information needed on <i>C. varia</i> .
<i>Hedysarum</i>	<i>coronarium</i>	seed	<i>H. coronarium</i> seed germination not change (98%) after 13 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Lathyrus</i>	<i>odoratus</i>	seed	P50 = 40 y (Walters <i>et al.</i> , 2005).
<i>Lespedeza</i>	<i>cuneata</i>	seed	P50 = 43 y (Walters <i>et al.</i> , 2005).
<i>Lotus</i>	<i>cornicularis</i>	seed	P50 = 68 y (Walters <i>et al.</i> , 2005).
<i>Lupinus</i>	<i>angustifolius</i>	seed	P50 = 41 y (Walters <i>et al.</i> , 2005).
<i>Medicago</i>	6 species	seed	[Includes: <i>M. falcata</i> ]. P50: <i>M. sativa</i> = 93 y (Walters <i>et al.</i> , 2005). <i>M. arborea</i> seed had 100 % viability after 15 % RH and 4 weeks at -20°C (RBG Kew, 2016). <i>M. scutellata</i> seed had germination change from 99 to 95% after 13 y under international standard seed bank conditions (RBG Kew, 2016). <i>M. rigidula</i> seed had germination change from 97 to 99% after 13 y under international standard seed bank conditions (RBG Kew, 2016). <i>M. truncatula</i> seed germination remained 100% after 11 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Melilotus</i>	<i>officinalis</i> (syn: <i>alba</i> )	seed	P50 = 63 y (Walters <i>et al.</i> , 2005).
<i>Onobrychis</i>	<i>viciifolia</i>	seed	P50 = 34 y (Walters <i>et al.</i> , 2005).
<i>Ornithopus</i>	<i>sativus</i>	seed	<i>O. sativus</i> seed germination not change (100%) after 13 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Prosopis</i>	5 species	seed	[Includes: <i>P. affinis</i> , <i>P. alba</i> , <i>P. chilensis</i> , <i>P. nigra</i> , <i>P. pallida</i> ] All these species are held in long-term storage (see RBG Kew, 2016).
<i>Pueraria</i>	<i>phaseoloides</i>	seed	<i>P. phaseoloides</i> average germination changed from 93 to 88% after 10 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Trifolium</i>	15 species	seed	[Includes <i>T. agrocicerum</i> ] 9/15 species with P50s: <i>T. alexandrinum</i> = 70 y; <i>T. ambiguum</i> = 86 y; <i>T. hybridum</i> = 29 y; <i>T. incarnatum</i> = 59 y; <i>T. pratense</i> = 43 y; <i>T. repens</i> = 77 y; <i>T. resupinatum</i> = 199 y; <i>T. subterraneum</i> = 73 y; <i>T. vesiculosum</i> = 46 y (Walters <i>et al.</i> , 2005). <i>T. alpestre</i> seed germination changed from 89 to 98 % after 13 y under international standard seed bank conditions (RBG Kew, 2016). <i>T. angustifolium</i> seed germination of 97% after 12 y under international standard seed bank conditions (RBG Kew, 2016). <i>T. arvense</i> seed germination changed from 93 to 98% after 13 y under international standard seed bank conditions (RBG Kew, 2016). <i>T. rueppellianum</i> seed at 15 % RH tolerated 37 d at -20°C with 100% viability (RBG Kew, 2016). <i>T. semipilosum</i> seed at 15 % RH tolerated 1 month at -20°C with 100% viability (RBG Kew, 2016). Seed viability constants: <i>T. subterraneum</i> , Ke 7.21 ; Cw 3.51 ; Ch 0.04; Cq 0.0004 (RBG Kew, 2016)
<b>Forage – grass</b>			
<i>Andropogon</i>	<i>gayanus</i>	seed	<i>A. gayanus</i> seed is held in long-term storage (RBG Kew, 2016).
<i>Agropyron</i>	2 species	seed	P50s: <i>A. cristatum</i> = 65 y; <i>A. desertorum</i> = 45 y (Walters <i>et al.</i> , 2005).
<i>Agrostis</i>	2 species	seed	[Includes: <i>A. tenuis</i> ]. P50: <i>A. stolonifera</i> = 232 y (Walters <i>et al.</i> , 2005).
<i>Alopecurus</i>	<i>pratensis</i>	seed	<i>A. pratensis</i> germination changed from 75 to 100% after 14 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Arrhenatherum</i>	<i>eliatius</i>	seed	<i>A. eliatius</i> seed germination remained at 96% after 12 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Dactylis</i>	<i>glomerata</i>	seed	P50 = 65 y (Walters <i>et al.</i> , 2005).

Contd.

Crop	Genus	Material for storage	Example of preservation*
<i>Festuca</i>	6 species	seed	2/6 species with P50s: <i>F. arundinacea</i> = 25 y; <i>F. rubra</i> = 25 y (Walters <i>et al.</i> , 2005). <i>F. gigantea</i> seed germination changed from 94 to 93% after 12 y under international standard seed bank conditions (RBG Kew, 2016). <i>F. heterophylla</i> seed germination changed from 88 to 100% after 11 y under international standard seed bank conditions (RBG Kew, 2016). <i>F. ovina</i> seed germination changed from 95 to 96% after 12 y under international standard seed bank conditions (RBG Kew, 2016). <i>F. pratensis</i> seed germination changed from 94 to 95% after 12 y under international standard seed bank conditions (RBG Kew, 2016).
<i>Lolium</i>	5 species	seed	[Includes: <i>L. hybridum</i> ]. 4/5 species with P50s: <i>L. multiflorum</i> = 43 y; <i>L. perenne</i> = 41 y; <i>L. rigidum</i> = 82 y; <i>L. temulentum</i> = 263 y (Walters <i>et al.</i> , 2005).
<i>Phalaris</i>	2 species	seed	<i>P. aquatic</i> seed germination remained c. 88% after 12 y under international standard seed bank conditions (RBG Kew, 2016). <i>P. arundinacea</i> seed held international standard seed bank conditions (RBG Kew, 2016).
<i>Phleum</i>	<i>pratense</i>	seed	P50 = 32 y (Walters <i>et al.</i> , 2005). Seed viability constants: <i>P. pratense</i> , Ke 9.571; Cw 5.262; Ch 0.04; Cq 0.0004 (RBG Kew, 2016).
<i>Poa</i>	3 species	seed	P50: <i>P. pratensis</i> = 54 y (Walters <i>et al.</i> , 2005). [Includes: <i>P. alpina</i> ; <i>P. annua</i> ]. <i>P. alpina</i> seed germination changed from 95 to 100% after 12 y under international standard seed bank conditions (RBG Kew, 2016). <i>P. annua</i> seed no problem for long-term storage under international standard seed bank conditions (RBG Kew, 2016).
<i>Tripsacum</i>	<i>laxum</i>	seed	Evidence needed.
<b>Forage – other</b>			
<i>Atriplex</i>	2 species	seed	Seeds of <i>A. halimus</i> are held in long-term storage (RBG Kew, 2016). Seeds of <i>A. nummularia</i> survive 14 days cryopreservation (Touchell and Dixon, 1993).
<i>Salsola</i>	<i>vermiculata</i>	seed	<i>S. vermiculata</i> seed maintained viability after 7 y storage at -15°C (Kay <i>et al.</i> , 1988) Summary: 76 genera (NB <i>Lathyrus</i> and <i>Agropyron</i> are listed under both Food and Forage; <i>Solanum</i> is listed twice for potato and eggplant).

\*Seed in the Walters *et al.* (2005) study was stored dry for >10 years at 5° C, plus c. 25 years at -18°C. P50 = estimated half-life. When P50 values were not available, information was drawn from the Seed Information Database, with information shown generally as average responses (RBG Kew, 2016).

apple, advantage is taken of adaptation to cope with cold winters as dormant buds can be cryopreserved, although very high levels of recovery growth are not yet guaranteed for all accessions and there is variability in response between years.

In summary, a range of techniques and protocols are available for the long-term *ex situ* preservation of the Annex I crops and crop complexes, but inter-specific and intra-specific variability in responses highlights the need to look beyond the one-size-fits-all approach to plant genetic resources *ex situ* preservation.

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## Plant DNA Resources in RIKEN BRC to Bridge the Gap between Gene Function and Phenotype

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National Bioresource Project started in 2002 by the support of Ministry of Education, Culture, Science, Sports and Technology (MEXT) in Japan. The important purpose of this project is to collect, preserve, and distribute bioresources (such as experimental animals, plants and microbes etc.) that are essential experimental materials for life sciences research. Developing fundamental technologies to improving the value of each bioresources, and enriching genome information are also important target of this project. At the same period, the Experimental Plant Division of RIKEN BioResource Center (RIKEN BRC: <http://epd.brc.riken.jp/en/>) was established in the RIKEN Tsukuba Institute in 2001 to promote resource activities of *Arabidopsis* plant (Kobayashi, 2011). *Arabidopsis* is one of the most well-analyzed experimental model plants. In the end of 20<sup>th</sup> century, the entire genome sequence of *Arabidopsis* has been completely characterised (The Arabidopsis Genome Initiative, 2000). Since then, *Arabidopsis* genome sequence data create massive amount of useful and desirable information by several omics-based analyses. Huge quantities of genome resources are also developed by international *Arabidopsis* research community. In RIKEN BRC, we collect seeds of RIKEN *Arabidopsis* transposon-tagged mutants, activation-tagged lines, FOX (full-length cDNA overexpressor gene) hunting system, and natural accessions, as well as *Arabidopsis* suspension cell culture T87 cells. We also collect DNA resources such as RIKEN *Arabidopsis* full-length cDNA clones (RAFL clones). Here, we introduce outline of our full-length cDNA resources and give detailed description about our approach to bridge the gap between gene function and phenotype.

### RIKEN *Arabidopsis* Full-length cDNA (RAFL) Clones

Unlike in the case of partial cDNA libraries, developing full-length cDNA libraries are very useful for further analyses of gene function, and also large-scale gene discovery projects, or other -omics research (Seki *et al.*, 2009).

Several techniques have been established to prepare enriched full-length cDNA libraries (Seki *et al.*, 1998). The usefulness of full-length cDNAs has been confirmed in humans, mice, and in various plants such as *Arabidopsis* (Seki *et al.*, 2002). RIKEN *Arabidopsis* full-length cDNA (RAFL) have been developed by RIKEN Genome Sciences Center. RAFL clones have been utilised as standard clones in *Arabidopsis* community. Currently, more than 200,000 clones are available from RIKEN BRC.

### Other Full-length cDNA Clones in RIKEN BRC

Full-length cDNA projects were performed in many plant species in Japan (Umezawa *et al.*, 2008, Aoki *et al.*, 2010, Abe *et al.*, 2011). In RIKEN BRC, we collect full-length cDNAs of model plant species. Recently we started the distribution of approx. 40,000 clones of *Brachypodium distachyon* full-length cDNAs. Approximately 150,000 clones of *Physcomitrella patens* full-length cDNAs, 20,000 clones of Poplar full-length cDNAs, 20,000 clones of *Manihot esculenta* full-length cDNAs, 40,000 clones of *Striga hermonthica* full-length cDNAs are also available from RIKEN BRC. In all case, we perform the end sequencing of every clones to confirm the absence of contamination or mishandling before shipping.

### SABRE Database to Cross-Search Plant Genetic Resources through Publicly Available *Arabidopsis* Information

After genome sequencing project, international *Arabidopsis* research community started 'Arabidopsis 2010 Project' (Ausubel, 2002) to investigate the function of about 27,000 entire genes of *Arabidopsis*. As a result, huge amount of information about each *Arabidopsis* gene function have been obtained. To utilise this useful information about *Arabidopsis* gene function for other plant species, RIKEN BRC have developed SABRE (Systematic Consolidation of *Arabidopsis* and other Botanical REsources) database (Fukami-Kobayashi *et al.*, 2013). In SABRE (<http://sabre.epd.brc.riken.jp/>)

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SABRE2. html), plant cDNA clones are linked to TAIR (The *Arabidopsis* Information Resource) gene models and their annotations through sequence homology. Approximately 1.5 million plant cDNA information from the National BioResource Project (NBRP) are integrated in SABRE database, and these data are composed of cDNA information from 14 plant model plant species (*Arabidopsis*, barley, cassava, Chinese cabbage, lotus, morning glory, poplar, *Physcomitrella patens*, *Striga hermonthica*, soybean, *Thellungiella halophila*, tobacco, tomato and wheat).

### Fox Hunting System to Bridge Between Gene Function and Phenotype

FOX hunting system (Full-length cDNA Over-eXpressing gene hunting system) have been developed in RIKEN Plant Science Center to create gain-of-function mutant lines caused by ectopic expression of full-length cDNAs (Ichikawa *et al.*, 2006). Approximately 10,000 *Arabidopsis* full-length clones were over-expressed in plants under the control of cauliflower mosaic virus 35S-promoter and created more than 10,000 transgenic lines. These lines were deposited in the RIKEN BRC and available for phenotypic screening. We also collect and distribute FOX hunting lines, overexpressing rice full-length cDNA in *Arabidopsis* plants.

### Conclusion

We recently collected and started the distribution of *Arabidopsis* TAC (transformation-competent artificial chromosome) clones (Shibata *et al.*, 2000). TAC clones can accept large genomic DNA fragment in *Agrobacterium tumefaciens* as well as *Escherichia coli*. Therefore, TAC clone can be directly used for *Agrobacterium* mediated *Arabidopsis* transformation, and accelerate genetic complementation to understand *Arabidopsis* gene function. We also started distribution of the RIKEN *Arabidopsis* Transcription Factor (RARTF) ORF clones. The cDNAs encoding transcription factor are distinctly important DNA resources. However, all of these cDNA set were found in RAFL clones. Accordingly, RARTF clones were PCR amplified using *Arabidopsis* genome information (Iida *et al.*, 2006). Fujita *et al.* (2007) have performed the mini scale fox hunting system, over-expressing stress inducible *Arabidopsis* transcription factor genes. They successfully isolated the stress tolerance related transcription factor genes. Strategic development of DNA Resources would enable us to bridge the gap between gene function and phenotype.

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## Securing Plant Genetic Resources for Perpetuity through Cryopreservation

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As the expanding world population increasingly depends on a dwindling number of cultivars and of crop species, so the concomitant loss of crop diversity increasingly threatens global food security. Therefore crop diversity and associated wild relatives urgently need to be conserved for future generations.

Seed conservation is the most used *ex situ* method to conserve crop germplasm. However, it is not an option for those crops that are sterile (do not produce viable seeds, like banana), or produce only recalcitrant (non-storable) seeds (like cocoa and coconut). Nor is it an option for species where specific gene combinations need to be maintained during propagation (many fruit species such as apple and potato). In such cases, vegetative material needs to be maintained in the field or in *in vitro* collections (micro plants grown in test tubes). Even under reduced-growth conditions, maintaining *in vitro* collections is still labour intensive, and there is always the risk of losing accessions due to contamination or human error. Moreover, *in vitro* material of some species is subject to somaclonal variation (spontaneous mutations whose frequency is generally increased during *in vitro* culture). Cryopreservation, or storage of biological material at ultra-low temperatures, is therefore preferred for the long-term conservation of plant genetic resources of vegetatively propagated crops.

### Cryopreservation

The main issue when exposing biological tissues to low temperatures is the formation of lethal ice crystals during cooling or thawing. Crystals thus formed penetrate membranous cell structures like nuclear and plasma membranes, causing irreversible damage, and a loss of their semi-permeability. The only way to avoid the formation of ice crystals in a solution is vitrification. Vitrification or glass transition refers to the transformation

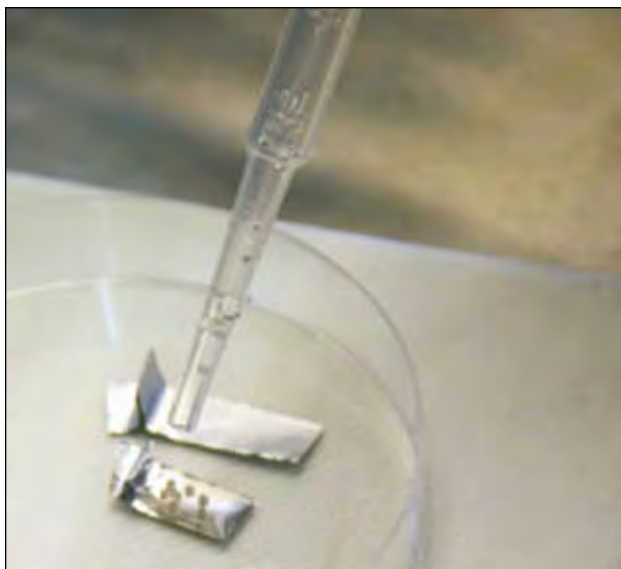
of a glass-forming liquid into a glass. Two requirements must be met for a liquid to vitrify. The solution must be both: i) rapidly cooled, and ii) “glass forming” or highly concentrated. These two requirements form the basis for developing efficient cryopreservation techniques (Panis *et al.*, 2001).

Roughly, three main cryopreservation protocols can be distinguished for hydrated tissues: i) the “classical” slow freezing protocol (currently mainly applied to non-organized tissues such as cell suspensions and calluses) (Withers and King, 1980); ii) the encapsulation/dehydration method that relies on synthetic seeds (Fabre and Dereuddre, 1990), and iii) the methods relying on the application of highly concentrated vitrification solutions such as PVS2 (Plant Vitrification Solution 2) (Sakai *et al.*, 1990). The latter two methods are more suitable for organised plant tissues such as shoot cultures.

The droplet vitrification protocol was established because it combines the application of highly concentrated cryoprotective vitrification solutions (often PVS2) with ultra-fast freezing and thawing. Such high freezing and thawing rates are obtained by transferring the biological material (often meristems) to small strips of aluminium foil and exposing them directly to liquid nitrogen (Panis *et al.*, 2005) (Fig. 1).

The droplet vitrification protocol can now be considered as the first “generic” cryopreservation method for plant tissues, as it has now been successfully applied to different tissues (shoot cultures/embryos) and a wide variety of plant species from different climatic environments. Moreover, the technique is less cumbersome in its application compared with other cryopreservation methods and no sophisticated equipment is needed (Panis *et al.*, 2011).

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**Fig. 1.** Excised banana meristems (each 1 mm<sup>2</sup>) are transferred inside a droplet of vitrification solution onto a strip of aluminium foil for droplet vitrification (Photo credit: Bioversity International/N. Capozio)

### Applications of Cryopreservation in Crop Germplasm Collections

Currently, many of the institutes that maintain large collections of vegetatively-propagated crops species use

droplet vitrification to store their valuable germplasm for the long-term (Table 1).

The largest collection of cryopreserved, vegetatively-propagated plant material is kept at the USDA-ARS National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, CO. Since 1993, dormant apple buds of 2,302 accessions have been cryopreserved (Volk *et al.*, 2015). The largest cryopreserved collection of *in vitro* shoot-tips comprises currently about 1,454 potato accessions and is stored at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben (Keller *et al.*, 2016). Other large collections of *in vitro* shoot tips are Bioversity International's International Transit Centre (ITC), in Leuven, Belgium (950 banana accessions) (Fig. 2) and the Centre for International potato (CIP) 's genebank, in Lima, Peru (1450 potato accessions) (CIP, 2016).

### Safety Back-up of Cryopreserved Collection

Until now each institute that stores cryopreserved crop germplasm has applied its own conservation standards with respect to 'what is a good regeneration rate to consider the germplasm safely stored'. Some of them consider a crop accession as safely cryopreserved for the long-term provided the regeneration rate of the

**Table 1.** Cryopreservation methods used in world's largest crop genebanks for storing their vegetatively-propagated germplasm

Institute	Country	Crop	Cryopreservation Method
Bioversity International, Leuven	Belgium	Banana	Droplet vitrification
Crop Research Institute, Prague	Czech Republic	Potato, garlic, hops	Droplet vitrification
International Center for Tropical Agriculture (CIAT), Cali	Colombia	cassava	Droplet vitrification Encapsulation/dehydration
International Institute of Tropical Agriculture (IITA), Ibadan	Nigeria	Yam, banana, cassava	Droplet vitrification
International Potato Center (CIP), Lima	Peru	Potato	Straw vitrification Droplet vitrification
Julius Kühn-Institut (JKI), Institut für Züchtungsforschung an Obst, Dresden	Germany	Strawberry/ Fruit trees	Vitrification Dormant bud freezing
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department, Gatersleben	Germany	Potato, garlic, mint	Droplet freezing Droplet vitrification
National Agrobiodiversity Center (NAAS), RDA, Suwon	South Korea	Garlic	Droplet vitrification
Tissue Culture and Cryopreservation Unit, NBPGR, Delhi	India	Banana, chives, medicinal plants, berries, fruit trees.	Vitrification Droplet vitrification Slow freezing Dormant bud freezing
National Institute of Agrobiological Sciences (NIAS), Tsukuba	Japan	Mulberry	Dormant bud freezing
USDA-ARS, Fort Collins and Corvallis	USA	Citrus species, grape, garlic, mint, fruit trees.	Vitrification Droplet vitrification Slow freezing Dormant bud freezing

representative sample is at least 30 %. Others use 40%, 60% or even 80% as cut-off.

Bioversity International accepts a banana accession as safely stored provided: i) Three successful independent repetitions are executed, and ii) for each successful repetition stored in the cryotank, the ITC [International Transit Centre (previously called INIBAP)] is 95% confident that at least one plant can be regenerated (Dussert *et al.*, 2003). As an additional “security” measure, one replicate set of all accessions has been transferred under frozen conditions to the Institut de Recherche pour le Développement (IRD), Montpellier, France using a dry shipper. This ‘black-box’ back-up at IRD is located 1000 km from Leuven thus reducing the risk of germplasm loss due to political and/or environmental hazards.

Bioversity International proposes creating a Global CryoVault for a range of vegetatively propagated crops. The proposed location will be in Leuven, Belgium, although other locations are being considered. This Global CryoVault, will ensure that a copy of cryopreserved crop samples conserved elsewhere in the world will be safely stored. It will act as a complementary facility to the Svalbard Global Seed Vault, in the Arctic tundra, which conserves crops that reproduce through ‘storable’ seeds. With these two facilities, the majority of existing crop diversity – tens of thousands of species and varieties of all food crops and wild relatives – will be preserved for present and future generations.

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**Fig. 2.** A box of Cryotubes, each tube containing 10 cryopreserved banana meristems retrieved from liquid nitrogen storage at Bioversity International Transit Centre ITC (Photo credit: Bioversity International/B. Panis)

## Expanding Applications of Cryobanking: Meeting Challenges for Effective Long-term Storage

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ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) leading the plant cryobanking efforts since 1987 equipped with large capacity cryotanks have successfully banked non-orthodox seeded species, prioritized orthodox seeds, pollen, dormant buds and genomic resources at ultra-low temperatures with retesting data generated for 28 years of cryostorage. Expansion of activities while extending the cryobanking facilities to ICAR-National Bureau of Agriculturally Important Microbes (ICAR-NBAIM) has led to microbes cryobanking. Wide scope exists for expansion of basic cryo-principles to store endangered exceptional species that produce few seeds, tropical and sub-tropical oilseeds especially orchids, species with inherently short-lived seeds (Type I seeds), despite being orthodox in nature, neglected and underutilised plant species (NUS), with focus on species of biodiversity hot-spots in the moist tropics as well as biotechnologically important cell lines and transgenics. Co-cryopreservation of orchid seed and fungal symbionts/mycorrhizal fungi for the conservation of endangered orchids successfully demonstrated in few studies needs to be taken up. With the concept of cryopreservation of phyto-diversity encompassing algae and cyanobacteria besides the higher plants, greater expansion is envisaged. With the state-of-the art cryotechnologies existing for aquatic species and veterinary animals, as recently reviewed in International Conference, “Low Temperature Science and Biotechnological Advances” held by ICAR-NBPGR, India in April 2015, expansion of large scale cryobanking is imminent. Studies on cellular impact of cryoprotocols and changes in gene expression in relation to cryopreservation are ongoing in different labs of the World. Broad range of technical solutions to permit establishment of more efficient and user-friendly protocols for cryobanking of ‘hydrated sensitive’ materials are now available and work is being expanded.

### Cryobanking the Plant Genetic Resources

Cryopreservation as a strategy to extend viability of conserved biological materials, for theoretically ‘indefinite’ periods, has been widely acknowledged. Germplasm/species/ organisms selected and prioritised for cryostorage are determined by each Cryogenebank keeping in view the technical, practical and economic aspects of cryopreservation. The priority for cryobanking depends upon the designed infrastructure, scope of expansion, networking with other institutes with common goals and policy statement of the organisation or the country. Several countries of the world have adopted large scale banking for long-term conservation of germplasm as zygotic seeds and its components, pollen, dormant buds, *in vitro* raised explants with each institute/ organisation focusing on each type separately and in few cases all the forms in the same bank. National Center for Genetic Resources Preservation (NCGRP), USA, besides being a seed bank, is also a repository for animal genetic resources in the form of semen and plant genetic resources as graftable buds or *in vitro* explants. ICAR-NBPGR, New Delhi is yet another Institute cryoconserving, plant origin explants, microbes and genomic resources.

### Plant Cryobanking at NBPGR: Seeds, Embryos, Embryonic Axes, Pollen, Dormant buds and Genomic Resources

With proven wide applicability of cryotechniques in plant sciences, diverse germplasm of national and international importance is being cryopreserved in National Cryogenebank at ICAR-NBPGR with emphasis on non-orthodox spp. (intermediate and recalcitrant species). Over a span of 28 years, cryoprotocols have been developed, in most cases on species-specific basis, using explants like seeds, embryos, embryonic axes, pollen and dormant buds of the difficult-to-store types.

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Orthodox seed species belonging to threatened and endangered plant species with critically small population size, wild and weedy relatives of crop plants, genetic stocks including core collections of crop diversity have been selectively cryostored. The Cryogenebank Facility with six cryotanks of extra large capacity (1000 lits LN) can hold quarter million samples. Presently it holds about 42,000 containers (cryovials of various capacities, polyolefin tubings of different diameter) storing more than 11,700 accessions belonging to about 794 plant species (Anon., 2015). Cryopreservation protocols have been standardised primarily for tropical species of horticultural, plantation, agro-forestry and industrial importance. Base collection of tropical and temperate fruits, plantation crops and agroforestry species e.g. of *Citrus* (25 species), *Piper nigrum*, *Buchanania lanzan*, *Prunus armeniaca*, *P. dulcis*, *Morus* (13 spp.), *Mangifera*, *Jatropha*, *Pongamia* and *Azadirachta* germplasm has been established in various forms like seeds, embryos, embryonic axes, dormant buds, pollen and genomic resources (Chaudhury and Malik, 2014). Cryobanking of non-orthodox (recalcitrant and intermediate) seeds is intensively undertaken (Chaudhury and Malik, 2010). The aim had been to recover plants from seeds, embryos, embryonic axes after various storage periods, bring back to room temperature without any damages-structural and physiological and obtain plantlets without an intervening callus to ensure genetic integrity of the conserved germplasm. Techniques have already been optimised to recover plantlets without an associated callus. Despite best efforts not all species under threat and those with recalcitrant seeds can be assisted by conservationists. Specialised techniques of desiccation-freezing, vitrification, encapsulation-dehydration and step-wise slow freezing are applied to difficult-to-store species.

Basic cryobiological investigations involving biochemical, biophysical and ultra-structural markers of desiccation and freezing sensitivity are being pursued (Chaudhury *et al.*, 2011; Malik *et al.*, 2011). Cryoconserved samples randomly tested for various periods upto 25 years (Choudhary *et al.*, 2013; Chaudhury and Malik, 2014) and after 28 years storage (pers. comm.) have shown retention of viability values comparable to that in initial samples. Recently cryobanking of genomic resources comprising mainly cloning vectors, cloned genes and promoters, different DNA libraries, BAC, YAC, PAC clones etc. of plants, animals and microbes

has been initiated. In addition, HRD training component is executed through the Centre of Excellence (CoE), recognised by ICAR and Bioversity International, for conduct of international trainings on *in vitro* conservation and cryopreservation. Seven international trainings have been conducted and so far more than 100 trainee participants of the World have been trained with three in collaboration with Royal Botanic Gardens, Kew, UK.

### Microbes Cryobanking at NBPGR

Cryopreservation of microbes leads to extended viability (several years) and prevents genetic and physiological changes expected during long-term culture of actively growing organisms. For microorganisms which are prone to cryogenic-injury, specific protocols have been designed to ensure optimal cryopreservation (Smith and Ryan, 2012). Microbes are reportedly successfully cryobanked including 4,000 species of fungi, belonging to over 700 genera. However, some microbes e.g. bacteria *Helicobacter*, fungi e.g. members of *Basidiomycota* and *Chromists* etc. are found to be preservation recalcitrant. To carry out research on the interactions between microbes in plants and algae, whether beneficial or pathogenic, requires utilisation of cryopreserved microbial cultures. Throughout the world most of the reputed microbial resource centres (eg. ATCC, BCCM-LMG, CABI, NES-MCC, etc) have adopted the cryopreservation technology.

At NBPGR cryopreservation has been attempted with *Micrococcus luteus*, *Agrobacterium tumefaciens*, *Acetobacter pasteurianus*, *A. baumannii*, *Escherichia coli*, *Enterobacter cloacae*, *Lactobacillus acidophilus*, *Klebsiella pneumonia*, *Cellulomonas flavigena* and *Brevundimonas diminuta* selected from genepool of NBAIM. Pure cultures of the bacteria inoculated in different glycerol concentrations were cooled slowly and cryobanked at -196°C. The viability of the bacteria was checked at regular time intervals and found satisfactory.

### Expanding Applications to Seeds of Orthodox and Rare and Endangered Plant Species, Transgenic Plant Materials, Mycorrhizal Fungi and Bryophytes

Cryopreservation has proven effective for futuristic programmes. For terrestrial native orchids which are dependent on mycorrhizal associations with fungi for survival in field, conservation of both is essential. Long-term *ex situ* storage of orchid seed and fungal

symbionts for the conservation of endangered orchids have increasingly being attempted using cryopreservation (Batty *et al.*, 2001; Sommerville and Offord, 2014). Pritchard and Nadarajan (2008) and Cruz-Cruz *et al.* (2013) have strongly suggested cryopreservation to be appropriate for integrating as an extra insurance policy for storing all orthodox seeds with sub-sample of each to be cryostored, in addition to the samples stored under classical gene bank conditions of -20°C. There are several examples to illustrate the application of cryopreservation techniques to preserve rare and endangered species for various higher plants, including orchids, bryophytes and ferns.

Reports exists for successful cryopreservation of transgenic materials containing different target genes in *Oryza sativa*, *Triticum aestivum*, *Carica papaya*, *Citrus sinensis*, *Pyrus pyrifolia*, *Betula pendula*, *Papaver somniferum* (Cho *et al.*, 2007; Van Eck and Keen, 2009; Wang *et al.*, 2012). Expression of foreign genes remained unaffected and the productive ability of cryopreserved cells containing recombinant proteins was similar to that in non-cryopreserved cultures.

### Looking Ahead for Expanding Applications of Cryopreservation

The future cryobanking activities need a fresh look based on experiences gained by laboratories that are adopting large scale banking. Regular brainstorming with International and national experts at ICAR-NBPGR in recent times contributed to refine and fine tune the concepts, practical approaches to match them with the expectations from the current researches. Laboratories are striving to put in correct perspectives the role of cryopreservation to handle the challenges faced by biodiversity conservation at national and international levels.

The issues needing focus are :

- Role of conventional storage and trends in *ex situ* conservation using cryopreservation techniques;
- Networking approaches – nationally and internationally;
- Best practice, knowledge transfer and infrastructure;
- Need to initiate banking even before techniques are perfected for high survival;
- Competitive basic research to maximize storage success:
  - a. Systems biology research to identify suitable markers;
  - b. Identifying the model species for basic studies on desiccation tolerance and for freezing tolerance.
- Promoting cryobiology work in the national and international arena through trainings.

### Exploring Wide Applications

Basic research on conservation are challenging as additional data from seed banks emerge and several crops especially wild species and crop wild relatives are left behind in our conservation efforts or show less than expected longevity under -20°C storage. Desiccation sensitive plant materials exist in about 47% of plants of tropical moist forest needing cryobanking. Recalcitrant seeded species, the main targets for cryobanking, may be close to one quarter of the World's higher plants (Walters *et al.*, 2013) and hence pose challenge to conservationists in this regard.

### Aquaculture Cryopreservation

Sperm cryopreservation, mainly of commercially important fish such as salmonids, carps, sturgeons, catfish, herring, cod and sea bass, has been achieved for several fish species with 10% to 85% post thaw fertility where lesser results are achieved with fresh water species in comparison to marine species. Advances in research on cryopreservation of fish oocytes and embryos are very limited and success is still not much encouraging and needs expansion.

### Animal Species Cryopreservation Especially Related to Endangered and Threatened Species

There is increasing interest in maintaining genetic diversity for future conservation of wild endangered animal species. The preservation of valuable breeds using assisted reproductive technologies is limited and is assisted by cryopreservation of biological materials especially gametes from such species. Several animal species classified as threatened, vulnerable and endangered are often difficult to breed both in captivity and under natural conditions and cryobanking assists in restoration of species in large numbers.

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## In Vitro Propagation and Conservation of Tropical RTBs

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Staple clonally propagated crops such as root, tuber, banana and plantain (RTBs) crops are subjected to genetic erosion and variety loss under the pressure of many factors. The combination of these includes habitat loss and climate change including increased pest and diseases incidence. Safeguarding genetic resources of those food security crops for future use is critical through ensuring their sustainable conservation and use, for global food and nutrition security.

The first conservation system for RTBs crop diversity is *in situ* especially for crop wild relatives. But *in situ* conservation of these world important food crop gene pools is highly under-resourced and inadequately managed. Therefore, *ex situ* conservation is key to holding materials in genebanks or seed banks (conserved as seeds, field plants, vegetative shoots *in vitro* or cryopreserved materials) that are also backed-up in another location.

In general, clonally propagated crops (produce very few seeds, are vegetatively propagated for breeding reasons and/or species that require a long life cycle to generate breeding and/or planting materials) such as RTBs, essentially, are not conserved as orthodox seeds. The germplasm is either conserved as live plants in fields, as potted plants in enclosed structures or different plant parts are conserved into *in vitro*, either in slow growth (medium term) or cryopreservation (long-term). Traditionally, the conservation and maintenance of root and tuber crops is done in field conditions. Many technical guidelines and training manuals exist for the management of RTBs germplasm collections held in field genebanks (Reed *et al.*, 2004a; Geburek and Turok, 2005). However, major challenges of field conservation of RTBs are long reproductive cycles, associated with low multiplication rate, high cost, pest and diseases, mislabelling and duplication leading to germplasm losses. These disadvantages are magnified by climatic factors.

*In vitro* biotechnological approaches are reliable, complementary and/or alternative system to support multiplication, safer and longer conservation RTBs genetic diversity, and their sustainable utilisation. They have the potential to address future, technical, scientific, economical and environmental demands on RTBs (Pilatti *et al.*, 2011).

### *In vitro* Propagation of RTB Crops

The major advantage of *in vitro* propagation of RTBs material is the potential of large multiplication for the seed system, e.g. as an efficient and cost-effective propagation system (Asiedu *et al.*, 1998; Quin, 1998; Thro *et al.*, 1999). The latter authors report two projects in Latin America that used cassava *in vitro* culture to address priorities of small-scale cassava farmers. Cassava propagation is generally done using lignified stem cuttings (Thro *et al.*, 1999). The multiplication rate is as low as 1:10 compared to at least 1: 100 in some cereals, creating a bottleneck for transfer and adoption of new varieties.

Tissue culture techniques have been used for RTB multiplication *via* organogenesis, mainly from younger and vigorous mother plants that might allow higher micropropagation rate (Mitchell *et al.*, 2006). For yams, pre-formed meristems (Malaurie *et al.*, 1995a, b), shoot organogenesis from immature leaves (Kohmura *et al.*, 1995), roots (Twyford and Mantell, 1996) shoot/nodes culture and microtuber formation (Balogun *et al.*; 2006; Ovono, 2007; Salazar and Hoyos, 2007) have been used to initiate *in vitro* multiplication. When the culture medium is supplemented with gibberellin inhibitors, shoots from nodal explants in (Poornima *et al.*, 2007) and numerous axillary nodes (Bimbaun *et al.*, 2002; Balogun, 2005) were reported in many yam species.

Among the various *in vitro* propagation techniques for RTBs, synthetic seeds (Standardi and Piccioni, 1998)

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can be cited and recently somatic embryogenesis. Somatic embryos were induced from leaf tissues of *D. rotundata* in culture medium containing 2,4-D and incubated in darkness. However, low induction frequencies (<30%) were recorded and protocols need to be optimized. In *D. alata* and *D. opposita*, embryogenic cell masses were induced from root explants in liquid MS supplemented with 2,4-D and cultured in light (Twyford and Mantell, 1996; Nagasawa and Finer, 1989). Germination of somatic embryos of *D. alata* increased in the presence of GA<sub>3</sub> (Deng and Cornu, 1992; Twyford and Mantell, 1996). Plantlet recovery from somatic embryos of *D. rotundata* was enhanced at 4.5% sucrose but not affected by benzylaminopurine (Okezie *et al.*, 1994; Pandro *et al.*, 2011). These reports pointed out probable genotype-dependent protocol for yam embryogenesis.

Photoautotrophic propagation (PAP) is another system that should be used for RTBs. In PAP system, explants are directed towards autotrophy while in culture by reducing or completely substituting sucrose with carbon dioxide. Plantlet growth is enhanced in photoautotrophic more than heterotrophic conditions if environmental control is adequate (Hazarika *et al.*, 2003; Xiao, 2004; Afreen, 2005). However, after about four weeks, plants may stop responding to carbon dioxide and growth is reduced due to slight closing of stomata on the bottom of the leaves as plants sense high CO<sub>2</sub>. However, use of CO<sub>2</sub> enrichment for two weeks, then a week off intermittently will ensure higher yields as the plant continuously seeks as much CO<sub>2</sub> as possible (Andrew, 2002). In potatoes, much work has been done on PAP and photo-mixotrophic propagation (Mohamed and Alsadon, 2010; Santana *et al.*, 2012).

The use of temporary immersion bioreactor systems (TIS) allowed handling of the culture by robotization, while optimizing multiplication rate of plantlet and microtuber production and sprouting (Cabrera *et al.*, 2011; Watt, 2012). In TIS, growth is enhanced (Escalona, 2006) since there is lack of continuous immersion in liquid/semi-solid culture medium; contamination is controlled while aeration is timed. In addition, microtubers from TIS can be grown on the field and used in original seed production programmes. Enhancement of growth in TIS can to the exploration of medicinal secondary metabolites production, like yam steroidal diosgenins (Raju and Rao, 2012). Shoots grown in TISs had enhanced growth and the leaves had higher

photosynthetic pigment content than other techniques (Jova *et al.*, 2011, 2012; Cabrera *et al.*, 2011).

### **In Vitro Slow Growth Conservation and Cryopreservation**

Slow growth conservation leads to the reduction of loss risks associated with the field banks, and constitute a viable alternative to complement and reduce the large size required for field banks. RTBs genebanks around the world have *in vitro* tissue culture facilities as a complementary conservation system, giving the possibility to clean the germplasm from diseases and pest via meristem culture and/or coupled with other cleaning methods. This conservation method requires technical expertise, facilities and operating budget. Though, they are generally more economical and less risky in a long-term perspective; as compared to field collections. Plant tissue culture is a powerful tool for safer and faster way to multiply large quantity of material for distribution, duplication in other genebanks and international exchange (easier plant material transport); and also for breeding purposes. Slow growth storage is however for short to medium term conservation, after which the plantlets are subcultured when signs of deterioration/necrosis are visible (Balogun, 2009). The conservation of RTBs needs small quantity of material and allows longer duration between two regenerations or subcultures, using slow growth storage. The principle is to place the *in vitro* plantlets under slow growth conditions, through adaptation to physical factors (light, temperature, culture medium, growth retardants). According to Ng and Ng (1997), 47 countries were holding cassava collections but only 12 maintained *in vitro* facilities for conservation. Many of these laboratories combine *in vitro* techniques target for pathogen cleaning with rapid multiplication and genebank conservation. *In vitro* conservation of cassava is still far less common than field conservation. The largest national *in vitro* collections are held in Brazil and Argentina. There appear to be very few RTBs *in vitro* genebanks in Africa. International collections are held at CIAT, CIP, Bioversity International and IITA, while all other *in vitro* genebanks have a national or regional focus.

Cryopreservation, almost systematically associated with *in vitro* conservation, is another conservation method for RTBs germplasm. It allows maintenance of plant material at ultra-low temperature (in liquid

nitrogen at  $-196^{\circ}\text{C}$ ) using cryogenic techniques. At such low temperature, plant cell biological activities and metabolism are stopped, eliminating the need to regularly rejuvenate or regenerate the plant. It is currently a supplementary tool to improve conservation of germplasm in a longer-term perspective. Cryopreservation is the most reliable technique for long-term storage of plant genetic resources (Popov *et al.*, 2005). It avoids the disadvantages of irreversible loss of totipotent competencies caused by *in vitro* ageing process (Benson, 2008), time and labour consumption. Many studies confirmed that it's economically more competitive compared to other conservation systems (Harvengt *et al.*, 2004; Reed *et al.*, 2004a; Keller *et al.*, 2008). Cryopreservation helps to overcome many of *in vitro* maintenance disadvantages such as labour-intensive sub-culturing, potential elimination of pathogens and somaclonal variation related to multiple subcultures. It also ensures the safe long-term conservation of genetic resources. Thus, cryopreservation techniques have been increasingly used for long-term storage. In the last 25 years, several cryogenic techniques have been developed, especially those based on vitrification method (the transition of water directly from the liquid phase into an amorphous or "glassy" phase, whilst avoiding the formation of crystalline ice) such as encapsulation-dehydration, preculture-dehydration, and encapsulation/vitrification. Therefore, the main requirement for using cryopreservation method is that it should be simple, economical, reproducible and should allow relatively high regrowth rate Leunufna and Keller (2003).

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## Conservation and Management Approaches of Fish Genetic Resources in India: Present Status and Future Outlook

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Conservation of aquatic biodiversity is important from the fact that significant share of the genetic resources for food is still contribute from the wild due to low domestication level in fisheries sector. Conservation needs must be aimed towards preserving existing biodiversity and also the evolutionary processes that foster the biodiversity. The approaches towards fish genetic resource conservation and management are as follows.

### Documentation of Fish Diversity

Knowledge of species, genetic stocks and ecological roles is necessary for the management of ecosystems and habitats as well as to the identification of important genomes and genes. Identification, cataloguing and prioritisation of species are important tasks in conservation. The ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR) has developed a database on Indian fish diversity comprising of 2,936 indigenous (1,887 marine, 113 brackish water and 936 freshwater fishes, besides 462 exotic finfishes. In addition, the database is also available with consolidated lists of freshwater fishes found in the Western Ghats and north eastern hill region biodiversity hot-spots. Check-lists of macro-fauna and flora of Gulf of Mannar Biosphere Reserve (3,065 species) and the Ramsar Site-Vembanad Lake (185 species) have been prepared.

### In situ Conservation

*In situ* conservation programmes for fish germplasm resources need the integration of knowledge on fish and habitat diversity, habitat utilisation, biology including genetic structure, life history traits as well as human interference and other socio-economic issues. The *in situ* conservation allow continued co-evolution wherein the wild species may continue to co-evolve with other forms, maintain genetic diversity of species and evolutionary adaptations.

National parks and biosphere reserves provide less expensive protection for the wild relatives than *ex situ* measures. Such conservation efforts can be meaningful only with people's participation through mass awareness programmes and involving the stake holders. In India, the protected area covers about 5.2% of the total land area, including 5,456 sites, and nine threatened fish species inhabiting these areas.

### Stock Replenishment and Enhancement

Captive breeding programmes have become the major tool used to compensate the declining fish populations and simultaneously to supplement as well as enhance yields of wild fisheries. Although culture, breeding and larval rearing technologies for the major carps have been developed for several decades, but many non-conventional freshwater fish species having commercial value are yet to achieve the demonstrated status. These include *Chitala chitala*, *Ompok pabo*, *O. pabda*, *O. malabaricus*, *Labeo dussumieri*, *Semiplotus semiplotus*, *Clarias dussumieri*, *Channa diplogramme*, *Anabas testudineus*, *Nandus nandus*, *Cirrhinus reba*, *Barbodes carnaticus* and *Puntius sarana*. These captive bred seeds can be used for ranching into natural waters. The programme on mass-scale breeding, seed production and ranching of important threatened endemic fish species through establishment of live germplasm resource centers in three locations, initially, is a significant step forward towards conservation. More regional live germplasm resource centers in different agro-climatic zones may be established in collaborative mode to accommodate more species.

In such stocking programmes, scientific knowledge on the genetic structure of the original wild population is crucial to avoid any long-term negative impact due to mixing with captive bred seed. Therefore, appropriate blood-stock population of genotyped individuals should

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be the source of seed used for ranching. NBFGR, in a joint programme with the Regional Agricultural Research Station (RARS), Kumarakom, Kerala successfully carried out stock-specific, breeding-assisted river ranching of two fishes (*Horabagrus brachysoma* and *Labeo dussumieri*) in Kerala; the landings of *H. brachysoma* after two years increased from 1.8% to 11 % and that of *L. dussumieri* showed an increase from 0.68% to 3.9% of the total landings from the Vembanad Lake and adjacent rivers in the state.

### Concept of State Fish

An innovative approach to fish conservation by declaring a State Fish was adopted for the first time in the country at NBFGR in 2006. This involved integration of the key stakeholders in the conservation plan where 17 states of the country have become partners with NBFGR in developing strategies for conservation and enhancement of their selected State Fish in order to achieve the real time conservation success.

### Ex situ Conservation–Cryopreservation of Fish Gametes and Embryos

Storage of fish spermatozoa, eggs and embryos without loss of viability is of considerable value in aquaculture and conservation. In India, NBFGR is the primary organisation carrying out fish sperm cryopreservation for long-term gene banking. The fish sperm cryopreservation needs development of species-specific protocols. Such protocols are developed through experimental standardization of various parameters, after the captive breeding protocol is developed. This becomes a bottleneck due to protracted breeding season and low domestication of most of the aquatic species, especially marine fishes. Nevertheless, in all such cases, time available in a year for conducting experiment is small and determined by breeding cycle of the species. In view of the constraint, it is essential that candidate species for sperm cryopreservation are prioritized. Species-specific sperm cryopreservation protocols have been developed for 28 species.

Fish gamete cryopreservation research still faces an important challenge in the form of long-term storage of fish eggs and embryos except the minute fertilized abalone eggs. Development of fish cell lines, embryonic stem (ES) cells and germ cells from Indian fishes and cloning technology as an alternative to long-term storage of finfish eggs and embryos has been emphasized. Embryonic stem (ES) cells are pluripotent stem cell lines that are derived

from early embryo and these cells can differentiate to become any tissue in the body. Successful protocols for grafting of embryonic cells to host embryos, for germline transmission of desired genome, can be instrumental in evolving effective programmes for rehabilitation of endangered species.

### Tissue Banking

Tissue banking is a fast mode of storing the biological material for longer durations and it can be used to retrieve genetic information and genetic manipulation studies in future. Nearly, 15,000 tissue accessions of freshwater and marine fish species collected from mainland and island ecosystems are maintained in the tissue bank of NBFGR.

### Molecular Tools to Complement Taxonomy and Evolutionary Linkages

DNA based approach to taxon identification which exploits diversity among DNA sequences and can be used to identify fishes and resolve taxonomic ambiguity including discovery of new species. These approaches offer simple, rapid and reliable means of identifying not only whole fish, but fish fragments, eggs and larvae. DNA barcodes (based on Cytochrome Oxidase I) of more than 600 finfish species reported from India so far. Establishing evolutionary linkages using molecular tools could be of immense utility in sustainable exploitation, management and conservation of Indian fish species. However, it is necessary that such effort is intensified through initiation of a mission mode programme to barcode all available finfish and shellfish species available in the country.

### Genetic Characterisation

The primary objective of the genetic characterisation is to assess the distribution and pattern of genetic variability at intra as well as inter-specific level populations, through the use of identified genetic markers. The conclusions from genetic diversity data have varied application in research on management and conservation of fish species, to understand the pattern of migration of fish stocks, nature of breeding populations and also in taxonomy/systematics. The choice of markers is crucial in achieving precise information that is useful for desired application. Several marker types soluble proteins, gene products (allozymes), nuclear and mitochondrial DNA markers have been used. Microsatellite DNA markers have been popularly used for the purpose. A concerted

effort made at different ICAR Institutes over last 10 years has provided description of genetic variation and population structure for about 30 prioritized fish and shellfish species from their major range of natural distribution. Distinct population structure was observed in many of these species indicating that propagation assisted restoration programmes must be stock-specific to replenish declining populations.

### *Exotics and Quarantine*

Many introductions of exotic species for fisheries and aquaculture diversification have been successful; but others have resulted in highly publicised failure, generating controversy over protection of native biodiversity, spread of pathogens and diseases. To safeguard our indigenous fish genetic resources from infectious exotic diseases and to develop effective protocols for fish quarantine adequate facilities and expertise have been developed in the country over the years. Rapid diagnostic capability for detecting the eleven fish OIE (The World Organization for Animal Health or Office International des Epizooties) listed pathogens using molecular and immunological tools are available today. Success has also been achieved in developing

monoclonal antibodies against *Labeo rohita*, which will be extremely useful in serodiagnostics for pathogen surveillance in aquaculture of Indian major carps. We have been able to establish referral laboratory for all OIE listed pathogens in India. The National Repository of Fish Cell Lines established at NBFGR with financial support of DBT possessing 50 fish cell lines is a significant step forward for undertaking research on fish virology in coming years.

### **Conclusion**

The conservation needs must be aimed towards preserving existing biodiversity and also the evolutionary processes that foster biodiversity. The conservation of fish diversity and resources of the country requires concerted efforts by integrating capture, culture fisheries and environmental programmes using latest technological innovations. Suitable programmes on the priority areas in consortia mode involving different research organisations, developmental agencies and community and stakeholder participation will certainly generate more results with respect to sustainable utilisation of fish genetic resources and management fisheries.

## Complementary Strategies for Conservation of Animal Genetic Resources and Global Innovation Challenges

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### Global Challenges and Trends

The livestock sector is facing major challenges in order to reach global food security and to supply animal products for a growing global population (UN, 2012). At the same time, the livestock sector will have to anticipate climate change (Aby and Meuwissen 2014; IPCC, 2014) through both mitigation and adaptation strategies. Sustainable growth and sustainable intensification strategies are needed to meet the predicted future demands taking advantage of innovation and implementation of new technologies in the livestock sector. Genetic improvement of livestock has played an important role in enhancing the efficiency and sustainability of animal production during the past decades and will continue to be relevant in order to deal with future challenges.

International flows of animal genetic resources have continued to expand over recent years, driven by the increasing demand for higher-output animals and major developments in livestock management and reproductive biotechnologies. Introduction of new technologies in advanced animal breeding programmes has resulted in high genetic gains. Global distribution of improved genetics has accelerated, although the introduction of highly specialised, improved genetics to more extreme and variable production circumstances is not always sustainable. A still growing proportion of livestock products is produced by highly specialised livestock breeds, and local breeds have been gradually replaced by more widely used specialised breeds. The Second State of the World's Animal Genetic Resources (FAO, 2015) showed that 17% of the world's livestock breeds is classified as being at risk of extinction.

### Complementary Conservation Strategies

Multiple publications have indicated that complementary *ex situ* and *in situ* conservation strategies are needed to support and to promote the conservation and sustainable use of genetic resources. Both type of strategies have

clear advantages and disadvantages with respect to specific conservation objectives, and individual strategies can include a wide range of activities to support the conservation and sustainable use of genetic resources. And, very important, there is a variety and large number of actors, contributing to or leading particular conservation strategies.

*In situ* conservation strategies support the maintenance of livestock populations in the environment where the breeds have developed, and in this context it is important to establish and implement sustainable breeding and genetic improvement programmes. The complementary *ex situ/in vitro* strategy (cryoconservation and storage of frozen genetic material) is particularly an important back-up strategy for long-term conservation of between and within breed genetic diversity.

For animal genetic resources (AnGR) most of the European countries report *in situ* and *ex situ* conservation activities for livestock breeds, but there are also gaps in (the breed coverage of) conservation programmes (FAO, 2015). The Second State of the World's AnGR (FAO, 2015) shows that *in vitro* gene banks have been established by 64 countries and a further 41 countries are planning to do so. However, many of these gene banks are in early stages of development and most collections have gaps in their breed coverage.

An integrated *in situ/ex situ* conservation strategy requires a common understanding of conservation priorities at local, national and European level. However, often neither *in situ* nor *ex situ* conservation strategies may have a high priority in national policies; stakeholders or user groups may have different interests resulting in different conservation priorities. Coordination at national, regional and global level is needed to strengthen (linkages between) *ex situ* and *in situ* conservation efforts, as it was also indicated in the the Global Plan of Action for Animal Genetic Resources (FAO, 2007).

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## Developments in Animal Breeding

Application of new technologies in animal breeding, including genomic selection and advanced reproductive technologies, will continue to play an important role in meeting future challenges for the livestock sector. Genomic selection, which enables prediction of the genetic merit of animals from genome-wide SNP markers, has already been adopted by dairy industries worldwide and is expected to double genetic gains for milk production and other traits. Use of whole-genome sequence data could further accelerate these developments (Hayes *et al.*, 2012). Genomic selection reduces the generation interval and has clear advantages for traits that cannot be measured on the selection candidates themselves, for traits that are difficult to measure or traits expressed late in life. Genomic selection also has the advantage to start a genetic improvement programme in an un-pedigreed population, and has the potential to be applied across breeds, in non-mainstream breeds, or in cross-bred small-holder cattle populations in developing countries (Brown *et al.*, 2016). However, extensive validation of the association between genotypes and phenotypes will always be needed first.

The livestock sector has entered the area of ‘precision breeding’ (Flint and Woolliams, 2008). Developments in genomics and the possibility of generating full DNA sequence information at low cost, will make it possible to unravel the genetic mechanisms of traits in livestock breeds. Detailed knowledge can then be used to make ‘interventions’ at DNA level, either through genomic selection, genomic introgression methodologies (Aby and Woolliams, 2014) or through emerging genome editing techniques (Jenko *et al.*, 2015). Modification of the genome using genome editing techniques has the potential to introduce specific phenotypes in commercial livestock populations, on the basis of increased knowledge of genome function and genetic mechanisms. Expected growing demands for animals that are robust, well-adapted to climatic extremes, or to climate-related disease challenges, could trigger such developments. Genome or gene editing techniques – combined with advanced reproductive technologies may change the animal breeding landscape in the future.

## Gene Banks – Their Current and Future Roles

Countries are implementing the Global Plan of Action for Animal Genetic Resources (FAO, 2007) and are developing *ex situ/in vitro* conservation programmes

(Paiva *et al.*, 2014; FAO, 2015), complementary to *in vivo* conservation. Gene banks are important sources of genetic variation to ensure long-term conservation of within and between breed genetic diversity and to support the maintenance of genetic diversity in *in situ* livestock populations. Moreover, genebanks have shown to be a crucial resource for building reference populations and training sets for genomic selection. For the future, gene bank collections are expected to become increasingly relevant for research and development purposes.

Establishment of national farm animal gene banks often serves multiple objectives and gene banks differ in strategies, types and size of collections and organisation (Hiemstra *et al.*, 2014). In general farm animal gene banks are organised by breed, and the concept of maximising/optimising genetic diversity stored for each breed is widely accepted. However, it can be argued that more emphasis should be put on characterisation and conservation of specific, unique phenotypes, across breeds (Leroy *et al.*, 2015).

Gene bank collections could be better evaluated in terms of the genetic diversity currently present in genetic collections, for a range of traits. More research on functional genomics could provide a better understanding of the relationship between genotype and phenotype, for example to assess the adaptation of individuals to environmental variation, in particular in the context of climate change.

Whereas methods have been developed to optimise the management of genetic variability of *in situ* populations either under selection or under conservation, few scenarios have been tested in order to (re)introduce diversity or ‘lost’ alleles in animal production systems through the use of gene banks (Leroy *et al.*, 2011). New techniques, including genomic introgression (Wall *et al.*, 2005, Yazdi *et al.*, 2008) and also gene editing could provide opportunities to (re)introduce rare or lost alleles or particular traits.

Long-term conservation of farm animal genetic diversity is primarily a government responsibility, but breeding industry is also interested in gene bank collections for future breeding and research, in particular to better understand the genetic background of traits across breeds and environments. Breeding industry will have to take responsibility for long-term conservation of genetic diversity in their own breeding populations or breeding lines, and at the same time they could contribute

to the long-term conservation of farm animal genetic diversity in national gene banks. Further involvement of breeding associations, breeding industry, farmers' networks and other actors in the establishment of gene bank collections should be promoted, and more could be done with regard to the practical utilisation of gene bank collections in breeding and research (EC, 2016).

### International Collaboration as a Network of Gene Banks

In Europe, the EUGENA initiative is developing the European Gene Bank Network, with the support of the European Regional Focal Point for Animal Genetic Resources (<http://www.rfp-europe.org/>). Currently there is limited information available about gene bank collections at national level and most gene banks are currently more active in storage than in distribution of genetic resources. At present, data on gene banks are scattered and incomplete. The current lack of information severely limits the transfer into current breeding programmes of genebank material which has been collected in the past. From this perspective there is a need for gene-banking data information systems that connect cryopreserved samples with a precise and comprehensive description of phenotypic and genomic information.

Groeneveld *et al.* (2016) also highlighted the importance of biobanks to meet future demands in livestock production. Well-established domesticated animal biobanks and integrated networks harbour immense potential for great scientific advances with broad societal impacts, which are currently not being fully realised.

IMAGE (Innovative Management of Animal Genetic Resources), a research project funded by the EU Horizon 2020 programme (<http://imageh2020.eu>), will support the development of the network of farm animal gene banks in Europe (EUGENA). The general aims of IMAGE are to enhance the use of genetic collections in Europe, to upgrade animal gene bank management, and to demonstrate the benefits brought by gene banks. IMAGE distinguishes two main types of genetic collections, germplasm collections (reproductive material) and genomic collections (DNA and tissues for research purposes). Both types of genetic collections are not very well connected. There is a need to facilitate access to (information about) genetic collections across Europe for research collaboration, conservation and breeding of

transboundary breeds and for future breeding in general. IMAGE will take advantage of rapid developments in genomics and bio-informatics and will further develop reproductive technologies.

### Conclusion

The global livestock sector is facing major global challenges and at the same time farm animal genetic diversity is under threat. Novel breeding approaches and complementary *in situ* and *ex situ* strategies are needed to deal with those challenges, and international collaboration and exchange of genetic resources has been important and will continue to be important for the future.

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## Experiences on Conservation of Indian Dairy Animal Biodiversity

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India is home to about 8% of the world's biodiversity on just 2.4% of global area and has one of the highest diversity of ecosystems in the form of forests, wetlands, grasslands, marine areas, deserts, glaciers, mangroves among others (8). Biodiversity is crucial to the alleviation of poverty, due to the basic goods and ecosystem services such as agriculture, forestry, fisheries and tourism, on which more than seventy percent of country's 1.3 billion people depend on for their livelihood. Sustainable development cannot be achieved if biodiversity is compromised by development efforts. With regard to Farm animal Biodiversity status in India, 40 breeds of cattle and 17 of buffalo among total 163 registered breeds of different species besides populations/breeds of other species which have not been classified in breeds and registered so far. Population of many indigenous breeds is declining due to large increases in human population, urbanization, mechanization and increased food demands over-time leading to change in the utility pattern of animal genetic resources (8). Endangered status of cattle breeds like Krishna Valley (7) and similar other breeds warrant immediate steps for correction. Small-scale livestock keepers and pastoralists over centuries have developed animal breeds that are well suited to local agro-climatic conditions of scarce food and water resources, frequent draughts, hardy and resistant to many tropical diseases (9). In spite of they being low producers (although many having potential of enhancing production) can continue producing meat and milk in areas where modern, imported breeds succumb without expensive housing, feed and veterinary care (7). Social changes have greatly influenced Animal Genetic Resources (AnGR) because present generation is not keen to continue their ancestral occupation of rearing livestock in migratory system of grazing (2). This calls for an immediate action for systematic conservation, genetic improvement and sustainable utilisation of indigenous livestock breeds.

### Need of Conserving Biodiversity

The task of conservation, management and evaluation of vastly distributed native animal genetic resources is of gigantic magnitude for the country especially when the animal holding per family is one or two (1). Since the genetic improvement through selection is limited to organized herds and concept of conservation of AnGR is poorly understood by development agencies and farmers, undertaking suitable programmes of survey, conservation, characterization and evaluation of different types of animal genetic resources by cooperation of various agencies such as Indian Council of Agricultural Research (ICAR) institutes, universities, research centres and Animal Husbandry Department (both Central as well as States), NGOs, animal rearing communities etc. becomes essential. Farming community expresses that strains of many cattle and buffalo breeds exist but are not being studied and recognized (3,4). Strong backing from Govt. and technology is needed (6).

### Status of Dairy Animal Conservation and Improvement

While reviewing the progress of ongoing programs on conservation of threatened breeds, the important gaps identified are the absence of participation of livestock keepers, inadequate multiplication, production and dissemination of germplasm of breeds from established or strengthened nucleus farms, poor impact on conservation of breed, inadequate infrastructure development for production of semen and AI facilities under field condition, delays in implementation of programs, poor technical monitoring etc. The need is felt for adequate funding in consonance with technical program and activities, strengthening institutional frameworks, sensitization of field functionaries, formulating breeding policies for conservation and development of breeds, empowerment of technical monitoring and implementing committees, their accountability and exit plan with self

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sustainability approach. The breed conservation units must act as demonstration cum training centers for the livestock keepers and other stake holders engaged in management of AnGR.

*In situ* and *ex situ* conservation are best ways of preserving/conserving a breed and if the sample size is fairly large, it can maintain sufficient genetic diversity in the breed population. The models of conservation are developed by NBAGR for providing technical inputs and incentives to the farmers/breeders of the breeding tract and can be adopted through the State Agricultural and Veterinary universities/State Animal Husbandry Departments/ICAR Institutes and NGOs (5). Ex-situ conservation through frozen semen, embryos, Somatic cell lines etc. at National Animal Gene/Somatic cell Bank established at NBAGR are useful.

### Constraints Faced

Since the priority of state animal husbandry department is more for improvement of productivity rather than conservation of AnGR, uncontrolled breeding at farmers' herd leads to difficulty in implementing conservation and improvement programs. This would result in dilution of genetic purity of the breeds. Inadequate capital resources, non availability of genetically superior breeding males, infrastructure for Artificial Insemination (A.I.) and animal health support, lack of Breed Societies or associations and patronage, lack of legal framework/Act for implementation of breeding policies and performance recording in the field and legislation for protection of Animal Breeds and Animal Keeper's rights, habitat erosion e.g. squeezing grazing lands, absence of regular system of monitoring of the breeds at risk, lack of farmers awareness about the long-term impact of conservation of biodiversity and no or negligible immediate financial benefit also make them less interested, entire funding and implementation by government agencies and negligible participation by farmers and private sector, make the implementation of conservation program more difficult and not sustainable.

### Suggestions for Future Approach to Sustenance of Biodiversity

The suggestions for sustenance of biodiversity include undertaking sample survey at periodical interval for determining breed population and for preparing breed watch lists, establish/strengthen and accredit breed specific nucleus farms of all stake holders to produce genetically superior males for breeding, characterization

and documentation of lesser known breeds/populations for breed registration, formation of breed organizations, value addition through identification and exploitation of breed specific unique products, finding niche markets for these products for ensuring survival of breeds. Unraveling of unique genes and bio-prospecting the special utility traits like disease resistance, adaptation to harsh climatic conditions and low input, bio-molecules, products, etc. and their commercialization for enhancing utility of local breeds, documentation of traditional knowledge (TK) regarding customary practices, innovations, etc. related to indigenous breeds need to be collected, evaluated, validated and commercially exploited to benefit the communities rearing these animals, strengthening National Gene Bank, formulating comprehensive scheme on conservation and sustainable utilisation of threatened breeds in the country covering the species of cattle and buffalo, conserve local breeds through sustainable use before they become endangered, finding niche markets for their products is one possible way of ensuring the survival of these breeds, and enabling the people who keep them to earn more from their existing lifestyle etc. are some of the ways suggested.

### Conclusions

India is home to 8% of the world's biodiversity on just 2.4% of global area and status wise includes Farm Animal Biodiversity resources of 40 breeds of cattle, 17 of buffalo among total 163 registered breeds of different species. Biodiversity is crucial to alleviation of poverty, due to the basic goods and ecosystem services it provides. Social changes have greatly influenced Animal Genetic Resources (AnGR) because of present generation not keen to continue their ancestral occupation of rearing livestock in migratory system of grazing. Emphasis need be given on breed development, their conservation, genetic improvement and sustainable utilisation, in-situ and ex-situ conservation models developed by National Bureau of Animal Genetic Resources (NBAGR), developing Artificial Insemination network in the country etc. Constraints include – absence of pedigree breeding, improper data recording, selection limited to organized herds only, poor understanding of AnGR conservation concept by development agencies and farmers, inadequate availability of superior genetic material, lack of Breed Societies, coordination among various agencies, inadequate capital resources, lack of legal framework/Act for implementation of breeding policies and performance recording, squeezing grazing lands

etc. Suggestions include undertaking regular periodical sample surveys, preparing breed watch lists, establish / strengthen and accredit breed specific nucleus farms for production of genetically superior males, characterize and document lesser known breeds/populations for breed registration, formation of breed societies to help AnGR, value addition through identification and exploitation of breed specific unique products and finding niche markets for them, documentation of traditional knowledge (TK), strengthening National Gene Bank by collection of the germplasm of economically important and endangered livestock breeds and their utilisation, undertaking comprehensive scheme on conservation and sustainable utilisation of threatened breeds in the country covering the species of cattle and buffalo.

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## Farm Animal Genetic Resources: Evaluation and Conservation in India

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Present farm animal diversity has evolved together with development of human society since ancient time. The agriculture and food requirements of the society have laid down the basis for the domestication of diversified farm animals and development of specialized populations called breeds and their production systems. This treasury in numbers of breeds of farm animals could be accumulated over the centuries by the judicious selection by human to molding them for specific purposes coupled with natural selection favoring the traits for adaptation to specific geo-climatic conditions. Our country is more privileged to possess large farm animal diversity, considering its vast geographic and ecological regions along with diversified necessity of the farmers of the region. In fact, Indian subcontinent remained as a major hotspot for the domestication of a number of farm animal species including humped cattle, buffalo, goat and red jungle fowl. Today, the country possess 512 million population of 10 livestock species – cattle, buffalo, sheep, goat, pig, horse, donkey, camel, yak and mithun and 729 million poultry distributed over a large range of geographical, ecological and climatic regions. Statistics shows that the country stands second in total livestock population in the world including first in buffalo comprising about half of the buffalo of the world, second in cattle and goat, comprising about one-sixth cattle and goat population of the world, third in sheep and fifth in chicken populations.

Nonetheless, livestock dynamics always keep changing, although immense increase has been seen for a number of farm animal species after Independence, the

overall livestock population decreased by about 3.33% over the previous census during year 2007-2012. Decline was much apparent in cattle (-4.1%), yak (-3.64%), sheep (-9.07%), goat (-3.82%), donkeys (-27.17%), camels (-22.63%), pigs (-7.54%), among different livestock species. In same duration, there is an increase in buffalo (2.3%), Mithun (12.88%), horses and ponies (2.12 %), mules (43.47%) and fowls (12.13%) populations.

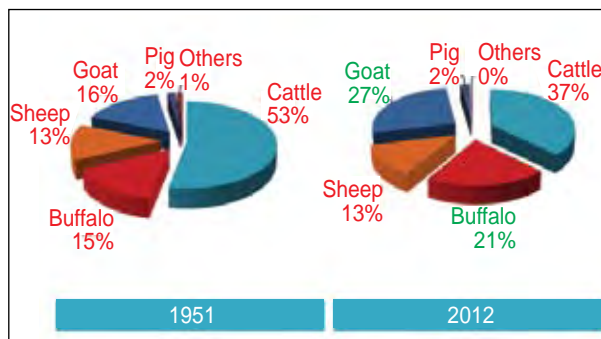
### Characterisation and evaluation of Farm animals

With the efforts of hundreds of years, a large number of livestock breeds specialized for specific production system has been evolved worldwide, possible with one of the most numbers in Indian subcontinent. At present in India, there are 160 registered indigenous breeds of farm animals - 40 for cattle, 13 for buffalo, 26 for goat, 42 for sheep, 6 for horses and ponies, 9 for camel, 6 for pig, 1 for donkey and 17 for chicken, so far. In recent past, majority of the so far registered livestock breeds have been characterised and also developed all type of physical and genetic database for these breeds. Although, the population recognized as registered breeds does not account even 30 percent of total livestock population of the country, there is still large proportion which is to be characterised, so far. There is a large gap for population under defined breed particularly for cattle (75%), goat (62%) and horses (80%). Among these, 31 breeds of different farm animal species were registered in last ten years only, from different parts, particularly remote of the country. Although, this trend seems increasing in coming years, there are still many more specialized

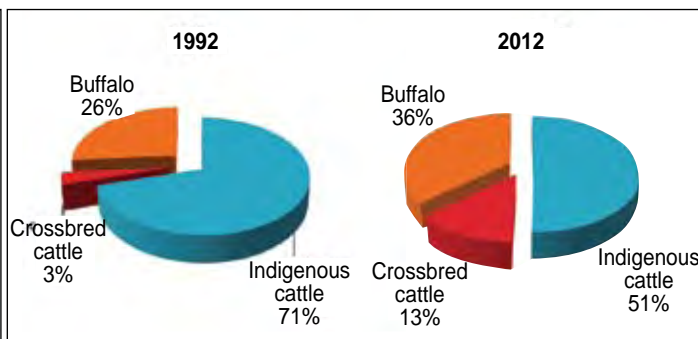
**Table 1. Livestock population and breeds in India and share in world**

Species	Population 2012 (million)	% of world population	Breeds	% of world breeds
Cattle	190.9	14.7	40	1.2
Buffalo	108.7	57.2	13	7.3
Goat	135.17	16.7	26	1.9
Sheep	65.07	6.8	42	1.6
Pig	10.29	1.1	6	0.15
Poultry	729.2	4.5	17	0.63

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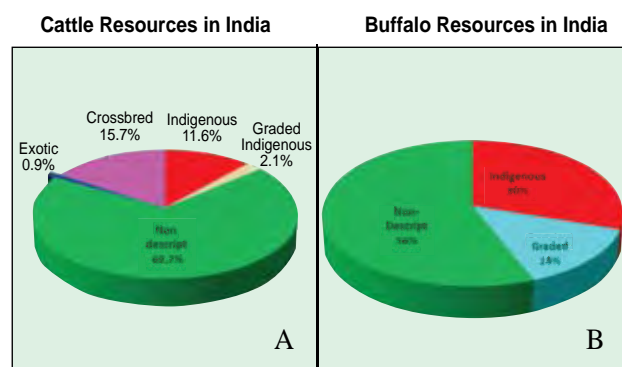
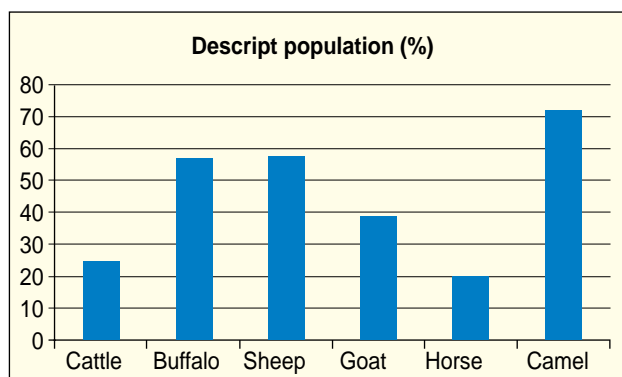
**Fig. 1. Proportion of different livestock species in India during year 1951 and 2012**



**Fig. 2. Pie diagram showing proportion of bovine species in year 1992 and 2012**

populations, as having breed potential. Still, the country has at least three to four less number of breeds in every million livestock in world.

Each animal population is specific combinations of alleles forming specific genepool, which serves particular purpose for mankind in their defined geographical region. Similarly, our diverse livestock species with unique characteristics such as sustenance on low quality feed and fodder, better thermo-tolerance and resistance to tropical diseases and parasites may avert the future



**Fig. 3. A) Descript population (in %) of different livestock species, B) Descript and other categories of cattle and buffalo genetic resources of India**

concerns arising from climate change. Indian bovines are very well acknowledged for tick resistance, protozoan diseases and parasitic resistance. Sahiwal, Gir, Ongole cattle and most of the best buffalo breeds were imported by many countries of the world for these traits. The Indian native cattle viz. Tharparkar, Rathi, Gir, Nagori, Kankrej etc. can survive in arid agroclimatic region. With changing global climate, these traits are going to be more important for sustainable animal production. Buffalo germplasm is known for high fat rich milk; drought tolerant, adapted to hot-dry climate and excellent draft power. Traits like prolificacy, meat quality, and parasitic resistance are also unique to Indian sheep and goat breeds. Further evaluation of the traits related to adaptation – disease resistance, heat tolerance, better thriving ability is also needed for different indigenous livestock breeds. Most of the information related to these traits and attributes are objectively described in literature, however, quantification of such traits is much required and justified with data. Performance of population in their production environment as well as outside also needs to be compared for complete evaluation of the breed characters. Finally the molecular basis of these traits and specificities should also be explored. Although sporadic research on this aspect is being carried out, more efforts are needed to unravel the genetic basis of thermotolerance, disease resistance, prolificacy and to validate the claims.

### *Declining Breed Populations – a Cause of Concern*

Presently, the population of a number of registered breeds is declining. The cattle in Indian sub continent have been bred to produce males for draught power which was vital for agriculture. Mechanization has replaced animal draught power and low milk yield of our cattle

breeds except Gir, Sahiwal, Tharparkar, Kankrej, etc. put majority of them in risk category. The reasons behind the decrease of most of the indigenous populations vary in general, but most evidently are decreasing utility; increasing pressure for production, low productivity. Consistently high demand with changing production scenario has derived the preference for highly specialized breeds, which has attributed to the loss of genetic diversity of the livestock in general, and of indigenous breeds in particular. Low productivity of indigenous livestock can be adjudged as biggest reason for threatening of a breed, further hampering the conservation efforts as such breeds are not economical as farmers point of view. It is well evident that there is high rate of loss in the indigenous livestock biodiversity in the country. Still, a large number of livestock breeds with unknown population status may be at risk. Serious threat has been observed in indigenous cattle genetic resources, particularly those which are not good milk yielder. More presence of certain exotic cattle breeds-HF, Jersey and their crossbreds, has laid down the foundation of dependency on certain type of breeds only. Likewise, about 22 states have adopted Murrah for crossbreeding of their native buffalo stock. Possibly trend seems worsen in coming years for few of the breeds evolved for drafting.

### Causes of Genetic Erosion in Domestic Animals

Three factors are considered as being largely responsible for the declining genetic diversity of livestock:

- Destruction of the native habitats of livestock breeds.
- The development of genetically uniform livestock breeds.
- Farmer and/or consumer preferences for certain varieties and breeds (and changes in these consumer preferences over time).

Among these, commercial interests are considered as the most important pressure on livestock diversity. Important factors in determining the direction and nature of change include: growth performance (productivity), pest and disease resistance, ease of handling, adaptation to current levels of technology, and to a relatively minor extent consumer choice.

### Breeds which are Facing Threats

- Most of the draft cattle breeds like Krishna Valley, Nagori, Khillar, Bargur, Amritmahal, Punganur, Ponwar, etc.

- Many of the buffalo breeds like Bhadawari, Toda, Surti are facing threat as Murrah is being used as improver breed throughout the country due to increased demand of liquid milk.
- Due to very little value for the wool from the Indian breeds and scarce grazing resources most of sheep breeds are losing ground. The sheep are being maintained as meat animal but has to compete with goat which are more prolific and have an advantage over sheep for value of meat in large part of country.
- All most all the native breeds of chicken face extinction due to over emphasis on commercial chicken farming.
- The pack animal species like camel, equines, donkey, Yak etc. face threatened due to their very limited utility and changing production systems.

**Table 2. Indigenous breeds/populations showing declining trends**

Species	Breeds/populations
Cattle	Vechur, Punganur, Krishna Valley, Bargur, Ponwar, Binjharपुर, Red Sindhi, Sahiwal, Tharparkar, Amritmahal, Kasaragode Dwarf, Tarai, Kumauni
Buffalo	Chilika, Toda, Bhadawari, Nili-Ravi, Manda, Kujang
Sheep	Ganjam, Bhakarwal, Gurej, Karnah, Niligiri, Poonchi, Pugal, Magra
Goat	Attapady Black, Chegu, Jamunapari, Beetal, Surti, Changthangi
Camel	Marwari, Mewati, Kutchi, Double humped camel
Horse	Bhutia, Manipuri, Zanaskari, Spiti, Marwari, Kathiawari, Malani
Chicken	All indigenous breeds of poultry except Aseel and Kadaknath

### Conservation of Farm Animal Diversity

Realization that animal genetic resources are at risk of being lost has stimulated national livestock conservation efforts. The need for conservation is based on economic, cultural, and ecological values; unique biological characteristics; shifts in market demand; and research needs. A first step in assessing genetic conservation needs is development of baseline information on population and genetic relationships. It is clear that livestock breeds are not biological taxa but rather represent the outcome of social processes. They are therefore unlikely to survive outside the social contexts and production systems that formed them. However, these losses weaken the potential of breeding programmes that could improve hardiness of livestock. Traditional pastoralists have often tended to foster biodiversity, in both plants and animals. Many

pastoral societies have developed elaborate systems that result in the preservation of genetic resources. Pastoralists have deliberately developed livestock to meet different needs and conditions.

Commercial breeds of livestock possess greater genetic variability than most crop varieties do. Diversity allows intensification of selection within breeds to be a fruitful approach for improving livestock productivity. However, if continued emphasis on breed replacement and increasing selection intensity (e.g. for greater productivity) take place at the expense of maintenance of genetic diversity, including the advantages of disease resistance and environmental adaptation, there may be significant long-term costs. As an example, Holstein cattle have become the pre-eminent dairy breed worldwide and have enjoyed sustained improvements in milk production potential, but only at the cost of declining genetic diversity within the breed.

Given the above conditions, there are two areas in which to base conservation efforts. These consist of developing a conservation infrastructure (a public service) and breeder actions (a private-sector activity). Nongovernmental organizations have to play a key role in the conservation of indigenous breeds, and their engagement is likely to continue by assisting breeders with technology transfer. Conservation infrastructure consists of a set of actions taken by the public sector for the public good. These actions include development of cryopreserved germplasm reserves that can be used to regenerate the breed, reduce inbreeding levels, and use molecular genetic tools to evaluate genetic diversity and/or genes of interest. A sufficient quantity of semen and, potentially, embryos should be collected to regenerate the breed if necessary and to relieve potentially high levels of inbreeding.

*In situ* maintenance of the genetic diversity is the responsibility of the breeders. To aid in conserving indigenous breeds, there is a need to develop market for indigenous breeds that provides breeders with an economic incentive for raising respective breed. Breeder, participation in the breed association provides a linkage for technology transfer and marketing activities.

Some of the biotechnologies offer tremendous potential to address real problems facing farmers in developing countries. For example, the area of genomics, allowing the identification and characterisation of individual genes influencing traits such as disease or stress resistance, growth rate or yield, promises to

be of great value. The genetic material (genomes) of several hundred species, including mammals, plants, fish, bacteria and viruses, has already been sequenced or sequencing is in progress and the information generated from genomics studies in other fields, such as human medicine or basic science, may also be useful for the application of genomics to food and agriculture.

### ***In vivo Conservation Models***

- Establishment of nucleus herd in the tract with field agency (ONBS mode).
- Raising of breeding males from elite females.
- Production, processing and cryopreservation of semen from breeding males.
- Registration of farmers' animals and recording for desirable traits by animal keepers.
- Incentive for retaining the animals in shape of feed/mineral mixture/de-worming/vaccination etc.
- Health care and management help.
- Use of elite breeding males in farmer's herds/flocks either by AI or NS.
- Improvement and conservation of various breeds in the field.
- Creation of breed societies to take care of the conservation and improvement of breed/population after the project period.
- Value addition, creation of niche marketing and branding of animal products having specialty of a breed.

### ***With Organized Farm***

- Unrelated young male progeny of elite animals from the breeding tract to be procured and maintained at the organized farm.
- A total of 2000 semen doses per male to be produced and 1000 semen doses from each male to be added to NBAGR genebank.
- Utilisation of frozen semen doses in the field through state agencies for breed improvement in the tract.

### ***With Livestock Keepers***

- 100 unrelated elite females in the farmer's herds, spread over the breeding tract to be identified and registered.
- Part of the maintenance cost of these elite females to be given to the farmer/stakeholder.

- Minimum of 50 unrelated males to be produced from these elite females.
- Incentive per male to be given to the farmer for raising the male progeny till maturity.
- These males to be used for further breeding in the tract.

Various conservation programmes being executed by the agencies is yielding little of the desired results as these are not able to improve the profits from the uneconomic breeds/species of livestock. Conservation is a long-term activity and the benefits are not generally appreciated by the planners and the masses as one cannot account these benefits in short term foreseeable time. Thus conservation activities have to be undertaken with long-term commitment in form of finances as well as continuity of the programmes. Most of the time the conservation/improvement in livestock resources is compared with plant resources which are entirely different and due to long generation interval and per year gains are very nominal.

### Conservation Programmes

A number of programmes are running which are directly or indirectly involved with the indigenous livestock breed conservation. These programmes should give more emphasis to the indigenous livestock breeds and population, covering large number of breeds.

1. Conservation of threatened breeds (endangered breeds of livestock and poultry)
2. All India Coordinated Research projects (cattle, buffalo, sheep, goat, pig and poultry)
3. Network projects (different breeds of livestock and poultry)
4. Mega Seed projects (Sheep and pig)
5. National project for cattle and buffalo breeding (NPCBB)
6. Rashtiya Gokul Mission
7. Registration of livestock and poultry breeds
8. National plan for action of AnGR
9. National Kamdhenu Breeding centre

### Factors Affecting Conservation

Indigenous breeds tend to contain high levels of genetic diversity and capabilities to be adapted to specific environments, being especially important in environmentally marginal areas. Developing countries typically rely on landraces for much of their production. They are important genetic resources, representing an insurance policy against uncertain markets and environmental conditions for food and agriculture in the future. Characteristics of the indigenous breeds (low growth rate, lower level of production) imply that the potential for altering gross income is lower than more prevalent breeds under current marketing conditions. However, adaptation to the environment and reproductive performance may alter this situation. Short-term ownership negatively affects breed conservation by creating an unstable situation for maintaining or increasing animal numbers. However, it is doubtful that any effective selection will be implemented; therefore, the population may behave as if it is a randomly mated population, with minimal loss of alleles due to selection. With the relatively small total population size and small individual flock sizes, genetic drift is an important factor affecting within-breed genetic diversity. With the small flock/herd sizes, one should expect random gene frequency changes that are cumulative over generations.

No doubt, the country possess the farm animal germplasm and their diversity as one of the best in the world – the evaluation of their characteristics and assessing true socio-economic importance and values are the responsibility of research institutions and other government agencies concern with. Although, the government for the development of indigenous livestock has initiated a number of programmes, these programmes needs to enlarge its cover to larger number of livestock breeds and populations. Similarly, efforts for conservation of our valuable germplasm in collaboration with government agencies and farmers are also needed to be enhanced – in fact identifying and demonstrating the unique characteristics, assessing economic contribution of indigenous livestock resources would also help in making indigenous farm animal resources more valuable, leading to their sustainable use and conservation.



## Responsible Fisheries for Resource Conservation

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The 20<sup>th</sup> century had seen an increase in the fishing capacity, in terms of the number of vessels, the increase in size of the vessels and advances in the electronic and navigational equipment coupled with easily manufactured and durable fishing nets. The effect of these innovations sequentially, was initially the increase in fish landings, then the plateauing of catches and, the myth of inexhaustibility of fishery resources in the sea was proven wrong. A large number of stocks world over have declined and the status of many of the stocks now are alarming. Overfishing, irresponsible and destructive fishing practices, and illegal, unreported and unregulated (IUU) fishing have long been recognized as leading causes that have reduced biodiversity and modified aquatic ecosystem functioning (Boehlert, 1996; Jackson *et al.*, 2001; Lotze *et al.*, 2006; Worm *et al.*, 2006).

In India about 1,94,490 fishing crafts of various sizes and classes are under operation in marine fisheries, consisting of 72,559 mechanised, 71,313 motorised and 50,618 non-mechanised fishing vessels (CMFRI, 2012). The existing numbers now is in excess by a factor of 3.8 for mechanized vessels and motorized vessels by 4.8 as per optimum fleet size estimates by Kurup and Devaraj (2000).

The term bycatch refers to the non-targeted species retained, sold or discarded for any reason. Global bycatch by the world's marine fishing fleets was estimated at 28.7 million t in 1994, of which 27.0 million t (range: 17.9-39.5 million t) were discarded annually and shrimp trawling alone accounted for 9.5 million t (35%) of discards annually (Alverson *et al.*, 1994). Average annual global discards, has been re-estimated to be 7.3 million t, during 1992-2001 period (Kelleher, 2004). Davies *et al.* (2009) redefined bycatch as the catch that is either unused or unmanaged and estimated it at 38.5 million tonnes, forming 40.4% of global marine catches.

In India, Pillai (1998) reported that among the bycatch, about 40% is consisted of juveniles. Kelleher (2004) has estimated total bycatch discards in Indian fisheries at 57 917t, which formed 2.03% of the total

landings. Juveniles contributed 36% of the discards (15.9% of total catch) in single day fishing and 78% (23.5% of total catch) in multi-day fishing conducted during 2001-02 in Karnataka (Zacharia *et al.*, 2005). Najmudeen & Sathiadhas (2008) have estimated the annual economic loss due to juvenile fishing by trawlers, purse seiners, ring seiners and mini-trawlers together, along the Indian coast at US\$19 445 million yr<sup>-1</sup>. Pramod (2010) has estimated the bycatch discards of Indian trawlers as 1.2 million t. Dinesh babu *et al.*, 2013, studied the Low value bycatch (LVB) composition of catches along the east and west coasts of India report that the all India contribution of LVB increased from 14% in 2008 to 25% in 2011.

Globally, shrimp trawling contributes to the highest level of discard/catch ratios of any fisheries, ranging from about 3:1 to 15:1. Trawl fisheries for shrimp and demersal finfish alone, account for over 50% of the total estimated global discards. Shrimp trawling contributes the most to the bycatch among the different fishing systems in India (Boopendranth *et al.*, 2008).

Overfishing as a result of increased capacity both in terms of number of fishing vessels and the installed engine power and bigger fishing nets which often do not conform to the legal sizes and designs stipulated, is one of the most important reasons for overfishing.

There is a considerable reduction in percentage of discards by trawlers operated along Indian EEZ and the discards in Indian trawl fisheries in 2011 was less than 10% (Dineshbabu *et al.*, 2013). This reduction in bycatch discards both locally and globally, in recent years could be attributed to (i) increased use of bycatch reduction technologies, (ii) anti-discard regulations and improved enforcement of regulatory measures, and (iii) increased bycatch utilisation for human consumption or as animal feed, due to improved processing technologies and expanding market opportunities, which encourage the fishermen to land the catch which consists predominantly of the juveniles of commercially important species, which is a very serious issue in fisheries.

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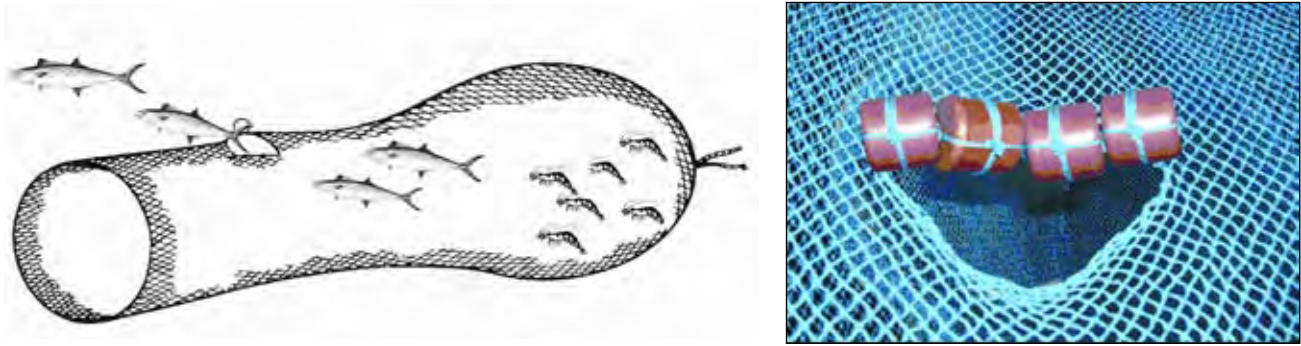


Fig. 1. View of Bigeye BRD in the trawl codend showing escaping finfishes (left) and Bigeye BRD attached to the codend (right)

Though the use of legal gears is mandatory, the adoption has been very poor. Recent studies indicate that investing to achieve sustainable levels of fishing by strengthening fisheries management, financing a reduction of excess capacity on the conventional resources and adoption of responsible fishing regime are required to rebuild the overfished and depleted conventional fish stocks (Worm *et al.*, 2006, 2009). Development, field testing and adoption of responsible fishing technologies for different gears assume great significance in this context.

Devices developed to exclude endangered species like turtle, and to reduce non-targeted species are collectively known as Bycatch Reduction Devices (BRDs). A large number of technologies have been developed and field tested to reduce the incidence of bycatch in trawlers, which generates largest quantity of bycatch in India (Boopendranth *et al.*, 2008, Boopendranath and Pravin, 2009). A number of BRDs such as Rectangular Grid BRD, Oval Grid BRD, Bigeye BRD, Fisheye BRD, Juvenile Fish Excluder cum Shrimp Sorting Device (JFE-SSD), Radial Escapement Device (RED), Sieve net BRD and Separator Panel BRD have been developed and field tested in Indian waters (Boopendranath *et al.*, 2008, 2012) (Fig. 1).

The Juvenile Fish Excluder cum Shrimp Sorting Device (JFE-SSD), an International Smart Gear-2005 price winning design (WWF, 2012) developed at Central Institute of Fisheries Technology (CIFT) (Cochin, India) which brings down the bycatch of juveniles and small sized non-targeted species in commercial shrimp trawl and at the same time enables fishermen to harvest and retain large commercially valuable finfishes and shrimp species (CIFT, 2007) was also successfully demonstrated (Fig. 2). CIFT Semi-pelagic Trawl System (CIFT SPTS) has been developed as an alternative to shrimp trawling in the small-scale mechanized trawl sector (CIFT, 2011).

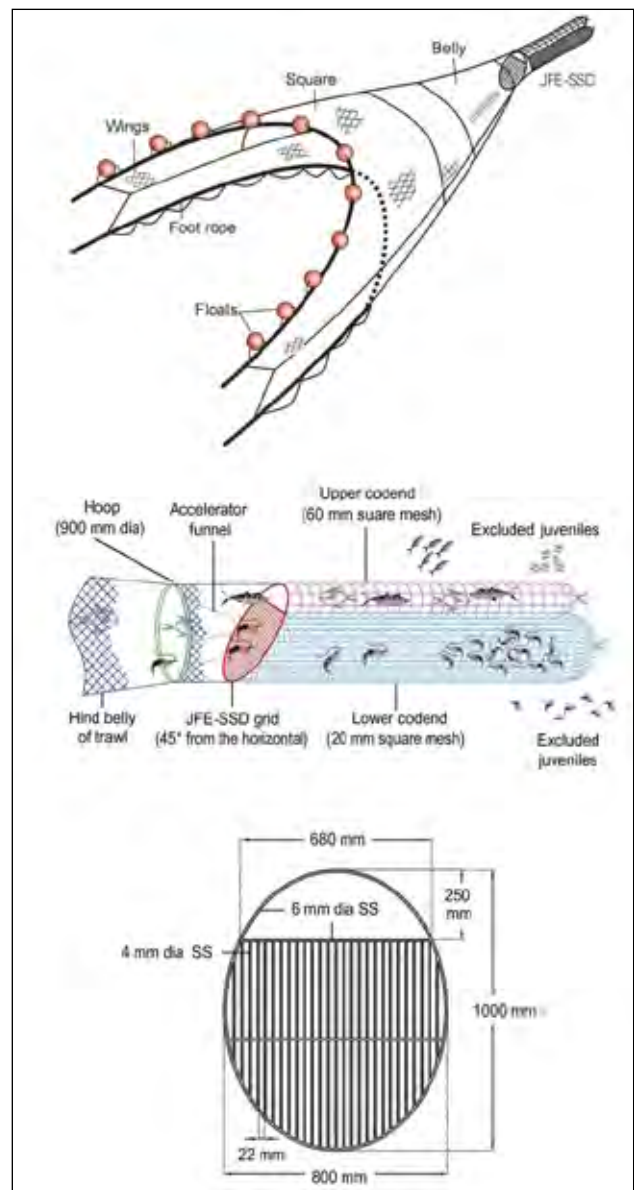


Fig. 2. The Juvenile Fish Excluder cum Shrimp Sorting Device (JFE-SSD)

Use of square mesh codends and square mesh windows that help in the release of juveniles is another promising technology developed and popularized by CIFT (Madhu *et al.*, 2015; Madhu *et al.*, 2010). Large mesh purse-seines for capture of large pelagic species, BRDs for release of Hilsa juveniles, circle hooks for longlining (Kumar *et al.*, 2013), optimized trap designs for lobster and fish are other technologies developed by CIFT for targeted fishing in the Indian waters.

Use of responsible fishing gears which reduce the incidence of juveniles and utilisation of the bycatch, by grading it into different categories like, for conversion to supplements, fish meal and as a source of pharmaceuticals and nutraceuticals, need to be explored. Changes will have to be made in the treatment of bycatch onboard, like sorting and preservation of the bycatch component if sourced for pharmaceuticals, need to be attempted.

## Conclusions

Fishing industry is facing major environmental, economic and social issues. The volume of landings has been declining and has plateaued for several years due to depletion of fish stocks. To address the overexploitation of fisheries resources, management tools have been developed and additional measures, including incentives to improve the selectivity of fishing gear, the obligation to change fishing location, the reduction of by-catch and the gradual reduction of discards have been worked out but have shown lower adoption in the Indian scenario.

Improvements that can be made for processing the incidental catches for by-product up gradation in an environmental and economic perspective to reduce the impacts also need to be addressed.

Adoption of ecosystem based fisheries management which incorporates responsible fishing practices along with strict implementation measures would help in protecting and restoration of biodiversity and enhance the resilience of the fish stocks and ecosystem services.

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## Birds and Insectivory: Agrobiodiversity Implications in Sustainable Agriculture

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Birds and insects have one common feature; both are winged and capable of flight. This makes them together an interesting biota-complex for further studies in terms of insectivory. Insectivorous birds have often kept a strong check on insect pests that are explosive in their breeding especially in mono-cropping situations. Major insectivorous birds belong to the families Accipitridae, Aegithinidae, Apodidae, Campephagidae, Cisticolidae, Hirundinidae, Laniidae, Meropidae, Monarchidae, Motacillidae, Muscipidae, Paridae, Picidae, Rhipiduridae, Sylviidae, Turidae and Upupidae (Ali, 1979)

It has been realized that birds which feed on invertebrates, specially insects, contribute immensely to their population dynamics (Verghese and Sriharan, 1993). Many a times birds are not specific predators and are invariably generalists and this is often thought of as a disadvantage in conventional biocontrol where parasitoids with narrow host range are used on target pests (Ali, 1979; Verghese and Sriharan, 1993). Birds are not amenable for inundative releases in areas of prey (pests) as they cannot be reared and introduced in a field especially with biocontrol as the focus. Therefore, the best options would be habitat management and conservation of birds by enhancing roosting, resting, feeding and breeding niches. For many practitioners of pest management this can be helpful since birds and insects have common behaviour in terms of aerial motion leading to habitat-overlap which plays a significant role in influencing agriculture pest scenario in agro-ecosystems (Canaday, 1996). Documentation of insectivory in India began with Mason and Lefroy's (1912) book (now not available in print) where birds were dissected and their gut analyses for insects were carried out. This is still the only major documentation available for us. With strict schedules banning wildlife (birds included) killing, gut analysis in birds is ruled out. This study therefore, has used archived notes of the first author to extrapolate the bird biodiversity trends over decades *vis-à-vis* pest diversity.

### Materials and Methods

This study was conducted in north Bangalore in horticultural and wetland ecosystems. The main crops grown here are an array of fruits vegetables and paddy. The area roughly measured around 400 acres of varying crops and soil types. The study began in 1975 and the documentation was done till 2015 lasting about 40 years. Main documentation was on the bird species diversity that was regularly carried out by irregular roving surveys along a definite transect path that encompassed all areas of the area. To facilitate documentation, the survey was done on a bicycle or on foot. A pair of 7×35 field glasses which was zoomable x 50 was used. The data base of pests on the crops of which the first author was part of, was used to extrapolate pest diversity. The first author maintained the notes. Analyses were done to see the diversity of bird's on a temporal scale at approximate temporal yearly mid points in 1985, 1995, 2005 and 2015 (Table 1). The change in habitat structure, cultivation practices, use of inputs especially insecticides were also recorded. From the data collected, the impact of insectivory in terms of bird diversity decline on pest resurgences was extrapolated. These observations and inferences were facilitated by two authors' being in the entomology division of the Indian Institute of Horticultural Research Hessarghatta, Bangalore, India.

### Discussion

In the present study, it was evident that in a span of four decades the biodiversity of insectivorous birds (Species Richness, S Table 1, Fig. 1) declined from 55 to 40 species in one decade and to 32 and further to 25 by the third and fourth decades respectively. There have been intensive agri-horticultural operations in the study area which probably caused the decline (Hallman *et al.*, 2014). Regular uses of insecticides have had a negative effect on the insectivorous birds especially the fantail flycatchers, iora, white-eye etc (Verghese and Sriharan,

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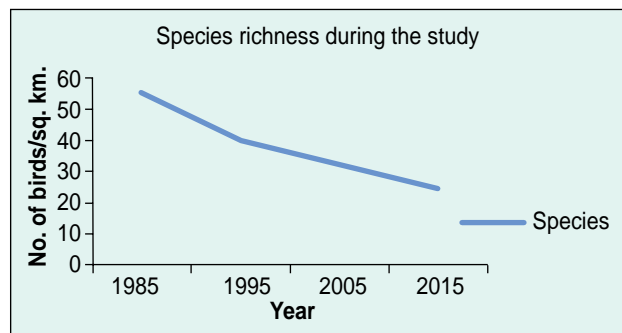
Table 1. List of insectivorous birds and their diversity

Sl No	Common name	Scientific name	Density No/sq km			
			1985	1995	2005	2015
1	Grey Heron	<i>Ardea cinerea</i>	3	1	x	1
2	Little Egret	<i>Egretta garzetta</i>	18	10	4	4
3	Pond Heron	<i>Ardeola grayii</i>	22	16	4	2
4	Cattle Egret	<i>Bubulcus ibis</i>	17	20	24	30
5	Night Heron	<i>Nycticorax nycticorax</i>	6	x	x	x
6	Shikra	<i>Accipiter badius</i>	1	1	x	x
7	Black-Winged Kite	<i>Elanus caeruleus</i>	1	1	x	x
8	Merlin	<i>Falco columbarius</i>	1	x	x	x
9	Black Winged Stilt	<i>Himantopus himantopus</i>	4	x	x	2
10	Red-Wattled Lapwing	<i>Vanellus indicus</i>	2	2	2	2
11	Wood Sand Piper	<i>Tringa glareola</i>	8	3	x	x
12	Little Stint	<i>Calidris minuta</i>	4	x	x	x
13	Spotted Owlet	<i>Athene brama</i>	3	2	2	1
14	Indian Nightjar	<i>Caprimulgus asiaticus</i>	2	1	1	x
15	Asian Swift	<i>Apus apus</i>	20	16	16	x
16	Indian Roller	<i>Coracias benghalensis</i>	2	1	1	1
17	White Breasted Kingfisher	<i>Halcyon smyrnensis</i>	2	1	1	1
18	Green Bee-Eater	<i>Merops orientalis</i>	37	28	28	18
19	Hoopoe	<i>Upupa epops</i>	2	1	1	x
20	Golden-Backed Woodpecker	<i>Dinopium benghalense</i>	2	x	x	x
21	Wood Shrike	<i>Tephrodornis pondicerianus</i>	1	x	x	x
22	Common Iora	<i>Aegithina tiphia</i>	2	x	x	x
23	Brown Shrike	<i>Lanius cristatus</i>	1	1	1	x
24	Black Drongo	<i>Dicrurus macrocercus</i>	8	6	6	4
25	White-Browed Fantail Flycatcher	<i>Rhipidura aureola</i>	2	1	1	x
26	House Crow	<i>Corvus splendens</i>	48	52	52	21
27	Jungle Crow	<i>Corvus macrorhynchos</i>	150	176	176	254
28	Great Tit	<i>Parus major</i>	2	1	1	x
29	Indian Bushlark	<i>Mirafra erythroptera</i>	2	x	x	x
30	Ashy-Crowned Finched Lark	<i>Eremopterix griseus</i>	3	2	2	x
31	Barn Swallow	<i>Hirundo rustica</i>	4	4	4	x
32	Red-Rumped Swallow	<i>Cecropis daurica</i>	12	4	x	x
33	Reed Warbler0	<i>Acrocephalus scirpaceu</i>	1	1	x	x
34	Blyths Reed Warbler	<i>Acrocephalus dumetorum</i>	1	x	x	x
35	Leaf Warbler	<i>Phylloscopussp</i>	1	1	1	1
36	Jungle Prinia	<i>Prinia sylvatica</i>	2	x	x	x
37	Ashy Prinia	<i>Prinia socialis</i>	2	2	4	2
38	Common Tailor Bird	<i>Orthotomus sutorius</i>	2	2	1	1
39	Jungle Babbler	<i>Turdoides affinis</i>	7	8	12	7
40	White-Eye	<i>Zosterops palpebrosa</i>	2	1	1	x
41	Jungle Myna	<i>Acridotheres fuscus</i>	18	24	36	48
42	Common Myna	<i>Acridotheres tristis</i>	22	26	28	31
43	Pied Bush Chat	<i>Saxicola caprata</i>	2	x	x	x
44	Indian Robin	<i>Copsychus fulicatus</i>	2	x	x	x
45	Tickells Flower Pecker	<i>Dicaeum agile</i>	4	8	14	18
46	Purple-Rumped Sunbird	<i>Leptocoma zeylonica</i>	2	1	x	x
47	Purple Sunbird	<i>Cinnyris asiaticus</i>	8	12	10	6
48	House Sparrow	<i>Passer domesticus</i>	300	x	x	x
49	Streaked Weaverbird	<i>Ploceus manyar</i>	28	x	x	x
50	Baya Weaver Bird	<i>Ploceus philippinus</i>	44	x	x	x
51	White Wagtail	<i>Motacilla alba</i>	3	2	1	1
52	Pied Wagtail	<i>Motacilla maderaspatensis</i>	4	2	2	2
53	Yellow Wagtail	<i>Motacilla flava</i>	2	1	1	1
54	Grey Wagtail	<i>Motacilla cinerea</i>	1	1	1	1
55	Indian Pipit	<i>Anthus rufulus</i>	1	1	x	x
Biodiversity- Species richness			55	40	32	25

**Table 2. Major insect pests of mango and corresponding insectivorous birds**

	Insect pests, 1978- 1990	Insect pests, 1991-2015	Insectivorous birds
Hoppers	<i>Idioscopus niveosparsus</i> <i>Amritodus atkinsoni</i>	<i>Idioscopus niviosparsus</i> <i>Idioscopus clypealis</i> <i>Idioscopus nagpurensis</i> <i>Amritodus atkinsoni</i> <i>Amrasca splendens</i>	Leaf warbler White-eye <b>X</b> Tailor-bird Ashy-wren warbler Tickells Flowerpecker Grey-tit <b>X</b> Rufous-tailed flycatcher <b>X</b> Iora <b>X</b> Golden-Backed Woodpecker <b>X</b>
Cerambycid borer	<i>Batocera rufomaculata</i> (only in neglected orchards)	<i>Batocera rufomaculata</i> , <i>Glenea multiguttata</i> Buprestids (now also in young trees)	
Lepidopterans	<i>Acrocercops</i> sp <i>Parasa lepida</i> <i>Chumetia transversa</i> <i>Euthalia garuda</i>	<i>Citripestis eutrapphera</i> <i>Orthaga exvinacea</i> <i>Citripestis eutrapphera</i> <i>Dudua aprobola</i> <i>Chumetia transversa</i> <i>Nanaguna breviascula</i> <i>Penicillaria jacosatrix</i>	Leaf warbler White-eye <b>X</b> Tailor-bird Ashy-wren Warbler Tickells flowerpecker Grey-Tit <b>X</b> Rufous-tailed Flycatcher <b>X</b> Iora <b>X</b> Green Bee-eater Hoopoe <b>X</b> Wood-shrike <b>X</b> Black Drongo Jungle Babbler Jungle Myna Common Myna House Sparrow <b>X</b>

X= Not recorded between 2001- 2015 period.

**Fig. 1. Decline in species diversity of insectivorous birds during the span of study (1985 to 2015)**

1993). The authors have found dead flycatchers in the mango groves. It was observed that decline of habitats like scrub jungles brought decline of shrikes, shikras, finche-larks, etc.

In the wetlands, the clearing of bull rush reeds caused the disappearance of the weaver- birds and likewise many other wetland birds like sand pipers, stints, water edge feeders like the yellow wagtails and grey wagtails declined. The decline of the house swift, house sparrow and swallows are inexplicable. In the

study it is therefore difficult to explain the increase in insect pestilence, due to bird diversity decline, except, accept the trend that insect pests have increased perhaps also because of bird decline.

However, documentation of insect pests and birds showed that there was a perceptible increase in insects in 1991 to 2016 period as against the number of insects prior that. For example, from 1978 to 1990, there were 8 major insect pests in mango in Bangalore while in the past two decades their numbers have increased to more than 15 species of pests probably due to decreased diversity of insectivorous birds (Table 2; Fig. 3).

It was interesting to know that 13 birds were highly sensitive, seven were moderately sensitive, 13 birds were sensitive, 17 birds were tolerant and four had adapted to the human/ farmer interferences and pressures.

Among the adaptable birds, the cattle egret, jungle myna, jungle crow and Tickells flowerpecker are the ones that in spite of farming interventions survived due to availability of roosting, feeding and breeding niches (Fig. 2).

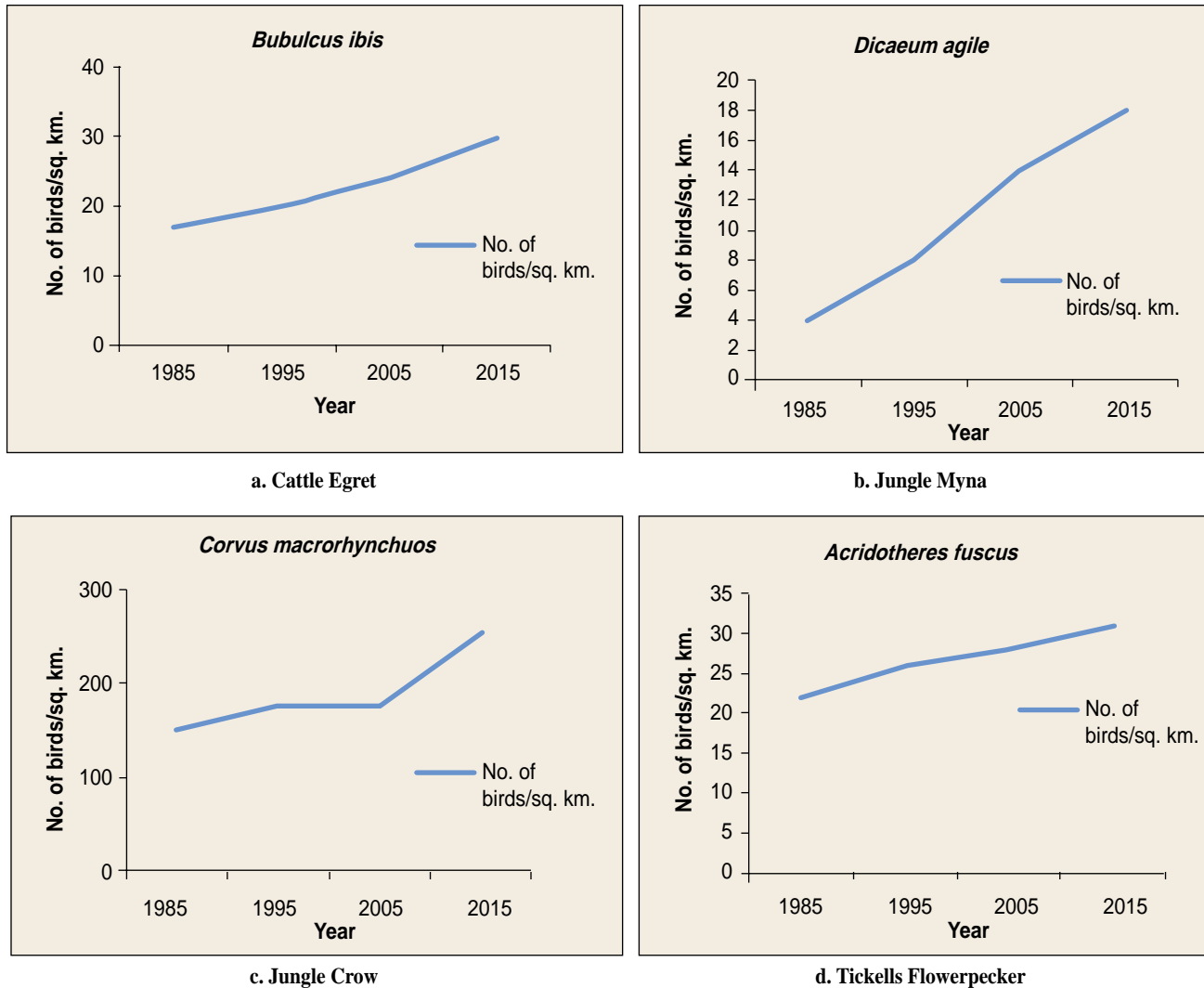
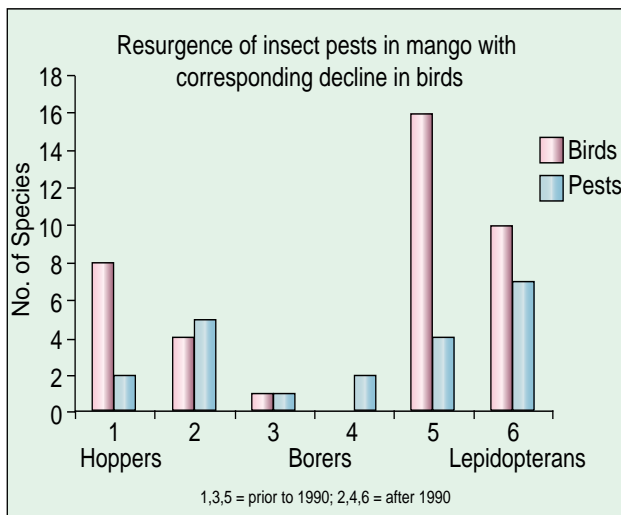


Fig. 2. (a, b, c, d) – Trend in population of birds that adapted to changes in farming ecosystems



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## Biodiversity of Fleshy Fungi, their Conservation and Applications for Human Welfare

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Mushrooms have a long history of use in oriental traditional medicines and healthy food. The biodiversity of mushroom is attracting common man and scientific community due to its attractive colour, fascinating shapes, sizes and spontaneous appearance. Nature is the biggest source of all microbial diversity including fleshy fungi or mushrooms. Mushroom biodiversity is very much influenced by biotic and abiotic factors. deciduous and evergreen forest with plenty of organic matter and less soil cover by grasses, shrubs and weeds favours profuse growth of mycorrhizic and lignicolous mushrooms like *Russula*, *Lactarius*, *Pleurotus* and *Lentinus* etc. Dense growth of grasses and weeds favours less growth of saprophytic and mycorrhizal mushrooms. India has enormous biodiversity of mushrooms in the world and several regions have so far been not explored for their flora and fauna. There has been enormous loss of mushroom biodiversity due to forests fires, grazing by domestic animals, environmental pollution, non-scientific methods for the collection of fruiting bodies for consumption and trade. Every year 15 billion trees are lost due to human activities and the world has lost almost half of the total forest cover from the day of civilization. The loss of forest has affected the mushroom biodiversity especially mycorrhizic, ligninolytic and saprophytic mushrooms which are mainly dependent on forest trees for their growth, spread and survival. Several ligninolytic and mycorrhizic fleshy fungi like *Pleurotus*, *Lentinus*, *Hericium*, *Amanita*, *Russula* and *Lactarius* are fast disappearing from their natural habitat. The loss of humus and organic biomass with soil erosion is also responsible for biodiversity loss due to fluctuation in soil temperatures and poor humidity in the microclimate of forest ecosystem. Several mushroom species are also reported endophytes of forest trees benefitting their host trees of vital nutrients and vitamins. However, studies on endophytes of Indian plants and trees have not been thoroughly studied. The studies on mushroom biodiversity of India started by Berkeley in 1851-52 and several fleshy fungi were reported from north western

Himalayas. Earlier attempts to study the mushroom biodiversity were confined to only academic interest in the form of dried specimens in various herbaria.

The systematic collection, conservation, identification and exploitation of mushrooms started with the establishment of ICAR-Directorate of Maize Research (ICAR-DMR), Solan as National Mushroom Research and Training Centre in 1983. Today more than 3,500 specimens of wild edible, non-edible, medicinal, wood rotting, poisonous and mycorrhizal mushroom specimens and about 3,000 tissue cultures have been conserved in the genebank of ICAR-DMR, Solan. The studies on mushroom biodiversity has not progressed much due to limited collection period (only during monsoon season), difficult terrain, danger of wild animals, lack of trained men power, non availability of literature and scientists working in isolation.

### Importance of Mushroom Biodiversity

- (a) *Mushrooms as Source of Nutrition and Vitamins:* Due to exotic flavour and texture mushrooms are liked by the mankind from the earliest civilization. Several mushroom species like *Pleurotus*, *Lentinus*, *Termitomyces*, *Morchella*, *Scleroderma*, *Phellorinia*, *Podaxis*, *Schizophyllum*, *Sparaciss* etc. are consumed by local people in Chhattisgarh, Maharashtra, Rajasthan, Jharkhand and most of the states in north east of India. Edible mushrooms are rich in potassium and poor in sodium which is good for persons suffering from high blood pressure. Mushrooms are good source of quality protein and amino acids like lysine, tryptophan, methionine etc. they are also rich in Vitamin-B complex including folic acid and Vitamin-B12. Mushrooms are the only source of Vitamin-D in vegetables. We have found very high Vitamin-D contents in most of the cultivated edible mushrooms and recently cultivated one of the tropical indigenous species *Macrocybe gigantea* is a rich source of Vitamin-D. We can augment Vitamin-D in mushrooms by exposure to

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UV light and sunlight. The mushroom growers are enriching their product for Vit-D content by artificial methods. Due to alkaline in nature and high fiber they are suitable food for persons suffering from hyperacidity and constipation. Mushroom mycelia due to absorption of various elements like selenium, copper, iron can be used for fortification of food items.

- (b) *Mushrooms as a Source of Novel Metabolites*: Out of one lakh metabolites 33,000 are from fungi and with the loss of every species we are losing a good source of bioactive compounds. Only a little fraction of mushroom species have been evaluated for bioactive compounds. Medicinal mushrooms have an established history of use in traditional oriental therapies. Historically, hot water-soluble fractions (decoctions and essences) from medicinal mushrooms were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated mushrooms such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga), and many others have been collected and used for hundreds of years in Korea, China, Japan and eastern Russia. Mushroom metabolites are increasingly being utilized to treat a wide variety of diseases, particularly as they can be added to the diet and used orally which could be efficient in possible treatments of diseases like allergic asthma, food allergy, atopic dermatitis, inflammation autoimmune, joint inflammation such as rheumatoid arthritis, atherosclerosis, hyperglycemia, thrombosis, HIV infection, listeriosis, tuberculosis, septic shock, and cancer. Major substances with immune modulatory and/or antitumor activity have been isolated from mushrooms mainly polysaccharides (in particular  $\beta$ -D-glucans, polysaccharopeptides (PSP), polysaccharide proteins, and proteins, triterpenes, lipids, statins and phenols. A new class of fungicide-strobilurin which is a broad spectrum fungicide has been extracted from a mushroom species *Strobilurus tenacellus*.
- (c) *Agro and Forest Waste Recycling*: Several saprophytic and wood rotting mushrooms due to production of extracellular enzymes specially oxidases and peroxidases are able to degrade cellulose, hemicellulose, lignin and release glucose

which can be utilized by the secondary microbes for faster degradation. We have utilized several agro and forest wastes like mentha, dried leaves of poplar, pipal, mango, pine for oyster mushroom cultivation. Mushroom species with faster mycelia growth can be used for glucose production and bio ethanol.

- (d) *Animal Feed*: Mycelia colonized wastes are more digestible as animal feed than fresh straw and also helps in preventing parasites of animals due to mushroom metabolites.
- (e) *Bioremediation*: The extracellular enzymes like laccase, aryl alcohol oxidase, ligninase and several other peroxidases which produces free radical oxygen are able to myco remediate contaminated sites with phenols, chloro phenols, TNT, poly aromatic hydrocarbons and azo dyes. Mycelia colonized straw can be also used to increase humus contents for water absorption and retention.
- (f) *Biological Control*: Some mushrooms have the potential as antifungal due to chitinase and proteases production. Although their potential as antibacterial and antifungal has not been thoroughly investigated. Members of *Issaria* and *Beauveria* are largely used to control various insect pests.
- (g) *Other Industrial Applications*: Mushrooms can be exploited for producing nano particles of oxalates, iron, zinc etc. The culture conditions to produce nano particles have to be standardized. Almost all kinds of colors like blue, pink, red, black, purple, yellow have been found in mushroom fruit bodies. These colors are also present in mycelia. There is a need to standardized methods to produce maximum pigments either in liquid broth or on cereal grains for producing organic colors for use in food industry and dying of cloths.

### Culture Conservation

It is easy to maintain cultures of sporulating fungi but most of mushrooms do not produce any spores and only the mycelia culture are to be conserved. Moreover it has been our experience that mushrooms which grows at sub tropical and tropical conditions like *Calocybe indica*, *Volvariella volvacea*, *Auricularia* spp, *Macrolepiota* sp, *Ganoderma lucidum* some *Morchella* and *Pleurotus* spp. are sensitive to low temperature conditions (less than 10°C) and often do not revive on sub-culturing due to

low temperature sensitive proteins. Such cultures are to be conserved at 15-17° C and sub-cultured regularly after 60-70 days. Lyophilized cultures of mushrooms on jowar grains are a better substrate for maintain cultures for a long time without the loss of natural vigour. Similarly mycorrhizal fungi when conserved in the genebank either the cultures cannot be revived or they loss their mycorrhization potential. Not only the survival but to obtain cultures of *Cantharellus* spp., *Russla* spp., *Lactarius* spp., *Termitomyces* spp. and *Amanita* spp is very difficult and specific growth media are to be prepared with specific carbon and nitrogen sources, vitamins, micronutrients and suitable pH. More than 5000-6000 species belonging to basidiomycetes, (45 genera) some ascomycetes (18 genera) and few zygomycetes (members of the genus *Endogone*) are reported as ectomycorrhizal (Smith and Read, 1997, Futai *et al.*, 2008). Members of *Cantharellus*, *Russla*, *Lactarius*, *Cortinarius*, *Inocybe*, *Suillus*, and *Amanita* are well recognized as ectomycorrhizal fungi. All the cultures at ICAR-DMR are maintained at +4° Cel in cold room, at 15-17° C in incubators and in mineral oil at room temperature. However, the cultures of mycorrhizal fungi should be maintained *in situ* or myco-silviculture to maintain their vigour. We need “Fungal Gardens” in tropical, sub-tropical and temperate forests with all the common trees under protected conditions and the inoculums multiplied in bulk is put under host trees for ECM fungi. Cultures of saprophytic and mycorrhizal fungi in the genebanks conserved for a long a time in the same culture medium may not be useful for their beneficial utilisation as the looses their potential to secrete extracellular enzymes for utilisation of natural substrates. Some of the culture banks are using systemic fungicides to prevent mould infestation which are responsible for mutation and ultimately loss of original culture.

### Future Plan to Conserve Mushroom Biodiversity

- (a) We are discussing more about biodiversity but we have very limited expert taxonomist to study the biodiversity. The mushroom biodiversity is little bit more complicated because mushrooms can be collected only for a limited period (during rainy season) and due to perishable nature they have to be studied and conserved immediately. Moreover, dangers from wild animals, snakes, leeches, etc. make mushroom collection very difficult and unattractive. We should strengthen our future team of mushroom taxonomists by utilising the experts available in the country for giving training for 30-45 days.
- (b) Advance centre for mushroom biodiversity should be established in any central university, Indian Council of Agricultural Research institute or in Council of Scientific and Industrial Research labs with full research facility for traditional taxonomy substantiated by molecular tools.
- (c) We should develop networking research projects on mushroom biodiversity with minimum 15-20 scientists /researchers to document the entire diversity of our country before we lose our national mushroom wealth. We should also study the medicinal, nutritional and novel compounds from the entire collections.

The biodiversity of common edible and medicinal mushrooms like *Agaricus*, *Pleurotus*, *Volvariella*, *Termitomyces*, *Lepista*, *Flammulina* and *Cordyceps* will be presented. The diversity in *Amanita* spp. which has both edible and poisonous members will be also presented.

## Conservation of Pollinator Diversity in the Era of Neonicotinoids

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More than 80 per cent of the approximately 350,000 species of flowering plants in the world depend partially or completely on insects, mostly bees, for sexual reproduction. This interaction between plants and bees is the basis for sustaining much of the biodiversity on Earth. Pollinators are vectors of genetic exchange.

Although honey bees constitute major pollinators of several cultivated crops, they are always considered as important in managed pollination. However, several species of cultivated crops depend on non-*Apis* bees, which are solitary. In fact, there are more than 800 species of non-*Apis* bees in India (~20,000 species in the world), which are wild pollinators of several crops and wild plant species. The survival of many species of plants in the wild, in short the plant diversity itself, depends on visits to their flowers by these bees.

In recent years, there has been a concern about decline in pollinator populations at the global level. The possible reasons attributed for this are, loss of habitats – including nesting sites for bees, loss of flora that sustain bee populations, monocropping, mismatch between flowering phenology and bee activity and excessive use of chemicals in agriculture (Potts *et al.*, 2013). Of these, indiscriminate use of herbicides and insecticides has been considered to be major.

### Beginning of the Era of Neonicotinoids

Use of insecticides has been a more common method of pest control. By 1980s many insect pests had developed resistance to the then available chemicals in the market belonging to organophosphates, carbamates and pyrethroid groups (Alyokhin *et al.*, 2008). Against this background, insecticides belonging to neonicotinoid group were projected as having several advantages, including selective toxicity to arthropods, high persistence, systemic nature, versatility in application (especially as seed treatments), high water solubility, and assumed lower impacts on fish and other vertebrates.

Understandably, neonicotinoids have been the most widely used class of insecticides worldwide since the 1990s. Their application ranges from plant protection and veterinary pest control to invertebrate pest control in fish farming. Neonicotinoids are systemic insecticides. Irrespective of the mode of application and route of entry, they translocate to all parts of the plant, making the entire plant toxic to the insects that feed on it. The discovery of imidacloprid by Shinzo Kagabu, followed by its introduction to the market in 1991, started the era of neonicotinoid class of insecticides (Tomizawa and Casida, 2011). Soon other insecticides followed – thiamethoxam and Clothianidin in 1999 (Maienfisch *et al.*, 2001). By 2010, neonicotinoids ruled the market and became the most widely used insecticides globally (Casida and Durkin, 2013) with a few more insecticides of the same group quickly added (acetamiprid, thiacloprid, dinotefuran and nitenpyram). Of all these, imidacloprid became more popular since it was available in several formulations as foliar sprays, soil application and seed treatment, and became a panacea for almost all pest problems on a variety of crops. No wonder this insecticide became most popular among farmers worldwide, and probably also resulted in over use or misuse of the formulations. Today there are over half-a-dozen multinational pesticide companies formulating insecticides of this group and competing in the market. Neonicotinoids have been registered in more than 120 countries. With a global turnover of over €1.5 billion, they represent more than 25% of the global market for insecticides.

### Are Neonicotinoids harmful to bees?

Though many insecticides are known to be unsafe to bees, more recently, the use of neonicotinoids has been specifically pointed out as a factor that might contribute to declines of both managed and wild bees (Goulson, 2013). Neonicotinoids are used on crops in three ways. There are formulations that are used for seed treatment,

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some are used for soil application and as foliar sprays on several crops. Even when they are used for seed treatment, low concentrations are subsequently found in the nectar and pollen of the crop, which is in fact surprising. Thiamethoxam used for seed dressing was present in all pollen samples and a majority of nectar samples collected from oilseed rape ( $3.26 \pm 2.16$  ng/g in pollen,  $3.20 \pm 4.61$  ng/g in nectar) (Bonmatin *et al.*, 2014; Botias *et al.*, 2015). Studies have also found that foraging bees brought back to the nest pollen and nectar contaminated with on average 71.8 ng of neonicotinoids/day (Botias *et al.*, 2015).

### What is the effect on bees?

Neonicotinoid contamination need not always result in death of bees. More often bees get sublethal doses. Sub-lethal doses of 1-24 µg/kg and 0.1 - 20 ng/bee have been shown to impair navigation, foraging behaviour, feeding behaviour, and olfactory learning performance in honeybees (Decourtye, 2004). The result is that a foraging bee will not be able to navigate and find its way back home leading to a sudden decline in the population of a bee colony. Further it has also been shown that the foraging bees do not avoid visiting neonicotinoid sprayed plants (Kessler *et al.*, 2015).

### Are neonicotinoids responsible for CCD?

Colony Collapse Disorder is the sudden disappearance of worker bees from their hives, resulting in collapse of the colony. In the US, between 2005 and 2015, millions of colonies were affected. One of the reasons for CCD is attributed to the extensive use of neonicotinoids. Though there has been a lot of debate on the issue (Lundin *et al.*, 2015), several developed countries have banned or brought neonicotinoids under restricted use. And, there is hardly any data on the effects of neonicotinoids on Indian bees.

### Conservation of pollinator diversity

Conserving pollinator diversity is important for our own survival, because we depend more directly on this ecosystem service, as many agricultural crops rely to some degree on pollinators for setting seeds that we use for our consumption. Nearly 70% of 1330 tropical crops have shown improvement in fruit or seed quality and quantity with bee visitation (Roubik, 1995) and it is estimated that about one-third of our food is derived

from pollination service provided by bees (Klein *et al.*, 2007). Globally the annual value of pollination service by insects to cultivated plants is estimated to be around €153 billion (Gallai *et al.*, 2009). Wild pollinators are as important as managed bees in terms of the economic value of their role (Kleijn *et al.*, 2015) and hence it is necessary to take suitable measures to conserve both *Apis* and non-*Apis* bees. Conservation measures should include identifying and protecting nesting habitats, development of floral calendars and making available resources after the cropping periods for sustaining populations and of course identifying and popularizing safer pesticides or pest control methods.

We do not know whether the era of neonicotinoids will continue or whether the bees will survive the era.

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## Microbial Conservation Strategies and Methodologies: Status and Challenges

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Microorganisms, ‘*silent and unseen majority of life*’, are one of the earth’s greatest treasures around us because they have the largest genetic and metabolic diversity which can be protected and utilized for sustaining plant productivity, ecological balance and human life. Researchers are working on exploration and conservation of the microbial universe for the future applications. These microbial strains could be exploited for several biotechnological purposes in industry, medical, pharmaceutical and agriculture sectors. Today, almost no branch of science e.g., engineering, agriculture, or medicine is untouched with microbial interventions. If we go back in the history of microbial ecology, Beijerinck (1913) quoted a most famous maxim in microbiology “*everything is everywhere, but, the environment selects*”, suggests (Baas Becking, 1934) suggesting that microorganisms do not exhibit any biogeographical pattern and, hence, these are ubiquitous and cosmopolitan. However, some microbes are inhabitant to specific environments therefore; there is no question of extinction of microorganisms. However, this is not absolutely factual and some microbes do face real threats of their continued existence due to habitat and climatic aberrations. Contrary to first part of the maxim ‘*everything is everywhere*’, microbes do exhibit biogeographical pattern. For example (1) a group of bacteria inhabiting leaf surface indeed appear ubiquitous but some microbes exhibit localized endemism and show absence of panmixis (Gerstein and Moore, 2011); (2) many pathogens have well-defined distribution in animals, humans and crops. Second part of the maxim ‘*the environment selects*’, means some microorganisms live in very specialized habitats and loss of habitat may cause extinction of the associated microorganisms. Curtis (2006) issued a call to arms for microbial ecologists—it’s time to start thinking big and requires worthy attention to conserve microbes

at risk of extinction. Therefore, the conservation of microbial diversity in the environment has been realised by scientists and researchers through understanding and application of rRNA gene barcoding and use of operational taxonomic units (OTUs) coupled with next generation sequencing (NGS). It is high time to address on the knowledge gaps and communication barriers that currently lags amongst microbiologists, microbial ecologists and conservation biologists while undertaking microbial conservation. With this background, here we will highlight strategies of microbial conservation and challenges associated with it.

### Strategies of Microbial Conservation

Innumerable microbial species are supposed to exist, however only 1-10% species are characterised, preserved and utilised for various purposes. In order to fully utilise the genetic resources of the microbes which are still uncharacterised, we need to conserve microorganisms in their habitats. Microbial conservation strategies applied “*in situ*”, “*ex situ*” and “*in-factory*” form of conservation. “*In situ*” (‘on site’, ‘in place’) conservation links the microbes in their natural habitats and is the most appropriate way of conserving viable populations in their ecosystem and natural habitats. “*Ex situ*” (off site) conservation preserves and maintains the distinct wild/isolated/cultivated species and their genetic resources in artificial media and are taxonomically well described. “*In-factory*” form of conservation is an intermediate form of conservation and mainly used by the agro-industrial sectors.

#### a. *In situ* Conservation

*In situ* conservation methods have the potential for long-term preservation of ecosystems, species, and populations under conditions of continuing adaptations. *In situ* conservation is essential in places whose microbiome had not been adequately inventoried. *In*

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*in situ* conservation is a set of conservation techniques involving the designation, management and monitoring of biodiversity within the context of the ecosystem in which it is found. *In situ* management approaches can either be targeted at populations of selected species (species-centred), or whole ecosystems (Heywood and Dulloo, 2005). Traditionally, protected areas have been seen as the cornerstone of *in situ* conservation. Conservation approaches that are more adaptable to individual situations and applicable beyond protected areas, are being increasingly applied. Convention on Biological Diversity (CBD), a treaty declared in June 1992 at the Rio de Janeiro Earth Summit, recognized the importance of conservation of biodiversity, however microbial diversity were not rightly addressed in CBD and therefore, in subsequent years GW Griffith advocated Global Strategies for Microbial Conservation (GSMC) (Griffith, 2012). Extinction of specific microbes inhabiting in specific habitats particularly those which are on the verge of extinction are need to be conserved on priority basis. The following are some specific examples which would highlight the challenges and importance of *in situ* conservation of habitats for conserving associated microbes for future application.

1. In tropics, soil bacterial diversity is maximum with neutral soil pH, and lower in acidic and alkali soils, make these areas more like desert for soil bacteria, whereas grassland and desert soils have much higher bacterial diversity than tropical rainforest soils. Soil texture, chemistry, moisture, and a multitude of other factors vary with depth, providing unique, vertically-stratified habitats for soil-dwelling organisms. Several studies have shown that soil organisms vary with soil depth, from fungi or bacteria (Parker, 2010). These examples show the relationships between soil diversity and soil functions, as well as the biogeographical patterns that are poorly understood and pose a challenge in *in situ* conservation.
2. Some novel anaerobic fungi specific to the hindgut have been reported from critically endangered animals like Somali wild ass and red Kangaroo (Liggenstoffer *et al.*, 2010). Recently, a new anaerobic fungus (*Oontomyces anksri* gen. nov., sp. nov.) from the digestive tract of the Indian camel (*Camelus dromedarius*), has been reported and raising the possibility that this fungus may be specific to camelids (Dagar *et al.*, 2015). In case of plant, *Goodyera pubescens*, an evergreen terrestrial orchid, interacts with the same fungal partner during periods of modest environmental variation but is able to switch to a different fungal partner under extreme environmental perturbations (McCormick *et al.*, 2006). These are some examples where environment selects a particular type of microbes which may extinct on extinction of larger partners and advocate habitat conservation.
3. Some of the extreme ecosystems such as desert soil crust, hydrothermal vent, hypersaline areas, hot springs, cryoconite hole on glacier and unusual habitat do support occurrence of specific type of microbes that need to be conserved.

### b. *Ex situ* Conservation

Microorganisms are being conserved in laboratories using specialized methods for their present and future application. At an international level, the World Federation for Culture Collections (WFCC) which is a federation of the International Union of Microbiological Societies (IUMS) and a commission of the International Union of Biological Sciences (IUBS) with responsibility for the promotion and development of collections of cultures of microorganisms and cultured cells. There are many microbial resource centres for conservation in many countries of the world, where microbes are collected, identified, characterised and conserved as per the guidelines of best practice of the Organisation for Economic Development and Cooperation (OECD). Further, the aims of Budapest Treaty is to conserve the patented microbes in the bioresource centres (repository) like DSMZ, KACC, MTCC, MCC etc. The microbes of specific nature can be protected and utilized in sustainable way in these repositories. National Agriculturally Important Microbial Culture Collection (NAIMCC), ICAR-NBAIM is a Designated Repository (DR) recognised by National Biodiversity Authority (NBA), Government of India, for conservation of agriculturally important microbial wealth of the country.

In *ex situ* methods of conservation, the microbial activities are reduced or stopped by imposing conditions. There are several methods employed for the preservation and maintenance of microorganisms, for example, sub-culturing, preservation on agar beads, use of mineral oils, silica gel storage, spray-drying, fluidized bed drying, cryopreservation, lyophilization (freeze-drying), L-drying, desiccation, induced anhydrobiosis,

sterile distilled water and gelatin discs. Preservation by cryopreservation and lyophilization is considered as the most valuable and widespread methods available to achieve long-term, stable storage of microorganisms. Some microorganisms are recalcitrant either to lyophilisation or cryopreservation and have a greater challenge for preservation of such microorganisms through different approaches. *Ex situ* conservation is rarely enough to save a species from extinction.

### c. In-factory Conservation

*In-factory* method of microbial conservation means keeping them in normal conditions of use. Two different ways of conservation can be implemented: *dynamic* and *static conservation*. *Dynamic conservation* does not impose significant restrictions on the use of strains, except for the introduction or mixing with cultures of different origin. *Static conservation* is very restrictive and tries to maintain strains under conditions such as to avoid any kind of changes.

### Conclusion

Despite of the fact that microorganisms play a pivotal role in ecosystem function and human life, a large proportion of these '*tiny life form*' have been basically ignored so far. In general different microorganisms often require special preservation methods for ensuring their optimal viability, storage, and purity. For protection point of view and to minimize the possibility of cultures being lost or contaminated therefore it is suggested to preserve the strains by at least two different procedures. Of which one should be based on freeze-drying (lyophilization) or storage in liquid nitrogen (cryopreservation) where applicable; these are the best methods for minimizing the risks of mutation or genetic change. Despite of above established protocols, only some culturable microbes have been preserved in some repositories located in different parts of the world. It is likely that there could be large gaps in understandings on methodology followed in preservation, knowledge of the distribution and abundance of microorganisms. A comprehensive strategy should focus on the conservation of microorganisms in their habitats with extreme environmental conditions and endangered plants and animals harbouring specific microbes. Hence, global strategies for conservation of hidden microbial treasure must come from microbiologist, researchers, and research institutes and must be supported

by government as a national policy to sustain life on the planet earth. It is worth quoting tenet of Tom Curtis which indicates importance of conservation of nitrifying bacteria '*if the last blue whale choked to death on the last panda, it would be disastrous but not the end of the world. But if we accidentally poisoned the last two species of ammonia oxidizers, that would be another matter. It could be happening now and we wouldn't even know*'. Subsequently, recent discoveries of two processes namely, anammox (anaerobic ammonium oxidation) and comammox (one step aerobic ammonium oxidation by single bacterium; Nitrospira) are evidence of continued ignorance of many microbial processes. This ignorance argue for maximizing conservation of microorganisms and also a challenge of what and how to conserve in this dynamic and rapidly evolving microbial world.

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## Fighting Climate Stress with Orphan Legumes

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Crop species which are adapted to hot and dry climates will become increasingly important as the world warms. The Kirkhouse Trust (KT) has supported research on legumes because of their importance in providing high quality protein in the diets of resource-poor farmers. Among these crops are many stress tolerant legume species found in India and Africa, that are relatively minor, neglected crops. A new programme called 'Stress Tolerant Orphan Legumes' (STOL) for KT aims to support systematic studies of their potential to address the loss of agricultural productivity in areas of the globe that are suffering the greatest climatic stresses.

A major region for our focus will be the semi-arid Sahel which stretches as a belt 100-600 km in width across the entire African continent south of the Sahara desert from Senegal in the West to the Red Sea coast in the East. The population of the Sahel, around 100 m, is already experiencing the effects of failed crops; many go hungry and as a result, many of the migrants we now see attempting to reach Europe are from the countries of the Sahel. The population is projected to treble to 300 m by 2050; there will be another 200 m mouths to feed. Other desert regions, such as the Thar desert of the North-West of the Indian sub-continent and the Kalahari of southern Africa are similarly stressed.

There are clear signs that climate change is already having severe effects on the agriculture of the Sahel: crops such as cowpea, an important staple, which thrived in the region, are now failing as a result of the changed climate. In addition to direct effects of heat and drought on crops, the region is suffering soil loss due to the southward spread of the Sahara. Over-grazing, intensive cultivation, removal of tree cover, poor water management have all been cited as reasons for this loss; the fact remains that large tracts of land are being taken out of production.

What can be done to mitigate this looming disaster? We see three ways in which adoption of stress tolerant legume species may help farmers fight the ravages of

climate stress: production of grain to feed the family and provide income; fodder and forage to feed livestock; ground cover to remedy soil degradation. For this reason, our research programme will include a diversity of species; we intend to study around ten species in our initial survey. Some of the crops may prove to be multipurpose, providing grain and fodder for example; others may be specialised for one function such as soil remediation.

Our choice of crop species is determined by factors such as the location at which they are found and the opinions of experts. KT has direct experience of *Dolichos lablab*, moth bean, horsegram, rice bean and marama bean. Others have suggested Bambara, Lima bean, greengram, pigeon pea and tepary bean. Controls will be cowpea and common bean for regions where these crops are already grown. We plan to carry out field trials in a number of locations in stressed regions to compare the responses of the diverse species and up to 50 accessions of each species. The first stage, which is already under way is to gather the accessions needed for the study. A second stage, again already under way is multiplication to generate enough seed for field trials. The planned field trials will be carried out by agronomists. Inputs of local farmers should guide the progress of the studies; they will tell us if the unfamiliar species outperform the cowpea that they are accustomed to, and how the new crops may fit into their systems. We anticipate that very few of the species will pass the first trials.

Kirkhouse Trust has had a decade of experience in region-wide breeding programmes for the improvement of two legume crops in Africa: the West African Cowpea Consortium (WACC), working in seven countries, has generated a number of improved cowpea lines; the African Bean Consortium (ABC) working in five countries has introduced a number of improvements to common bean. This experience has taught us that coordinated efforts in well organised consortia are the key to effective progress across the large areas represented by African countries. This is the model we

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intend to follow for our STOL programme. However, we can see that the geographical reach must be even wider for the STOL programme than those of the WACC and the ABC. We hope to find common ground with the growing cooperation between India and Africa by forming a joint programme embracing centres in the two regions; we have plans for such collaborations.

As may be expected, different countries have different procedures for accessing their genetic resources, so this process is taking some time and effort. We would be very grateful for any help in obtaining the materials for the studies. The need is urgent!

## Use of Agrobiodiversity in Adapting to Climate Change

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Globally, the rapid demographic changes occurring largely due to increasing population pressure and more recently attributed to climate change also, are likely to increase the demand for food and energy many folds. The implications of climate change are serious for agriculture, and this has brought agrobiodiversity in the forefront for dealing with climate change related events viz. rise in temperatures, changes in precipitation patterns, rise of sea levels, increase of greenhouse gases especially carbon dioxide, untimely droughts and floods, etc. The declining precipitation (both wet & dry) in the recent years and occurrence of frequent drought years has affected the agriculture and rural communities adversely. The data generated through scientific research suggests that this trend will become more pronounced in future. The most vulnerable population to this change would be poor and small holder farmers particularly those residing in Africa, Latin America and some parts of Asia. Many mitigation and adaptation measures might be beyond the reach of people living in these regions due to severe resource constraints. It is also cautioned that significant agrobiodiversity has already been lost from many production systems leaving them impoverished, vulnerable, dependent on external inputs and increasingly unsustainable. Therefore, small holder agriculture, which is >50% world over and even much higher in developing countries is tend to obscure central role in food production and key environmental systems. Traditional agrobiodiversity, which have until now less economic value, would greatly help people to cope with the changing climatic situations. The continued use and adaptive management of agrobiodiversity is central to sustainable production for improving the livelihoods, food security, and health of poor farmers.

### Impacts of Climate Change

The climate change is expected to impact species both positively and negatively. Consequently, the species that have long generation times, poor dispersal, specialists/narrow climatic tolerance, isolated populations and genetically impoverished are at more risk than those

that have short generation times, good dispersal, broad climatic tolerance and are generalists. Scientists using different models have predicted that climate change would lead to development of novel climates, and/or disappearance of existent climates. When climatic factors such as temperature and precipitation change beyond the tolerance of a species phenotypic plasticity, the inward and outward movement of species resulting in change in species composition is inevitable (Parmesan and Yohe 2003). Though evidence of climate related biodiversity loss remains limited, a large number of plant and animal species are reported to be moving to higher latitudes and altitudes (Pimm *et al.* 2006, Rana and Sharma 2010). While studying climate change on the upward shift of species in the Indian Himalayan region of India, Rana and Sharma, 2010 reported that many species e.g. *Aconitum heterophyllum*, *Lilium polyphyllum*, *Sorbus lanata*, *Swertia chirayita*, *Androsace* spp., which were abundant in 1902 (Collet 1902) in and around Shimla hills were observed in 2010 in the localities mentioned but are instead found at 200-600 m above higher elevation. Species in transition zones between subalpine and alpine are especially vulnerable to climate change as they have limited scope to move further and bound to extinct due to absence of suitable migration corridors (Pounds *et al.* 2006, Rana *et al.*, 2010). In agriculture, overall production, productivity and genetic diversity of species are also going to be affected. It is expected that some crops like wheat, rice, apple, oats may experience significant reduction while maize, pearl millet, sunflower, chick pea, soybean etc. may show some yield advantage. On the contrary, many traditional varieties, which contain significant genetic diversity may adapt to changed environments but some varieties having unique traits but narrow range of genetic variability may need support of active breeding to enhance their adaptability to the changed production conditions.

Increased temperature and water stress will also cause significant losses in production and productivity of

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livestock, especially in tropical and semi-arid production environments. Lower yields are predicted for dairy cattle as elite cattle species such as cross bred Karan-Swiss and Karan-Fries are more sensitive to heat. Buffalo being a less thermo-tolerant species will be affected more than cattle. The animal species adapted to extreme cold environments will be most affected due to warming and are bound to go extinct due to lack of migration corridors. Rising of temperature in rivers, lakes and oceans means less food and less oxygen for marine and freshwater fish populations (James *et al.*, 2008). Fish species such as snow trout and *Schizothorax* sp. generally move towards high torrential stream for breeding but increase silting and construction of dams have damaged their breeding habitats in the Himalaya (Sehgal, 1988). Changes in species interactions e.g. the utilization of a new host plant, maladaptive early hatching before host bud burst, phenological mismatches for pollinators are likely to affect the insect fauna. Microbial diversity has substantial effect on production due to changes in water availability, soil moisture retention and in the rate of aquifer recharge.

### Adaptations through Agrobiodiversity

Agrobiodiversity enable us to cope with the consequences of changing environments. This capacity is an outcome of genetic diversity and its continued cultivation in the farmer's fields where it is exposed to a wide range of agricultural and ecological conditions and organism's interaction with its environment. Therefore, adaption of plant species to stress environments is different kind of genetic engineering achieved through traditional breeding and continued exposure of species to different stress environments. Besides, social institutions and women in particular whose practices and experiences often embedded in the cultures indigenous peoples constitute an important element in the strategies to cope with and adapt to climate change. However, the information on the importance of agrobiodiversity and the ways it is used by farming communities is scattered, thus need to be documented and appreciated.

To adapt to climate change, farmers usually make changes in crop production practices such as changes in varieties, crops and crop combinations, alterations in agronomic and seed storage practices, etc. In recent times, participatory plant breeding based agricultural research has also become increasingly important. Diversifications of existing genetic diversity using climate analogues, crowd sourcing approach have also been advocated

as adaptation measures. These methods help farmers to ensure that their preferred materials give better return and remain adapted to changing production environments. Community seed banks and organic seed banks are being promoted at community level as seed insurance during weather uncertainties. Many civil society, farmer organization and public institutions such as MSSRF, Gene Campaign, Navdanya, Seva Mandir, Honey bee network, NBPGR, Bioversity International, etc. have been establishing community seed banks across India. The Potato Park in Peru is a locally managed community conserved area provides an important resource for coping with change and livelihood needs of Andean communities.

Most indigenous and traditional farming systems use agroforestry based land use systems and the same land management unit as agricultural crops and/or animals, either in some form of spatial arrangement or temporal sequence. It contributes to better utilization and conservation of soil and water resources, provide buffer to farmers against climate variability helps in income generation and local nutritional benefits. Examples include development of mango production systems in Bangladesh and planting of orange and lemon trees planted in Baharwala, Pakistan to promote agroforestry and multiple cropping, medicinal and multipurpose tree species in hill slopes in Western Himalayan region of India (Ensor and Berger, 2009, Rana *et al.*, 2010).

Agrobiodiversity occurring in the wild has also helped poor communities during food crisis arise due to extreme of climate change particularly drought and floods. For instance, Rendille pastoralists in northern Kenya and nomadic and gaddi tribes in Trans Himalyan region, which generally rely on their herds of camel, cattle, sheep and goats for their primary means of subsistence, look out for wild fruits and vegetables for consumption. Similarly, people inhabiting in the extreme environments of cold and hot arid regions of world collect edible plants from wild prior to expected extreme weather events and then preserved for use later (Rana *et al.*, 2009).

Animal husbandry is one of the most important components of integrated farming systems, and has been considered as one the important adaptations to climate change. There are a number of breeds of sheep, goats and ponies which are endemic to and are able to withstand hazards of mountainous areas. The important breeds of sheep and goat are Rampur Bushair, Gaddi, Biangi, Gurez, Karnah, Bhakarwal, Poonchi, Kashmir

Merino and Changthangi among sheep; Chamba, Gaddi, Pashmina and Chegu among goats and Spiti and Chummarti among ponies. Homestead based enterprises such as dairy, goatery, piggery, poultry, fishery, etc. provide additional insurance and capacity to cope with climate change particularly for small holders' agriculture in rural and tribal areas.

Soil and water management techniques such as micro-catchment rainwater harvesting systems in forms of terraces, earth or rock bunds, tied ridges, rock dikes, stone lines, planting pits or basins, etc. may not seem directly linked to agrobiodiversity maintenance and use, they can certainly increase the extent and use of agrobiodiversity. Aymara communities in the Andes have a long established system of rainwater harvesting in the mountains and pampas based on constructing small dams (qhuthañas) which have helped them to respond to the increased desertification of the last 50 years. Revival of a traditional rainwater harvesting system called Johads in Rajasthan and khtarees, kund in Western Himalaya have been revived as these systems recharge ground water and improve forest growth, while providing water for irrigation, wildlife, livestock and domestic use.

In addition to crop, livestock and soil biotic diversity, traditional agro-ecosystems all possess large amounts of associated diversity of plant and animal species. Mycorrhizae are beneficial associates of plants that influence plant growth in terms of increased nutrient and water absorption from the soil, protection from pathogens, increased tolerance to soil toxins, and root elongation (Podila and Varma, 2005). The ecosystem services rendered by pollinators are of great importance providing not only pollination of useful plants but also, in the case of bees, useful products and income.

## Conclusion

The conservation and use of genetic resources will remain essential for improving productivity in agriculture and sustaining human existence and well being. Given that global food security depends significantly on production in more industrial agriculture, it is relevant to note the important contribution of agricultural biodiversity to global food production as well as to sustainable livelihoods is more on traditional agricultural systems (Brush and Meng 1998). It is, therefore, inappropriate to promote large-scale abandonment of biodiverse agriculture. There is a need to develop new crop and livestock varieties, use of multiple varieties or the continuing maintenance of traditional materials (crop and

livestock varieties) adapted to changed (and changing) environmental conditions; maintenance and use of agrobiodiversity, both within production existing systems and through its deployment in different production systems and diversifying livelihoods. Promotion of integrated farming systems, improving water infiltration and water retention capacity, maintenance of high levels of soil organic matter, improved and accessible infrastructure and market systems, and alternative livelihoods options are needed to cope with new climatic regimes in the times to come. Blending traditional skills, knowledge, practices and experiences with new scientific developments is need of the hour.

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## Post-disaster Revival of the Local Seed System and Climate Change Adaptation: A Case Study of Earthquake Affected Mountain Regions of Nepal

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The devastating earthquake that hit Nepal on 25 April 2015 most severely impacted rural farm households particularly in remote and risk-prone mountainous regions. Here, farmers' food security is highly dependent on the use of self-saved and locally exchanged seeds of traditional crops and crop varieties. The official estimate by the Government of Nepal indicated that stored food grains and seeds amounting to more than NPR 8 billion (US\$80 million) were lost and about 60% of households' food and seed stocks were completely destroyed by the mega earthquake and subsequent aftershocks (FAO, 2015).

Seed is at the heart of restoring food security for farmers and their families in disaster affected areas. Seed aid interventions are commonly used in post-disaster agricultural relief operations. However, they are not always successful as they are complex to organise and manage, context-specific, often implemented in a hurry and without the required experience and expertise in seed systems and seed security analysis (Sperling *et al.*, 2006). To respond to the immediate disaster caused by the earthquake, significant efforts were made by the national government, international donors and non-government organisations to rescue human beings and provide immediate relief materials to the disaster affected households. However, no immediate initiatives were made in affected areas to rescue seeds and endangered native crop varieties as a means to quickly revive and strengthen the local seed system. Considering the critical role of local crop diversity and the local seed system in remote and risk-prone mountainous regions, Bioversity International in partnership with the National Gene Bank and LI-BIRD (Local Initiatives for Biodiversity, Research and Development) implemented a seed rescue and seed

recovery programme immediately after the disaster with the funding support of the Global Crop Diversity Trust and the Genetic Resource Policy Initiative-Phase 2 project (Bioversity International, 2016). The output of the research activities carried out in the last one year to revive local seed system capacity in earthquake affected areas to adapt to changing climatic conditions in 7 of the 26 most affected districts as identified by the Post Disaster Need Assessment (PDNA) of the Government of Nepal (NPC, 2015) are presented here.

### Methodology

This study was carried out in earthquake affected districts of the central and western mountains of Nepal involving rescue seed collection missions, conservation, characterisation, multiplication, seed exchange and repatriation of collected seeds. The collection missions were carried out in 7 earthquake affected districts including 326 farmers in 22 village development committees (VDCs) representing 2-4 VDCs from each district within the mountain landscapes (high mountains, middle mountains and lower hills). The study employed several tools and techniques including literature review, participatory rural appraisal, consultation meeting with district agricultural development offices (DADO) and other stakeholders to locate earthquake affected villages and communities and identify specific sample households and vulnerable farmers to undertake collection missions. Sample seed collection and survey of farmers with specifically designed passport data format were carried out including 138 farmers from 4 districts (Gorkha, Lamjung, Dhading and Manakwanpur) representing western cluster (Gorkha epicenter) and 188 farmers from 3 districts (Dolakha, Ramechhap and Sindhupalchowk)

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representing eastern cluster (Dolakha epicenter). At the community level, participatory rural appraisal tools (PRA) such as transect walk, focus group discussion, four cell analysis and participatory seed exchange (PSE) were employed to assess local crop diversity, identify rare and endangered landraces and exchange and distribute farmer preferred seeds of local crop varieties among communities in the affected areas to safeguard diversity and rebuild the local seed system. The collected seeds are currently processed, characterised, multiplied and conserved in the National Gene Bank for future availability to adapt to changing climate conditions in disaster affected areas.

## Results and Discussion

The post-earthquake assessment showed that the severely affected areas are mostly the mountainous regions of western and central Nepal that have diverse landscapes with wide variation in cropping patterns, crop diversity and disaster impact in terms of crop cultivar loss. Altogether, a total of 764 samples of 47 crop species covering cereals, pseudocereals, vegetables, oilseeds and spices were collected from 7 earthquake affected districts representing western cluster (4 districts) and eastern cluster (3 districts). About 80% of the collected germplasm was processed for conservation in the National Gene Bank. The collected seed samples and survey information from sample farmers were processed and analysed for their use in conservation, multiplication and distribution back to affected communities. The earthquake led a major loss of diversity of local crop varieties due to destruction of storage structures, burial of stored seeds and damage of agricultural lands. However, the major perceived causes of genetic erosion occurring in the surveyed areas and germplasm at risk are the *ad hoc* distribution of large amounts of improved, untested seeds as relief material from external agencies, the sudden migration of farmers after the disaster and attraction of rural farm households towards other alternative income generating options. About 10% of the collected samples were endangered varieties that need special efforts for future conservation and use. To organise the rescue collection of endangered landraces by visiting household after a disaster turned out to be a challenging task due to the heavy damages caused in the communities, the state of shock of people affected, and the hesitance to share with other the few possessions left in the hands of affected farmers. More specific study findings by cluster are presented below:

### I. Western Cluster Representing Gorkha Epicenter

From the western clusters (4 districts), a total of 320 accessions of 47 species of crops (cereals, legumes, vegetables, oilseeds, spices) were collected of which 185 were common (no risk of extinction), 55 were endangered, 19 were rare based on distribution and 61 were at risk of loss due to the earthquake. The earthquake led to many crop landraces acquiring the endangered status. The remaining seeds were rescued and sent for conservation in the National Gene Bank. Of the total collection, the highest numbers of endangered seed samples were collected from Gorkha with 28, followed by Dhading (25), Makwanpur (14) and Lamjung (12).

### II. Eastern Cluster Representing Dolakha Epicenter

In the eastern cluster, a rescue collection was undertaken in the three most severely hit districts (Sindhupalchowk, Dolakha and Ramechhap) where 444 samples of seeds were collected representing 46 crop species covering major cereals, legumes, vegetables, oilseeds and spices (Sthapit and Gautam, 2016). Of these, 78% of the samples were processed for conservation in the National Gene Bank for safety duplication. Participatory Seed Exchange (PSEs) were organised in 6 VDCs of the three districts in December 2015 as part of the post-earthquake seed recovery programme. In the PSEs, 485 farmers brought 2,058 samples of seeds from three districts to share and 503 farmers took 1,249 samples of seeds from the seed exchange process. Legumes, vegetables and cereals were most prominent in the seed exchange programme. On average, 41% of the varieties were cultivated by less than 5% of the households in each of the locations that are considered to be endangered. Over 98% of the exchanges consisted of locally adapted varieties not in the national official list of 605 notified varieties of Nepal. The PSEs also highlighted the pivotal role of women farmers in seed conservation and exchange.

## Conclusions

The most endangered and valuable local seeds of traditional crops were rescued from earthquake affected areas, characterised and conserved in the National Genebank. Farmer's preferred ones are multiplied and shared with disaster affected local communities through PSE and diversity kits distribution. Over 90% of the

collected and shared seeds in the earthquake affected local communities were not in the official national notified list of varieties in Nepal, which demonstrates the valuable complementary role PSE can play to strengthen the local seed system. Some of the rescue collected seeds and those shared back with local communities were also rare, endangered and not in the collection list of the National Gene Bank of Nepal. The process of rescue collection and PSEs adopted after disaster have improved *ex situ* and *on-farm* conservation and enhanced access of locally adapted crop seeds and varieties not readily available in the market. This has also helped to restore lost diversity, revive and strengthen the local seed system and safeguard biodiversity of native crops to adapt to more extreme, changeable and uncertain climatic conditions facing the Nepal Himalayas. The strategy has been useful in building national capacity and resilience to cope with future disasters and laying a foundation for community seed banks. Based on the experience and lessons learned from this study, we recommend collection, conservation and repatriation of farmer preferred seeds regularly through participatory seed exchanges. We also recommend to link the National Gene Bank to the establishment and operations of (newly formed) community seed banks in disaster prone areas

as a building block toward an integrated seed system and to be able to more effectively adapt to changing climate and market needs.

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## Global Challenges and Urgency for Partnerships to Deploy Genetic Resources

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The gene bank collections are believed to be “humanity’s most valued assets”, “repositories safeguarding agricultural heritage”, and “essential to adapt agriculture to a rapidly changing environment”. Yet gene banks are the ultimate source of global gene diversity, breeders seldom access this resource considering it to be of little relevance to their current breeding needs (Global Crop Diversity Trust, 2006). The *on-farm* diversity as well as genetic variability maintained in gene banks represents important reservoir in form of wild relatives, landraces and traditional cultivars that have been evolved in specific environments under particular set of climatic conditions. Several initiatives have been undertaken at global level focusing wheat pre breeding, example UK’s WISP (<http://www.nottingham.ac.uk/wisp/index.aspx>) and CIMMYT’s Seeds of Discovery (SeeD) project (<http://seedsofdiscovery.org/>). Despite challenges, there opportunities exist that can greatly help in successful utilisation of gene bank resources for crop breeding purposes, for example, increasing cost efficiency of high density genomics techniques/methods, availability of enormous germplasm diversity and a dynamic partnership between national and international stakeholders. These opportunities can be successfully utilized to fill the gap between diverse germplasm resources and the breeding pipelines through performing large scale coordinated pre-breeding efforts. Pre-breeding is a broad term refers to all pre-requisites of crop varietal improvement programmes. This paper presents pre-breeding efforts made at CIMMYT in collaboration with gene bank and NARS to mobilize the under-utilized diversity to breeding pipelines.

### Genotypic and Phenotypic Analysis of Gene Bank Accessions for Pre-breeding

A set of 1,500 wheat gene bank accessions including landraces, FIGS and synthetics were characterised by GBS technology at CIMMYT following a two-step complexity reduction method. Restriction was followed by amplification and sequencing using Illumina Hiseq

2500 and allele calling was performed by a proprietary analytical pipeline developed by DArT P/L (Li *et al.*, 2015, Vikram *et al.*, 2016). A core set of 200 accessions was formulated and utilized in developing wheat pre breeding germplasm resource. Core set of the Mexican wheat landrace has been formulated through using large scale genotypic and phenotypic information as well as a unique methodology explained by Vikram *et al.* (2016) to maximize the rare allelic variations (Data source: [www.seedsofdiscovery.org](http://www.seedsofdiscovery.org)).

### Wheat Pre-breeding Germplasm Development and Phenotypic Evaluation

A top cross population development strategy was followed for pre-breeding population development. Linked top cross population (LTP) panels were developed in such a way that each panel had a series of top cross populations in which each top cross was linked with another one via common elite parent and therefore top crosses were linked making a panel called, LTP. A total of 15000 wheat pre-breeding lines were evaluated for plant type and yellow rust in CIMMYT’s El Batán research farm. For yellow rust evaluation methodology reported by Hao *et al.* (2011) was followed and visual selection for plant type was done in such a way to maintain diversity simultaneously keeping other parameters (height, flowering, yield parameters etc.) similar to adapted varieties.

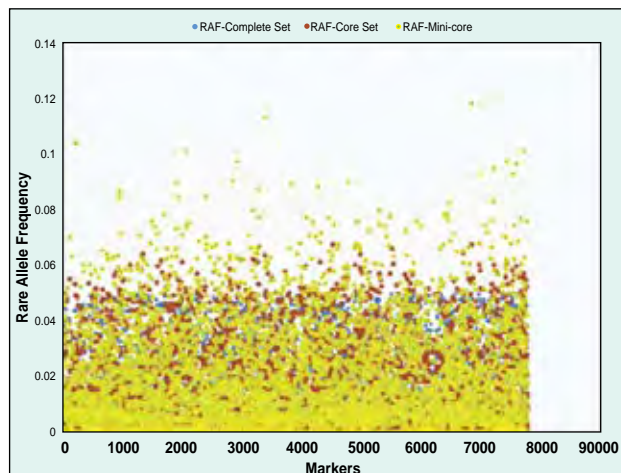
### Results and Discussions

The complexity of wheat genome and a narrow genetic base (compared to its progenitors) due to its recent origin of bread wheat is a major challenge. A systematic and focused effort with clear vision in mining genes/alleles from the available genetic stocks can be the most feasible and safest approach for development of climate resilient wheat cultivars. A core reference set of 232 genotypes was selected from total of 8,000 Mexican bread wheat landraces harboring 62.87% of the rare alleles of the complete set. Interestingly, frequency of some of the rare alleles (311 alleles) rose above 0.05% in the core

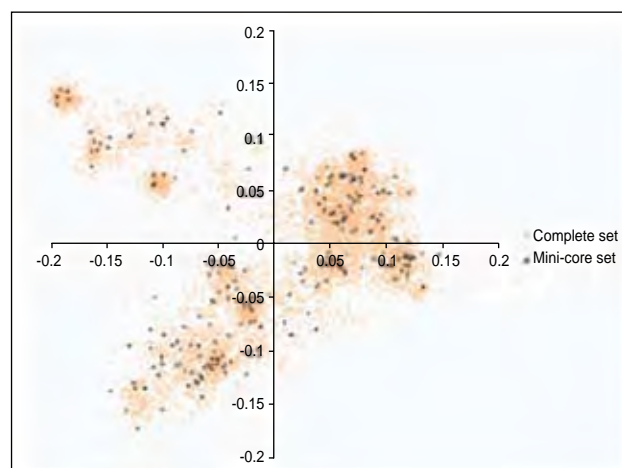
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set (Fig. 1 and 2). This core reference set has already been shared with partners in India, Mexico and Kenya for evaluation under disease pressure.

For pre-breeding purpose synthetics as well as FIGS germplasm sets were also selected as allele donors. A similar strategy was followed to select accessions for use in pre-breeding. Genotypes from different genetic spaces have been selected for their deployment in pre



**Fig. 1.** Frequency of rare alleles (of complete set) in three populations: complete set, core reference set and mini-core reference set. Rare allele frequency and individual markers (7,775 SNPs) are plotted on the Y and X axes, respectively. The figure also shows that the frequency of some alleles (which were <0.05 in the complete set) increased in core and mini-core reference sets. Details of core sets have been explained by Vikram *et al.* (2016) (Data source: [www.seedsofdiscovery.org](http://www.seedsofdiscovery.org)).



**Fig. 2.** Multidimensional scaling graph showing the relative distribution of complete and mini-core reference set accessions of Mexican hexaploid landraces (Data source: [www.seedsofdiscovery.org](http://www.seedsofdiscovery.org)).

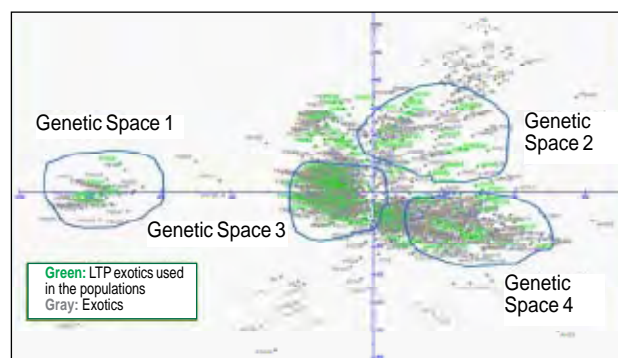
breeding (Fig. 3). These germplasm sets can be very efficiently utilized by wheat breeders in their varietal improvement programmes. Breeders from different parts of world have been involved and trait donors will be provided to them upon request.

Two complimentary pre-breeding strategies were followed, one incorporating general diversity and other one to introduce trait based diversity. International wheat pre breeding nurseries are being shared with the NARS in different countries (Fig. 4). Preliminary evaluation results identified disease resistant genotypes as compared to the local checks (CIMMYT, Unpublished).

A comprehensive strategy is being followed in CIMMYT's wheat pre-breeding initiative ([www.seedsofdiscovery.org](http://www.seedsofdiscovery.org)) for leveraging gene bank and derived resources for achieving short-, medium- and long-term impacts. The global wheat community has encouraged these ongoing largescale-unique efforts and breeders from South Asia and other parts of world are welcomed to leverage resource and deliver impact. The wheat landrace core sets/pre breeding germplasm resources have been shared with NARS/ARIs in Mexico, India, Iran, Pakistan, China, Kenya and USA. Efforts are made to enhance partnerships among stakeholders for maximizing the resource leverage and ensure impact.

## Conclusion

An extra ordinary support of Mexican government to CIMMYT's Seeds of Discovery-wheat project enabled sequencing of nearly 40% CIMMYT's wheat GeneBank, large scale phenotypic characterisation (Data Source: [www.seedsofdiscovery.org](http://www.seedsofdiscovery.org)) and establishes a wheat pre breeding pipeline for the wheat breeders in Mexico



**Fig. 3.** Principal component graph showing distribution of exotics (gray) in different genetic spaces and selected accessions (green) from them. Exotics include landraces, FIGS and synthetics (Data source: Sehgal *et al.*, 2015).



**Fig. 4.** Wheat pre-breeding nurseries planted at NARS site in Mexico (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias-INIFAP, Mexico) and India (Borlaug Institute for South Asia-BISA, India)



**Fig. 5.** Pre-breeding lines developed after selecting for heat-drought-plant type-diseases, available for breeders upon request

and abroad. Partnership among national and international organizations is urgently required to leverage these resources for expanding the genetic base of cultivated wheat, thereby delivering high impact at the farmer field level.

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## Rediscovering our Future: How Neglected and Underutilized Biodiverse Foods can Nourish the Planet

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The food systems we rely upon to feed the planet are under scrutiny and the results coming forth are not favouring continuing the status quo. Globally we rely on 12 crop and 5 animal species for the majority of our diet, while throughout the millennia humans have used thousands of species for nourishment. Dietary factors are the number one risk factor in the global burden of disease. These risk factors include low intakes of fruit, vegetables, nuts and seeds, and high intakes of processed meat, sodium and sugar sweetened beverages (Global Burden of Disease Study, 2015). Diet related diseases such as diabetes, high blood pressure and stroke are predicted to rise while two billion people worldwide are deficient in vitamins and minerals (IFPRI, 2016). The global food system is a major contributor to large environmental footprints, including biodiversity loss, greenhouse gas emissions, water shortages, ecosystem pollution, and land degradation (Tilman and Clark, 2014).

One approach that can help food systems better ‘tap into food biodiversity’ as a basis for healthier, diverse diets is to enhance the scientific evidence that demonstrates that biodiversity is important for improved dietary diversity and nutrition (Fanzo *et al.*, 2013; Hunter *et al.*, 2015). Increasing the scientific body of knowledge on the significant intraspecific differences in the nutrient content of most plant-source foods and other relevant food biodiversity (FAO/INFOODS, 2013a, b) is one example of how to do this. However, we still know so little about the nutritional value of most of the world’s food biodiversity (Hunter *et al.*, 2016). In order to address this gap countries such as Brazil, Kenya, Sri Lanka and Turkey and others are undertaking comprehensive nutritional analysis, as well as documenting associated traditional knowledge, of significant numbers of neglected and underutilized food crops and species in order to compile national food

biodiversity information systems and which contribute to the global FAO/INFOODS Food Composition Database for Biodiversity (FAO/INFOODS), (2013b).

For example, Brazil is in the process of establishing the nutritional composition data of over 70 native species including baru (*Dipteryx alata*), buriti (*Mauritia vinifera*), cagaita (*Eugenia dysenterica*), mangaba (*Hancornia speciosa*) and pequi (*Caryocar brasiliense*), Umbu (*Spondias tuberosa*) from the Caatinga biome and cupuaçu (*Theobroma grandiflora*) and pupunha (*Bactris gasipaes*) among others. Kenya is focusing on indigenous leafy greens such as the Spider plant (*Cleome gynandra*), African nightshade (*Solanum scabrum*) and jute mallow (*Corchorus olitorius*) as well as fruits, insects, indigenous poultry, mushrooms and the small dried lake fish, known locally as omena (*Rastrineobola argentea*). Wild edible plants that are still widely consumed are being analysed in Turkey. Golden thistle (*Scolymus hispanicus*), chicory (*Cichorium intybus*), fennel (*Foeniculum vulgare*), wild radish (*Raphanus raphanistrum*), glasswort (*Salicornia europaea*), black-eyed and yellow-eyed cowpea (*Vigna unguiculata*) and red pine mushroom (*Lactarius deliciosus*) are being analysed for their macro- and micro-nutrient content. In Sri Lanka, seven traditional rice varieties (*Oryza sativa*)—Suwandel, Kalu heenaty, Kuruluthuda, Madathawalu, Pachchaperumal, Pokkali and Suduru Samba, five banana varieties (*Musa* spp.)—Ambul, Seeni, Kolikuttu, Anamalu and Rathabala, four varieties of yam (*Dioscorea* spp.)—Rajaala White, Rajaala purple, Kukulala and Walala, one variety of finger millet (*Eleusine coracana*), two varieties of eggplant (*Solanum melongena*)—Wambatu, and Talanabatu—and one variety of jackfruit (*Artocarpus heterophyllus*) have been selected as target species for food composition analysis largely for their food security and marketing potential.

Improving access to this kind of knowledge can make it easier for policy makers, researchers and

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practitioners to facilitate change in food systems and diets in a variety of ways. For example in Brazil, it has helped realize the endorsement of a new public policy, Ordinance No. 163 'Brazilian Sociobiodiversity of Native Food Species of Nutritional Value', which for the first time formally and legally recognizes the economic and cultural value of 64 native species which are of importance to communities and family farmers. Such recognition can greatly facilitate the integration of these nutritious species into value chains, markets, public food procurement and school feeding programmes and contribute to more diverse diets. Likewise in Kenya, this kind of information has been used to mobilize the promotion of African leafy vegetables into school feeding programmes. Among the 10 priority actions identified by the Global Panel on Agriculture and Food Systems for Nutrition for transforming food systems and diets is the recommendation to institutionalize high-quality diets through public sector purchasing power including ensuring the provision of high-quality diets to schools, hospitals and prisons (Global Panel on Agriculture and Food Systems for Nutrition, 2016).

Such knowledge and information can also help add value and identify markets for food biodiversity. In Sri Lanka, under the brand name "Hela bojun-True Sri Lankan taste", nine market outlets, supported by the BFN Sri Lanka project, for the sale of traditional foods are serving freshly-prepared local food biodiversity and empowering rural women across Sri Lanka to earn a living while conserving and protecting biodiversity and making healthy food available at competitive prices. Whereas in Turkey, major supermarket companies such as Metro and Migros are exploring markets for wild food biodiversity species such as golden sow thistle as well as landraces of cultivated einkorn wheat.

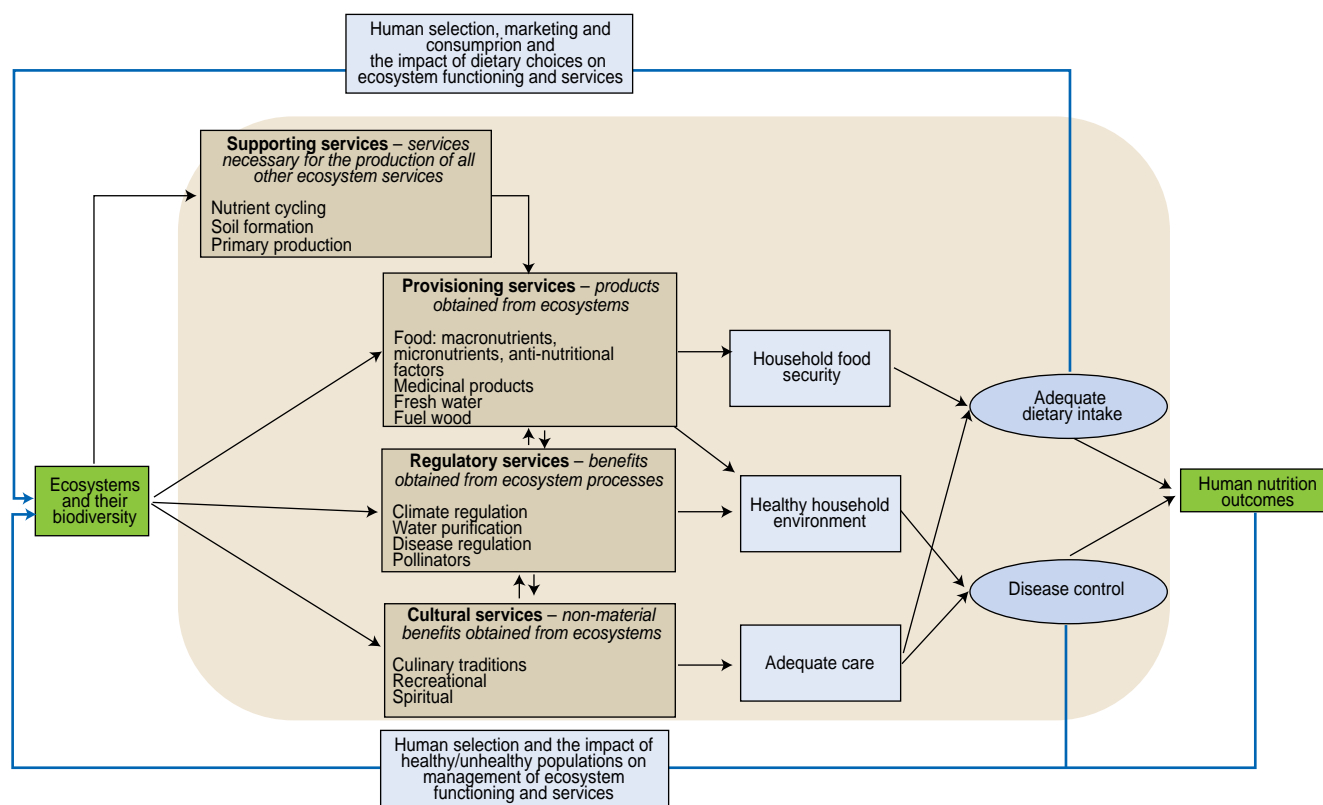
Nutrition-sensitive landscapes (NSL) initiative is another example of promotion of biodiverse foods for improved nutrition. NSL is based within the context of systems research and focuses on the synergies, relationship trade-offs and feedback loops that exists between different food system factors and elements (Fig. 1). While the NSL approach brings attention between the nexus of human, landscape and ecosystem nutrition and health, it does not assume that a local food system or local food environment has the capacity to completely to satisfy all nutritional needs. However, it does allow emphasis on diversifying production systems within a landscape, while managing and supporting other

ecosystems functions that directly contribute to both human health and environmental sustainability.

This multi stakeholder initiative, combining efforts from multiple CGIAR, International research and local expertise has been piloted in Kenya and Vietnam. In both sites a household surveys were undertaken that included agriculture production and nutrition dimensions and measured biodiversity and the source of all food consumed within each household. The nutrition survey was repeated over two seasons to capture seasonal variation in the diet.

Lists of locally available, yet underutilised (defined as being produced by less than 50% of surveyed households) species from the food groups that had low consumption in the diet were compiled. A key set of these crops were selected based on the community's preferences, and together barriers to production and consumption of these species were identified. Local communities then shared experiences about possible innovative solutions to overcome these barriers, in a way that empowered communities to produce and consume selected species and provide useful ecosystem services to the landscape, throughout the year. Local innovations on best-management practices, as well and adapted local recipes and nutrition education was delivered to communities through village level diversity clubs affiliated by local health workers. Local nutrition education material was adapted to integrate useful information about local agrobiodiversity that could directly contribute to improving the diversity of their diet, including seasonal availability and planting calendars, nutrition education, recipes and crop management practices. End line assessments for these sites are scheduled by December 2016 and mid-line evaluations have so far found that just over 70% of club participants are regularly implementing the new skills and knowledge gained during the clubs (including cultivating diverse foods in the home garden, and preparing new recipes using these foods for the household).

From the wealth of neglected and underutilized plant species (NUS) recorded from every region (Padulosi *et al.*, 2013; RBG Kew, 2016), research and development should strategically invest on 'low hanging fruits' crops where gap filling in knowledge and synergy building across isolated projects can lead to important outcomes in short time. To that end, important opportunities do exist across each major food crop category viz. cereals (fonio, minor millets), pseudo-cereals (Andean grains), pulses



**Fig. 1. Nutrition sensitive landscapes conceptual framework: a combination of the Millenium Ecosystem Assessment Framework and UNCIEF framework of nutrition determinants (Adapted from Remans and Smukler, 2013)**

(bambara groundnut, tepary bean), vegetables (African leafy vegetables) and fruit species (tropical fruit trees). Issues hampering the satisfactory deployment of these species in local and national food systems range from limited availability of germplasm and lack of improved varieties, poor access to good quality seed, drudgery in cultivation and processing operations to dis-organized value chains, poor entrepreneurial skills of value chain actors, poor awareness among consumers over their nutritional benefits and absence of a supportive policy environment for their use enhancement (Padulosi *et al.*, 2013, Sthapit *et al.*, 2016). In the real world, crop diversification using locally adapted varieties is becoming increasingly vital to farmers to allow them to cope with climate change. Food systems offer ample opportunities for improving both supply and demand of nutrient-dense underutilized species leveraging also consumers' interest for healthier food which can connect them to cultures and territory identity. One of the strongest instruments for moving forward such a vision of resilient, diverse, healthy and sustainable food systems are Government

policies on Agriculture and Food Systems for Nutrition (Global Panel, 2014; 2016) and to that regard worth of appreciation is the amendment made by India of its National Food Security Act in 2013 (Ministry of Law and Justice, 2013) aimed at including also coarse cereals (minor millets) in the National Public Distribution System.

Though different in scale and scope, these examples demonstrate how food biodiversity, or the rich and vast array of plant and animal species used for food, can be promoted in local and national programmes as the building blocks for diets that can sustain both human and planetary health.

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## Harnessing Traditional Foods for Nutrition and Health

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Agricultural diversification and innovation should be defined and suited for each location to achieve sustainable production and food security. From this viewpoint, traditionally important crops and the recipes using these crops are worthy of attention because of their tight linkage with people's livelihoods and culture, and eminent suitability to the agricultural environment.

In many African countries, there are many traditionally important crops such as sorghum, millets, yam, cowpea, banbara groundnuts, banana and plantain etc. They possess various unique characteristics that enable them to become suitable to regional and local environments. The importance of these crops are primarily attributed to their favourable agronomical characteristics which provide stability and resilience to the existing agricultural systems of the region.

Take cowpea [*Vigna unguiculata* (L.) Walp.] as an example; it is a major grain legume widely cultivated especially in a range of agro-ecological zones in West Africa. With its favourable agronomical characteristics, such as tolerance to drought and low soil fertility, nitrogen-fixing ability, and adaptability to different cropping systems, this crop exhibits resilience in agricultural systems under severe and unstable growth environments, particularly in resource-poor areas. For these reasons, farmers have been cultivating cowpea in combination with other crops to maximise the productivity of their limited land and resources. It is a shared axiom that diversity among crops enhances both the health and the function of complex biological systems in agriculture. As an important component crop of the system, cowpea plays an significant role in increasing the stability and resilience of agricultural systems in the region.

On the other hand, with their long cultivation histories, each traditional crop has formed a deep linkage with the peoples' lives and culture in the region. Variation in food species included in the diet has been associated with nutritional adequacy and food security. Cowpea is considered important not just as food, but also as

a key protein source, especially when consumption of animal proteins is precluded from the diet because of inaccessibility, poverty or dietary preferences. With its rich micronutrient and vitamin contents, this crop can supplement the staple crops (cereals and tubers) for more nutritionally balanced diets. Another important role of cowpea in the region is the production of green pods and grains of early maturing varieties; these supply farmers with food and cash income that would enable them to survive the "hungry period" when local food supplies are depleted before the other crops reach maturity.

In West Africa, cowpea is served on a daily basis in various forms, making the dishes appealing and interesting (Fig. 1). Philipps *et al.* (2003) mentioned over 50 different dishes of cowpea in both whole grain and milled forms. Its green leaves and pods are also consumed as vegetable. Strong preferences for grain quality which affect the market value reflecting deep rooted cultural traditions has been reported (Coulbaly and Lowenberg-DeBoer, 2002; Faye *et al.*, 2004). Interestingly, the situation of cowpea contrasts greatly with that of soybean which is relatively new to the region. Although soybean is also rich in protein and is now widely cultivated in the region, there are only a few ways of processing it for direct human consumption.



**Fig. 1. Various cowpea dishes in northern Nigeria**

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Like cowpea, traditionally important crops play critical roles in peoples' livelihoods attributable to their favorable characteristics and deep linkage with traditions in the region. These crops do not only improve stability and resilience of the agricultural systems in each region, but also provide local solutions to diet-related nutrition and health problems such as nutrient deficiencies and obesity.

However, despite the recognition of the importance of traditional crops and traditional foods in various aspects, a global trend toward simplification of agricultural systems and food composition prompted by increasing reliance on a suite of truly global crop commodities has been observed; this implies a narrowing of diversity of both crops and foods in the region. The changing food demand patterns of urban populations have also had negative effects on the consumption of these traditional crops and foods. With the loss of functions of these crops, the ensuing narrow diversity-based food security has certainly impacted the nutrition and health of the people, mostly the rural poor who have limited options.

How could we effectively utilise the valuable functions of traditional crops and traditional foods to secure the nutrition and health of the people in the region? Considering the recent rapid economic growth in Africa, "quality" of agricultural products could very well be the key to the future of agricultural development of the continent. With regards to traditional foods, their selection and consumption are generally dictated by social reasons that are founded on regional cultures, particularly those that involve customs and prestige. In addition to "quantity" in crop production, more attention should be placed on improving the "quality" of the traditional crops and their products to enhance consumption and promote utilisation. Given adequate motivation of consumers regarding consumption and of farmers to produce traditionally important crops, we

can expect a wide range of traditional foods to become available resulting in diversification of food supply and increase in dietary choices, and consequently to the further improvement of nutrition and health situation in the region.

To drive further innovation for food security in the region, effective utilisation of existing wide genetic diversity of these crops in both crop improvement and utilisation, taking into consideration indigenous preferences and knowledge in the region, is called for. Such would in turn strengthen their role as traditional crops, and eventually will contribute to the improvement of the livelihoods of the people. However, still, there is a large gap existing between the actual potential that these crops hold and the levels of investment they have so far received despite their crucial contribution to achieving household food security and poverty eradication. To fully utilise the great potential of these crops and traditional foods with the aim of bolstering the livelihoods in the region, a multi-level approach is needed, ranging from conservation of genetic diversity, characterisation and utilisation in breeding and processing, and understanding local knowledge and preferences.

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## Medicinal Plant Diversity for Health, Wellness and Livelihood in Indian Perspective

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India is bestowed with 45,000 plant species out of which about 15,000-20,000 plants are known to have medicinal properties and about 7,000-7,500 are being used in Indian System of Medicine (ISM). Medicinal and aromatic plants being natural, non-narcotic, easily accessible, affordable and changing concepts from 'health to healthy living' and balance between 'mind and body' have revived this sector. Besides, their role in prevention as well as curing of human health problems, MAPs are also a source of significant livelihood opportunities for many rural communities, especially, primitive forest-dwellers, landless poor and marginalized farmers. In spite of scope, importance and awareness and several scientific innovations in the field of MAPs, still more than half of the global population does not have access to the primary health care facilities.

India is still careworn to reduction of child mortality, maternal health, HIV/AIDS, malaria, and other diseases and more than 50% child births unattended by skilled health worker, 16% children are undernourished, 40% child deaths occur in the first month after birth and among these 25% deaths are due to malaria alone in the world. This is mainly due to poor accessibility of healthcare services. High prices of medicines are also considered as main barrier to use of medicines and health products. Due to these, the whole world is looking for an easily accessible, affordable, effective medicine as an alternative to allopathic medicines which are costly having side effects and becoming non-responsive in several diseases. Various human migration and settlements in the history led to discover new and new plants of medicinal values and their distribution over wider areas. Further, increased knowledgeable and consciousness among the consumers about health during the 1980s and 1990s, aroused the interest in organic and natural foods using medicinal and aromatic plants. But the sector is plagued by loss of biodiversity, lack of standards formulations and finished

product ingredients. This is one of the major hindrances for sustainable growth of this sector and can partly be attributed to the absence of any recognized central or regional authority with this responsibility.

There is huge diversity of medicinal plants in India. According to FAO report Himalaya, Southern India and North eastern India is the largest MAPs species inhabiting centres.

**Table 1. Region wise number of medicinal plant species in India**

Geographic region	Estimated no. of medicinal plants sp.
Trans Himalayas	700
Himalayan	2500
Desert	500
Semi-Arid	1000
Western Ghats	2000
Deccan Peninsula	3000
Gangetic Plain	1000
North-East India	2000
Islands	1000
Coasts	500

Development of standardized, synergistic, safe and effective traditional herbal formulations with robust scientific evidence can also offer faster and more economical alternatives. For instance, Ayurvedic texts include thousands of single or poly herbal formulations which have been rationally designed and have been in therapeutic use for many years. There are an estimated 119 medicines derived from phytochemicals which occupy 74% space of modern medicines.

Providing future healthcare will be an extremely complex, technology and capital-intensive process. Better validated pre clinical targets with proof-of-concept of better efficacy and safety of drugs can, however, mitigate such attrition risks. The most of the work in this field has remained within the clinics of traditional practitioners or confined to academic research laboratories and not

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**Table 2. Drugs Derived from Wild Plants**

Plant	Location	Drug	Use
Willow	Worldwide	Aspirin	Fever and pain
Cinchone	Tropics	Quinine	Malaria
Rosy periwinkle	Madagascar	Vinblastine Resprine	Leukemia
Pacific yew tree	Pacific Northwest	Taxol	Ovarian cancer
Opium poppy	Eurassia, Africa	Morphine	Pain
Curane	Amazon	Tubocurarine	Muscle relaxant
Snakeroot	India	Roserpine	Hypertension
Forsk. glow	Eurassia, Africa	Digoxin	Cardiac anty

taken seriously by industries that are strong in research and development. Therefore, path-breaking initiatives are crucially important. The Government project should be formulated by integrating biomedicine, modern sciences and traditional medicine is indicative of a trend where traditional sciences like Ayurveda are increasingly embracing the scientific evidence-base and the spirit of robust research.

The future will be more problem some and complex with full of all type of healthcare glitches. The problem will be more aggravated due to changing personal and global perspectives of living and non-living factors. Most important factors are, burgeoning human population for which providing healthcare itself will be a daunting

task; changing food habits leading to several new health problems; changing climate which spawned several new disease causal organisms; changing life style; progression of disease susceptible genes in the pool; dwindling biodiversity and the increasing pollution. There is a huge challenge to development of technologies for gearing up supplies via large-scale cultivation and appropriate post-harvest technologies and establish the safety, quality and efficacy of the traditional health products. This again calls for immediate research into development of location specific high yielding and better quality varieties of MAPs, quality planting materials and standardization of agro-technologies and agro-economics.



## Biodiversity, Food and the Future: India

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Every academy of sciences in the world has agreed for more than 15 years that foods derived from transgenic crops not only are perfectly safe for human health, but that in fact they had nothing in common by virtue of which they could even theoretically pose any common danger. Despite this sturdy scientific consensus, India and many other countries continue to delay the approval of such crops. Arguments about them have become politicized and are being used for fund-raising purposes by organisations such as Greenpeace, and by individuals who stridently condemn them even though there is literally nothing concrete to condemn. The world faces a number of really serious problems, but this is not one of them. It is time to move on.

How did we get to this point? Approximately 200 years ago, when Britain was consolidating its conquest of India, the countries of the world and their colonies had expanded to take up all of our planet's ice-free land. The human population of the world had reached one billion people, with the population of India about 270 million of them. Most of the history of the world since has involved fighting for territory and resources, and, tragically, about 200 million people have died in wars during these centuries. Meanwhile, the global population has expanded to 7.4 billion people and that of India to 1.3 billion. By the year 2050, world population is projected to grow to 9.9 billion people, adding roughly 250,000 net per day, and that of India to 1.7 billion. There is much wishful thinking about stabilization sooner, but the sheer numbers of people and the time it takes to reach the end point of existing trends indicates that stabilization will require decades of gradually slowing growth ([www.prb.org](http://www.prb.org)). What effect are these numbers of people having on our planetary home?

Global Footprint Network ([www.footprintnetwork.org](http://www.footprintnetwork.org)) estimates that the people of the world together are consuming about 164% of our planet's sustainable productivity. In other words, it would require about 64% more sustainable productivity than exists on earth for us to attain collective sustainability. We could

achieve stability by attaining a level population, socially justifiable levels of consumption, and improving our technology, but we are not gaining on the problem at present. In the 1960s, we were using about two-thirds of total sustainable productivity, with the subsequent doubling of our population and greater increases in consumption have sent us well past our planet's total capacity.

We have reached this point because of our cultivation of crops, starting some 12,000 years ago. By that time, our species was 200,000 years old; it had reached Eurasia and Australia about 60,000 years earlier. Once stored food was available, people could settle and form villages, towns, and cities, in which individuals who lived earlier as hunter-gatherers could adopt individual specialized professions and build our modern civilization. The more people, and the more they subdivided the world, the more was the competition and warfare.

Some countries consume more than their share of the world's productivity. Since our use already exceeds the total amount of productivity available, a given country can increase its standard of living only through improved technology and resource management or by taking something away from another country. India's Ecological Footprint per Person has doubled since 1961, while its population has grown from 460 million to 1.3 billion people – nearly tripling, so that the average footprint per person (amount of consumption available per person) has decreased. Clearly, some citizens are enjoying an enhanced standard of living, while most are not benefiting from the economic boom. It will require incredibly careful management of natural capital for India to shift from an economy that has grown at the expense of its environment to one that flourishes by nurturing and preserving it.

In the same period of time, China's Ecological Footprint per Person has tripled, while its population has doubled – and much of the growth has taken place in a period of nearly stable population. Individuals in China have become markedly better off during this

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period, but in the case of both China and India, much of the improvement is fueled from abroad.

For India, achieving its global emissions goals and meeting the targets set in Paris in December 2015 is proving difficult, with multiple missions and agencies involved in the national effort to lower emissions and achieve increased energy efficiency. Globally, it may already have become impossible to hold global temperature increases within the 2°C target set at the Paris meeting. Irregular monsoons, glaciers melting, the intensified use of water that accompanied the Green Revolution in India, and the projected 2 meter sea level rise in the remainder of this century will certainly adversely impact agricultural productivity in India and as well as globally.

India is a net exporter of food, the world's seventh largest, but still runs a substantial negative trade balance. Even with no increase in population, India would still need to increase its agricultural production greatly to feed its people adequately. From the time of the Bengal Famine (1943) to the start of the Green Revolution (1967), millions of Indians starved to death, and there are now three times as many people to feed as there were during that period. The Green Revolution resulted in increasing croplands, double-cropping through intensive irrigation, and introducing improved genetic strains of crop plants. The population was increasing more rapidly than food production, however, during this whole period. No level of agricultural productivity can feed a continually increasing population.

To feed all its people, the world will need to attain a level human population that might not be as large as the one we have now. Consumption levels will need to be adjusted by the principles of social justice; women and children will need to be empowered if social justice is to be attained. Technology will need to continue to be improved as one element of the equation. Obsolete economic theories that assume that the goods we get from nature are as expandable as, for example, labour, will need to be modified to take into account the conditions of the actual planet that we inhabit. On a finite planet, it verges on immorality to assume that economic prosperity can be attained by adding more children to a given population. In India, this is vividly illustrated by the falling rates of consumption of the poor during the past two decades of prosperity for a part of the population.

In improving agricultural productivity, it seems a mystery to the world that India, the greatest beneficiary

of the Green Revolution, has been so oddly backward in adopting current improvements in crop genetics. Genetically modified (transgenic) crops provide a concrete example. The struggles that have taken place in a bureaucratic setting that very often failed to take into account the findings of science have done nothing but contributed to the hunger of the Indian people, and it is heartening that the barriers are starting to break down, although very slowly. We can only wish that ways may be found to accelerate the process for the good of the Indian people. Among the problems has been the fight against utilizing Golden Rice; those who have opposed its introduction and widespread use should realistically be held responsible for the deaths of hundreds of thousands of children and the blindness of many more each year – and without a shred of factual evidence against its rapid introduction.

Human pressures on the environment, exacerbated by inefficient agriculture and urban spread, are also causing what will in the future doubtless be seen as the most serious problem of our time, the extinction of perhaps half of all living species of organisms during the course of this century. India's biodiversity is among the world's richest, with most species still unknown in all but a few groups, such as vertebrates and plants. Meanwhile, bureaucratic snarls and lack of interest retard both the efficient acquisition of knowledge about these organisms and their conservation. We derive all of our food and most of our medicines from them, and that we are just beginning to understand the ways in which they function to protect the water and the soils that support us and provide the beauty that enriches our lives, our inefficiency and stubbornness in getting on with the job seems incredible. No wonder that Harvard University's E.O. Wilson has termed the loss of biodiversity that we are driving as the sin for which our descendants are least likely to forgive us.

There is no future for India or any other nation if we do not find better ways to cooperate with one another to fashion a sustainable planet while there is still some room to maneuver. In doing so, we must embrace both the principles of science and the moral precepts that we have developed over the years to find peace based on mutual love and mutual accommodation. The question is really not whether we can do better – we simply must.



## Utilizing Genetic Diversity in CIMMYT Global Wheat Breeding Programme

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Wheat is grown on over 215 million hectares worldwide and provides about 20% of calories and proteins to humans. About 1.6% annual productivity increase is needed to meet the future demands caused by population and prosperity growth. The global wheat cultivation expands to very diverse environments exposing the crop to a range of biotic and abiotic stresses and therefore enhancing genetic yield potential together with resistance/tolerance to key stresses and nutritional/processing quality are important breeding objectives for CIMMYT and National breeding programmes. Being an allohexaploid, wheat possesses a wide genetic diversity in the progenitors of the three genomes and has the ability to absorb chromosomal segments of varying sizes from related species and genera. Systematic incorporation of alien chromosome segments possessing numerous race-specific resistance genes to important diseases, e.g. rusts, has been a great example of enhancing the genetic diversity in cultivated wheat germplasm pool.

The existing genetic diversity and targeted incorporation of new diversity for traits that have promise to enhance productivity are of paramount importance to breeding programmes for continuing to make genetic progress. Because grain yield, abiotic stress tolerance and durable resistance to diseases and pests are under complex and quantitative genetic control, most breeding programmes are often reluctant to utilise landraces or other diverse genetic resources as identifying and extracting minor or small effect genes are tedious process often requiring 2 or 3 complete breeding cycles before competitive varieties can be developed. To overcome this limitation trait/gene based pre-breeding is an attractive approach to enrich the most recent breeding germplasm pool. The success of pre-breeding depends on a reliable phenotypic selection of the trait or linked molecular marker based incorporation of targeted genes.

CIMMYT spring wheat breeding programme continually incorporates new genetic diversity for different traits or genes present in diverse breeding

materials available to CIMMYT, e.g. winter/facultative wheat germplasm and synthetic wheats. Utilisation of synthetic wheats in wheat breeding has facilitated transferring diversity from the D-genome progenitor *Triticum tauschii* for resistance to diseases, pests and tolerance to heat and drought stresses. It is now common to find synthetic wheat derived varieties grown by farmers.

About half of the catalogued genes conferring resistance to stem (black) rust and leaf (brown) rust were transferred to wheat from related species and genera. In case of stripe (yellow) rust about 20% genes are derived from alien origins. Some resistance genes were identified in landraces indicating the importance of continued search for the existing diversity in wheat gene pool. Deployment of these single race-specific resistance genes to rust or powdery mildew fungi, irrespective of their origin, leads to rapid evolution and selection of virulence. A majority of the race-specific genes effective against the Ug99 race group of stem rust fungus are of alien origin and some important genes like *Sr24* and *Sr36* were overcome by the new variants of Ug99. The improper utilisation of resistance genes that is common at present highlights the important issue of protecting the incorporated disease resistance diversity through proper utilisation as multiple combinations of resistance genes. Developing diagnostic molecular markers for resistance genes located in alien chromosomal segments has been easier than for genes of wheat origin. The longevity of these resistance genes can be enhanced by ensuring that all released varieties have at least three effective resistance genes and these genes are not deployed singly, which has not been possible so far. As some rust resistance genes have been cloned and new techniques allowing accelerated cloning of race-specific resistances genes offer great promise, use of multiple *cis*-genic cassettes concept has been proposed. These cassettes where multiple genes will inherit together can simply breeding and selection by using a single molecular marker or selection for

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resistance in field conditions. However, it is not known yet whether *cis*-genic wheat varieties will be acceptable to consumers.

Utilisation of genetic diversity for multiple minor, slow rusting resistance genes with additive effects has proven to be an excellent alternative in achieving durable resistance to wheat rusts and other important diseases. Diversity for these genes can be found in improved wheat varieties as well as landraces. The identification of three pleiotropic multi-pathogen resistance genes, viz. *Lr34/Yr18/Sr57/Pm38/Sb1/Bdv1*, *Lr46/Yr29/Sr58/Pm39*, and *Lr67/Yr46/Sr55/Pm46*, and the cloning of two of them shows their novel nature in conferring disease resistance. The slow rusting gene *Sr2*, transferred to hexaploid wheat from tetraploid emmer wheat, is also known to confer durable resistance to wheat stem rust fungus including the Ug99 race group. Various genetic and mapping studies have shown that combinations of any of these well characterised resistance genes with 3 to 4 additional minor genes, often mapped as quantitative trait loci; result in conferring a high level of durable resistance that is comparable to immunity. Resistance to wheat rusts and other important pathogens in a large proportion of CIMMYT wheat germplasm that is distributed annually worldwide is often based on diverse combinations of these genes.

With climate change new, more aggressive and virulent races of common wheat pathogens are expected to emerge and migrate to new areas. Moreover, new pathogens can also evolve and cause severe damage to wheat crop. One example is the wheat blast disease, first detected in Brazil in the 1980s, then spread to

some other countries of South America, and in 2016 reported to have migrated to Bangladesh in South Asia causing severe losses in affected areas. Identification and utilisation of genetic resistance in improved wheat germplasm, landraces, related species and genera are likely to play a crucial role even though only moderate resistance has been identified so far.

A recent and successful utilisation of genetic resources is in enhancing grain Zn and Fe concentrations in wheat under the HarvestPlus project. The modern semi-dwarf wheat varieties usually have moderate levels of these micronutrients with limited diversity. Extensive search for genetic resources with high Zn and Fe identified some tall varieties, landraces, spelt wheat (*T. spelta*), tetraploid wheat (*T. dicoccum*), and some synthetic wheat accessions. Targeted breeding initiated about a decade back to develop high yielding wheat germplasm with enhanced Zn content and adaptation in South Asia. The non-destructive, high-throughput phenotyping for Zn is now possible by using a XRF machine and calibrations that accurately determine grain Zn and Fe concentrations. High yielding competitive wheat varieties with 20-40% higher grain Zn concentration could be developed; one variety 'Zinc Shakti' (Chitra) released and disseminated to thousands of farmers in eastern Gangetic plains of India. This is a great example of fast delivery of end-product from utilisation of genetic resources. In addition, various genomic regions involved in increasing grain Zn were identified and this diversity will be useful in continuing to increase grain Zn further. Enhanced grain Zn is often associated with enhanced grain Fe and thus, a further value is added.

## Indian Wild Rice: Diversity, Population Structure, Trait Value and Relation with Cultivated Rice

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Wild relatives of crop plants are rich in mutations for adaptive agronomically important traits, which could be used in crop improvement for sustainable agriculture under global climatic change. However, human developmental activities pose serious threats to the natural habitats leading to erosion of genetic diversity of wild rice populations. The germplasm utilisation strategies involve collection, characterisation and evaluation to understand the underlying morphological and molecular variations for the adaptive agronomic traits. India has a huge wealth of untapped wild rice resources and to explore the variation present in these we made hasty expeditions around diversity hot-spots in nine different eco-geographical regions of the country and collected more than 600 accessions wild rice *Oryza nivara*/*Oryza rufipogon* representing wide range of ecological niches. Enormous variation was observed among these accessions on evaluation for 46 morphological descriptors. Cluster analysis based on Euclidean distance matrix revealed three major morphological groups but these did not correspond to their geographical origin. Genome-specific *pSINE1* DNA markers revealed that all the accessions belonged to AA genome and these can be easily used for introgression of useful genes in the cultivated rice (*Oryza sativa*) genome. Further characterisation using ecotype-specific *pSINE1* markers classified these accessions into annual (for *O. nivara*), perennial (for *O. rufipogon*), intermediate (mixed type) and unknown types not described earlier. Principal component analysis revealed continuous variation for the morphological traits in each ecotype group.

Genetic diversity analysis using genome wide multi-allelic SSR markers clustered these accessions into three major groups. Analysis of molecular variance (AMOVA) in relation to the eco-geographical origin revealed that 68% of the genetic variation was inherent amongst individuals while only 11% variation was due to geographical regions. Though there was significant

correlation between genetic and spatial distances of the accessions. Model based population structure analysis using genome wide unlinked bi-allelic SNP markers revealed three sub-populations which we have designated 'Pro-Indica', 'Pro-Aus' and 'Mid-Gangetic' populations, which showed no correspondence to the ecotypes or *O. nivara*/*O. rufipogon* distinctions. Wide eco-geographical distribution of the Pro-Indica and Pro-Aus sub-populations indicates a more fundamental grouping based on the ancestry. The Pro-Indica and Pro-Aus populations are closely related to 'Indica' and 'Aus' groups of rice cultivar, respectively and hence may represent their ancestral wild progenitors, whereas the Mid-Gangetic population was distinct from major cultivated rice groups.

Soil salinity covers a large part of the arable land of the world and is a major factor for yield losses in salt-sensitive crops, such as rice. Rice productivity is adversely affected by salt stress prevalent in about 30 percent of the cultivated land. For developing salt-tolerant rice varieties through conventional breeding or biotechnological interventions, there is an urgent need to identify natural allelic variations conferring salt tolerance. We screened a large number of these wild rice accessions collected from different agro-climatic regions of India for growth under salt stress. Further, 95 representative accessions were sequenced for members of HKT ion transporter family genes by employing Ion Torrent PGM sequencing platform. Haplotype analysis revealed that specific haplotypes, namely H5 of the *HKT1;5* genes and H1 of the *HKT2;3* were significantly associated with high salinity tolerance. This was the first report of allele mining of eight members of HKT gene family among wild rice germplasm reporting a salt tolerant allele of the *HKT2;3*. The *HKT1;5* gene also showed a salt tolerant allele from wild rice. Phylogenetic analysis based on the nucleotide sequences of these genes showed a different grouping of the HKT family

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genes as compared to the known protein sequence based classification. Different gene families that respond to salinity have been identified in rice, but limited success has been achieved in developing salt-tolerant cultivars. Therefore, 21 salt stress-responsive candidate genes belonging to different gene families were re-sequenced to analyse their genetic variation and association with salt tolerance. The average single nucleotide polymorphism (SNP) density was 16 SNPs per kbp amongst these genes. The identified nucleotide and haplotype diversity showed comparatively higher genetic variation in the transporter family genes. Linkage disequilibrium (LD) analysis showed significant associations of SNPs in *BADH2*, *HsfC1B*, *MIPS1*, *MIPS2*, *MYB2*, *NHX1*, *NHX2*, *NHX3*, *P5CS1*, *P5CS2*, *PIP1*, *SIK1*, *SOS1*, and *SOS2* genes with the salt-tolerant phenotype. A combined analysis of SNPs in the 21 candidate genes and eight other HKT transporter genes produced two separate clusters of tolerant genotypes, carrying unique SNPs in the ion transporter and osmotic adjustment related genes. Haplotype network analysis showed that all the major alleles and few minor alleles were distributed over distant geographical regions. Minor haplotypes may be recently evolved alleles and subsequently migrated to distant geographic regions through human intervention, representing recent expansion of Indian wild rice. The analysis of genetic variation in different gene families identified the relationship between adaptive variations and functional significance of the genes. Introgression of the identified alleles from wild relatives will enhance the salt tolerance and consequently rice production in the salinity-affected areas.

Drought is a serious constraint to rice production globally that can be addressed by deployment of drought tolerant genes. *OsDREB1F*, is a potent drought tolerance transcription activator gene. We sequenced the *OsDREB1F* gene from diverse wild rice collections for allele mining and association study in a set of 136 wild rice accessions and four cultivated rice. This analysis led to identification of 22 SNPs with eight haplotypes based on allelic variations in the accessions used. The nucleotide variation based neutrality tests suggested that the *OsDREB1F* gene has been subjected to purifying selection in the studied set of rice germplasm. Six different *OsDREB1F* protein variants were identified on the basis of translated amino acid residues. Five protein variants were truncated due to deletions in coding region and the genotypes carrying these were found susceptible

to drought stress. Association study revealed that three coding SNPs of this gene were significantly associated with drought tolerance. Three-dimensional homology modeling helped understand the functional significance of this potentially useful allele for drought tolerance in rice. The natural allelic variants mined in the *OsDREB1F* gene can be further used through translational genomics for improving the water use efficiency in rice.

Single nucleotide polymorphism (SNP) is the most abundant DNA sequence variation present in plant genomes. We designed and validated a unique genic-SNP genotyping chip for genetic and evolutionary studies and molecular breeding applications in rice. The chip incorporates 50,051 SNPs from 18,980 different genes spanning 12 rice chromosomes, including 3,710 single-copy genes conserved between wheat and rice (CSCWR), 14,959 single-copy genes unique to rice (SCR), 194 agronomically important cloned rice genes (AGCR) and 117 multi-copy rice genes (MCR). Assays with this 50K SNP chip showed high success rate and reproducibility because of the SC gene based array with no sequence redundancy and cross-hybridization problems. The chip was used successfully to study genetic diversity and phylogenetic relationship of rice cultivar groups and *O. nivara*/*O. rufipogon* wild rice accessions. The origin and domestication of rice has become a subject of considerable debate of late. Rice cultivars have been categorized based on isozymes and DNA markers in to two broad groups, 'Indica' and 'Japonica'. Among other well-known groups of cultivated rice varieties, 'Aus' is closer to Indica and 'Aromatic' including Basmati is closer to Japonica, while deep-water rice varieties belong to both 'Indica' and 'Japonica' cultivar groups. This analysis based on the genome wide high-density genic-SNP haplotype analysis confirmed that the Aus and Indica groups of rice cultivars are nested in their respective wild rice progenitor populations and are more distant from each other than their wild rice progenitor. Similarly, a broad analysis including more wild accessions from the North-Eastern regions of India, South-East Asian countries and Chinese wild rice accessions shows that even the japonica and aromatic groups of rice cultivars have closely related wild rice ancestors in the wild rice accessions from South Asia and may have polyphyletic origin different from Aus and Indica groups of rice cultivars but in the Indian Subcontinent and South-East Asian regions.

We analyzed haplotype networks and phylogenetic relationship in a diverse rice germplasm including a large set of *O. nivara*/*O. rufipogon* wild rice accessions and representative varieties of different cultivar groups, based on red pericarp, grain size and eight starch synthase genes to study the phylogenetic relationship of these genes in wild and cultivated rice. Our results with *RC*, *GS3*, *GBSSI*, *SSSI*, *SSIIa*, *SSIIb*, *SSIIIa*, *SSIIIb*, *SSIVa* and *SSIVb* genes also suggested a polyphyletic origin of the cultivated rice. It further showed that *O. nivara*/*O. rufipogon* accessions from different eco-geographical regions of India have the ancestral haplotypes of all the ten genes and in most cases the alleles in the rice cultivars were shared by wild rice accessions which could be their probable progenitors. Our study shows a complex pattern of migration of domestication alleles

from wild rice to different rice cultivar groups. These findings will be useful in understanding the domestication history of rice and utilisation of wild rice germplasm in genetic improvement of rice cultivars.

In addition to the evaluation for abiotic stress tolerance and analysis of the origin of cultivated rice, the new cultivated Indian wild rice accessions have also been evaluated for resistance to a range of biotic stresses including, bacterial leaf blight, blast and sheath blight as well as productivity related traits such as fertility restoration, hybrid vigour and improved plant types. The analysis has shown that our wild rice collection is tremendously rich source of genes for these important traits. Efforts are now on for the introgression of these novel genes in to cultivated rice for practical utilisation for enhancing rice productivity and production stability.

## Application of Genomics to Enhance Utilisation of Plant Genetic Resources

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Genomics may greatly enhance utilisation of plant genetic resources (Brozynska *et al.*, 2016). This is especially critical with the prospects of major climate change (Aberton *et al.*, 2016). New cereal genotypes are needed to deliver desirable nutritional and functional characteristics in the environments of the future (Henry *et al.*, 2016). Analysis of wild and domesticated genetic resources may identify new sources of genetic variation for breeding. Genomic analysis of cereal (especially rice and wheat) genetic resources may contribute significantly to global food security. Whole genome sequencing and transcriptome sequencing can contribute to discovery of valuable new genes and alleles. Utilizing plant genetic resources requires analysis of large amounts of genomic data (Rossetto and Henry, 2014).

### Rice

Sequencing of the genomes of wild rice (*Oryza*) populations has revealed new ancestral populations that widen the effective genepool of rice for breeding. These newly identified genetic resources will provide a new source of diversity for use in breeding for disease resistance and tolerance to climate change in rice. These resources and genomics tools facilitate the development of rice genotypes to satisfy the quality requirements of increasingly discerning rice consumers (Anacleto *et al.*, 2015).

#### *The AA genome primary genepool of rice*

The primary genepool of rice includes the wild *Oryza* species with AA genomes that are inter-fertile with domesticated rice. Analysis of the whole chloroplast genome of these species has been used to define their evolutionary relationships (Wambugu *et al.*, 2015). Diverse grain quality attributes may be found in this genepool (Wang *et al.*, 2015). This genepool also provides a source of biotic and abiotic stress tolerance genes for current and future environments.

#### *New sources of genetic diversity for rice*

Recent research has identified large poorly characterised populations of *Oryza* in northern Australia (Brozynska

*et al.*, 2014). Phylogenetic analysis of the AA genome taxa indicates that they are sister groups to the clade including domesticated taxa suggesting that this region may be an important centre of diversity for rice. Reference genome sequences for these taxa have been produced recently as a tool to facilitate analysis of diversity in these wild populations.

### Wheat

Sequencing of the transcriptome of developing seeds of diverse wheat germplasm has revealed genetic diversity that explains differences in carbon assimilation (Rangan *et al.*, 2016), flour yield in milling and bread quality on baking (Furtado *et al.*, 2015). Together these gene discoveries offer a significant opportunity to accelerate the rate of genetic gain in wheat breeding.

#### *Carbon assimilation*

Photosynthesis in the pericarp of wheat proceeds by a C<sub>4</sub> pathway. Genetic variation in grain photosynthesis by this recently defined pathway (Rangan *et al.*, 2016) may explain differences in yield due to re-fixation of carbon respired to support protein and starch biosynthesis during endosperm formation. Selection for enhanced photosynthesis in the grain may result in higher yields in environments limited by harsh conditions during late grain filling. The influence of heat stress on expression of this pathway and variation within the wheat genepool is being studied.

#### *Flour yield*

The yield of flour obtained when wheat is milled is controlled by genes that have been identified by analysis of levels of expression in wheat genotypes differing in flour yield. This discovery will avoid the need to have large amounts of grain to assess flour milling performance and provide tools for early generation selection.

#### *Breadmaking quality*

A highly differentially expressed gene encoding a small sulphur rich protein in wheat endosperm may explain differences in the breadmaking qualities of wheat

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(Furtado *et al.*, 2015). However, this gene may not be important for products such as chapatti. Knowledge of this gene may allow the development of wheat genotypes with acceptable end use quality at much lower grain protein contents improving the nitrogen use efficiency of the crop. The combination of selection for flour yield and bread quality will ensure wheat genotypes can be selected with acceptable grain quality. A modest number of genetic loci may require selection to ensure wheat breeding delivers acceptable end use quality allowing more selection pressure for grain yield.

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## Genome Sequencing to Unlock the Potential of African Indigenous Fruit Tree Species

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Sub-saharan Africa is endowed with several indigenous fruit tree species (IFTs) that were traditionally consumed in the region especially by children. Some of these fruits are rich in various vitamins and minerals (Stadlmayr *et al.*, 2012) important in fighting hidden hunger, a common problem in Sub-Saharan Africa (SSA) where there is over-reliance on staple food diets. Due to their nutritional qualities, some of these tree species e.g. *Tamarindus indica*, *Sclerocarya birrea*, *Adansonia digitata*, and *Dacryodes edulis* have attracted global markets (Rachel, 2015) and hence are potential income earners. However, this potential remains under-utilised. Unlike the tropical fruits from other regions that have undergone selection and improvement and are widely grown on farms, these IFTs continue being sourced from the wild. Being native of the land, IFTs also help in preserving local culture, cuisines, biodiversity and the societal heritage. With the continued forest and natural parklands degradation, the survival of these IFTs is threatened and much worse, the existing intra-species diversity is under peril. Due to social-cultural issues the introduced tropical fruit tree species such as mangoes, pineapple, bananas, avocados and pawpaw account for the largest amount of fruits grown and consumed in SSA; there are minimal attempts to grow and improve the African IFTs. Other factors that have limited the cultivation of African IFTs include the presumed trees' long reproductive cycle (some of the species are said to take about 10-20 years to the first fruiting); lack of suitable planting material, propagation techniques and poor productivity which is linked to lack of investment in African IFTs research. An IFT research geared towards global market development must respond to these issues. There is a need to develop African IFTs cultivars with improved reproductive cycles, propagation techniques, customer preferred fruit characteristics, improved productivity and tolerance to diseases and pests.

### Employing Genomics to Accelerate IFTs Improvement

One of the keys to make the IFTs economically viable so that they can contribute to the economic resilience of the local communities is to make them yield high and better in nutrition and quality and also develop value chains and new markets. The new-age tools like genomics, phenomics, association mapping, etc. can immensely help to improve yield, quality and nutrition of the IFTs. These tools are already being applied to improve world's major food crops and some industrially important tree species such as eucalypts (Silva-Junior *et al.*, 2015; Muller *et al.*, 2015), *Populus trichocarpa* (Geraldes *et al.*, 2013) and oil palm (Singh *et al.*, 2013; Teh *et al.*, 2016).

With genome sequencing of the indigenous African tree species, the generated information can be used to accelerate the breeding of these species to overcome key limitations that have slowed their adoption in farming and utilisation. The African Orphan Crop Consortium (AOCC; <http://africanorphan crops.org/about/>) genome sequencing initiative is founded on such platform. Examples of Important traits for improvement in some of the African IFTs include:

1. *Adansonia digitata*: increased leaf production fruit traits (yield, pulp quality), fast growth
2. *Tamarindus indica*: fruit yield and pulp quality, fast growth, tree architecture
3. *Sclerocarya birrea* (dioecious species): fruit yield and pulp quality, sugar content in fruits, kernel oil, tree size and architecture
4. *Dacryodes edulis* (dioecious species): fruit yield and pulp quality, fast growth

For the dioecious species, genome sequencing can be employed to develop sex-specific markers which can

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be useful in early sex-screening so as ensure the correct female/male ratios and to avoid incurring expenses of raising male-only population.

### Conclusion

Unravelling the genome sequences of the African IFTs lays a foundation to their future improvement and sustainable utilisation of important African fruit diversity before it is lost. Being the custodian of this diversity, African national governments need to play a bigger role in development of such initiatives as the AOCC as well as encouraging the use of the generated genome information in their country's IFT breeding programmes.

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## Genome Analysis of Rice Genetic Resources for the Identification of Novel Genes and Alleles for Biotic Stress Resistance

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Rice (*Oryza sativa* L.) is one of the important crops which is the source of staple food for more than 3 billion people all over the world. Rice is subjected to various biotic and abiotic stresses during different stages of growth and development which directly affect its yield. Among the biotic stresses, various insect pests and diseases such as rice blast, bacterial leaf blight, brown leaf spot and sheath blight are severe constraints for yield of rice plant. Among the biotic stresses rice blast disease caused by *Magnaporthe oryzae* is the most dreaded disease which infects rice plant at all developmental stages affecting mainly leaves, collars, nodes, panicles, shoots and roots. Deployment of resistance genes for the management of rice blast disease is one of the best options available for resource poor farmers (1). The cultivation of different plant species by human beings has lead to the evolution of superior genotypes with improved agronomic traits over a long period. However, owing to their inferior agronomic traits, wild germplasm of most of the cultivated crop plants has been unattended and yet to be exploited for crop improvement. Wild germplasm of rice are 'treasure trove' as they harbour many useful traits, such as tolerance to biotic and abiotic stresses particularly disease resistance. Many important genes have been identified and cloned from wild species of rice. These include resistance genes for rice blast *Pi9(t)* and from *O. minuta*, *Pi-40(t)*, *Pir2-1(t)*, *Pi54rh* and *Pid-A4* from *O. australiensis*, *O. rufipogon*, *O. rhizomatis* and *O. rufipogon* respectively. We cloned *Pi54* (2) blast resistance gene from rice line Tetep and its orthologues *Pi54rh* (3) and *Pi54of* (4) were later cloned from wild species *O. rhizomatis* and *O. officinalis* of rice, respectively. Once genes are cloned and functionally validated, their alleles and orthologues can be cloned and characterised from landraces and wild species of rice using allele mining approach. Allele mining is mainly used to find the novel variant of that gene and identification of signatures within the genes. Single nucleotide polymorphisms (SNPs) are hailed as

the unique identifiers of choice in plant genetic analysis because of their co-dominant inheritance, biallelic nature, chromosome specific location and abundance in the genome. In addition, they are highly amenable to automation and have the ability to reveal hidden polymorphisms giving higher resolution.

In rice, the greatest allelic diversity has been commonly observed in the accessions of *Oryza* spp. Using PCR-based approach, we have analyzed lines of *O. sativa* for mining alleles of three blast resistant genes (*Pita*, *Pi54*, and *Piz(t)*). Allelic variants of the broad spectrum blast resistance gene, *Piz(t)* have been analyzed from 49 rice lines (comprising of Indian local landraces and cultivated varieties) selected after phenotyping across three blast hot-spot regions of the India (5). Nucleotide variations in terms of SNPs (Single Nucleotide Polymorphism) and InDels were higher in the *Piz(t)* locus analyzed in this study. SNPs in the form of transitions were more frequent than the transversions in the *Piz(t)* orthologues. Based on nucleotide polymorphism, 46 haplotypes have been identified, with major haplotypes forming three main haplogroups. Allelic variants of the broad spectrum blast resistance gene, *Pi-ta* have been analyzed from 48 rice lines (local land races and cultivated varieties) selected after phenotyping these lines across three eco-geographical blast hot-spot regions (6). Besides, *Pi-ta* orthologue sequences of 220 rice accessions belonging to wild and cultivated species (*O. rufipogon*, *O. barthii*, *O. glaberrima*, *O. meridionalis*, *O. nivara*, *O. glumepatula* and *O. sativa*) were retrieved from the database were also included in the study for a better *evo-devo* perspective of the diversity present in the gene and the selection pressures acting on this locus. Nucleotide variations (SNPs and InDels) were higher in the intronic region compared to the coding region. Based on nucleotide polymorphism, 64 haplotypes have been identified, with major haplotypes forming eight main haplogroups. The variants for rice blast resistance gene *Pi54* from a panel of 92 rice genotypes were also prospected (7).

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The *Pi54* alleles of landraces harbour substantially higher polymorphism as compared to the *Pi54* alleles of cultivated species, because of heterogenous nature of land races. Among the *Pi54* alleles of *indica* and *japonica* species the diversity was low in the alleles of *japonica* species. In the haplotype network, the 50 identified haplotypes were clustered in five major haplogroups and the rest as minor haplogroups. Based on indels and SNPs allele based markers were designed for their use in rice improvement programme using marker assisted selection.

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## Towards the Third Report on the State of the World's Plant Genetic Resources for Food and Agriculture

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Plant genetic resources for food and agriculture (PGRFA) form the biological basis of world food security and directly or indirectly support the livelihoods of every person on Earth. Ever since hunter-gatherers realized some 12,000 years ago that they could save and plant seeds from season to season, the sum of the world's plant genetic resources for food and agriculture has been expanding. Over the millennia, farmers learned to save seeds from the crops they deemed easiest to process or store, those most likely to survive growing seasons or even those that simply tasted the best. More than 7,000 species of plants have been cultivated for food and agricultural purposes. Many remain important to the food security of local communities. However, it is estimated that only 30 crops now provide 95 percent of human food-energy needs and just five of them – rice, wheat, maize, millet and sorghum – provide about 60 percent. Given the significance of this relatively small group of crop species to global food security, it is of pivotal importance to conserve the diversity within them – which is often immense. The number of distinct varieties of the rice species *Oryza sativa* is estimated to be more than 100 000. Farm communities in the Andes cultivate more than 175 locally named potato varieties. It is this within-species diversity that allows crops to be cultivated in a range of different regions, in different climates and in different types of soil.

### The State of the World's Plant Genetic Resources for Food and Agriculture

*The State of the World's Plant Genetic Resources for Food and Agriculture*<sup>1</sup> (First Report), presented in 1996 by FAO to the Fourth International Technical Conference on Plant Genetic Resources (ITC), was the first comprehensive worldwide assessment of the state

of conservation and use of PGRFA. Prepared from 158 country reports and with the input of numerous scientists and other specialists, the report identified gaps and constraints in national and international efforts to safeguard PGRFA and to use them to ensure food security and sustainability of agriculture. The Fourth International Technical Conference on Plant Genetic Resources welcomed the First Report and adopted, in response to its findings, a "Leipzig Declaration" and the rolling Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture, a policy framework that, together with the periodic assessment of the state of the world's plant genetic resources, forms part of the a global information system and is recognized as "supporting component" of the International Treaty on Plant Genetic Resources for Food and Agriculture (Treaty).

12 years later, a new assessment, *The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture*, was prepared (Second Report). It was presented to the Commission on Genetic Resources for Food and Agriculture (Commission) and its Intergovernmental Technical Working Group on Plant Genetic Resources for Food and Agriculture (Working Group), for their review, and published in all UN official languages in 2010/2011.<sup>2</sup> While focusing on changes and developments that occurred since 1996 and resulting gaps and needs, the Second Report provides an updated assessment of the status and trends of PGRFA. The Commission endorsed the report as "the authoritative assessment of this sector".

### Global Plan of Action

Both reports generated global policy responses. In response to the findings of the First Report, the

<sup>1</sup> <ftp://ftp.fao.org/docrep/fao/meeting/015/w7324e.pdf>.

<sup>2</sup> <http://www.fao.org/docrep/013/i1500e/i1500e00.htm>.

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Commission negotiated the rolling *Global Plan of Action on the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture* (GPA), which was adopted by 150 countries at the ITC in 1996. In response to the Second Report the Commission revised the GPA. In November 2011, the FAO Council, on behalf of the FAO Conference, adopted the *Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture* (Second GPA), a revised GPA with updated priorities for the conservation and sustainable use of PGRFA.<sup>3</sup>

The Second GPA is a framework, guide and catalyst for action at national, regional and international levels to create an efficient system for the conservation and sustainable use of PGRFA, including seed systems. It provides a comprehensive and flexible tool for countries to adopt policies and programmes for the conservation and sustainable management of PGRFA, and calls for strengthening capacities and linkages among all stakeholders through a combination of appropriate policies, use of scientific information, farmers' knowledge and joint action. Updating the rolling Global Plan of Action also strengthens the implementation of the Treaty to which the GPA contributes as a supporting component.<sup>4</sup>

### Monitoring the implementation of the Second GPA

Following the adoption of the Second GPA,<sup>5</sup> a new framework for monitoring the implementation of the 18 priority activities of the Second GPA through a country-led participatory process has been set up. The monitoring framework is based on a Reporting Format,<sup>6</sup> a questionnaire which serves to collect the information needed to elaborate 63 indicators and 3 Higher-order Composite Indices, as adopted by the Commission.<sup>7,8</sup>

With the aim to facilitate periodic reporting by countries on their efforts to implement the Second GPA, FAO launched in 2015 the Reporting System for the Second GPA, a web based system accessible through

the World Information and Early Warning System on PGRFA (WIEW)<sup>9</sup> by officially nominated National Focal Points (NFPs)<sup>10</sup> and stakeholders.

In addition to responding to the Reporting Format which is based on the 63 agreed indicators, NFPs also provide an expert judgement on the level of achievement for each of the indicators. The NFP expert judgements are used to elaborate Higher-order Composite Indices (HCIs) for each of the three PGRFA targets adopted by the Commission:

#### Target 1–PGRFA Conservation

By 2020, an increasing proportion of the genetic diversity of cultivated plants and their wild relatives, as well as of wild food plant species is maintained in situ, on farm and ex situ in a complementary manner;

#### Target 2–PGRFA Sustainable Use

By 2020, there has been an increased use of plant genetic resources for food and agriculture to improve sustainable crop production intensification and livelihoods while reducing genetic vulnerability of crops and cropping systems; and

#### Target 3–PGRFA Institutional and Human Capacities

By 2020, many more people are aware of the values of plant genetic resources for food and agriculture and institutional and human capacities are strengthened to conserve and use them sustainably while minimizing genetic erosion and safeguarding their genetic diversity.

Data reported by NFPs and international organizations are used to produce an assessment of the implementation of the Second GPA.

A first assessment of the implementation of the Second GPA, still based on a limited number of country reports, was presented recently on the occasion of the Eighth Session of the Working Group.<sup>11</sup> The Working Group took note of the results of the assessment and recognized the potential of the monitoring framework and the Higher-order Composite Indices for providing

3 CL 143/REP, paragraph 43.

4 International Treaty on PGRFA, Article 14.

5 <http://www.fao.org/docrep/015/i2624e/i2624e00.pdf>.

6 CGRFA-15/15/Inf.9

7 CGRFA-14/13/Report, Appendix C.

8 Background Study Paper No. 67. Higher-Order Composite Indices for Plant Genetic Resources for Food and Agriculture Targets.

9 <http://www.fao.org/wiews/en/>.

10 <http://www.fao.org/agriculture/crops/thematic-sitemap/theme/seeds-pgr/gpa/national-focal-points/en/>.

11 CGRFA/WG-PGR-8/16/Inf.1 Assessment of the implementation of the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture 2012–2014

a synthetic view of the global status of the conservation and use of PGRFA. The Working Group also expressed concern about the high number of accessions due for regeneration but without the necessary budget coverage and recommended that the Commission continue monitoring the issue closely. With the monitoring framework the Commission will be in a position to review the global status of PGRFA on a regular basis and to target action and new initiatives on actual gaps and needs. It is, of course, essential that countries and other stakeholders report regularly and take action, as appropriate, in response to Commission instruments and recommendations.

### Preparation of the Third Report

In 2011, the Commission agreed, through its Multi-Year Programme of Work, that a Third Report on the State of the World's PGRFA (Third Report) be prepared and that the monitoring of the implementation of the Second GPA be fully integrated with the preparatory process for the Third Report.

The proposed timeline for the preparation of the Third Report, as reviewed and revised by the Working Group, at its last session, is as follows:

The deadline for countries to comply with the first round of reporting, covering activities undertaken to implement the Second GPA between January 2012 and June 2014, will be extended until the end of 2017. Through the on-line WIEWS Reporting System for the Second GPA, countries that have not yet done so will be able to complete the Reporting Format and provide NFP ratings on the level of achievement of the Second GPA indicators.

Guidelines for the preparation of country reports contributing to the Third Report will be made available in all UN languages by FAO, following their review by the Working Group in 2018 and their final endorsement by the Commission in 2019.

The second round of reporting, covering activities undertaken between July 2014 and December 2019, is scheduled for 2020. During this year countries will also be asked to produce a synthetic Country Report on the State of PGRFA following the guidelines endorsed by the Commission.

A draft Third Report will be presented to the Eleventh Session of the Working Group in 2022 and

will be presented for endorsement by the Commission to its Nineteenth Regular Session (2023).

The Third Report will follow the structure of the Second GPA and will thus be quite different from previous reports.

The introductory chapter will provide the context for the Third Report. It will present a critical review of relevant developments of global, regional, and national importance that impact on the management of PGRFA. This chapter will also discuss emerging challenges and opportunities including science and technology improvements, intellectual property rights regimes, public-private partnerships, the roles of civil society that may have evolved since the Second Report will be treated. The introduction will also include a summary of the most relevant policies and a section on genetic erosion and vulnerability of PGRFA.

The second chapter will focus on *in situ*, on-farm and *ex situ* conservation and explore how and to what extent the different forms of conservation may better complement and support each other. The status of crop wild relatives, wild food plants and landraces will be analysed based on an overview of the current level of conservation, use and erosion with an emphasis on the gaps and needs going forward.

The third chapter of the Third Report will cover sustainable use of PGRFA and will document the potential of PGRFA as actually harnessed and made available to farmers. Country-level information on plant breeding capacity, formal and informal seed systems and the status of crop diversification and neglected and underutilized species will give an important picture of recent achievements in the use and deployment of PGRFA and of the level of PGRFA vulnerability.

The fourth and last chapter of the Third Report will address institutional and human capacities for PGRFA conservation and sustainable use. The chapter will focus on the roles of national policies, legislation, economics, infrastructure, education, etc. for the management of PGRFA.

### Towards Monitoring the Implementation of SDG Target 2.5

The new framework for monitoring the implementation of Second GPA will allow FAO to assist countries in monitoring activities contributing to the implementation

of Sustainable Development Goal (SDG) target 2.5.<sup>12</sup> As a custodian of the related indicator, FAO will be responsible for collecting and analysing data, contributing to annual SDG progress reports and for establishing

partnerships with other international agencies and relevant stakeholders that support country implementation of the SDGs.

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<sup>12</sup> **Goal 2.** End hunger, achieve food security and improved nutrition and promote sustainable agriculture; **Target 2.5:** By 2020, maintain the genetic diversity of seeds, cultivated plants and farmed and domesticated animals and their related wild species, including through soundly managed and diversified seed and plant banks at the national, regional and international levels, and promote access to and fair and equitable sharing of benefits arising from the utilization of genetic resources and associated traditional knowledge, as internationally agreed.

## Digital Technologies to Modernize Effective and Efficient Use of Plant Genetic Resources

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The genetic diversity of crop plants and their relatives is the raw material of plant breeding. Since the beginning of agriculture, farmers have attempted to identify and use the available variants to their advantage. More recently, plant breeders have identified and recombined useful variants to produce improved landraces and cultivars. Over the past 20 years, a series of technical innovations have opened opportunities to improve the efficiency and effectiveness of plant breeding programmes. Commercial seed companies in developed countries and in some developing countries have adopted these innovations. This paper will discuss some of these opportunities and obstacles to their deployment in the context of developing countries. The presentation will be illustrated by examples from projects supported by ACIAR.

### **From phenotype to genotype: cheaper, faster, whole genome, DNA profiling**

DNA analysis technologies developed in the 1990's such as microarrays dramatically increased the throughput and reduced the costs of identifying and scoring genetic variants (1). For the first time it became practical to analyse the whole genome rather than a small set of genetic markers. Single Nucleotide Polymorphism chips assaying in parallel 9,000 to 90,000 markers are now widely used (2). Improvement in cost and throughput of DNA sequencing is now making it one method of choice to identify and score genetic variants. For species with large genomes, genotyping by sequencing (3), where a reproducible fraction of the genome – 1 to 10% - is sequenced, allows to determine marker profiles with a density of thousands to tens of thousands markers. The process costs about 5,000 USD for 96 samples. For species with small genomes such as rice, whole genome sequencing can be used. Prompted by technological development, genetic data production services have become available commercially. For most breeding programmes, it is now more cost-effective to outsource high density data production to commercial facilities,

than to produce marker data in house. Data quality from commercial facilities is also higher.

The characterisation of the whole genome using high density marker profiles is now facilitating the use of agrobiodiversity in breeding. Traits of interest can be mapped in wild relatives, in landraces, or in elite cultivars and markers then used to transfer the traits into elite varieties (4).

### **Accurate phenotyping: electronic field data capture, electronic experiment management**

The effectiveness of a breeding programme relies on its ability to generate high quality phenotypic data. The use of drones and other high throughput measurement methods increase the demand for rigorous management of field trials and experiments. Electronic tools to manage experiments and to capture field data are available, and help to reduce the error rate. A common source of error is the loss of accurate sample tracking. The use of barcodes to label plants and experimental plots, while not a complex technological innovation, reduces sample tracking errors. Handheld devices (smartphones and tablets) able to read barcodes and record field phenotypic data have now become affordable for most breeding programmes. These tools increase the productivity of staff and make their job more enjoyable. Larger experiments become possible, thereby increasing the potential genetic gain. Examples of Applications specifically developed for capturing plant phenotypic data on handheld devices include FieldScorer (5) and KDSmart (6).

### **Using data productively: data storage, management and analysis**

While data can now be acquired or purchased more efficiently, their effective application requires a dedicated data management system. These systems need to respond specifically to breeders needs and be easy to use so that breeders adopt them readily. Their key functionalities include managing:

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- Lines, seed stocks, pedigrees, families;
- Field experiments;
- A large range of phenotypic data;
- Ideally genotypic (genetic marker) data: this is proving to be challenging.

Systems should be able to relate the various types of data and to interface seamlessly with data analysis tools for example: to map Quantitative Trait Loci; to identify genomic regions and markers associated with specific traits; to assist in the design of breeding strategies; or to decide which parents to cross. Several systems available to diverse breeding organisations have been designed over the past 15 years, each with their own strengths and limitations. Examples include the Breeding Management System of the Integrated Breeding Platform (7) initially developed under the Generation Challenge Programme initiative of the CGIAR, the commercial software Agrobase from Canadian company Agronomix (8), or KDDArT from Australian company Diversity Arrays Technology Pty Limited (9). The International Rice Research Institute has been developing its own system to manage rice breeding. The hardware required to use these systems has become affordable and with the development of cloud computing and the increasing availability of internet connectivity, many breeding programmes in developing countries can now use a breeding information management system. A would-be user of these systems should review their performance and their suitability for the specific purpose and context of the organisation. This presentation does not present an exhaustive view of available systems.

One institutional advantage of adopting a breeding information management system is to provide a vehicle for effective cooperation between breeders, pre-breeders and the gene banks. Within one organisation, it also ensures better collective memory of experiments, materials and results. A well-managed system reduces the risk of losing information through natural disasters. Adoption of data management systems will have a profound influence on how breeding programmes run.

Cooperation between breeding organisations will be facilitated if the systems they use can communicate and exchange information. Since multiple systems are currently used, future development will need to ensure their ability to exchange information.

### **Modelling the Genotype by Environment by Management Interactions to Breed Better Varieties**

The use of plant and crop simulation is another area where digital tools are impacting plant breeding. Models such as APSIM (10, 11) are parameterised using high quality environment data (soil and climate) and crop performance measured under various environments and management strategies. Once properly established, models can simulate the effect of the new traits introduced by breeders, for example different maturity dates, different timing of using water, tolerance to high temperature, etc. (12). The increased sophistication of the models allows for predictions in the various environments so that breeders can estimate the value of specific traits in specific environments, before starting the breeding project. Integrating molecular genetic data, models can also guide parent selection before crosses are initiated (13).

### **From Genotype to Phenotype: Novel Approaches to Breeding**

Similarly to the trend in other areas of research (for example in clinical research or economic research), the high density plant genotypic and phenotypic data now available lends itself to analysis using machine learning-based algorithms. Discoveries and inferences can be made with limited prior hypothesis or knowledge. Using Genomic Selection (14), experimental validation in the field can then be focused and more efficient. The phenotypic data collected limit the scope of this approach. No doubt the eye of the breeder in the field will remain an important tool to detect and exploit unexpected variation!

### **Modernising Breeding Programmes**

Most of the technologies discussed here can increase the performance of breeding programmes in developing countries. Their costs are manageable, but their adoption may be limited by institutional obstacles and the lack of awareness, knowledge and skills in many plant breeders. Building institutional capacity to consider, and then to adopt, innovation is therefore a critical first step.

One limitation is the ability of breeding programmes to acquire goods and services, especially when it involves the use of foreign currency. Even when data produced commercially is of better quality, higher utility and lower

cost than data produced in-house, some institutions will have difficulty accessing the small amount of foreign currency required. To benefit fully from innovations, breeding programmes will need reliable access to some high quality consumables (for example barcode labels and barcode printers, good quality seed bags), regular software maintenance and upgrades, and genotyping services.

Another limitation is the requirement for increased communication and cooperation between members of a breeding team: breeding is becoming more multidisciplinary. Successful programmes will integrate the contribution of multiple experts and will use these new tools to design the breeding programme itself. This will require institutional evolution in managing human resources, updating reward systems for individual performance, and recognising the contribution of multiple actors to the successes. Metrics of success may need to be updated: genetic gain of released varieties rather than number of releases needs to be considered. Compared to the traditional situation of a lone breeder responsible and recognised for the release of an improved variety, this may prove a challenge for some organisations.

## Conclusion

While the technical innovations discussed above can increase the performance of breeding programmes, they are not sufficient to guarantee impact. Beyond technological innovations, impactful breeding programmes respond to clearly articulated demands from farmers. Defining the farmers' demands and needs, and the corresponding traits that improved varieties must have, remains one of the most demanding task of a plant breeder, and the most critical to ensure impact in the field. The impact pathway of an improved variety is also critically dependent on an effective seed system to make the improved varieties available to farmers. The impact pathway also requires an effective communication or extension system to make the farmers aware of the new varieties. Responsiveness to farmers' demand, ability to supply the seeds of improved varieties, and effective

dissemination of the relevant information are challenges that have to be met for breeding organisations to fully capture the benefits of agrobiodiversity in the improved varieties they release.

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## Genetic Improvement of Production and Adaptive Traits in Livestock

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### State of Genomics Research in Livestock

In the previous century, technological advances like artificial insemination (Foote, 2002) and best linear unbiased prediction (Henderson, 1975) drove dramatic improvements in livestock species. For example, milk production has more than doubled (Abdallah and McDaniell, 2000). These genetic advancements have helped world farmers to generate more food more efficiently to feed the global population. However, to continue this trend additional improvements must be made.

Since the turn of the century, we have witnessed some dramatic changes in genetic improvement of livestock species. The advent of having access to a sequenced/assembled genome for species of interest has fueled these advancements (Elsik *et al.*, 2009; Groenen *et al.*, 2012; ICGS, 2004). The second major advancement was the development of genomic selection technologies (Meuwissen *et al.*, 2001). Finally, genome editing has great potential to alter what is possible (Kim, 2016).

For many species, their genome has been assembled and annotated – Chicken (ICGS, 2004), Cattle (Elsik *et al.*, 2009) and Swine (Groenen *et al.*, 2012). Technologies have been developed to genotype 10's of thousand of variants in thousands of individuals – SNPchips. Because of reference genomes and advances in sequencing technology, variant discovery, primarily single nucleotide variants and small insertions/deletions, is relatively easy. For example, the 1000 bulls project has identified and analyzed more than 25 million variants in cattle for association with traits and a similar project in sheep identified more than 46 million variants. These projects have just begun to identify the genetic variation that exists in livestock species.

For many years, marker assisted selection was practiced, but it wasn't until Meuwissen, Hayes and Goddard (Meuwissen *et al.*, 2001) proposed genomic selection that real progress in genetic selection using

molecular markers was even possible. Today, genomic selection is being practiced in all livestock species (Meuwissen *et al.*, 2013). The dairy industry has aggressively implemented genomic selection, with more than 1 million head of Holstein being genotyped in the United States. The dairy industry has gone as far as performing ovum pickup in pre-pubertal heifers, and genotyping *in vitro* fertilized embryos, to decide what embryos to implant in recipients. Genomic selection has dramatically decreased the generation interval in dairy cattle. Another leap forward was with the implementation of single-step GBLUP (Misztal *et al.*, 2009), in which genetic evaluation could utilise both genotyped and un-genotyped individuals in a single analysis. It was then possible to harness the power of both genomic selection and traditional quantitative genetic evaluation. Today, phenotypic not genotypic information is the rate limiting step to genetic progress. The genomic tools are in place. Now advancements in phenotype collection need to occur to fully utilise the genetic potential of livestock.

### On-going Research Projects

The genome sequencing projects brought livestock research communities together on a scale never seen before. They ushered in the era of large collaborative multi-disciplinary research projects designed to tackle a problem larger than what one research lab could undertake. These projects have become to some extent the new norm. While there are more projects than can be summarized here, a couple projects will be discussed to high-light some unique aspects that they bring to the community.

The 1000 Bulls project (citation; <http://www.1000bullgenomes.com/>) (Daetwylwe *et al.*, 2014) is the largest collaborative genome re-sequencing project in livestock. Researchers from around the world have re-sequenced more than 1,100 bulls. Variants were discovered in each of these individuals using

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a common variant detection pipeline. Run4 data has been imputed into more than 58,000 head and has been used in the analysis of stature (hip height) (unpublished results). Pooling of resources from across the world has allowed a project of this scope to be conducted. Project members have access to genotype information on all of the re-sequenced individuals to date. This provided an incredibly powerful resource for future research.

The Functional Annotation of Animal Genomes project (FAANG; <http://faang.org/>; (Tuggle *et al.*, 2016; Andersson *et al.*, 2015) aims to identify all functional elements in animal genomes. This project seeks to expand upon the human ENCODE (Consortium, 2012) and modENCODE (Celniker *et al.*, 2009) projects by identifying functional elements throughout. More than 300 researchers world-wide are working to set standards for sample collection, metadata, assays, bioinformatic pipelines, and data integration. It is expected that this project will have positive benefits on annotation of genomes, understanding gene function and predictive biology, which will continue to grow over time. This is an example of pre-competitive research that will have a powerful impact on biology.

The ADAPTmap project (<http://www.goatadaptmap.org/>) is an international effort to coordinate independent genomics based projects on goats. It is interesting in that it involves coordination of projects ranging from genomic selection for improved milk production on commercial populations to surveying of genetic diversity throughout the world. A goal of the project is to disentangle the genetics of adaption of goats throughout the world.

These projects and others like them will have a positive impact on both scientific knowledge and in meeting the challenge of feeding the world in the future.

### **Traits of Interest**

Traditionally, genetic improvement has focused on easy to measure traits that have an economic impact on livestock, e.g. yearling weight in beef, number of eggs laid in chicken, milk yield in dairy cattle, growth rate in swine to name a few. The impact of genetics on these production traits cannot be under estimated, for what has occurred in nothing short of incredible. Furthermore, these production traits will continue to be selected on well into the future.

Unfortunately, elite genetics do not fair well in many parts of the world. Selection for traits of economic importance has adapted these animals to their environment. In order to thrive in other environments other adaptive traits need to be selected upon. Disease resistance is a trait that could have a dramatic impact upon livestock production world-wide. However, disease resistance/susceptibility traits are not easy to measure. What constitutes a sick animal? How do you objectively measure incidence and severity? New technologies are needed to drive genetic progress in these traits. Unfortunately, the heritability of many disease resistance trait is low, which means that selection for improvement will be slow as well. Furthermore, the number of livestock diseases that we know about continues to increase. Taken together, disease resistance is a topic in need to increased emphasis.

Climatic stress is one of the main limiting factors of production efficiency in many areas of the world and is expected to become even more of a limitation due to climate changes associated with global warming. Substantial differences in thermal tolerance exist among breeds and among animals within breeds indicative of opportunities to improve it by selection. However, many of the physiological pathways underlying thermotolerance are interconnected with metabolic changes and nutrient partitioning resulting in suboptimal productivity. Unfortunately, this is a trait that we do not fully understand the metabolic/physiological mechanisms at play, which makes it hard to know whether the right phenotypic data is being collected. We have room for a lot of improvement in this trait.

There are a myriad of other traits, e.g. feed efficiency, green house gas production, reproduction to name only a few, that the livestock industry needs to select for improvement. Many of these traits are hard to measure and as a result are costly to measure. Thus, there is a real need to invest in development of new measurement technologies to take what is hard and expensive to measure today to become cost-effective in the future.

### **Limitations to Genetic Progress and Conservation of Genetic Diversity**

In many parts of the world, the basic infrastructure needed for genetic progress just does not exist. In many places, it is not possible to measure a trait of interest (e.g. no scale to collect an animal weight or no way to cost effectively measure something like methane

production), record it (i.e. there is no organized data recording site), and then use it in a genetic evaluation (i.e. there is no organized genetic analysis provider; e.g. a breed association). This all starts at the level of the producer. There needs to be a basic understanding of the economic benefit of genetic selection. Someone can invest all they want into the newest latest genomic technology, but unless a strong foundation for genetic improvement is in place, real genetic progress throughout the country will not take place. This is a fundamental difference between livestock and plant species. Genetic improvement of plant species can be accomplished in limited locations and then improved seeds can be distributed and rapidly deployed. In livestock, hundreds of thousands of producers own the genetic resources. A large percentage of them need to be actively involved for genetic progress to occur. Generally in livestock, genetic improvement has been implemented in larger operations first. Then as the technology costs drop due to volume application, smaller and smaller operations can benefit. Smaller producer will be able to benefit from the ability to have access to genetically superior animals that they can breed their animals too.

The tools exist today in some species like cattle, pigs, sheep, goat and chicken to utilise genomic technologies, i.e. SNPchips, for conservation of genetic diversity. Most of these resources have been used to large surveys of the genetic diversity differences between breeds. Once a breed has been characterised, these same SNPchips can be used to minimise the inbreeding within a limited population (Saura *et al.*, 2013). Along with an estimate of the effective population size of the breed, selective breeding can be employed to minimise inbreeding and maximise the potential to conserve the breed. Unfortunately, this process is expensive requiring a long-term investment in the conservation of that breed. Thus, a well thought out breeding goal and plan need to be in place to maximise the return on investment.

## Conclusions

Many livestock species have a wealth of genomic and quantitative genetic tools that can be used for genetic improvement or conservation of genetic diversity. Therefore, it is possible to accomplish most breeding goals/plans if sufficient resources or economic incentives are in place to drive them forward.

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## Molecular Traceability of Spatial Genetic Diversity for Sustainability of Fish Genetic Resources: A Perspective for India

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Sustainability of aquaculture sector is considered pivotal for future nutritional security, to produce 40 million ton required from 2030 onwards. The management and improvement of fish genetic resources, holds the key to enhance production while overcoming the constraints of finite resources. In this context, it becomes imperative that the genetic variability in natural populations of aquaculture species is documented so as to plan its use in domestication, genetic improvement and for conservation of the natural genepool. A population of a species is composed of genetic stocks, which are locally evolving units and develop attributes of adaptive significance to the diversified local environments. Such intraspecific variability in the species can be a source of useful characters with potential application in domestication and genetic improvement of the species. The inadequate knowledge on genetic stocks of cultivable species of fishes is a major constraint in blue growth of aquaculture (CGRFA, 2016) and this is in contrast to the scenario in domesticated animals and plants, where breeds/ varieties etc are well documented. Therefore, bridging this knowledge disparity between fisheries and other agriculture sectors is necessary for implementing common guidelines on issues related to biodiversity, genetic erosion in farmed populations, IPR protection and technological advancements.

This presentation addresses a concept on the use of standardized molecular markers (Masih *et al.*, 2014) as traceability tools, to decipher the distribution and pattern of genetic variability. Such traceability tools can be applied to the wild and farmed populations, for varied objectives. The application of these tools to the wild population will discover the different sub-populations, needing strategies for *in situ* conservation, sourcing of the animals to implement improvement programmes. The known sub-populations can be evaluated, *on-farm*, for evaluation of production performance traits such as growth, reproduction, disease and any other adaptive trait

of economic significance. In a way, this could establish the knowledge of genetic stocks which exhibit variation in their phenotypes and production traits (as breeds in livestock animals).

In the aquatic species, phenotypic variation and production performance is not directly evident and may need indirect methods like scale reading for growth and image analysis for the phenotypes. However, results from such indirect analysis on the performance may be a comparative indicator of phenotypes but possibly not true reflection of their performance under controlled farmed conditions. Molecular markers could be useful to provide direct assessment of genetic divergence and the identified genetic stocks can be assessed for performance in culture. In a study carried under Outreach project on Fish Genetic Stocks, Nuclear (Microsatellite DNA) and partial mitochondrial genes (Cytochrome b and ATPase 6/8) were analyzed for wild populations of Indian carps and catfish and indicated the existence of genetic divergence in wild populations. An example of spatial distribution of genetic variability in wild population of two species, *Labeo rohita* and *Clarias magur* with contrasting biology is presented here. *Labeo rohita*, a carp, is native of rivers in Indo-gangetic region and found and dispersed in fast flowing rivers. *L. rohita* is the icon of Indian polyculture. In the contrast, catfish *C. magur* lives in stagnant puddles and may be dispersed during floods. The *Labeo rohita* (N=925, 19 localities) samples were collected from four different river basins, include Indus, Ganges, Brahmaputra of Indo-gangetic region and Mahanadi river system flowing through peninsular India. For *C. magur* (N=792, 16 localities) were collected from northern, western and peninsular India. In the two respective species, all the three class of markers used, provided similar results. *Labeo rohita* population had low genetic differentiation (>8%) while high genetic differentiation (26%) is seen in the *C. magur* population. The Bayesian analysis on spatial scale did

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not confirm existence of river wise genetic stocks in *L. rohita*, rather admixture of divergent clusters in different rivers was evident. This admixture may be the result of ancestral genotype or gene flow happening in continuous river systems. In *C. magur*, high genetic differentiation was evident and spatially differentiated clusters were existing.

The identified genetic clusters based on standardized molecular markers become appropriate candidates for aquaculture improvement programmes.

On the other hand, the domesticated populations in aquaculture (Indian major carps and shrimp in India), possibly held with small-scale farmers or hatcheries, which could possibly have differentiated genetically from their wild ancestors, need to be identified on spatial scale. Such farmed populations after several generations of domestication might have lost some alleles or acquired new useful alleles. The ignorant management practices or use of unauthenticated broodstock (as considered in shrimp) might cause genetic erosion and high propensity of disease risks. The traceability will help to develop framework of policies and guidelines, with an objective to preserve genetic diversity than eroding it. This will open up new avenues for the preservers of diversity, to trade the genetic products (such as improved seed, broodstock) at national, regional and international level. Trading of genetic products is happening in livestock but not so prevalent in aquaculture yet. However, the spread of *Litopenaeus vannamei* (white leg shrimp), showed that this is possible as broodstock worth 50 million USD was traded from Hawaii, during 2015. This presentation proposes a concept, "The GRA (Genetically Responsible Aquaculture) plan": in this context, with an aim to policy change for preserving aquaculture genetic diversity.

'The GRA plan' is novel and yet not tested, nevertheless, system changes envisaged here, is a necessity for sustainable intensification of aquaculture. The scenario emerging from the Asian shrimp culture in the last two decades, also indicates this lesson. The authors were encouraged to frame this plan with the letters from two shrimp farmer associations in India to NACA (Network of Aquaculture Centers in Asia-Pacific), recognizing that the shrimp post-larvae needs innate fitness, often eroded, when unknown pathogens co-exist in pond culture post SPF (Specific Pathogen Free) stage. The GRA plan will enable formation of farm genetic-exchange networks (Doyle *et al.*, 2015),

novel for aquaculture, but resembling farm associations for terrestrial crops and livestock sectors, that conserve genetic diversity, indefinitely within regional "meta-populations". Misrepresented, diluted and inbred seed is threatening the livelihoods of small-hold aquaculture in Asia-Pacific. Protocols using novel, genetic-marker-based "certificates of authenticity" will be developed to prevent misrepresentation, while opening new global opportunities for small enterprises to use genetic diversity for trading new genetic products and commodities (e.g. certifiably "fair trade").

In this endeavour, three aspects critical for implementation of the plan are:

1. *Traceability Protocols and Genetic Diversity Indices:* Demonstrating that the traceability tools and genetic diversity indices assays, are available, performed easily in routine laboratories and low in expenses, the farming community is likely to accept. Development of tools is critical and can be overcome, though initial molecular research could be intensive. It needs to establish standardized species-specific marker set with the reference levels of genetic standards. This standard marker set protocol can be improvised to accomplish in the existing diagnostic facilities used in shrimp aquaculture. This will enable the wide use of genetic standards assessment tools in seed and broodstock, verifying origin, strategizing breeding plans and securing the fair trade. This can provide rapid appraisal of farm-level inbreeding and regional genetic diversity and help in avoiding using broodstock and stocking seed from inbred sources. This can contribute to a good disease mitigation with genetic fitness and support the existing pathogen control mechanisms like SPF protocols.
2. *Empirical Data on Magnitude of Genetic Erosion, Consequent Production Performance Losses and Linkage with Socio-economic Impact on Small-scale Farm-holds:* This critical information is vital to press for a systematic improvement in aquaculture broodstock and seed management system in developing countries. This will facilitate taking steps for implementing the new procedures as part of GAP standards. The benchmark survey and analysis in this start-up proposal (in selected regions) will give an insight on the genetic erosion and resulting income losses due to underperformance of seed, disease susceptibility and unfair trade practices due to misrepresented seed and broodstock. This will be

linked to socio-economics, impact on woman and children, in farming house-holds.

3. *Information Management for National/Regional Broodstock Improvement Banks or Network:* National Broodstock Bank or Network (NBIN) is a knowledge driven strategy for long-term preservation of on-farm genetic diversity, maintain genetic gains, and establish informed breeding plans and exchange of genetic material. Information management will be a crucial element in developing and sustaining such operational networks. This could be spatially distant farms linked through establishing IT based database, accessible through single nodal point maintained regionally or nationally and provide genetic information of the broodstock available.

### Conclusion

In view of the above, standardized molecular markers for cultivable species are important and need to be established with the genetic indices. This can be used for varied objectives of knowledge development for use

in genetic management of wild relatives and genetic fitness of farmed populations.

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## Genomic Resource Development for Aquaculture Species in India

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India is the second highest cultured global fish producer country and freshwater aquaculture contributes to over 95 percent of the total aquaculture production. It comprises of the culture of carp fishes, catfishes (air breathing and non-air breathing), freshwater prawns, pangasius and tilapia. In addition, in brackishwater sector, the aquaculture includes culture of shrimp varieties, mainly the native giant tiger prawn (*Penaeus monodon*) and exotic whiteleg shrimp (*Penaeus vannamei*). Thus, the production of carp in freshwater and shrimps in brackishwater form the bulk of major areas of aquaculture activity (FAO, 2016, [http://www.fao.org/fishery/countrysector/naso\\_india/en](http://www.fao.org/fishery/countrysector/naso_india/en)). Among the carps, major contribution is by three species of Indian major carps, viz., catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), (contributing about 70-75% ), supplemented by three exotic species viz., silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*). It is followed by exotic Pangas catfish, *Pangasianodon hypophthalmus* (*Pangasius sutchi*), and freshwater prawn (*Macrobrachium rosenbergii*) from freshwater aquaculture and shrimps from coastal farming. Among the brackishwater/marine finfish species, the Asian seabass (*Lates calcarifer*) and cobia (*Rachycentron canadum*) are considered as among the most important candidate species suitable for farming in ponds and cages in brackish and marine water ecosystem. (<http://www.ciba.res.in/index.php/candidate-species>). In addition, important food fishes of cold waters are mahseer and schizothoracids belonging to the indigenous species and trouts among the exotic varieties, contributing around 1% to the food basket.

Over the past 10-15 years, rapid development in the area of agricultural genomics has taken place in India, focussed on innovative genomic applications towards improving the important production and performance traits. Genome research requires the development of a number of resources that facilitate both structural and functional analysis of the genome. For this purpose, wide-ranging genomic tools are now been generated in

fish species. These include large number of molecular markers including genetic sequence polymorphisms, Cell lines, Expressed sequence tags (ESTs), transcriptomes and Gene expression profiles under different conditions, Genetic/ linkage and physical maps and Whole genome sequences resources. This wealth of new genomic resources has caused a paradigm shift in research, by reducing the dependence on the model systems and working directly on the aquaculture species.

### Whole Genome Sequencing and Development of Allied Genomic Resources

Whole genome sequencing of two commercially important fish i.e. *Labeo rohita* (rohu) and *Clarias batrachus* (magur) has been in progress in a network project mode (DBT, New Delhi sponsorship) involving ICAR-NBFGR, ICAR-CIFA, AAU Anand and ICAR-IASRI ([www.nbfgr.res.in](http://www.nbfgr.res.in)). Under this initiative, multi platform whole genome sequencing up to more than 100x coverage in both rohu (genome 1.4 GB) and magur (1 GB) has been accomplished using more than five sequencing platforms and the *de novo* assembly is in progress ([http://mail.nbfgr.res.in/RohuMagur\\_Genome/index.html](http://mail.nbfgr.res.in/RohuMagur_Genome/index.html)). Complete mitogenomes of both the species have been deciphered. A 5x coverage BAC library in magur has been developed. In addition, heterozygous SNPs and SSRs, Repeat elements, genes involved in anti-microbial peptide family and miRNAs mining have been done. A total of 483830 SNVs were identified from the assembled contigs in all the three populations of *C. batrachus*.

Programmes on whole genome sequencing of an the anadromous hilsa shad, *Tenualosa ilisha* and Indian white shrimp, *Penaeus indicus*, have been initiated under the ICAR Consortium Research Platform (CRP) on Genomics in 2015, for structural and functional genomics in these two species with commercial potential to discover variants of genes, from hitherto unexplored germplasm (ICAR, <http://www.icar.org.in/en/node/10866>).

An important genome resource with chromosome level assembly of whole genome sequence of an important

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candidate species, the Asian seabass (*Lates calcarifer*) was generated, which would serve for development of genomic assays for establishing molecular aquaculture in this species as well as related species (Vij *et al.*, 2016).

### Trait Associated Transcriptome Profiling

Transcriptome/EST sequencing can boost genetic and genomic research of target species and genetic mechanism underlying the production and performance traits can be studied. It is also a fast and efficient means for novel genes identification and genetic marker development.

The Indian major carp, *Labeo rohita*, genomic resources for reproduction-research were generated through transcriptome profiles from twelve-tissues from pre-spawning rohu by mRNAseq and identified identified 940 reproduction-related genes (Sahu *et al.*, 2015). In an another study by a collaborative project of ICAR-CIFA and Nofima Ås Norway, transcriptome profiles were used for identification of expressed genes and polymorphisms in lines of rohu selected for resistance or susceptibility to an important bacterial pathogen, *Aeromonas hydrophila* (Robinson *et al.*, 2012).

The mechanism of abiotic stress tolerance has also been studied through transcriptome studies. In Indian catfish, *Clarias magur*, a hypoxia tolerant species, the genomic resources pertaining to molecular processes facilitating its adaptation to hypoxia stress were generated. The analysis of differential expression of transcripts from six tissues suggest that *C. magur* combat low O<sub>2</sub> level by, in part, through transcription factors, altering the expression of a series of genes involved in metabolism, signaling and immune responses, interlinked with other several pathways (Mohindra *et al.*, 2016). In the hypoxia susceptible fish, *Catla catla*, oxidative stress conditions were defined (Singh *et al.*, 2016) and the important role of TLRs and NOD receptors signaling pathway in sterile inflammation and pathobiology of fish in hypoxic stress was observed and thus their role in abiotic stress management in aquaculture (Basu *et al.*, 2016). Differentially expressed genes from shrimp (*Penaeus monodon*) in response to low and high salinity stress were also identified through EST analysis (Shekhar *et al.*, 2013, 2014). Transcriptional response to heat shock in liver of snow trout (*Schizothorax richardsonii*) through deep RNA sequencing characterised the genes and molecular pathways involved in heat shock response (Barat *et al.*, 2016).

The black tiger shrimp, *Penaeus monodon*, is the second-most widely cultured prawn species in the world.. However, disease and poor reproductive maturation in captivity is one of the serious threats to sustainability of the shrimp farming industry. The information on host immune gene response to WSSV pathogenesis was studied by Shekhar *et al.* (2015) through microarray analysis with Shrimp cDNAs of 40,059 unique sequences. It revealed the altered expression of numerous genes represented diverse functions such as immune response, osmoregulation, apoptosis, nucleic acid binding, energy and metabolism, signal transduction, stress response and molting (Shekhar *et al.*, 2015). Another study by Soonthornchai W (2016) to understand the immune responses in the stomach of black tiger shrimp (*Penaeus monodon*) during acute Hepatopancreatic Necrosis Disease (AHPND), caused by a pathogenic strain of *Vibrio parahaemolyticus*, differentially expressed transcripts (DETs) in the stomach during strain 3HP infection was examined using Ion Torrent sequencing.

### Genetic/Linkage Map and Mapping QTL for the Traits

Linkage map with wide marker coverage is an essential resource for genetic improvement study for any species. Genetic linkage map of *Labeo rohita* was developed by placing 68 microsatellite markers (Sahoo *et al.*, 2015) spanning a sex-averaged total length of 1462.2 cM, in 25 linkage groups. A linkage map of 3193 transcribed single nucleotide polymorphisms in rohu was constructed (Robinson *et al.*, 2014) and QTL associated with resistance to *Aeromonas hydrophila*, with 21 SNPs mapping to ten linkage groups showed significant associations with the traits hours of survival and dead or alive.

To scan the *P. monodon* genome for loci associated with resistance to white-spot syndrome virus and markers associated with sex determination, a high density linkage map for black tiger shrimp (*Penaeus monodon*) was developed under a collaborative project of ICAR-CIBA and Nofima Ås Norway, based on genotyping array containing 6,000 cSNPs (Baranski *et al.*, 2014) and a total of 3959 SNPs were mapped to 44 linkage groups. Linkage groups were identified to contain QTLs significantly associated with hours of survival after white spot syndrome virus infection and SNPs significantly associated with sex (Baranski *et al.*, 2014).

## Mitogenomes

Mitochondrial genome sequences have been extensively used for population genetics/genomics, evolutionary and phylogenetic studies and mitogenomes have been sequenced for *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Labeo calbasu*, *Labeo gonius*, *L. frimbriatus*, *Clarias magur* (earlier name *C. batrachus*), *Channa marulius*, *Chitala chitala*, *Pangasius pangasius*, *Puntius denisonii*, *P. chalakkudiensis*, *Schizothorax richardsonii*, *Tor putitora*, *Tor tor* and many other species.

## Development of Genomic Resources for Utilisation in Genetic Differentiation of Wild Fish Populations

Development of genetic/genomic resources for utilisation in estimation of genetic variation, genetic differentiation and population structure for wild populations of aquaculture as well as potential aquaculture fish species have been studied at ICAR-NBFGR and other ICAR fisheries institutes using molecular markers including mitochondrial sequences and SSR (gene associated as well as anonymous) markers across the natural range of distribution in Indian rivers. These species include *Tor putitora*, *Tor tor*, *Catla catla*, *Cirrhinus mrigala*, *Labeo rohita*, *L. calbasu*, *L. dero*, *L. dyocheilus*, *L. dussumieri*, *Clarias batrachus*, *Chitala chitala*, *Tenualosa ilisha*, *Puntius denisonii*, *Horabagrus brachysoma*, *Gonoproktopterus curmuca*, *Channa marulius*, *Macrobrachium rosenbergii* and *Etroplus suratensis*. Using the genomics tools as well as the morphological characters, attempts are made to study the phylogeography and delineate evolutionary significant units or genetic stocks existing in natural population.

## Genomic Resource Databases

The generation of vast amounts of biological data has fuelled the need for computational systems and techniques to manage and analyse such data, which has led to the development of bioinformatics tools. The concept of public accessibility of data ('open data') is generally adopted for all genomics projects and most genomics data are posted online in publically accessible databases. Genomic resource Databases under the Scheme 'Centre for Agricultural Bioinformatics: Fisheries Domain' at ICAR-NBFGR, Lucknow have been prepared and major ones are Fish Karyome, The Fish Barcode Information System (FBIS) for Indian fishes; Fish and Shellfish

Microsatellite Database for Indian Fishes and Fish Mitogenome Resource (FMiR).

## National Repository of Fish Cell Lines (NRFC)

NRFC is located at ICAR-NBFGR, Lucknow, which was established with the financial support from Department of Biotechnology, Govt. of India, New Delhi. NRFC aims to receive, authenticate, store and supply the cell lines to the scientific community for R & D work. NRFC also aims to provide support for training and education to stake holders and to serve as a National Referral Centre of Indian and exotic fish cell lines in the country. At present, 50 fish cell lines are being held at NRFC ([www.nbfgr.res.in](http://www.nbfgr.res.in)).

## Future Perspective

The vast information being generated through the aquaculture fish genome projects, in the form of a vast array of structural and functional genomics data, would form a baseline data for further research on genetic improvement for production and performance traits, including fish health management. Identification of genetic markers surrounding quantitative trait loci (QTL) forms the basis for MAS. These molecular markers can help in genetic management of the species to optimize the production. Genomics can help to overcome problems related to infectious diseases by better understanding host defence systems and identifying QTL or candidate genes. Further, the use of SNPs associated with disease/immunity related traits in aquaculture species can facilitate the selection of fish with superior genetic material as well as a better understanding of host-pathogens interaction and disease resistance. This genome information may uncover a mine of genetic information in the form of gene and regulatory sequences, SNPs, STRs etc., which can further be used for documenting genetic variability and identifying DNA regions for early and efficient selection.

In aquaculture, with the passage of time, significance of wild fish genetic resources is enhanced, as sources of genetic diversity for use in fish breeding programmes and related research of fish genomics technologies. The genetic diversity is needed for meeting the future challenges of climate change, new diseases or further improving the production traits. Thus for future sustainability and profitability of aquaculture, it is important to characterise and conserve the wild genetic resources.

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## Insect Genetic Resources–Innovations in Utilisation

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Insects can be a problem or an opportunity. Though the proportion of useful and harmful insects is low within the class Insecta, it is now widely acknowledged that interactions in the insect community impact the density and diversity of economically important insects. Generally the emphasis is on economically important insects as they visibly and directly influence mankind, while the number of agriculturally important insects that need to be documented far outweighs the mere quantum of economically important insects. Insect biodiversity is instrumentally important not only for the production of food, but for other ecological services as well, including the recycling of nutrients, regulation of microclimate and suppression of undesirable organisms. In the United States alone, pollination by bees accounts for over US\$9 billion of economic revenue and some estimates indicate that over 1/3 of the human diet can be traced directly or indirectly to bee pollination. There are two key gaps in understanding and utilizing the positive aspects of insect diversity: a general neglect of insects in biodiversity research and an overemphasis on their negative impacts in all other biological research areas.

### Macrobiotics for Pest Management

ICAR-National Bureau of Agricultural Insect Resources, Bangalore is constantly in quest of potential natural enemies/biological control agents for tackling major pests which damage economically important cash, horticultural and polyhouse crops. With the current demand for organic, pesticide-residue free commodities, the search for bio-agents has become more intense. The pre-requisite of any bio-control programme is to have a large-scale supply of beneficial agents. Biological control has gained maximum acceptance in India through use of *Trichogramma* species against several lepidopteran pests. The egg parasitoid *Trichogramma chilonis* Ishii is generally mass produced on the eggs of rice moth *Corcyra cephalonica* (Stainton). Recent studies indicate



**Fig. 1.** *Trichogramma chilonis* parasitizing eri silkmoth egg

that the production of *T. chilonis* on eri silkmoth *Samia cynthia ricini* eggs is a farmer friendly system (Fig.1) and it could potentially yield trichogrammatids with superior biological attributes. The quality of *T. chilonis* mass produced on eri silkmoth eggs was assessed in the field against early shoot and internode borers which attack sugarcane and their performance was recorded to be on par with those reared on rice moth eggs (Table 1). This mass production system proves to be economical and feasible to be adopted by farmers (Lalitha *et al.*, 2013).

Predators are also effectively used in pest management. Augmentative releases of the Australian lady bird beetle, *Cryptolaemus montrouzieri* have led to successful suppression of mealybugs on grapevine, guava and custard apple. Chrysopid predators are used for effective management of aphids. *Amphiareus constrictus* (Stål), biology of which has been less studied, was successfully reared in the laboratory on the eggs of *C. cephalonica* without any plant substrate. The mass reared *A. constrictus* was evaluated against the tomato pinworm *Tuta absoluta* (Meyrick) and the brown plant hopper *Nilaparvata lugens* Stål and was found to be a

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**Table 1. Percent pest incidence, Percent pest intensity, Infestation index and Percent parasitism by *T. chilonis* and yield in different treatments**

Treatments	Pest incidence ** (%)	Pest intensity ** (%)	Infestation Index*	Parasitism ** (%)
Control plot	84.4 (71.4)b	9.7 (18.3)b	8.5 (2.9)b	0.65 (5.6)b
<i>T. chilonis</i> reared on Esw eggs released plot	60 (53.9)a	5.4 (13.0)a	4.5 (1.9)a	9.79 (16.4)a
<i>T. chilonis</i> reared on Corcyra eggs released plot	54.7 (48.3)a	6.6 (13.4)a	5.2 (2.1)ab	7.65 (15.3)a
LSD at 5%	16.88	4.26	0.8	4.79***

promising bioagent. Recent outbreak of the litchi stink bug, *Tessaratomia javanica* (Thunberg) (Hemiptera: Tessaratomidae), in Jharkhand has generated a pressing need for developing suitable mass rearing protocols for the parasitoids associated with this pest. *Anastatus acherontiae* Narayanan, Subba Rao & Ramachandra Rao (Hymenoptera: Eupelmidae) and *A. bangalorensis* Mani & Kurian, egg parasitoids of *T. javanica*, were reared successfully on eggs of eri silk worm.

### Role of Endosymbionts in Improving Fitness Attributes and Insecticide Degradation

Insect health is strongly influenced by the composition and activities of resident endosymbionts. They alter the reproductive mode of their hosts and provide protection from natural enemies, including bacteria, viruses, protozoa, and parasitic insects and various other plant allelochemicals (Huang *et al.*, 2012; Kikuchi *et al.*, 2012; Werren, 2012). The symbiotic infection confers not only insecticide resistance but spread of the resistant trait in the insect populations. Role of endosymbionts in fitness attributes (enhanced adult longevity, fecundity, parasitism / predatory potential, temperature tolerance and insecticide degradation) of *Trichogramma*, *Chrysoperla* and *Cotesia plutellae* was established at NBAIR. DNA sequence data for the endosymbionts were also generated.

Chrysopids are found to harbour many endosymbiotic yeast and bacteria in the gut as well as in diverticulum. These yeasts may be commercially exploited for the mass rearing programmes. *Enterobacter asburiae* associated with the gut of larva of *Chrysoperla zastrowi sillemi* degrades the acephate and indoxacarb by imparting resistance to larvae against acephate and Indoxacarb.

Acephate-degrading bacterial isolates *Bacillus cereus* (PXBC.Or), *Enterobacter asburiae* (PXE), and *Pantoaea agglomerans* (PX-Pt.ag.Jor) were isolated from the larval gut of diamondback moth. Apart from the insect esterases, bacterial carboxylesterase may aid in the

degradation of insecticides in DBM. This information would be useful for synthesis of new molecule having different modes of action for suitable pest management strategies against DBM.

### Bt as Insect Derived Resources for Pest Management

At NBAIR novel *Bacillus thuringiensis*-based formulations have been developed. Presently more than 300 *Bt* strains have been characterised for their cry gene profile. Strains of *Bt* for coleopteran and dipteran pests have been identified. Cloning, Expression and Bioassay of Vip3A Protein for broad spectrum activity has shown promising results against *Spodoptera litura* (Rangeshwaran *et al.*, 2016). Further work on classification of the wide diversity of *Bt* strains, characterisation of anti-Hemipteran strains of *Bt*, cry protein with cytotoxic activity against specific human cancer cells, and deciphering the natural role of *Bt* in the environment are in progress.

### Exploitation of Endophytism of Entomofungal Pathogens for Insect Pest Management

Entomofungal pathogens such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium* sp., *Paecilomyces* sp. which are generally used as foliar spray are found to occur naturally as endophytes in plant tissues. Presently, these isolates are being tested for their ability to establish as endophytes in crop plants and to exploit their endophytism as a novel delivery mechanism for managing the insect pests especially borer pests.

Six indigenous strains of *Beauveria bassiana* (NBAIR-Bb-5a, 7, 14, 19, 23 and 45) which were established as endophytes in stem and leaf tissues of maize (Fig.2) (Renuka *et al.*, 2016) and sorghum (Anon, 2016) were evaluated and Bb-5a isolate was found to be most effective against stem borer (*Chilo partellus*) in maize and sorghum by significantly reducing the pest infestation (by 60-70%) and increasing yield by 20% (Anonymous 2016). Multi-locational field trials are being

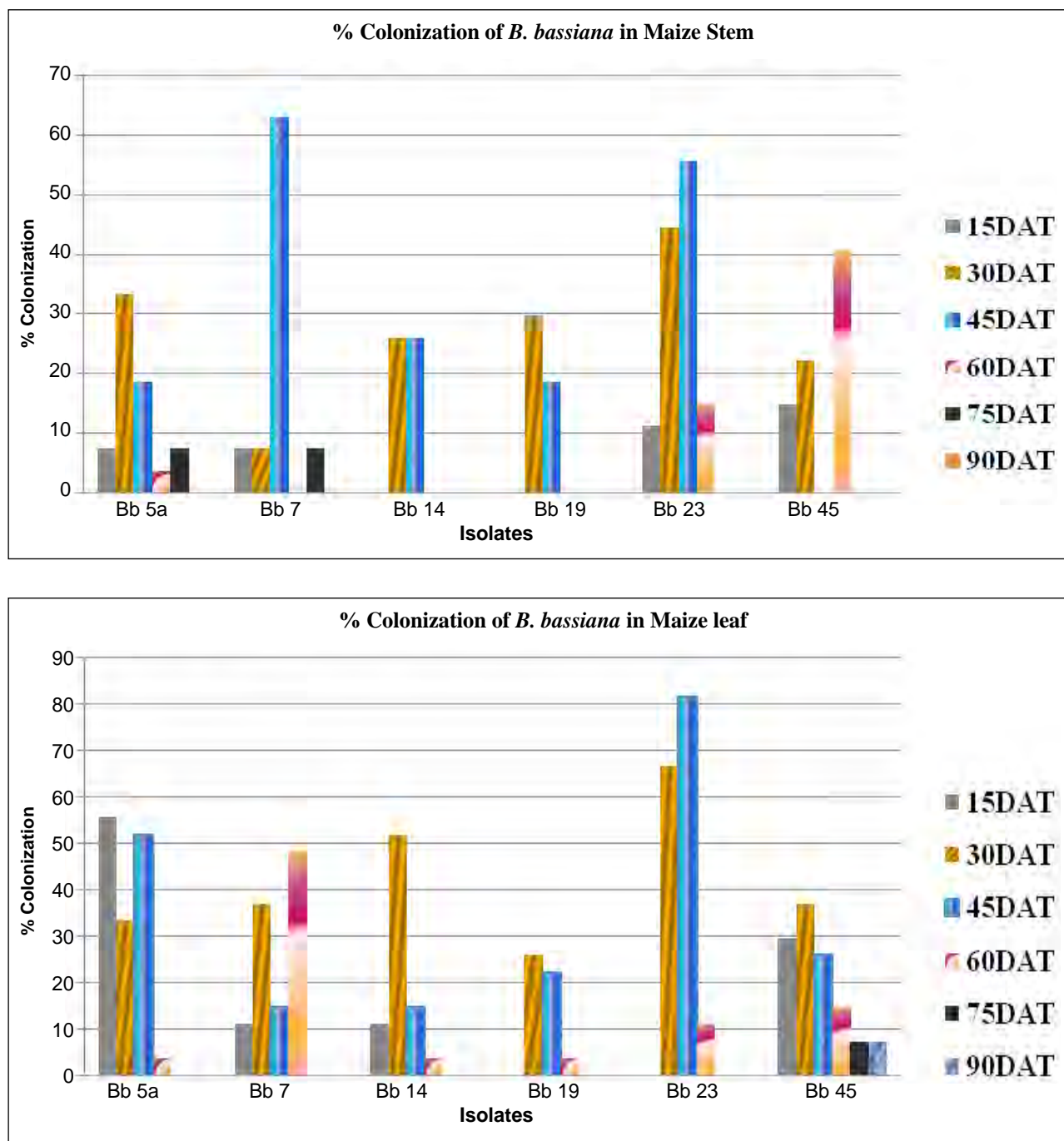


Fig. 2. Endophytic colonization of *B. bassiana* in Maize Stem and Leaf tissues (DAT: Days after treatment) (Renuka *et al.*, 2016)

attempted through AICRP Biocontrol & AICRP on maize. In cabbage and cauliflower, isolates of *B. bassiana* (Bb-5a, Bb-45 & Bb-68) and *M. anisopliae* (Ma-4, Ma-6 & Ma-35) have been established as endophytes and are being tested against *Plutella xylostella* (B. Ramanujam, Personal Communication).

### Entomopathogenic Nematodes for Pest Management and Antagonists for Management of Plant Parasitic Nematodes

Diversity mapping and molecular identification have been done for 40 strains of EPNs (*Heterorhabditis* and *Steinernema* spp.). DNA barcoding has been

done for 12 EPN strains (Nagesh *et al.*, 2013). Under pathogenomics of entomopathogenic nematodes against *G. mellonella*, NBAIR has sequenced whole genome & transcriptomes for four indigenous bacterial symbionts (*Photorhabdus indica*, *P. bacteriophora*, *H. sp.*, and *Xenorhabdus sp.*) and identified some genes responsible for virulence through insecticidal, antibiotic and phase transmission properties. NBAIR has developed solid-state and diphasic fermentation and post-production technologies for contamination-free antagonistic fungi for the management of root-knot and cyst nematodes (Nagesh *et al.*, 2007, 2013) and Indian patent rights have been granted to NBAIR on *A simple and novel design for small-scale solid state mass production unit for antagonistic fungi* (Indian Patent No. 275009).

Research efforts at NBAIR evolved *in vivo* production and formulation technologies for entomopathogenic nematodes with 10-12 months shelf-life, established their field efficacy against whitegrubs in arecanut, sugarcane, groundnut, maize, cardamom, redgram, ginger, eggplant etc. (Indian Patent application no. CHE/3490/2010). These technologies have been licensed to 14 commercial companies and currently in use over 20,000 ha across the country. Further, these technologies enabled win-win Private–Public Partnerships on EPN production and utilisation with a production of about 100 metric tonnes of WP formulation during 2015-16 alone.

## Conclusion

Promising species/strains of macrobials and microbials have been identified for several of the notorious pests and diseases infesting crops. Simple and novel farmer friendly technologies have been standardised by NBAIR for the production of these potential bio-agents. At this juncture, armed with these technologies, focus should be

on popularising the concept of biological control among farmers and motivating them to adopt this eco-friendly bio-approach as an important component of IPM.

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## Exploiting the Essence of the Microbial Diversity of the Rann of Kutch for Devising Strategies for Salinity and Drought Tolerance

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It is predicted that by the year 2050, the challenge of providing food security to around 9.6 billion world population will be acute. In a rapidly changing climate scenario, there will be increase in frequency and intensity of stresses, particularly drought, indicating likely shift of vast arable land further to the kitty of already existing 40% of global land under arid and semi-arid areas. The extent of salinity affected area is also likely to increase further from the existing 20% of irrigated lands of the world. Thus, to bridge the demand and supply gap of food grains for feeding nearly 9.6 billion projected world population by 2050, we need to produce more in arid-, semi-arid and rain-fed systems and the areas affected by salinity need to be brought under cultivation. Rain-fed agriculture has further setbacks owing to recurrent and prolonged droughts forcing it to become over-dependent on groundwater for irrigation thereby depleting the aquifers and soil moisture. At the same time with predicted increase in the mean sea level due to global warming, more area in the coastal areas is likely to be engrossed by sea water rendering them unfit for cultivation.

It is known that for one gram of carbon gain, plants need to transpire 1.7 l of water. In a hypothetical situation, if the total biomass (yield and plant biomass) of a target crop is predicted to be around 7 tonnes/ha, there will be likely requirement of around 6800000 l of water to meet the transpirational demand of the plants. This luxury is unlikely to be available during acute drought and salinity stress. Therefore, strategies would have to be thought off as to how the carbon gain can be maximised even when there is drastic reduction in the transpirational loss of water by regulating the stomatal opening.

To cope with these situations, genetic enhancement is considered to be a major option, yet spectacular progress in developing truly drought- and salinity-tolerant cultivars have not been made. The bottleneck of such efforts, probably, has been their inability to provide a

solution to the reduced photosynthesis during drought- and salinity-stress, and compensate them suitably, when plants resort to minimize stomatal opening or even shut them down during daytime to reduce transpirational losses or outflow of water resulting in drastic reduction in photosynthesis and carbon gain. If the situation persists for a long period, unless alternative methods are made available for carbon gain, the plants will eventually die due to lack of sufficient reserves to overcome the situation.

Under saline situation, if a crop is continuously irrigated with 4 EC (Electrical Conductivity) saline irrigation water, an estimated amount of 6000 kg of Na will be accumulated inside plant tissues/ha, if no control mechanisms exist in plants to reduce the entry of Na or detoxify it. This would make almost all the plants susceptible to salinity. But that does not happen. To reduce the inflow of Na inside the plant tissues, the plants resort to minimizing the transpiration by regulating the stomatal behaviour. This happens at the cost of drastically reducing the photosynthesis and carbon gain and enhancing photorespiration, especially in C<sub>3</sub> crops, reducing the net carbon gain further. Moreover, plants will also reduce the influx of Na by activating the sodium pumps in the root so that most of the Na is removed from the incoming solute. Still whatever amount of Na will enter the plant tissues will be good enough to impair the cellular machineries.

Therefore, the question remains as to how the concerns of salinity and drought can be addressed so that more area can be brought under cultivation to sustain agricultural growth. This needs stable signatures/traits that might have transferred evolutionarily across domains of life which can impart reasonable level of salinity and drought stress tolerance. Such 'treasure trove' might be present in a system which would have evolved and witnessed all events of evolution and has transferred the stable signatures/traits into new and newer forms of life in each step of evolution. As salinity tolerance

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is almost synonymous to drought tolerance, clues can be obtained from strains of extreme halophilic bacilli and their derived genera, archaea, and fungi that survive the extremities of salinity as prevalent in the otherwise undisturbed ecosystem of the Rann of Kutch in Gujarat with the history of stable evolutionary lineages. While studying the diversity of the extreme halophiles, we found that these groups of organisms can survive and perpetuate their races at upto saturated levels of NaCl concentration, the most inhospitable level of possible salinity.

The sequencing of the genomes and metagenomes of the representative organisms and samples of the Rann of Kutch and their diversity provided the much needed information about the strategies required to be adopted to circumvent such level of stresses and the ways and means of gaining valuable carbon required for growth and multiplication when there is very limited availability of nutrients and carbon sources in the growth environment. Invocation of the Darwinian 'survival of the fittest' has much relevance today than ever before to understand the mechanisms of osmo- and desiccation-tolerance.

Genes reported to have capability of imparting osmotolerance could not be detected in metagenomes and many of the genomes of the organisms obtained from the Little and Great Rann of Kutch. Then how did all these organisms survive in such inhospitable salinity? Critical evaluation of the sequence data of the metagenomes revealed that there are more than 20,000 species of organisms that can survive and multiply in more than 35% of salt concentration. When the salt concentration reaches around 50% level, the species richness reduced to around 15,000 species with predominance of extreme halophilic archaea. Contrary to the reported involvement of methyl aspartyl pathway in helping *Haloarcula marismortui* to thrive in the extreme salty settings prevalent in the Dead Sea, we found possible involvement of serine-glyoxylate, acetyl CoA, methyl malonyl- and ethyl malonyl-CoA pathways in a novel haloarchaeon and metagenomes in imparting salinity tolerance by helping the organisms to gain carbon and channelizing the intermediates to other anabolic reactions resulting in net positive carbon balance. This begins with the uptake of dissolved CO<sub>2</sub> as HCO<sub>3</sub><sup>-</sup> which is then converted into phosphoenolpyruvate → oxaloacetate → malate → pyruvate/ phosphoenolpyruvate → glyoxylate → serine → by key enzymes like phosphoenolpyruvate carboxylase, malate dehydrogenase, isocitrate lyase,

malate synthase, decarboxylases, serine hydroxymethyl transferase, etc. in a series of reactions to capture carbon from the environment and also to utilise respiratory CO<sub>2</sub> and eventually diverted them for synthesis of key intermediates required for sustaining life processes.

What is important is to have the intact network of the pathways and the expression of all the necessary enzymes in coordination. In addition to carbon gain, the organisms expressed Na/K/H pumps to remove most of the Na trying to enter into cytoplasm with solute. All the machineries required for such expression are present in bacilli and fungi (archaea-eubacteria-eukaryote pipeline) obtained from the Rann of Kutch. The big question is whether such pathways have been acquired by plants while transition from aquatic to terrestrial ecosystems during the process of acclimatization and evolution and divergence? If yes, whether it would be feasible to identify all these footprints or orthologs in plants so that crop plants truly tolerant to salinity can be identified which can address the issue of reduced photosynthesis during salinity stress by ensuring steady supply of carbon through alternative route/mechanisms to anabolic reactions to occur leading to net positive carbon gain.

The blue green algae like *Synechocystis*, a marine alga, has unique feature of concentrating CO<sub>2</sub> often called Carbon Concentrating Mechanism (CCM) to ensure continuous and steady supply of CO<sub>2</sub> around RUBISCO to ensure unabated photosynthesis during entirety of daytime for maximizing fixation of carbon required for rapid growth and development. Evolutionarily, C<sub>4</sub> and Crassulacean Acid Metabolism (CAM) are other CCMs wherein photosynthetic metabolism have evolved from C<sub>3</sub> background during the process of evolution and acclimatization to environmental extremities and are reported to be water-use efficient and drought tolerant as compared to C<sub>3</sub> crops. Taking clues from the CCMs from the photosynthetic prokaryotic background, of late, scientists are thinking of transferring the entire photosynthetic machinery of CAM into C<sub>3</sub> background which is being thought of as a solution for improving the much-needed water-use efficiency (WUE) of carbon assimilation and for improving drought-tolerance, exploiting the nocturnal CO<sub>2</sub> fixation and daytime CO<sub>2</sub> re-assimilation by inverse opening/closing of stomata. This will ensure reasonable photosynthesis behind closed stomata, during daytime, in drought-stress condition. However, there are enormous difficulties in stably

transferring genes of all the modules of CAM into  $C_3$  background because of little understanding about stomatal control and mesophyll succulence.

Though the mechanisms of net carbon gain in photo- and non-photosynthetic prokaryotic, lower group of non-photosynthetic eukaryotes, and photosynthetic plants would be different, evolutionary clues obtained

from the photosynthetic prokaryotes would suffice the future strategies for ensuring drought and salinity tolerance in plants by ensuring steady supply of carbon through alternative means to ensure photosynthesis to continue even behind closed stomata during daytime in acute stress.

## Innovations in Insect Pest Management: Interventions Using Insect Derived Volatiles

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Insects release volatiles known as semiochemicals which include intraspecific volatiles (pheromones) and interspecific volatiles (allelochemicals which include kairomones, synomones and allomones). The pheromones are secreted by the individuals of the same species, either males or females or both or even the developing instars. These pheromones are used successfully for the management of insects on various crops.

### Material and Methods

The pheromones especially, sex pheromones are usually secreted by females from their glands located in their last abdominal glands or aggregation pheromones by males from thoracic glands. Usually these glands are tweezed out and their chemical profiles are created. Electrophysiological instruments such as electroantennogram (EAG), Gas chromatography coupled electroantennogram (GC-EAD), Gas chromatography coupled single sensillar recorder (GC-SSR) and analytical instruments such as Gas chromatography mass selective detector (GC-MS), Nuclear magnetic resonance (NMR) and Fourier Transformed Infra Red Detector (FTIR) are used for the isolation, identification and structural elucidation of the pheromonal compounds. The identified compounds are synthesized using organic synthesis process and their efficacy was confirmed through behavioural assays using wind tunnel, olfactometers and field cages. Dispensers were used for sustained release of pheromones at the dose simulating natural the field releases from the individuals.

### Results and Discussion

Pheromones are of different types such as alarm pheromones, brood pheromones, contact sex pheromones, defense substances, marking pheromones, recognition pheromones, trail pheromones, sex pheromones and aggregation pheromones. Among them sex pheromones and aggregation pheromones are widely used in the insect pest management.

Several species of insects are monitored by keeping the pheromones in the field or green houses/net houses. The catches in monitoring traps will help in decision making process such as the application of insecticides or release of biological control agents. Effective monitoring of the pests is achieved through the use of appropriate dispensers containing the exact blend of the pheromone compounds, placed in optimum quantities of traps, which are spread one to two per acre at appropriate places in the fields (Witzgall *et al.*, 2010; Colazza *et al.*, 2007). Quarantine monitoring of invasives such as fruit flies are effectively done using parapheromones such as methyl eugneol or cue lure. Monitoring of spread or dispersal and social auditing of the effectiveness of weed biological control agents are also done using the pheromones.

The use of pheromones for mass trapping has led to reduction in the pest load of rice infesting viz. *Scirpophaga incertulas* (Walker), (Cork *et al.*, 2005). In the management of brinjal, installation of 25–30 traps/ac, containing a blend of E-11-hexadecenyl acetate and E-11-hexadecenol (100:1) along with insecticidal sprays such as neem, effectively managed *Leucinodes orbonalis* (Guenee) (Cork *et al.*, 2005; Rath and Dash, 2005).

Mating disruption is accomplished by inundating the whole field with the pheromones that results in the confusion of males in locating and mating with the females which drastically reduces the egg load in the fields. Commercial formulations such as PB-ROPEL, Isomate M Plus, Isomate M 100, Isomate OFM, RAK1+2 dispensers, or RAK1+2R Isonet and Biodegradable dispensers are a few examples of pheromone dispensers used for successful mating disruption in species such as *Cydia pomonella* and *Cydia moelsta* (Denis and Schiffermüller) (Angeli *et al.*, 1999; Molinari *et al.*, 2000). Mass trapping and mating disruption can easily be integrated with insecticides, biological control, and are pollinator friendly.

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Polymorphic geographical populations of pests like *Helicoverpa armigera* (Hubner) which exhibit various blend ratios do exist within short geographical ranges, necessitating discovery of appropriate blends of pheromone.

Aggregation pheromones are secreted from the thoracic glands, mostly in coleopteran and heteropteran insects. These are effectively used in attracting both the males and females and thus reducing the population levels. Mass trapping of coffee stem borer, *Xylotrechus quadripes* (Chevr) and red palm weevil, *Rhynchophorus ferrugineus* Olivier was done using their aggregation pheromone. The number of traps used in mass trapping are usually more and both the sexes were normally trapped, for eg, 10 traps per acre each containing 75 mg of pheromone was used for successful management of *X. quadripes*.

Several species of beetles use antiovipositing pheromone which release message to the conspecific females to avoid those trees oviposited by the females. Verbanone is one such substance which was commercially exploited to reduce the infestation by *Dendroctonus ponderosae* Hopkins (Fettig, 2013). Frontalin at higher doses and methylcyclohexanone (MCH) (3-methylcyclohex-2-en-1-one) also act as antiaggregation pheromones and are used for species such as *Ips pini* Say (Miller, 2001; Ross *et al.*, 1996).

The natural unsaturated hydrocarbons present on the cuticle of several lepidopteran insects comprise of compounds such as octacosane, pentacosane, hexacosane, tricosane, hexatriacontane, and docosane which increased the activity of egg parasitoid, *Trichogramma* spp. (Paul *et al.*, 2002; Paramasivan *et al.*, 2004; Yadav *et al.*, 2008).

The alarm pheromone ( $\beta$ -farnesene) and sex pheromone of aphids [(+)-4aS, 7S, 7aR] nepetalactone] are involved in cross-talk with aphid predators or parasitoids (Micha and Wyss, 1996; Powell *et al.*, 1993; Hardie *et al.*, 1991).  $\beta$ -farnesene is commercially used as attractant for lady bird beetles.

## Conclusion

In India, the use of pheromone in pest management is rather very less and farmers are to be educated on the importance of pheromones in management of pests. Pheromones may be incorporated easily in the integrated pest management and accordingly package of pest

management to be revised. Semiochemicals comprising of pheromone and kairomone act as important tools in the conservation biological control and these technologies need to be commercialized.

Electronic nose or electronic sensors need to be developed which should be able to identify the presence of calling females in the fields which makes the management practices easy.

Technologies need to be refined with the use of state of art dispensers and environment friendly precision traps for the economical use of pheromones.

Pheromones for insects of Indian origin (such as cashew stem borer, *Plocaederus ferrugineus* (L.) and mango stem borer, *Batocera rufomaculata* De Geer) need to be isolated, identified, and synthesized for commercialization. Pheromones for sucking pests such as leaf hoppers, aphids, mealybugs, leafminers also need to be identified and exploited for monitoring purpose.

Several alien pests such as coconut borer *Brontispa longissima* (Gestro) are expected to invade India in the future, it will be appropriate to isolate, identify and synthesize and share with the countries where the potential of invasion looms over.

Infrastructure facilities need to be strengthened and shared between ICAR and agricultural universities for the semiochemical research for the meaningful development of semiochemical technologies.

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## Insights from the Genome and Genome Informatics of Agriculturally Important Microorganisms

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Microorganisms have been evolving on earth for at least 3.8 billion years. However, unlike animal or plant diversity, complete map of microbial diversity in a particular ecosystem is not available. Over the past few years, high-throughput sequencing has been revolutionized by the flooding of several next-generation sequencing (NGS) platforms. High-throughput next generation sequencing of microbial genomes and transcriptomes has changed the face of present era's microbiology. With the adventures of new technologies and methodologies, now it became possible to sequence a large microbial genome/ transcriptome within few days. Microsatellites such as simple sequence repeat markers (SSRs) are dispersed throughout the genome/ transcriptome which can be located and characterised through using the tools of genome informatics. It became easier to compare the multiple genomes through genome informatics. Genome wise comparison reveals the similarity and differences among the different organisms. Moreover, the recombination causes frequent genome rearrangements, horizontal transfer introduces new sequences into bacterial chromosomes, and deletions remove segments of the genome. These technologies are far more powerful than any existing molecular tools in diversity mapping and genome comparative analysis. However, our ability to accurately analyze these data relies on well-characterised, foundational reference data of microbial genomes. These sequencing techniques provide unique opportunity for researchers to build fundamental knowledge on microbial communities in the environment and understanding their role in management and protection of a biological system.

The high-quality throughput genome sequencing could be used to collect the real information and to utilise these insights in improving the important metabolic pathways to harness their potential in agriculture. A quick search of Genome online database (GOLD) showed that about 56,000 bio-samples, 19,200 studies, 56,000 sequencing projects and 39,400 analysis

projects have been performed for microorganisms from the extreme environments. However, genome and transcriptome sequencing of the agriculturally important microorganisms (AIMs) is highly overlooked throughout the world; especially in India. However, several microbial genomes including archeal genomes sequenced in the country have given insights which can be utilized to decipher the coding sequences of key enzymes of different metabolic pathways, and changes in their expression profiles for better production of useful compounds/metabolites. Efforts are ongoing to perform the depth analysis of microbial genome sequences, and consequently to predict the sequences and structures of important proteins. The nucleotide sequences for key enzymes of different pathways can be utilized for the prediction of corresponding protein sequences and their structures through molecular modeling and simulations.

ICAR-NBAIM has sequenced the genomes of nine important bacterial strains that were previously characterised as potential nitrogen fixer rhizobia, stress mitigating salt / temperature tolerant organisms. Genome architecture of micro-organisms is the key targets to know that how the antibiotic resistance is being transferred or how the lysogenic phage get inserted into the bacterial genome and add the unusual characteristics. The comparison of whole genome sequence of six species of *Mesorhizobium* have shown that maximum numbers of singletons were found in *M. ciceri* ca181 and least in *M. australicum* 2075. Whole genome phylogeny analysis by different methods revealed that *M. loti* MAFF303099 and *M. huakuii* 7653R are in monophyletic clade and has a close relationship *M. ciceri* ca181. *Nif* gene analysis inferred that these are conserved during evolution. *M. ciceri* ca181 has maximum unique genes among six bacteria. The comparative whole genome analysis of these six *Mesorhizobium* has led to the identification of several proteins which are specific to particular strain and their roles are specific. In another study on the genome

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of a salt tolerant bacterium *Exiguobacterium profundum* PHM11, the presence of lysogenic phage DNA at five different places in the chromosome was recorded and recognized the portions of phage DNA which encode for the key proteins involved in the transport across the plasma membrane. We identified a *virD4* gene which is an ATPase that helps in transport across the membrane. Not only genome, but also the transcriptome analysis of microbial genomes are emerging area, and somehow related to the genome informatics. Researchers are also trying to elucidate that how the salt or drought stress can affect the plant physiology or how these stressors induced effects can be mitigated through microbial inoculations. Further, NGS driven amplicon based metagenomics have revolutionized the field of microbiology by providing a culture independent technique to identify and assess microbial diversity. The ability to sample environments for complex fungal metagenomes is rapidly becoming a reality and will play an important part in efficient utilisation of fungi for agricultural, industrial and climate management purposes. However, the metagenomic approach still has inherent limitations as it cannot distinguish live from dead or active from inactive microbial cells.

Genome and genome informatics, therefore, is an emerging area in the agricultural science. It could be only a possible way to get the real inferences which are

present in the genomes of AIMs which can be utilized to decipher the coding sequences of key enzymes of different metabolic pathways, and changes in their expression profiles when coming in the contact with agricultural crops. Different aspects of agricultural biotechnology have added their inputs; however, plant/microbial genomics and genome informatics has contributed significantly. Besides these contributions, still numbers of questions have remained to be answered such as (a) can *in silico* tools be helpful in deciphering the coding sequences of regulatory enzymes involved in the key metabolic pathways (b) how the expression profiles of important genes of key pathways or whole genome network of a crop get changed, when it is grown in stressed soils/environments, or when crops come in contact with pathogens/beneficial microorganisms (c) what changes in the amino acids during divergent evolution led to the increased pathogenicity of microorganisms against agricultural crops? (d) how the carbon fluxes or biomass production get changed with the inoculation of stress mitigating plant growth promoting rhizobacteria (PGPRs) (e) based on *in silico* results, could it be possible to *in vitro* optimize the sequence / codon of a stress mitigating enzyme through modern approaches of synthetic biology and genetic engineering to get the improved crop production/varieties?



## Navigating the International Exchange of Plant Genetic Resources Amidst Biosecurity Challenges: Experiences of IITA in Africa

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### **International Exchange of Germplasm is Vital for the Sustainable Development of Global Agriculture and Conservation of Biodiversity**

International exchange of genetic resources as botanic seeds, plants or plant parts suitable for propagation has played a crucial role in the growth of international and national genebanks; development of improved high-yielding nutrient-rich crop varieties resilient to pests, diseases, and abiotic stresses; international trade in planting materials; and even international research collaborations. The frequency of international exchange of germplasm is on rise due to the growing demands of global food systems, international research partnerships, and trade linkages. Studies on the origin and use of food crops indicated that 68.7% of national food supplies are derived from crops with a foreign origin and emphasize the importance of the inter- and intra-continental exchange of germplasm (Khoury *et al.*, 2016). The 11 CGIAR genebanks with 730,000 accessions in 35 collections manage the world's largest and genetically most diverse collections, and have distributed ~380,000 samples to over 120 countries between 2012 and 2014 for their own research and also to meet the demands of national crop improvement programmes (CGIAR 2014; Galluzzi *et al.*, 2015). The 11 CGIAR genebanks were also credited with the highest annual number of international germplasm exchanges, especially under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). National climate change adoption policies on broadening genetic diversity for resilient cropping systems and the prevention of biodiversity loss are also spurring germplasm exchanges. Landraces and wild germplasm are mainly exchanged by the genebanks; improved varieties and research material (training sets, international evaluation trials, pre-breeding populations, etc.) are mainly exchanged by the breeding programmes, and both the private and public sectors are involved in the distribution of plant propagative

materials for commercial and non-commercial uses. Various mechanisms are in place to facilitate germplasm exchange between countries. Phytosanitary cleanliness is the key criterion for a decision on the export or import of germplasm.

### **Germplasm Exchange is a Risky Affair and Regulated through Quarantine Controls**

Exchange of germplasm carries simultaneous risks of moving harmful pests and pathogens between geographies and introducing them in new territories where they were not known to exist (e.g., intercontinental spread of *Banana bunchy top virus*). High risk of biotic agents spreading through plant and plant-propagation materials was recognized in the nineteenth century as a consequence of damaging outbreaks caused by pests and pathogens introduced with the germplasm (Klinkowski, 1970). The Irish famine due to the epidemic of potato late blight (*Phytophthora infestans*) in the 1840s was a notable example of catastrophic damage by an introduced pathogen from South America. Sensing similar risks, quarantine measures were enacted in 1873 for the first time in Germany and the UK as a legal measure for the inspection of potato for Colorado potato tuber beetle imported from the USA. Quarantine measures were subsequently adapted by several countries and eventually by the mid-twentieth century they had been established as a global norm.

Quarantine procedures continue to evolve to prevent every possible biotic risk from consignments of propagative and non-propagative plant products. Despite comprehensive measures, pests and pathogens find their way into new territories (Waage *et al.*, 2006) resulting in serious economic damage. For instance, introduced insects and pathogens in the USA were estimated to cause annual losses worth US\$40 billion (Pimental *et al.*, 2005). More recently, the introduction of *Maize chlorotic mottle virus* (MCMV) in Kenya

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resulted in the loss of production worth over US\$188 million between 2012 and 2014 (de Groote *et al.*, 2016). The MCMV subsequently spread to other countries in East Africa, establishing a major threat to maize in the African continent (Mahuku and Kumar, 2016). A few plausible reasons for the incursion of biotic agents despite quarantine controls could include insufficient capacity limiting rigorous phytosanitary controls, insufficient knowledge on biological risks, and biophysical factors that are beyond human control. An analysis of 1,300 known invasive pests and pathogens and the risks they pose to 124 countries has identified developing countries, especially those in sub-Saharan Africa, (SSA), as the most vulnerable because of weak capacities to prevent or manage biological threats (Paini *et al.*, 2016). Stringent inspections by both exporting and importing parties could reduce the risk of biotic agents spreading along with the germplasm and compensate for weak controls along the distribution network.

### **Germplasm Exchange from International Institute of Tropical Agriculture (IITA) is Vital for Ensuring Food Security in SSA**

IITA in Nigeria is one of the CGIAR centers with an international genebank conserving germplasm of its six mandate crops: banana and plantain (*Musa* spp.), cassava, cowpea and wild *Vigna* species, maize, soybean, and yam. Since its establishment in 1967 the center has collected and conserved over 33,000 accessions of landraces and wild relatives representing global collections (cowpea and yam) as well as regional resources (cassava, maize, *Musa*, and soybean). The center also has a vibrant international breeding programme developing improved varieties of its six mandate crops. Both the genebank and the breeding programmes frequently exchange germplasm for research and other uses. Generally landraces and wild relatives are exchanged from the genebank; breeding programmes often exchange parental lines, pre-breeding populations, inter-specific hybrids, and inbred lines, and share international multilocal trials; seed system programmes acquire pest-free material for the bulk propagation of planting materials; germplasm or plant products are exchanged for research use. IITA conserves and distributes cowpea and *Vigna* spp., maize, and soybean as botanic seeds, germplasm of cassava, *Musa*, and yam as in vitro tissue culture plants or plant propagules such as stems, corms, or tubers.

### **The Germplasm Health Unit (GHU) of IITA Facilitates the Safe Exchange of Germplasm to End-Users**

The majority of the IITA operations are in developing and less developed countries that lack adequate quarantine services to ensure the safe exchange of germplasm. Following the recommendations made in August 1993 during the Sixth International Plant Protection Congress in Montreal the CGIAR centers, including IITA, have established a dedicated GHU to ensure the exchange of germplasm that meets desired phytosanitary standards. The GHU facilitates the production, maintenance, and exchange of pest-free germplasm in accordance with the international requirements on plant protection advised by the FAO-International Plant Protection Convention (IPPC), the African Union Inter-African Phytosanitary Council (AU-IAPSC) and National Plant Protection Organizations (NPPOs). The Unit operates within the framework of the procedures for the introduction and export of germplasm established by the Government of the host country. For instance, all the exchange operations of IITA's activities in Nigeria are organized in accordance with the legislation of the Nigerian Agricultural Quarantine Service (NAQS).

GHU performs plant health indexing, seed treatment, and phytosanitation of clonal crop germplasm and ensures compliance with the national regulations on germplasm introductions and exports. GHU conducts research to develop technologies and strategies for improved phytosanitary cleaning of germplasm, better diagnostics for virus indexing, and efficient schemes for seed health inspection. GHU also participates in the surveillance schemes to generate baseline knowledge on pest and pathogen distribution in the target countries and this knowledge is used to establish inspection schemes.

GHU procedures are frequently updated in accordance with new knowledge on pest and pathogen occurrence in hosts and geographies. Increasing reports of new viruses, many of which were discovered based on the small RNA sequencing using NGS technologies (Roossinck *et al.*, 2015) are posing new challenges to phytosanitary decision-making. The NGS-based discovery of viruses in some clonal crop germplasm considered to be virus-free is exposing the limitations of current procedures. New schemes are being developed to standardize the virus indexing of clonal crop germplasm

using NGS-based methods for a high-level assurance of health status.

GHU adopts the most stringent standards to monitor germplasm imported to and exported from IITA. Germplasm of clonal crops is exchanged in the form of virus-indexed in vitro plants generated from meristem tip cultures subjected to thermo- or chemotherapy procedures. Certified in vitro plants are used for propagation under pest-free conditions (contained screen houses) to generate vegetative plant parts (mini-stem cuttings for cassava; mini-tubers for yam; corms for *Musa*) for distribution to countries/partners that cannot accept in vitro plants owing to a lack of facilities for propagation or post-flask management.

GHU annually facilitates germplasm exchange with 40 to 50 countries; the majority of them are in Africa. Maize is the germplasm most frequently exchanged, followed by cassava, cowpea and other *Vigna* species, soybean, and yam. *Musa* germplasm exchange is pacing since the development of a new decision scheme in 2015 for distributing *Musa* germplasm with integrated badnavirus genomes that were blocked from distribution. GHU proactively engages with stakeholders to appraise and understand the needs and possibilities for facilitating germplasm exchange within the permissible rules.

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## Converging Provisions of CBD and WTO to Ensure Agricultural Biosecurity

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Biosecurity is a strategic and integrated approach that encompasses the policy and regulatory frameworks (including instruments and activities) for analysing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment. Biosecurity covers food safety, zoonoses, the introduction of animal and plant diseases and pests, the introduction and release of living modified organisms (LMOs) and their products (e.g. genetically modified organisms), and the introduction and management of invasive alien species. Thus biosecurity is a holistic concept of direct relevance to the sustainability of agriculture, and wide-ranging aspects of public health and protection of the environment, including biological diversity. The overarching goal of biosecurity is to prevent, control and/or manage risks to life and health as appropriate to the particular biosecurity sector. In doing so, biosecurity is an essential element of sustainable agricultural development.

The approach of ensuring biosecurity mainly emancipates from the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement); the Convention on Biological Diversity (CBD) and the Cartagena Protocol on Biosafety (Cartagena Protocol); the Codex Alimentarius Commission (Codex); the Office International des Epizooties (OIE, or World Organization for Animal Health); and the International Plant Protection Convention (IPPC). The SPS Agreement provides for a unified approach to the different sectors of Biosecurity. The approach is centred on harmonization through international standards, science-based risk assessment and minimization of interference with international trade. While traditional sanitary and phytosanitary controls were designed to ensure efficient production through the protection of natural resources, modern controls tend to integrate these concerns into a wider spectrum of issues, such as preservation of the environment and protection against the loss of biodiversity.

The CBD which is the key global instrument on the conservation and sustainable use of biological diversity and the fair and equitable sharing of the benefits from the use of genetic resources has provisions that relates to biosecurity. The agrobiodiversity which includes the components of biological diversity that are essential for feeding human populations and improving the quality of life (encompassing the variety and variability of ecosystems, animals, plants and micro-organisms, at the genetic, species and ecosystem levels) are threatened by invasive species, pests and diseases and thus are directly in the purview of biosecurity.

Article 8(h) of the CBD requires contracting parties to prevent the introduction of, and control or eradicate, those alien species which threaten ecosystems, habitats or species. The multiple impacts of invasive alien species (IAS) call for coordinated international action to minimize their environmental as well as economic effects. Toward this end, the CBD and IPPC have been working cooperatively in several ways. The CBD Conference of the Parties and the IPPC have collaborated on the preparation of a supplement to International Standards of Phytosanitary Measures (ISPM) No. 11 (Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms, LMOs) in order to incorporate risks to biodiversity posed by IAS that are considered plant pests. In this regard, the IPPC standard is relevant to the regime regulating LMOs under the Cartagena Protocol. The protocol establishes an informed agreement procedure for ensuring that countries are provided with information in advance, including an assessment of risks to biological diversity, necessary to make informed decisions before agreeing to the import of such organisms into their territory. In the assessment of risks to biological diversity, ISPM No. 11 thus can be applied for LMOs that are categorized as plant pests. Further collaboration has taken place in the revision of ISPM No. 3 (Guidelines for the export, shipment, import and release of biological control agents and

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other beneficial organisms) in order to manage risks to biodiversity that beneficial organisms may generate.

There are other international instruments of trade which form a part of the Biosecurity related regulatory framework and impinges upon biodiversity to some extent in one way or the other. For instance, the OIE Terrestrial Animal Health Code, the Aquatic Animal Health Code and their respective Manuals for Diagnostic Tests outline import and export procedures to avoid disease spread and structures for the communication of epidemiological information. Several Codex documents are also relevant, including the Principles for Food Import and Export Certification and Inspection; Guidelines for the Design, Operation, Assessment and Accreditation of Food Import and Export Inspection and Certification Systems; and Guidelines for the Exchange of Information between Countries on Rejections of Imported Food.

To prevent the introduction and mitigate the impacts of invasive alien species specifically an Inter-Agency Liaison Group has been formed for which the Strategic Plan for Biodiversity 2011-2020, and in particular Aichi Target 9, provides the main focus of the work of this group. This group comprises of the seven biodiversity-related conventions viz. the CBD; the Convention on Conservation of Migratory Species; the Convention on International Trade in Endangered Species of Wild Fauna and Flora; the International Treaty on Plant Genetic Resources for Food and Agriculture; the Ramsar Convention on Wetlands; the World Heritage Convention and the IPPC. The Liaison Group meets regularly to explore opportunities for synergistic activities and increased coordination, and to exchange information. The secretariats of the international organizations related to trade, biosecurity and biodiversity that participate in the liaison group meetings as core members also include the FAO, WTO, OIE, the International Civil Aviation Organization, International Maritime Organization and the International Union for the Conservation of Nature. This in fact is a step forward in converging the biosecurity and biodiversity agendas of WTO and CBD and enhance coherence and cooperation in implementation. However, with increasing population, pest outbreaks and economic pressures it remains to be seen as how effectively the convergence can be achieved for developing an operational framework for ensuring agricultural biosecurity along with conserving the agro biodiversity. The global community seems to

be getting geared up as the Eight Trondheim Conference on Biodiversity held in June 2016, brought together decision makers and experts from around the globe to discuss interrelationships between agriculture and biodiversity and how their policies can address problems and provide solutions for the achievement of mutually supportive and sustainable outcomes. This is going to be further deliberated in the thirteenth CoP meeting of CBD at Cancun, Mexico and may prove to be a positive way forward in converging the provisions of CBD and WTO that includes those of biosecurity.

Risk analysis is the basis for the establishment of sanitary and phytosanitary measures for the import of plants, animals and foods, and the concepts are the same across these sectors. Thus, risk analysis is one common thread among the many international instruments relevant to Biosecurity. But although international standard-setting and cooperation are important, the establishment, implementation and monitoring of Biosecurity in agriculture is a matter for national governments. How to implement a Biosecurity approach at national level is a challenge for developing and Least Developed Countries given the variety of legislative and policy provisions that are scattered across the Ministries. At national levels generally the controls and authorities for Biosecurity matters tend to be scattered over a variety of ministries, including the ministries of agriculture, health, environment and trade and industry. The objective of Biosecurity is to draw together relevant regulatory authorities or to create coordinating mechanisms to streamline approaches to managing biological risks. Therefore to implement the necessary coordination, countries must look closely at their national legal frameworks. This will aid in implementing the most efficient institutional set-up while also protecting rights and establishing responsibilities in a way that is conducive to the active participation of public authorities, the private sector and consumers.

To sum up the convergence of provisions of WTO and CBD at regulatory and operational level needs to be further deliberated upon and expedited at both national and international level to ensure biosecurity for useful plants, animals, fisheries and microbes. The countries need to simplify the regulatory mechanisms to achieve this on the ground and should not get overwhelmed or overpowered by a plethora of global regulatory instruments that are often generic in nature.

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## Plant Quarantine System for PGR in India

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Exchange of plant genetic resources (PGR) has contributed significantly towards crop improvement and increased crop production in the country. However, many pests have also moved across the countries along with planting material. Plant quarantine is a government endeavour enforced through legislative measures to regulate the introduction of planting material, plant products, soil and living organisms, etc. in order to prevent inadvertent introduction of pests (including fungi, bacteria, viruses, nematodes, insects and weeds) harmful to the agriculture of a country/state/region, and if introduced, prevent their establishment and further spread.

The historical Irish famine of 1845, caused by late blight of potato (*Phytophthora infestans*) introduced from Central America; powdery mildew (*Uncinula necator*), root eating aphid (*Phylloxera vitifolia*) and downy mildew (*Plasmopara viticola*) of grapes into France in quick succession in mid 19<sup>th</sup> Century from America; coffee rust into Sri Lanka in 1875 and its subsequent introduction into India in 1876 are prominent examples that clearly demonstrate that introduction and establishment of quarantine pests into new areas can severely damage the crop production and economy of a region/ country. Like-wise, in India also, a number of exotic pests got introduced along with imported planting material causing serious crop losses from time to time. These include the recently introduced tomato pin worm *Tuta absoluta* in 2014, Jackbeardsley mealybug (*Pseudococcus jackbeardsleyi*) in 2012, papaya mealy bug (*Paracoccus marginatus*) in 2007, fluted scale on citrus introduced from Sri Lanka in 1928; San Jose scale in apple in 1930s; bunchy top of banana introduced from Sri Lanka in 1943; the golden nematode infesting potatoes introduced in 1960s from UK and the noxious weed, *Lantana camara* introduced in 1809 from Central America are glaring examples that clearly demonstrate that introduction and establishment of quarantine pests including weeds into new areas can severely damage the crop production and economy of a region/country. These introductions highlighted the fact that increased

international travel and trade had exposed the country to the danger of infiltration of exotic pests harmful to our agriculture.

With the liberalization of trade under World Trade Organization (WTO), the quarantine set-up including legislation and infrastructure of the country has been reviewed. As far as legislation is concerned, the Destructive Insects and Pests (DIP) Act was legislated by the British government ruling India in 1914 which was retained revising it as per requirements over the years through various amendments. However, after the WTO came into force, India legislated the Plant Quarantine (Regulation of Import into India) Order in 2003, henceforth referred to as the PQ Order. The Directorate of Plant Protection Quarantine and Storage (DPPQS) of the Ministry of Agriculture and Farmers Welfare is the nodal agency for implementation of PQ Order. ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) is the nodal agency for PGR management in the country, has been empowered under the PQ Order for issuance of Import Permit and to undertake quarantine processing of all imported PGR including transgenics and trial material meant for research. Besides, NBPGR also tests samples of bulk imports sent by DPPQS for presence of exotic pests.

ICAR-NBPGR is well equipped with most modern quarantine facilities including a Containment Facility of Level 4 (CL- 4) for quarantine processing of transgenic germplasm in a risk-free manner. ICAR-NBPGR also has a well-equipped quarantine station at Hyderabad, which mainly deals with the quarantine processing of PGR meant for Southern India including State Agricultural Universities, ICAR institutes, private industry and international institutes viz., International Crop Research Institute for Semi-arid Tropics (ICRISAT), CIMMYT, AVRDC and CIP, Peru.

### Procedure for Import of PGR

Research institutions of public and private sector interested in importing plants or planting material should request ICAR-NBPGR for Import Permit (IP), which

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is not transferable. Phytosanitary Certificate is also a statutory requirement issued by the country of export and is a proof that the consignment has been examined according to the requirements of the importing country and found to be free from the quarantine pests. On arrival of the plant material, they are carefully processed. In case material is found to be infected/ infested with pests, all efforts are made to salvage the material. Only in rare cases, when the material cannot be salvaged it is incinerated. In case post-entry quarantine (PEQ) examination of the imported material is required, it is done at PEQ greenhouse facilities, at NBPGR, New Delhi, its Regional Station, Hyderabad and ICRISAT and also at the indenter's PEQ growing facility.

### Quarantine Processing of PGR

In case of PGR including transgenics, the samples size is generally small and the entire sample is examined for presence of pests and transgenics are also tested for ensuring the absence of terminator gene technology (embryogenesis deactivator gene) which is a mandatory requirement. During quarantine processing, seeds and plant material are examined for the presence of unwanted weed seeds, plant debris, soil clods, insect and mite pests, plant parasitic nematodes and pathogens including fungi, bacteria, viruses, etc. The external feeders and other incidental insect pests infesting the planting material are easily detected visually either by the naked eye or with the help of magnifying glass or stereoscopic binocular microscope. Presence of nematodes is indicated during visual examination by the observation of galls or swellings on roots, tubers and rhizomes; white, yellow or brown pinhead sized round bodies adhering to roots; swollen or malformed leaf, stem or other tissues or root lesions or unusual root proliferation. Fungal infection is indicated by the presence of sclerotia, smut balls, malformed seeds and fungal fructifications on seed surface. Presence of yellow discolouration around the hilum is suggestive of bacterial infection. The discoloured, deformed and shriveled seeds are removed during dry seed examination as these seeds may carry seed transmitted pathogens. The seeds of quarantine weeds for India also need to be detected in imports. Specialized tests used for detection of different groups of pests and all efforts are made to salvage the infected/ infested material before release

### Interceptions in Imported Material

Over the years, during quarantine processing, a large

number of pests have been intercepted in germplasm and other research material. Over the years, during quarantine processing, a large number of pests have been intercepted in imported PGR and other research material. The significant interceptions made which are yet not reported from India include fungi like *Claviceps purpurea* in seeds of wheat, barley, *Peronospora manshurica* on soybean from several countries, *Fusarium nivale* on wheat, and barley from Germany, Italy, Hungary, Sweden and UK, *Uromyces betae* on sugarbeet from Belgium, Germany, Italy, UK and USA, *Phoma lingam* on *Brassica* from several countries, *Phomopsis longicolla* on *Glycine max* from USA, bacteria like *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. from Canada, France, Pakistan, Sweden, Taiwan, UK and USA, viruses like *Barley stripe mosaic virus* on wheat from USA, *Broad bean stain virus* on *Pisum sativum* from Spain and *Vicia faba* from Syria and Bulgaria, *Cowpea mottle virus* on *V. subterranea* from Ghana and *V. unguiculata* from Philippines, *Raspberry ring-spot virus* on soybean from AVRDC (Taiwan), Sri Lanka, Thailand, USA, etc. and *Cherry leaf roll virus* on *Glycine max* from Taiwan, Sri Lanka, Thailand and USA and *Phaseolus vulgaris* from Colombia. Insects like *Acanthoscelides obtectus* in *Cajanus cajan* and *Phaseolus vulgaris* from Brazil, Colombia, Italy and Nigeria, *Anthonomus grandis* in *Gossypium* spp. from USA, *Ephestia elutella* in *Macadamia* nuts and *Vigna* spp. from USA, *Quadrastichodella eucalyptii* in *Eucalyptus* from Australia and nematodes like *Heterodera schachtii* from Denmark, Germany and Italy, *Ditylenchus dipsaci*, *D. destructor*, *Rhadinaphelenchus cocophilus*, etc. in soil clods and plant debris, and weeds like *Cichorium spinosum* and *Echinochloa crus-galis*, which are not yet reported from India.

The pests intercepted can be categorised as (i) many that are not known to occur in India; (ii) have different races/biotypes/strains not known to occur in India; (iii) are present on a new host or are from a country from where they were never reported before; (iv) an entirely new pest species hitherto unreported in science or (v) are reported to be present in India but with a wide host range. Interceptions, especially of pests and their variability not yet reported from India signify the importance of quarantine in preventing the introduction of destructive exotic pests. The third and fourth category of pests are not expected in the sample as per the risk analysis which is literature-based and since no records are available on

the pest/host their presence is unexpected and important from quarantine view point. The last category - pests with a wide host range are critical and could become invasive in case they find suitable biotic and abiotic environment. Such interceptions signify the success of quarantine, otherwise, these pests would have entered the country and played havoc with the plant biodiversity and agriculture.

### Issues in International Exchange of PGR

There are a number of issues related to quarantine in exchange of plant germplasm both legislative and quarantine methodology. The national quarantine legislation has classified all imports as:

- Prohibited plant species (Schedule IV);
- Restricted plant species where import is permitted only by authorized institutions (Schedule V);
- Restricted plant species permitted only with additional declarations of freedom from quarantine/regulated pests and subject to specified treatment certifications (Schedule VI) and;
- Plant material imported for consumption/ industrial processing permitted with normal Phytosanitary Certificate (Schedule VII).

Under the PQ order, PRA has been made mandatory for all material being imported into the country other than those present in Schedules V, VI and VII. The various schedules V, VI and VII of the PQ Order give lists of crops for which a generic PRA is given and detailed PRA is not required. In case of PGR, a large number of species of cultivated crops (and their wild relatives/ land races) with useful traits are imported. Such wild relatives, land races of germplasm whose pest profile is not adequately recorded hampers PRA preparation and consequently their import. However, in 2007 the legislation has been amended whereby ICAR-NBPGR is empowered to undertake PRA for germplasm material for pest free import. This is more relevant in the present context when access to germplasm is becoming more and more difficult under the Convention on Biological Diversity, 1992.

Another difficulty, which is faced during import of certain material, is the additional declarations being sought under the Schedule VI. Many countries from where a pest is not reported are unable to certify in the phytosanitary certificate the freedom from those pests. The recent amendments of the PQ Order have resolved

this problem to an extent by giving country specific requirements under additional declarations. This would greatly help the indentors in procuring germplasm of their interest from varied sources. Besides, NBPGR has also been empowered to relax certain conditions for import in specific cases where the material being imported is of utmost importance for the country.

The technical issues include issues pertaining to quarantine processing are as follows:

- Sample size based non-destructive procedures are required for detection
- A repository of antisera needs to be established as diagnostic reagents such as antisera for viruses/ bacteria are often not available for exotic pests.
- Expertise is also required in the field of taxonomy and biosystematics to identify unknown/new pests or strains.
- Highly sensitive and practically feasible molecular techniques are required for the detection of new pests/ races/ biotypes/strains/pathotypes etc.
- Need to consolidate the taxonomic information and prepare digitized keys for quick and reliable identification of insect pests is reference collections for exotic insect pests and identification keys are not readily available.
- Non-destructive and eco-friendly salvaging techniques are required.
- Post-entry quarantine testing at NBPGR and release from indexed virus-free plants may take one crop season.
- Strengthening of post-entry quarantine facilities at public and private stakeholders.

Websites like <http://www.plantquarantineindia.nic.in>, consisting of national database on legislation, quarantine procedures, methodologies, plant quarantine alerts, etc. designed by Directorate of Plant Protection, Quarantine and Storage (DPPQS) are available. However, an internet-based portal mechanism for exchange of official information to facilitate communication among countries needs to be developed. Also, availability of databases on quarantine pests and endemic pests would simplify the work of the quarantine personnel. Such lists of potential quarantine and endemic pests of different crop groups are being compiled at ICAR-NBPGR which would act as a ready reckoner.

It is clear that under the present international scenario, the quarantine specialists have a major role to play not only in promoting and facilitating the export and import in the interest of their respective nations but also in protecting the environment from the onslaughts of invasive alien species. The importance of quarantine has increased manifold in the WTO regime and adopting

not only the appropriate technique but also the right strategy for pest detection and diagnosis would go a long way in ensuring pest-free exchange of germplasm and transparency in international exchange, and is considered the best strategy for managing transboundary movement of pests.



## GM Diagnostics as an Aid to Strategic Genebank Management

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India has rich genetic diversity in cultivated crops and their wild relatives. It is one of the 12 mega-biodiversity centres and one of the Vavilovian Centres of Origin of Crop Plants. About 29% of flowering plants occurring in India are endemic and three of the 25 hot-spots of biodiversity exist here. While harnessing the benefits of genetically modified (GM) crops, for better yield, nutritional quality and resistance to biotic and abiotic stresses, it is important to safeguard the genetic diversity. Inadvertant and accidental occurrence of GM events may lead to adventitious presence of transgenes in the *ex situ* collections conserved in the genebanks. The genebanks collect, conserve and provide the genetic resources to the breeders, hence maintaining the purity of genetic identity of the germplasm is of critical importance. Therefore, all possible efforts should be made to prevent the unintentional introgression of transgenes into the conserved samples.

Systematic germplasm management procedures and practices vary from crop to crop, in terms of mode of pollination, breeding system, and whether the crop is annual or perennial. The available techniques and procedures do not enable the complete exclusion of unintentional presence of transgenes, in genebank accessions and therefore do not provide an absolute guarantee, without testing every single seed or plant that any given accession is free from transgenes. Only the best management practices and strategies will achieve the required degree of statistical probability to the effect that an accession in the genebank does not include unintentional presence of transgenes.

### Global Initiatives

International Maize and Wheat Improvement Centre (CIMMYT) has shared its expertise and advice with the Mexican institutions to formulate guidelines for maize conservation and related aspects and to conduct gene flow studies at Oaxaca and Chipas regions of Mexico. To discuss various strategies for handling the unintentional presence of transgenes in germplasm

collections, a workshop to develop policies for addressing the possibility of adventitious presence of transgenes in CGIAR *ex situ* collections was organised by the International Plant Genetic Resources Institute (IPGRI) at Rome in 2004. The workshop aimed mainly to evolve the technical consensus for developing an appropriate approach to handle the probability of unintentional introgression of transgenes into *ex situ* collections in the genebanks. The technical and economic information associated with institutional strategies and practices for collecting, managing and distributing materials to reduce this risk, including screening for GM elements and associated costs so as to formulate an action plan to minimize the adventitious presence of transgenes need to be worked out and shared with the genebanks.

### Future Strategies and Thrust Areas

The genebanks need to take proactive steps to determine the risk of unintentional presence of transgenes in *ex situ* collections. Information needs to be compiled addressing the crop-specific guidelines for best genebank management practices. The major area of unintentional introduction of transgenes is the collection and acquisition stage since the genetic resources may have been exposed to gene flow beyond the control of the genebanks. The strategies therefore need to minimize the gene flow of the transgenes at these stages. As part of their risk analysis, when collecting or acquiring new accessions by other means, the genebanks should ensure the following aspects before testing: (i) Whether transgenic events (commercial or research) in the relevant taxa are likely to be present in the area of exploration/collection; (ii) The distance between the collecting site and areas where transgenic events (commercial or research) are being cultivated; (iii) Whether germplasm providers can give adequate documentation of their germplasm management practices with respect to the material in question.

With already conserved germplasm accessions, genebank testing procedures need to be guided by the following criteria: (a) No testing would be required

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when: (i) there are no transgenic events (for commercial or research) in the relevant taxa; (ii) there were no transgenic events (commercial or for research) in the relevant taxa at the time of acquisition (e.g. *Bt* cotton prior to March 2002 in India); (b) there are transgenic events (for commercial or research) present, however, proper management practices have been followed and documented in the management of the accession.

A database on the global and national status of GM research and development for crops need to be maintained by the genebanks.

### Issues and Gaps in GM Detection

There are several issues pertaining to the development of methods for GM testing which need to be addressed:

- (i) Self certification/disclosure from source countries or personnel
- (ii) Obtaining gene sequences for synthesizing probes and primers from the developer of GM crop in case of indigenously developed GM crop and from the importer in case of imported GM crop
- (iii) Classifying areas and crops as per the possibility of contamination: certain hot-spots, where the possibility of accidental contamination by transgenic seeds is possible have to be identified
- (iv) Cost of detection: less for protein detection in the field, though developmental cost for the kits is high; moderate, if it is for one gene and one sample in case the primer/probe(s) of the transgenes are already available and high if several samples are to be analyzed and it is not known as to which transgene should be analyzed and fresh primer/probe(s) need to be designed and synthesized.

### Initiative at National Level

National Gene bank at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), the second largest genebank in the world, conserves more than 0.4 million accessions conserves of field and horticultural crops and their wild relatives. India being rich in agrobiodiversity, efforts for efficient management of germplasm are being made. For GM-free conservation, efficient strategy is to check the unintentional introgression/adventitious presence of transgenes in *ex situ* collections employing DNA-based GM diagnostics (Tiwari and Randhawa, 2010).

The expertise and capacity for DNA based

GM detection has been strengthened and upgraded significantly at ICAR-NBPGR during the last decade. Cost-effective strategies based on GMO matrix, polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP) and multi-target real-time PCR system, which facilitates testing of GM events have been reported by GM detection laboratory. PCR and real-time PCR based diagnostics for *Bt* crops, commercialized (*Bt* cotton) or under field trials (*Bt* okra, *Bt* rice, *Bt* eggplant) in the country, have been developed, which could be employed for monitoring adventitious presence of transgenes in respective crops (Randhawa *et al.*, 2010). To confirm the GM status of a sample irrespective of specific crop and GM trait, a hexaplex PCR-based screening assay targeting marker genes (*aadA*, *bar*, *hpt*, *nptII*, *pat*, *uidA*) commonly employed in the GM events, was developed (Randhawa *et al.*, 2009). GMO matrix of 141 GM events of 21 crops with 106 genetic elements was developed as a decision support system to check for authorized GM events (Randhawa *et al.*, 2014a).

A TaqMan® real-time PCR based multitarget system simultaneously detecting 47 targets for six GM crops was developed (Randhawa *et al.*, 2014b). Besides detection of commercialised GM cotton events, the system allows detection of five GM maize events (Bt11, Bt176, MON810, MON89034, TC1507), six cotton events (MON531, MON15985, GFM-cry1A, MON1445, MON88913, Widestrike), rice (Liberty Link), soybean (GTS40-3-2) and wheat (MON71800) using event-/construct-specific assays. LAMP assays were employed to detect commonly employed transgenic elements, *CaMV* 35S and *FMV* promoters, *aadA*, *nptII* and *uidA* marker genes, and *cryIAc*, *cry2Ab2* and *cp4-epsps* genes to check the GM status of the unknown samples (Randhawa *et al.*, 2013; Singh *et al.*, 2015). Event-specific LAMP assays have been reported for detection commercialized *Bt* cotton events, Randhawa *et al.*, 2015).

To ensure GM free conservation preliminary studies for checking adventitious presence of transgenes in *ex situ* collections of brinjal (150 accessions), cotton (280 accessions), maize (200 accessions), and okra (50 accessions) employing PCR and Real Time PCR based markers were undertaken. The strategy for selecting these accessions was (i) collection sites in proximity with the regions where field trials of specific GM

events of a particular crop were conducted (ii) the year of collection either after commercialisation of the particular crop or the year after which field trials of GM have been conducted. None of the tested accessions of these crops showed adventitious presence of transgenes; Bairwa *et al.*, 2016; Randhawa *et al.*, 2015; Parimalan *et al.*, 2015.

Case studies for monitoring of adventitious presence of transgenes in brinjal, cotton, maize and okra, employing DNA based markers could be used as models for monitoring other major crops with rich diversity or having the Centre of Origin in a particular country, where field trials of GM crops are being conducted in close proximity of those areas rich in their biodiversity.

Meticulously planned introduction of GM crops with effective risk assessment/management strategies and conservation of GM free *ex situ* collections in the National genebanks would ensure purity of conserved germplasm and safeguard the rich biodiversity.

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## Rust, Risk, and Germplasm Exchange: The Borlaug Global Rust Initiative

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Global food security depends on the free movement and open sharing of plant genetic resources. Some of the best examples of what can be achieved are the dwarf varieties of wheat and rice that contributed to the Green Revolution in India—genetic resources that came from Mexico, Japan, China, India and elsewhere.

Plant breeders and seed industries have contributed to the prosperity of the world's agricultural sector by identifying and deploying genetic resources through the processes of selective breeding. They have improved yield, disease resistance, and resistance to abiotic stresses, including many traits influenced by climate change. Their work has played a huge role in rural development and food security.

### The Borlaug Global Rust Initiative

The Borlaug Global Rust Initiative (BGRI) and the Durable Rust Resistance in Wheat (DRRW) project that serves as the BGRI's secretariat are excellent examples of the importance of free germplasm exchange. Beginning in 1999, the ability of the world's farmers to meet current and future demand for wheat was gravely threatened by new strains of stem rust disease emerging out of East Africa. Stem (or black) rust of wheat, caused by the fungus *Puccinia graminis*, is the most feared of the rusts of wheat. Stem rust can rapidly reduce a healthy looking crop to a black tangle of broken stems and shriveled grain in a matter of weeks. Historically, wheat farmers have suffered enormous losses from rust diseases: from the Romans who prayed to a "stem rust god" called Robigus, to US and Canadian farmers in the 1950s, who saw yield losses of 40% across spring wheat growing areas. The success of wheat breeders led by Norman Borlaug protected wheat farmers from the scourge of rust diseases from the middle to the end of

the last century, and certainly contributed to the stable yield gains of the Green Revolution.

**The Problem was Urgent:** about 90% of the wheat grown around the world was vulnerable to severe damage to the new types of stem rust disease emerging out of East Africa. Stem rust variant Ug99, identified in Uganda in 1999, was the only known type of *P. graminis* that has virulence against historically durable resistance genes used in breeding programmes, notably *Sr31*.

**The Problem was Spreading:** stem rust Ug99 was spreading out of East Africa via airborne spores. In early 2007, Ug99 was detected in Yemen and Sudan. Ug99 is now known to be as far north as Iran. Based on prevailing wind patterns, the spores are likely to migrate to other regions of Asia including India and Pakistan, although exact timing of dispersal is difficult to predict. Colonization of North Africa by Ug99 is also likely. International travelers could also spread rust spores on their clothing: similar rapid spread by (inadvertent) human transport has been documented for other plant diseases, including yellow rust of wheat.

**The Problem is Becoming More Severe:** stem rust is continuing to evolve in East Africa. New derivatives of Ug99 have been isolated that can overcome resistance genes *Sr24* and *Sr36*, both important sources of disease resistance for international breeding programmes.

**The Problem is Being Solved:** breeders do have access to a limited number of undefeated stem rust resistance (*Sr*) genes from various wheat varieties, landraces and wild relatives. Some of these sources of resistance are "minor" genes that are additive and need to be deployed together in one variety to provide effective protection (also known as Adult Plant Resistance, APR). A small group of elite high-yielding breeding

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lines possessing resistance are being tested already in several countries. Other sources, particularly from wild relatives, need to be “cleaned up” by having linked deleterious traits (linkage drag) removed before they can be used effectively in wheat breeding programmes. Marker assisted technologies can speed up these breeding approaches. The DRRW project (2008-2016) and its subsequent project, Delivering Genetic Gain in Wheat (DGGW) (2016-2020), envisions multiple approaches to achieve long-lasting stem rust resistance, from traditional breeding, to marker assisted selection (MAS) and high-end basic science explorations. This combination of approaches has been judged highly likely to succeed by a panel of 10 diverse external experts who reviewed the proposal for the Bill & Melinda Gates Foundation and the Department for International Development of the UK (DFID) who fund the DRRW and DGGW.

**Resource-poor Farmers are Especially Vulnerable:** commercial wheat farmers can access chemical protection against rust diseases, although available fungicides are expensive (estimated at \$40 per crop cycle to protect one hectare in Kenya) and pose risks to human health and the environment. Chemical protection offers, at best, a limited short-term fix and is unlikely to be accessible to vulnerable resource-poor farmers in countries like Ethiopia, Pakistan, India and Nepal in a timely manner. We can protect the livelihoods and food security of these farmers by supporting development and delivery of improved, resistant wheat varieties that do not require additional inputs for crop protection.

**Protecting Wheat Supplies is Important for Global Food Security:** wheat represents approximately 30% of the world’s production of grain crops. The FAO predicts that 598 million tons of wheat will be harvested this year on 220 million hectares of land. Nearly half of that production will be harvested in developing countries. On average, each person in the world consumes 68.2 kilograms of wheat each year. That equates to about 630 calories per day per person, or 1/3 to 1/2 of the minimal energy requirements of most adults—numbers that are even higher in many developing countries.

The DRRW and the DGGW breeding efforts to improve wheat focus on Africa, a continent that imports about 9 million tons of wheat a year (more than 80% of its wheat needs)—a gap predicted to increase steadily in the future. Poor consumers in Africa and elsewhere are particularly vulnerable to price increases

related to wheat—vulnerability that contributes to social instability.

Increases in wheat productivity were a major driver for the Green Revolution, especially in India and Pakistan. In India, wheat yields grew from a total production of 12 million tons (MT) in 1965, to 76.37 MT in 2000. The Government of India has projected that wheat demand could grow to 109 MT by 2020. Crop shortfalls are also occurring in other key wheat producing nations, such as Australia, where recurring droughts in recent years have reduced production.

Using a very conservative estimate of 10% loss in regions hit by Ug99, annual global losses could be devastating in the absence of germplasm and effective breeding programmes. The largest target market would be the 50 million farming families in the Indo-Gangetic plain who rely on wheat production, who would stand to lose over 7 million tons of annual production (\$2.3 billion) for each 10% drop in yield. The reality could be much more frightening since much higher losses are possible. Between Rabat and Vladivostok, there are over 100 million hectares of wheat under cultivation, most of it genetically susceptible to Ug99. The 1953 rust epidemic in North America resulted in average yield losses of 40% across US and Canadian spring wheat growing areas.

### Constraints on Germplasm Exchange

Increasingly, however, what was previously a free and open exchange of wheat germplasm has become mired in forms and legal documents associated with the multilateral Convention on Biological Diversity<sup>1</sup> and its series of attendant treaties and conventions—particularly the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), the Nagoya Protocol<sup>2</sup>, and the Access and Benefit-sharing Clearing House (ABS)<sup>3</sup>. Increasingly, the burden is on the researchers to determine that the germplasm they are deploying has been acquired legally.

The Convention on Biological Diversity affirms that states (countries) have sovereign rights over their own biological resources. Each state draws up its own

1 Convention on Biological Diversity <https://www.cbd.int>

2 Nagoya Protocol <https://www.cbd.int/abs/>

3 Access and Benefit-sharing Clearing-House: <https://www.cbd.int/abs/theabsch.shtml>

regulations. Some are particularly draconian, protective and punitive.

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation to the Convention on Biological Diversity aims to share the benefits arising from the utilisation of genetic resources in fair and equitable ways. It was ratified on 12 October 2014. Since then, it has become the means by which plant breeders and the seed industry are increasingly subject to legal wrangling.

The Access and Benefit-sharing Clearing-House (ABS Clearing-House, within the Nagoya Protocol) is a platform for exchanging information on access and benefit sharing established by Article 14 of the Protocol, as part of the Convention on Biological Diversity. The ABS Clearing-House is a key tool for facilitating the implementation of the Nagoya Protocol, by enhancing legal certainty and transparency on procedures for access and benefit-sharing, and for monitoring the utilisation of genetic resources along the value chain, including through the internationally recognized certificates of compliance.

“Legal certainty,” “access and benefit-sharing” and “monitoring the utilisation of genetic resources along the value chain” brings international lawyers, accountants and bankers with little to no background in plant breeding onto the playing field of crop improvement as referees.

Previously plant breeders in the CG system and public sector universities freely exchanged germplasm for plant improvement and did not assess monetary “value” along the “value chain” particularly since some of the “value” did not become evident until genes were used in combination, stacked and/or otherwise proven in performance trials. But, today, without the right “forms”

all along the value chain, anyone from CEOs of seed companies to researchers employed by public research institutions can be charged and arrested for violating any one of many protocols.

There are certain provisions of the Convention on Biological Diversity that have merit. It is an important tool to protect various bio-resources for end uses like: drugs, industrial enzymes, food flavors, fragrance, cosmetics, emulsifiers, oleoresins, colors, extracts, and genes used for improving crops and livestock by means other than conventional breeding or traditional practices.

But stringent regulations and country-specific control are stifling the germplasm exchanges critical to agriculture and horticulture, including development of hybrids, introgression breeding for introgressing transgenic traits, and seed production including hybrid seeds. It is not only the improved seeds that are subject to regulation, but isolates of country-specific disease organisms such as Ug99 stem rust that move between collection sites worldwide and biosafety testing labs in Minnesota, Denmark and Canada, and that are subject to the Convention on Biological Diversity because they are “bio-resources.”

### **Sustaining the Delivery of Genetic Gain in Wheat**

Exchanges of seed and germplasm and even disease organisms between and among countries are critical to global plant breeding programmes. Wheat germplasm and pathogen exchanges have been essential in the Durable Rust Resistance in Wheat (DRRW) project and will be even more so under the Delivering Genetic Gain in Wheat (DGGW) project as plant breeders broaden their investigation to deliver new traits for wheat that fight diseases, pests, drought and other challenges brought about by global climate change.

## Dimensional Analysis of International Regulations of Biological Matter. Genetic Resources and Agrobiodiversity

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Developments in biotechnology, molecular genetics, intellectual property regimes and access to genetic resources and related information over the last two decades are rapidly changing the conditions for public research. The emerging “proprietary science landscape” is comprehensive (Table)

Adding the Nagoya protocol to the above presented box gives indeed food for thought as regards how all these agreements—several of them legally binding—may work in everyday life for scientists, farmers and in trade. These conditions are global and comprise an *evolving new global legal regime related to all biological matter*. Here we will refer mainly to three international treaties (several others also matter see box above) that have considerable bearings on public research, namely:

- the Biodiversity convention/CBD + the Nagoya Protocol
- the WTO-agreement and its annex on trade related intellectual property rights/TRIPS

- and the FAO international treaty on plant genetic resources for food and agriculture/FAO-IT.

The two latter have (different) provisions for intergovernmental enforcement and sanctions, while the first (CBD) leaves this subject to national legislation. In short the CBD means nationalization of genetic resources (previously seen as part humankind’s common heritage), TRIPS sets minimum standards for what must be protected as intellectual property/IP and the FAO-treaty stipulates multilateral access and benefit sharing/ABS rules for some 50 crop genera of high country interdependence and for global food security. In short the new subsequent regulatory regimes on access and ownership means an enclosing of the biological and genetic commons. In this zero sum game the public domain is continuously reduced as more and more of the commons are proprietized. Thus a move from being free public good to private, corporate or state property. Biological common rights are thus replaced with regulated/discriminating access.

**Table**

CBD 1992/93	TRIPS WTO 1994	FAO-IT PGRFA 2001/2004	UPOV 1978/91	WIPO ICGRTKF 2000	Cartagena Biosafety Protocol/ 2000
National sovereignty SNL Art 15 PIC/MAT Art 8j Protection of TK ABS - Bonn Guidelines/ Nagoya protocol	Mini-standard for IP (time limited) Art 27.3(b) <i>Sui generis</i> –protection Microorganism Microbiological processes Plant varieties  Patent Copyright Trademark Trade secret Industrial designs Integrated circuits Databases * Novelty/ non prior art * Innovative step * Industrial appl'n Morality, Ordre public	Multilateral access to ca 50 crop genera Standard MIA Art 9.3 Farmer’s Rights SNL Farming system knowledge Landraces Farmer varieties SNL	Plant Variety Protection PVP (time limited) * New * Distinct * Uniform * Stable Common knowledge Public domain Extant varieties Farmer’s privilege SNL Breeder’s Exemption SNL	Protection of TK CO/DO in IP-application International ABS regime Standardized PIC/MAT regime	Transboundary movement of LMOs Art 10 Right to delay import subject to further scientific biosafety check Art 17 Unintentional transboundary movements and emergency measures  IPPC/1997 Art V, VI, VII regarding phytosanitary requirements in shipping of plant material

Source: CG Thornstrom, 2012

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The emerging new legal regimes have deep impact on the freedom to operate/FTO for public science. In fact for public (and in fact also private sector) research we can summarize this in an equation: IP+ABS=FTO or in other words –*proprietary science*. Thus if we marry IP with ABS, how to create a viable offspring that has considerable FTO for science and scientists? In the following we will focus mainly on plant genetic resources. Animal genetic resources fall under CBD provisions while human genetic resources is subject to different other legal provisions mainly conventions and protocols under the World Health Organization.

### Moving into a global legal can of worms?

In a study (CGIAR GRSS 2010:21) the following observation was made as regards microbial and insect genetic resources “... more than 53,000 accessions of living microorganisms or cell cultures and an astounding collection of insects and other arthropods numbering about 420,000 are collectively held in the CGIAR Centers. The ... survey also contacted 28 worldwide bio resource centers, of which 26 responded showing more than 7 million accessions of living and nonliving materials.” So which is the *legal status* of these 7 million accessions in the context of CBD, FAO-IT, TRIPS and UPOV? The GRSS-study gives the following answer (Ibid page 22): “...most collections of non-crop genetic resources are used by researchers to develop their activities and sometimes specimens are exchanged with partners, without due regard to IP or the access and benefit sharing regulations. Can these accessions be regarded as Global Public Goods? Or are they only genetic resources for current research? It seems that a serious discussion is needed as basis for further investment on conservation, capacity building, and infrastructure or to establish connections with local institutions and international repositories for the maintenance of the specimens that are indispensable for the research projects.”

The CBD is one of the fastest approved international legally binding agreements. Negotiations started late 1987 and the final text was endorsed in Rio 1992 and entered into force in December 1993. The CBD places every living cell and its derivatives on the planet (with the exception of humans) under national sovereignty where access by other parties to the Convention is subject to the PIC/MAT procedures as adopted in different national legislation. The negotiations for an exception covering plant genetic resources for food and agriculture started already in 1983 (10 years before) and resulted in the

FAO-IT 2001 which entered into force in 2004. In the history of international treaties the link between CBD and FAO-IT is an almost hilarious one. Policy making is an extremely complex game, especially when advanced genetics, evolution/genetic drift and legal matters are to be usefully –and fairly politically correctly- combined. To expect that the discussions around genetic resources for food and agriculture led by national ministries of agriculture (starting on larger scale in the 1970’s) would have influenced the discussions on implementing benefit sharing under CBD would be naïve. And still: during the late part of that period a similar negotiation process was on, led by national ministries of the environment to establish another internationally binding agreement which adopted in October 2010 by parties of the CBD – the Nagoya-Cali Protocol on Access and Benefit Sharing (GRSS 2010:3). The CGIAR GRSS study (2010:70) makes the following observation of the Protocol’s text: “While a certain degree of creative ambiguity is a hallmark of international accords, the text of the Nagoya protocol has left experts puzzled about what exactly has been agreed on for many critical issues, including the substantive and temporal scope of the agreement and the application of the definitions – derivatives and utilisation, giving rise to a range of partially conflicting interpretation.”

### Agrobiodiversity and the Nagoya Protocol

Agrobiodiversity concerns biological material and associated knowledge regulated under half a dozen different international treaties. Here it is extremely important to note that all the different regulatory regimes use concepts that either overlap or criss-cross over different legal regimes. The UPOV–and FAO-IT legal languages are specific as regards the concepts **scope of protection** and **rights conferred** (*Seeding solutions* vol 2 page 10 and 95) and very different from the more political languages used in the CBD and the Nagoya protocol. In the European Commission process (September 2016) the Nagoya–protocol assumes six ‘biological categories’: cosmetics, plant breeding, biocontrol, pharmaceuticals, food and feed, biotechnologies. But these general concepts do not lend themselves easily to legal specifications. Living biological matter: plants, animals, microbes etc are all part of evolution and their life cycles do certainly not respect certain given national borders in their life cycles. Further inherited traits are not necessarily specific for a certain species. Still further it is unclear whether the definition of genetic resources in the Nagoya protocol

includes sequenced data deposited in public international databases. On top of this biological and medicinal model organisms are international – and thus outside the Nagoya provisions. Wild relatives of cultivated crops fall under the FAO-IT. But for example India urged in early 2012 that such material collected in India after enforcing of the Nagoya protocol should be made available on Nagoya-provisions – not those of the FAO-IT. Still another challenge is to clear and safely beyond any doubt make sure that a microorganism is unique on a unique sovereign country's territory. While in reality microorganism through winds, ocean currents, international trade (fruits/vegetables), migration of insects, migrating birds, animals and humans continuously cross national borders it is in reality very difficult or completely impossible to identify a single national origin – as assumed/required in the Nagoya protocol.

Further the Nagoya protocol assumes (like the CBD article 8j) easily identifiable traditional groups, that are in full legitimate control of their stable homelands and which 25 years after the entering into force of the CBD all of them now have effective smart/smooth PIC/MAT-procedures for Prior Informed Consent and Mutually Agreed Terms. In reality so far very few such smoothly organized procedures are in place in most indigenous communities or most countries. Many countries members of the Andean Pact have enacted draconian access legislation. The challenge is how to find smooth and effective PIC/MAT-procedures when each member country may have 20-50 indigenous groups/tribes with their own 'national' territories. And where the same "Nagoya material" may occur on the territory of several of these groups/tribes/countries. So far few or even no successes have been achieved at the scale expected in national and international legislation. Thus the COP of the CBD has over decades made few successes. The Nagoya protocol is a politically negotiated compromise. Focusing on static/stable mixes of stable species. Assuming stable and easily defined local communities and effective government structures. In reality impossible to translate into workable and effective access/transfer procedures that respect interests of local communities and increase and facilitate scientific and commercial exchange. Another challenge is the concept of **intangible genetic information** making it possible to digitalize a whole genome and "beam" it to another location – thus no longer in need of the biological matter *in situ* as the CBD/Nagoya assumes. Given these realities my own

university Swedish University of Agricultural Sciences proposed to the Swedish government that Sweden shall **revoke** ratification of Nagoya protocol.

### Concluding Remarks

The CBD is one of the fastest negotiated international agreements. It took less than 6 years and adopted in Rio 1992. It took another two decades to negotiate the subsequent Nagoya protocol. May introduction of the concept **agrobiodiversity** facilitate overview and rationalization/slimming of obligations under different treaties? The FAO-IT and the Global Diversity Conservation Trust have still not yet reached enough long-term and secured funding. And the CGIAR is facing rapidly shrinking core funding through its "Window 1". The upcoming 1<sup>st</sup> International Agrobiodiversity Congress scheduled for ICAR in New Delhi in November 2016 may be a proper and timely forum to reconsider the overloaded and underfunded international regulatory regimes now increasingly blocking scientific progress globally. Here I would recommend a recent PhD-Thesis ca 450 pages (2014) by Dr Frantzesca Papadopoulou at the Dept of IP and Market law Stockholm University Faculty of Law entitled: **Opening Pandora's Box Exploring Flexibilities and Alternatives for Protecting Traditional Knowledge and Genetic Resources under the Intellectual Property Framework** (available at: <http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A694432&dswid=-4339>).

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### Footnote: Agrobiodiversity and antagonistic threats

In January 2009 the European Commission published its report on Chemical, Biological, Radiological and Nuclear security. The Swedish Ministry of Rural Development instructed SLU to appoint a classified group of senior scientist tasked to look further into possible antagonistic threats – called bioterrorism to Swedish food and agriculture. The present author was–due to his studies of agricultural research in Germany during the Nazi-era 1933-45 tasked to start and chair this group 2009-2013 and of which he still is a member. For details please consult: <http://online.liebertpub.com/toc/bsp/11/S1>

## Indian Initiatives on Farmers' Rights

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Plant Genetic Resources (PGR) are the heritage of humankind and the foundation for attaining food, nutritional and health security. Before 1993, the PGR were shared freely among countries for the betterment of human beings, till concerns of conservation of biological diversity were raised by the Convention on Biological Diversity (CBD). Consequently, many issues regarding the rights of the farmers as conservers, protectors and developers of PGR in the biodiversity hot-spots, the researchers and breeders who invest on development of improved varieties with superior traits, the users of PGR and intellectual property rights related matters emerged.

India is a signatory to both CBD and World Trade Organization (WTO) conventions. The Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) is an International Agreement administered by the WTO that sets down minimum standards and regulations for many forms of intellectual property (IP) as applicable to WTO Member Nations. Nations seeking to obtain easy access to the numerous international markets opened by the WTO must enact the strict intellectual property laws mandated by TRIPS. The WTO under the Article 27.3 (b) of the TRIPS, for the protection of plant varieties provided different options namely by patents, by an effective *sui-generis* system or a combination of both. As a corollary to this, India opted for the *sui-generis* system for the plant varieties giving importance to farmers' rights compared to the provision of the International Union for the Protection of New Varieties of Plants (UPOV). With intensive and extensive national level consultations and dialogues, the Government of India enacted the "Protection of Plant Varieties and Farmers' Rights Act (PPV&FR Act)" in 2001. The PPV&FR Act recognises the multiple roles of farmers with respect to their contributions made in conserving, improving and making available PGR for the development of new plant varieties and also evolvers of farmers' varieties.

India is the first country to provide substantial rights to farmers and registration of their varieties is one of them. The PPV&FR Act seeks to address the rights of plant breeders and farmers on equal footing. The other provisions related to farmers' rights are presented in Fig. 1 and the Indian PPV&FR Act is a model in protecting the interest of the farmers of the agrobiodiversity rich countries.

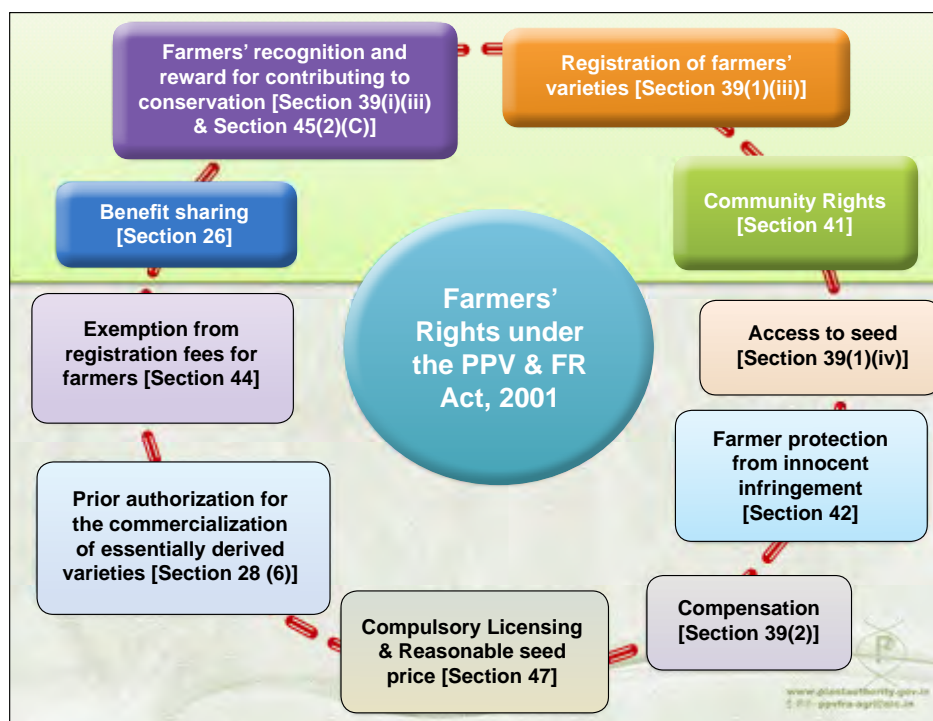
### Why to Protect Farmers' Varieties?

Farmers' varieties are outcome of centuries efforts by the farmers/tribal communities, who selected the plants of economic importance from the wild species/relatives and landraces. Through continuous efforts, from the PGR available in particular agrobiodiversity hot spot regions, these varieties were selected and conserved dynamically and possess climate resilience traits which is the need of the hour. Some varieties in different crop species are tolerant to biotic and abiotic stresses besides their suitability to contingency planning in case of weather aberrations. Some of the varieties possess medicinal, nutraceutical, therapeutic and pesticidal values. Varieties such as "*Kasala*" in rice possess a gene "*Pstoll*" which fixes the phosphorus from phosphorous poor soils. Some important farmers' varieties with their unique traits are presented in Table 1. These varieties are unique genetic stocks in developing improved varieties/germplasm. Under post WTO era, where restrictions on free exchange of PGR is imposed worldwide, the dynamic conservation, promotion and protection of PGR in the form of local/farmers' varieties achieves greater importance.

### National Gene Fund for Promoting PGR Activities

On the basis of richness of agrobiodiversity i.e. number of crop species, crop varieties, wild relatives of various crop species cultivated, social relevance and ancientness of the agriculture, wild relatives of crop species occurring in the region, number of species domesticated and the uniqueness of the agro-ecosystems, the PPV&FR

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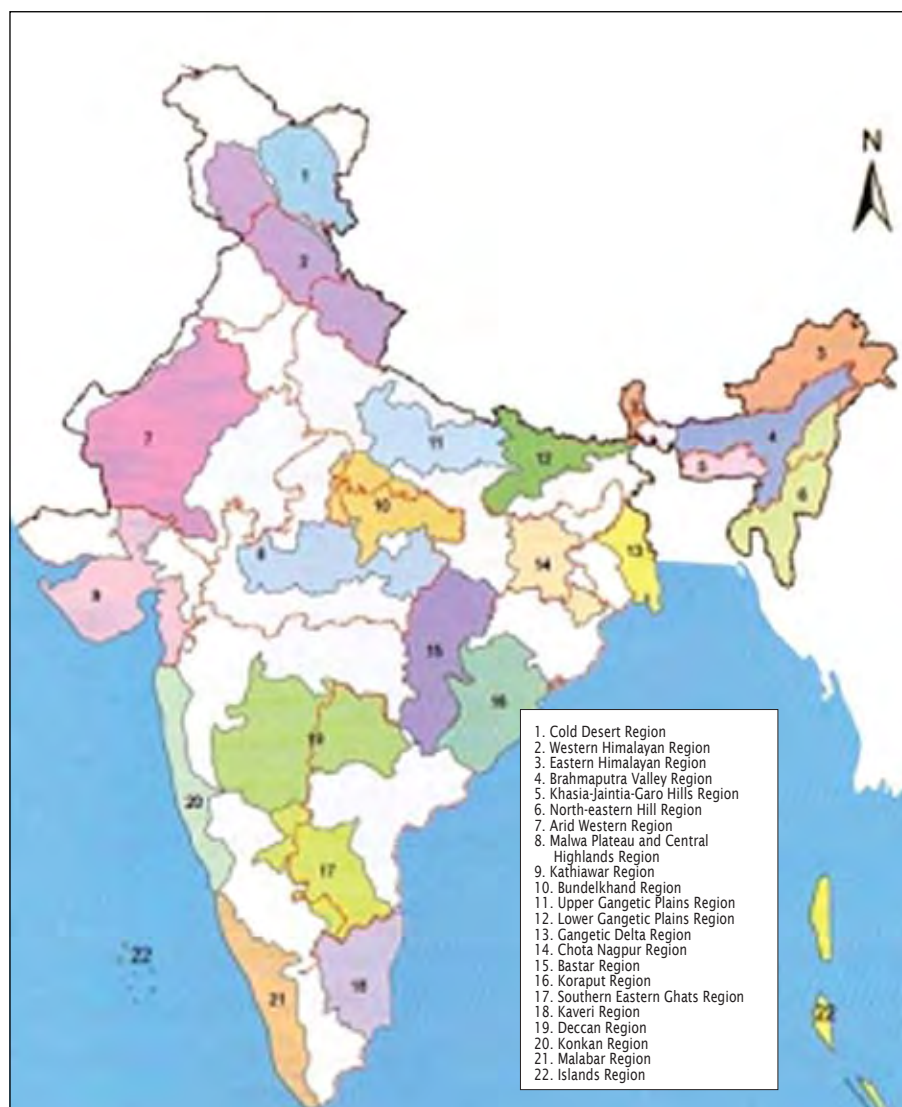
**Fig. 1. Provisions related to Farmers' Rights**

**Table 1. Farmers' varieties with their unique traits**

Farmers' Variety (crop)	Unique Traits
Navara-Rice	Medicinal: curing circulatory, respiratory digestive, nervous system ailments and pest resistance
Borah Rice	Absence of the need for cooking, instant cooking
Pokkali Rice	Salt resistance
Kasalath Rice	Pstol1 gene
Chennellu Rice	Curing stomach ulcer
Thondi Rice	Drought tolerant
Pokkali Rice	Salt resistance
Jugal, Sateen-Rice	Resistance to BLB & BPH, multiple grains
Rajmudi Rice	Quality rice
Sarvat Rice	Antidote to Poisonous snake bite
Bunny grass	Resistant to salinity (Rann of Kutch)
Karchia Local Wheat	Salt tolerance
Naga Chilli (Bhut jholakia)	Pesticidal value
Dagadi-Sorghum	Best quality Roti
Pundya-Sugarcane	Table purpose
Kali tur-Pigeon pea	Resistant to drought, SMV, Good quality
Jhakrana Bajra	Drought tolerant

Authority has identified 22 agrobiodiversity hot-spot regions in India viz., 1. Cold Desert Region, 2. Western Himalayan Region, 3. Eastern Himalayan Region, 4. Brahmaputra Valley Region, 5. Khasia-Jaintia-Garo Hills Region, 6. North-eastern Hill Region, 7. Arid Western Region, 8. Malwa Plateau and Central Highlands Region, 9. Kathiawar Region, 10. Bundelkhand Region, 11. Upper Gangetic Plains Region, 12. Lower Gangetic Plains Region, 13. Gangetic Delta Region, 14. Chotanagpur Region, 15. Bastar Region, 16. Koraput Region, 17. Southern Eastern Ghats Region, 18. Kaveri Region, 19. Deccan Region, 20. Konkan Region, 21. Malabar Region and 22. Islands Region (Andaman & Nicobar Islands, Lakshadweep) (Fig. 2).

Farmers who have been engaged in conservation and preservation of plant genetic resources (PGR) of landraces and wild relatives of economic Plants and their improvement through selection and preservation in these identified 22 agrobiodiversity hotspots, receive recognition and rewards from the National Gene Fund. This provision, when taken in conjunction with the provisions relating to the farmers' privilege, is similar to the concept of Farmers' Rights contained in the International Treaty on Plant Genetic Resource for Food and Agriculture (ITPGRFA) (Article 9.1).



**Fig. 2. Agro biodiversity hot spot regions of India**

The National Gene Fund receives contributions from central government, national and international organizations and other sources [section-45 (1-d)]. The gene fund also receives funds from benefit sharing [section-45 (a)] from the breeder of the variety or an essentially derived variety registered under the Act or propagating material, of such variety or essentially derived variety as the case may be the compensations deposited [section-41 (4)] and the annual fee payable to the Authority by way of royalty [sub-section (1) of section-35 (d)]. The expenditure of the fund are earmarked for the payment of benefit sharing, compensation required for use of genetic material towards evolution of new and essentially derived variety, to meet expenditure incurred

for conservation and sustainable use of genetic resources and for framing of schemes related to benefit sharing. The gene fund is also used for capacity building on *ex situ* conservation at the level of local body, particularly in regions identified as agrobiodiversity hot-spots and for supporting *in situ* conservation. Thus, in this way it can be considered to be a national equivalent to the global benefit sharing fund operating within the ITPGRFA.

### **Plant Genome Saviour Awards, Rewards and Recognition**

The gene fund is also utilised to support and reward farmers, particularly the tribal and rural communities engaged in conservation, improvement and preservation

of genetic resources of economic plants and their wild relatives, particularly in areas identified as agrobiodiversity hot-spots (22 Agrobiodiversity hot-spots distributed over 7 agro-geographical zones (Fig. 2). Recognising the important contribution of farmers and farming communities and their role in enhancement of quality in research and development in agriculture and to energise and implement Rule 70(2) (a) of PPV&FR Rules, 2003 and the provision of section 45 of PPV&FR Act, 2001, the PPV&FR Authority in consultation with the Government of India instituted the Plant Genome Saviour Community Awards (maximum of five awards per year consisting of a citation, a memento and cash of One million rupees each). Since 2009-10 this award has been granted to 15 communities from different agrobiodiversity hot-spot regions. Government of India has notified the Protection of Plant Varieties and Farmers' Rights (Recognition and Reward from the Gene Fund) Rules, 2012, whereby a farmer who is engaged in the conservation of genetic resources of landraces and wild relatives of economic plants and their improvement through selection and preservation and the material so selected and preserved has been used as donors of gene in varieties registerable under the PPV&FR Act, 2001 shall be entitled to Plant Genome Saviour Farmer Reward (maximum of 10 rewards per year comprising of a citation, memento and cash of Rupees 0.15 million each). Already twenty farmers have been awarded with Plant Genome Saviour Farmer Reward. Another award namely Plant Genome Saviour Farmer Recognition (maximum 20 recognitions per year) consists of a citation and memento and a cash of Rupees 0.10 million each. Nineteen eligible farmers have been recognised with this award for their PGR conservation efforts.

### **Supporting Plant Genome Saviour Awardee Communities**

PGR conservation, protection and promotion for sustainable use are being practiced by farmers and their families since ancient time. This has allowed them to cultivate a large number of local varieties in different crop species of economic importance. This is how India has been regarded as one of the mega bio-diversity centres in the world. To support the activities of PGR, the PPV&FR Authority has selected the Genome Saviour Awardee Communities to support their efforts of saving local varieties and landraces. As climate change has a

significant impact on agricultural production, growing local varieties which have a high degree of genetic diversity is highly important because these varieties have the ability to better withstand and adapt to environmental stresses and change. Setting up community seedbanks may help farmers to acquire varieties that are adapted to local conditions; these varieties may not be accessible through formal seed systems, may be costly or may suffer from erratic supplies. To make available the quality seeds of popular local varieties/planting material through informal seed chain, the PPV&FR Authority is promoting "Community Seed Bank Concept" for field crops and "Community Nursery Bank/Community Clonal Gene Bank" for vegetables, fruits and trees, medicinal and aromatic plants and fodder grasses at different Agro-climatic bio-diversity hot-spots where improved varieties have not made impact on production and productivity. PPV&FR Authority has identified regions in agrobiodiversity hot-spots and mainstreaming of farmers' varieties is being taken up by following unique maintenance breeding programme for the supply of seed/planting material.

### **Training and Awareness Programmes**

The Authority, since inception, took initiatives to popularise provisions of the Farmers' Rights provided in the PPV&FR Act to the civil societies and grass-root workers. There exists a close linkage with the farmers, researchers, plant breeders, intellectuals, scientists, students, NGOs, and public and private organizations active in this area. The PPV&FR Authority has been releasing funds for training, awareness and capacity building on the provisions of the PPV&FR Act, 2001 including Farmers' Rights, Breeders' Rights, and Researchers' Rights involving different stakeholders viz. ICAR Institutes, State Agricultural Universities (SAUs), Krishi Vigyan Kendras (KVKs), NGOs and other Government Departments for the farmers, researchers, plant breeders, intellectuals, scientists and students etc., and also for creating awareness through participation in agricultural fairs, kisan melas, kisan utsav, farmers' forum etc. In different regional languages bulletins have been prepared and distributed. Frequently asked questions have been prepared with answers and distributed to clear the doubts. Street Drama CDs have also been prepared and distributed. The details of training programmes organized by the PPV&FR Authority are presented in Fig. 3.

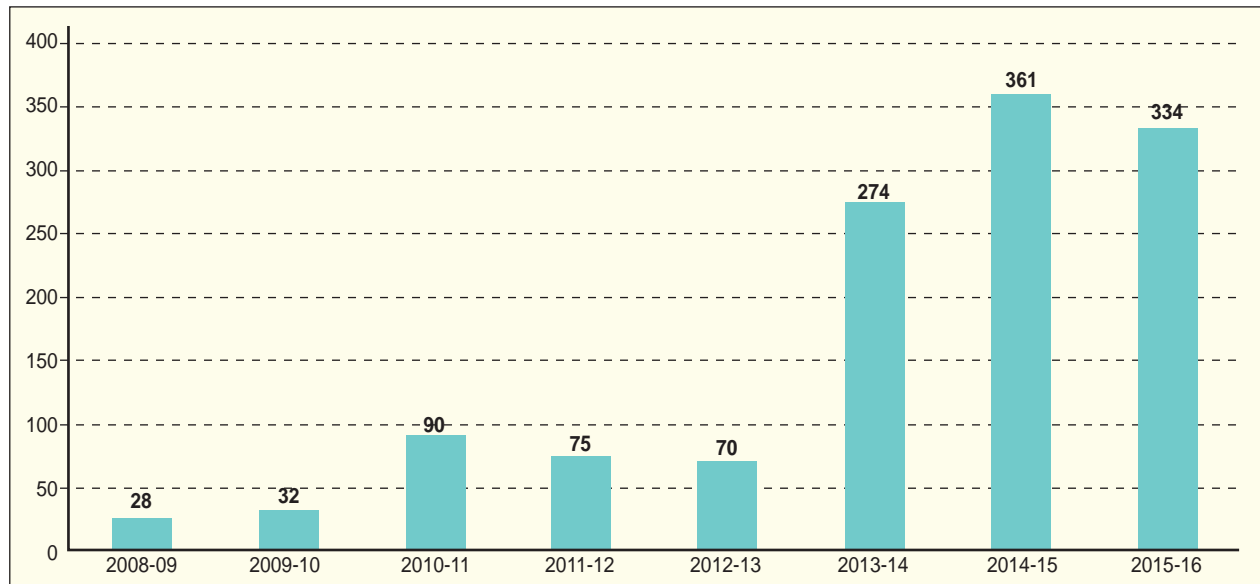


Fig. 3. Details of training programmes organized by the Authority

### Impact of Training and Awareness Programmes in Registration of Varieties and Response from Plant Genome Saviour Awards

The filing of applications for registration of farmers' varieties (Fig. 4) which commenced from 2007 indicated inconsistent trend. For the first three years it was in ascending trend with sudden increase in filing 127 applications during 2009. However, the trend in 2010 was

far from optimal with only four applications being filed. On the contrary, during 2011, there was a sudden rise in the filing of applications by 939 while in 2012, there was a sudden drop by filing only 302 applications. Further during 2013-14 the Authority launched a programme "Take it to the Farmer, The Farmers' Rights through awareness" involving National Agricultural Research System (NARS). As a result during 2013, there was a

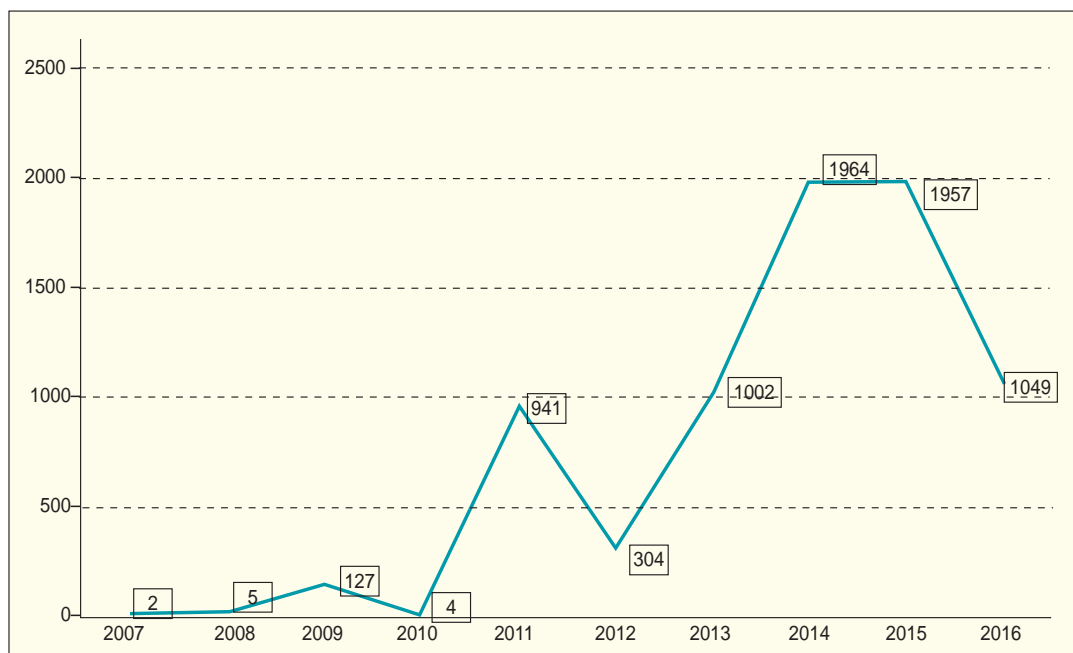


Fig. 4. Yearwise trend of filing application of farmers' varieties for IPR

**Table 2. Details of applications received for the “Plant Genome Saviour Awards”**

S. No.	Name of the Award	2007-08			2008-09			2009-10			2010-11			2011-12			2012-13			2013-14			2014-15		
		Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given	Total Application Received	State Participated	Total Award Given
1.	Plant Genome Saviour Community Recognition Certificate	-	5	5	15	-	4				19	11	7												
2.	Plant Genome Saviour Community Award							20	11	2	19	11	4	27	11	4	28	15	5	26	14	5 for Community Award Approved by the Selection committee	14	10	
3.	Plant Genome Saviour Farmers' Reward													30	13	10	80	20	10	78	16	3 Applications for Reward approved by the Selection Committee	46	10	Under process
4.	Plant Genome Saviour Farmers' Recognition													30	13	15			4			11 Application for Recognition approved by the Selection Committee	46	10	

record number of 1001 applications received and the same trend is being continued in 2014, 2015 and till October, 2016 with receipt of record number of applications of 1964, 1957, and 1221 respectively. The Authority has also taken initiative in documentation, indexing and cataloging of Farmers' varieties. The compendiums of registered farmers' varieties have been published.

The awareness programmes also made good impact on the receipt of applications for genome saviour awards also. The details of the applications received and states participated is presented in Table 2. In the beginning the response from the farmers was very poor. But as a result of awareness programmes as many as 108 farmers from 21 biodiversity rich states participated and filed applications for awards.

### Notification of NARS as Centres for Compensation under Section-41 of the PPV&FR Act

With the support from NARS, arrangements are being made to notify the centres under section-41 of the PPV&FR Act, so that any village or local community in India can file in any centre notified in the Gazette of India, the claim for compensation against registered breeder if the contribution of the community is significant in the evolution of such registered variety.

Thus India has taken many unique initiatives to implement the provisions provided as Farmers' Rights in the Act and to conserve the PGR for sustainable use in the days to come.

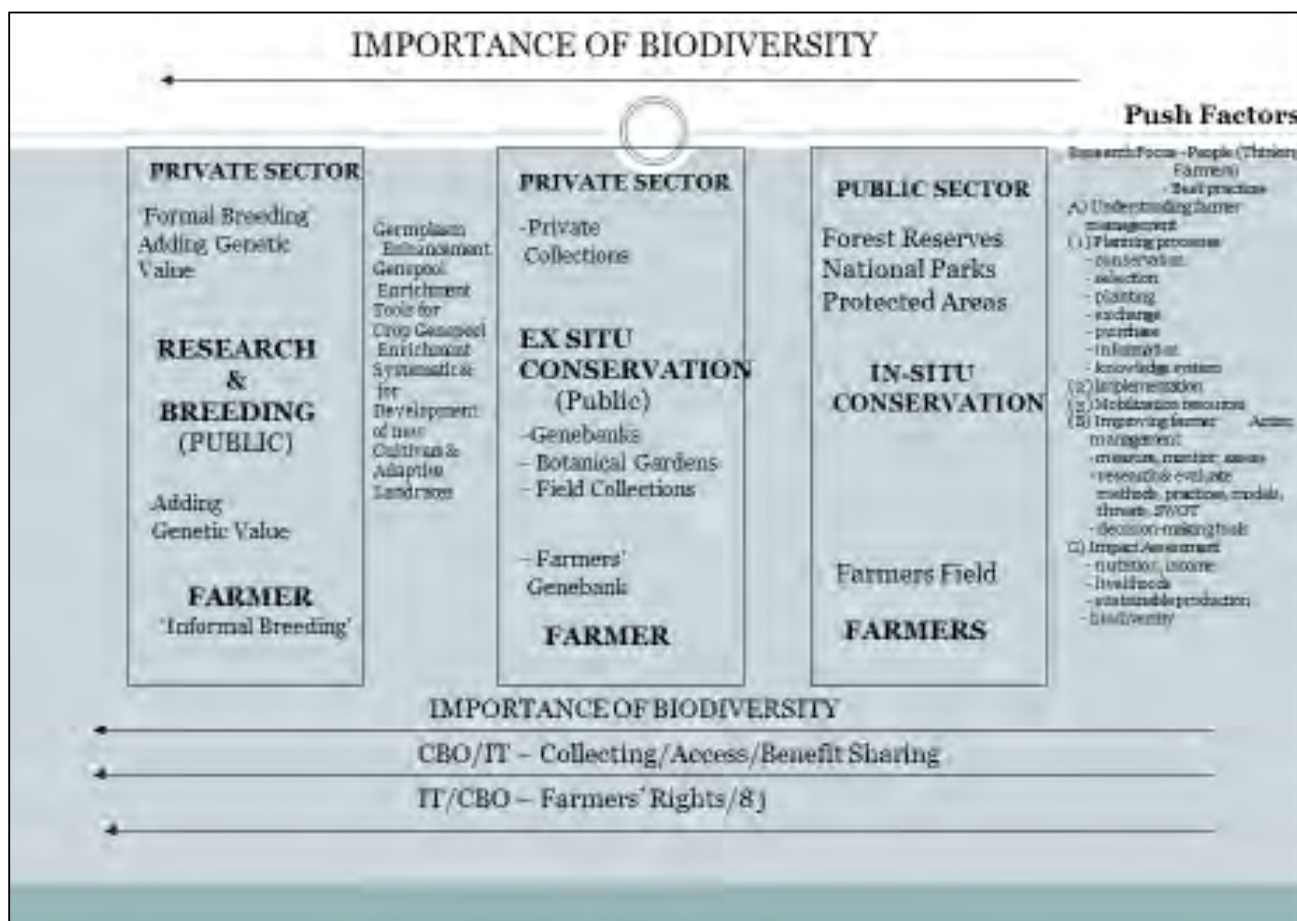
## The Role of ITPGRFA in Agrobiodiversity Management: Future Strategies and Actions

**Lim Eng Siang**

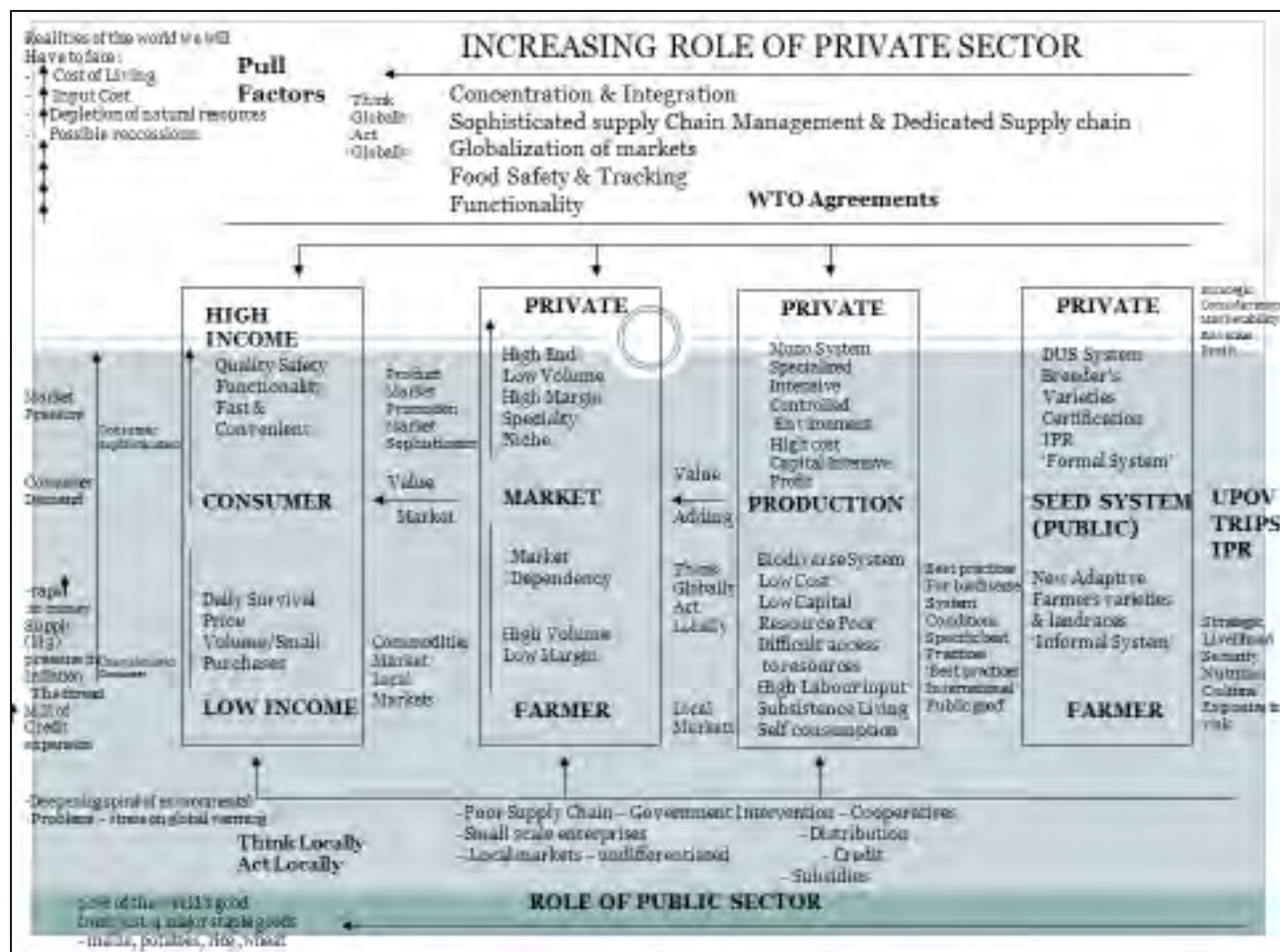
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One of the serious threats to agrobiodiversity is the standard-based modern industrial food system. The market forces of this food system linking use to conservation are pushing the approaches to agrobiodiversity management in the field. Various global efforts have been made to promote agrobiodiversity management. One such efforts is the adoption of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) in 2004. The Treaty recognises the important role of custodian farmers in maintaining, adapting and promoting agrobiodiversity. As such, one of the strategic directions of the ITPGRFA

is the encouragement of custodian farmers to produce desired agrobiodiversity products and services at the field level. These desired products and services are those which are i) produced by farmers, ii) for the farmers, iii) for conservation and sustainable use of PGRFA, iv) for food security and v) for improved livelihoods. The ITPGRFA encourages such desired products and services through various provisions of the Treaty as well as through the Benefit Sharing Fund (BSF), in particular Article 9 of the Treaty on Farmers' Rights, Article 6 on Conservation, Article 6 on Sustainable Use, Article 13 on Benefits Sharing, and Article 18 on Funding Strategy. The



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BSF seeks to invest in high impact projects supporting farmers in developing countries conserve crop diversity in their fields and assisting farmers and breeders globally adapt crops to changing needs and demands.

So far the results on the implementation of the various provisions in the Treaty for improvement on agrobiodiversity have not been encouraging. Contracting Parties are not making an active effort to implement Farmers' Rights and other provisions of the Treaty for the improvement of agrobiodiversity. The BSF has limited funding and since 2008, 52 projects have been approved for implementation over three project cycles.

What next for the role of the Treaty in Agrobiodiversity Management? Can an alternative biodiverse-food system be developed? How can agrobiodiversity be improved through use in the food system as well as through non-use? Can agrobiodiversity be pushed through the food system? Can consumption patterns and lifestyle be

changed to pull agrobiodiversity through the food system? So far global efforts have been to push agrobiodiversity through the food system. However, market forces in the food system are pulling away from agrobiodiversity. Therefore the role of the Treaty in agrobiodiversity management is getting more challenging and difficult.

The BSF has to play a bigger and more effective role to create the demand or pull factors in improving agrobiodiversity management in food and non-food uses. Among pull factors that can be created or developed are: i) developing the supply and value chains for the biodiverse food system, ii) strengthening the local seed systems for *on-farm* management of diversity in agricultural systems, iii) improving the biodiversity-based safety net for food security and livelihoods through better understanding of the desired products and services for biodiversity and ecosystem functioning.

## Access and Benefit-Sharing of Animal Genetic Resources: About the Need to Think Out of the Box

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When the United Nations Convention on Biological Diversity (CBD) was first conceived in 1992, it put biodiversity under national sovereignty and sought to reward and incentivize conservation of wild biodiversity in the “South” by making the “North” share the benefits from its commercialization. As soon as the focus shifted to plant genetic resources (PGR) for food and agriculture, it was realized that this principle could not be easily transferred to crops and other farmed plants, as there were often multiple places of origin and crops had been developed incrementally by series of actors. In response, the global community created the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) which establishes a multi-lateral system, Farmers’ Rights and set up the Seed Vault in Svalberg where seeds are systematically put in long-term storage so as to be available in the future.

Animal genetic resources (AGR) for food and agriculture present yet a third scenario that will require its own tailor-made version of Access and Benefit-Sharing to achieve the goals of the CBD (Koehler-Rollefson and Meyer, 2014). The purpose of this paper is to provide guidance to decision makers about how to achieve the goals of the CBD with specific reference to animal genetic resources as well as the Global Plan of Action on Animal Genetic Resources.

### Animal Genetic Resources

For the sake of simplicity, we can classify AGR into two types (although there are many intermediate types as well):

1. A small number of breeds or strains with vast outputs of one specific product, but equally enormous requirements in terms of feed, veterinary care and artificially optimised and stabilised conditions. These breeds have been developed by companies or breeders’ associations, with the help of performance recording and statistical programmes.
2. A multitude of breeds with lower output, but that are multi-functional and bred to take advantage of natural environments and, in the case of pastoral breeds, specifically for capitalizing on environmental variability (Kraetli, 2008). They are the products of networks of breeders with a common culture who inhabit the same landscape, who exchange animals amongst each other according to customary rules and who keep no written records of their animals’ performance, although they know them and their genealogies intimately.

It is these latter types of animals that humanity will need for adapting to climate change, for a green economy, and for reducing the climate impact of agriculture. Many of them have been created and developed by pastoralists whose cultures revolve around their animals. Others—especially poultry and pig breeds—are the product of indigenous and smallholder communities.

### Pastoral Herds: Mobile Banks of Fitness Genes and Knowledge

Because of their adaptation to harsh climates, pastoralist herds are repositories of fitness genes, especially genes for physiological adaptations to extreme weather and “unconventional feed stuffs”, i.e. native vegetation with high fibre and mineral content

But they are more than just assemblages of genes. They also represent knowledge, not only the knowledge of their keepers, but also learned behaviour of animals that is passed on from one generation of livestock to the next: how to make use of natural environments, both individually and in group as a socially organized population. Survival and performance under extreme conditions is thus not just a matter of physiological traits and instinct but also of learned behaviour.

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Table 1. Biocultural Protocols prepared by livestock keeping communities, finalized and in preparation

Community	Breeds	Country	Status
Raika	Camel, Nari cattle, Boti sheep, Sirohi goat	India	finalized
Banni Maldhari	Banni buffalo	India	finalized
Rebari and Jatt	Kutchi and Kharai camels	India	finalized
Bargur Hill cattle	Bargur Hill Cattle	India	finalized
Attappady goat breeders	Attapatty goat	India	finalized
Pullikulam cattle breeders	Pullikulam cattle	India	finalized
Pashtoon	Various breeds	Pakistan	finalized
Samburu	Red Maasai sheep	Kenya	finalized
Golla	Ganjam goat	India	In preparation
Kuruba	Kuruba shepherding system, incl. Deccani breed	India	In preparation
Kangayam cattle breeders	Kangayam cattle	India	In preparation
Malgaddi	Brela camel	Pakistan	In preparation

### Adapted Breeds Need to be Conserved in their Original Environment

Pastoralist herds represent living heritage that cannot be preserved out of context and in a freezer, but can only be conserved for the future in situ by people, by the herding communities that have developed and stewarded them over centuries. Defreezing semen or embryos, even if they have the right constellation of genes, would not result in the herds of livestock that in the presence convert into proteins for human consumption the vast rangelands found in the most climatically volatile regions of the planet.

### Implications for an Access and Benefit-sharing Regime

In order for humanity to have continued access to these (genetic) resources at any time in the future, it has to ensure that they are conserved and managed sustainably in their respective ecological and social contexts. And ensuring this must be the central aim and issue of any access and benefit-sharing regime for animal genetic rather than focusing only on the detail of material transfer agreements. We must understand access not from the narrow perspective of contracts between specific providers and commercial users, but in the wider sense of ensuring access to a pool of genes long into the future. If we fail, we will lose one of our most valuable assets for adapting to climate change, as well as for food production in harsh environments and with minimal inputs.

### Strengthening the Role of Communities as Keepers of Living Gene Banks

The Nagoya Protocol for Access and Benefit-Sharing, concluded in 2012, includes the provision for countries to support Community Protocols in which communities detail the genetic resources and traditional knowledge that they are the custodians of, as well as the conditions under which they would give prior informed consent and provide access to their genetic resources and traditional knowledge (FAO, 2015). This represents a fantastic opportunity for countries to better understand their animal genetic resources, the social contexts in which they exist, the threats that they are exposed to and thereby laying the foundation for their long-term conservation.

### Biocultural Community Protocols

Livestock keeping communities in several countries have already taken the initiative to develop such Biocultural Community Protocols in which they explain their situation and outline the conditions under which they can continue to act as stewards of and will be able to ensure access in the future (Koehler-Rollefson *et al.*, 2012). By and large these correspond to the points already summarised in the Declaration on Livestock Keepers' Rights ([www.pastoralpeoples.org/docs/LKRdeclaration.pdf](http://www.pastoralpeoples.org/docs/LKRdeclaration.pdf)) that was an eventual output of the Interlaken Process that culminated in the Global Plan of Action for Animal Genetic Resources (Koehler-Rollefson *et al.*, 2010).

So far this remains a scattered and underfunded effort which urgently needs to be expanded, with the

goal of eventually creating a global *in-vivo* “Community Breed Repository” as the animal equivalent to the Global Seed Vault and whose benefits can be shared by all of humanity.

### Acknowledgements

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## Access to Genetic Resources and Benefit Sharing: Indian Experience

**Rai S Rana**

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‘Access and Benefit Sharing’, ABS in short, refers to access to genetic resources (and traditional knowledge associated with them) and fair and equitable sharing of benefits (generated from their use by researchers, breeders and biotechnologists, and also by developers and manufacturers of bioresource-based products) with the country of origin/ rightful owners. This topic, along with its relevance to adaptation to climate change, is centre stage these days. The legally binding Nagoya Protocol on ABS, adopted in 2010 and ratified presently by 87 Parties, has more clearly defined and strengthened this concept derived from Article 15 of the Convention on Biological Diversity.

India is contracting party to several international treaties impacting on ABS.

**International Undertaking on Plant Genetic Resources for Food & Agriculture (PGRFA)**, established by the FAO in 1983, and based on the principle that PGRFA were common heritage of mankind, was the first voluntary international agreement on sharing of PGRFA. India was among the 113 nations who signed and adhered to this International Undertaking. This era was followed by granting of intellectual property rights (IPR) over the products of research on genetic resources. Working mostly in developed countries and making use of PGRFA obtained from developing countries, IPR holders appropriated all the resulting benefits to themselves.

**The TRIPS Agreement under WTO (1995)** provided that the Members may exclude plants and animals (other than microorganisms) from patenting but any country excluding plant varieties from patenting must provide for an effective *sui generis* system for their protection.

**The Convention on Biological Diversity (CBD):** It is a legally binding comprehensive treaty adopted in June 1992, sought to balance the rights of providers of genetic resources with those of their users (including the breeders and other researchers). It also marked the end of the ‘common heritage’ concept of genetic resources by recognising sovereign rights of States

over their natural resources within their boundaries and affirming that the authority to determine access to genetic resources rested with the national governments subject to national legislation. The FAO also revised the text of its 1983 International Undertaking on PGR to bring its provisions in harmony with those of the CBD and then adopted it on 3 November, 2001 as the legally binding **International Treaty on Plant Genetic Resources for Food & Agriculture (ITPGRFA)**. This Treaty presently covers designated accessions of 64 Annex-1 food and forage crops (which are in public domain and managed by the national governments) and upholds the Farmers’ Rights subject to national legislation. Unlike the bilateral system of agreements under CBD for access to genetic resources, this Treaty provides for a Multilateral System (MLS), based on Standard Material Transfer Agreement.

**The Nagoya Protocol to CBD on ABS** is the new legally binding international treaty specific to ABS and provides implementation mechanism for the benefit sharing provision under the Convention’s Article 15, setting out core obligations on the Parties to develop provider as well as user country measures so as to ensure that genetic resources are not used without prior consent of the countries of origin. The ABS-Clearing House offers assistance in monitoring the process.

### Legal Framework for ABS in India

#### *Focus on Conservation and Sustainable Use, Supported by ABS Provisions*

Implementing the CBD gained momentum soon after its entry into force in December 1993 as several nations passed legislation to claim sovereign rights over their natural bioresources. India enacted the Biological Diversity Act (BD Act) in 2002 and framed Rules for its implementation in 2004 in pursuance to its commitments under the CBD. This Act provides legal framework for implementing the CBD and to affirm India’s sovereign rights over granting access to its naturally occurring

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bioresources (and associated traditional knowledge) and for setting terms for sharing of benefits arising from their utilisation.

Four agencies are working in partnership in implementing this Act: the Central Government, the National Biodiversity Authority (NBA), established by the Central Government; the State Biodiversity Boards (SBBs), established by the State Governments; and the Biodiversity Management Committees (BMCs) at the local level, set up by every Local Self-Government body. They have well defined, non-overlapping and mutually supportive roles.

The BD Act follows a common but differentiated approach to deal with applications received from Indian citizens/entities and those from foreign persons/entities for the regulated four activities. 1) A foreign person/entity cannot access bioresources [and associated traditional knowledge (TK)] occurring in India for research/bio-survey and bio-utilisation for research/commercial use without prior approval of NBA. Indian researchers/entities, however, are permitted access for research without any approval but they are required to intimate the concerned SBBs, from where the bioresources are accessed, and enter into a benefit sharing agreement prior to commercial utilisation. 2) All persons/entities applying for intellectual property rights (IPR) on products/processes, based on research on India's bioresources and associated TK, require prior approval of NBA. 3) In addition, all scientists/institutions, intending to transfer results of their research on bioresources occurring in India to foreigners/entities for monetary or other gains, are required to take prior approval from NBA.

4) The foreigners/entities intending to transfer already accessed bioresources to another foreigner/entity (Third Party Transfer) also require prior approval of NBA for this purpose (See Box 1). The differentiation between foreigners/entities and Indian applicants ensures that all the foreigners/entities deal with a single window system.

### Some Exemptions Provided under the BD Act

- Local people and communities of the area including growers and cultivators of biodiversity and persons practicing indigenous medicine.
- Accessing bioresources for conventional breeding or traditional practices in use in any agriculture, horticulture, poultry, dairy farming, animal husbandry, bee keeping, etc.
- International Collaborative Research Projects conforming to the guidelines issued by the Central Government in November, 2006 for this purpose.
- Breeders applying for variety protection under the PPV&FR Act, 2001.
- Normally Traded Commodities, notified u/s 40, when traded as commodities.
- Access to designated accessions of notified Annex-1 crops under the ITPGRFA.

The NBA takes help of several Expert Committees (ECs)/Core Expert Groups who provide guidance on specific issues/topics. Eight such committees/core groups are functioning at present involving a pool of over 170 experts drawn from different specializations across the country. EC on ABS, for example, scrutinises applications

**Box 1. Activities covered under the BD Act and the authorities for granting approval**

Purpose for use of bio-resources and/or traditional knowledge associated thereto	Authority for granting approvals	
	For Indian citizens/entities	For persons/entities as defined u/c 3(2) [foreigners]
1. a) Research	No approval required	NBA
b) Bio-survey & Bio-utilisation	SBB	NBA
c) Commercial utilisation		
<b>Special Forms:</b>		
<b>1A</b> For a trader/manufacturer	NBA	NBA
<b>1B</b> For emergency research abroad		
<b>1C</b> For depositing microbial sample in a foreign repository		
2. Transfer of results of research on bio-resources to entities defined u/s 3(2)	NBA	NBA
3. Seeking intellectual property rights on bioresource-based products	NBA	NBA
4. Transfer of already accessed bio-resource to entities defined u/s 3(2)	Not relevant	NBA

for access by foreigners/entities, IPR, research results transfer and third party transfer of accessed bioresources. EC on agrobiodiversity has helped in implementing the facilitated access to designated accessions of notified Annex-1 crops under the ITPGRFA and a Core Group is presently assisting implementation of the Nagoya Protocol.

### Implementing the BD Act

Following NBA's establishment in 2003, SBBs have been constituted in all the 29 States. Nearly 40,000 BMCs have also been formed across 26 states and guidelines for preparing People's Biodiversity Registers were updated in 2013 leading to preparation of over 2,500 such registers by BMCs staking claim on their bioresources. Threatened species have been notified in 16 states and two union territories u/s 38 while the Guidelines for Identification of Biodiversity Heritage Sites have been developed and seven such sites have been notified in three states u/s 37.

Responding to the suggestions from stakeholders, formats for the four broad categories of legal agreements have been revised. Out of 1,221 applications received by the NBA so far including 219 that were closed, 814 have been approved under different categories and 247 benefit sharing agreements have been signed with the applicants on mutually agreed terms. National Biodiversity Fund and State Biodiversity Funds, amounting to nearly INR 360 million, are now available for claim of the identified beneficiaries to support conservation related activities including capacity building and raising awareness about the BD Act (See Box 2). In addition, SBBs have also

received nearly INR 40 million through registration fees and benefit sharing.

Guidelines on Access and Benefit Sharing Regulations were notified in November, 2014 accelerating the benefit sharing process. Seventeen States have now framed their *sui generis* Biodiversity Rules and Regulations and 16 SBBs have been supported for ABS activities under 4 externally funded projects. Winners and runner ups of India Biodiversity Awards, 2016 (including the new category on Good Practices for ABS Mechanisms) were honoured on the International Day for Biodiversity this year (See Box 3).

### Some Notable Features of the Indian ABS System

NBA is an autonomous public-funded institution and it has received full support from the Central Government, particularly in policy guidance and making rules and regulations. It is essentially an inter-ministerial apex decision-making body assisted by five technical experts (as non-official members) and has been meeting at regular intervals with its 39<sup>th</sup> meeting held on 14 October, 2016.

Regular consultations, held by the NBA with different relevant ministries (having jurisdiction over various components of biodiversity) and major stakeholders, have helped in clarifying several contested issues and in developing implementable procedures. Dialogues with the DIPP and the Indian Patent Office, the CSIR, the ICAR and the DBT have proved to be particularly useful. Active partnership developed by NBA with 29 SBBs, aided by national and regional level workshops, has also helped greatly in accelerating the ABS system in recent years.

In addition, the Guidelines on ABS Regulations (2014), well received by most of the stakeholders, have really galvanised the ABS system. Responding to requirements of the business organisations (and also many other sectors like agriculture, biotechnology, herbal drug manufacturers and pharmaceuticals) and issuing helpful notifications, like those on Guidelines on ABS and Normally Traded Commodities, have turned out to be a win-win situation.

The BD Act, though basically designed for implementing provisions of the CBD, has come out as an overarching ABS legislation by providing effective linkages to other relevant national Acts and also for working as the enabling legislation for several

**Box 2. Some outstanding contributions to the National Biodiversity Fund**

Contributors paying the Benefit Sharing	Amount (INR in million)
Seaweed: PepsiCo India Holdings (P) Ltd., Gurgaon PSS Ganesan & Sons, Tuticorin Britto Sea Foods, Chennai	4.250
Cattle embryos: Brasif S. A., Brazil	10.764
Neem leaves: Bio India Biologicals, Hyderabad	0.055
Red Sander Wood: 35 Bidders in public auction of Red Sander Wood seized by the Andhra Pradesh state government under the Wild Life (Protection) Act [CITES]	339.600

**Box 3. India Biodiversity Awards 2016: Successful and Replicable Mechanisms for ABS**

In India, nearly 300 million people are dependent on biodiversity for subsistence and livelihoods. This dependence is particularly critical for the tribal people who reside in forest areas and collect non-timber forest produce, including plants of medicinal value, to augment their income. India Biodiversity Awards 2016 included a category on 'Successful Mechanisms for Access and Benefit Sharing' to identify good practices that have been developed and adopted in this context.

The Award Winner was the Gram Moolgai Co. Ltd. who, working in Katni District of Madhya Pradesh, had developed and successfully adopted a fair benefit sharing mechanism, much earlier to the Central Government's notification of Guidelines on ABS Regulations in November, 2014. This company traded in medicinal plants by purchasing them from the collectors, mostly the poor tribal families living on the fringe of Bandhavgarh National Park, and supplying to a well known drug manufacturer. These collectors were registered by the company for this purpose and were shown the price paid by the manufacturer. Raw bioresources were purchased by the company from the collectors at that rate after a deduction of 30%, as the overhead charges including the profit margin, and this offered price was nearly 20% higher than the prevailing market rate. The company also employed these local communities in cleaning, grading, packaging and storing them prior to their supply to the manufacturer promoting thereby sustainable use practice with fair benefit sharing.

international treaties and protocols.

Guidelines on International Collaborative Research Projects, notified in 2006, have helped the researchers in availing the exemption provided for this purpose u/s 5 of the Act. Exemption for designated accessions of Annex-1 crops was also notified in December, 2014 to facilitate access under the ITPGRFA. To assist the commerce sector, 385 Normally Traded Commodities were exempted from the Act in April, 2016 u/s 40 as long as they are traded as commodities (not as bioresources for research). **Fifteen institutions have also been notified** as the national designated repositories u/s 39 and Guidelines on their mandates and safekeeping of deposited specimens/samples have been issued in March, 2016. All these guidelines and notifications are available on NBA's website for easy access to them.

**The Way Ahead**

While national ABS legislations of most other biodiversity-rich countries appear to target primarily the bio-survey and bio-utilisation of their bioresources by foreign companies, the Indian national legislation focuses on promoting conservation and sustainable use of bioresources linked to fair and equitable sharing of benefits generated from their commercial utilisation. The BD Act has stood the test of time as an umbrella legislation for not only implementing the CBD and its Nagoya Protocol on ABS but also the ITPGRFA. Its implementation has been effectively linked with other relevant national legislations like the Patents Act, 1970 and the PPV&FR Act, 2001. An on-line application system is to be launched soon to further

improve efficiency and effectiveness of the national ABS system.

Notification of the ABS Guidelines Regulations in November, 2014 has remarkably speeded up the Act's implementation process as also the notification on exempting a large number of plants, normally traded as commodities (not as bioresources), u/s 40 to support the foreign trade sector. More effort is underway to include animal and marine products under this exemption.

There are still some areas where interpretation/perceptions about the BD Act's provisions differ among the stakeholders such as the interpretation of certain terms defined under the Act and the scope of exemptions provided therein. To create increasing awareness, however, a section on 'Frequently Asked Questions' is displayed on the NBA's website for guidance and 'sensitisation workshops' are also being organized regularly. The Act's implementation is still weak where it matters most, i.e., in involving and empowering of the local communities for decision making but an Expert Committee's recent suggestions on this aspect are expected to help energising the national ABS system at the grass root level.

Investing in biodiversity conservation is the first step to safeguard the natural bio-wealth for use of future generations. According to an estimate prepared by the BIOFIN project team, India funded core biodiversity schemes and related projects amounting to nearly INR 108 billion during the year 2012 and launched the India Business & Biodiversity Initiative offering avenues for investment to the corporate sector as part of its social responsibility.



## Conservation and Use of Genetic Resources through Implementation of Suwon Agrobiodiversity Framework and Partnerships in Asia and the Pacific

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Agrobiodiversity forms the foundation of sustainable agricultural growth and development. Plant genetic resources for food and agriculture (PGRFA) provide the biological basis for agricultural production and food security. The plant genetic diversity allows crops and varieties to adapt to ever changing conditions and to overcome the constraints caused by biotic (pests, diseases) and abiotic (drought, heat, flood) stresses. However, the genetic diversity has been eroding at an alarming pace due to developmental activities, climate change and ill-planned use leading to irreversible and irreparable loss. Genetic erosion of plants occurs through replacement of local varieties of crops, and other cumulative effects from invasive alien species, pests, weeds, diseases, land use change and environmental degradation. The world's aquatic diversity is threatened by over-exploitation of fish genetic stocks and environmental pollution.

The conservation, sustainable use, and fair and equitable sharing of benefits from their use, are both an international concern and an imperative as these are the objectives of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which is in harmony with the Convention on Biological Diversity (CBD). In reaffirming the context of the sovereign rights of states over their biological resources and the interdependence of countries regarding PGRFA, the second Global Plan of Action (GPA) of FAO addressing plant genetic resources for food and agriculture, adopted by FAO Council, is an appropriate manifestation of the international community's continued concern and responsibility in this area. The Second Report on the State of the World's PGRFA has shown that interdependence on plant genetic resources is increasing at a global level. However in many countries, efforts on conservation, *on-farm* management, crop improvement and seed systems are insufficiently integrated to adequately address present and future challenges, particularly food security, sustainable development and climate change

Consequently, managing PGRFA has become a collective global responsibility and international cooperative efforts are essential to enhance conservation through exchange and utilisation. National and international genebanks have been working towards collecting and conservation of genetic resources for food and agriculture. Effective use of genebank materials for breeding purposes is, however, extremely low, due to existence of technical and policy barriers and constraints.

### Suwon Agrobiodiversity Framework

As a part of the ongoing efforts, and in recognition of 2010 as an International Year of Biodiversity, APAARI had organized an International Symposium on "Sustainable Agricultural Development and Use of Agrobiodiversity in the Asia-Pacific Region" from October 13-15, 2010 in Suwon, Republic of Korea (Mathur *et al.*, 2011). This was done in partnership with Rural Development Administration (RDA), Republic of Korea; Global Forum for Agricultural Research (GFAR); Bioversity International, FAO and other International Centers such as CIMMYT, ICARDA, ICRISAT, IRRI, ILRI and AVRDC. The symposium provided an excellent opportunity to review, identify and redefine the role and directions of agricultural R&D, especially in the context of conservation through use of valuable agrobiodiversity for sustainable agricultural development. It also helped in agreeing on a 'Way Forward' for the access and benefit sharing of valuable genetic resources. The agrobiodiversity research and development framework for the Asia-Pacific region, adopted during the symposium provided a strategic approach, towards both management and use through regional collaboration and partnerships among stakeholders. The framework published as "The Suwon Agrobiodiversity Framework" agreed by national partners to be used for developing their own national programmes for conservation and sustainable use of plant genetic resources. The following are the key elements of the Suwon Agrobiodiversity Framework.

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### (a) Challenges and Opportunities

The Asia-Pacific region is the center of diversity of many important species of crops, livestock and many other bio-resources. Resource poor producers in the region are largely dependent on the agrobiodiversity of staple crops, minor crops, their wild relatives and other species of plants and animals for their food security and livelihood. It is also evident that the contribution of agrobiodiversity in ensuring sustainable and productive agriculture remains vital. The reservoir of genetic resources remains a main resource for food security, and equally important for improving nutrition, product quality, product diversification and food safety. The genetic diversity in both indigenous and introduced species has been enhanced through extensive exchange of germplasm within the region.

However, while the threats to these resources are growing, the efforts to conserve and use genetic diversity are insufficient in the region. Also, the traditional knowledge, associated with the use of old varieties/landraces, has remained undocumented and is rapidly vanishing. Therefore, reduction of agricultural biodiversity can significantly increase the vulnerability of farmers and existing agro-ecosystems. Therefore, initiatives through conservation and use of agrobiodiversity must respond to these challenges.

Responding to the emerging challenge of climate change, greater access to a range of varieties that can help farmers deal with drought or flood, will be required. Exploring the genetic resources available will require new tools (Genomics, GIS, ICT), technologies and innovative approaches for their conservation and use.

All these challenges are compounded by the continuing loss of genetic diversity of plants, livestock and aquatic resources. Hence, effective conservation and sustainable use of available genetic resources becomes a major priority in the region.

### (b) Integrated Approach

The Suwon Framework proposed an integrated approach which seeks to ensure the continued availability of critical genetic resources not only for the improvement of agricultural productivity and resilience of the production systems but also to improve the quality of the supply chains through effective collaboration of different stakeholders working on a broad range of genetic resources. It also builds on current partnerships and eco-regional experiences involving national and international

organizations and for integrating partnerships across the different sectors involved in genetic resources. The approach draws lessons from existing collaboration between different CGIAR centres, AIRCA members, NARS, Regional Fora and many other stakeholders in the region – a collaboration that needed to be strengthened to a higher level of effectiveness and accountability.

This integrated approach would intrinsically be more effective in the long run since it brings together work on microbes, bios, crop plants, forest trees, livestock and fish genetic resources. It should also combine research on genetic, biological, agronomical, socio-cultural, market, trade, economic and policy aspects. It would encourage development of national plans, focussing not only on major crop commodities but also on other neglected and underutilized minor crops, livestock, aquatic resources and tree and forest species. The approach encourages the different organizations and local communities to work in partnership for collective actions. This approach will maximise the resources and opportunities to have an agile response to new, yet unforeseen developments in understanding diversity and promoting its use through research, conservation, evaluation and documentation.

### (c) Research and Development

Various focus areas of research and development as proposed in the Suwon Agrobiodiversity Framework are as below:

- Studies to enhance use of genetic resources through sub-set approaches
- Pre-breeding and participatory breeding to enhance utilisation of genetic resources in commodity improvement programmes
- Strategies and technologies to enhance *in situ* and *ex situ* conservation through use
- Assessment of the agrobiodiversity richness and the status in terms of economic, social and cultural (traditional knowledge) factors
- Interdisciplinary studies on the invaluable ecosystem services for agriculture that agricultural landscapes, forests and other mainly wild ecosystems provide
- Information systems and tools for data exchange
- Supportive policies, laws and strategies to enable enhanced PGR exchange and use

### (d) Areas of Regional Collaboration

The important areas of regional collaboration indicated

in the Suwon Agrobiodiversity Framework are given below:

- Developing national agrobiodiversity plans and integrating them into regional and global collaborative frameworks
- Increasing R&D collaboration on agrobiodiversity conservation and use in the region
- Increased sharing of information and data on genebank collections
- Strengthening agrobiodiversity capacity, education and public awareness
- Enhancing exchange and use of genetic resources
- Facilitating involvement of stakeholders in strengthening agrobiodiversity conservation and use

#### **(e) Collaboration and Partnerships**

The Suwon Agrobiodiversity Framework builds on partnerships and eco-regional experiences involving national and international organisations and for integrating partnerships across the different sectors of genetic resources. The vision of the proposed framework approach draws lessons from collaboration between different CGIAR centres, NARS, regional fora and all the stakeholders in the region—a collaboration that needs to be strengthened to a higher level of performance and accountability.

With a view to enhance conservation and use of these genetic resources, APAARI, in collaboration with its stakeholders, especially Bioversity International and other CGIAR Centers, viz., CIMMYT, IRRI, ICRISAT, ICARDA, CIP, ICRAF, CIFOR ILRI, World Fish Center, IFPRI, IWMI, International Research Centers, viz., AVRDC, ICBA, ICIMOD, CFF, CABI, other international organisations as FAO, GFAR, GFRAS, Regional Fora, and the National Agricultural Research Systems (NARS) continue to review the role and direction of agricultural R&D to efficiently address above challenges.

Four sub-regional PGR networks engaged in promoting regional collaboration for strengthening PGRFA conservation and use include: (i) South Asia Network on Plant Genetic Resources (SANPGR), (ii) the East Asia PGR Network (EA-PGR), (iii) Regional Cooperation for Plant Genetic Resources in Southeast Asia (RECSEA-PGR), and (iv) Pacific Plant Genetic Resources Network (PAPGREN). In addition, there

were also several commodity focused PGR networks like the Banana Asia-Pacific Network (BAPNET), the International Coconut Genetic Resources Network (COGENT), Cereals and Legumes Asia Network (CLAN), and the International Network for the Genetic Evaluation of Rice (INGER).

#### **Actions Required to Implement Suwon Agrobiodiversity Framework**

The following actions are needed in ensuring optimal participation of different actors, and the building of new partnership opportunities:

- Benefitting from the new tools and technologies through new alliances among researchers working on plant and animal breeding, molecular biology, bioinformatics and biometrics that integrate genetic resources, genomics and genetic improvement programmes;
- Laying focus on genetic resources in different CGIAR research programmes for better integration into national plans and regional and global collaborative frameworks, to avoid gaps and overlaps;
- Enhanced regional crop improvement programmes and PGR networks to ensure capacity development and improved exchange of materials and their use in the Asia-Pacific region;
- Network activities to focus more on underutilized and neglected species in the region.
- The different sub-regional and crop networks would also be more sustainable if linked with regional or global initiatives, such as those of APAARI and other regional and global fora. These networks need to be revitalised;
- Strengthening partnerships with CSOs and the private sector to contribute more effectively towards public awareness, education and policy advocacy; and
- Finally, there is a need to form new partnerships involving farmers and other stakeholders who ultimately guard the agrobiodiversity and its associated knowledge.

#### **Moving Forward with the Framework**

Concerted efforts needed to move forward with the implementation of Suwon Agrobiodiversity Framework are as follows:

- Encourage national systems to adopt the framework

in their national agrobiodiversity work plans

- Encourage donor/funding organisations to use the framework as the basis in supporting initiatives on agrobiodiversity
- Strengthen capacity of sub-regional and genetic resources networks to follow through the framework
- Develop regional collaborative projects based on the framework
- Catalyse national systems in enhancing their breeding programmes through the use of germplasm, and
- Support activities that promote the use of under-utilised crops

### Priority Activities Undertaken

#### (a) *Priority projects identified during Kuala Lumpur Workshop*

As a follow-up of Suwon Agrobiodiversity Framework, a Regional Workshop on Implementation of Suwon Agrobiodiversity Framework, was organized at Kuala Lumpur, Malaysia on November 4-6, 2011 (APAARI, 2012) in which the following priority projects were identified:

- Enhanced utilisation of germplasm for sustainable crop production – for Pacific countries
- Acquisition of crop wild relatives and accessing novel alleles
- Increasing availability and accessibility to the rich agrobiodiversity for conservation and improvement of livelihoods of farmers
- Enhancing use of underutilised species for improved livelihoods and diversified diets
- Agrobiodiversity transition and deficits: understanding and managing changes in diversity and local thresholds to the sustainability of ecosystem services

#### (b) *Matching Seeds for Needs: PNG-Bioversity Efforts*

The following activities were undertaken with joint efforts of PNG and Bioversity International under the programme “Matching Seeds for Needs”:

- Crop suitability models for taro and sweet potato for current and future climatic conditions
- Identification of crop production areas most

threatened by climate change

- Identification of varieties adapted to future climatic conditions
- Development of improved seed multiplication and delivery systems
- Improved genetic production potential of staple crops in Papua New Guinea (PNG)

### Recent Initiatives by APAARI in Strategic Partnership and Networking

In recently developed APAARI Vision 2030 (APAARI, 2016) and thereby articulated Strategic Plan–2017-22, APAARI has assigned a high priority in terms of its time, effort and resources in developing and implementing partnership and networking programmes to: i) effectively manage and utilise agrobiodiversity and ii) promote and improve the application of advanced biotechnologies and regulatory systems in agri-food systems in Asia and the Pacific region. This will be undertaken through strengthening of agri-food research and innovation systems. The *modus operandi* will be the collective actions between APAARI, agri-food research and innovations systems, their partners and other stakeholders, including governments, CSOs (NGOs, FOs), the private sector and regional and global agencies. The major focus of these efforts will be on:

- Conservation and use of underutilised, marginalised and neglected species;
- Scientific research, technological advancement, innovation, economic and policy considerations, and regional and global treaties and arrangements covering various aspects of agrobiodiversity;
- Looking at comprehensive dimensions of agrobiodiversity covering not only plants but also animals, fishes, microbes, bios, insects, etc.

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## A Resource Manual for Resilient Seed Systems

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### Smallholder Farmers' Seed Systems under Pressure

Have you ever wondered how farmers in developing countries get their seed? If, in your imagination, you see them buying certified seeds from what is known as the 'formal seed system', you are very likely to be wrong. Estimates suggest that 60-90% of the seeds on which smallholder farmers in developing countries depend is saved *on-farm* or obtained through local distribution channels, such as exchanges between farmers, community sharing systems, and local markets. Women farmers play key roles in farmer seed systems, although they are often overlooked by researchers and development personnel, policies, and programmes.

This enduring high level of seed flows among farmers masks the fact that, almost everywhere, local seed systems face challenges. Agricultural intensification and commoditization, privatization of natural resources, and the strong concentration and expansion of corporate power in the life science industries (including the seed industry) are contributing to a decline in collective local management of plant genetic resources for both conservation and sustainable use.

Many farming households have become more individualised in terms of decision-making and deployment of knowledge, labour, capital and seeds. Traditional seed exchange relationships have become weaker in many areas. Farming practices are becoming more market oriented, and this increased involvement in markets has both benefits and costs depending on local context. Large-scale rural-to-urban migration is contributing to a decline in farming in many countries or transforming small-scale family farming into contract farming. It is also leading to the feminization of agriculture, increasing the workload and responsibilities of women in many regions. These trends are affecting local seed production, selection, storage, distribution, and exchange practices, for example, through substitution of local varieties with hybrids that can be easily purchased at local markets.

Climate change has begun to put additional pressure on farmers' seed and food production systems and on the multiple functions that they fulfill. In many areas, farmers continue to maintain crop diversity, but a significant reduction in the number of crops as well as area planted is occurring. Findings from the field indicate a decline in diversity of local varieties in many countries. Future impacts of climate change are expected to become more pronounced in many parts of the world, forcing farmers to change their practices and causing them to search for information about crops and varieties better adapted to new weather dynamics.

### Resilient Seed Systems

Bioversity International supports research on resilient seed systems to address a number of challenges: food insecurity, malnutrition, poverty, and climate change (Vernooy *et al.*, 2016).

Research results from around the world suggest that providing farmers with better access to crop and varietal diversity can strengthen their capacity to adapt to climate change. Under supportive policy and socio-economic conditions, such strengthened capacity could contribute to greater food availability throughout the year, the production of more nutritious and healthy crops, and income generation.

Climate and crop modeling tools are increasingly used to predict the adaptive capacity of a given crop to expected changes in climate. The results of these modeling exercises can be used to design strategies to access and use crops and crop varieties that are expected to be better adapted to future climate changes in specific locations. The results of these modeling exercises, complemented by additional research (e.g., local crop pest and disease studies, lessons learned from past crop-improvement efforts, technology adoption studies) will help researchers, gene bank managers, extension agents, and farmers gain access to potentially useful plant genetic resources through the multilateral system of the International Treaty on Plant Genetic Resources

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for Food and Agriculture or other means. Once obtained, these “new” plant genetic resources can be evaluated in target environments through on-farm experimentation over one or more cycles.

Over the past three years Bioversity International has collaborated with partners in various countries to design and implement a comprehensive capacity-building strategy to access and use plant genetic resources more effectively in the context of climate change adaptation. Countries include Benin, Bhutan, Burkina Faso, Costa Rica, Côte d’Ivoire, Guatemala, India, Madagascar, Nepal, Rwanda, South Africa and Uganda. This led to the development of a resource manual for resilient seed systems.

### Content

The methodology for building more resilient seed systems includes the following eight steps; for each one the manual includes a specific module:

- **Module 1: *Situational analysis and planning***—explains how to work with farmers and other stakeholders to determine baseline conditions in a community in terms of seed systems and climate change; how to set priorities and objectives; and how to plan research into development intervention.
- **Module 2: *Data preparation and selection of software***—introduces useful tools, such as DIVA-GIS, Maxent, and Climate Analogues, and explains how to prepare relevant data for a comprehensive climate change analysis.
- **Module 3: *Climate change analysis and identification of germplasm***—describes the steps in climate change analysis in the context of impact on agriculture and seed systems and how to identify germplasm suitable for the future climate.
- **Module 4: *Germplasm acquisition***—introduces the International Treaty on Plant Genetic Resources for Food and Agriculture, and explains how to acquire new germplasm while protecting traditional knowledge and taking into account the phytosanitary aspects of seed production and distribution.
- **Module 5: *Field experimentation***—presents a number of methods for participatory crop evaluation with the newly acquired germplasm in local environments and farmers’ fields.
- **Module 6: *Germplasm conservation***—discusses

specific aspects of the conservation of the newly tested germplasm and the various ways in which it can be multiplied.

- **Module 7: *Participatory evaluation***—presents a global method for evaluating the research process with farmers, gene bank managers, extension agents, and other stakeholders.
- **Module 8: *Knowledge sharing and communication***—is about sharing research results with the participants as well as with others involved or potentially interested in the results.

### Users and Uses

The resource manual is intended for:

- Plant breeders, researchers, gene bank managers, and policymakers with an interest in plant genetic resources
- University lecturers and advanced students with an interest in agricultural development, adaptation to climate change, and seed systems
- Others involved in the strengthening of farmers’ seed systems and their capacity to adapt to climate change

The resource manual can be used as:

- ***A one-stop shop***—for finding selected, easily accessible resources to support research on climate change adaptation by strengthening seed systems
- ***A learning aid***—to build capacity in facilitating, conducting, and participating in such a research process
- ***Pedagogical material***—for higher education classes or on-the-job training workshops

The interactive web-based version of the manual is available at: <http://www.seedsresourcebox.org>. The hardcopy version is available at: <http://www.bioversityinternational.org/e-library/publications/detail/resource-box-for-resilient-seed-systems-handbook/>.

### Experiences from the Field

In Burkina Faso, researchers from the Université de Ouagadagou used parts of the manual to obtain to acquire new millet accessions that are better adapted to the changing climate. They are currently planning on-farm experiments in three contrasting agro-ecological regions of the country and have already mobilized

farmers interested to take part in the research. They have collected weather data of the last 30 years to underpin their analysis about future trends. They are now in the process of identifying promising new accessions from both inside and outside Burkina Faso that respond to current and future climate changes.

In Bhutan, the country's two only plant breeders and staff of the National Biodiversity Centre identified five different agro-ecological sites in the country for the analysis of future climate changes based on the presence of weather stations (availability of longer term weather data) and on-farm conservation programmes implemented in the past or present (availability of farmers interested and experienced in on-farm crop experimentation). Based on the new knowledge and skills acquired, they recently completed climate change scenario analyses using DIVA-GIS and the climate analogue tool for each site for the major crops in Bhutan (rice, maize, potato and chilli) taking 2030 as reference year. The team is producing a synthesis of the results at this moment to decide about which possible analogue sites to target for further analysis and possible acquisition of promising germplasm.

In Guatemala, staff of the Instituto de Ciencia y Tecnología Agrícolas (ICTA) has used the new knowledge and skills gained to realise collection, characterisation and conservation projects for bean, maize, *Amaranthus*, *Capsicum*, cassava and several tubers. They also produced distribution maps of the accessions collected in the country and maps of potential collection sites of accessions still to be collected. These maps will be used for the planning of future collection missions.

In Rwanda and Uganda, following the training in

the classroom, a team of scientists and extension agents interacted with farmers and their communities in selected sites to identify local climate related challenges. Climate change scenario analysis resulted in the identification of present and future analogue sites. Using DIVA-GIS and crop suitability modelling applied to beans (a key crop for farmers' livelihoods) the team then identified bean accessions with good climate adaptation potential from three sources: (i) the national gene banks in Rwanda and Uganda, (ii) communities in both countries, and (iii) international genebanks. In 2014, the first phase of participatory field trials with farmers was done using materials from the national genebanks and locally adapted material was realised. A total of 20 varieties in each country were evaluated (and ranked) by farmers for climate resilience and other desirable traits. Accessions from international genebanks have been requested and once acquired will be multiplied and tested in the field hopefully in 2015. In addition, farmers from community seedbanks in Rwanda and Uganda organised an exchange visit during which they identified varieties of beans that they would like to exchange. Modalities for this exchange are still being worked out by the two countries.

### Providing Feedback

The Bioversity International research team that developed the resource manual would be very pleased to receive your feedback on the content and practical use you have made of the manual. Please send your comments to [bio-policy@cgiar.org](mailto:bio-policy@cgiar.org)

### Reference

- Vernooy R, G Bessette, P Rudebjer and G Otieno (eds) (2016) Resource box for resilient seed systems: handbook. Bioversity International, Rome, Italy. <http://www.bioversityinternational.org/e-library/publications/detail/resource-box-for-resilient-seed-systems-handbook/>.

## Biosafety Issues and Preparedness for GMOs

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Rapid advances in biotechnology have opened up countless opportunities for practically all sectors of economy, such as pharmaceutical and drugs, agriculture, industrial processes, environment and biodiversity conservation. In modern biotechnology, a Genetically Modified Organism (GMO) is that in which the basic genetic material (DNA) has been artificially altered or modified to improve the attributes or make it perform new functions. Common GMOs include agricultural crops that have been genetically modified for greater productivity or for resistance to pests or diseases e.g. Bt cotton, incorporating a gene from a bacterium *Bacillus thuriangiensis* effective against the American Bollworm, a major pest on cotton. In the context of International Agrobiodiversity Congress, the focus of this paper is on GM crops only.

Although the development of GM crops using recombinant DNA techniques is relatively recent, their applications and use is increasing because of advantages over the conventional crops. However as more and more GM crops are being released for field-testing and commercialization, concerns have been expressed regarding the risks arising from their use due to potential risks to both human health and environment. These apprehensions arise because GM technology crosses the species barrier as compared to classical selection techniques, thereby permitting the gene transfer among microorganisms, plants and animals. There is no evidence that any unique hazards exist in the development of GM crops, because of novel combinations of genes. It is not true that all GM crops are toxic or are likely to proliferate in the environment. However, specific crops may be harmful by virtue of novel combinations of traits they possess. This means that the concerns associated with use of GMOs can differ greatly depending on the particular gene-organism combination and therefore a case-by-case approach is required for assessment of safety concerns.

Evaluating the safety of a GM crop is a comprehensive process that involves several steps. Systematic safety

assessment methodologies are in place that have been agreed on years of consultations under the aegis of international organizations and agreements viz. FAO, WHO, Codex Alimentarius, OECD and Cartagena Protocol on Biosafety. The potential changes introduced using genetic engineering are assessed using comparative risk assessment approach. The underline assumption of this comparative approach is that traditionally cultivated crop has a history of safe use and thus serves as the comparator. As a consequence, safety assessment process gives conclusion on whether or not the GE plant is as safe as its conventional non-GE counterpart. Safety assessment studies required for commercial release of a GE plant comprise of food and feed safety assessment and the environmental risk assessment coupled with information through the molecular characterization of the GE plant and characterization of the expressed, transgenic proteins.

Safety assessment requirements have led to the development of regulatory regimes in various countries for research, testing, safe use and handling of GM crops and their products. Regulatory framework in India governing GM crops is based on the "Rules for the manufacture, use, import, export & storage of hazardous microorganisms, genetically engineered organisms or cells, 1989 notified under the Environment (Protection) Act, 1986. These rules are the apex rules for regulation of all activities related to genetically engineered organisms and products thereof. These rules are implemented by the Ministry of Environment, Forest & Climate Change (MoEFCC), the Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India and State Governments. Six competent authorities and their composition and roles have been notified under the rules. These include Recombinant DNA Advisory Committee (RDAC), Institutional Biosafety Committee (IBSC), Review Committee on Genetic Manipulation (RCGM), Genetic Engineering Appraisal Committee (GEAC), State Biotechnology Coordination Committee (SBCC) and District Level Committee (DLC). Various

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sub-committees and Expert committees are set up by RCGM and GEAC on a case by case basis and comprise of experts from various disciplines drawn from public sector institutions to prepare and review various guidelines and biosafety data. Central Compliance Committees are also set up for monitoring of confined field trials on case by case basis.

A series of biosafety guidelines have been issued by the regulatory authorities to deal with various aspects such as contained research, confined field trials, food safety assessment, environmental risk assessment etc. with a view to minimize any adverse impact that the GE plants may have. GMOs and product thereof would have on the environment as well as human and animal health. These include:

- Recombinant DNA Safety Guidelines, 1990
- Revised guidelines for research in transgenic plants & guidelines for toxicity and allergenicity evaluation of transgenic seeds, plants and plant parts, 1998
- Guidelines and Standard Operating Procedures (SOPs) for Confined Field Trials of Regulated, Genetically Engineered (GE) Plants - 2008
- Guideline for the Monitoring of Confined Field Trials of Regulated, Genetically Engineered Plants, 2008
- Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants, 2008.
- Protocols for Food and Feed Safety Assessment of GE crops, 2008
- Guidelines and handbook for Institutional Biosafety Committees (IBSCs), 2011

In continuation to these efforts, MoEFCC in association with the Department of Biotechnology (DBT) have prepared a set of three documents to strengthen the environmental risk assessment of genetically engineered plants in India. These include Guidelines for the Environmental Risk Assessment of Genetically Engineered Plants, 2016; Environmental Risk Assessment of Genetically Engineered Plants: A Guide for Stakeholders and Risk Analysis Framework, 2016.

The ERA Guidelines for GE Plants provided a comprehensive, and science-based framework for identification of potential harms, collection of relevant

scientific data pertaining to the nature and severity of any harms, and characterize the level of risk posed by GE plants. The accompanying Guide for Stakeholders has been prepared to provide additional explanatory material, illustrative examples, and references to scientific literature to provide a better understanding. The Risk Analysis Framework (RAF) describes the principles of risk analysis to be used by the Regulatory Agencies to protect human health and safety, and the environment. RAF also includes concepts related to, risk management, and risk communication in addition to risk assessment. The three documents put together provides a practical elaboration of risk assessment framework included in the Indian regulations in conjunction with Annex-III of the Cartagena Protocol on Biosafety, to which India is a Party.

Besides other issues, the guidelines deal with potential effects on biodiversity and ecosystems. The comparison of phenotypic and agronomic characteristics of the GE plants is used to determine whether they are substantially different from those of the know GM plant and whether any of these difference could have significant adverse environmental impacts on biodiversity.

**Table 1. An indicative list of GE plants under research and development/ field trials in India**

S.No.	Plant	Trait
1.	Banana	Antimicrobial peptide (AMP) gene
2.	Brinjal	Insect resistance
3.	Cabbage	Insect resistance
4.	Castor	Insect resistance
5.	Cauliflower	Insect resistance
6.	Chickpea	Abiotic stress tolerance, insect resistance
7.	Corn	Insect resistance, herbicide tolerance
8.	Cotton	Insect resistance, herbicide tolerance
9.	Groundnut	Virus resistance, abiotic stress tolerance
10.	Mustard	Hybrid seed production
11.	Okra	Insect resistance
12.	Papaya	Virus resistance
13.	Pigeonpea	Insect resistance
14.	Potato	Tuber sweetening, fungal resistance
15.	Rice	Insect resistance, diseases resistance, hybrid seed production, nutritional enhancement
16.	Rubber	Abiotic stress tolerance
17.	Sorghum	Insect resistance, abiotic stress tolerance
18.	Sugarcane	Insect resistance
19.	Tomato	Insect resistance, virus resistance, fruit ripening
20.	Watermelon	Virus resistance
21.	Wheat	Effect of mutant strains Azotobacter

As on date Bt cotton is the only GM crop approved for commercial cultivation in India. The total area under Bt cotton has increased from 0.05 million hectares in 2002 to 11.6 million hectares in 2014. Bt cotton is cultivated in more than 90% of the area under cotton.

Several public and private sector institutions are involved in the research and development of GE plants in India. More than 20 plants with varying traits such as hybrid seed production, insect resistance, herbicide tolerance etc. are under various stages of field trials.

As per the regulatory requirements, developers of GE plants from both public and private sector test their products according to the biosafety regulatory

requirements which include detailed documentation of testing. Regulatory authorities undertake thorough analysis of the data and the protocols used to ensure the validity of the results. In view of the above, India is well prepared to take forward the GE plants under research and development through the biosafety regulations for the benefit of society and farmers.

### References

- MoEF and BCIL, 2007, Biosafety Information Kit
- MoEFCC and BCIL, 2015, Biosafety Resource Kit
- MoEFCC and DBT, 2016, Environmental Risk Assessment of Genetically Engineered Plants



## Utilization of Crop Wild Relatives in the Breeding Programmes: Progress, Impact and Challenges

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The Crops Wild Relatives (CWR) includes wild forms, progenitors and those species that are closely related to cultivated crops. The concept of CWR is relative in the sense that all the related species may not have equal potential as gene donors to crops (Maxted *et al.*, 2006); their relative status is very often inferred rather than based on direct evidence (Heywood *et al.*, 2007). The gene pool system devised for understanding genetic relationship between crops and related species by Harlan and de Wet (1971) facilitated beneficial gene and traits transfer from wild to cultivated. Historically, the conservation of plant genetic resources (PGR) has focused almost explicitly on cultivated plants (Maxted *et al.*, 2008), however, in the recent past the importance of CWR have been recognized globally for breeding high yielding varieties to feed increasing population and to tolerate variety of stresses arising due to climate change. Thus, future crops species will need to be able to thrive in a drier, warmer, and more variable climate and in an environment increasingly populated by new pathogenic organisms. To meet these challenges we need broader crop gene pools and CWR has greater role to play in it.

### Crop Wild Relatives in India

India is one of the 12-mega diversity centres, accommodates part of four hot-spots—the Himalaya, Western Ghats, Indo-Burma and Sundaland out of 35 identified the world over and is immensely rich in agricultural biodiversity. Indian gene centre is rich in domesticated crops diversity having 168 species out of 2489 species distributed in 12 regions of diversity of cultivated plants (Zeven and de Wet 1982). Among CWR, 326 documented species have originated and/or developed diversity in different phyto-geographical regions of India (Pandey and Arora, 2004). However, Pradheep *et al.*, 2014 have made a pragmatic exercise to further shortlist and update the CWR of 168 native

crops, which resulted into 817 taxa belonging to 730 species, including wild/weedy form(s) or populations of 142 crop species, occurring in India (Table 1).

### Importance of CWR

The increasing genetic uniformity of crop varieties combined with climate change effects makes crops more vulnerable to various biotic and abiotic stresses. There had been examples of large scale devastations of crops due to genetic uniformity, for instance potato famine of the 1840s due to late potato blight epidemic across Ireland, Europe and North America; 1970s southern corn blight outbreak in the US maize and rice losses due to blast in the Philippines, Indonesia and India leading to the great Bengal famine. Intensive modern breeding efforts have contributed to a narrowing of the gene pool by concentrating more on favorable alleles already present in early domesticates (Debouck, 1991). CWR are therefore important for maintaining genetic diversity for and preventing such losses, which may have serious consequences for food, nutrition and environmental security. Advancement in the molecular biology in the study and utilization of species as gene source has made CWR a priority in PGR management and crop improvement. At the same time, post-CBD concerns of habitat loss, genetic erosion, policy issues related to access and benefit sharing and sovereign rights have also come in the forefront.

### Utilization in India Breeding Programmes

Despite having valuable genes with immense value for crops improvement and adaptation to changing environmental conditions, utilization of CWR has enjoyed a great success only in few crops, while disappointing for numerous others. Many genes are still lies untapped in these genetic resources, presumably due to the lack of useful genetic information and genetic bottlenecks as well. Scientists, the world over have argued that breeders were not fully exploiting the potential of

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**Table 1. Summary of crop-group-wise native CWR occurring in India**

Crop-group (crops**)	No. of CWR species*	Taxa	Crop-group (crops**)	No. of CWR species*	Taxa
Cereals and millets (13)	72 (2)	83	Vegetables (25)	76 (11)	87
Pseudocereals (3)	13 (1)	13	Spices and condiments (12)	50 (7)	54
Grain legumes (10)	49 (4)	57	Ornamentals (13)	141 (61)	152
Oilseeds (4)	9 (1)	10	Medicinal & aromatic plants (20)	70 (19)	81
Fibres (5)	18 (3)	20	Plantation crops (3)	12	14
Forages (16)	58 (14)	63	Others (8)	35 (7)	39
Fruits and nuts (36)	127 (12)	144	Crops: 168	730 (142)	817

\*Figures in parenthesis is crop species with wild/weedy form(s) or populations occurring in India, which are also included for counting as CWR;

\*\*One crop may involve more than one species

CWR; historically they relied on searching genes for beneficial traits associated with certain CWR rather than searching more generally for beneficial genes. Hajjar and Hodgkin (2007) while reviewing the utilization of CWR in 20 years found that over 60 wild species were used in 13 major crops and >100 beneficial traits derived from them.

Although, utilization of CWR in crop improvement is limited still many wild species have used successfully. Some of the examples include; use of *Oryza nivara* genes provided long-lasting resistance to grassy stunt virus (Brar and Kush, 1997) and use of *O. spontanea* as the source of wild abortive cytoplasmic male sterility, which has provided the cornerstone for today's hybrid rice (Li and Zhu 1988). Many unique traits such as weed competitiveness, drought tolerance and the ability to grow under low input conditions has been transferred from *O. glaberrima* to *O. sativa* and combined with high yield to develop NERICA (NEw RIce for AfriCA), which is high yielding, drought and pest resistant and adapted to the growing conditions of West Africa (Sarila and Mallikarjuna, 2005). Molnar-Lang *et al.* (2013), have given comprehensive review on intergeneric breeding of *Hordeum* species (cultivated and wild) with wheat. They found that wheat-barley hybridization has the potential to incorporate earliness, salt and drought tolerance, desired amino acid integration, and high tillering ability of barley into wheat, whereas the favourable characteristics of stem strength and winter hardiness of wheat can be introgressed into barley. Resistance to stem and leaf rust have been transferred from *Agropyron elongatum* and *Aegilops umbellulata* in wheat (Prescott-Allen and Prescott-Allen 1986) and spring wheat germplasm lines derived from *Aegilops tauschii* provided or resistance to Hessian fly (Suszkiw 2005). The most well known introgression is the rye (*S. cereale*) 1RS translocation that harbors genes involved in multiple disease resistance (*Pm8/Sr31/Lr26/Yr9*; Mago *et al.*, 2005) and improved

root structure (Sharma *et al.*, 2011), as well as additional positive agronomic characteristics (Rajaram *et al.*, 1983). Also, using marker assisted selection, one APR gene for leaf rust and stripe rust has been transferred from *T. monococcum* to bread wheat WL711 and one gene for leaf rust has been transferred to PBW343 background (Singh *et al.*, 2007).

In potato, *Solanum demissum* provided resistance to late blight (National Potato Council 2003) while in tomato >40 resistance genes have been derived from *S. peruvianum*, *Solanum pennellii* Correll var. *pennellii*, *Lycopersicon cheesmanii*, *L. pimpinellifolium* for traits such as increased soluble solid content, fruit colour, and adaptation to harvesting (Rick and Chetelat 1995). Broccoli varieties producing high levels of anti-cancer compounds have been developed using genes obtained from wild Italian *Brassica oleracea*. The species of *Solanum* complex such as *S. incanum*, *S. viarum*, *S. melongena* var. *insanum*, *S. khasianum* have provided gene for resistance to *Fusarium* wilt, bacterial wilt, frost tolerance and fruit & shoot borer. The wild species of okra *Abelmoschus tuberculatus* to YVMV and wild cucumbers *Cucumis hardwickii* and *C. callosus* have resistance to downy mildew and fruit fly, *Cucumis melo* var. *chito* for *Fusarium* wilt resistance.

Among pulses, *Vigna tribolata*, *V. mungo* var. *sylvestris*, *V. radiata* var. *sublobata* have provided resistance to yellow mosaic virus, *V. vexillata* has high protein and resistance to cowpea pod sucking bug and buchids and is crossable with *V. unguiculata* and *V. radaita*. Cytoplasmic male sterile systems were developed for pigeon pea exploiting the cross-pollination mechanism and utilizing wild *Cajanus* species (Mallikarjuna *et al.*, 2012). High protein and seed size breeding lines such as HPL 2, HPL 7, HPL 40 and HPL 51 were developed from *C. sericeus*, *C. albicans* and *C. scarabaeoides* (Saxena *et al.*, 1987, Jadhav *et al.*, 2012). *Phaseolus*

*coccineus* is a source of resistance to anthracnose as well as root rots, white mold, and BYMV in common bean (Sharma and Rana, 2012). In chickpea, productivity enhancement related traits have been introgressed from *C. reticulatum*, *C. echinospermum*, (Sandhu *et al.*, 2006; and for resistance to *Ascochyta* blight, pod number and short internode from *C. reticulatum*, *C. echino* (Singh *et al.*, 2015). *Cicer microphyllum* have been identified to carry genes for cold hardiness, drought tolerance and seeds/pod (Rana *et al.*, 2009). Stable Recombinant Inbred Lines (RILs) were developed for resistance to rust, powdery mildew and pod number from *Lens orientalis*, *L. odomensis* and *L. ervoides* (Singh *et al.*, 2013)

Gene sources have also been identified in *Sesamum laciniatum* to leaf phyllode, *S. malabaricum*, *S. mulyanum* and *S. alatum* for powdery mildew; *Linum perenne* for drought and cold hardiness, *L. grandiflorum* for linseed bud fly and alternaria blight; *Brassica oxyrhina*, *Moricandia arvensis*, *Trachystoma balli*, *Diplotaxis catholica* as sources of CMS, *B. tournefortii*, *Diplotaxis acris*, *D. harra*, *Eruca sativa* for drought tolerance in *Brassica*; *Corchorus aestuans*, *C. tridens*, *C. fascicularis* resistant to Jute semi-looper and *Macrophomina* sp., *C. olitorius* for yellow mite, *C. capsularis* for stem rot and anthracnose, *C. depressus* for drought tolerance and *Erianthus* sp. have gene for cold and drought tolerance, high yield for *Saccharum* improvement.

Among fruits, *Malus baccata* in apple; *Pyrus pashia* and *P. pyrifolia* in pear; *Prunus cerasoides* in cherry and *P. mira* in peach are used as rootstocks with multiple disease and insect resistance and drought tolerance. In Citrus, *Citrus jambhiri*, *C. limonia* and *C. karna* are used as rootstocks for cultivated species and are tolerant to citrus tristeza virus and most promising rootstocks for mandarin, orange and Kinnow in the lower hills. *Musa acuminata* ssp. *burmannica* found resistant to leaf spot and Foc wilt and *Rhodochlamys* such as *M. laterita*, *M. velutina*, *M. ornate* and *M. aurantiaca* have resistance to leaf spot, wilt and tolerance to nematodes. *Phyllanthus acidus* has tolerance for rust and frost. *Vitis parviflora* showed multiple disease resistance while *V. himalayana* is cold hardy, drought tolerant and late ripener, hence escape fruit cracking in rainy season in grapes.

## Conclusion

Wild species/species complexes that have studied and utilized are few considering the large number of

native economically important taxa in India. Also, species introduced for their potential importance are another large resource, which needs to be conserved on a priority basis as future introductions are likely to be few especially since these are now exchanged only with prior consent and under material transfer and benefit-sharing agreements. To cope with problems arising from intensive modern agriculture and climate change, it is essential to maintain genetic diversity within crop gene pools. In the past crop breeders had been struggling with the problem of linkage drag while dealing with CWR. Nevertheless, now the advances in DNA sequencing technology particularly combination of de novo sequencing and resequencing are being used efficiently to explore useful genetic variation in CWR. Therefore, plant breeders have to work closely with genetic engineers to develop new cultivars. There is need to design altogether new crops, plant types and varieties, which have the ability to perform well under adversities. Breeders should be well equipped with the genes to keep themselves one-step ahead of the rapidly evolving pests and diseases and even new climate regimes. The value of diversity in crops is similar to the value of a diversified portfolio to an investor. If one stock fails, the investor does not lose everything. Nature does not produce individual plants resistant to all diseases, pests and environmental stresses, and neither can the breeder. It is natural genetic diversity, in which many individuals, each somewhat different genetically, thrive together, allows a population to withstand challenges to its survival and produce traits of needs.

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## Insect Biodiversity, Ecosystem Services and Sustainable Agriculture

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The conflict for food between man and insects is as old as agriculture itself. Insects by far outnumber all other forms of animal life and they have been in existence for more than 400 million years in comparison to man who inhabited this planet just two-four million years ago. Hexapods are one of most resilient and successful having survived three mass extinction and witnessed the birth and death of dinosaurs. The diversity of insects can be gauged by the fact that there could be one billion insects per ha in a tropical forest and constitutes 55% of all animals by weight. They are found in deserts, mountain tops in evergreen forests and sea. They could be a mere 0.1mm or to 300 mm. While some are aquatic, other are terrestrial and often crop and tree dwelling. One of the unique features of insects is their reproductive potential. A queen termite can lay ~30000 eggs per day whereas, a human female in her lifetime can give birth to a maximum 25 children. Similarly, metamorphosis conferring several advantages to insects to tie over adverse ecological conditions have rendered insects by far to outnumber all other forms of life. Insects were the first to conquer air much before birds and mammals. The insect flight is a marvel of nature. They fly with four wings, unlike an aeroplane, which has two. While a horsefly can fly at 150 km/hour, a human being can maximum run 30 miles. The mastery of communication to find a potential mate or food or the presence of coordinated 1000 eyes have rendered them masters of evolution. The ability of insects to produce light is phenomenal as it is 99% energy efficient. The chemical ecology or communication through chemicals or social livings or chemical warfare or mimicry were mastered by arthropods 100s of millions of years ago. They were the one of first to use tools much before we started using sickle and hammer. They have migrated 1000 of miles to capture the entire world building termite mound or a tailored leaf or a honey coomb each an engineering marvel, surpassing the best architect. Insect societies still hold an example to the civilized world, the essence of living. They give us very useful products

like silk and honey and regulate our pests and diseases. Unfortunately, less than 1% compete with us but we have misunderstood the entire group of insect we have branded as our enemies. As very few are crop pest and vector of animal diseases. Let's not forget that they are excellent biocontrol agents, essential for nutrient cycling, essential for pollination and agriculture, necessary for crop diversity, one of the important source of food web in food chain, provide silk and honey besides stabilizing the ecosystem.

Ecosystem services as a concept was ingrained in the thought process and wisdom but over years the complexity of this holistic science has not been effectively documented. Unlike physical sciences, this requires a different approach and statistical validation over long term. ES is critical and is receiving emphasis more due to impact of agrochemicals than otherwise. Besides pollination and biological control, the decomposition of the dead and dead material, recycling of the waste, maintaining an ecological balance at different trophic levels, maintaining soil health, pollination of crop wild species and weeds providing seeds and berries for birds is critical in supporting regulating different services. Thus, any disturbance to insect population will impact pest infestation, pollination, predation and decomposition which will impact a chain of communities and agroecosystem.

Insects form an important linkage in food chain. It is now recognized insects contribute to not only decomposition but by the regulating services suppress pathogens of both livestock and humans. They provide services by conversing organic waste into nutrition rich humus and larvae of these insects are rich animal feed for chicken, pig and animal culture. In fact, bees as pollinators personify panda or tiger in educating people to support conservation.

Often ecosystem services are weighed on a scale of economic benefits alone. While economic return is a prerequisite for policy makers to make an investment,

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it would be unfortunate if ecosystem services suffer purely due to lack of scientific and economic assessment. The recent outbreak of insect transmitted diseases such as dengue, chikungunya reflects a need for scientific assessment of both urban and rural ecosystems, factors influencing epidemiology, food chain and food web and above all crop diversity as male mosquitoes are exclusively plant feeders.

In recent years, the infestation of stem borers on mango, citrus, cashew, pomegranate, grapes, coffee etc. is on the increase. The same is true of shot hole borers (Scolytidae) which are emerging a serious threat on coffee, grapes, pomegranate and other forest crops. Our export of any commodity on wooden crates infested with these borers pose a challenge to our international trade. Besides, many of these pests are extremely difficult to manage. A close perusal indicates that one of the reasons for their outbreak is deforestation. Thus, sustainable agriculture requires sustainable forest and not monoculture. Diversity is the essence of life.

Recently, the word *Soil Health* is often discussed. Soil health and soil health cards measuring just nutrient status are different. Arthropods measuring 0.2 to 10 cm inhabit soil and play a significant role in converting dry matter to humus. In fact, this is a prerequisite for microbes to enact their role. The greatest contribution

to agricultural biotechnology *Bacillus thuringiensis* is a soil dwelling bacterium. There are a host of others that need to be discovered. The world is beginning to appreciate the role of entomopathogenic nematodes in soil and their importance in pest control. From grasshopper eggs to a million others, soil is the source of survival.

Let us remember the words of George Washington who said *Nothing in my opinion is as valuable to a country as the top six inches of soil*. Indirectly, he was talking of the ecosystem services of soil arthropods and others along with microorganisms that are critical to soil health. Many are microscopic but their role is invaluable. Only one when we appreciate their direct and indirect benefits to the ecosystem, when sustainability is given more or at least equal importance as productivity and profitability, sustainable agriculture is possible. It is not just a matter of proof of concept, as the concept itself is complex and multidimensional. It is a matter of integrating learning with knowledge and wisdom.

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## **We Manage What We Measure: An Agrobiodiversity Index to Help Deliver the Sustainable Development Goals**

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### **Introduction**

Over the past century, farmers and breeders have used genetic diversity to breed high-yielding varieties. The Green Revolution brought new varieties and production methods to developing countries, with significant results. The number of undernourished people diminished from 35% in 1969-71 to 15% in 2010 (FAO, 2011), while global production of major crops increased dramatically, particularly in developing countries, where production went from under 1 billion tonnes to over 27 billion tonnes (idem).

However, these achievements have come at a price. The estimated limitations for keeping the planet safe have been exceeded for two boundaries which are closely related to the effects of modern agriculture: loss of genetic diversity and biochemical flows of phosphorous and nitrogen (Steffan et al., 2016; Rockström et al., 2014).

The second price was that – while the production of major crops increased – nutrition was largely left out of the equation. Today, six of the top 11 risk factors (such as child and maternal malnutrition, high blood pressure, high body mass index and high cholesterol) driving the global burden of disease are related to diet (Global Burden of Disease study 2013, in Global Hunger Index 2014). Around 800 million people suffer from insecure food supplies, while 2.1 billion people are obese or overweight (Ng, Fleming, et al., 2014; FAO: The State of Food and Agriculture, 2014). At the same time, 2 billion people lack essential vitamins and minerals critical for growth and development, such as vitamin A, iron and zinc (FAO, 2013). It is important to note that often these forms of malnutrition co-exist. In South Asia, the prevalence of wasting among children – at almost 15% – is so severe that UNICEF has declared that it is approaching the level of a critical health problem. Stunting among children in the region has decreased by 37% since 1990, but is still the highest in the world at

63 million. Overweight has risen 120% among children since 1990 – now the second highest in the world.

The third price we paid for the achievements of the Green Revolution was a narrowing of the genetic diversity in our production systems which in turn led to a narrowing of our global diet. From the 391,000 documented plant species, 5,538 have been counted as human food (Royal Botanic Gardens Kew, 2016). Out of these, just three – rice, wheat and maize – provide more than 50% of the world's plant-derived calories (FAO Animal Production and Health Guidelines, 2015).

Our global diets are becoming more homogenized with declining intake of nutritious pulses, fruits and vegetables, (Khoury et al., 2014). The on a global level the production of fruit and vegetables, nuts and seeds falls about 22% short of nutritional needs of the population. Shortfalls of these nutritious crops are even higher in developing countries. On the other hand, globally we are overproducing meat by 468%, while meat production falls short in many developing countries, including in South East Asia.

### ***Sustainable Development Goals***

Global leaders created the Sustainable Development Goals because they recognized that the challenges facing our world could not be tackled by developed or developing countries alone, nor by national governments and international agencies alone. Last year, the leaders of all 193 countries of the United Nations signed the Agenda 2030, committing themselves to achieving 17 Sustainable Development Goals (SDGs). The SDGs recognize that global problems are interconnected, and that considering only one problem at a time – for example, looking at food without considering nutrition or sustainable production systems – is no longer an acceptable way forward.

We need to find solutions which find synergies between different sectors so that we can feed the

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world (SDG 2), while addressing malnutrition (SDG 3), promoting sustainable production and consumption (SDG 12), adapting to the demands of climate change (SDG 13), and protecting the natural resource base on which all else depends (SDG 15).

### *Putting Options Back on the Table*

In this paper we outline one way to do this – by taking a more systemic look at the elements and activities that interact to provide our food. Food systems are a critical agent of a world transition to global sustainability. At the moment we are ‘locked in’ to a model of agriculture (IPES Food, 2016), which is both responding to and creating homogenized diets. The narrowing of our diets has also reinforced the narrowing of global research in the vast diversity of crops, leading to reduced interest in improving, commercializing and conserving this diversity. This in turn has led to fewer varieties of seeds on the market in many countries. As a result, farmers have fewer options which closes the cycle back to increasingly homogenized agriculture. It is difficult to break this feedback loop: but current measures – such as yield per hectare, calories produced, percentage of land under modern varieties, number of accessions – do not take the other goals, such as nutrition or resilience or climate adaptation into account. (IPES-Food, 2016).

New metrics are needed that can meaningfully juxtapose dimensions of agricultural biodiversity: nutritious, diverse diets; productive and resilient farms and landscapes; farmers’ access to quality, diverse seeds; conservation of agrobiodiversity for current needs and future options. Bioversity International is creating an index, the Agrobiodiversity Index, with this aim in mind.

There is an urgent need to find a way to measure and understand biodiversity in a quick, rapid and less expensive way; we need to go beyond just numbers and influence policymakers’ and private sector’s actions and decisions on best practices to foster diversity. Expected benefits are to be able to identify and steer opportunities for change towards sustainable food systems, and to be able to better measure and manage progress towards global targets such as the Sustainable Development Goals and the Aichi Biodiversity Targets of the Convention on Biological Diversity. Private companies and finance institutions are also interested in its applicability to measure the sustainability of investments, green bonds and company purchasing policies.

Of the huge, and growing, number of existing datasets collected at different scales across different dimensions, we ask: How to choose those to use in the Agrobiodiversity Index? Which are the salient aspects that need to be measured and compared? For food production we ask: how does agricultural biodiversity add simultaneously to the resilience, productivity and low environmental impacts of food production? For food consumption we ask: how does agricultural and wild biodiversity contribute to healthy, diverse diets? The third dimension asks: how are seed systems organized and supported to deliver the diversity needed for production and consumption? Finally, the fourth dimension asks: How to make sure that a wide enough pool of agricultural biodiversity remains available to serve today’s production and consumption needs and those that will arise to cope with changing demands of climate, land integrity and human nutrition?

Within each dimension, we scanned the scientific literature to identify evidence for the most salient aspects of each dimension with respect to agricultural biodiversity.

For consumption of healthy diverse diets, we bring together the scientific evidence that links using food biodiversity to diversification of diets to improved nutrition. We looked at both consumption via own production (or collected from the wild) and food purchases in markets. We looked at the role agricultural biodiversity can play in improving year-round nutrition.

For resilient production systems, we examine the pathways through which agricultural biodiversity drives critical ecological processes (e.g. soil structure maintenance) and allows a landscape simultaneously to provide multiple benefits to people (e.g. nutritious foods, income, natural pest control, pollination, water quality).

We also bring together the uses of agricultural biodiversity to increase resilience to farms, communities and landscapes; and how using it more effectively and more sustainably can help to maintain and increase the flow of services and benefits that agricultural biodiversity provides to communities.

For seed systems, we reviewed the five key functions of seed systems – facilitating access, production and distribution, innovation, regulation and conservation – and examined the evidence for how more diversified

seed systems can enhance sustainable food systems. We assessed what we know about the role policy and regulation can play in ensuring farmers have access to the options they need.

For conservation, our research indicates that many potential benefits of agricultural biodiversity to sustainable food systems are not realized because of poor conservation, lack of information and inadequate or restrictive government policies. We explored the evidence for successful conservation schemes, which safeguard the genetic diversity in places where it has evolved, backs it up in ex situ facilities for posterity, and makes it readily accessible and available for use.

In each of these four areas, we not only examined the relevant scientific literature, we also evaluated the various metrics used in each area, identifying which are efficient and effective measures of agricultural biodiversity. An assessment of the evidence and available metrics provide a starting point for identifying indicators for the Agrobiodiversity Index, which will be tested and validated throughout 2016 and 2017. A resulting book, forthcoming in 2017, will provide an overview of evidence which scholars and practitioners alike will find useful in our joint quest to use agricultural biodiversity in food systems that are sustainable.

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**Title Page:** The title page of the manuscript should be the first page and should include the title, names and addresses of the authors, abstract and keywords.

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**Materials and Methods** should include relevant details on the nature of material, experimental design, the techniques employed and the statistical method used. For well-known methods, citation of reference will suffice.

**Results and Discussion** should be clear to readers in different disciplines. Units of measurement should be SI.

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2. Withers LA and F Englemann (1998) In vitro conservation of plant genetic resources. In: A Altman (ed.) *Agricultural Biotechnology*. Marcell Dekker Inc., New York, pp 57-58.
3. WOI (1985) *The Wealth of India - Raw Materials. A Dictionary of Indian Raw Materials and Industrial Products - Raw Material Vol 1: A* (Revised). Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 513 p.
4. Engels, JMM and V Ramanatha Rao (eds) (1988) *Regeneration of seed crop and their wild relatives*. Proceedings of a Consultation Meeting, 4-7 December 1995. ICRISAT, Hyderabad, India and IPGRI, Rome, Italy, 167 p.

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