

Indian Journal of Plant Genetic Resources

Vol. 11

No. 2

1998

- Agro-biodiversity and Intellectual Property Rights (IPR) related issues
P.L. Gautam and A.K. Singh ... 129
- Biosafety concerns in biological resource management
K.P.S. Chauhan ... 153
- Plant quarantine as an aid to safer exchange, conservation and utilization
of plant genetic resources
P.C. Agarwal, Baleshwar Singh and P.L. Gautam ... 165
- Collection of rice germplasm from Malabar, Kerala
K. Joseph John and M. Abdul Nizar ... 173
- Genetic variability for morpho-physiological components of yielding
ability in rainfed rice (*Oryza sativa* L)
S.G. Hegde and C.S.P. Patil ... 183
- Correlation and path analysis in induced mutants of wheat (*Triticum
aestivum* L)
M. K. Rana and G. S. Sethi ... 189
- Variability in pea germplasm for some agromorphological traits
S. Sardana, Dinesh Kumar, Poonam Suneja and S. Kochhar ... 197
- Genetic divergence in tomato
S.N.S. Chaurasia and Major Singh ... 203
- Genetic divergence in *Leucaena* varieties using non-hierarchical euclid-
ean analysis
K.C. Chauhan and R. Hosalli ... 207

(Continued on back cover page)

Published by

INDIAN SOCIETY OF PLANT GENETIC RESOURCES

NBPGR CAMPUS, NEW DELHI 110 012, INDIA

AGRO-BIODIVERSITY AND INTELLECTUAL PROPERTY RIGHTS (IPR) RELATED ISSUES

P.L. Gautam¹ and A.K. Singh²

National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

Biodiversity refers to variability of living organisms and ecosystems, while agro-biodiversity to variation in agriculture related organisms and their ecosystems. They are the keys to sustainable socio-economic development. The realisation of importance of variation among the species and among the individuals of the same species was the beginning of farming. The developing countries are rich in biodiversity. India is one of the twelve centres of megabiodiversity. The plant biodiversity originating from developing countries has been the basis of crop improvement, in addition to its other benefits as source of food, fuel, fibre, medicines etc. However, the benefit accrued from the use of biodiversity has mainly been harnessed by technologically and economically rich developed countries and has not been fairly shared with provider gene-rich countries. Several international initiatives, such as CBD and its mechanisms, FAO Commission and its mechanisms, TRIPS under GATT, UPOV etc. are debating to reach an international consensus on conservation, use and fair and equitable sharing of benefits arising from the use of biodiversity. Consequently, genetic resources that were considered as "common heritage of humankind" until December 1993, have become national property, with State's having sovereign rights to determine access and use. On the other hand, TRIPS agreement demands member countries to provide protection to all inventions, including those of agricultural products and processes. As the earlier property rights and variety protection acts, do not recognise the contributions of farmers and local communities in conservation, sustainable use and providing of these genetic resources, the gene-rich developing countries need to take appropriate steps for documenting the indigenous knowledge related to genetic resources conservation and use, and bring them under intellectual property rights on one hand and develop a *sui generis* property right system on the other for variety protection recognising these contributions. The present article discusses these issues in detail and provides status of, and insight into different international initiatives.

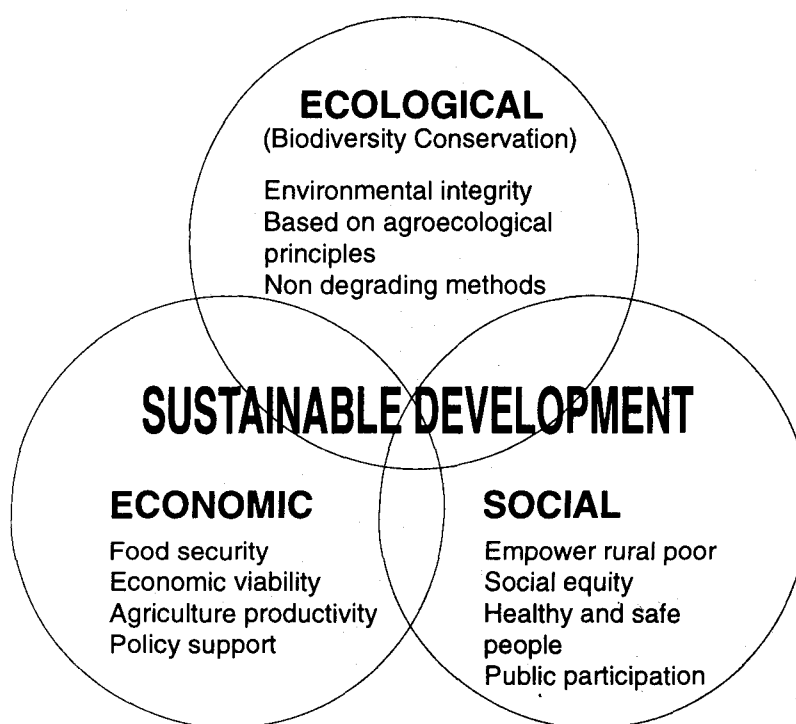
Key words : Biodiversity, agro-biodiversity, Intellectual Property Rights, access to plant genetic resources, benefit sharing, *sui generis* system

Elements of Agro-biodiversity

Biodiversity is the variability among the living organisms and diversity within species, between species and ecosystems. It provides a range of benefits to humanity. Agro-biodiversity is an evolutionary divergent line of biodiversity

¹ Director, NBPGR; ² Visiting Scientist/Expert (Currently Project Coordinator, Pigeonpea, IIPR, Kanpur)

concerned with agro-ecosystems, and variation in agriculture related plants, animals, fish, insects and other flora and fauna in agro-ecosystems, as well as elements of "natural" habitats that are part of food production cycle. It has multiple, economic, ecological and social benefits and is a crucial and integral component of sustainable development as illustrated in Figure 1. Agro-biodiversity gives farming systems and taxa, the means to recycle nutrients, reduce pest, and disease problems, control weeds, maintain good soil and water conditions, handle climate stresses, while producing agricultural commodity necessary for human survival. Thus, it is "cornerstone stability" and a basis of livelihood and of sustainable development (GRAINS, 1994).



Source : Thrupp, 1998

Fig. 1. Agrobiodiversity and sustainable development

Agro-biodiversity in retrospective

Early farmers, from the beginning of civilisation, realised the value of diversity of species and varieties. The evolution of farming systems and practices, along with farmers' experimentation and trading practices of seeds allowed the production of an immense diversity among plants of economically important species. Plant collecting - also referred as "gene prospecting" began very early. The earliest record of plant collection goes back to 2500 BC, when

Sargon crossed the Tanras ^{ou} ~~maintains~~ to Antolion Pleatu to collect figs, Viner Xdives and to 1945 BC, with Egyptians collecting plants from various parts of the world for food, medicine, and other purposes (Juma, 1989). In Europe during colonial rule, wealth was gained by exploiting plant genetic resources from overseas; collection and movement of seeds was associated with expansion of political control and trade. This was one of the motives of most of explorers. Continuation of global collection expedition and advent of new techniques of plant propagation, conservation and exchange of seed increased. The botanical gardens became the new centres of adaptation, conservation/maintenance and disseminating tropical crops (Juma, 1989). Explorers also continued to seek control of these resources in the tropics.

A breakthrough in the understanding of the principle of inheritance following Mendel's discovery in mid of 19th century added another dimension to value of plant diversity and became foundation of breeding programs to overcome yield reducing factors and to increase productivity using the available variation for such factors.

In the 20th century, N. Vavilov, a renowned Russian Botanist carried out systematic plant collection, pioneering research and conservation of plant diversity. He developed the theory about the origin of domesticated species. He also recognised the value of diverse multi-cropping farming practices being used by traditional farmers to buffer against risk and to sustain yields. He launched numerous collection expeditions worldwide to collect germplasm and established a seed bank in 1940 at St Petersburg; that still exists as N.I. Vavilov Institute, and contains some 380,000 specimens from more than 180 places in the world. Vavilov and others, O.H. Frankel, J.R. Harlen, J.G. Hawkes, A.C. Zeven, P.M. Zhuovsky etc. revealed the importance of diversity in breeding high yielding varieties and to overcome different yield reducing factors. Vavilov recognised eight centres of origin (as quoted by Harlen in 1971), Zeven and Zhukovsky (1975) 12 and Hyes (1992) 14 (as cited by Thrupp 1998); India happens to be one of these centres.

The gene-rich Indian sub-continent

Indian sub-continent entails a wide spectrum of life forms; its cultural, ethnic diversity includes over 550 tribal communities of 227 ethnic group, spread over 5,000 forest villages, agro-climatic situations vary from humid tropical to semi-arid, temperate and alpine, and agrarian habits range from highly mechanised, near mono-specific cropping to nomadic cultivation and even gatherers. The sub-continent is bestowed with immensely rich landrace diversity in major agricultural and horticultural crops, due to native farmers' conscious/unconscious selections, inherited and perpetuated over generations.

The Indian sub-continent has great variation in agro-climatic conditions and distribution of flora. On the basis of distribution of flora it has been divided into eight regions: 1) Western Himalayas, 2) Eastern Himalayas,

3) Central Himalayas, 4) Assam plains, 5) Gangetic plains, 6) Indus plains, 7) Deccan, and 8) Malbar (as cited by Arora 1991). Based on physiographic, climatic and cultural features, eight agro-climatic regions were initially recognised, however, in 1990, based on further micro-climatic consideration they have been increased to 20 (Sehgal et al. 1990). Further, the Planning Commission has demarcated the country into 15 agro-climatic zones.

The sub-continent is the centre of domestication and diversification of several forest plant species, forages, grasses, shrubs, herbs, ornamentals etc. The floristically very rich India has about 141 endemic genera belonging to over 47 families of higher plants (MoEF, 1997). Of the 4,950 endemic species, the largest number (about 2532) species are located in Himalayas followed by peninsular region (1,788 species) and Andaman Nicobar islands (185 species). It also holds half of the world's aquatic floral diversity. It is a treasure house of wild economic plants, particularly wild edible and medicinal plants, which are largely utilised locally or in several *Ayurvedic* preparations, which can be safely termed as commercially under-utilised (Gautam, 1997).

About 44 major or minor crop species were domesticated in India (Table 1). India is known to have more than 17,500 species of higher plants (MoEF, Pers. Comm.) including 168 major and minor crop species (NBPGR, Pers. Comm.: Table 2) and 334 of their wild relatives (Table 3). Additionally, the native tribes use another 1,532 wild edible plant species. These include 145 species of roots and tubers, 521 of early vegetables/green, 101 of bulbs and flowers, 647 of fruits and 118 of seeds and nuts. In addition, nearly 9,500 plant species of ethno-botanical uses have been reported from the country, of which around 7,500 are of ethno-medicinal purposes and 3,900 are multipurpose/edible species (NASS 1998)

Table 1. Cultivated crop species originated in India

Crop Group	Crops
Cereals	Rice, Little millet
Grain Legumes & Oilseeds	Black gram, green gram, Mothbean, Pigeonpea, Horse gram, Sarson
Vegetables	Egg plant, Cucumber, Ridge gourd, Pointed gourd, Snake gourd, Round gourd, Kundru, Arbi, Jamikand, Leafy mustard
Fruits	Citrus, Banana, Mango, Jamun, Kronda, Khirni, Phalsa, Bael, Wood apple, Jack fruit
Spices & Condiments	Ginger, Turmeric, Cardamom, Arecanut, Black pepper betel leaf, Cinnamon, Dalchini
Others	Sugarcane, Sunhemp, Tree cotton, Jute, Bamboos, Tea, Dhaincha

Arora, 1991 and personal communication

Table 2. Cultivated crop species grown in India

Crop groups	Number
Cereals	7
Legumes and oilseeds	13
Vegetables	29
Fruits	22
Fibre and fodder plants	18
Spices and condiments	17
Medicinal and aromatic plants	20
Others	42
Total	168

Source : Personal communication

Table 3. Wild relatives of crop species found in India

Crop groups	Number
Cereals and millets	51
Grain legumes	31
Oilseeds	12
Fibre plants	24
Fruits	109
Vegetables	54
Spices and condiments	27
Others	26
Total	334

Source : Arora, 1991

India is also rich in animal diversity. A total of 81,000 animal species are recorded in India, which represents 6.24% of the faunal species recorded in the world (MoEF, Pers. Comm.: Table 4). It houses perhaps the world's largest diversity of livestock, with some 26 breeds of cattle, 40 of sheep, 20 of goats, and 18 of poultry. World's all the eight buffalo breeds are found in the country. The wild fowls provide valuable genetic stocks for poultry improvement, particularly for incorporating disease resistance in the improved breeds (Gautam, 1997).

Table 4. Number of animal species in India and the world

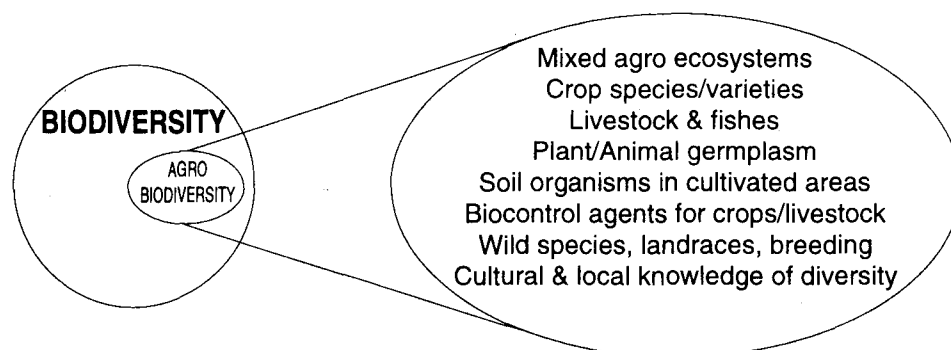
Taxa	Species		Percentage of India to the world
	India	World	
Protista	2,577	31,290	8.24
Mollusca	5,050	70,000	7.21
Arthropoda (Insecta, Crustacea etc.)	60,383	1,065,000	5.67
Other invertebrates (including Hemichordata)	8,329	87,121	9.56
Protochordata	116	2,173	5.34
Pices	2,546	21,723	11.72
Amphibia	206	5,145	4.00
Reptilia	485	5,680	8.54
Aves	1,228	9,672	12.69
Mamalia	372	4,629	8.03
Total	81,292	1,302,433	6.24

Source : UNEP-GBA (1995), (MoEF 1998)

Scope of agro-biodiversity

Agro-biodiversity consists of many dimensions of diversity related to agriculture, at the genetic, species and agro-ecosystem levels (Fig. 2). It encompasses variability of

- Genetic resources that are essential living material of plants and animals
- Edible plants/food crops, including landraces, cultivars, hybrids etc.
- Livestock and edible fish/aquatic organisms
- Soil organisms that are vital to soil fertility, structure, quality and soil health
- Insect, fungi and bacteria, that naturally occur in and around farms, which serve predators and natural enemies for pests and diseases to domesticated plants and animals
- Agro-ecosystem components and types, that are vulnerable for nutrient cycling, stability and productivity
- "Wild" resources (species and elements) of natural habitat and landscapes, that can provide services relevant for agricultural development



Source : Thrupp, 1998

Fig. 2. Conceptual view of agrobiodiversity

Thus agro-biodiversity does not contain only the variability of species but also the associated traditional knowledge for managing biological resources i.e. the ways farmers have used the biological diversity - of crops, trees, soils, animals, insect and biota - for sustainable conservation and food production.

Benefits of agro-biodiversity

There are approximately 75,000 species of edible plants worldwide (Wilson 1988), but over the course of human civilisation, only about 7000 plant varieties has been used for food (Juma, 1989). There is also a remarkable diversity and abundance of insects, fungi and other organisms that are valuable to the productivity of agro-ecosystems. The concept also includes habitats and species outside farming systems that benefit agriculture and enhance ecosystems functions, such as, sources of host plants for natural enemies and predators of agriculture pests. In order to increase production and food security some wild plant species are also used. In sum, agro-biodiversity clearly has multiple proven benefits - for productivity, sustainability, and food security at all scales and types of agricultural systems, and it is a vital part of diversity in general. Hence, Agro-biodiversity can benefit as follows

- Increase productivity, yields and food security (consequently economic returns)
- Reduce pressure of agriculture on fragile areas, forests and endangered species
- Build stability, robustness, and sustainability of farming systems
- Contribute to sound pest and disease management and sustainable intensification
- Conserve soil and increase natural soil fertility and health
- Diversify products and income opportunities from farms
- Reduce/spread risks to individuals, communities, and nations

- Help maximise effective use of resources and environment (restore ecological health)
- Reduce dependence on external inputs
- Increase nutritional values, provide sources of medicine and vitamins
- Conserve ecosystem structure and stability of species diversity

Use of agro-biodiversity in sustainable development

The component of agro-biodiversity used in development of new plant varieties or hybrids is called genetic material. It is any material of plants, animals, and microbes or of other origin containing functional units of heredity. It includes seeds, semen, tissue, cells, pollens, DNA molecules etc. The use of the genetic material (genetic variation) as source of gene(s) in the genetic improvement of crop species/domesticated animals to produce high yielding varieties, hybrids, breeds etc. for sustainable increase in food production, food security and poverty alleviation is increasing, to effect ecologically sustainable economic and social development. Hence, the flow of genetic material of actual and potential value is also on the increase. A few examples of utilisation of Plant Genetic Resources in conventional breeding that made significant impact on food production are :

The Japanese primitive dwarf wheat *Norin 10*, which was introduced into America in 1946, played an important role in efforts to improve wheat varieties. The selected dwarf cultivars reacted well to fertilizers giving higher yields. Semi-dwarf wheat varieties occupied 60% of wheat acreage in the USA (Brady, 1985). A Turkish wheat collected by Harlen in 1948, which was excluded because of poor agronomic traits, proved very useful because of having genes for resistance to *Puccinia striiformis*, *Tilletia caries* (35 strains), *T. foetida*, *T. controversa* (10 strains). This variety was also tolerant to some species of *Urocystis*, *Fusarium* and *Typhula*. Therefore, it was used in resistance breeding to increase resistance of wheat varieties against these parasites (Esquinas-Alcazar 1987).

In tomato, several species have been used in breeding programmes to select varieties resistant to parasite fungi, (*Lycopersicum hirsutum*, *L. peruvianum* and *L. pimpinifolium*), virus (*L. chilense* and *L. peruvianum*), nematodes (*L. peruvianum*), insects (*L. hirsutum*), for improvement of fruit quality (*L. chmielewskii*) and adoption to unfavourable condition (*L. cheesmanii*) (Esquinas Alcazar, 1987). Two species from Peru were used in US to increase pigmentation and solid matter content, which resulted in an annual gain of US \$ 5 million to canned tomato industry in US (Sasson, 1996). The conservation of diversity within a species is indispensable to future utilisation. For example, one single population of *Oryza nivara* has been used to select rice varieties with resistance to grassy stunt virus (Esquinas-Alcazar, 1987). There are many more such

examples in corn, rice, groundnut, rubber, alfalfa etc. proving that accession of genetic resources has been the basis of improving and selecting crop varieties to increase productivity either through conventional breeding, culture of plant cells, tissue and organ or gene transfer.

Similarly, according to a 1988 consultancy report of McAlpine, Thorpe and Warriar (U.K), the market potential for herbal drugs in the "Western" world could range from 4.9 billion to 47 billion US \$ in the next 10 years (i.e. by 2000). This expected growth is due to paradigm shift in consumer preference to plant derived drugs and that pharmaceutical companies are continuously seeking new compounds for medicine to improve their competitiveness (Komen, 1991). World Health Organisation (WHO) estimated that about 80% of people in developing countries still rely [low price] on plant derived drugs, (Sasson 1996).

Despite the clear economic significance of plant genetic resources, the countries, communities, individuals providing these genetic material or knowledge about the use of these resources did not receive adequate benefit from their commercial and industrial uses. This is not only inequitable, but also undermines the efforts of conservation of biological diversity for sustainable use. Such situation led to realisation for the development of control mechanisms to regulate the access and use of genetic resources, indigenous knowledge on conservation and use, and varieties developed through human innovation to ensure incentives and fair and equitable sharing of benefits between stakeholders.

Intellectual Property Rights (IPRs)

The introduction of new era of national sovereignty over genetic resources under CBD requires a new relationship among countries of origin (provider) and the user of genetic resources. Country of origin is not only entitled to share the benefit arising from the use of these resources, but is provided with the legal basis on which the demand can be made for this entitlement. The key to implement the new relationship is exercising control over access to agro-biodiversity. However, this is not simple control because of intangible nature of value of these resources and presents unique difficulties. One of the best ways of regulating access to plant genetic resources and agricultural products to internalizing intangible benefits is via IPRs. This is due to flexibility to private property rights, minimal involvement of government, and psychological advantage of absolute ownership. There are three broad categories of IPRs:

1. **Industrial property rights** covering IPRs such as patents, trademarks, and industrial design;
2. **Copyright** and related rights covering artistic and literary works performances, broadcast etc. and

3. IPRs that do not fit to this classification are called *sui generis*, meaning one-of-its-kind; they include layout design of Semi-conductor chips and plant breeders' rights etc.

International mechanisms concerned with plant biodiversity and intellectual property rights

For a long time in international law, genetic resources were viewed as a "common heritage of humankind" and thus to be made available to all. Several international mechanisms and bodies of the United Nations were activated for conservation, and sustainable and equitable use of agro-biodiversity, yet the Convention on Biological Diversity (CBD) and its mechanisms like, the Conference of Parties (CoP), Subsidiary Body for Scientific, Technical and Technological Advice (SBSTTA), interim funding mechanism, the Global Environment Facility (GEF), and the Food and Agriculture Organisation of the United Nations (FAO) inter-governmental Commission on Genetic Resources for Food and Agriculture (CGRFA) and its mechanism, FAO International Undertaking on Plant Genetic Resources (IUPGR) and FAO's Global Plan of Action (GPA) constitute the main institutional components of the global system. In addition, Trade-Related Aspects of Intellectual Property Rights (TRIPS) under General Agreement on Tariffs and Trade (GATT) of the World Trade Organisation (WTO) and International Union for the Protection of New Varieties of Plants (UPOV) are the other two agreements concerned with patenting and plant variety protection.

Convention on Biological Diversity

The CBD is a legal binding agreement signed in Rio de Janeiro at the earth summit in 1992. It has 170 countries and the European Union as parties. The objective of the CBD are the conservation of biodiversity, the sustainable use of its components, and fair and equitable sharing of the benefits from the use of genetic resources.

The resulting agreement establishes a range of general, flexible obligations that emphasise national action within national jurisdiction for conservation and sustainable use, in recognition of sovereign rights to set environmental policy and exploit natural resources. It has also set up a frame work for international exchange of genetic resources.

The Convention through Article 15 recognises sovereign rights of States over natural resources. The authority to determine access to genetic resources rests with the national governments and is subject to national legislation, ensuring thereby that benefits derived from their use are shared equitably. The important provisions of CBD on access to genetic resources and benefit sharing are as follows:

Access to genetic resources : The Convention calls on to the Parties (national governments) to establish conditions that facilitate access to genetic resources for environmentally sound use and promote the sharing of benefits arising from their use. In other words, source countries are obliged to ensure that their policy, administrative, legal and constitutional frame does not impose restrictions that run counter to the objectives of the Convention.

Access on mutually agreed terms : Article 15(4) of the Convention requires that access to genetic resources, where granted, should be on "mutually agreed terms" (MAT). However, this sets out a number of conditions, Prior Informed Consent (PIC); a fair agreement on mutual terms; agreement on sharing of benefits arising from use of the resources; participation by the party providing genetic resources in scientific research utilising genetic resources in question; transfer of technology to provider and assurance that resources will be put to environmentally sound use. The concept of "mutually agreed terms" establishes new participatory relationship among country of origin, provider and user of genetic resources.

Prior Informed Consent (PIC) : Article 15(5) of the Convention states that "access to genetic resources shall be subject to Prior Informed Consent of the Contracting party providing such resources, unless otherwise determined by that Party". PIC gives the providing Party the authority to require potential user to obtain authorisation, and provide information that may determine access, such as, which resource, how and by whom they will be used etc., thereby providing the providing Party an opportunity to become equal partner and monitoring the use

Fair and equitable sharing of benefits : Along with the requirement of access, Convention also establishes the principles of fair and equitable sharing of benefits derived from the use of genetic resources. These can be scientific and technological knowledge, skill enhancement, up-front payment on collection of genetic resources and royalties on product developed. However, Convention does not provide explicit guidelines, on how the benefit are to be shared and has left the Parties to develop legislative, administrative or policy measures to ensure that benefit actually reach intended beneficiaries.

The CBD's Conference of Parties in its second, third and fourth meetings (CoP decision II/5, III/11 and IV/6) has emphasised that agro-biodiversity must receive adequate attention in the member nations development plans. Unfortunately, some key issues, such as pre-CBD *ex situ* collections held at botanical gardens, herbaria and zoo etc. and the collections held by International Agricultural Research Centres (IARCs) of the Consultative Group on International Agricultural Research (CGIAR) "in trust of humankind" under agreement reached with FAO in 1994 with open access to *bona fide* users and Farmers' Rights are still outstanding.

International Undertaking on Plant Genetic Resources/FAO Commission on Genetic Resources for Food and Agriculture

In 1983, the FAO Conference established the first permanent intergovernmental forum (The Commission on Plant Genetic Resources) in UN system to deal with question concerning plant genetic resources. It also adopted a formal framework: the IUPGR. The Commission aims to establish a Global System with the objectives to ensure the safe conservation and promote the availability and sustainable utilisation of plant genetic resources for food and agriculture for present and future generations, by providing a flexible framework for sharing benefits and responsibilities. As part of the Global System and its framework for implementation of activities, the IUPGR is currently being adapted/negotiated to harmonise with the CBD provisions (FAO, 1997). Another element of Global System is the Global Plan of Action (GPA) for the Conservation and Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture. It is adopted by more than 150 countries and 40 organisations. It has programmes and activities aimed at filling gaps, overcoming constraints and facing emergency situations identified in the FAO report on the state of the world's plant genetic resources. The Global Plan of Action has 20 priority activities areas divided in to four groups, in situ conservation and development, ex situ conservation, utilization of plant genetic resources and institution and capacity building. IUPGR is a non-legally binding agreement in contrast with other agreements/conventions.

CBD has left several issues of relevance to the IU and FAO, including the question of Farmers' Rights and the status of pre-CBD *ex situ* collections. Three outstanding issues dominate the current IU negotiations: scope and access, benefit sharing, financial mechanisms and Farmers' Rights.

Trade Related Intellectual Property Rights Systems (TRIPS) under the World Trade Organisation (WTO)

The TRIPS Agreement is one of the agreements of the WTO adopted under the General Agreement on Tariffs and Trade (GATT). It entered into force in 1995.

The TRIPS Agreement is innovative from both a trade and intellectual property rights (IPR) perspective. Article 27 of TRIPS in paragraph 1 provides in part that the "patents shall be available for any invention, whether products or process, in all fields of technology". Article 27.3 (b) of TRIPS provides that members may exclude from patentability-

"Plants and animals other than micro-organisms, and essentially biological process for the production of plants or animals other than biological and micro-biological process. However, members shall provide ~~for~~ the information for the protection of plant varieties either by patents or by an effective sui generis system or by any combination thereof"

The requirement of IPR protection for plant varieties - in the form of either patents or a *sui generis* system - will also necessitate legislative changes with a number of WTO Members. Under Article 65, developing economies and least developed economies are allowed a grace period of four and 10 years respectively, to implement these provisions.

TRIPS article 27.3(b), which concerns life patents and plant variety protection, provides that its provisions, "shall be reviewed four years after the date of entry into force of WTO agreement". The first review is due in 1999.

The TRIPS Agreement also ensures a universal, minimum level of protection of commercial marks such as trademarks and geographical indications. For the first time in an international law, trade secrets have also been accorded the status of IPRs.

International Union for the Protection of New Varieties of Plants (UPOV)

UPOV is an intergovernmental organisation established by the International Convention for the Protection of New Varieties of Plants (the "UPOV Convention") with the purpose of ensuring that member States acknowledge the achievements of breeders of new plant varieties, by making available to them an exclusive property right, on the basis of a set of uniform and clearly defined principles. The exclusive rights provide an incentive to breeders for genetic improvement and development of new varieties and hybrids in agriculture, horticulture and forestry (Anonymous 1997). The right is not just a reward for further investment, but also requires the breeders to maintain the variety and allow him/her to participate actively in the distribution, thus ensuring that past investments are not wastes (Anonymous 1997). The UPOV convention was signed in 1961, came into force in 1968 and was revised in 1972, 1978, and 1991. The 1978 version was in force until April 1998 (Watal 1998a). It has 32 members as of May 1997, most of which are from developed world. UPOV encourages the adoption of *sui generis* laws for protecting new plant varieties by creating distinct system outside of patent law (Bragdon and Downes, 1998). There are four version of UPOV. All four versions require that variety be new, distinct, uniform and stable in order to be eligible for protection.

Countries which are signatory to these agreements must align national policy and legislation with them. However, the agreements emerged from different debates have different goals and objectives and in some instances may be incompatible in their requirements. Language is often very vague or deliberately non-definitive which leads to multiple interpretations depending on prospective. Neither TRIPS nor UPOV address genetic resources *per se* and thus silent on issues such as sovereignty, ownership, access, benefit sharing, technical problems, and funding. Efforts to harmonise several of these accords

are underway; some of the differences on specific issues between above agreements are given in the Table 5.

Table 5. Differences on specific issues among various international agreements

ISSUE	INTERNATIONAL AGREEMENT			
	CBD	IUPGR	TRIPS	UPOV
National sovereignty	Reaffirms	Reaffirms	Do not address	Do not address
Access to genetic resources	With PIC and on mutual agreement	On mutual agreed terms and on mutual exchange	Do not address	Do not address
Benefit sharing	One of its major objectives	As component of farmer's rights	Do not address	Do not address
Intellectual property rights	Acknowledges but without guide lines	Acknowledges in agreed interpretations	Wants PVP through patent or <i>sui generis</i> system or a combination	It is the objective
Funding	Exist as concept	Under discussion	Silent	Silent

Agro-biodiversity/Agricultural Products and IPRs

The IPRs were developed in industrial world mainly to protect, and provide incentives for human creativity. These rights provide monopoly to the holder to control the use, dissemination of products or processes that are novel, unique, useful and satisfy the test of incentiveness. The IPRs most suited for goods or services produced in agricultural are patents, plant breeders' rights, trademarks, geographical indications and trade secrets.

Patents : Patents universally give the patentee the right to prevent third party from making, using or selling the patented product or process. Patents however, have to be disclosed to the public through the patent documents. This enables researchers to develop further useful products or services. Patentable products have to meet the criteria of patentability i.e. novelty (not known before), non-obviousness (involve inventive step), and usefulness (industrially applicable).

Plant breeders' rights (PBRs) : These IPR fall in-between industrial (patent) and *sui generis*. They are little weaker than patent protection in that right holder can only prevent third party from commercially exploiting the

protected material. Realising the fact that developing new breeds requires much efforts and resource, that developed product can be multiplied fast providing insufficient benefit to recover investments, and to keep the tempo of genetic improvement of crop species for increase in food productivity, PBRs were developed in developed economies. Convention of the Union for Protection of New Varieties of Plants (UPOV) prepared first text in 1961. Until recently, it had two exceptions compared to monopoly rights-

1. "Farmers' Privilege", which amounts to farmers right to save seeds for replanting and
2. Right for use of protected varieties in research & development

["UPOV 1978" could be considered as a model for developing countries to frame their own legislation, provided it is otherwise TRIPS-compatible. Such law can contain provisions for "breeders' exemption"- allowing the use of the protected variety for breeding purpose, or "farmers' privilege" allowing farmers to retain seed for their own use or for across-the-fence non-commercial exchange or sales of seeds (Watal 1998b)].

However, in 1991, at the request from Plant Breeders of developed economies, UPOV made two amendments to match PBR with industrial patents protection. These may have some discouraging effect on agro-biodiversity conservation and on economics of rural and farming communities (Johnston and Yamin, 1997). The amendments are-

1. Breeders authorisation for propagation of material, and
2. Limiting the "Farmers privilege", i.e. to save seeds for further planting and innovation

This means that farmers have to take breeder's consent for handling the protected varieties and pay royalties for each activity of commercial benefit. It would restrict them from taking further innovation that they have been doing from centuries and put them at an economic disadvantage. However, recently farmers have been allowed to propagate the saved seeds for their farm. Thus, PBRs have come close to patents and may have adverse effect on conservation and use of agro-biodiversity. The PBRs do not recognise the contributions of country of origin, local communities and farmers, which have spent time and resources identifying, conserving and providing genetic resources, and nor do they address the problems of genetic erosion and increased vulnerability in farmers' fields caused by encouragement of monoculture and uniformity of released varieties and the abuse of market by multinationals.

Trademarks : They are generally used in commerce, but can be applied to both agriculture and industry. For instance, trademarks are used to market seeds or spraying services. The essential purpose of the trademark is to

distinguish the goods or services from another. Such protections prevent wrongful use of commercial marks. These, need to be renewed from time to time.

Geographical indications : These are marks associated with products originating from a country, region or locality where the quality, reputation or other characteristics of the product are essentially attributable to its geographical origin. Most geographical indications relate to agricultural products or those derived from them, as in the case of wine, spirits, and tea etc. Plant varieties developed with traditional knowledge and associated with a particular region can also be protected as geographical indications.

Trade secrets : The agriculture sector used trade secret protection to protect for instance, hybrid plant varieties. Trade secret can be protected against third party misappropriation through laws relating to unfair competition or to restrictive trade practices or to contract law. It does not have obligation to disclose the inventive or creative ideas to society.

Farmers'/Community rights : There is at present no widely accepted legal system, which recognises the right to own natural resources, traditional varieties and traditional knowledge etc., though such concepts as "Farmers' Rights", "Community Rights", "Traditional Resources Rights" and "Informal Innovation System Rights" are debated internationally. For example, **community Rights** envisage to recognise the rights of local communities over 'Common property' e.g. pastures, forests, water bodies and the knowledge on their conservation and use, to ensure equitable benefit sharing, and provide powerful incentives to these communities to conserve biological resources. Whereas, **farmers' rights** are conceived as a rights of compensation arising from the past, present and future contributions of farmers in conserving, improving and making available plant genetic resources, particularly from centre of origin/diversity. These can be thought as type of IPR and attempt to create a property right over the intellectual or intangible contribution of farmers to current agricultural wealth.

TRIPS requirement for IPR

TRIPS require all member countries to establish an IPR regime, though the countries are free to determine the method of implementing the provisions within their own legal system and practices (Watal 1998b). The patentability of plant genetic innovation is under Article 27(1). Member countries have option to exclude innovation on grounds of *ordre* public, morally and for protection of environment Article 27(2), and option to exclude plants Article 27(3)b. Consequently, every country needs to consider carefully the relationships between conventional IPRs and *sui generis* IPRs they wish to develop. IPR are also complicated by social, ethical and cultural differences of stakeholders,

which range from "no living thing should be patented" to "everything under the sun should be protected".

Intellectual property rights, access and benefit sharing

There are basic differences in existing IPR regimes and agro-biodiversity source countries desire to develop control on access to plant genetic resources for sharing of benefit accruing from their use. For example :

- Parties that have patented biotechnology's are hesitant to share these technologies with countries that do not have same level of IPR protection.
- The current IPR system protects and rewards some type of innovation but not others; particularly the informal innovation carried out collectively by the people in conservation and making agro-biodiversity available for use.
- IPR systems make no provisions for compensating the owners of wild genetic resources, though in many cases they are the basis for development of commercial product.
- They do not recognise community ownership (to reflect contributions and needs of indigenous people/communities to protect genes, which are found in more than one area).
- Do not require holder to share benefits.
- Economic theory of property rights stipulates to provide incentives (for conservation of biodiversity), and that the holders should be able to implement decisions concerning the use of the resources.
- Applicable to limited period like leases and not suitable for collective ownership.

This situation has raised difficulties in developing new IPR systems as per the TRIPS provisions. However, few following adherences could bring IPR more in line with benefit sharing provisions of the Convention.

1. full disclosure regarding the source of material or country of origin,
2. adhere to access regulations of relevant countries (PIC of the country providing genetic resources) as well as informed consent of its local and indigenous communities or the industry codes,
3. encourage benefit sharing.

Thus, establishing linkage between IPRs, and genetic resources access regulations and benefit sharing. This would also help to overcome monitoring of enforcement problems. Additionally, one will also have to keep in mind the following points :

- The legal, social and economic condition of particular country or the regions as no IPR can satisfy varying demands;

- have to be flexible as seen in the development of PBRs, where international community has recognised the farmers' rights, in line of provisions of Convention;
- Valuation of the genetic resources, as the flow of genes is two ways. For example, CGIAR network has significant contribution in green revolution. If IPR were established to internalise the externalities of genes, developing countries should be ready to pay to developed countries.

It is worth pursuing for IPRs in plant genetic resources, as they are the only mean, to develop dominions on genetic resources of local people as required under Article 8(j) of the Convention. Developing economies will have to consider ways and means to harmonise their new IPRs with conventional IPRs practised by other economies.

Limiting the scope of conventional IPRs

Two critical issues of relationships between conventional and the *suigeneris* IPR are the scope of the property rights, and subject matter covered by conventional IPRs.

Subject matter : Scope of conventional IPR should be carefully defined to avoid ambiguity and overlap with *sui generis* rights. TRIPS have several exceptions for flexibility and Parties may exclude from protection, plants and animals other than microorganisms and biological process other than microbiological process. Thus providing opportunities to exclude from *sui generis* system a wide range of products, including natural genes, and to impose clauses of source, utility and safety requirement.

The property rights : The importance of scope of subject matter would be reduced if it were possible to limit the property right created by the conventional IPR. If conventional IPR provided ownership to innovation and did not allow ownership of the unimproved germplasm used, then *sui generis* and conventional IPR could cover the same subject without conflict.

Currently conventional IPR grant absolute ownership, but this is not possible for biotechnological applications and would need frank disclosure of the genetic resources of a product and warranty in IPR application that user has complied with all the relevant access requirements. Such requirement is inherent in the Convention provisions for PIC in Article 15.4. Additionally, at present PIC as per Article 15.1 is under the control of country of origin, whereas, it should equally be an obligation of the recipient country.

Operating the sovereign rights and IPRs together

For simultaneous operation of these two aspects of agro-biodiversity, one has to workout an approach that will take care of all dimensions. This would need effective integration and harmonisation of all related Sectors. India does have an established system of variety protection, the available system of variety development, testing and yield trials is proposed to be reoriented to meet the dual needs of distinctness, uniformity and stability (DUS) and Value for Cultivation (VCU) testing, as followed by EC countries. This may help in protecting the varieties developed in country and drawing intellectual benefit from them. The proposed Plant Variety Protection Act should have provision for protection of farmers rights and of those who have over time conserved and improved agro-biodiversity. There are some attempts of benefit sharing. For example, Tropical Botanical Garden Research Institute (TBGRI), Kerala has decided that royalties arising out of a drug based on information provided by tribals, would split in a ratio of 10:5:5 among TBGRI, researchers and tribals, respectively. It is clear that it needs mutual respect and trust between provider and user, requires appropriate valuation of biodiversity used, as envisaged in CBD at the national, regional, species and intraspecific level and documentation of indigenous knowledge, for which internal efforts are necessary. However, all these efforts have to be done effectively to harness maximum benefit from our strengths and by overcoming weaknesses.

Scenario in India

The TRIPS under GATT agreement endeavour to honour the individual creativity, who are limited in number and mostly, belong to the developed nations. Whereas the CBD endeavours on confirming sovereign rights of individual nations on genetic wealth, by recognising their contributions in conservation of natural wealth and the creativity of unlimited number of informal innovators of traditional knowledge. Both are right at their place for achieving the set objectives. India is signatory to both and is in a unique position because of its strength in both intellect and agro-biodiversity and should strive for maximum gains using both systems.

TRIPS covers most areas of intellectual property rights. India is yet to legislate on several innovations, including geographical indications, protection of new varieties of plants etc. The Indian patent Act 1970 does not allow products patents in pharmaceuticals, food and agro-chemical. However, until the patent laws are changed, as per the TRIPS the developing countries are expected to provide for a pipe line protection "in form of mail box". This would accept patent applications for products relating to pharmaceuticals and agricultural chemicals from January 1995. Review of present Patent Act expects a number of changes.

- Product patent to be followed in all fields of technology without exception.
- All patents will have uniform duration for 20 years.
- Compulsory licences may be given on merit basis, though patent holder will be given opportunity for hearing.
- No discrimination between imported and domestic products on issue of working of patent.
- The burden of proof to shift to alleged infringer.

In recent dispute at the WTO between USA and India. Panel recommended to the Dispute Settlement Body to request India to bring transitional regime for patent protection of pharmaceutical and agricultural chemical products in conformity with its obligations under TRIPS agreement. Recently Government of India (GoI) has become a member of Paris Convention and has also joined the Patent Co-operation Treaty. A bill on the Patent Act with suitable amendments has also been introduced in the Parliament.

In India, both proposed draft, National Biodiversity Action Plan and the Conservation of Biological Diversity Act have provisions to recognise community rights. They do not provide details regarding the nature of these rights. There is also a cautious move towards transfer of control over natural resources to local communities through *Panchayat* system (Kothari, 1997).

The initiative for introduction of plant breeders' rights were made by the private seed companies in India in the late 80's, after the adoption of new seed policy in 1988. With this policy, GoI liberalised the import of seeds for joint ventures, including hybrid seeds. However, this was seen as an attempt by Northern Multinational Enterprises to privatise genetic diversity of south (Shiva, 1991). Attempts of Ministry of Commerce that *sui generis* system can be devised to take care of the national interests and clarification offered by the Director General, GATT in an article that UPOV 78, which has both farmers' and breeders' privilege could reasonably constitute an effective *sui generis* protection failed to assuage these fears. Nevertheless, there is another group of agricultural scientist and organisations, who believe that India with its intellect can turn TRIPS proposal to its advantage.

Given the farmers' and public reaction on plant variety protection, GoI prepared draft legislation for debate in 1994. But this was bitterly criticised, although it tried to balance with inclusion of provisions of community and farmers' rights and extensive provisions on compulsory licencing (Srinivas, 1994). This was revised, but failed to get acceptance. Some felt that India has given up the concept of farmers' rights or their conviction was that farmers' rights have to be juxtaposed to IPR granted to seed companies in the same legislation (Watal, 1998a).

Another IPR issue related to agriculture sector that has raised considerable concern in India is the patenting of 'Basmati' rice and few other agriculture products in the US. There is a wide spread belief that unless there is a domestic *sui generis* legislation to protect geographical indications, these marks can not be protected in other countries. TRIPS does allow WTO members to deny protection to geographical indications that are not protected in the country of origin, but it has to be brought into domestic law. The problem is that the certification marks system or even any *sui generis* legislation requires the definition of the particular product. For instance, what are the agreed characteristics of 'Basmati' rice?

In India, the debate on the implications of the final GATT round and patenting of microorganisms and products like, Turmeric, Basmati rice etc. has generated strong reaction against extension of IPRs to life forms and related technologies. In order to fulfill the obligation of Article 27 (Section 5) of TRIPS Agreement of WTO, the Department of Agriculture & Co-operation, Ministry of Agriculture is formulating a legislation for Plant Varieties and Farmers' Rights Protection with following objectives (Pers. Comm.) -

1. Stimulate investments for research and development both in the public and private sectors for development for new plant varieties by ensuring appropriate returns on such investments; and
2. Facilitate the growth of the seed industry in the country through domestic and foreign investment, which will ensure the availability of high quality seeds and planting material to Indian farmers.

While providing for an effective system of protection of plant breeders' rights, the proposed legislation also seeks to safeguard farmers' and researchers' rights (Pers. Comm.). It also contains provisions for safeguarding the larger public interest.

Salient features of the proposed legislation are :

- Extends to all categories of plants but shall not include micro-organisms
- For eligibility, a variety must be new, distinct, uniform and stable
- The protection shall be 18 years for trees and vines, and 15 years for other plants
- The concept of breeder's right envisages that breeders authorisation would be required for production and commercial sale of reproductive or propagating material of a protected variety
- The Plant Variety Protection and Farmers' Rights Authority will also set up to perform all function relating to the protection of plant varieties

- The proposed legislation also provides for the setting of an Appellate Tribunal to hear various appeals under proposed Act
- The legislation also covers provision for compulsory licencing in public interest, this can be revoked whenever material requirement is not met
- The farmers will enjoy all traditional rights to save, use, exchange, share and sell their produce of protected variety, but will not be able to sell branded seeds of the protected variety.
- The right for research for non-commercial purpose are also protected.

This proposal is under consideration of government for approval of parliament.

REFERENCES

- Anonymous 1997. Background document on UPOV provided to FAO.
- Arora, R.K. 1991. Plant Diversity in the Indian Gene Centre. *In*: R.S. Paroda and R.K. Arora (eds) Plant Genetic Resources, Conservation and Management. Concept and Approaches, p. 25-44, IBPGR, Regional Office for South and Southeast Asia, New Delhi.
- Brady, N.C. 1985. Agricultural Research in US Trade. *Science*. **218**: 847-853.
- Bragdon, Susan B. and D.R. Downes. 1998. Recent policy trends and developments related to conservation, use and development of genetic resources. *Issues in Genetic Resources* No. 7, June 1998, IPGRI, Rome, Italy, 42 p.
- Esquinas-Alcazar, J.T. 1987. Plant Genetic Resources: a base for food security. *Ceres, The FAO Review* **118**: 39-45.
- FAO. 1997. Progress report on the Global System for the conservation and sustainable utilisation of plant genetic resources for food and agriculture. Commission on Genetic Resources for Food and Agriculture, Seventh Session, Rome.
- Gautam, P.L. 1997. Agro-biodiversity in the Indian gene centre and conservation strategies. NBPGR-NAAS Workshop on national concern for conservation, management and use of agro-biodiversity. Shimla, India 15-16 October 1997.
- Harlen, J.R. 1971. Agricultural origin: centres and non-centres. *Science* **174**: 468-474.
- GRAIN 1994. "Biodiversity in Agriculture: Some Policy Issues". IFOAM Ecology and Farming, January, 14
- Johnston, S. and F. Yamin. 1997. Intellectual property rights and access to genetic resources. *In*: J. Mugabe, C.V. Barber, G. Henne, L. Glowka and A.L. Vina (eds) Access to Genetic Resources, pp. 245-270, ACTS press, Nairobi, Kenya.
- Juma, C. 1989. The Gene Hunter : Biotechnology and the scramble for seeds. Princeton NJ : Princeton University Press, p. 14, 41.
- Komen, J. 1991. Screening plants for few drugs: Biotechnology and Development. *Monita*. **9**: 4-6.
- Kothari, A. 1997. Access to genetic resources and benefit sharing: Options for action in India. *In*: J. Mugabe, C.V. Barber, G. Henne, L. Glowka and A.L. Vina (eds.) Access to Genetic Resources, pp. 201-218, ACTS press, Nairobi, Kenya.

- MoEF, 1997. National Action Plan on Biodiversity. Government of India, Ministry of Environment & Forests, New Delhi.
- MoEF, 1998. Implementation of article 6 of the Convention on Biological Diversity in India, National Report (Interim).
- National Academy of Agricultural Sciences (NAAS) 1998. Conservation, Management and Use of Agro-biodiversity. NAAS workshop held at Central Potato Research Institute, Simla, 15-16 October 1997. NAAS, New Delhi, 10 p.
- Sasson, A. 1996. Biotechnologies and the use of Plant Genetic Resources for Industrial Purpose : Benefit and Constraints to Developing Countries. *In*: F. di Castri and T. Younes (eds) Biodiversity Science and Development: Towards New Partnerships, pp. 469-487, CAB International, Wallingford, U.K.
- Sehgal, J.C., D.K. Mandal, C. Mandal and S. Vadi. 1990. Agro-ecological regions of India : NBSS Tech. Bull. No. 24, NBSS & UP, Nagpur, 73 p.
- Shiva, V. 1991. Biotechnology Development and Conservation of Biodiversity. *Economics and Political Weekly*, p. 2740-2746.
- Srinivas, R.K. 1994. Power Without Accountability: Draft Bill on Plant Breeders' Rights'. *Economics and Political Weekly*, p. 729-730.
- Thrupp, L.A. 1998. Agricultural Biodiversity and Food Security: Predicament, Policies, and Practices, World Resource Institute (Publication draft). 68 p.
- Watal, J. 1998a. Intellectual Property Rights in Indian Agriculture. Seminar Presented at Indian Council for Research on International Economic Relations, 9 July 1998.
- Watal, J. 1998b. The TRIPS Agreement and developing Countries- Strong, weak or Balanced Protection. *The Journal of World Intellectual Property* Vol. I(2): 281-307.
- Wilson, E.O. 1988. "The Current State of Biological Diversity". *In*: Biodiversity, (ed. E.O. Wilson) Washington DC : National Academy Press, 15 p.
- Zeven, A.C. and P.M. Zhukovsky. 1975. Dictionary of cultivated plants and their centres of diversity. PUDOC, Wageningen. 219 p.

BIOSAFETY CONCERNS IN BIOLOGICAL RESOURCE MANAGEMENT*

K.P.S. Chauhan

Ministry of Environment and Forests, Government of India,
Paryavaran Bhawan, C.G.O. Complex, Lodhi Road,
New Delhi 110 003

The development of transgenies and their release into both contained and open environments have raised concerns relating to their direct or indirect impacts on management of biological resources and human health. Following UNCED (1992), the non- legally binding International Technical Guidelines for Safety in Biotechnology were finalized in 1995 under the aegis of UNEP. In the meanwhile, the 2nd Conference of Parties of the Convention on Biological Diversity (CBD) decided to constitute an Open-ended Ad Hoc Working Group to develop a 'Protocol on Biosafety (BSWG)'. This paper highlights the basic elements consolidated by this Group as of now, and which are to be finally negotiated in the forthcoming meeting in late August, 1998.

Key words : Biosafety, biotechnology, living modified organisms, protocol, biological resources, conservation, biological diversity

The rapid unfolding of vast opportunities for applying the tools and techniques of biotechnology in the areas of agriculture, health, industry and environmental pollution have aroused tremendous and wide ranging expectations. These technologies have the potential to provide more abundant and nutritious food, new medicines including therapy for genetically induced diseases, new environment-friendly products, and the means to clean up industrial pollution of water and soil. Some technologies are also being applied in the assessment, monitoring, management and sustainable utilisation of biological resources.

As is increasingly realised, it is necessary to create the supporting infrastructure and a congenial environment to capitalise on the potential of the new biological technologies (Brenner, 1995). It has also been acknowledged that the release of Living Modified Organisms (LMOs) in a contained or open environment could pose risks which would have various direct and indirect impacts. If the benefits of biotechnology are to be optimised without affecting the environment, effective biosafety regulations must be developed based on

*The views expressed in this paper are the author's and not necessarily that of the Government of India

sound scientific principles (Persley, *et al.* 1992; Walsh, 1993; Krattiger and Lesser, 1994).

Dealing briefly with the potential of biotechnology and its impact on the environment, in particular on biological resources, this paper traces the various developments that have taken place in developing biosafety measures, including the finalisation of the 'International Technical Guidelines for Safety in Biotechnology' (UNEP, 1995). The paper also highlights the basic elements which are being considered for inclusion in the proposed 'Protocol on Biosafety' under the aegis of the CBD, and are currently being negotiated by the Contracting Parties.

IMPACTS OF BIOTECHNOLOGY

Biotechnology provides a range of tools and methods for assessment, monitoring and managing biological resources, such as clarifying taxonomic and evolutionary relationships among groups of organisms, and assessing the effects of ecosystem disturbance on components of biological diversity and biological processes. The tools of biotechnology could also be utilised for *in situ* conservation i.e. assessment of optimal or minimal population size, and *ex situ* conservation i.e. enhancing the quality of characteristics and efficiency, through compact storage of DNA libraries and sequence databases (Apples *et al.* 1995). The tools current employed for the sustainable use of genetic resources are in the area of breeding, genetic engineering, the development of novel genes and gene products, and environmental remediations. Some areas of application of biotechnology in utilisation of biological diversity, as identified by Montagu *et al.* (1995), are given in Table 1.

a) Direct Impacts :

The introduction of any LMOs in a biological community can have various undesirable impacts (Tzotzos *et al.* 1995) :

- displacement or destruction of indigenous/endangered or endemic species;
- exposure of species to new pathogenic or toxic agents;
- pollution of the gene pool;
- loss of species diversity; and
- disruption of energy and nutrient cycling.

These impacts are largely ecological and evolutionary and can be scientifically assessed and tested through simulated conditions. As part of an overall risk assessment strategy, the main problems arising from direct impact could be dealt with, including the processes of introgression, weediness,

Table 1. Areas of application of biotechnology in the management of biological resources

*	Using technologies as sources of:
-	proteins and peptides
-	lipids and fatty acids
-	carbohydrates
-	secondary metabolites for pharmaceuticals
*	Genetic engineering, breeding and <i>in vitro</i> culture systems can be used to enhance agronomic performance.
*	Improving environmental conditions through :
-	identification of soil microorganisms and determination of best combinations for soil rehabilitation
-	use of plants to mitigate heavy metal pollution
-	engineering key genes in bacteria for pollutant degradation
-	improving plant microbe symbiotic systems for waste water treatments
-	production of biosurfactants
*	Enhancing the efficiency of microorganisms in industrial processes such as :
-	microbial-enhanced secondary recovery of oil from reservoirs
-	bioleaching: microbiological extraction of metals from low grade ores
-	production of industrial enzymes
-	production of endogenous products e.g. antibiotics

(Source : Montagu *et al.* 1995)

pathogenicity, altered nutrient cycling etc. However, there is an inadequate understanding of the possible direct impact of LMOs on soil micro flora and fauna (Angels, 1994 and Morra, 1994) and the potential of virus-resistant plants on the host range of some viruses (Rissler and Mellon, 1993).

b) Indirect impacts :

The possible number and types of indirect effects of biotechnology could be immense. These effects are mostly socio- economic in nature and can be of major importance particularly to middle to low-income developing countries where people are dependent on biological resources for subsistence. Indirect impacts may be secondary or tertiary effects. Tzotzos *et al.* (1995) has listed some indirect impacts :

- Pressure on natural habitats because of the increasing value of genetic resources;

- Lack of immediately perceivable incentives for conservation;
- Moral/ethical problems of ownership of genetic resources and benefit-sharing;
- Increase in agricultural productivity;
- Replacement of traditional landraces; and
- Decline or opportunities loss for disadvantaged groups in areas of marginal production.

In addition, various sociological and socio-economic impacts could also be visualised with these impacts depending on the type of LMO, kind of release, and the precaution taken for any harmful effect.

PUBLIC UNDERSTANDING ON THE NEED FOR BIOSAFETY MECHANISM

The public debate on the applications of biotechnology has been marked by apprehensions of two kinds. The first is that there may be adverse impacts on the environment and human health. The second is the fear that control of the new technologies could give some nations or groups the power to use this in unfair ways.

The public perception of biotechnology as unpredictable and dangerous was highlighted by Perlas (1993) through examples such as the creation of a 'Super Aids Virus', super pigs and cows reporting of serious ailments using a genetically altered version of L-tryptophan and insulin; the use of biotechnologically developed bovine growth hormone (BGH); stunting in corn and other crops due to the presence of *Clavabacter xyli*, a vector used to transfer BT endotoxin gene; the requirement of six times more pesticides for Unliver cloned oil palms; novel mutant carps, catfish, trout and salmon polluting native species; and illegal trials on pseudo-rabies in Argentina. This short list of unpredictable, and negative impacts of biotechnologies clearly shows the possibility of the adverse impact of biotechnologically developed products on human health and the environment. The views of Holmes (1993), Williamson (1991) and Ellstrand and Hoffman (1990) further support these perceptions.

Berg *et al.* (1974) voiced their concern about the potential biological hazards with respect to r-DNA experimentations. This led to the finalisation of 'The Guidelines for Research Involving r-DNA' by the US Government. By the mid- 1980s, however, the context of biotechnology had shifted from research to commerce. An intense debate ensued between molecular biologists and ecologists, on controversial issues regarding risk assessment related to the release of new biotechnology products into the environment (Krimsky, 1991). The debate was also joined by prominent environmental groups, politicians,

Table 1. Areas of application of biotechnology in the management of biological resources

*	Using technologies as sources of:
	- proteins and peptides
	- lipids and fatty acids
	- carbohydrates
	- secondary metabolites for pharmaceuticals
*	Genetic engineering, breeding and <i>in vitro</i> culture systems can be used to enhance agronomic performance.
*	Improving environmental conditions through :
	- identification of soil microorganisms and determination of best combinations for soil rehabilitation
	- use of plants to mitigate heavy metal pollution
	- engineering key genes in bacteria for pollutant degradation
	- improving plant microbe symbiotic systems for waste water treatments
	- production of biosurfactants
*	Enhancing the efficiency of microorganisms in industrial processes such as :
	- microbial-enhanced secondary recovery of oil from reservoirs
	- bioleaching: microbiological extraction of metals from low grade ores
	- production of industrial enzymes
	- production of endogenous products e.g. antibiotics

(Source : Montagu *et al.* 1995)

pathogenicity, altered nutrient cycling etc. However, there is an inadequate understanding of the possible direct impact of LMOs on soil micro flora and fauna (Angels, 1994 and Morra, 1994) and the potential of virus-resistant plants on the host range of some viruses (Rissler and Mellon, 1993).

b) Indirect impacts :

The possible number and types of indirect effects of biotechnology could be immense. These effects are mostly socio- economic in nature and can be of major importance particularly to middle to low-income developing countries where people are dependent on biological resources for subsistence. Indirect impacts may be secondary or tertiary effects. Tzotzos *et al.* (1995) has listed some indirect impacts :

- Pressure on natural habitats because of the increasing value of genetic resources;

- Lack of immediately perceivable incentives for conservation;
- Moral/ethical problems of ownership of genetic resources and benefit-sharing;
- Increase in agricultural productivity;
- Replacement of traditional landraces; and
- Decline or opportunities loss for disadvantaged groups in areas of marginal production.

In addition, various sociological and socio-economic impacts could also be visualised with these impacts depending on the type of LMO, kind of release, and the precaution taken for any harmful effect.

PUBLIC UNDERSTANDING ON THE NEED FOR BIOSAFETY MECHANISM

The public debate on the applications of biotechnology has been marked by apprehensions of two kinds. The first is that there may be adverse impacts on the environment and human health. The second is the fear that control of the new technologies could give some nations or groups the power to use this in unfair ways.

The public perception of biotechnology as unpredictable and dangerous was highlighted by Perlas (1993) through examples such as the creation of a 'Super Aids Virus', super pigs and cows reporting of serious ailments using a genetically altered version of L-tryptophan and insulin; the use of biotechnologically developed bovine growth hormone (BGH); stunting in corn and other crops due to the presence of *Clavabacter xyli*, a vector used to transfer BT endotoxin gene; the requirement of six times more pesticides for Unliver cloned oil palms; novel mutant carps, catfish, trout and salmon polluting native species; and illegal trials on pseudo-rabies in Argentina. This short list of unpredictable, and negative impacts of biotechnologies clearly shows the possibility of the adverse impact of biotechnologically developed products on human health and the environment. The views of Holmes (1993), Williamson (1991) and Ellstrand and Hoffman (1990) further support these perceptions.

Berg *et al.* (1974) voiced their concern about the potential biological hazards with respect to r-DNA experimentations. This led to the finalisation of 'The Guidelines for Research Involving r-DNA' by the US Government. By the mid- 1980s, however, the context of biotechnology had shifted from research to commerce. An intense debate ensued between molecular biologists and ecologists, on controversial issues regarding risk assessment related to the release of new biotechnology products into the environment (Krimsky, 1991). The debate was also joined by prominent environmental groups, politicians,

corporations and trade unions. This led to the formulation of a 'coordinated Framework for Regulation of Biotechnology' by the US Government. Subsequently, biosafety guidelines were developed by the OECD and European Union (EU) and its member countries. Several surveys conducted to analyse public perceptions revealed that they were very similar irrespective of the geographical situations. However, there were also considerable differences between EU countries (OTA, 1987), Hoban and Kendall (1992) and Marlier (1992).

GLOBAL STATUS OF LEGISLATIVE AND REGULATORY MECHANISMS FOR BIOSAFETY

The current global status of biosafety regulations has been extensively analysed by Virgin *et al.* (1995). As per their analysis, 24 countries with high to high-middle income economies have laws and regulations in place taking into account the specific concerns arising from new recombinant techniques. The EU countries have instituted new laws, which are similar in scope requirements and impacts.

In developing countries, the situation is significantly different. In Latin America, Argentina, Brazil, Mexico, Chile, Costa Rica and Cuba have regulatory mechanisms in place. The African continent is represented only by South Africa and Egypt. Kenya, Zimbabwe and Nigeria are at various stages of drafting regulations and are likely to finalise these very soon. In Eastern Europe, Hungary has an ad hoc review process, and Russia has submitted a biosafety law for official approval. Of the developing countries in Asia, only India, China, Thailand and the Philippines have guidelines. Malaysia is preparing new legislation, and Indonesia is in the process of drafting (Table 2).

It may be stated that the implementation of these biosafety regulations varies from developed countries to developing countries, from being very rigidly effective to non-effective because of the lack of a well-defined institutional structure. It is also pertinent that the guidelines evolved by most developing countries are very similar in scope and requirements, and have been adopted as a part of their National Environment Acts. However, these provisions are inadequate in respect of modalities/protocols for access to and transfer of biotechnology on 'Mutually Agreed Terms', procedures for 'Advanced Informed Agreements' and procedures for risk assessment and management (Chauhan, 1996).

Virgin *et al.* (1995) also studied the rate of adoption of guidelines by different countries. Analysis revealed that 67 per cent of countries with high to upper-middle income economies and 12 per cent of lower-middle to lower income countries have regulatory procedures in place. It is expected that less than 30 per cent of the lower-middle to lower income countries will have

Table 2. Status of adoption of biosafety regulations in different countries

Industrialised Countries:	Australia, Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Luxembourg, New Zealand, Norway, Portugal, South Africa, Spain, Sweden, Switzerland, The Netherlands, United Kingdom, United States
Developing Countries:	Argentina, Brazil, Chile*, China*, Costa Rica*, Cuba*, Egypt*, Hungary**, India*, Indonesia**, Kenya**, Malaysia**, Mexico, Nigeria*, Philippines*, Russia**, Thailand*, Zimbabwe**, **

* Lower-Middle to Low Income Economy

** Currently drafting regulations

(Based on Virgin *et al.* 1995)

biosafety procedures by the year 2005. The accrual of benefits from biotechnological applications would be facilitated by the harmonised adoption of biosafety regulations.

POST-UNCED SCENARIO

The issues relating to safe application of biotechnology in relation to conservation and sustainable use of biological resources found prominent place in the negotiations towards finalising the text of the CBD, which was adopted at the UNCED Earth Summit held at Rio de Janeiro in Brazil in June 1992. Chapter 16 of Agenda 21, which deals with 'Environmentally Sound Management of Biotechnology', specifically seeks to ensure safety in biotechnology development, application, exchange and transfer through international agreement on principle to be applied on risk assessment and management. Articles 8(g) and 19(3) & (4) of the CBD also address the issue of safety in biotechnology. Article 8(g) calls upon each Contracting Party to "establish or maintain means to regulate, manage or control the risks associated with the use and release of LMOs resulting from biotechnology which are likely to have adverse environmental impacts that could effect the conservation and sustainable use of biological resources, taking into account the risks to human health".

Keeping this in view, the Governing Council of the United Nations Environment Programme (UNEP) "affirmed the desirability of UNEP contributing to international efforts on biosafety, including the development of international guidelines (decision 18/36 B)" which may be used by the national governments, inter-governmental, private sector and other relevant organisations to provide safety in biotechnology.

The non-legally binding 'International Technical Guidelines for Safety in Biotechnology (ITGSB)' were finalised, in 1995, under the aegis of UNEP,

through extensive discussions among most countries which had ratified the CBD. Six chapters of these Guidelines spell out various elements dealing with general principles, assessment and management of risks, mechanisms at national and regional level for providing safety, information supply and exchange, and capacity building. These are based on common elements and principles derived from relevant national, regional and international instruments, regulations, and guidelines. The Guidelines also address the human health and environmental safety of all types of applications of biotechnology, from research and development to commercialisation of biotechnological products containing or consisting of LMOs. The adoption of these guidelines is expected to facilitate Governments in taking appropriate actions towards developing mechanisms for evaluating biosafety, identifying measures to manage foreseeable risks, and monitoring research and information exchange, all of which improve safe application of biotechnology (UNEP, 1995). This would also facilitate the implementation of Article 8(g) in those countries which have ratified the CBD.

The general principles followed in the Guidelines are based on identifying any hazards: assessing and managing the risks (case by case and in a step-wise manner); and monitoring. The Guidelines provide details relating to the assessment and management of risks, safety mechanism at national and regional levels, safety mechanism at international level using information supply and exchange, and capacity building. It also highlights the step-wise approach and provides a framework on each aspect which is to be developed by the countries either based on already existing mechanisms or totally new ones. It is expected that implementation of these Guidelines will provide an impetus to the uniform development of capacity at the international level for risk assessment and management associated with the release of LMOs arising from biotechnological applications and harmonisation as emphasised by the UNIDO (UNIDO, 1990).

ON-GOING EFFORTS FOR DEVELOPING BIOSAFETY PROTOCOL

Article 19(3) of the CBD, dealing with handling of biotechnology and distribution of its benefits, states that "The Parties shall consider the need for and modalities of a protocol setting out appropriate procedures, including, in particular, advance informed agreement, in the field of safe transfer, handling and use of any LMOs resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity".

Further, Article 19(4) states that "Each Contracting Party shall, directly or by requiring any natural or legal person under its jurisdiction providing the organisms referred to in paragraph 3 above, provide any available information about the use and safety regulations required by that Contracting Party in handling such organisms, as well as any available information on the potential adverse impact of the specific organisms concerned to the

Contracting Party into which those organisms are to be introduced". In the second meeting of the Conference of Parties to the CBD, held in 1995, it was decided to establish an Open-ended Ad Hoc Working Group (BSWG) under the Conference of Parties to develop a protocol on biosafety taking into account these two paragraphs of Article 19, the principle enshrined in the Rio Declaration on environment and development, and in particular the precautionary approach contained in Principle 15. In this regard, this Working Group has had four meetings (most recently in February, 1998), and has consolidated the text of

Table 3. Summary of the Major Articles of the Proposed 'Protocol on Biosafety'

a) Some Major Articles

Article 3. Application of Advance Informed Agreements (AIA) Procedure	Article 15. Unintentional Transboundary Movement
Article 4. Notification Procedure for AIA	Article 16. Emergency Measures
Article 5. Response to AIA Notification	Article 17. Handling
Article 6. Decision Procedure for AIA	Article 18. Competent Authority Focal Point
Article 7. Review of Decision Under AIA	Article 19. Information Sharing/Biosafety Clearing House
Article 8. Notification of Transit	Article 20. Confidential information
Article 9. Simplified Procedures	Article 21. Capacity Building
Article 10. Subsequent Imports	Article 22. Public Awareness/Public Participation
Article 11. Bilateral and Regional Agreements	Article 23. Non-Parties
Article 12. Risk Assessment	Article 24. Non-Discrimination
Article 13. Risk Management	Article 25. Illegal Traffic
Article 14. Minimum National Standards	Article 26. Socio-economic Considerations
	Article 35. Monitoring and Compliance

b) Other items (text to be consolidated and negotiated)

Preamble, Objectives and Jurisdictional Scope	Accession
Use of terms/Definition	Depository
Relationship with other international agreements	Reservation and declaration
Entry into Force	Review and adoption
Settlement of disputes	Authentic Texts
Financial issues	Annexes
Right to Vote	Withdrawal; and Signature

some major articles (Table 3) dealing with various substantial matters. In addition, negotiating countries would need to finalise legal definitions of various crucial terms on the basis of mutual agreement. These terms include: LMOs, Transboundary Movement, Transfer, Safe Transfer, Competent Authority, Familiarity, Adverse Effects, Contained Use, Intended/Deliberate Use, Unintended Release, Focal point, Risk Assessment, Risk Management, Modern Biotechnology, Advanced Informed Agreement, Prior Informed Consent, Minimum National Standards, Biosafety, Limited Trial, Handling of LMOs, Use of LMOs, Centres of Origin, Centres of Genetic Diversity, Compensation, Open Environment, Open Field Trial and Accidents.

The majority of countries believed that 'AIA' procedures constituted a very important part of a protocol dealing with transboundary movement of LMOs, taking into account the provisions of the Basel Convention and the operational guidelines and principles developed by the Forest Stewardship Council. The procedures should also include the notification of components, and must deal with transboundary movements of LMOs and data relevant to safety and information contained therein. The other important aspect associated with this is capacity building, which must be an integral part of the notification in a manner that the AIA mechanism is workable and practical.

The priority activity regarding relevant categorisation of LMOs resulting from modern biotechnologies is to establish a clear understanding of, and early agreement on, the classes of organisms under consideration in the negotiation process. An agreed categorisation would help to establish which existing international agreements might be applicable to some categories of LMOs and relevant to developing a protocol on biosafety. In addition, categorisation according to the degree of assessed potential risk to biological diversity would appear relevant in considering AIA procedures. Apprehensions have also been raised that risk classification for LMOs would be unrealistic, as biosafety risks associated with a given LMO would be different under different geographical, ecological and climatic conditions.

The fourth meeting of the BSWG, held in February, 1998, has consolidated the substantial text of the major Articles of the proposed 'Protocol on Biosafety'. However, most of the contentious issues remain bracketed, for further extensive negotiations. This has to be accomplished in the forthcoming meeting of this Group in late August, 1998. The agreed text has to be presented in the ordinary or extra-ordinary session of the next COP.

It is now the special responsibility of global molecular biologists and ecologists to facilitate the negotiation process, so that the protocol on biosafety can be finalised early. This would help in the suitable development of biotechnology to meet the objective of sustainable development including global food security on the one hand, and the sustenance of biological diversity for

future use on the other. This is one of the most difficult challenges, for the entire global scientific community, because the development of a protocol on biosafety will be turning point of our modern times. It is expected that the same spirit that brought about agreement on the complex issues contained in the CBD would also be evident in finalising the protocol on biosafety.

REFERENCES

- Angels, J.S. 1994. Release of transgenic plants: biodiversity and population level considerations. *Molecular Ecology* 3 : 45-50.
- Appels, R., A.G. Young, G.T. Tzotzos, B.A. Barlow, and C. Simoens. 1995. Biotechnology applications in biodiversity assessment and management. In: V.H. Heywood (ed) Global Biodiversity Assessment. Cambridge University Press, Cambridge.
- Berg, P., D. Baltimore, H.W. Boyer. S.N. Cohen, R.W. Davis, D. S. Hogness, D. Nathans, R. Roblin, J.D. Watson, S. Weissman, and N.D. Zinder. 1974. Potential biohazards of recombinant DNA molecules, *Science* 185: 303.
- Brenner, C. 1995. Technology Transfer: Public and private sector roles. In Kommen, J., J.I. Cohen and S.K. Lee (eds) Turning Priorities into Feasible Programmes: Proc. of a Regional Seminar on Planning, Priorities and Policies for Agricultural Biotechnologies in Southeast Asia. Intermediary Biotechnology Service/Nayang Technological University. The Hague/Singapore.
- Chauhan, K.P.S. 1996. Legal requirements for fulfilling obligations under the Convention on Biological Diversity. *Indian J. Pl. Genet. Resources* 9(2): 183-191.
- Ellstrand, N.C. and C.A. Hoffman, 1990. Hybridisation as an avenue of escape for engineered genes. *Bioscience* 40. 438-442.
- Hoban, T.J. and P.A. Kendall. 1992. Consumer attitudes about the use of biotechnology in agriculture and food production, US Deptt. of Agriculture, Raleigh, North Carolina.
- Holmes. B. 1993. The Perils of Planting Pesticides, *New Scientist* 28 August, pp. 34
- Office of Technology Assessment (OTA). 1987. New Developments in Biotechnology-Background Paper; Public Perceptions of Biotechnology, OTA-BP-45, United states Govt. Printing Office, Washington D.C.
- Krattiger, A.F. and W.H. Lesser. 1994. Biosafety - an environmental impact assessment tool and the role of the Convention on biological diversity. In: A.F. Krattiger, W.H. Lesser, K.R. Miller, Y. St. Hill and R. Senananayake (eds) Widening Perspectives on Biodiversity, IUCN, Gland, Switzerland and International Academy of the Environment, Geneva, Switzerland.
- Krimsky, S. 1991. Biotechnics and society - The Rise of Industrial Genetics. Praeger Publisher, New York.
- Mailier, E. 1992. Eurobarometer 35.1: Opinions of Europeans on Biotechnology in 1991. In: J. Durant (ed) Biotechnology in Public: a Review of Recent Research. Science Museum for the European Federation, Biotechnology, London.
- Montagu van M., J.M. Tiedje, D. Powell, C. Simoens, G.T. Tzotzos, and B.A. Barlow. 1995. Application of biotechnology for the utilisation of biodiversity. In: V.H. Heywood (ed.) Global Biodiversity Assessment. Cambridge University Press, Cambridge.

- Morra, M.J. 1994. Assessing the impact of plant products on soil organisms. *Molecular Ecology* 3: 53-55.
- Perlas, N. 1993. When what could go wrong did go wrong. Third World Resurgence, Penang, Malaysia. p. 8.
- Persley, G.J., L.V. Giddings and C. Juna. 1992. Biosafety: The safe application of biotechnology in Agriculture and the Environment. International Service for National Agricultural Research. The Hague.
- Rissler, J. and M. Mellon. 1993. Perils Amidst the Promise: Ecological risks of transgenic crops in a global market. Union of Concerned Scientists, Cambridge, Mass.
- Tzotzos, G.T., W.H. Lesser, P.J. Powell, and P.J. Dale. 1995. Impacts of biotechnology on Biodiversity. In: V.H. Heywood (ed.). Global Biodiversity Assessment, Cambridge University Press, Cambridge.
- UNEP. 1995. International Technical Guidelines for Safety in Biotechnology. United Nations Environment Programme. Nairobi, Kenya.
- UNIDO. 1990. An International approach to biotechnology safety. p. 43-47, UNIDO, Vienna
- Virgin, I., R.J. Fredrick and S.C. Ramachandran. 1995. Impact of International Harmonisation on biosafety regulations in biotechnology. Proc. on biosafety regulations in biotechnology pp. 115-125. Bangkok. Thailand.
- Walsh, V. 1993. Demand, public markets and innovation in Biotechnology. *Science and Public Policy* 25 : 138-156.
- Williamson, M. 1991. Biocontrol risks. *Nature* 353 : 394.

PLANT QUARANTINE AS AN AID TO SAFER EXCHANGE, CONSERVATION AND UTILIZATION OF PLANT GENETIC RESOURCES

P.C. Agarwal, Baleshwar Singh and P.L. Gautam

National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

Exchange, conservation and utilization of Plant Genetic Resources (PGR) are the key factors towards providing food security to humankind & animals in the next century. Globally there are several instances of plant disease epidemics through introduced seed and other planting materials. In India, more than 60,000 samples each year are processed for quarantine examination for the detection of exotic pests and pathogens and weeds by employing various techniques such as joint visual inspection, washing test, incubation test, serological examination, nematode extraction techniques, X-ray radiography and post entry isolation growing etc. Such examination resulted in the detection of several pests and pathogens of very high quarantine significance including those not known to occur in India. Some of these are as follows : fungi-*Peronospora manshurica*, *Fusarium nivale*, *Uromyces betae*, *Claviceps purpurea* etc.; nematodes- *Ditylenchus destructor*, *D. dipsaci*, *Heterodera gottingiana*, *H. schachtii* and *Rhadinaphelenchus cocophilus*; insects- *Acanthoscelides obtectus*, *Bruchidius halodendri*, *Carpocarpa pomonella*, *Ephesia elutella*, *Eriococcus araucaria*, *Sitophilus granarius* etc. On an average 88 per cent of the infected/infested samples were salvaged using various techniques such as hot water treatment, fumigation, ethyl alcohol wash, acid-treatment and pesticidal treatments etc. In this paper we are discussing in depth only fungi intercepted.

Key words: Germplasm, quarantine, interception

Population pressure particularly in developing countries emphasizes the need for sustainable efforts in crop improvement strategies, a wide genetic diversity in various crops is the backbone to such efforts. To build such diversity, introduction of Plant Genetic Resources (PGR) from different countries of the world is of paramount importance. However, such introductions always carry an element of inadvertent introduction of exotic pests and pathogens into new areas. Severe crop losses are reported in India due to inadvertent introduction of pests and pathogens viz., *Phytophthora infestans* (Late blight of potato, 1883), *Hemileia vastatrix* (Coffee rust, 1879), *Plasmopara viticola* (Downy mildew of grapes, 1906), *Synchytrium endobioticum* (Potato wart, 1953), *Peronospora destructor* (Onion downy mildew, 1977), *Plasmopara halstedii* (Sunflower downy mildew, 1985), *Quadraspidiotus perniciosus* (San Jose Scale, 1910), *Icerya purchasi* (Fluted scale, 1912), *Cydia pomonella* (Codling moth, 1919), *Globodera rostochinensis* (Golden nematode, 1961) etc.

MATERIALS AND METHODS

During last one decade (January, 1987- December, 1996) a total of 7,12,295 germplasm samples of various agri-horticultural crops and their wild relatives (including international trials) were received and processed for quarantine clearance. Exotic samples constituted 6,87,203 and those meant for export were 25,292 (Table 1). Five thousand seven hundred twenty one Phytosanitary Certificates were issued. The samples processed were comprised of true seeds, bulbs, nuts, cuttings, rhizomes, suckers, rooted plants etc. After visual examination all the germplasm samples were subjected to detailed examination for the detection of associated pests and pathogens by employing various techniques such as washing test, blotter test, infectivity test, serological testing, X-ray radiography, nematode extraction techniques, growing in post entry quarantine nursery (PEQN) (for chemically treated seeds) and grow out tests in environment controlled Poly- house/glass-house (for legumes). The whole procedure of examination is presented in Fig. 1. Germplasm samples found infected/infested/contaminated were salvaged by employing various techniques such as mechanical separation, hot water treatment (HWT) at 50-52°C for 20-30 minutes, ethyl alcohol wash (Agarwal *et al.*, 1990b), acid wash (Ram Nath *et al.*, 1986), standard fumigation and pesticidal treatments.

All the paddy germplasm introduced (> 10,000 samples per year) in the country was given prophylactic HWT at 52°C for 30 minutes.

RESULTS AND DISCUSSION

Interception of pathogenic fungi

A number of pathogenic fungi of high quarantine significance were intercepted in various germplasm material which include: *Peronospora manshurica* (Naum.) Syd. (Downy mildew of soybean), *Uromyces betae* Tul. ex Kickx. (Sugarbeet rust), *Fusarium nivale* (Fr.) Ces. (Snow mould of cereals), and *Claviceps purpurea* (Fr. ex. Fr.) Tul. (Ergot of cereals), not yet reported from India; *Drechslera maydis* (Nisik.) Subram. & Jain (Maydis leaf blight of maize), *Phoma betae* Frank (Black leg of sugarbeet) and *Puccinia carthami* Corda (Safflower rust) are known to possess a large number/more virulent races; *Botrytis cinerea* Pers. ex Pers. (Grey mould fungus), *Fusarium solani* (Mart.) Sacc. (Wilt fungus) *Colletotrichum dematium* (Pers. ex Fr.) Grove (Anthracnose fungus) and *Macrophomina phaseolina* (Charcoal rot fungus) are known to have a wide host range.

Downy mildew of soybean is not yet reported from India. However, it was intercepted on seeds of soybean imported from various countries (Mukewar *et al.*, 1980 and Agarwal *et al.*, 1990 a). Agarwal and Singh (1998) reported that while processing of 10,336 soybean samples during 1978-1995 downy

mildew was intercepted in 1514 samples from 15 countries. *P. manshurica* was also intercepted on seeds imported from Malaysia (Agarwal and Khetarpal, 1985) and Indonesia (Anitha *et al.*, 1993) where it was not reported as well.

Table 1. Quarantine clearance of seed and other planting material processed during 1987-1996

Year	Total No. of Samples Received	Total No. of Samples Imported	Total No. of Samples Exported	No. of Samples Found Infected/Infested /Contaminated	No. of Samples Salvaged
1987	86,922	83,945	2,977	3,198	3079
1988	67,424	65,311	2,113	3,006	2709
1989-90	72,899	69,380	3,519	1,538	1487
1990-91	62,136	60,503	1,633	889	862
1991-92	62,041	60,017	2,024	1,702	1692
1992-93	78,922	74,392	4,530	1,020	1013
1993-94	70,511	68,520	1,991	2,384	2369
1994-95	67,655	64,564	3,901	566	555
1995-96	72,990	71,121	1,869	899	894
1996-97	70,995	69,450	1,545	1,590	1567
Total	7,12,495	6,87,203	25,292	16,792	16,227

Uromyces betae (beet rust) which was intercepted from Bulgaria, Denmark, Germany, USA and U.S.S.R. during 1987-96 is not yet reported from India. *U. betae* was intercepted for the first time in 1983 in seeds imported from USA and Italy (Agarwal *et al.*, 1984). Later, a quarantine treatment was developed by Ram Nath *et al.*, 1986. They have found concentrated sulphuric acid highly effective in eliminating the inoculum from seed. Seed-borne rust spores can cause epidemics in new areas (Emdal and Foldo, 1979).

Fusarium nivale, the snow mould fungus is a serious pathogen of cereals and grasses, was intercepted in a large number of wheat germplasm samples received from U.K. during 1988 (Dev *et al.*, 1989); infection level was as high as 70% in some of the samples. *F. nivale* is reported to cause total loss of winter wheat and rye in parts of U.S.S.R., Japan, Canada and Central Europe (Blomqvist, 1970; Jamalainen, 1970). Fungicidal resistant strains are also reported in *F. nivale* (Olvang, 1984).

Claviceps purpurea, the causal agent of ergot of cereals was intercepted on a wide range of hosts i.e. *Aegilops* spp., *Agropyron* spp., *Avena sativa*,

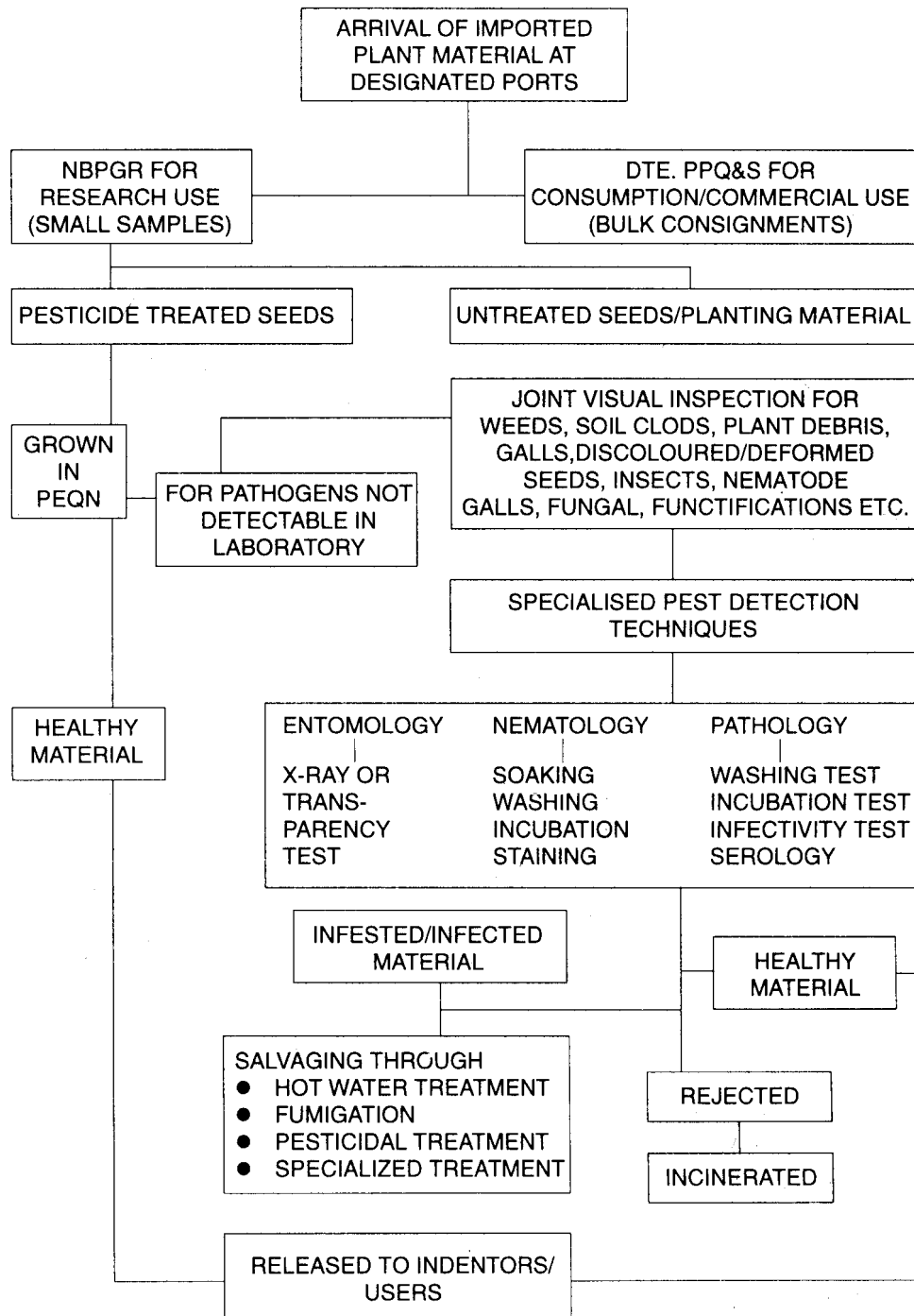


Fig. 1. Quarantine processing of imported plant germplasm

Festuca spp., *Hordeum vulgare*, *Lolium* spp., *Secale cereale*, *Triticum* spp., etc. The destructive ergot pathogen of rye, has caused epiphytotic conditions on *Agropyron repens* in America and Russia. However, it was not reported to attack this crop in India.

Teliospores of *Puccinia carthami* were detected in seeds received from a number of countries (Canada, China, Ethiopia, and USA). *P. carthami* has caused severe epiphytotics in USA during 1949 and 1950 (Thomas, 1952). Also a number of races are reported in this fungus.

Neovossia indica, the causal agent of karnal bunt of wheat was detected in seeds of wheat from Mexico, Nepal, Pakistan, and U.S.S.R., is a serious pathogen. European and Mediterranean Plant Protection Organization (EPPO) has declared *N. indica* to be a pathogen of high quarantine importance (Anonymous, 1979). Recently it has also been reported from a few states of USA and it has been listed as a very high risk pathogen, since the US trade is very badly affected due to infection of *N. indica*.

Drechslera maydis, the Maydis leaf blight fungus detected on maize and sorghum from Thailand and USA, respectively, has a devastating race i.e. race T which has caused the great epidemics of 1971 in U.S.A. due to use of cytoplasmic male sterile (cms) lines.

Botrytis cinera, the grey mould fungus which was detected in a number of crops has a wide host range. It has caused heavy crop losses to chickpea crop in states of Bihar, Haryana, Punjab and U.P. (Lambat *et al.*, 1985). However, *B. cinera* is considered as a common saprophyte in European countries.

Fusarium solani, *Macrophomina phaseolina* and *Colletotrichum dematium* are known to possess a wide host range. These three pathogens have been intercepted on a large number of hosts from different countries.

Fungi observed in PEQN were loose smut (*Ustilago tritici*) in wheat and barley, covered smut (*U. segetum* var. *hordei*) in barley, rust (*Puccinia carthemi*) in safflower, stripe disease (*Pyrenophora graminea*) in barley and leaf blight (*Drechslera sorokiniana*) in wheat.

Interception of pathogenic bacteria

Xanthomonas campestris pv. *campestris*, black rot of crucifers was detected in *Brassica* spp. from several countries; *X. oryzae* pv. *oryzae* was detected in paddy seeds from Philippines; and *Pseudomonas syringae* pv. *syringae* was detected in *Hibiscus cannabinus* seeds from Bangladesh (Singh *et al.*, 1995).

Interception of viruses

Plant viruses detected in legumes and cereals were: barley stripe mosaic virus in wheat (USA); pea seed-borne mosaic virus in pea (Netherlands and Syria), in faba bean (Syria); bean common mosaic virus in soybean (USA); soybean mosaic virus in soybean (Argentina, Philippines and USA) and yellow mosaic virus in french bean (Syria).

Interception of insects and mites

The insects and mites of high quarantine significance intercepted in exotic germplasm include; *Acanthoscelides obtectus* in *Cajanus cajan* (Brazil and Colombia), in *Phaseolus vulgaris* (Italy and Nigeria); *Anthonomus grandis* in *Gossypium* spp. (USA), in *Hibiscus* spp., *Sesamum* spp., *Vigna unguiculata* (Italy and Zambia); *Ephestia elutella* in *Macadamia* spp. (nuts) and *Vigna* spp. (USA) and *Quadrastichodella eucalypti* in *Eucalyptus* spp. (Australia).

Interception of nematodes

More than 65 spp. of plant parasitic nematodes were intercepted. Among the major interceptions, nematodes not reported in India include : *Heterodera schachtii*, *H. glycines*, *Ditylenchus dipsaci*, *D. destructor* and *Rhadinaphelenchus cocophilus*.

REFERENCES

- Agarwal, P.C. and R.K. Khetarpal. 1985. Interception of *Peronospora manshurica* on soybean seeds imported from Malaysia. *FAO Plant Prot. Bull.* **33**: 49.
- Agarwal, P.C., A. Majumdar, U. Dev, Ram Nath and R.K. Khetarpal. 1990a. Seed borne fungi of quarantine importance in exotic germplasm of soybean (*Glycine max*). *Indian J. Agric. Sci.* **60**: 361-363.
- Agarwal, P.C., Ram Nath, U. Dev and A. Majumdar. 1990b. Quarantine salvaging of safflower seed infected with safflower rust (*Puccinia carthami*). *FAO Pl. Prot. Bull.* **38**: 141-143.
- Agarwal, P.C., Ram Nath and A.K. Lambat. 1984. Interception of *Uromyces betae* in imported sugarbeet seeds. *Indian J. Pl. Prot.* **12**: 123-124.
- Agarwal, P.C. and B. Singh. 1998. Interception of *Peronospora manshurica* (Naum.) Syd. in soybean germplasm introduced from 1978-97. *Indian J. of Pl. Genet. Resources* **11**: 31-34.
- Anitha, K., P.C. Agarwal and Ram Nath. 1993. Interception of *Peronospora manshurica* (Naun.) Syd. in soybean seeds imported from Indonesia. *FAO Newsletter APPPC* **36**: 1.
- Anonymous 1979. Data sheets on Quarantine organisms EPPO List A2, *Neovossia indica*. European and Mediterranean Plant Protection Organization, Paris.
- Blomqvist, H. 1970. Wintering of field crops in Finland. *Ann. Agric. Fenn.* **9**: 345-346.
- Dev, Usha, A. Majumdar, P.C. Agarwal and Ram Nath. 1989. Interception of *Fusarium nivale* in exotic wheat (*Triticum* spp.) germplasm, *Indian J. Agri. Sci.* **59**: 602-603.

- Emdal, P.S. and N.E. Foldo. 1979. Seed-borne inoculum of *Uromyces betae*. *Seed Sci. and Technol.* 7: 93-102.
- Jamalainen, E.A. 1970. Studies on *Fusarium* fungi in Finland. *Suomalaisen Tiedeakatemia Toimituksia (Sarja IV. Biologica)* 168: 54-56.
- Lambat, A.K., Ram Nath, P.C. Agarwal, R.K. Khetarpal, Usha Dev, P. Kaur, A. Majumdar, J.L. Varshney, P.M. Mukewar and Indra Rani. 1985. Pathogenic fungi intercepted in imported seed and planting material during 1982. *Indian Phytopath.* 38: 109-111.
- Mukewar, P.M., Ram Nath, A.K. Lambdat, U. Kapoor, R.K. Khetarpal and Indra Rani. 1980. Interception of *Peronospora manshurica* in imported seed of soybean. *Seed Res.* 8: 267-270.
- Olvang, H. 1984. Benomyl resistance in *Gerlachnia nivalsis*, 1. Survey of diseased plants in the field. *Zeitsch. Fur Pflanzenkran. Und Pflanzensch.* 91: 294-300.
- Ram Nath, P.C. Agarwal, U. Dev and A.K. Lambat. 1986. Quarantine treatment of sugarbeet seed infected with rust (*Uromyces betae*). *FAO Plant Prot. Bull.* 31: 205-207.
- Singh, B., K. Anitha, P.C. Agarwal and Ram Nath. 1995. Interception of *Pseudomonas syringae* pv. *syringae* on *Hibiscus cannabinus*. *Indian Phytopath.* 48: 352.
- Thomas, C.A. 1952. Transmission of safflower rust on treated seed. *Phytopath.* 42: 108-109.

COLLECTION OF RICE GERMPLASM FROM MALABAR, KERALA

K. Joseph John and M. Abdul Nizar

National Bureau of Plant Genetic Resources
Regional Station, Vellanikkara, Thrissur 680 654 (Kerala)

In an exploration undertaken in northern districts of Kerala, parts of South Canara and Coorg districts of Karnataka, 104 accessions of rice comprising 60 landraces and 24 wild rice samples falling under four species were collected. Some of the very rare landraces which are at the verge of extinction, such as 'Kurichyar', 'Karimpalar', 'Mavilan', 'Jathikurichyar', 'Wynadan chetty', 'Gounder' and 'Naikkar' could be collected. The collection included special purpose local landraces as detailed in the text.

Key words : Rice, wild rice, landraces, germplasm

Malabar is considered as one of the centres of diversity of rice and the antiquity of rice cultivation here dates back to 3000 B.C. (Manilal, 1990). The world renowned 'Pattambi' rice varieties which have contributed sources of resistance to many IRRI varieties are all pureline selections of landraces hailing from the erstwhile Malabar Province (Sukumara Dev, *et al.*, 1993). Diversity at ecosystem level, interspecific levels in addition to ethnic diversity is highest in this small stretch of land. Attempts have been made in the past to explore the region and 895 accessions of rice including 184 landraces and 213 accessions of wild rices were collected (NBPGR 1986-89; Vaughan & Muralidharan, 1988). To fill in the gaps in landraces collection the present programme was executed.

MATERIALS AND METHODS

Coastal and midlands of Thrissur, Palakkad, Malappuram, Kozhikkode, Kannur, Kasaragod, hill district of Wynad and adjoining areas of Coorg and South Canara of Karnataka were explored (Fig. 1). In a 12 day collection programme in December 1996, 104 accessions were collected from 62 sites. Random samples in the case of wild rice and biased samples in the case of landraces were collected (Chang, 1976; Chang *et al.*, 1972). In addition to passport data, ethnobotanical notes on special uses of landraces were recorded. A part of the seed samples were sent to the medium term storage of NBPGR,

New Delhi and a small part was raised in pots during December 1996-April 1997 for characterization purpose.

RESULTS AND DISCUSSION

Rice is grown throughout the region from coastal lowlands below sea level to 1500 m above MSL in Wynad. The mode of cultivation followed is diverse. The 'Punam valal', - a cut and slash type of cultivation in forest clearings (now practiced in rubber plantations in the juvenile stage), 'Modan' or upland rainfed, 'Oruppu'- of one crop in a year in terraced hills, 'Iruppu' - of two crops per year in planes, 'Pokkali' of saline coast lands, 'Puncha' of shallow waterbodies and 'Kayal' lands and 'Koottumundakan' or mixing of 'Virippu' (Kharif) and 'Mundakan' (Rabi) cultivars together so as to get two harvests from one sowing are all practiced to a limited extent, though irrigated transplanted rice is the main rice ecosystem.

Characteristics of important landraces collected are given in Table 1. Field observations and chracterization revealed wide variability for yield and yield contributing characters besides morphological traits. Plant height varied from 60-12 cm, number of tillers 5-18, days to maturity 90-130, panicle length 15-34 cm, number of primary branches 5-13 and number of grains per panicle from 36-230. Majority of the cultivars have red bold grains used for parboiling, though medium bold and short round were also present. Farmers reported between 6-23 q/ha yield for these landraces. All land races showed fertility except 5 lines as IC 203807, IC 2038081, IC 203808, IC 203811 and IC 203812. Only given lines showed white seed coat colours all others were red.

Landraces are still popular for their palatability and reliable yield performance even under low input conditions. Wynad and Coorg districts are still dominated by few traditional landraces like 'Marathondi', 'Thonnuran', 'Veliyan', 'Keduveliyan', ('Keerubeliya') and scented rices like 'Gandhakasala', 'Jeerakasala', 'Mullanchanna' and 'Rajani'. The cultivar 'Mullanchanna', in addition to scented quality, is preferred for "uppuma" and rice flour preparations. In other districts, 'Kaima' is the most popular red bold rice of irrigated transplanted belt. It fetches a premium price as it is used by rice mill owners to impart a better look to the lot by mixing 30% of this to other improved varieties.

Cultivation of medicinal rice 'Navara' has become commercial in some parts of Malappuram district where three crops per year are taken as irrigated transplanted rice whereas traditionally it is grown as rainfed upland rice by 'Kurichya' tribe in Kannavam forest. This high value crop, in addition to being an important ingredient of 'navarakkizhi' ('Panchakarma') in Ayurvedic treatment, is being used as weaning food. 'Chennellu', another landrace of

Table 1. Characteristics of important landraces collected

Sl. No.	IC No.	Landrace name	District & State	Plant height (cm)	Panicle length (cm)	Grains per panicle	Grain size	Husk colour	Remarks
A. IRRIGATED TRANSPLANTED FIRST SEASON CROP									
1	203786	Varukkunna nellu	Kasaragod, Kerala	85	24	117	Short bold	Gold & or gold furrows	Excellent popping quality; also suitable for II & III crops; rare
2	203825	Aryan	Malappuram, Kerala	90	25	108	Long bold	Gold & or gold furrows	Best cooking quality as parboiled rice; frequent
3	203794	Sonabitha	South Canara, Karnataka	75	23	96	Long bold	Brown furrows	Good yielder under low input organic farming; common in South Canara
4	203817	Thonnuran thondi	Wynad, Kerala	90	26	103	Long bold	Straw	Stable yielder, excellent as parboiled rice; frequent
5	203785	Nagari	Kasaragod, Kerala	90	28	146	Long bold	Straw	Excellent yielder; frequent
6	203744	Kochuvithu	Malappuram, Kerala	63	22	128	Medium slender	Brown furrows	Good yielder, rare
7	203746	Padannavella	Malappuram, Kerala	68	23	172	Long bold	Straw	Good yielder, rare
8	203750	Thavalakkannan	Malappuram, Kerala	70	20	78	Long bold	Brown furrows	Good yielder, still popular
9	203755	Omanakuttan	Malappuram, Kerala	75	22	107	Long bold	Straw	Suitable for both transplanting and broadcasting
10	203757	Aryankali	Malappuram, Kerala	70	20	72	Long bold	Brown furrows	Rare

(Contd. to next page)

B. IRRIGATED TRANSPLANTED SECOND SEASON CROP

1	203795	Kaulkaima	South-Canara, Karnataka	92	24	172	Medium slender	Straw	Excellent yielder; secondary branches many; attractive panicle; popular in South Canara
2	203790	Kaima	Kasaragod & Kannur, Kerala	76	25	148	Long bold	Straw	Good yielder; excellent parboiled rice; most popular landrace of north Kerala
3	203751	Ennapatta	Malappuram, Kerala	68	20	86	Long bold	Straw	Panicle just exerted; average yielder; rare
4	203782	Veluthamundodan	Malappuram, Kerala	86	23	120	Long bold	Straw	Good straw and grain yielder; rare
5	203789	Puncha	Kasaragod & Kannur, Kerala	78	23	96	Long bold	Straw	Good yielder; common landrace of Malabar
6	203791	Undakaima/Urunkaima	Kasaragod & Wynad, Kerala	90	22	109	Short bold	Straw	Round grains, good yielder; rare

C. UPLAND RAINFED DIRECT SEEDED ('PUNAM' CULTIVARS)

1	203793	Chennel	Kasaragod & Kannur, Kerala	108	28	194	Long bold	Straw	Excellent yielder as upland as well as irrigated transplanted rice; good straw yield also; landrace of "Naikkar" tribe; nearly extinct
2	203771	Karuthanavara	Kannur, Kerala	78	21	68	Long bold	Black	Compact panicle with few or no secondary branches; poor yielder; medicinal, high value crop
3	203821	Karakkozhivalan	Kasaragod, Kerala	86	25	108	Long bold	Straw	Primary branches terminate at the same level; good for parboiling and rice flour preparation
4	203779	Keeripallan	Kasaragod, Kerala	112	29	182	Long bold	Straw	Kernel tip black dotted, good for rice flour; rare

(Contd. to next page)

5	203772	Vellakoyala/ Veluthon	Kannur, Kerala	112	27	178	Medium slender	Straw	Thick culm; good straw and grain yield; nearly extinct
6	203769	Palkaima	Kannur, Kerala	93	27	188	Long bold	Straw	Excellent cooking quality; nearly extinct
7	203792	Veluthadichal	Malappuram, Kerala	98	24	140	Long bold	Straw	Compact panicle; good straw yield; nearly extinct
8	203766	Ambaladan	Kannur, Kerala	120	28	188	Medium slender	Straw	Thick culm; good straw yield; open panicle; nearly extinct
9	203819	Chuvannachoman	Malappuram, Kerala	92	30	126	Long bold	Straw	Nearly extinct
10	203820	Karuthachoman	Malappuram, Kerala	92	21	63	Long bold	Black	Compact panicle; sterile glume slightly black; nearly extinct
11	203770	Kallarikoyala	Kannur, Kerala	114	32	144	Long bold	Brown (tawny)	Sterile glumes purplish; husk black tinged; secondary branches many; excellent yield; almost extinct
12	203765	Chingappadan	Kannur, Kerala	60	20	77	long bold	Straw	Average yield; nearly extinct
13	203823	Keeriputhada	Kasaragod, Kerala	82	26	116	Long bold	Brown (tawny)	Flag leaf extra long; primary branches long nearly reaching the tip; rare
14	203776	Mundodan	Malappuram, Kerala	106	24	108	Long bold	Straw	Fairly good yield; excellent cooking quality; rare
15	203815	Chembery collection	Kannur, Kerala	114	32	230	Long bold	Brown furrows	Excellent yield; landrace of "Karimpalar" tribe; rare
16	203767	Veluthanavara	Kannur, Kerala	102	25	103	Long bold	Brown furrows	"Navara" used for cooking purpose; nearly extinct
17	203818	Mullankoyala	Kannur, Kerala	90	27	120	Long bold	Straw	Awned; nearly extinct

(Contd. to next page)

D. HIGHER ALTITUDE WYNAD AND COORG COLLECTIONS

1	203809	Mattathondi	Wynad, Kerala	92	25	112	Long bold	Straw	Good yielder; rare
2	203799	Onavattan	Wynad, Kerala	82	23	116	Long bold	Brown furrows	Hairy husk; apiculus red; flat grooved grains; fairly good yielder; rare
3	203812	Veliyan/ Kirubeliya	Wynad, Kerala/ Coorg, Karnataka	80	28	92	Long bold	Straw	Most common landrace of Wynad; poor adaptability outside Wynad
4	203808	Chomala	Wynad, Kerala	78	27	139	Long bold	Straw	Poor adaptability outside Wynad; very rare; nearly extinct
5	203807	Marathondi	Wynad, Kerala	88	25	128	Long bold	Straw	Secondary branches many; fairly good yielder; common
6	203788	Thonnuran	Wynad, Kerala	80	26	113	Medium slender	Brown furrows	Fairly good yielder; short duration; common
7	203813	Jeerakasala/jeera kasanna	Wynad, Kerala	80	29	158	Short slender	Straw	Scented rice; poor adaptability in plains
8	203810	Rajani/Channa	Wynad, Kerala	78	27	113	Medium slender	Straw	Scented rice; common in Wynad
9	203801	Gandhakasala	Wynad, Kerala	82	34	172	Short slender	Straw	Most popular scented rice of Wynad; poor adaptability outside Wynad
10	203811	Mullanchanna/ Soojichanna	Wynad, Kerala	60	15	36	Short bold	Straw	Red, awned, scented rice; very poor adaptability outside Wynad; rare
11	203804	Koduveliyan	Wynad, Kerala	90	24	98	Long bold	Straw	Fairly good yielder; common

medicinal value is grown by 'Mavilan' tribe who administer popped rice soaked water to diarrhoea patients.

'Punam' cultivation as well as the landraces for this ecosystem is almost extinct due to non availability of forest land for this purpose. However, few 'Kurichya' families in Kannavam forest still practice this in small plots with their traditional landraces line 'Chingappadan', 'Ambaladan', 'Vellakkoyala', 'Kallarikkoyala', 'Mullankoyala', 'Palkaima', 'Velutha navara' and 'Karutha navara' out of emotional attachment for their traditional cultivars. Broadcasting is done before 'Vishu' (First of the Malayalam month 'Medam') in order to avail the entire growing season. 'Kallarikkoyala' is an ecotype specifically for laterite soils whereas 'Palkaima' has the best cooking quality. 'Chennel' is another 'punam' landrace still grown by 'Naikkar' community in the remote hills of Kasaragod district bordering Karnataka.

In Kasaragod - Bandadka route, a popping quality rice 'Varukkunna nellu' is grown for home consumption. besides its popping quality, it is said to be good for 'payasam' (sweet meat) and rice flour. In the irrigated transplanted belot, good straw yielders are preferred in the second season and 'Cheruvellari' is one such landrace. Collections like IC-203785 ('Nagari'), IC- 203783 ('Undakaima'), IC-203795 ('Koulkaima'), IC-203793 ('Chennel') and IC-203815 (Chemberi collection) are good yielders comparable with control (28 q/ha).

Wild rice species *Oryza meyeriana* ssp. *granulata* grows in abundance in Karulai range of Nilambur forest in newly cleared forest coups and young teak plantations. *O.officinalis* ssp. *malampuzhensis* is also seen growing on the banks of streams and rivulets in the same forest range confirming the earlier report (Vaughan & Muralidharan, 1986). *O.sativa* f. *spontanea* showing varying degrees of introgression is found growing in the entire coastland from Thrissur to Mangalore. *O.rufipogon* is seen restricted to wetlands in Thrissur and Malappuram districts.

CONCLUSION

Though landraces are still popular, as rice cultivation is less remunerative in Kerala due to many reasons, people go for other crops like cassava, vegetables, ginger, banana, coconut and arecanut after reclaiming paddy fields or prawn culture in 'pokkali' lands. So the threat is more to ecosystem diversity which in turn will lead to genetic erosion.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, NBPGR for the facilities provided and to the Officer in Charge, NBPGR Regional Station, Thrissur for his constant encouragement and support. The help of Dr. S. Mohan, Head, Dept. of Botany,

Govt. Brennan College, Thalassery and Dr. T.N. Ravindran, Lecturer in Botany, Sir Syed College, Taliparamba in collection of grain samples is gratefully acknowledged.

REFERENCES

- NBPGR. 1986, 1987, 1989-90, 1990-91, 1992-93. Annual Reports of NBPGR Station, Thrissur. 1986, p. 9-10; 1987, p.8; 1988, p. 7-8; 1989-90, p.7; 1990-91, p.6; 1992-93, p. 3-6.
- Chang, T.T. 1976. Manual on the genetic conservation of rice germplasm for evaluation and utilization. IRRI, Los Banos, Philippines.
- Chang, T.T., S.D. Sharma, C.R. Adair and A.T. Perez. 1972. Manual for field collectors of rice. p. 32. IRRI, Los Banos, Philippines.
- Duncan A. Vaughan and V.K. Muralidharan. 1988. Collecting wild relatives of rice from Kerala State, India. *FAO/IBPGR. Plant Genetic Resources Newsletter* 75/76 : 45-46.
- Manilal, K.S. 1990. Ethnobotany of the rices of Malabar *In Contribution to Ethnobotany* of India. pp 243-253. Botanical Survey of India.
- Sukumara Dev, V.P., C.A. Rosamma and C.R. Elsy. 1993. "Samridhiyude Vithukal" (in Malayalam). Kerala Agricultural University, Thrissur (Kerala).

CORRELATION AND PATH ANALYSIS IN INDUCED MUTANTS OF WHEAT (*TRITICUM AESTIVUM* L.)

M. K. Rana¹ and G. S. Sethi

Department of Plant Breeding and Genetics,
Himachal Pradesh Krishi Vishvavidyalaya,
Palampur 176 062 (Himachal Pradesh)

True breeding mutants of two improved wheat cultivars 'CPAN 1922' and 'HPW (DL)30' were grown in the M4 generation in an RBD with three replications. Estimates of variability, heritability and genetic advance as well as correlation coefficients and path coefficients were worked out for the mutants of each cultivar. Wide range of variability was observed for plant height, grains/spike and harvest index. High heritability coupled with high genetic advance was observed for grain yield. Grain yield showed positive association with tillers per plant, grains per spike, 100-grain weight and harvest index in the 'CPAN 1922' mutants, whereas 100-grain weight and harvest index did so in 'HPW (DL) 30' mutants. Path-analysis revealed the importance of harvest index, 100-grain weight and tillers per plant in the mutants of 'CPAN 1922' and 100-grain weight and harvest index in the mutants of 'HPW (DL) 30' for increasing the grain yield.

Key words: *Triticum aestivum*, mutants, variability, correlations, path-analysis.

Breeders select superior genotypes for crop improvement based on their phenotypic expression. As variation in a character is an interplay of heritable and non-heritable agencies, selection based on merely phenotypic expression is misleading. In the present investigation, therefore, an attempt has been made for partitioning overall variability into its heritable and non-heritable components and genetic parameters like genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), broadsense heritability (H) and genetic advance (GA) were studied for 9 characters which could be useful in developing reliable selection indices.

¹Currently at NRC on DNA Fingerprinting, NBPGR, Pusa Campus, New Delhi 110 012

MATERIALS AND METHODS

The experimental material for this study comprised of 35 true- breeding mutants of wheat (20 of 'CPAN 1922' and 15 of 'HPW (DL) 30') induced by using gamma irradiation (10 kr, 20 kr and 30 kr). These mutants, in the M4 generation, were grown in an RBD with three replications at the Experimental Farm of the Department of Plant Breeding & Genetics, HPKV, Palampur. Each entry consisted of one-metre row plot with a row-to-row and plant-to-plant spacings of 60 and 2.5 cm, respectively. The data on 10 randomly chosen plants were recorded for grain yield and its component traits (harvest index, 100-grain weight, grains per spike, spikelets per spike, spike length, tillers per plant, plant height and days to maturity). Coefficients of variation, heritability (broad sense) and genetic advance were worked out following methods given by Burton and De Vane (1953) and Johnson *et al.* (1955). The phenotypic and genotypic coefficients of correlation were computed as suggested by Al-Jibouri *et al.* (1958) and the path coefficient analysis of various traits was done following Dewey and Lu (1959).

RESULTS AND DISCUSSION

The mutants of both the cultivars showed a wide range of variability for all the characters under study. Mean, its standard error and various parameters of genetic variability are presented in Table 1. Wide differences between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) revealed that tillers per plant and grains per spike in both 'CPAN 1922' and 'HPW (DL) 30' mutants were highly influenced by the environment. An almost close resemblance between the corresponding estimates of PCV and GCV for rest of the traits suggests that the environment had little role in their expression.

Grain yield per plant in the CPAN 1922' mutants and spike length, 100-grain weight and grain yield in the 'HPW (DL) 30' mutants (Table 1) had high heritability (> 80%) coupled with high genetic advance (> 30%). High heritability coupled with moderate genetic advance (20-30%) was observed for harvest index in 'HPW (DL) 30' mutants which according to Panse (1957) may be due to dominance epistatic effects and least effect of additive genes. However, reverse (moderate heritability i.e. 50-80% and high genetic advance i.e. > 30%) was true for 100-grain weight in the CPAN 1922 mutants and for tillers per plant in the 'HPW (DL) 30' mutants. Moderate heritability and genetic advance were observed for tillers per spike in the 'HPW(DL) 30' mutants which indicates that these characters are governed by both additive and non- additive gene effects. So, selection will be more effective for the improvement of these traits. High heritability coupled with low genetic advance

Table 1. Genetic parameters of variability for nine characters of 'CPAN 1922' and 'HPW (DL) 30' mutants

Character	CPAN 1922						HPW (DL) 30			
	Mean	SE ($\pm$)	PCV (%)	GCV (%)	H	GA	Mean	SE ($\pm$)	PCV (%)	GCV (%)
Days to maturity	156.13	0.41	1.15	1.05	84.30	2.00	148.23	0.27	0.73	0.66
Plant height	75.53	1.49	10.30	9.74	89.00	18.93	8.80	1.95	8.47	7.48
Tillers per plant	1.75	0.09	17.38	14.84	72.95	26.11	1.90	0.19	28.32	22.61
Spike length	8.65	0.31	10.03	7.84	61.10	12.63	9.86	0.30	21.91	21.26
Spikelets/spike	14.82	41.34	12.72	8.90	48.90	12.82	14.50	0.73	15.43	12.75
Grains per spike	41.34	3.00	18.22	13.18	52.32	19.64	42.70	2.89	19.29	15.32
100-grain weight	3.26	0.21	21.55	18.47	73.46	32.61	3.73	0.17	26.29	25.17
Harvest index	32.69	1.04	10.55	8.99	72.70	15.80	37.43	1.00	13.07	12.22
Grain yield	2.32	0.16	29.65	27.12	83.66	51.10	2.79	0.07	33.64	33.37

SE = standard error of mean; PCV = phenotypic coefficient of variation; GCV = genotypic coefficient of variation; H = Heritability; GA = genetic advance.

was observed for days to maturity and plant height in the 'CPAN 1922' mutants, but only for days to maturity in the 'HPW (DL) 30' mutants. Low heritability and genetic advance were observed for spikelets per spike in the 'CPAN 1922' mutants which may be due to genes having non-additive effects.

The estimates of phenotypic and genotypic correlation coefficients revealed that genotypic correlation coefficients, in general, were higher than the corresponding phenotypic ones, indicating the inherent association among various traits studied in the 'CPAN 1922' and 'HPW (DL) 30' mutants (Table 2). Grain yield showed positive association with tillers per plant, grains per spike, 100-grain weight and harvest index at the phenotypic level in the 'CPAN 1922' mutants, whereas, 100-grain weight and harvest index did so in the 'HPW (DL) 30' mutants. Among the yield components, harvest index with grains per spike and 100-grain weight and spikelets per spike with spike length in the 'CPAN 1922' mutants showed positive correlation. Spikelets per spike had negative associations with 100-grain weight while positive association of grains per spike with spikelets per spike and harvest index with 100-grain weight was discernible in the 'HPW (DL) 30' mutants. Grain yield has been found to have positive association with tillers per plant, 100-grain weight (Paroda and Joshi, 1970) and grains per spike (Jain *et al.*, 1975). The number of spikelets per spike has been found to be positively correlated with spike length and grains per spike and negatively with 100-grain weight (Khamankar, 1986).

For efficient indirect selection and for identifying the yield components, it becomes imperative to know the causal factors for the observed association between two characters. At the phenotypic level, harvest index had the maximum positive direct effect, followed by days to maturity, tillers per plant, 100-grain weight and spike length, whereas plant height, spikelets per spike and grains per spike had negative direct effects on grain yield per plant in the 'CPAN 1922' mutants (Table 3). The direct positive effects of tillers per plant and 100-grain weight have been reported through path-coefficient analysis earlier as well (Jain *et al.*, 1975). Paroda and Joshi (1970) have also reported negative direct effect of grains per spike. In the 'HPW (DL) 30' mutants spikelets per spike had the maximum direct positive effect, followed by 100-grain weight, days to maturity, plant height and harvest index, whereas spike length, tillers per plant and grains per spike had negative direct effects (Table 4).

The positive correlation of tillers per plant with grain yield in the 'CPAN 1922' mutants appears to be the result of its high positive direct effect and positive indirect effect via harvest index (Table 3). The high positive indirect effect of grains per spike via harvest index was responsible for its positive correlation with grain yield. The positive correlation of 100-grain weight

Table 2. Phenotypic (P) and genotypic (G) correlation coefficients in M4 mutant of 'CPAN 1922' (below diagonal) and 'HPW (DL) 30' (above diagonal)

Trait		Days to maturity	Plant height	Tillers per plant	Spike length	Spikelets per spike	Grains per spike	100-grain weight	Harvest index	Grain yield
Days to maturity	P		0.031	0.475	0.041	-0.138	0.120	-0.083	0.435	0.335
	G		0.123	0.728	0.041	-0.215	0.178	-0.138	0.494	0.357
Plant height	P	0.341		0.233	0.426	-0.116	-0.187	0.485	0.451	0.474
	G	0.413		0.061	0.436	-0.339	-0.437	0.619	0.566	0.540
Tillers per plant	P	0.078	-0.002		-0.192	0.284	-0.090	-0.156	0.349	0.382
	G	0.214	-0.317		-0.299	0.268	-0.199	-0.209	0.476	0.489
Spike length	P	0.001	0.217	0.054		0.393	0.299	0.014	-0.128	-0.145
	G	-0.088	0.249	-0.204		0.371	0.261	0.036	-0.126	-0.160
Spikelets per spike	P	0.072	-0.132	0.153	0.552*		0.638*	-0.574*	-0.214	-0.276
	G	-0.013	-0.225	0.079	0.415		0.609	-0.656	-0.281	-0.366
Grains per spike	P	0.154	-0.054	0.120	0.022	-0.081		-0.012	-0.115	0.175
	G	0.230	-0.008	0.081	-0.282	-0.283		-0.014	-0.133	0.200
100-grain weight	P	0.231	0.095	0.194	-0.011	0.364	0.262		0.505*	0.602*
	G	0.278	0.106	0.134	-0.139	-0.443	0.403		0.559	0.629
Harvest index	P	-0.149	0.260	0.197	0.152	-0.011	0.482*	0.530*		0.827*
	G	-0.204	0.297	0.401	0.298	-0.112	0.897	0.719		0.896*
Grain yield	P	0.215	0.020	0.437*	0.004	-0.010	0.553*	0.587*	0.726*	
	G	0.278	0.021	0.525	-0.520	-0.170	0.849	0.723	0.923	

*Significant at 5% level.

Table 3. Estimates of direct and indirect effects at the phenotypic (P) and genotypic (G) levels in M4 mutants of 'CPAN 1922'

Trait		Days to maturity	Plant height	Tillers per plant	Spike length	Spikelets per spike	Grains per spike	100-grain weight	Harvest index
Days to maturity	P	0.406	0.139	0.032	0.000	0.099	0.063	0.094	-0.060
	G	-0.127	-0.052	-0.027	0.011	0.002	-0.029	-0.035	0.026
Plant height	P	-0.129	-0.379	0.001	-0.082	0.050	0.020	-0.036	-0.098
	G	0.129	0.313	-0.099	0.078	-0.070	0.002	0.033	0.093
Tillers per plant	P	0.020	0.000	0.259	0.014	0.040	0.031	0.050	0.051
	G	0.059	-0.087	0.274	-0.056	0.022	0.022	0.037	0.110
Spike length	P	0.000	0.022	0.005	0.101	0.056	0.002	-0.001	0.015
	G	0.059	-0.087	0.136	0.667	0.022	0.022	0.037	0.110
Spikelets per spike	P	-0.018	0.033	-0.038	-0.137	-0.248	0.020	-0.090	0.003
	G	-0.006	-0.133	0.040	0.208	0.501	-0.142	-0.222	-0.056
Grains per spike	P	-0.002	0.001	-0.001	0.000	0.001	-0.012	-0.003	-0.006
	G	0.088	-0.003	0.031	-0.108	-0.108	0.383	0.154	0.343
100-grain weight	P	0.044	0.018	0.037	-0.002	0.070	0.050	0.192	0.102
	G	0.339	0.131	0.050	0.063	-0.066	-0.209	0.471	0.339
Harvest index	P	-0.107	0.187	0.142	0.109	-0.008	0.347	0.382	0.720
	G	0.267	-0.054	0.079	0.107	0.080	-0.030	0.240	0.267
Correlation with Grain Yield	P	0.215	0.020	0.437*	0.004	-0.100	0.522*	0.587*	0.726*
	G	0.278	0.021	0.525	-0.520	-0.170	0.849	0.723	0.923

Underlined values denote direct effects and the remaining indirect effects.

*Significant at 5% level.

Table 4. Estimates of direct and indirect effects at the phenotypic (P) and genotypic (G) levels in M4 mutants of 'HPW(DL)30'

Trait		Days to maturity	Plant height	Tillers per plant	Spike length	Spikelets per spike	grains per spike	100-grain weight	Harvest index
Days to maturity	P	0.695	0.022	0.330	0.028	-0.096	0.083	-0.058	0.302
	G	0.609	0.075	0.443	0.025	-0.131	0.183	-0.084	0.301
Plant height	P	0.014	0.462	0.108	0.197	-0.054	-0.086	0.224	0.209
	G	0.186	0.510	0.092	0.658	-0.512	-0.660	0.935	0.855
Tillers per plant	P	-0.171	-0.084	-0.360	0.069	-0.102	0.032	0.056	-0.126
	G	-0.288	-0.024	-0.396	0.118	-0.106	0.079	0.083	-0.189
Spike length	P	-0.030	-0.309	0.139	-0.725	-0.285	-0.217	-0.010	0.158
	G	-0.056	-0.600	0.412	-0.377	-0.511	-0.359	-0.049	0.173
Spikelets per spike	P	-0.121	-0.102	0.250	0.346	0.880	0.562	-0.505	-0.188
	G	-0.129	-0.204	0.161	0.223	0.602	0.366	-0.395	-0.169
Grains per spike	P	-0.021	0.033	0.016	-0.053	-0.114	-0.179	0.002	0.020
	G	0.116	-0.286	-0.130	0.171	0.399	0.655	-0.009	-0.087
100-grain weight	P	-0.070	0.411	-0.132	0.012	-0.487	-0.010	0.848	0.428
	G	-0.028	0.128	-0.043	0.007	-0.136	-0.003	0.207	0.116
Harvest index	P	0.038	0.040	0.031	-0.011	-0.019	-0.010	0.045	0.088
	G	-0.051	-0.059	-0.50	0.013	0.029	0.014	-0.058	-0.104
Correlation with Grain yield	P	0.334	0.474	0.382	-0.145	-0.276	0.175	0.602*	0.827*
	G	0.357	0.540	0.489	-0.160	-0.366	0.200	0.629	0.896

Underlined values denote direct effects and the remaining indirect effects.

*Significant at 5% level.

appears to be the result of high positive indirect effect via harvest index, and a little positive direct effect. The high direct positive effect of harvest index and indirect positive effect via 100-grain weight led to its positive correlation with grain yield. Traits, namely, tillers per plant, grains per spike and 100-grain weight had high positive indirect effects via harvest index, indicating the importance of harvest index towards yield.

The positive correlation of 100-grain weight with grain yield in the 'HPW (DL) 30' mutants was due to its high direct positive effect and indirect positive effect via plant height (Table 4). The high positive correlation of harvest index with grain yield appears to be the result of its high positive indirect effects via days to maturity, plant height, spike length and 100-grain weight.

On the basis of the estimates of correlation coefficients and direct and indirect contribution of component characters towards grain yield, harvest index, 100-grain weight and tillers per plant in the mutants of 'CPAN 1922' and 100-grain weight and harvest index in the mutants of 'HPW (DL)' were found rewarding for increasing the grain yield.

REFERENCES

- Al Jibouri, H.A., P.A. Miller and H.P. Robinson. 1958. Genotypic and environmental variances and covariances in an upland cotton cross of interspecific origin. *Agron. J.* 50: 633-636.
- Burton, G.W. and E.H. De Vane. 1953. Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated material. *Agron. J.* 45: 478-481.
- Dewey, D.R. and K.H. Lu. 1959. A correlation and path coefficient analysis of components of crested wheat grass population. *Agron. J.* 51: 515-518.
- Jain, R.P., K.B. Singh and R.S. Malhotra. 1975. Path coefficient analysis in wheat. *Cereal Res. Commun.* 3: 121-125.
- Johnon, H.W., H.F. Robinson and R.F. Comstock. 1955. Estimate of genetic and environmental variability in soybean. *Agron. J.* 47: 314-318.
- Khamankar, Y.G. 1986. Evaluation of dwarf mutants of bread wheat. *Wheat Inform. Serv.* 63: 23-26.
- Panse, V.G. 1957. Genetics of quantitative characters in relation to plant breeding. *Indian J. Genet.* 17: 318-328.
- Paroda, R.S. and A.B. Joshi. 1970. Genetic architecture of yield and components of yield in wheat. *Indian J. Genet.* 30: 298-314.

GENETIC VARIABILITY FOR MORPHO- PHYSIOLOGICAL COMPONENTS OF YIELDING ABILITY IN RAINFED RICE (*ORYZA SATIVA* L.)

S.G. Hegde and C.S.P. Patil¹

Department of Genetics and Plant Breeding
University of Agricultural Sciences, Dharwad 580 005 (Karnataka)

Genetic variability parameters such as phenotypic and genotypic coefficients of variation (PCV & GCV), heritability and genetic advance (GA) for various morphological and physiological characters were estimated in 40 genotypes of rainfed rice (*Oryza sativa* L.). High estimates of PCV and GCV were observed for net assimilation rate, harvest index, absolute growth rate and crop growth rate, where as, moderate to high estimates of PCV and GCV were recorded for plant height, leaf area index, total dry matter, specific leaf weight and grain yield per plant, indicating wide variability in the material for these traits. However, there was little variability in the material for days to 50% flowering, total tillers, effective tillers, photosynthetic rate, spikelets per panicle, 1000-grain weight and panicle length. High heritability and high genetic advance indicate scope for improvement through simple selection for harvest index, 1000-grain weight, grain yield per plant and net assimilation rate.

Key words: Rainfed rice, variability, heritability, yield

The ideotype concept, as described by Donald (1968), tells about the importance of morphological and physiological characters in optimum combination to get the maximum productivity. The rainfed rice ecosystem has some production constraints such as rainfall pattern and limited radiation. The rice genotypes with better yielding ability under such situations should be physiologically efficient with optimum combination of morphological and physiological attributes of yield. Therefore, an integrated approach with major emphasis on physiological traits to breed varieties with increased yield potential has become absolutely essential. But the research work on the genetic variation for different physiological traits of rice are quite few. Keeping this in view, the present study was initiated to assess the extent of genetic variability,

¹Corresponding author: Professor of Forestry, College of Forestry, Sirsi 581 401 (Karnataka)

heritability and genetic advance for different morphological and physiological characters in rainfed rice.

MATERIAL AND METHODS

Forty genotypes of rainfed rice germplasm were evaluated in randomized complete block design with two replications at the Agricultural Research Station (Paddy), Sirsi (Karnataka), situated at an altitude of 619 m above mean sea level. Seeds were sown in nursery, and later 30 days old seedlings were transplanted in the main field. Each variety was grown in four rows of 2 m length with a spacing of 20 × 15 cms. Two plants were randomly selected from the central two rows in each plot for recording data on physiological characters whereas, five plants per plot were selected for recording yield data. The observations on days to 50% flowering were recorded on plot basis.

Analysis of variances was performed following standard procedures (Panse and Sukhtme, 1967). The phenotypic and genotypic coefficients of variability were computed according to the method suggested by Burton (1952) and heritability (broad sense) and genetic advance (GA) as per Robinson *et al.*, (1949).

RESULTS AND DISCUSSION

Significant differences were observed among genotypes for all characters except specific leaf weight at flowering and effective tillers per plant. High magnitude of variation in the experimental material was also reflected by wide range of values for almost all the characters (Table 1).

Estimates of coefficients of variability, heritability and genetic advance are also presented in table 1. The phenotypic coefficient of variation (PCV) in general was higher than genotypic coefficient of variation (GCV) for all the characters under study indicating the influence of environment on the manifestation of the characters. However, the difference between PCV and GCV was less for some characters, *viz.*, days to 50% flowering, photosynthetic rate, 1000-grain weight and harvest index, indicating low environmental influence and predominance of genetic factors controlling these traits. The quantum of genetic variation as indicated by GCV was highest for net assimilation rate (52.7%) followed by harvest index (44.2%), absolute growth rate (41.9%) and crop growth rate (41.05%). This indicated wide variation among the genotypes for the photosynthetic efficiency and partitioning of the dry matter produced. Considerable variability for harvest index was reported earlier also (Sheshshayee, 1987). Low values of GCV were obtained for other characters, *viz.*, days to

Table 1. Range, mean, variability, heritability and genetic advance values for different morpho-physiological traits in rainfed rice genotypes

Characters	Range	Mean	Phenotypic co- efficient of variation (PCV)	Genotypic co- efficient of variation (GCV)	Heritability	Expected genetic advance in % of mean
Days to 50% flowering	98.00-134.0	111.58	8.82	8.40	90.7	16.49
Total number of tillers	8.10-13.1	10.58	16.70	10.36	38.5	13.23
Plant height (cm)	36.50-123.0	83.79	24.40	21.19	75.4	37.90
LAI at 50% flowering	1.97-5.38	3.24	32.65	17.97	30.3	20.37
LAI at maturity	0.59-2.33	1.13	40.99	27.63	45.4	38.93
TDM at 50% flowering (g)	11.73-28.88	18.44	26.29	21.03	64.0	34.65
TDM at maturity (g)	16.27-36.61	23.20	20.46	16.29	63.4	26.72
SLW at 5-% flowering (mg cm ²)	3.51-7.53	4.73	24.01	11.37	22.4	10.99
SLW at maturity (mg cm ²)	6.74-32.01	14.24	49.49	33.72	46.4	47.33
AGR (g plant ⁻¹ day ⁻¹)	0.06-0.49	0.15	68.69	41.90	37.2	53.33
CGR (gm ⁻² day ⁻¹)	1.93-11.60	4.79	51.95	41.05	62.4	66.80
NAR (gm ⁻² LA dy ⁻¹)	0.70-8.23	2.72	60.73	52.72	75.4	94.11
Photosynthetic rate (μ mol. CO ₂ m ⁻² sec ⁻¹)	14.20-25.0	19.72	14.68	13.36	82.8	25.05
Effective tillers	6.60-11.7	8.69	19.14	9.03	22.2	8.74
Panicle length (cm)	12.90-20.0	15.76	13.94	7.43	28.4	8.18
Spikelets per panicle	43.90-122.1	65.36	27.95	16.93	36.7	21.12
Spikelet sterility (%)	15.66-43.60	23.83	28.50	25.21	78.2	45.95
1000-grain weight (g)	16.80-31.4	24.82	16.33	16.01	96.1	32.35
Harvest index (%)	6.99-54.2	27.27	46.87	44.92	91.9	88.70

50% flowering (8.4%), total tillers (10.3%) and effective tillers (9.03%) per plant, photosynthetic rate (13.3%), spikelets per panicle (16.9%), 1000-grain weight (16.9%) and panicle length (7.4%) indicating less scope for improvement of these traits through selection. Low variability for these traits were also reported in earlier studies (Talwar *et al.*, 1974; Sundaram *et al.*, 1988; Ganeshan and Subramanian, 1990). As against this, presence of moderate to high genetic variability for the remaining characters such as plant height, leaf area index, total dry matter, specific leaf weight and grain yield per plant would be helpful to exploit such variation by direct selection or by involving carefully chosen parents, from the material studied, in hybridization programme to generate more variability for further exploitation.

Heritability estimate was highest in case of 1000-grain weight (96.1%) followed by harvest index (91.9%) and days to 50% flowering (90.7%). Photosynthetic rate and grain yield per plant showed over 80% heritability. Earlier reports also indicated high heritability for 1000-grain weight (Kihupi and Doto, 1989 and Li *et al.*, 1991), days to 50% flowering and harvest index (Li *et al.*, 1991). High heritability along with high genetic advance was observed for harvest index, grain yield per plant and net assimilation rate. It is quite evident from these results that these traits are mainly governed by additive gene action. In case of days to 50% flowering, total tiller number, plant height, leaf area index at maturity, total dry matter at flowering and maturity, specific leaf weight at maturity, absolute growth rate, crop growth rate, photosynthetic rate, spikelet sterility and 1000-grain weight, moderate to high heritability coupled with moderate to low genetic advance was observed. This indicates that expression of these traits is governed by both additive and non-additive gene-action. The characters with low heritability and low genetic advance (effective tillers, leaf area index and specific leaf weight at flowering, panicle length and total spikelets per panicle) are presumed to be governed by non-additive gene action or the presence of high genotype-environment interaction. Similar results were also obtained in earlier studies (Maurya, 1976 and Paramasivan, 1980).

Considering all the points related to genetic variability discussed above, it may be concluded that traits like net assimilation rate, harvest index and grain yield can be improved more effectively by selecting superior phenotypes. Based on similar arguments, reasonably good progress can also be achieved in respect of plant height, total dry matter and crop growth rate.

REFERENCES

- Burton, G.W. 1952. Quantitative inheritance in grasses. *Proc. 6th Intern. Grassland. Congr.* 1: 277-283.
- Donald, C.M. 1968. The breeding of crop ideotypes. *Euphytica* 17: 385-403.
- Ganeshan, K. and M. Subramanian. 1990. Genetic studies of the F₂ and F₃ of tall × semi dwarf rice varieties. *Intern. Rice Res. Newsl.* 15: 4-6.
- Kihupi, A.N. and A.L. Doto. 1989. Genotypic and environmental variability in selected rice characters. *Oryzae* 26: 129-134.
- Li, Q.L., G.T. Li., G.Z. Guo., G. Jiang and Y. Zhu. 1991. Study on high yield breeding and genetic analysis of yield components of main rice cultivars in Jilin, China. *Hereditas* (Beijing). 13(5): 3-6.
- Maurya, D.M. 1976. Heritability and genetic advance in rice. *Oryza* 26(3): 238-242.
- Panse, V.G. and P.V. Sukhatme. 1967. In: Statistical methods for agricultural workers, ICAR, New Delhi p. 58-61.
- Paramasivan, K.S. 1980. Study of phenotypic and genotypic variability in quantitative characters in hybrids of tall and dwarf *indica* rice varieties. *Crop Improvement* 7(2): 141-142.
- Robinson, H.F., R.E. Comstock and P.H. Harvey. 1949. Estimates of heritability and degree of dominance in corn. *Agron. J.* 41: 353-359.
- Sheshshayee, M.S. 1987. Varietal variation in leaf area development, photosynthetic efficiency and their influence on biomass production and yield in rice (*Oryza sativa* L.) M.Sc (Agri.) Thesis, Univ. Agril. Sci. Bangalore.
- Sundaram, T., W.W. Manuel and S. Palaniswamy. 1988. Genetic variability and correlation coefficient in early rice. *Indian J. Agril. Sci.* 8: 629-630.
- Talwar, S.N., C.V. Dhulappanavar, K.G. Hiremath, and J.V. Goud. 1974. Genetic parameters for quantitative characters in paddy (*Oryza sativa* L.). *Mysore. J. Agril. Sci.* 8: 520-528.

VARIABILITY IN PEA GERMPLASM FOR SOME AGROMORPHOLOGICAL TRAITS

S. Sardana, Dinesh Kumar, Poonam Suneja and S. Kochhar

National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

A study was carried out on genetic variation of 69 accessions of pea germplasm of exotic and indigenous origin, for ten agro-morphological traits. Genotypic coefficient of variation revealed high to moderate variability for all the characters studied, except for days to 50% flowering and days to maturity, which showed low variability. Seed yield per plant, plant height, number of primary branches per plant and seed weight expressed high heritability (broad sense) coupled with high genetic advance; number of clusters per plant, number of pods per plant, pod length and number of seeds per pod showed moderate to high values whereas days to 50% flowering and days to maturity had low estimates. The wide genetic variability observed in the accessions for yield and yield related traits could be useful for breeding superior genotypes. EC-6620, EC-8257, IC-240775, IC-240779 and IC-209107 were identified as superior accessions for utilization.

Key words : Pea, germplasm, coefficient of variation, heritability, genetic advance

Pea (*Pisum sativum* L.) is known to have been domesticated and cultivated as a winter annual crop in the Mediterranean basin. Adaptation to relatively cool conditions has enabled its spread to areas beyond initial domestication. In India, this crop has a long history of cultivation, as *rabi* vegetable/pulse crop grown throughout the country. Success in varietal adaptation/improvement has been attained in the past from direct introductions, such as, the varieties Bonneville, Arkel and Lincoln, as vegetable types, or through introduction/breeding and selection, such as, the dual purpose variety Pusa Harbhajan or the seed type Rachna, among others. The National Bureau of Plant Genetic Resources presently holds over 1,050 accessions in its active collection and 1,488 accessions in the long term repository of National Gene Bank. The materials are shared with wide category of users, breeders, geneticists, scientists of other disciplines from various organisations, on request for their immediate and sustained use.

Nevertheless, further improvement for yield and other traits depends upon the amount of variability present in the breeding materials for the required traits. Germplasm provides the most valuable reservoir of variability,

on which direct selection can be made for evolving superior genotypes or it may be utilized as parent/donor in hybridization programmes. The present study attempts to find out initial variability parameters for some agro-morphological traits in respect of pea germplasm augmented from different eco-geographic sources within the country and from abroad.

MATERIALS AND METHODS

The experimental material for this study comprised 69 germplasm accessions (Table 1), including 34 indigenous collections from Rajasthan (18), Uttar Pradesh (12), Madhya Pradesh (3) and Bihar (1). The 35 exotic collections were introduced from USA (26), France (4), U.K. (4) and Holland (1). The experiment was laid at the NBPGR Experimental Station, Issapur, New Delhi, during *Rabi*, 1996, in a randomized block design, in paired row blocks replicated thrice. Four checks were used, viz. Rachna, DMR-11, Bonneville and Harbhajan.

Table 1. List of Indian and Exotic germplasm of pea

Sl. No.	Place of Collection/Country of Introduction	Accession numbers
1.	Bihar, India	IC 240779
2.	Madhya Pradesh, India	IC 240778, IC 209136, IC 209137
3.	Uttar Pradesh, India	IC 209117, IC 209119, IC 209120, IC 209122, IC 209127, IC 209131, IC 209132 IC 219020, IC 219026, IC 240777, IC 240780, IC 240781
4.	Rajasthan, India	IC 209092, IC 209093, IC 209095, IC 209096, IC 209102, IC 209103, IC 209107, IC 209108, IC 209109, IC 209110, IC 209111, IC 209112, IC 209113, IC 219005, IC 240773, IC 240774 IC 240775, IC 240776
5.	USA	EC 8257, EC 8495, EC 8762, EC 8763, EC 341765, EC 341766, EC 341771, EC 341787, EC 341793, EC 341986, EC 341988, EC 341998, EC 342007, EC 381953, EC 381854, EC 381855, EC 381856, EC 381857, EC 381858, EC 381859, EC 381860, EC 381861, EC 381862, EC 381864, EC 381865, EC 381866
6.	UK	EC 6620, EC 385244, EC 385245, EC 385246
7.	France	EC 269322, EC 269538, EC 269540, EC 269581
8.	Netherlands	EC 9123

Observations were recorded on five randomly selected plants in each replication, for 10 characters, viz days to 50 per cent flowering, days to maturity, plant height, number of primary branches per plant, number of

clusters per plant, number of pods per plant, pod length, number of seeds per pod, 100-seed weight and seed yield per plant. Genetic variability parameters were estimated, based on mean values, as per Singh and Choudhary (1979).

RESULTS AND DISCUSSION

Mean, range, mean sum of squares and standard errors of mean values for all the ten characters are presented in Table 2. The mean sum of squares were highly significant for all characters, indicating the presence of wide

Table 2. Mean, range, mean sum of squares (MSS) and standard error of mean

Sr. No.	Characters	Mean squares		Range			Mean $\pm$ SEM
		Treatment	Error				
	(df)	72	144				
1.	Seed yield/ plant(g)	173.57**	3.82	2.92	-	37.39	7.55 $\pm$ 1.59
				(IC 240781)		(EC 6620)	
2.	No. of primary branches/plant	18.87**	1.45	2.0	-	13.70	6.09 $\pm$ 0.98
				(IC240774)		(IC 240781)	
3.	No. of pods/ plant	2013.03**	364.33	19.30	-	122.00	63.21 $\pm$ 15.58
				(EC-381862)		(IC-209026)	
4.	No. of clusters/plant	1623.27**	192.81	17.00	-	106.00	54.18 $\pm$ 11.33
				(EC-385242)		(EC 8257)	
5.	Pod length (cm)	3.66**	0.17	4.40	-	10.60	5.97 $\pm$ 0.34
				(IC 240781)		(EC 342007)	
6.	No. of seeds/pod	4.37**	0.81	2.60	-	7.80	5.38 $\pm$ 0.73
				(IC 240775)		(IC 209102)	
7.	100-seed weight (g)	58.91**	0.01	4.35	-	26.50	17.52 $\pm$ 0.09
				(IC-240781)		(IC 209122)	
8.	Plant height (cm)	3171.58**	171.98	40.00	-	158.00	84.42 $\pm$ 10.70
				(EC 341793)		(IC 209122)	
9.	Days to 50% flowering	119.48**	16.37	77.00	-	109.00	98.65 $\pm$ 3.30
				(EC 381861)		(IC 240779)	
10.	Days to maturity	51.40**	0.63	137.0	-	156.00	150.38 $\pm$ 0.65
				(EC-381862)		(EC-8763)	

**Significant at 1% level

variability in the germplasm studied. Grain yield per plant showed a wide range (2.92-37.39 g); the minimum and maximum yield were recorded in accessions IC 240781 and EC 6620, respectively. Number of primary branches per plant ranged from 20 (IC 240774) to 13.70 (IC 240781), with a mean of 6.09. Pods per plant and number of clusters per plant also registered considerable variability which ranged from 19.3 (EC 381862) to 122.0 (IC 219026) and 17.0 (EC 385242) to 106.0 (EC 8257), respectively. Maximum pod length (10.6 cm) was recorded in EC 342007 and minimum (4.4 cm) in EC 8257 and IC 240781. The present set of germplasm possessed an average of 5.38 seeds per pod, which ranged from 2.6 (IC 240775) to 7.8 (IC 209102). IC 209122 exhibited maximum 100 seed weight (26.50 g) whereas a minimum 100-seed weight was exhibited by IC 240781 (4.35 g). Plant height varied from 40 to 158 cm, EC 341793 registering the lowest and IC 209122 the maximum values. However, narrow range was observed for days to 50% flowering (77-109 days) and maturity (137-156 days). Genotypes EC 381861 and EC 381862 were observed to be early types for flowering and maturity, respectively. EC 6620, EC 8257, IC 240779 and IC 209106 were observed to be the most notable accessions for seed yield per plant, 100-seed weight and plant height.

Seed yield per plant had highest coefficient of variation, both at the phenotypic and genotypic levels and the magnitude was considerably higher than the corresponding values at environment level (Table 3). Coefficient of variation was also high in 100- seed weight at phenotypic and genotypic level, with a negligible environmental value. Sizable coefficient of variation at

Table 3. Estimates of genetic parameters for some agro-morphological traits in pea germplasm

S.No.	Characters	Coefficient of variation			Heritability in broad sense (%)	Genetic advance as % of mean
		Phenotypic	Genotypic	Environmental		
1	Seed yield/plant	102.88	99.57	25.86	93.68	198.54
2	No. of primary branches/plant	44.19	39.54	19.74	80.05	72.87
3	No. of pods/plant	47.83	37.09	30.19	60.13	59.24
4	No. of clusters/plant	47.78	40.30	25.63	71.21	70.06
5	Pod length	20.20	18.94	7.01	87.95	36.59
6	No. of seeds/pod	26.27	20.26	16.75	59.48	32.19
7	100-seed weight	25.29	25.28	0.65	99.93	52.17
8	Plant height	40.55	37.46	15.53	85.32	71.27
9	Days to 50% flowering	7.22	5.94	4.10	67.73	10.07
10	Days to maturity	2.79	2.74	0.53	96.43	5.53

genotypic (18-40%) and phenotypic (20-48%) level was observed for number of clusters per plant, number of primary branches per plant, plant height, number of pods per plant, number of seeds per plant and pod length. However, pods per plant recorded high values for environmental component which calls for more systematic evaluation by the users of this germplasm set intending to manipulate the trait expression. In the present case, selection based on number of pods per plant may not be rewarding as compared with involving seed yield per plant and/or 100-seed weight.

Heritability estimates were high (> 80%) for 100-seed weight, days to maturity, seed yield per plant, pod length, plant height and number of branches per plant and moderate (50-80%) for number of clusters per plant, days to 50% flowering, number of pods per plant and number of seeds per pod. Reports of high heritability for yield and yield components in pea have also been reported (Singh, 1995, Kumaran *et al.*, 1995 and Vikas *et al.*, 1996). The high heritability was associated with high genetic advance as per cent of mean (> 50%) for seed yield per plant, plant height, number of primary branches per plant and 100 seeds weight, in conformity with the earlier similar observations of Solanki *et al.* (1988) and Dev *et al.* (1995).

Table 4. Donors for various morpho-agronomic characters in pea germplasm

Characters	Accessions					
	EC 6620	EC 8257	IC 240779	IC 209107	IC 240775	IC 209026
Seed yield/plant(g)	37.39	34.49	32.08	30.00	31.82	28.96
100-seed weight(g)	22.10	18.91	22.83	23.20	24.99	13.83
Plant height (cm)	115.00	125.00	124.33	77.67	68.00	158.00
No. of primary branches/plant	9.67	9.00	7.67	5.67	5.33	11.0
No. of clusters/plant	47.67	106.30	63.67	67.67	51.00	99.33
No. of pods/plant	48.00	104.00	70.67	85.67	69.33	121.67
Pod length(cm)	5.52	4.40	6.27	6.73	5.20	5.50
No. of seeds/pod	5.67	5.00	5.00	5.33	3.00	3.33
Days to 50% flowering	105.00	120.00	109.30	100.33	96.67	103.33
Days to maturity	155.00	151.00	154.00	152.00	149.00	149.00

The parallelism between the magnitude of heritability and degree of genetic gain has been due to the fact that additive genes play a predominant role. Presence of high and moderate heritability for days to maturity and days

to 50% flowering with low genetic gain suggested that high heritability did not necessarily lead to increased genetic gain unless sufficient genetic variability existed in the germplasm. Sardana and Sasikumar (1989) also reported high variability but low genetic gain for days to 50% flowering.

Occurrence of sufficient genetic variability for yield *per se* and its contributing traits, in the present set of germplasm can be exploited for pea improvement and also for characterization of traits during selection or validation. Seed yield per plant, plant height and 100-seed weight were observed to be most reliable selection parameters. EC 6620, IC 240779, EC 8257, IC 209107, IC 240775 and IC 209026 were recorded as superior lines, along with some others (Table 4) for yield *per se* and its component traits, which can be used in further breeding programmes.

ACKNOWLEDGEMENTS

Authors are highly thankful to Dr. P.L. Gautam, Director NBPGR and Dr. S.K. Mithal, Head, Germplasm Evaluation Division, NBPGR for providing facilities and guidance for this work.

REFERENCES

- Dev, H., K.B. Rastogi and K.S. Panwar. 1995. Assessment of variability in F5 generation of garden pea (*Pisum sativum* L.) *J. Hill Res.* 8: 265-26.
- Kumaran, S.S., S. Natarajan and S. Thamburaj. 1995. Genetic variability in pea. *South Indian Horticulture* 13: 1-2.
- Sardana, S. and B. Sasikumar. 1989. Genetic evaluation of field pea. *J. Hill. Res.* 2: 77-79.
- Singh, R.K. 1995. Genetic Variability and heritability studies in pea (*Pisum sativum* L.) *Crop Research* 10: 2.
- Singh, R.K. and B.D. Choudhary. 1979. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi.
- Singh, Vikas, S.P. Singh, Rajvir Singh and K. Singh. 1996. Variability and inheritance of some quantitative characters in Pea (*Pisum sativum* L.) *Annals of Biology* 12: 34-38.
- Solanki, S.S., P.K. Saxena and I.C. Pandey. 1988. Genetic Variability studies in pea under agroclimatic conditions of Western U.P. *Indian J. Hortic.* 45 : 300-303.

GENETIC DIVERGENCE IN TOMATO

S.N.S. Chaurasia and Major Singh

Project Directorate of Vegetable Research
1, Gandhi Nagar (Naria), Sunderpur,
Varanasi 221 005 (Uttar Pradesh)

Seventy one genotypes of tomato were evaluated during 1993-94 and 1994-95. Significant differences were observed for all the characters studied. Based on Mahalanobis D^2 values, genotypes were grouped into 9 clusters in 1993-94 & 10 clusters in 1994-95. Fruit weight showed maximum contribution to the genetic diversity in both years followed by plant height. Considering cluster distances and cluster means the genotypes KS-7, DVRT-2, Antey and PS-1 were recommended the best parents for hybridization.

Key words : *Lycopersicon esculentum* Mill, genetic, divergence, clustering, multivariate analysis, genetic drift, genetic existence, segregants

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable grown in India. Selection of suitable variety for different use from the diverse group of tomato genotypes is of great importance. In plant breeding improvement over existing varieties is a continuous process. A plant breeder is constantly engaged in making an effective choice of desirable parents having high genetic variability so that the desirable character combination may be selected for higher yield. The genetically diverse parents are likely to produce heterotic effects and desirable segregants. Thus the gene diversity has obvious importance. Multivariate analysis using Mahalanobis (1936) D^2 statistics is a powerful tool in quantifying the degree of divergence among the biological populations. The present study was carried out to identify suitable donors having wider genetic distance among tomato lines/genotypes for hybridization programme.

MATERIALS AND METHODS

Seventy one genotypes of tomato collected from different sources were grown during winter season of 1993-94 and 1994-95 at experimental farm of project Directorate of Vegetable Research, Varanasi. The material was planted in a randomised block design having 3 replications. Each plot consisted of 5 rows of 3 m length with spacing of 60 cm from row to row and 45 cm from plant to plant. The recommended agronomic practices were followed to raise a good crop. The observations were recorded on plant height (cm), number

of flowers per cluster, number of fruits per cluster, fruit set (%), node to fruit set, fruit length (cm), fruit diameter (cm), fruit size (cm²), fruit shape, number of fruits per plant, pericarp thickness (mm), number of locules per fruit, 10 fruit weight (g), fruit weight per plant (kg), number of nodes per branch, length of inter nodes (cm), and number of branches per plant. The multivariate analysis was done as suggested by Mahalanobis (1936) and genotypes were grouped into different clusters following Tocher's method as described by Rao (1952).

RESULTS AND DISCUSSION

The analysis of the variance for both the years revealed significant differences among the genotypes for all the characters studied indicating the existence of genetic variability among the genotypes. Based on the relative magnitude of the D^2 values all the 71 genotypes were grouped into 9 clusters in 1993-94 and 10 cluster in 1994-95 (Table 1). The distribution pattern of population showed that cluster-1 was the largest comprising 60 genotypes in 1993-94 and 57 genotypes in 1994-95 followed by cluster-2 having 3 genotypes in 1993-94 and 4 genotypes in 1994-95. The clustering pattern was not same in both the year as the lines like Deogiri, Pant T-1 make distinct cluster in 1993-94 but were grouped in cluster-1 in 1994-95. It may be due to the influence of genotype $\times$ environment interactions. The genotypes did not cluster according to their geographical distribution. Similar results have also been observed by Singh and Singh (1980). This may be due to the fact that genetic drift and human selection in different environment have caused greater diversity than geographic distances. Among all the genotypes KS-7, Punjab Chhuhara, PDVR-2, Antey, PS-1 and Pusa Hybrid-2 (male), displayed their separate genotypic identity by constituting different clusters in both the years. This could be due to different genetic make up as compared to other genotype.

The statistical distances represents the index of genetic diversity among the clusters. The intra cluster distances ranged from 0.00 (monogenotypic cluster) to 11.04 (cluster-2) in 1993-94 and from 0.00 to 11.99 (cluster-2) in 1994-95. The highest intra cluster values were recorded for cluster-2 followed by cluster-1 in both the years. The inter cluster values ranged from 14.38 (cluster-5, cluster-7) to 39.16 (cluster-8 and cluster -9) in 1993-94 and from 14.15 to 29.96 in 1994-95. The lowest inter cluster values was observed between cluster-5 and cluster-7 in 1993-94. and between cluster-1 and cluster-7 in 1994-95 which indicated that the genotypes of these clusters were quite close to each other. On the other hand highest inter cluster value was observed between cluster-8 and cluster-9 in 1993-94 and between cluster-4 and cluster 10 in 1994-95, which revealed that the genotypes from these two clusters could be used as donors in hybridization programme for obtaining wide spectrum of variation among the Segregants.

Table 1. Grouping of 71 genotypes of tomato in different clusters

Cluster No.	No. of entries	Group of Genotypes (1993-94)	No. of entries	Group of Genotypes (1994-95)
1	60	NDT-1, PDVR-4, TC-104, H-36, BT-17, NDT-6, Ageta-1, DVRT-2, Sel-10, Ageta-2, Marglobe, ATV-2, Sel.No.4, PDVR-5, Punjab Kesari, Desi Local, ACE, Sel. No 6, IP-11, KS-2, Kalyani Eunish, Sioux, H-24, Roma, BT-3, NDT-11, NDT- 96, Dhanshree, Bhagyashree, BT-(12-2), NDT-4, Arka Saurabh, Pant-T- 4, Pusa Hyb-2 (female), IP-10, KT-15, NDT-120, IP-6, Sel-7, Arka Vikas, Sel-2, PDVR-1, Anand T-1, Arka Vishal, IP-8, TC-1, Sel-16, Pusa Sel-4, IP-2, Sel-32, IP-4, Pusa Ruby, Field King, PDVR-3, Pant Bahar, KS- 17, Solan Gola, Sel-4,D VRT-1, KT-10.	57	NDT-1, PDVR-4, IP-11, Sel No-6, H- 36, Punjab Kesari, Marglobe, NDT-6, BT-(12-2), BR-17, Pusa Sel- 4, Kalyani Eunish, KT-15, PDVR-3, Ageta-1, TC-1,Pant Bahar, DVRT- 1, Sel-17, Ageta-1, Bhagyashree, Sel-14, Arka Saurabh, TC-104, NDT- 11, IP10, H-24, IP-4, Dhanshree, Ageta-2, Arka Vikas, Sioux, Pusa Ruby, Desi Local, PDVR-5, KS-17, ACE, KT-15, Sel.No-4, NDT-120, Sel- 2, DVRT-1, Sel-16, Sel-32, Pant T-1, NDT-96, UP-6, Sel-18, Sel- 4, Deogiri, NDT-4, IP-2, Solan Gola, Anand T-1, Field King, ATV-2, KS- 2, BT-3
2	3	Sel-18, Rutger, Floradale	4	KS-7, Punjab Chhuhara, KT-10, Pusa Hyb-2 (male)
3	2	KS-7, Punjab Chhuhara	1	Roma
4	1	PDVR-2	3	ATV-2, Rutger, Arka Vishal
5	1	Antey	1	Pant T-4
6	1	PS-1	1	DVRT-2
7	1	Pusa Hyb-2(male)	1	IP-8
8	1	Pant T-1	1	Antey
9	1	Deogiri	1	PS-1
10			1	PDVR-1

Apart from high genetic divergence the performance of genotypes and character with maximum contribution towards genetic divergence should also be given due consideration. The relative contribution of different characters towards the genetic divergence showed that 10 fruit weight had the maximum contribution in both the year (25.15% in 1993-94, 22.25% in 1994- 95) followed by plant height (15.2%, 14.37%). Cluster means were also calculated and compared which showed that in the year 1993- 94 cluster-2 and cluster-9 included the genotypes with dwarf in nature and high yield. Cluster-7 included single genotypes with dwarf and high yield. Though the cluster 4 and cluster-8 included tall genotypes but they showed poor yield.

Table 2a.. Intra and intercluster divergence in tomato in 1993-94

	Grp. 1	Grp. 2	Grp. 3	Grp. 4	Grp. 5	Grp. 6	Grp. 7	Grp. 8	Grp. 9
Grp.1	11.00	18.96	21.02	16.92	15.31	17.94	15.65	20.46	31.00
Grp.2		11.04	28.45	25.73	18.30	29.38	23.52	30.15	33.09
Grp.3			8.58	25.32	14.47	22.52	14.87	28.58	22.33
Grp.4				0.00	22.96	23.77	21.21	16.53	32.92
Grp.5					0.00	22.43	14.38	27.38	23.06
Grp.6						0.00	15.26	17.01	34.84
Grp.7							0.00	24.01	23.04
Grp.8								0.00	39.16
Grp.9									0.00

Table 2b. Intra and intercluster diversity in tomato in 1994-95

	Grp. 1	Grp. 2	Grp. 3	Gr. 4	Grp. 5	Grp. 6	Grp. 7	Grp. 8	Grp. 9	Grp. 10
Grp.1	11.43	16.695	14.747	19.279	15.718	17.056	14.148	15.742	17.992	18.988
Grp.2		11.991	16.422	25.562	21.373	18.92	23.405	14.768	17.681	19.052
Grp.3			0	26.367	16.263	20.295	19.222	18.977	16.584	17.084
Grp.4				10.887	26.58	26.217	19.569	20.377	28.221	29.945
Grp.5					0	15.784	14.466	24.742	20.926	16.343
Grp.6						0	18.478	21.448	19.992	12.741
Grp.7							0	21.924	22.221	22.395
Grp.8								0	19.811	21.939
Grp.9									0	15.814
Grp.10										0

Considering the cluster distances and cluster means the genotypes KS-7, DVRT-2, Aney and P.S-1 are recommended the best parents for hybridization owing to its high genetic distance. The other parents may be chosen from cluster-1, as per requirement. The choice of such diverse genotypes as donor hybridization is more important for varietal improvement.

REFERENCES

- Mahalanobis, D.C. 1936. On the generalised distance in statistics. Proc. Nat. Inst. Sci., India, 12 : 49-55.
- Rao, C.R. 1952. Advanced Statistical Methods in Biometric Research, John Wiley and Sons. Inc., New York.
- Singh, R.R. and H.N. Singh. 1980. Genetic divergence in tomato, Indian J. Agric. Sci. 50(8) : 591-594.

GENETIC DIVERGENCE IN *LEUCAENA* VARIETIES USING NON- HIERARCHICAL EUCLIDEAN ANALYSIS

K.C. Chauhan and R. Hosalli

Department of Tree Improvement and Genetic Resources,
Dr. YS Parmar University of Horticulture and Forestry,
Solan 173 230 (Himachal Pradesh)

Nature and magnitude of genetic divergence was assessed in 49 varieties of *Leucaena* using non-hierarchical cluster analysis for morphological and biomass traits. The population was grouped into 9 clusters. Maximum number of genotypes figured in clusters I and IX, four in II, and VI and one each in IV and VIII. Intra-cluster distance was found maximum for the cluster VI(2.01). Inter-cluster diversity was found maximum between clusters VI and V(11.69) and minimum between cluster III and IX (1.87). The geographical diversity was not related to genetic diversity. Clusters with small statistical distance were considered less diverse than those with large distances.

Key words : *Leucaena*, genetic divergence, cluster analysis, geographical diversity

Leucaena's which are known as "Alfa alfa of Tropics" are among the most versatile of tropical trees. They belong to the sub-family Mimosidae of family Leguminosae. As a leguminous plant native to the "New World", they have been long introduced to other tropical and sub tropical countries owing to its multifarious uses. Few tropical trees matched *Leucaena's* ability to grow fast, provide nutritious fodder, fuel wood, green manure and other useful products. This also helps in preventing the erosion and is used as a nurse crop, shade crop and wind breaks in plantations.

The more diverse the parents, within over all limits of fitness, the greater are the chances of obtaining higher amounts of heterotic expression in F₁'s and broad spectrum of variability in segregating generations. The use of Mahalanobis's D₂ statistics for estimating genetic divergence have been emphasized by many workers in many agricultural crops, but such studies in forestry are very limited. Among tree crops it has been used by Burley *et al* (1971) in *Eucalyptus camaldulensis*, Khosla *et al* (1979) in *Populus ciliata*, Pandey *et al* (1977) in *Bauhinia variegata*. Recently non-hierarchical euclidean cluster analysis derives the attention to overcome the limitations of Mahalanobis D₂ statistic suggested by Beale (1969) and Sparks (1973). Garg and Gautam (1997)

have attempted this method in wheat germplasm to identify the rust and powdery mildew resistant stock. The present investigation was aimed at ascertaining the nature and magnitude of genetic diversity present in a set of 49 *Leucaena* species/varieties.

MATERIALS AND METHODS

Seeds of different species/varieties of *Leucaena* were procured from CFI Oxford, University of Hawaii, IGFR Jhansi, PAU Ludhiana and CCS HAU, Hisar. Before sowing, the seeds were mechanically scarified by nicking the rounded end of the seed and soaked in cold water over night. The seeds were sown (after mixing with pure *Rhizobium* culture Agro forester TM, Tropical Seeds, Holualoa, HI96725 USA) in complete randomised block design with three replications. The spacing between and within the rows was kept 30 cm and 10 cm, respectively in one meter bed. Data on ten competitive plants was subjected to statistical analysis vis- a-vis non-hierarchical euclidean analysis. Seedlings per replications were randomly selected excluding the border ones. The observations were recorded on the selected individuals for morphological and biomass traits viz; germination, seedling height, collar diameter, inter-nodal length, number of nodes, number of leaves, leaf area, shoot and root fresh and dry weight. Mean data was recorded as suggested by Beale (1969) and Spark (1973).

RESULTS AND DISCUSSION

The analysis of variance showed highly significant differences in variety means for all the eleven characters under study which indicated that there existed a considerable inherent difference between these genotypes. Non-hierarchical euclidean cluster analysis proved useful for estimating the genetic divergence in 49 *Leucaena* genotypes which were grouped into 9 clusters (Table 1). The grouping indicated considerable amount of genetic diversity in the germplasm. Maximum species/varieties were in cluster 111 involving 12 genotypes followed by cluster II and VI had 44 each and cluster IV and VIII possessed one each. The table under reference revealed that some varieties from different locations are accommodated in the same cluster (K-636 Hawaii, K-8 Hisar and K-340 Jhansi) and thus indicated their close affinity. On the other hand varieties developed from the same area like K-39 Jhansi, CS 10-Jhansi were distributed to different clusters. These results show that geographical diversity may not necessarily be related to genetic diversity. These findings are in general agreement with the results obtained by Murty and Arunachalam (1966), Bhatt (1970) and Tewari (1970) in different agricultural crops; Pandey *et al* (1995) and Chauhan *et al* (1997) in forest tree species.

Table 1. Clustering pattern of 49 species/varieties on the basis of genetic divergence

Cluster number	Number of genotypes	Name of species/varieties
I	5	<i>L. salvadorensis</i> (ID)-34/88), <i>L. macrophylla nelsonii</i> , <i>L. salvadorensis</i> (ID-36/88), <i>L. hybrid</i> (ID-1/95), <i>L. trichodes</i>
II	4	<i>L. collinii</i> (ID-52/88), <i>L. pallida</i> (137/94), <i>L. salvadorensis</i> (36/88), <i>L. shannonii magnifica</i>
III	12	<i>L. pulverulentax</i> <i>L. leucocephala</i> (-75), <i>L. pulverulenta</i> (22/86), <i>L. leucocephala</i> (K-Ludhiana), <i>L. leucocephala</i> (S-22), <i>L. leucocephala</i> (K-8, Jhansi), <i>L. salvadorensis</i> (17/86), <i>L. leucocephala</i> (K-29, Jhansi), <i>L. leucocephala</i> (K-636, Jhansi), <i>L. leucocephala</i> <i>L. diversifolia</i> (K-743A, Ludhiana), <i>L. diversifolia</i> (45/87), <i>L. leucocephala</i> (K-39, Jhansi)
IV	1	<i>L. esculenta paniculata</i> (52/87)
V	7	<i>L. pulverulenta</i> (84/87), <i>L. leucocephala</i> <i>L. pulverulenta</i> (K-340, Ludhiana), <i>L. diversifolia</i> (83/92), <i>L. lempirana</i> and <i>L. multicapitulata</i>
VI	4	<i>L. diversifolia</i> (K-784, Jhansi), <i>L. esculenta paniculata</i> (79/92), <i>L. lanceolata</i> and <i>L. pulverulenta</i> (83/87)
VII	10	<i>L. collinsii</i> (51/88), <i>L. leucocephala</i> (S-10, Jhansi), <i>L. collinsii</i> (18/84), <i>L. leucocephala</i> (K-601, Jhansi), <i>L. leucocephala</i> (K-8, Hissar), <i>L. leucocephala</i> (K-340, Jhansi), <i>L. leucocephala</i> (K-636, Hawaii), <i>L. leucocephala</i> (nauni), <i>L. leucocephala</i> var <i>EL Salvadorensis</i> (Jhansi), and <i>L. leucocephala</i> (34/92)
VIII	1	<i>L. leucocephala esculenta</i> (47.87)
IX	5	<i>Leucaena hybrid</i> (K-636 × K-156 < Hawaii), <i>L. leucocephala</i> (S-24, Jhansi), <i>L. leucocephala</i> (K-636, Ludhiana), <i>L. diversifolia</i> (83/92) and <i>L. collinsii</i> (56/88)

Intra and inter-cluster distances are presented in table 2. This provided an index of genetic diversity among and within the cluster. The intra-cluster

Table 2. Inter and intra cluster distances in 49 *Leucaena* species/varieties

	I	II	III	IV	V	VI	VII	VIII	IX
I	1.74								
II	2.77	1.74							
III	3.47	4.87	1.65						
IV	9.35	7.18	10.37	0.00					
V	3.12	5.14	2.78	11.69	1.60				
VI	2.68	3.18	4.32	7.73	4.73	2.01			
VII	3.41	3.90	2.13	8.53	4.40	3.66	1.70		
VIII	7.82	6.01	9.13	2.52	10.35	4.30	7.23	0.00	
IX	2.20	4.18	1.87	10.31	2.26	3.51	2.87	9.03	1.30

value ranged from 0.00 (cluster VIII) to 2.01 (cluster VI). Maximum inter cluster distance (11.69) was found between cluster V and IV, suggesting wide diversity between the groups. The crosses between the genotypes from these clusters may give putative transgressive segregants. On the other hand, minimum distances occurred between cluster IX and IV (1.87) indicating the close relationship.

The intermediate value was recorded between cluster II and VI (6.01). The species/varieties included in these clusters were not as much diverse as in cluster V and IV but may be of high yielding potential. The genetic diversity among the parents to be included in hybridization programme has been greatly emphasized in agricultural crops. Therefore, during the selection of parents in forest tree species inter-cluster distances must be taken into consideration.

The comparison of cluster means for 11 characters are appended in table 3. It is apparent from the table that different characters showed considerable

Table 3. Cluster mean for 9 clusters of 49 *Leucaena* species/varieties

Character	I	II	III	IV	V	VI	VII	VIII	IX
Germination	36.67	35.21	37.17	51.67	26.07	35.42	46.75	62.50	27.67
Seedling height	77.26	98.97	59.87	140.9	36.49	75.14	83.63	117.5	65.89
Collar diameter	6.41	6.20	4.81	8.90	3.98	6.32	6.06	10.01	5.69
Inter nodal length	5.74	7.12	3.84	8.28	4.23	5.23	4.95	7.74	4.12
No. of nodes	15.36	16.97	19.34	22.47	15.92	15.85	20.37	20.37	16.57
No. of leaves	14.65	16.75	18.83	22.53	15.32	15.39	19.90	20.60	16.15
Leaf area	69.77	76.92	37.61	114.0	56.20	83.93	46.80	123.3	38.95
Shoot fresh weight	53.19	96.57	39.68	146.3	41.20	62.24	48.17	122.1	41.63
Shoot dry weight	28.19	45.21	16.83	70.56	18.68	33.43	24.18	56.18	22.92
Root fresh weight	7.27	8.25	7.86	20.74	4.80	14.06	9.95	19.11	7.36
Root dry weight	4.67	5.47	4.79	12.64	3.11	8.46	5.58	10.15	5.11

differences between the clusters. Maximum field germination was observed for cluster VIII followed by cluster VII. Seedling height, inter nodal length, no. of nodes, no. of leaves, shoot and root fresh and dry weight was found maximum for cluster IV followed by cluster VIII, Collar diameter and leaf area were recorded highest for cluster VIII followed by cluster IV. Among the 9 clusters, cluster IV and cluster VIII recorded the highest values. The crossing programme involving genotypes from these clusters could help in obtaining the wide spectrum of variability for the above mentioned characters in the subsequent generations.

REFERENCES

- Beale, E.M.L. 1969. Euclidean cluster analysis. Contributed paper to the 37th session of the International Statistical Institute.
- Bhatt, G.M. 1970. Multivariate analysis approach to selection of parents for hybridization aiming at yield improvement in self-pollinated crops. *Aust. J. Agric. Res.* **21**: 1-7.
- Burley, J., P.J. Wood and A. S.Hans. 1971. Variations in leaf characteristics among provenances of *Eucalyptus camaldulensis* Dehr. grown in Zambia. *Aust. J. Bot.* **19**: 237-249.
- Chauhan, K.C., A. Srivastava and R.C. Thakur. 1997. Analysis of genetic divergence in seed stands of *Bauhinia variegata* Linn. *Indian J. Pl. Genet. Resources* **10**(1): 115-121.
- Garg, D.K. and P.L. Gautam 1997. Genetic divergence studies in wheat germplasm using non-hierarchical euclidean cluster analysis. *Indian J.Pl. Genet. Resources* **10**(1): 11-15.
- Khosla, P.K., S.P. Dhall and D.K. Khurana 1979. Studies in *Populus ciliata* Wall ex Royle 1. Correlation of phenotypic observations with sex of trees. *Silvae Genet.* **28**: 68-77.
- Murty B.R. and V. Arunachalam 1966. The nature of genetic divergence in relation to breeding system in crop plants. *Indian J. Genet.* **26A**: 188-98.
- Pandey, D., S.K. Tewari and S. Tripathi 1975. Genetic divergence in *Populus deltoidae* Bart. *Indian J. Genet.* **55**(2) : 129-131.
- Park, D.N. 1973. Euclidean cluster analysis. *Algorithm. Appl. Statistics* **22**: 126-30.
- Tewari, S.N. 1975. Studies on genetic divergence in Barley (*Hordium vulgare* L.) 11. Proc. Int. Barley Genet. Symposium, Garchung, 821-31.

A COLORIMETRIC METHOD FOR ESTIMATION OF CAPSAICIN IN CHILLI FRUITS

S. Mandal, Poonam Suneja and D.K. Hore¹

National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

A simple colorimetric method involving sodium tungstate- phosphomolybdic acid reagent is described for determining the pungency, due to capsaicin present in oleoresin of chilli fruits. Capsaicin concentration of some locally available chilli fruits and its pericarp, after removal of seed were determined with reference to a calibration graph. Oleoresin nitrogen concentrations of some samples were also determined by conventional Kjeldahl's method and converted to capsaicin by suitable multiplication factor. The results are compared and discussed.

Key words : Capsaicin, chilli fruits, oleoresin, colorimetric method, kjeldahl nitrogen

The capsicums and chillies are used in food preparations in which the quality is of much importance. The quality is based on characteristics like pungency level, a bright red colour, a good flavour, a medium-sized fruit with a moderately thin pericarp, a smooth, glossy surface, less seeds in the fruit and a firm stalk. In pungent chillies that are to be used for the preparation of capsicum oleoresin, a high pungent flavour assumes more importance than the appearance. The pungent flavour being determined by the capsaicin content. Chemically capsaicin is N-3-methoxy-4-hydroxybenzyl-8- methylnon-trans-6-enamide. However, Benett and Kirby (1968), Kosuge and Furata (1970) and Masada *et al.* (1971) have reported that capsaicin is actually a mixture of atleast five closely related vanillyl amides as shown in Figure 1. Various methods are recorded in the literature for the quantitative estimation of capsaicin in capsicum fruits. Among these a gas-liquid chromatography method devised by Todd *et al.* (1977) also permits the resolution of individual capsinoids. Gonzalez and Tamirano (1973) developed a procedure based on soxhlet extraction of green chilli with isopropanol and ultraviolet absorbance at 281nm. Paper chromatography technique was applied for the purification of sample. Colorimetric method of North (1949) based on the colour reactions

¹National Bureau of Plant Genetic Resources, Regional Station, New Kench's Trace, Shillong 793013.

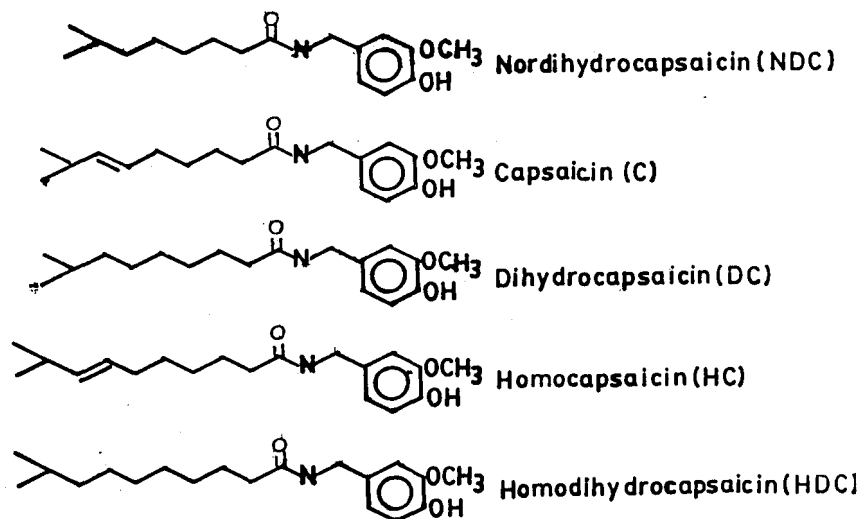


Fig. 1. Natural capsaicinoids

of capsaicin and phosphotungstic-phosphomolybdic acid. Selective solubility technique was followed in extraction procedure to reduce the phenolic interference. All these procedures are time consuming and is not suitable in germplasm evaluation programme. Moreover, Phosphotungstic-phosphomolybdic acid reagent of North (1949) when applied in the oleoresin extract, colour development was not observed even after 2 h of reaction.

In this paper, a method is described for the determination of the total capsaicin content of samples of *Capsicum* species that have different degrees of pungency. This method is based on an extraction of capsicum oleoresin by acetone followed by the colorimetric determination procedure involving sodium tungstate and phosphomolybdic acid in presence of sodium hydroxide with reference to a standard solution of capsaicin.

MATERIALS AND METHODS

Plant Material

Among five chilli fruits, sample no. 1, 2 & 4 were obtained from the National Bureau of Plant Genetic Resources, Regional Station, Shillong and sample no. 3 and 5 were procured from the local markets based on their morphological variations. All chemicals used were of analytical reagent grade. Standard capsaicin (8-Methyl-N-vanillyl-6-nonenamide) was purchased from

Sigma Chemical, USA. Whole fruit (without stalk) and seedless pericarp parts were ground in a Remi Anupam blender. Evaporation was done in a fumehood. The absorbance at 650 nm was determined by a Beckmann spectrophotometer Model 26. Nitrogen content of the samples were determined by the classical Kjeldahl method with kjeltec auto 1030 analyzer from Tecator, Sweden. The samples were digested in the presence of 1013 scrubber unit of Tecator, Sweden.

Extraction of oleoresin from chilli powder

Ground sample (0.2 g) was mixed with 10ml of dry acetone (25 g anhydrous sodium sulphate was added to 500 ml acetone atleast one day before use) for about 3 h by occasional shaking; filtered through Whatmann No. 1 filter paper to get the clear supernatant. Supernatant was divided in 3 parts of 0.5 ml each for colorimetric determination of capsaicin, and from the remaining supernatant 5.0 ml were taken for the estimation of oleoresin percentage.

Colorimetric determination of capsaicin

Supernatant containing oleoresin was dried on heating slab inside fume hood. To dry residue (oleoresin), 0.5 ml of 4% sodium tungstate was added and followed by 4.5 ml of 0.2% sodium hydroxide to dissolve the capsaicin present in oleoresin. In the above mixture 2 ml of 2% phosphomolybdic acid containing 1.2% sodium tungstate was added and kept for 2h, at room temperature to develop blue colour. The solution was filtered through Whatmann No. 42 filter paper and absorbance was recorded at 650 nm against a reagent blank. The relative concentrations of capsaicin present in ground sample and oleoresin are calculated by reference to calibration curve and oleoresin percentage, respectively.

Calibration of standard curve

Pure capsaicin (5 mg) was dissolved in 5 ml of 0.2% sodium hydroxide solution. This would serve as stock solution equivalent to 1000 ppm of capsaicin. Two ml of this stock solution was diluted to 10 ml with 0.2% sodium hydroxide to get standard capsaicin solution of 200 µg/gml. A series of concentrations containing 15, 20, 30, 40, 50, 60 and 70 µg/g capsaicin was prepared in 0.2% sodium hydroxide and colour was developed with sodium tungstate and phosphomolybdic acid as described earlier. From the absorbance values recorded at 650 nm, a standard curve plotting absorbance against capsaicin up to 70 µg was prepared and the quantity of capsaicin in the samples was determined from this calibration curve. One optical density at 650 nm was equivalent to 285.71 µg of pure capsaicin.

RESULTS AND DISCUSSION

Table 1 shows the relative concentrations of capsaicin and acetone extracted oleoresin percentages in five different chilli samples both in whole fruit and in pericarp.

Table 1. Quantitative determination of capsaicin in oleoresin of whole fruits and pericarp parts of some chilli samples with reference to pure capsaicin

Sample No.	Sample	Oleoresin (%)	Capsaicin (%)	
			Oleoresin	Dry powder
1	Whole fruit	11.97	2.842	0.3402
1(a)	Pulp	12.50	5.225	0.6532
2	Whole fruit	11.92	3.089	0.3683
2 (a)	Pulp	13.00	5.523	0.7181
3	Whole fruit	15.53	3.145	0.4880
3 (a)	Pulp	15.23	7.075	1.077
4	Whole fruit	15.45	3.719	0.5746
4 (a)	Pulp	16.00	6.802	1.090
5	Whole fruit	15.53	5.027	0.7810
5 (a)	Pulp	16.20	7.5405	1.221

Chilli contains up to 61 per cent seed in its fruit. In the present studies 11.92 to 15.53 per cent of oleoresin was recorded by using acetone as solvent for extraction. Naveş (1974) reported 8.7-16.5 per cent oleoresin and used dichloroethane as solvent. Szabo (1970) asserted that acetone is an excellent solvent for capsaicin and is the most frequency used water miscible solvent. Capsaicin concentration was found to vary between 0.3402 to 0.7810 per cent on dry weight basis on the whole fruit. Govindarajan and Ananthakrishna (1970) reported 0.12 per cent capsaicin concentration in Mysore variety chilli and 0.7 per cent for Guntur Variety. Generally pulp part of the fruit gives little higher percentage of oleoresin than the whole fruit; even in some cases (sample no. 3) whole fruit gives higher percentage of oleoresin than the pulp. But capsaicin concentration in pulp oleoresin always in higher proportion nearly 1.5 to 2.2 times when compared with the whole fruit oleoresin. This is mainly because, capsaicin oleoresin of whole fruit contains considerable amount of fixed oil originating mainly from the seed. Pearson (1976) reported a range of 15.5 to 27.0 per cent fixed oil in whole chillies. Chilli fruit of

sample no. 3 may have comparatively higher amount of oil percentage of the whole fruit as expected. It is well known that the seed of the fruit does not contain any capsaicin (Tice, 1933), therefore proportional increase in capsaicin concentration is not expected in the oleoresin extract of the whole fruit (sample No. 3).

Oleoresin of the whole fruit was found to contain 0.1444 to 0.2308% nitrogen. Nitrogen concentrations when converted to the capsaicin by multiplying a factor of 21.786 (Pure capsaicin contains 4.59% nitrogen), the calculated capsaicin percentages are very close to experimentally determined value of the presently described colorimetric method. This indicates capsaicin is the only nitrogenous substance present in the oleoresin of the fruit (whole as well as pulp) and reacts completely with the presently used colour reagent.

Non-capsaicin nitrogen of the residue (meal) obtained after oleoresin extraction with acetone, alongwith whole fruit nitrogen and seed nitrogen were also determined. The dry chilli fruit was found to contain an average of 2.1720 per cent nitrogen whereas 2.6508% nitrogen was present in seed. An average of 2.50 per cent nitrogen was found in residue after oleoresin extraction. This nitrogen concentration of the meal was found same, when ethyl acetate was used as the solvent for the extraction of oleoresin. Even further extraction with ethyl acetate does not change the nitrogen content value for the meal.

The present acetone extraction and colorimetric procedure has precision to satisfactory degree, accuracy and simplicity and suitable for germplasm evaluation programme for the capsaicin quantitation.

ACKNOWLEDGEMENTS

We are thankful to Director, NBPGR and Head, Division of Germplasm Evaluation, NBPGR, New Delhi, for providing necessary facilities during the course of present investigation.

REFERENCES

- Bennett, D.J. and G.W. Kirby. 1968. Constitution biosynthesis of capsacin *J. Chem Soc. (C)* 442-446.
- Gonzalez, A.T. and C.W. Tamirano. 1973. A new method for the determination of capsaicin in capsicum fruit. *J. Food sci.* **38** : 342-344.
- Govindarajan, V.S. and S.M. Ananthakrishna. 1970. Observations on the separation of capsaicin from capsicum and its oleoresin. *J. Food Sci. Tech.* **7** : 212.
- Kosuge, S. and M. Furata. 1970. Studies of the pungent principles of *Capsicum*. XIV chemical constitution of the pungent principle. *Agric. Biol. Chem.*, **34** : 248-258.

- Masada, Y., K. Hashimoto., T. Incue, and M. Suzuchi. 1971. Analysis of pungent principles of *Capsicum annum* by combined gas chromatography and mass spectrometry. *J. Food Sci.*, **36** : 858-860.
- Naves, Y.R. 1974. Technologie et Chimie des Parfums Naturels, Paris : masson et Cie.
- North, H. 1949. Colorimetric determination of capsaicin in oleoresin of Capsicum. *Analytical Chemistry*. **21** : 934-936.
- Pearson, D. 1976. The Chemical Analysis of Foods, 7th edn, London : J. and K. Churchill.
- Szabo, P. 1970. Production of paprika oleoresin. *Amer Perfumer and Cosmetic*. **85** : 39-42.
- Tice, L.F. 1933. 'A simplified and more efficient method for the extraction of capsaicin'. *Amer. J. Pharm.*, **105** : 320-325.
- Todd, P.H., M.G. Bensinger and T. Biftu. 1977. Determination of pungency due to capsaicin by gas-liquid chromatography. *J. Food Sci.*, **42** : 660-665, 680.

INFLUENCE OF STORAGE RELATIVE HUMIDITY ON VIABILITY OF *AMARANTHUS HYPOCHONDRIACUS*, *BRASSICA OLERACEA* AND *GLYCINE MAX*

Sanjeev Saxena, Kalyani Srinivasan, R.K. Tyagi and B.B. Singh

Germplasm Conservation Division,
National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

The storage temperature and relative humidity are two most important factors influencing seed viability. The critical moisture content is crop specific. The seeds equilibrate with the ambient humidity to particular moisture content at a particular temperature. The relationship between seed moisture and viability at different relative humidities and 35°C, was studied over a period of 150 days in *Amaranthus hypochondriacus*, *Brassica oleracea* and *Glycine max*. The three crops under investigation showed a rapid loss in the germination percentage at higher relative humidities of 90 and 75%. More pronounced effect was observed for *G. max* followed by *B. oleracea* and *A. hypochondriacus*. The proximate composition of the seeds is an important factor in determining the equilibrium moisture content of the seed.

Key words: Relative humidity, viability, storability, *Amaranthus hypochondriacus*, *Brassica oleracea*, *Glycine max*

The accessions received in a genebank are of different crops and many of them are from endangered populations. The determination of proper storage conditions is of prime importance to preserve this heritage for posterity. This assumes greater significance in the context of germplasm storage sites where controlled environmental conditions are not available. The early experiments on storage conditions of seeds showed that the storage temperature and relative humidity (RH) are important factors, which influence the seed moisture content and thus have a bearing on the storage life of the seeds (Roberts 1973).

To understand the exact relationship between the two variables the seeds of *Amaranthus hypochondriacus*, *Brassica oleracea* and *Glycine max* were allowed to equilibrate at different humidities. These samples were then stored at respective humidities at 35°C (commonly prevailing temperature in sub-tropical region) and the changes in the viability were periodically monitored over 150 days of storage.

MATERIALS AND METHODS

Seeds of *Amaranthus hypochondriacus* variety "Annapurna", *Brassica oleracea* variety "GA", and *Glycine max* variety "Gaurav" were procured from National Seeds Corporation, New Delhi.

The seeds were equilibrated to different moisture contents using various saturated salt solutions to 11, 32, 50, 75 and 90% relative humidities (RH) at 35°C temperature (Vertucci and Roos 1993). The equilibrated seeds were stored at respective RH at 35°C temperature. The viability was tested at regular intervals over a period of 150 days.

Seed moisture was determined by hot air oven method (ISTA, 1985).

Viability was assessed through germination test using 150 seeds in three replications and evaluated on the seventh day as per ISTA rules (ISTA, 1985).

RESULTS AND DISCUSSION

The moisture content to which the seeds of *A. hypochondriacus*, *B. oleracea*, and *G. max* equilibrated to various humidities is given in Table 1.

Table 1. Moisture content (Fw basis) in seeds of *Amaranthus hypochondriacus*, *Brassica oleracea* and *Glycine max* when equilibrated at 35 °C at different relative humidities for a period of 150 days

Relative Humidity (%)	<i>A. hypochondriacus</i>	<i>B. oleracea</i>	<i>G. max</i>
11	4.9	3.0	3.5
32	7.5	3.9	5.0
50	8.5	4.5	6.0
75	13.5	9.5	12.2
90	16.8	13.5	16.5

The change in the viability of the seeds of *A. hypochondriacus*, *B. oleracea*, and *G. max* when stored at 11, 32, 50, 75 and 90% RH at 35 °C for 150 days is shown in Fig. 1. The seeds of *A. hypochondriacus* could maintain 100% germination at RH 11%, 32% and 50% for 150 days. The viability declined to 8% in 115 days of storage at 75% RH whereas at 90% RH the seed viability declined to 3% after 90 days of storage. The viability of *B. oleracea* seeds declined to 60% during the total storage period at 11% RH, whereas at 32 and 50% RH the seeds could retain 80 and 60% germination respectively for this period. The viability decline is more pronounced at 75 and 90% RH where no germination was observed after 75 and 60 days of storage respectively. In *G. max* the viability of the seeds declined to 58% and 68% during the total storage period (150 days) at 11% and 32% RH respectively. The deterioration was faster at 50% RH leading to a decline in viability to 42% during the same storage period. Storage at 75 and 90% RH resulted in a total loss of viability in the seeds after 75 and 24 days of storage respectively.

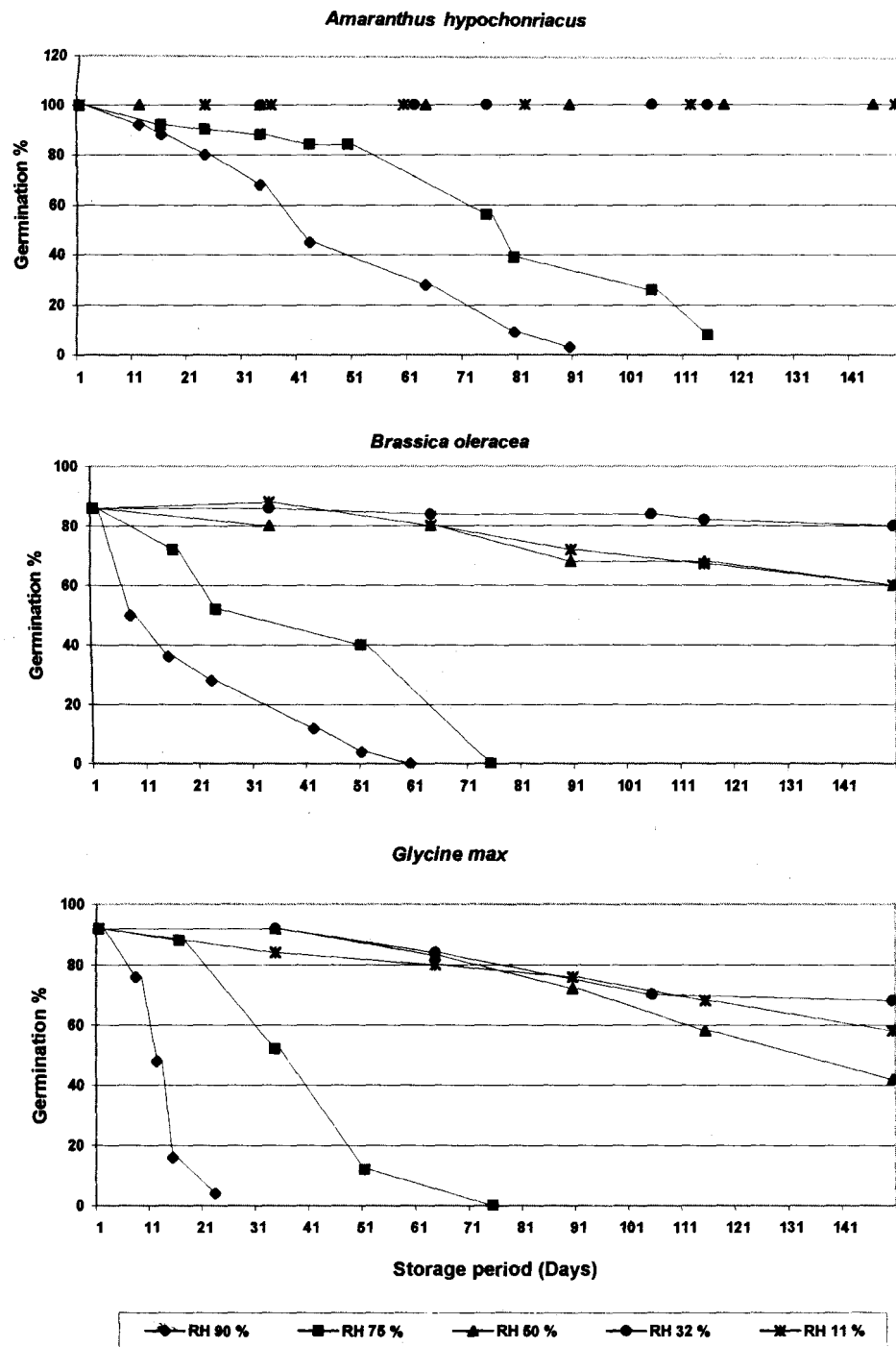


Fig. 1. Changes in the viability of seeds of *Amaranthus hypochondriacus*, *Brassica oleracea* and *Glycine max* when stored at 35°C at different relative humidities for a period of 150 days

It was found that the seed moisture content at a particular humidity varied for the three crops studied. The proximate composition of the seeds is an important factor in determining the equilibrium moisture content of the seed and similar differences have been reported for other crops (Vertucci and Roos 1993). The three crops showed a similar trend of seed deterioration wherein a rapid loss in the germination percentage was observed at higher RHs of 90 and 75 per cent. More pronounced effect was observed for *G. max* followed by *B. oleracea* and *A. hypochondriacus*. Relatively higher seed viability of 80 per cent and 68 per cent was observed in *B. oleracea* and *G. max* respectively at 32 per cent RH, as compared to 11 and 50 per cent RH. Non enzymatic auto oxidations leading to free radical production have been reported to result in loss of viability at lower moisture contents (Koostra and Harrington 1969). The differential response of the three crops may be attributed to the high oil content and the fatty acid composition of *G. max* and *B. oleracea* seeds, making them more prone to peroxidative changes as compared to the starchy seeds of *A. hypochondriacus*. It has been reported that the high linolenic acid content (50-60%) present in the oil of *G. max* renders it more vulnerable to enzymatic oxidations especially at higher moisture content. Consequently the production of highly reactive hydroperoxides reduce the storage life of seeds (Stewart and Bewely 1980, Buchvarov and Gantcheff 1984, Senaratana *et al.* 1988).

The results imply that maintaining the relative humidity in the storage environment of the seeds to a level of 30-35% can help in better short-term storage of seeds at places where the temperature is about 35°C. Moreover, in places where the ambient temperatures are lower it shall have additional benefits in improving the shelf life of the seeds.

REFERENCES

- Buchvarov, P. and T. Gantcheff. 1984. Influence of accelerated and natural ageing on free radical levels in soybean seeds. *Physiol. Plant.* 60: 5-56.
- ISTA. 1985. International rules for seed testing. Rules 1985. *Seed Sci. Technol.* 13: 57-64.
- Koostra, P.T. and J.F. Harrington. 1969. Biochemical effects of age on membranal lipids of *Cucumis sativus* L seeds. *Proceed. Int. Seed Test Ass.* 34: 329-340.
- Roberts, E.H. 1973. Predicting the storage life of seeds. *Seed Sci. Technol.* 1: 499-514.
- Senaratna, T., J. Gusse and D. Mckersie. 1988. Age induced changes in cellular membranes on imbibed soybean seed axes. *Physiol. Plant* 73: 85-91.
- Stewart, R.R.C. and J.D. Bewely. 1980. Lipid peroxidation associated with accelerated ageing in soybean axes. *Plant Physiol.* 65: 245-248.
- Vertucci, C.W. and E.E. Roos. 1993. Theoretical basis of protocols for seed storage II The influence of temperature on optimal moisture levels. *Seed Sci. Res.* 3: 201-213.

CONSERVATION OF BOTTLEGOURD GERMPLASM SEEDS

S.D. Doijode

Indian Institute of Horticultural Research,
Bangalore 560 089 (Karnataka)

Bottle gourd (*Lagenaria siceraria* Standl) germplasm is commonly preserved through the seeds. Seeds are short lived under ambient conditions. Seeds of cv. Arka Bahar with 7.5 per cent moisture was packed in kraft paper, polyethylene (300 gauge), glass and sealed laminated aluminium foil pouched and stored at ambient (16-35°C), low (5°C) and sub-zero (-20°C) temperatures for 5 years. Seed viability reduced to 50 per cent in paper and polyethylene bags after 1 year of ambient storage and none of the seeds germinated during second year of storage. However, the high seed viability was retained for 5 years at 5°C and -20°C. Polyethylene bags are effective in retaining high viability (> 90 %) at 5°C and laminated aluminium foil pouches at -20°C. There was increased leaching of electrolytes, soluble sugars and potassium for ambient stored seeds than low temperature stored seeds. Further, dehydrogenase activity lowered significantly in ambient stored seeds. Seed conservation in polyethylene bags at 5°C is effective for conserving germplasm for relatively shorter period (5 years). However, for long term storage, seeds to be conserved in laminated aluminium foil pouches at -20°C with high vigour.

Key words : Bottlegourd, *Lagenaria siceraria*, seed conservation

In bottle gourd, seeds are predominantly used for cultivation and it is valuable media for the genetic conservation owing to easy to use, inexpensive and genetically stable on storage. Seed loses viability rapidly under ambient conditions (Abdul-Baki and Anderson, 1972). Seed longevity is predominantly dependent on nature and age of seeds (Harrington and Satyati-Harjadi, 1966) and method of storage (Popovska *et al.*, 1981). Several factors contribute to the seed deterioration, among these high temperature and humidity are important which reduces the longevity period in cucurbits (Villareal *et al.*, 1972). Further, certain biochemical changes occur during deterioration like increase in leaching of electrolytes (Givelberg *et al.*, 1984) and sugars (Doijode, 1994) from seeds and decrease in enzymatic activity in seeds (Copeland, 1976). The present experiment was conducted with the view to increase seed storability by manipulating certain packaging and storage temperatures and to correlate biochemical changes with loss of viability.

MATERIALS AND METHODS

Seeds of bottle gourd cv Arka Bahar were extracted from fully matured fruits, dried in shade and the moisture content was estimated (7.5 per cent).

Seeds were packed in kraft paper, polyethylene (300 gauge), glass bottles and laminated aluminium foil pouches and stored at ambient temperatures (16-35°C), low temperature (5°C) and sub-zero temperature (-20°C) for 5 years. Seed viability was expressed in percentage of germination. One hundred seeds each in three replicates were germinated in rolled towel at 20-30°C an alternate temperatures for 16 and 8 hours respectively. Seedling vigour was measured by means of number of days taken for emergence and by seedling characters like shoot length, root length and dry weight. Eight days' old seedlings were dried in hot air oven at 60°C for 48 hours and dry weight was recorded. Seed vigour calculated by using germination speed, and expressed as sum of days taken for germination. Vigour indices I and II were expressed by multiplying percentage of germination with seedling length and dry weight respectively. For biochemical studies, seeds were surface sterilized with 0.1 per cent mercuric chloride, washed thrice with sterile water and surface dried with tissue paper. Seeds were soaked in 20 ml sterile water for 18 hours at 25°C for measuring electrical conductivity of seed leachates. Soluble sugars were analysed according to the method of Dubois *et al.* (1956). The dehydrogenase activity was measured by reduction of tetrazolium salt from stored seeds (Kittock and Law, 1968).

RESULTS AND DISCUSSION

Seed germination was significantly affected by storage temperatures (Table 1). About 40 to 63 per cent of seeds germinated when stored in

Table 1. Effect of storage temperatures and packaging on seed germination (%) during storage of bottle gourd seeds

Storage temp (°C)	Storage containers	Storage period (years)				
		1	2	3	4	5
Ambient	Kraft paper	47	0	0	0	0
	Polyethylene	40	0	0	0	0
	Glass	57	0	0	0	0
	Al. foil	63	0	0	0	0
5	Kraft paper	83	80	80	80	80
	Polyethylene	93	90	90	90	90
	Glass	95	93	93	93	90
	Al. foil	97	92	92	93	93
-20	Kraft paper	0	0	0	0	0
	Polyethylene	90	90	63	60	60
	Glass	92	93	90	94	92
	Al.foil	90	92	93	100	93
CD at 5%	7.2					

polyethylene and laminated aluminium foil pouches respectively from 1 year of storage under ambient conditions. Further, none of these seeds germinated during 2nd years of storage irrespective of storage containers under ambient seed storage. However, high seed viability was recorded in seeds stored at 5°C and -20°C (except in paper bags) after 5 years of storage. The germination was 93% in seeds stored in aluminium foil pouches followed by 90% in polyethylene bags stored seeds at 5°C. While it was decreased to 60% in seeds stored in polyethylene bags at -20°C after 5 years of storage. High seed germination was recorded in seeds stored in moisture proof containers like laminated aluminium foil pouches at -20°C even after 5 years of storage.

Seedling vigour viz., shoot and root growth and dry weight was greater for seeds stored in aluminium foil laminated pouches at sub-zero temperature (-20°C) during 5th years of storage (Table 2). Similarly, seed vigour was

Table 2. Seedling characteristics as affected by the temperatures and containers during storage of bottle gourd seeds

Storage temp (°C)	Storage containers	Coeff. of germination	Shoot length (cm)	Root length (cm)	Dry wt. (mg)
5	Kraft paper	20.4	5.6	13.8	33.2
	Polyethylene	19.4	5.9	15.3	34.9
	Glass	20.3	6.8	11.3	38.3
	Al. foil	21.0	7.1	12.3	35.1
-20	Kraft paper	0	-	-	-
	Polyethylene	17.8	4.6	9.9	22.6
	Glass	17.2	4.0	13.2	30.5
	Al. foil	18.7	7.5	14.3	31.9
Se m ±	0.593	0.922	1.089	3.398	
CD at 5%		1.827	2.843	NS	NS

greater for seeds stored in laminated aluminium foil pouches both at 5°C and -20°C (Table 3). There was quantitative difference for leaching of electrolytes from stored seeds at different temperatures (Table 4). The electrical conductivity of leachates from seeds under ambient temperature was 38% higher than the seeds stored at -20°C and 23% greater than 5°C stored seeds, and only 12% increase was observed between 5°C and -20°C stored seeds at the end of 5th year of storage. The soluble sugar content increased in leachates of ambient stored seeds up to 27%. There was sharp decline up to 96% in dehydrogenase activity in ambient stored seeds than low temperature stored seeds. Potassium content in leachates of ambient stored seeds was increased up to 42%. This

Table 3. Effect of temperatures and packaging on seed vigour during 5th years of storage

Storage temp (°C)	Storage containers	Vigour	Vigour index	Vigour Index II
5	Kraft paper	18.0	1681	2847
	Polyethylene	18.5	1986	3245
	Glass	19.6	1678	3590
	Al. foil	22.99	1904	3445
-20	Kraft paper	0	0	0
	Polyethylene	10.9	887	1352
	Glass	16.0	1576	2799
	Al.foil	18.5	2041	2986
SEm ±		1.24	142	325
CD at 5%		3.84	437	1002

Table 4. Biochemical changes in seeds and leachate as affected by temperatures during 5th years of storage

Storage Temp (°C)	EC (µv)	Soluble sugars (mg/g)	Dehydrogenase Activity (0D480)	Potassium (ppm)
Ambient	393 ± 4.5	1.22 ± 0.023	0.07 ± 0.009	59.0 ± 1.0
5	319 ± 8.5	1.02 ± 0.009	1.81 ± 0.128	45.5 ± 1.5
-20	285 ± 4.5	0.96 ± 0.019	1.97 ± 0.014	41.5 ± 0.5

suggests that decrease in seed viability be associated with increase in leaching of metabolites and decrease in dehydrogenase activity in seeds. Reduction in germination percentage was positively correlated with seed leachate for conductivity and soluble sugars (Shamasherry and Banerji, 1979). Ray and Gupta (1979) also noticed that conductivity of leachates and soluble sugars increased with increased in storage period. The greater leakage was attributed to disruption of cell membrane (Schoettle and Leopold, 1984) and it was believed to be a major factor associated with loss of seed viability (Madhusudanan and Johnson, 1990).

Bottle gourd seeds are poor storer and remain viable for one year under ambient storage conditions. The process of seed deterioration was slow down by lowering storage temperatures. Harrington (1972) opined that rate of seed deterioration was faster at higher temperatures and higher relative humidity and germination decrease slowly at cooler temperatures. The beneficial effect

of low temperature on seed storability was reported in other vegetable crops (Doijode, 1997). Lowering of seed vigour precedes the loss of viability and associated with increased leaching of metabolites from the seeds. The leaching of metabolites was greater in ambient stored seeds than low temperature stored seeds, which was inversely related to seed germinability. The greater amount of exudation of sugars or potassium from seeds on imbibition helps in distinguishing non- viable and viable seeds (Mckay, 1972). The measurement of dehydrogenase activity in seeds or potassium content in leachates can be used for quick prediction of seed viability in different seed lots. Seed stored under ambient conditions begins to deteriorate faster and show low germination and low vigour (Fonseca *et al.*, 1980). The choice of packaging material depends on temperature and period of storage. Polyethylene bags are ideal packaging for low temperature seed storages, and aluminium foil pouches are suitable for seed storage at sub-zero temperatures. Seed storage in polyethylene bags at 5° C or in laminated aluminium foil pouches at -20° C successfully conserves the germplasm of bottle gourd for 5 years without loss of vigour and viability.

REFERENCES

- Abdul-Baki, A.A. and J.D. Anderson. 1992. Physiological and biochemical deterioration of seeds. In seed Biology. vol. II p. 283-309. Academic Press, New York.
- Copeland, L.O. 1976. Principles of seed science and technology Burgers Publ. Co., Minnesota.
- Doijode, S.D. 1994. Biochemical changes and conservation of watermelon (*Citrullus lanatus* Thunb) germplasm. *Indian J. Plant Genet. Resources* 7(1) : 79-84.
- Doijode, S.D. 1997. Influence of temperatures and containers on the storage of chilli seeds. *Indian J. Plant Genet. Resources* 10(1) L 163-166.
- Dubois, M., K. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1958. Colorimetric method for deterioration of sugars and related substances. *J. Analyt. Chem.* 28 : 350-56.
- Fonseca, J.R., A.De.B. Freire, M.s. Freire and F.J.P. Zimmerma. 1980. Conservation of bean seeds under three method of storage. *Revista brasileira de Sementes* 2 : 19-27.
- Givelberg, A., M. Horowitz and A. Poljakoff-Mayber. 1984. Solute leakage from *Solanum nigrum* seeds exposed to high temperatures during imbibition. *J. Exp. Bot.* 35 : 1754-63.
- Harrington, J.F. 1972. Seed storage and longevity. Seed Biology vol. III pp 145-245, Academic Press, New York.
- Harrington, J.F. and S. Satyati-Harjadi. 1966. The longevity of vegetable seeds under high temperatures and high humidity. Proc. 17th Inter. Hort. Congr. Md. 1
- Kittock, D.L. and A.G. Law. 1968. Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agron. J.* 60 : 286-88.
- Madhusudhanan. K. N. and K.M. Johnson. 1990. Solute leakage from winged bean (*Psophocarpus tetragonolobus*) seeds. *Seed Sci. Technol.* 18 : 157-162.
- Mckay, D.B. 1972. Viability of seeds, Chapman and Hall Ltd., London, 1972.
- Popovska, H. P., M. Mihajlorski and M. Skopje. 1981. The influence of packing over germination of pepper and tomato seeds. *Acta. Hort.* 111 : 281-90.

- Ray, M.B. and K. Gupta. 1979. Effect of storage of rice seeds on solute leaching and nucleic acids synthesis. *Indian J. Agric. Sci.* **49** : 715-19.
- Schoettle, A.W. and A.C. Leopold. 1984. Solute leakage from artificially aged soybean seeds after imbibition. *Crop Sci.* **24**(5) : 835-38.
- Shamsherry, R. and D. Banerji. 1979. Some biochemical changes accompanying loss of seed viability. *Plant Biochem. J.* **6**(1) : 54-62.
- Villareal, R.L., J.B. Balagedan and A.D. Castro. 1972. The effect of packing materials and storage conditions on the vigour and viability of squash, potola and upto seeds. *Philippine Agriculture* **56** : 59-76.

MIDSTORAGE TREATMENTS FOR IMPROVING VIABILITY OF MUSTARD SEEDS

Kalyani Srinivasan, Sanjeev Saxena and B. B. Singh

Germplasm Conservation Division,
National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

The effects of Osmo and hydropriming on partially aged seeds of mustard variety *Varuna* were studied. Seeds were osmoconditioned in PEG solution of strength-0.75 MPa and hydroprimed by (a) immersing in water for 3 hours; (b) allowing slow absorption of water from moist muslin for 36 hours (c) equilibrating the seeds in water saturated atmosphere for 48 hrs at 25°C. The treated seeds were then equilibrated at 20°C and 45% RH for 48 hours to 6% moisture and packed in aluminium foil packets. Primed seeds were assessed immediately as well as after 10 months of storage at ambient conditions for their germination, mean germination time, leachate parameters, protein content and activities of amylase, acid phosphatase, peroxidase and catalase. Osmoconditioning and slow hydration treatments resulted in significant improvement in germination and vigour parameters in contrast to direct soaking of seeds in water. These observations are supported by less leakiness of electrolytes and UV absorbing material and increased activity of free radical scavenging and reserve mobilizing enzymes.

Key words : Priming, viability, storability, mustard

Genebanks often encounter seeds with low viability at times due to improper storage conditions prior to their arrival in the genebank. It is important for genebanks to work out treatments so that the viability of these seeds is improved for storage and for the seeds regenerated or multiplied. Mid-storage treatments by priming the seeds has been used in some crops for seed lots that have lost vigour due to improper storage conditions (Lush *et al.* 1981, Pan and Basu 1985). In these treatments the seeds are hydrated to activate pre-germination metabolism and then dried again. The treated seeds thus reach the same state of readiness and there is shortening of time taken for radicle protrusion by the slow or fast seeds in a population, leading to synchronised germination. However, there are few contradictory reports wherein a reduction in storability has been observed (Argerich *et al.* 1989; Nath *et al.* 1991).

The present experiment was conducted to study the ameliorating effect of various priming treatments on seeds of mustard variety *Varuna*.

MATERIAL AND METHODS

One year old seeds of mustard variety Varuna were procured from the National Seeds Corporation, Pusa Campus, New Delhi. The seeds were subjected to the following priming treatments :

- a) OP : Seeds were osmoprimed by spreading in a Petri dish lined with Whatman No. 1 filter paper soaked in PEG 6000 solution of - 0.75 MPa osmotic potential and incubated at 20°C for 72 hours (Michel and Kaufmann 1973). After conditioning, the seeds were thoroughly rinsed in distilled water to remove any trace of osmoticum and wiped free of water.
- b) HP : Seeds were hydroprimed by soaking in water at 25°C for 3 hours.
- c) MM : Seeds were placed between two moist muslins and wrapped within a thin polythene at 20°C for 36 hours.
- d) WS : Seeds were kept in a water saturated desiccator at 25°C for 48 hours.

The seeds from all the four treatments were then equilibrated at 20°C and 45% RH for 48 hours to 6% moisture content. One set of treated seeds from each treatment was tested immediately (PIE). Another set was sealed in tri-layered moisture proof aluminium foil pouches and stored at ambient conditions for 10 months and then tested to study the effect of priming on natural ageing (PNA). A set of untreated seeds served as control (C). Germination test was performed using 50 seeds incubated at 20°C. Germination count was taken every day considering the protrusion of the radicle as indication and is reported as the germination percentage (ISTA, 1985). Mean germination time (MGT) was calculated in days using the formula of Ellis and Roberts 1981. Root and shoot vigour were evaluated on the 7th day of planting the seeds. Twenty five seeds were weighed and soaked in 25 ml of deionised water at 20°C for 24 hours and electrical conductance (dS m^{-1}) was determined with a digital conductivity meter (Control Dynamics model APX 185E). The leachate was also used to determine the UV absorbance at 264 nm with spectrophotometer (DU 7400 Beckman). The hydrated seeds were used for estimation of soluble protein and enzyme analysis. Soluble proteins in the seed were extracted in 0.05M phosphate buffer pH 7 and estimated by Lowry's method (Lowry *et al.* 1951). Seed extract prepared in Tris - HCl buffer (0.05M, pH 7.6) was used for the assay of amylase (Bernfeld 1955) and peroxidase (Shannon *et al.* 1966). Extract in 0.05M phosphate bufer pH 7 was used for the assay of acid phosphatase (Leigh and Walker 1980) and catalase (Maehly and Chance 1967).

Data were analysed as a factorial design with a minimum of three replicates in all cases. All data were subjected to an analysis of variance and tested for significance.

RESULTS AND DISCUSSION

Effect of priming on germination, vigour and leachate parameters are presented in Table 1. The germination percentage was increased by MM and OP when seeds were tested immediately after priming and by all the treatments after natural ageing. There was a decrease in the mean germination time immediately after treatment. Maximum decrease was by OP and MM followed by WS and HP. There was no significant decrease by treatment HP when the seeds were tested after natural ageing. The root vigour was increased immediately after priming by OP, WS and HP. When seeds were tested after natural ageing all the treatments increased the root vigour, maximum by OP followed by WS, HP and MM. The shoot vigour was also increased by all the priming treatments in both the cases. The electrical conductance of the leachate was significantly decreased by treatments OP and MM immediately after treatment. After natural ageing treatment WS also significantly decreased the conductance. The UV absorbance of the leachate followed the same trend as the conductance. The efflux of potassium ions into the leachate was significantly decreased by all the treatments after ageing. The natural ageing of seeds resulted in the decrease of soluble protein content. Primed seeds had higher protein content immediately after priming and also after natural ageing. Amylase activity decreased during natural ageing. Treatments OP and MM increased the amylase activity immediately and also treatment WS after natural ageing (Fig. 1a). Similar trend was observed for phosphatase activity with exception of HP which also increased the activity after natural ageing (Fig. 1b).

Peroxidase activity also decreased due to natural ageing, treatment OP increased the activity immediately after priming (Fig. 1c). Seeds subjected to natural ageing retained higher peroxidase activity in all the treatments. Primed seeds showed higher activity of catalase before and after ageing. Catalase activity decreased due to natural ageing (Fig. 1d).

The seed quality had evidently deteriorated in control and treated seeds during the 10-month storage period as indicated by the decline in the germination percentage, and an increase in the MGT of aged seeds. The seeds primed by OP, MM and to some extent WS showed an improved quality immediately after treatment thus indicating the improvement in the storage life of the primed seeds. These priming treatments also increased the root and shoot vigour. Only the HP treatment was detrimental to seed quality probably due to the imbibitional injury occurring as a result of rapid uptake of water by the seeds (Ellis *et. al.* 1985) as the seeds in this treatment were directly soaked in water for hydration.

The electrical conductance of the leachate was significantly lower in the treated seeds after natural ageing. The maximum decrease was observed due

Table 1. Effect of priming on germination, vigour and leachate parameters immediately after priming (PIE) and after natural ageing (PNA) in mustard variety Varuna

Treatments	Germination		Vigour		Leachate		Soluble protein (mg 250 mg fw ⁻¹)
	Percentage	Mean Time (days)	Root (cm)	Shoot (cm)	UV absorbance (at 264 nm)	Conductance (dS m ⁻¹)	
PIE							
C	79	2.40	3.24	3.14	0.062	52.3	4.26
OP	91	1.11	6.24	4.42	0.053	42.0	5.15
HP	80	1.50	3.93	4.28	0.066	55.2	4.28
MM	92	1.17	3.52	6.91	0.056	45.4	5.31
WS	84	1.54	4.33	5.69	0.060	48.6	4.56
PNA							
C	60	2.52	2.41	1.92	0.126	114.6	2.74
OP	85	1.23	4.69	2.98	0.076	63.6	3.75
HP	72	2.53	2.96	3.71	0.127	118.0	3.13
MM	86	1.32	2.84	6.05	0.074	66.0	3.74
WS	71	2.19	3.93	4.71	0.083	87.4	3.14
LSD>0.05%	6.3	0.29	0.42	0.84	0.004	4.30	0.28

(C-untreated control; OP-Osmo priming at -0.75 Mpa; HP- Hydropriming by soaking in water 3 hours; MM-Equilibration in moist muslin for 36 hours; WS-Equilibration in water saturated desicator for 48 hours)

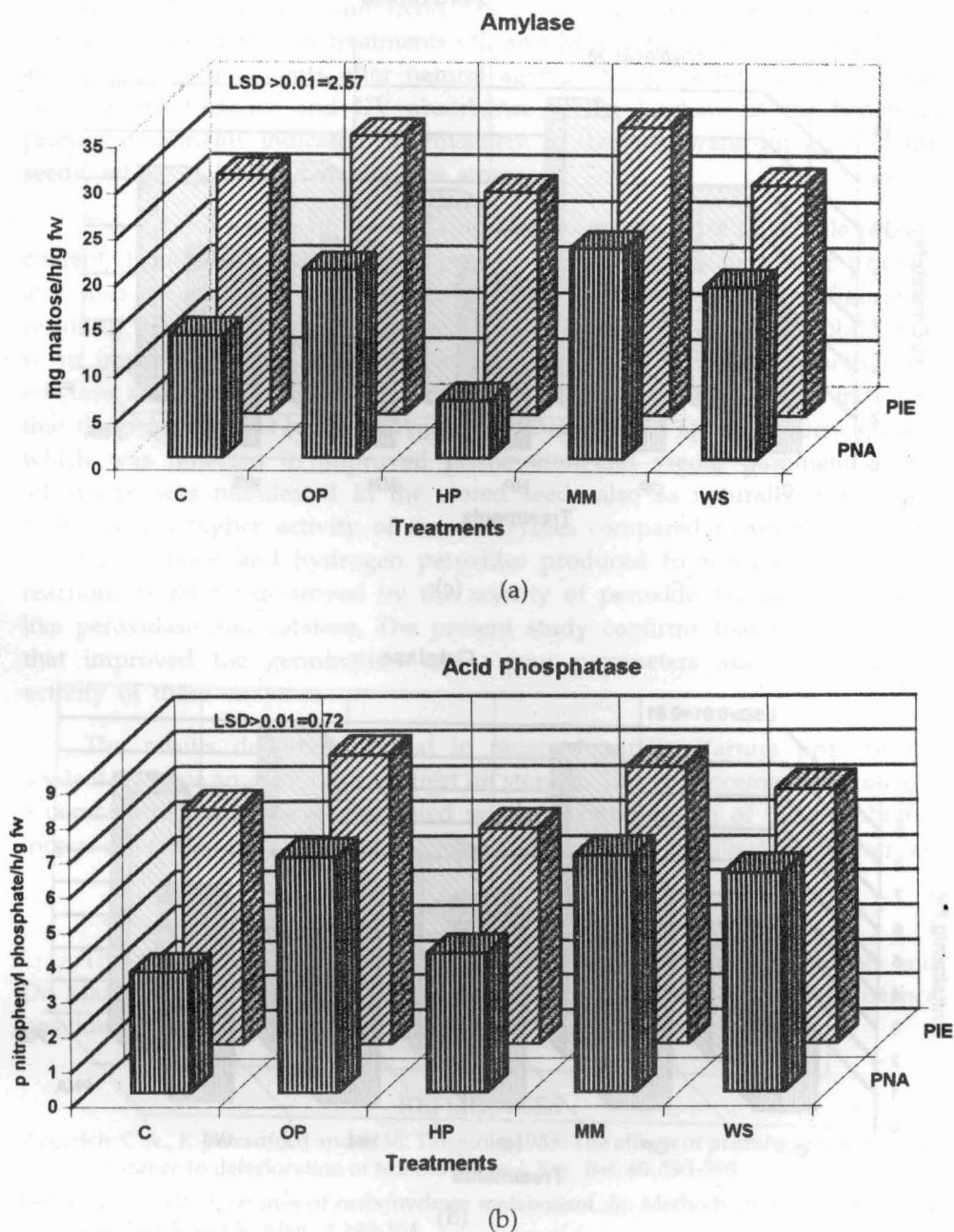


Fig. 1a&b. The effect of priming treatments on the activity of amylase, phosphatase, peroxidase and catalase in mustard variety Varuna. (C - untreated control; OP - Osmo priming at -0.75Mpa; HP - Hydropriming by soaking in water for 3 hours; MM - Equilibration in moist muslin for 36 hours; WS - Equilibration in water saturated desiccator for 48 hours). The seeds were tested immediately after priming (PIE) and after natural ageing (PNA)

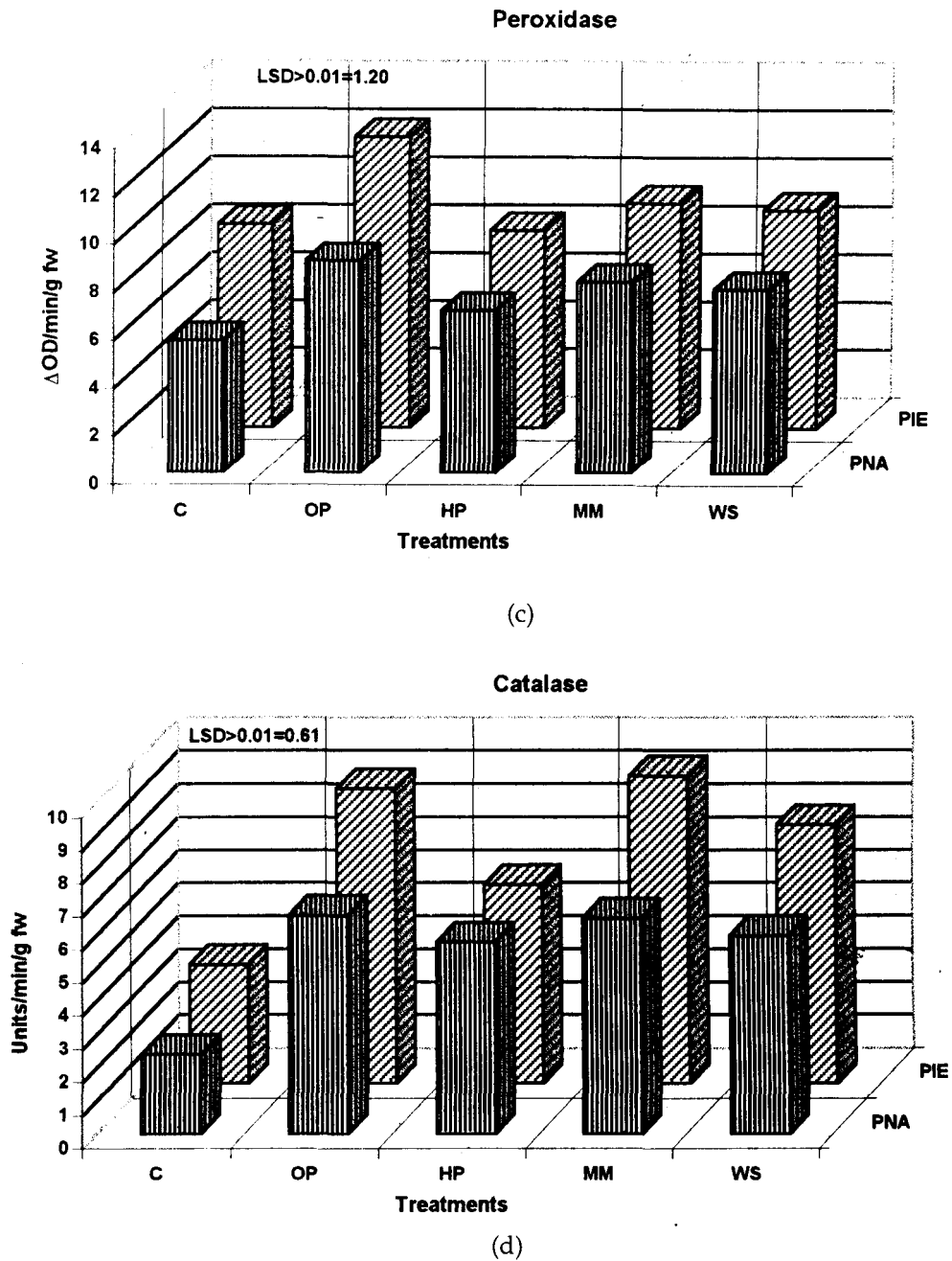


Fig. 1a&b. The effect of priming treatments on the activity of amylase, phosphatase, peroxidase and catalase in mustard variety Varuna. (C - untreated control; OP - Osmo priming at -0.75Mpa; HP - Hydropriming by soaking in water for 3 hours; MM - Equilibration in moist muslin for 36 hours; WS - Equilibration in water saturated desiccator for 48 hours). The seeds were tested immediately after priming (PNE) and after natural ageing (PNA)

to treatments OP and MM followed by WS and HP. The UV absorbency of the leachate followed the same trend. The potassium ion content in the leachate significantly decreased in treatments OP and MM immediately after treatment and in all the treatments after natural ageing. A significant decrease in the electrical conductance and UV absorbance of the leachate in the beneficial priming treatments indicated improvement in the membrane integrity of the seeds, which is retained during the storage.

The natural ageing of the seeds resulted in the decrease of soluble protein content. Primed seeds had higher protein content immediately after priming and also after natural ageing, probably due to membrane reconfiguration resulting in re-synthesis of membrane bound enzymes. Reserve mobilisation is an important prerequisite for seed germination. The increased activity of amylase and acid phosphatase enzymes in the beneficial treatments indicated that the primed seeds had an advantage of being ready for subsequent growth which was reflected in improved germination and vigour parameters. This advantage was manifested in the stored seeds also as naturally aged seeds maintained a higher activity of these enzymes compared to untreated seeds. The free radicals and hydrogen peroxides produced from various metabolic reactions could be destroyed by the activity of peroxide scavenging enzymes like peroxidase and catalase. The present study confirms that the treatments that improved the germination and vigour parameters also increased the activity of these enzymes.

The results demonstrate that in mustard variety Varuna priming the seeds does have an ameliorating effect on storage. This enhancement is probably a combination of priming stimulated repair/reconfiguration of the membrane, increased activity of scavenging enzymes and improved mobilisation of reserves.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, for providing the facilities and Dr Manju Uprety, Technical Officer, NBPGR, New Delhi for the assistance provided in conducting the experiments.

REFERENCES

- Argerich, C.A., K.J. Bradford and A.M. Tarquois. 1989. The effects of priming and ageing on resistance to deterioration of tomato seeds. *J. Exp. Bot.* **40**: 593-598
- Bernfeld, P. 1955. Enzymes of carbohydrate metabolism. *In: Methods in enzymology*, Ed. Collowick and Kaplan, **1** 149-154
- Ellis, R.H., and E.H. Roberts. 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. and Technol.* **9** : 373- 409
- Ellis, R.H., T.D. Hong and E.H. Roberts. 1985. Handbook of seed technology for genebanks. Vol. 1, Principle and methodology, 210 p. International Board for Plant Genetic Resources, Rome

- ISTA. 1985. International rules for seed testing. Rules 1985. *Seed Sci. and Technol.* **13**, 57-64.
- Leigh, R.A. and R.R. Walker. 1980. ATPase and acid phosphatase activities associated with vacuoles isolated from storage roots. *Planta* **150** : 222-229
- Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin - Phenol reagent. *J. Biol. Chem.* **193** : 265-275
- Lush, W.M., R.H. Goves and P.E. Kaye 1981. Presowing hydration- dehydration treatments and early seedling growth of wheat and ryegrass. *Aust. J. Plant Physiol.* **8**: 409-425.
- Maehly, A.C. and B. Chance. 1967. Methods of biochemical analysis Vol 1 (Ed. D. Glick) Interscience Publ. Inc. NY., p 357-424
- Michel, B.E. and M. K. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiol.* **51**: 914-916
- Nath, S., P. Coolbear and J.C. Hampton. 1991. Hydration dehydration treatments to protect or repair stored "Karamu" wheat seeds. *Crop Sci.* **31**: 822-826
- Pan, D and R.N.Basu. 1985. Mid storage and presowing seed treatment for lettuce and carrot. *Scientia Horticulturae* **25**: 11-19
- Shannon, L.M., E. Kay and J.Y. Low. 1966. Peroxidase isoenzymes from horse radish roots. I. Isolation and physiological properties. *J. Biol. Chem.* **241**: 2166-2172

Short Communication

EVALUATION OF CHILLIES GERMPLASM

S.K. Verma, K.C. Pant and K.C. Muneem

National Bureau of Plant Genetic Resources
Regional Station, Bhowali, Nainital 263 132 (Uttar Pradesh)

Key words : Chillies, germplasm, evaluation

Capsicum, native to the New World Tropics, now widely cultivated for use as spices or vegetables in the temperate zone. Chillies powder, red and cyenne peppers, tabasco, paprica, sweet or bell peppers and pimentos are all derived from the pod like berries of various species of *Capsicum*. The pungency of pepper is due to *capsaicin* or *capsicutin* ($C_{18} H_{27} NO_3$) a condensed product of 3-hydroxy-4 methoxy benzylamine and the vanilly amide of isodecylenic acid, contained in the placenta. It is grown in all parts of India including the districts of Uttar pradesh viz, Pithoragarh, Almora, Nainital and adjoining parts of *tarai* region of Kumaon and also Pauri, Uttarkashi and Dehradun districts in Garhwal hills. Chillies broadcasting cultivation still prevails in some hilly tracts. Gola-Khatima area is having *Jheen Mirch* (a wild form) and some tribal belts have *Longia Mirch* (*Capsicum frutescens*). The collections made from the local habitat (farmers' fields) of Kumaon Hills, from different state and private agencies during different explorations, and exotic collections from USA were included in evaluation programme and promising types identified for various traits for use in crop improvement programmes.

One hundred nineteen accessions were sown in nursery for raising seedlings and planted in an augmented randomised complete block design along with four checks P-1094, P-1130, P-1311 and P-1639 at NBPGR Regional Station, Bhowali, Nainital (UP). The inter row spacing was maintained at 50 cm and within row spacing at 30 cm respectively. All the standard practices recommended for the crop were followed (Muthukrishnan *et al.* 1986). The accessions were evaluated for qualitative (31) and quantitative (12) characters. The evaluation was conducted for three consecutive years, 1993, 1994 and 1995 at Bhowali (29 N latitude and 70 E longitude) at an altitude of 1600 m above sea level. The climate of this site is sub-temperate with minimum and maximum temperature ranging between $-2^{\circ}C$ to $32^{\circ}C$ and with an average annual rainfall around 1600 mm.

Table 1. Promising accessions identified in chillies germplasm

S.No.	Characters	Promising accessions
1.	Days to 50% germination > 80 days	EC-362911, EC-362931
2.	Leaf length > 12 cm	EC-362918, EC-362919, EC-362922, P-1939, P-2072, N-1149, N-1382
3.	Leaf width > 5 cm	EC-362919, EC-362925, EC-362930, EC-362938, P-1639, P-1713, P-1718
4.	Plant height > 60 cm	P-2072, N-1160, N-1382
5.	Days to 50% flowering < 150 days	P-32, P-64, P-173, P-290, P-241, P-272, P-249, EC-362905
6.	Days to 50% fruiting < 165 days	P-32, P-64, P-173, P-297, P-641, EC-362905
7.	Number of fruits/plant > 60	P-1399, EC-362903, EC-362916, EC-362930
8.	Fruit length > 9 cm	P-1311, P-2072
9.	Fruit width > 2 cm	P-840, KN-613, N-1149
10.	Fruit (10) green weight > 30 g	P-64, P-190, P-1258, P-2072, N-349, KN-613
11.	Fruit (10) dry weight > 30 g	P-64, P-100, P-1258, P-1639, N-349, N-368, N-867, N-1149
12.	Disease free accessions from leaf spot, powdery mildew and fruit rot	IC-99910, IC-99912, IC-92150, P-1718, P-1939, N-1015, EC-362901, EC-362910, EC-362913, EC-362925

The germplasm exhibited wide range of variability in plant height (50.0-86.0 cm), growth habit (prostrate, compact, erect), leaf and stem pubescence (glabrous, sparse, intermediate, abundant), seedling, leaf and stem colour (green, purple), density of branches, plant canopy (104.0-6348.0 sq cm), leaf length (4.2- 15.9 cm), leaf width (1.2-6.8 cm), flower colour, days to 50% flowering (142-172 days), pedicel position, number of pedicel per axil, days to 50% fruiting (161 - 220 days), fruit set, calyx margin and shape, annular construction at junction of calyx and peduncle, fruit position, fruit colour at immature stage (green, yellow, orange, red, purple, brown/black), fruit shape (elongate, oblate, round, conical, companulate, bell/blocky), fruit shape at peduncle attachment (truncate, cordate, lobate, acute, obtuse), fruit shape at blossom end (pointed, blunt, sunken), presence and absence of neck at base of fruit, fruit cross section (smooth, intermediate, corrugated), fruit pungency, number of fruits per plant (2.1-120.0), fruit length (1.6-12.4 cm), fruit width (0.2- 2.6 cm), fruit green weight/10 fruits (7.0-55.0 g), fruit dry weight/10 fruit (1.0-7.5 g), seed colour and pest and diseases of varying intensity.

Number of fruits per plant is the main character for increasing the fruit yield with other important associated characters e.g. fruit length, fruit width and fruit weight. Accessions P-64, P-190, P-1258, P-2072, N-349 and KN-613 showed higher fruit weight whereas accessions P-1399, EC-362903, EC-362916

and EC-362930 showed more number of fruits/plant which revealed that individual fruit weight and all the factors associated with fruit number are not having strong compensatory interactions (Lacy, 1973; Webb *et. al.* 1974; Mason *et al.* 1980). It is suggested that the promising genotypes (Table 1) could be used in hybridization programmes for selecting desirable segregants/cross combinations for the economic traits under study. Requests for small quantity of seed for the research purposes can be made to the Director, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110012 or to the Officer-In-Charge, NBPGR, Regional Station, Bhowali (Nighat) Distt Nainital U.P. 263132

ACKNOWLEDGEMENTS

Authors are thankful to Sh. R.R. Arya, Technical Assistant, NBPGR, Regional Station, Bhowali for technical help during the above study.

REFERENCES

- Lacy, C.N.D. 1973. Phenotypic correlation between vegetative characters and yield components in strawberry. *Euphytica* 22: 546-554.
- Mason, D.T. and N. Rath. 1980. The relative importance of some yield components in East of Scotland strawberry plantation. *Am. Appl. Biol.* 95: 399-408.
- Muthukrishnan, C.R.; T. Thangraj and r. Chatterjee 1986. Chillies and Capsicum in Vegetable Crops in India, Ed. T.K. Bose and M.G. Som. Naya Prokash, Calcutta 343-384.
- Webb, R.A., J.V. Purves and B.A. White. 1974. The component of fruit size in strawberry. *Scientia Hort.* 1: 321-330.

Short Communication

COLLECTING SAFFLOWER GERMPLASM FROM WEST BENGAL

N. Dikshit, P.S. Mehtre¹ and W.S. Yadav¹

National Bureau of Plant Genetic Resources,
Base Centre, Cuttack 753 006 (Orissa)

Key words: Safflower, *Carthamus tinctorius*, germplasm, collection

Safflower (*Carthamus tinctorius* L.) is one of the important oil yielding crops in India. It is mainly cultivated in Maharashtra, Karnataka and Andhra Pradesh. The other non- traditional areas of safflower cultivation are West Bengal, Orissa and base of the Himalayas where local variability still exists. Safflower initially grown for extraction of dyes and preparation of herbal medicine and a substitute/adulterant for saffron is now mainly grown for oil and other table preparations. In West Bengal, the crop has been cultivated for a long time. Now the farmers in West Bengal grow it as a border crop around potato and vegetable crops. It was learnt from cultivators that the crop was earlier grown as a sole crop for extraction of dye and for home made preparations like *murrhi* and *chalbhaza*. It is apprehended that the crop may be totally wiped out in future from these areas because of non-availability of market and non-availability of oil mills (local ghani) although there is possibility of using paddy fallow land for this crop. It was therefore thought necessary to collect and conserve the important genetic wealth from these areas of West Bengal which is in the verge of extinction.

The collection mission was undertaken in Midnapore, Bankura, Purulia, Birbhum, and Murshidabad districts in West Bengal during April 1997. The area explored falls between 21°.68' - 24°.85'N longitude 85°.80' - 38°50'E latitude. During the exploration, most of the areas of occurrence were covered. Passport data were noted at the collection site. Observations were taken as per the descriptors for safflower (IBPGR, 1983). Morphological and quantitative observations on the growth habit, spiny/non spiny, flower colour, location of branches on main axis, no. of primary branches, flower colour at blooming and fading and seed colour were recorded at the collection site whereas the

¹ All India Coordinated Research Project on Oilseeds (Safflower), Solapur 413 002 (Maharashtra)

metrical characters *viz.*, seed length, seed width, 100 seed weight were recorded in the laboratory. The oil content (%) was analysed at the Directorate of Oilseed Research, Hyderabad.

Considerable variability was observed in morphological and quantitative traits. The district-wise safflower collections includes Midnapore (7), Bankura (10), Purulia (1), Birbhum (2) and Murshidabad (4). These accessions were

Table 1. Range of variability for some important quantitative characters in Safflower germplasm

IC no.	Seed length (mm)	Seed width (mm)	100 seed weight (g)	Oil content (%)
IC-208230	6.34	3.94	3.15	20.2
IC-208231	6.44	4.15	2.54	19.5
IC-208232	6.58	3.78	2.29	16.5
IC-208233	6.54	4.14	2.90	24.0
IC-208234	6.10	4.25	2.60	N.R
IC-208235	6.24	4.24	2.95	16.5
IC-208236	6.00	3.80	2.59	N.R
IC-208237	6.13	4.03	2.29	N.R
IC-208238	6.28	3.94	2.71	22.6
IC-208239	6.78	4.75	2.55	21.5
IC-208240	6.00	3.80	2.18	N.R
IC-208241	6.50	3.95	1.60	N.R
IC-208242	6.10	3.88	1.72	N.R
IC-208243	5.65	3.75	2.46	22.0
IC-208244	6.55	4.08	3.82	24.5
IC-208245	6.30	4.25	2.62	25.5
IC-208246	6.64	4.64	4.34	20.5
IC-208249	6.85	3.95	3.58	21.5
IC-208250	5.44	3.90	2.75	25.5
IC-208251	5.94	4.00	2.93	25.5
IC-208252	5.90	3.80	2.29	20.3
IC-208253	5.90	3.64	2.98	25.3
IC-208254	6.04	3.94	2.73	25.5
IC-208255	5.40	3.54	2.63	24.5
Range	5.4-6.85	3.54-4.75	1.6-4.34	16.5-25.5
Mean	6.19	4.0	2.63	22.3
SE $\pm$ M	0.07	0.05	0.11	0.68
CV (%)	6.09	6.87	22.27	12.97

N.R. - Not recorded due to small quantity of seed

collected from a total of 22 diverse agroecological niches. The altitude of the areas varies from 60-345 metre above mean sea level (msl). The soil is mostly lateritic in hill zones and 10 am and sandy loan in Indo-gangetic plain regions. The growth habit varied from erect to bushy type. The leaves were mostly lanceolate, narrow with upper leave ovate. Most of the accessions had flower colour yellow during bloom stage and red, dark red and orange red at fading. Most of the accessions had medium size capitula having diameter less than 3.0 cm. Very few accessions were non-spiny or spine present at the tip of the leaf with the determinate type of growth. The seed colour was mostly white or cream colour with medium seed index and thick hull. Significant variation was observed among the quantitative characters. The plants were mostly tall or bushy type. The number of primary branches varied from 10-15 per plant, maturity 150-160 days, seed length 5.4-6.85 mm, seed width 3.54-4.75 mm, 100 seed weight 1.6-4.34 g and percentage of oil content 16.5-25.5%. The variation in days of maturity, no. of primary branches per plant and 100 seed weight also falls within the range of variability as reported by Mehtre and Hegde (1997). High seed weight was observed in IC-208246 (4.34 g) followed by IC-208244 (3.82 g) IC- 208249 (3.58 g) whereas high oil content of 25.5% was recorded in IC-208245, IC-208250 and IC-208251 followed by 24.5% oil content in IC-208244 and IC-208255. Coefficient of variation was observed high for 100 seed weight (22.27%) followed by oil content (12.97 %). This shows the diversity among the safflower accessions for 100 seed weight (Table 1).

In general, there was less incidence of serious pests like aphids and *Heliothes* and the growth of roots was not deep among the accessions collected from the targeted areas which may be due to availability of moisture at the surface level.

ACKNOWLEDGEMENTS

The authors are extremely thankful to Directorate of Oilseed Research, Hyderabad for analysis of oil content. The authors are also grateful to farmers of West Bengal for sparing local safflower germplasm.

REFERENCES

- IBPGR. 1983. Descriptors for Safflower, IBPGR, 22 p
- Mehtre, S.P. and D.M. Hegde. 1997. Genetic Resources of Safflower in India. Paper presented in the IVth International Safflower Conference held at Bari, Italy. June 2-7, 1997, p. 205-206

GENETIC DIVERGENCE IN HORSEGRAM (*MACROTYLOMA UNIFLORUM* (Lam) Verdc.)

Rajiv K. Sharma¹

Vivekananda Parvatiya Krishi Anusandhan Shala,
Almora 263 601 (Uttar Pradesh)

Key words : Horsegram, divergence, heterosis, genotypes

Horsegram, a traditionally very important pulse of South India, has been an important pulse of U.P. hills since time immemorial. To strengthen the ongoing breeding programme, several horsegram accessions were procured from different sources.

Fourty horsegram genotypes procured from National Bureau of Plant Genetic Resources (NBPGR), Regional Stations Bhowali, Akola and Shimla, University of Agricultural Sciences (UAS), Bangalore and four local collections were sown in a randomized complete block design with three replications. Data were recorded on days to initiation of twines (TI), 50% flowering (FL), maturity (MT), plant dry weight (g) six weeks after sowing (DW), plant height (PH), pod length (PL) in cm, number of pods per plant (PP), number of grains per pod (GP), 100-grain weight (GW) and grain yield per plant (YP) in grams. Each plot consisted of a row of 1.75 m length and the row to row spacing was maintained at 0.45 m. Data were subjected to analysis as per Mahalanobis (1936) and Rao (1952).

Studying variation among newly procured germplasm lines to generate information on the nature and magnitude of genetic divergence is quite logical. This helps breeders to choose diverse parents for purposeful hybridization (Arunachalam, 1981; Samsuddin, 1985). The significant treatment mean squares indicated potent variability among the genotypes studied. Based on D^2 values for all possible 780 cultivar pairs, the 40 cultivars were grouped into six clusters. This indicates the presence of a large amount of diversity among the

¹Present address: Division of Genetics, IARI, New Delhi

Table 1. Distribution of horsegram genotypes among different clusters

Cluster	No. of Genotypes	Genotypes
I	23	Arka local, Macintosh, Sapatu local, Sundar nagar local, P- 2056, NIC-2921, Bilaspur local, HPK-2, VLG-1, C-6-3-4, P-1610, Raigarh local, NIC-2913, P-1648, NIC-762, P-1192, S-67/14, PHG-20, Mandi local, P2030, NIC-2659, KN-825, P-1955
II	6	BU & 7-1, D-6-3-4, PLKV-38, DO-377, BDT-89-5, P-1659.
III	7	VLG-2, P-192, P-1910, Almora local-2, NIC-7321, NIC- 2757, R1711
IV	2	Tumkur local, Almora local-1
V	1	AU-6-4-2
VI	1	P-1668

Table 2. Intra and inter cluster distance (D^2) among six clusters in horsegram

Cluster	I	II	III	IV	V	VI
I	145.4	29.2	24.6	43.6	49.9	34.9
II		53.4	44.4	38.4	60.9	38.7
III			57.8	52.8	79.6	52.0
IV				8.6	66.2	57.6
V					0.0	116.0
VI						0.0

genotypes for all the traits studied (Table 1). Cluster 1 was the biggest with 23 genotypes, cluster II had six, cluster III had seven, IV had two and clusters V and VI had one genotype each. The intracluster distance ranged from 0.0 (clusters V and VI) to 145.4 (cluster I). The cluster I comprised of a big heterogeneous group of 23 genotypes. The maximum intercluster distance of 116.0 was recorded between clusters V and VI, followed by 79.6 between V and III suggesting wide diversity among these groups. On the other hand the minimum distance (24.6) between clusters I and III indicated their close relationship.

Based on cluster means, the important clusters are : III for pods per plant, I and VI for grain weight, I, III, IV and V for number of grains per pod, and III, V and I for grain yield. Crosses involving parents from divergent

Table 3. Means (M), standard errors (SE) and coefficient of variation (CV) for different characters in the six clusters

Character/ Cluster		TI	FL	MT	DW	PH	PL	PP	GP	GW	YP
I	M	45.3	72.9	123.3	6.7	82.9	4.7	29.6	5.8	3.2	3.4
	SE	0.7	0.3	0.6	0.2	6.3	0.01	1.2	0.01	0.1	0.3
	CV	7.8	1.9	2.5	17.0	36.6	5.1	18.8	5.7	13.7	36.3
II	M	47.3	73.9	120.2	7.0	92.5	4.5	15.6	4.9	2.8	0.1
	SE	0.4	1.1	1.8	1.1	6.8	0.1	1.2	0.2	0.1	0.2
	CV	2.3	3.8	3.7	38.8	18.1	7.5	42.2	8.4	9.2	62.5
III	M	47.2	72.5	117.2	7.8	83.4	4.5	49.7	5.7	3.0	3.7
	SE	1.4	0.4	3.4	0.4	3.0	0.1	4.4	0.2	0.1	0.4
	CV	6.7	1.5	7.7	13.8	9.5	5.4	23.3	8.9	11.3	28.8
IV	M	44.5	83.3	123.5	7.8	96.0	4.5	20.3	5.6	2.3	1.6
	SE	0.5	0.0	0.8	0.8	11.7	0.1	2.0	0.5	0.5	0.4
	CV	1.6	0.0	0.9	14.5	17.3	3.1	13.9	12.6	30.7	31.9
V	M	49.0	74.3	154.3	5.6	83.3	4.9	22.3	5.5	2.5	3.6
VI	M	36.7	72.3	111.3	8.6	79.0	4.1	13.3	5.3	3.3	0.7

clusters are expected to manifest maximum heterosis and also wide variability in subsequent segregating generations. The clusters comprising only one genotype with specific traits could also be exploited in a hybridization programme. Thus, crosses between the genotypes of cluster III and those of clusters I and V are expected to exhibit high heterosis and are also likely to produce new recombinants with desired traits.

REFERENCES

- Arunachalam, V. 1981. Genetic distance in plant breeding. *Indian J. Genet.*, **41** : 23-27.
- Mahalanobis, P.C. 1936. On the generalized distance in statistics, *Proc. Nat. Acad. Sci. India*, **2** : 49-55.
- Rao, C.R. 1952. Advanced Statistical Methods in Biometrical Research. John Wiley & Sons, New York.
- Samsuddin, A.K.M. 1985. Genetic diversity in relation to heterosis and combining ability in spring wheat. *Theor. Appl. Genet.*, **70** : 306-308

Short Communication

WILD GINGERS OF ANDAMAN AND NICOBAR ISLANDS

D.B. Singh, A. Subramaniam¹, P.V. Sreekumar¹ and T.V.R.S. Sharma

Central Agricultural Research Institute,
Port Blair 744 101 (Andaman and Nicobar Islands)

Key words : Wild ginger, Andaman and Nicobar Islands

The tropical rain forests of Andaman and Nicobar Islands harboring 4 species of medicinal and aromatic wild gingers namely *Zingiber odoriferum* Bl., *Z. Spectabile* Grioff, *Z. squarrosum* Roxb. and *Z. zerumbet* Rosc. ex Sm., which are generally found growing in shady humid places. Most of these wild gingers are possessing an eye-catching, colourful bracts in ovoid, conical or cylindrical spikes and are exhilarating the horticulturists to grow them as ornamental and decorative herbs in the gardens. These herbs are also being used for therapeutical purposes like that of the common, well-known ginger *Zingiber officinale* Rosc., a medicinal and a flavouring agent.

Zingiber odoriferum Bl. is a terrestrial, aromatic herb with 2-3 leaves in clumps, 3m and above in length. Leaflets sub-opposite, 25-30 pairs, spikes conical, crimson-red turning greenish-red up to 50 cm long. The dark red pouch like bracts are basally white, spoon shaped, acuminate, embedding with solitary cream-yellow flower on the bracteoles with mucilage. Corolla white or pale yellow. The seeds are black, like beads, ellipsoid 0.6 × 0.3 cm with carunculate base, can be collected after 2 months of its flowering during August-October. Spike turns to dark brown when fully ripen. The stem juice is being used as a tranquiliser for honeybee.

Distribution : South Andaman; Mini zoo, Haddo; Way to Aberdeen Bazar from BSI office, Haddo.

Zingiber spectabile Griff. is a robust, aromatic herb, leaves in clumps of more than 2 m long. leaflets 20-25 pairs. The spike is erect, cylindrical up to 40 cm long, Bracts scarlet in honeycomb form, turning to red as ripen. The solitary pale yellow flowers bloom for a day, lies in between the primary yellow bracts and the secondary bracteoles. Fruit is cylindrical, covered with

¹Botanical Survey of India, Port Blair 744 101

bracteoles in the pouch. Black beady eyes like ellipsoid seeds can be collected after 2 months of its flowering. Fruit wall remains persistent when dry. It could be grown as an ornamental herb.

Distribution : Chidiatappu to Kodaighat; BSI premise, Haddo.

Z. squarrosus Roxb. is a robust herb, 2.0-2.5 m tall; rhizome is covered with mucronate red sheaths. Leaves broadly lanceolate, acuminate, wavy margined, 35-50 cm long. Spikes obovate up to 8 cm long, bracts red pubescent, calyx tubular, pink, pubescent, corolla white, unequal, pubescent, pale red; flowering during August-October. The petioles are chewed when thirsty by the tribal 'Onges'.

Distribution : South Andaman; Dhanikhari; Little Andaman; Way to forest nursery.

Zingiber zerumbet Rosc. ex. Sm. is an aromatic herb, 1.3 cm in thick upto 1.5 m in height bearing 20-30 sessile leaflets. Spike conical or ovoid on stalks of 3-40 cm long clothed with long appressed obtuse greenish sheaths. Bracts of 2-4 cm are obovate with rounded apex and pale membranous margin, bright green at first and becoming red when fruiting. Flowers pale sulphur yellow. Seeds black, ellipsoid, oblong 2.5 mm × 4.0 mm. The rhizome is used like that of *Z. officinale* Rosc. a hot remedy for cough, asthma, worms, leprosy and other skin diseases. Boiled rhizome is given in pulmonary infections

Distribution : Dhanikhari, BSI experimental garden Haddo, BSI premises; Mini zoo.

Conservation measures : As part of *ex situ* conservation, the germplasm collection of the wild ginger have been introduced/maintained in the experimental garden-cum-arboratum of the Botanical survey of India, at Nayashahar near Dhanikhari, Port Blair, and also in the at experimental garden of Central Agricultural Research Institute, Port Blair.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P.K. Hajra, Director, Botanical Survey of India, Calcutta and Dr. A.K. Bandyopadhyay, Director, CARI, Port Blair for their encouragement.

REFERENCE

HOOI, K.S. 1995. Gingers from Belun. *Malayan Naturalist* 48(4) : 32.

Short Communication

TRANSGRESSIVE SEGREGATION AND SELECTION OF ZERO ERUCIC ACID STRAINS FROM INTERGENERIC CROSSES OF *BRASSICA*

Abha Agnihotri and Nutan Kaushik

Bioresources & Biotechnology Division,
TERI, Habitat Place, Lodhi Road, New Delhi

Key words : Rapeseed mustard, intergeneric hybridization, embryo rescue, erucic acid

Rapeseed mustard is the second most important edible oilseed crop in India. The Indian cultivated varieties have high erucic acid in the seed oil which is nutritionally undesirable (Gopalan et al. 1974, Sauer and Kramer 1983), and have low oleic acid while high is required for extended shelf life. 'Canola' is a trade name designating rapeseed having ideal fatty acids profile for human consumption (Downey 1990). In India, efforts were initiated during mid seventies resulting in identification/development of some 'O' erucic acid rapeseed mustard genotypes that need to be improved for their agronomic attributes (Malode et al. 1995). The available exotic 'Canola' cultivars could not be popularized in India due to late maturity and poor seed set (Anonymous 1995). The present work reports selection and development of nutritionally improved zero erucic acid rapeseed mustard strains, from the transgressive segregants of intergeneric crosses of *Brassica*, suitable for Indian agroclimatic conditions.

The advanced generation backcross derivatives of *B. juncea* derived from (*E. sativa* × *B. campestris*) × *B. juncea* (Agnihotri et al. 1990a) were selected for yellow seed coat and early maturity. The advanced generation backcross derivatives of *B. napus* derived from (*B. napus* × *Raphanobrassica*) × *B. napus* (Agnihotri et al. 1990b) were selected for shattering tolerance and early maturity. Single plant selections were made for four consecutive generations for phenotypically superior early maturing plants following the pedigree method. The plants were analysed for their fatty acids through bulk seed analysis, followed by half seed technique (Agnihotri et al. 1995). The single plant progenies, derived from the remaining half cotyledon of the identified zero

erucic acid *B. juncea* and *B. napus*, selected for desired proportions of oleic, linoleic and linolenic acids, were utilized for development of zero erucic acid strains following the pedigree method. Agronomical selections were made during four subsequent generations. The fatty acids composition of these advanced generation lines/strains were analysed by an improved GLC method (Kaushik and Agnihotri 1997) that confirmed the stability of zero erucic acid in these lines.

As evident from Table 1, these newly developed zero erucic acid strains are significantly superior in quality as compared to the Indian cultivars. They have zero erucic acid and high oleic acid, 40-50% in *B. juncea* and 45-70% in *B. napus*, as compared to the high erucic acid (40-50%) and low oleic acid (10- 25%) in the cultivated varieties. The essential fatty acid, linoleic acid, is much higher, 30-38% in *B. juncea* and upto 22% in *B. napus*, as compared to 10-15% in the national check varieties, Varuna and GSL-1. The oil content of some of these lines viz. TERI(OE)R05, R09, M08 and M10, is marginally higher than the national check varieties.

Table 1. Quality and agronomical characteristics of zero erucic acid strains of *Brassica* species

S. No.	Characters	<i>B. juncea</i> Strains					Varuna (NC)
		TERI(OE) M 07	TERI(OE) M08	TERI(OE) M 10	TERI(OE) M21		
1	Days to maturity	139	136	139	117		143
2	Plant height (cm)	177	179	177	167		170
3	No. of pods/plant	490	296	471	258		530
4	1000 seed weight(g)	2.89	3.66	2.88	3.63		4.29
5	Seed colour/size	Yellow/medium	Yellow/medium	Yellow/medium	Yellow/medium		Brown/medium
6	No. of seeds per pod	12	18	15	17		13
7	Yield (Q/ha)	17.61	18.76	18.30	18.32		20.38
8	Oil content (%)	42.8	45.6	46.1	43.7		44.4
9	Oleic acid (%)	34.47	45.0	50.0	40.6		11.42
10	Linoleic acid (%)	36.52	36.63	30.7	36.3		19.07
11	Linolenic acid (%)	16.49	13.84	10.4	15.2		10.52
12	Erucic acid (%)	0.0	0.0	0.0	0.0		48.43

<i>B. napus</i> Strains						
S. No.	Characters	TERI(OE) R03	TERI(OE) R05	TERI(OE) R09	TERI(OE) R15	GSL-1 (NC)
1	Days to maturity	136	141	149	118	153
2	Plant height (cm)	140	88	122	101	165
3	No. of pods/plant	291	212	255	210	308
4	1000 seed weight(g)	3.05	3.01	2.50	3.87	3.47
5	Seed colour/size	Brown/medium	Brown/medium	Brown/medium	Brown/medium	Brown/medium
6	No. of seeds per pod	22	20	24	24	17
7	Yield (Q/ha)	19.01	17.58	13.31	14.23	15.90
8	Oil content (%)	43.3	47.5	50.2	46.3	46.4
9	Oleic acid (%)	59.5	47.68	70.1	45.60	24.42
10	Linoleic acid (%)	20.9	22.73	13.3	22.50	14.67
11	Linolenic acid (%)	12.5	10.19	7.3	8.13	8.87
12	Erucic acid (%)	0.0	0.0	0.0	0.0	36.93
NC-National check variety						

These zero erucic acid strains have been evolved from the transgressive segregants of the backcross progenies of the intergeneric hybrids derived through *in vitro* embryo rescue, followed by callusing and secondary embryogenesis (Agnihotri *et al.* 1990a, b). Chromosome alterations in callus cultures is well documented in literature (Hayliss 1980, Shepard *et al.* 1980) and tissue culture conditions are known to cause increased genetic variations termed somaclonal variations (Lee and Philips 1987, Larkin and Scowcroft 1981, Zehr *et al.* 1987). There is a possibility that these embryos have undergone chromosomal changes/mutations during *in vitro* culture leading to the altered fatty acid biosynthesis.

A significant reduction in the days to maturity was observed in a large number of segregants. Some of the selected zero erucic acid *B. napus* [TERI(OE)R03 and R15] and *B. juncea* [TERI(OE)M08 and M 21] strains matured 17 to 35 days earlier than the national check varieties. Thurling and Kaveeta (1991) have reported introgression of early flowering and early maturity in *B. napus* from the primary and secondary gene pools of *B. campestris*. The parents involved in the intergeneric hybridization i.e. *B. campestris*, *E. sativa* and *Raphanus sativus* are early in maturity (90 to 120 days) as compared to the

rapeseed mustard cultivars (130 to 160 days) and seem to be responsible for the introgression of earliness in the transgressive hybrid progenies that were utilized for developing the early maturing zero erucic acid strains of *B. juncea* and *B. napus*. Further work is in progress to make selections for the improvement in economically important agronomic attributes to overcome the marginal yield penalty. These nutritionally superior quality strains provide useful gene pool that can be profitably utilized in further breeding programmes.

ACKNOWLEDGEMENTS

We thank NOVODB (Ministry of Agriculture), Gurgaon for financial assistance for advancing this work. We are grateful to Dr. R.K. Downey, Emritus Scientist, Agriculture and Agri-Food Canada Research Centre; Prof. N.B. Singh, ADG (OP), ICAR; Prof. Basudeo Singh, ED and Dr. J.P. Singh, Secretary, NOVODB; Dr. A.K. Singh, IARI, for valuable discussions. Technical assistance by Mr. Gautam Sarkar and Mr. Bir Singh is highly appreciated.

REFERENCES

- Agnihotri, A., V. Gupta, M.S. Lakshmikumaran, K.R. Shivanna, S. Prakash, V. Jagannathan, 1990a. Production of *Eruca- Brassica* hybrids by embryo rescue. *Plant Breeding* **104**: 281-289.
- Agnihotri, A., K.R. Shivanna, S.N. Raina, M. Lakshmikumaran, S. Prakash, V. Jagannathan, 1990b. Production of *Brassica napus* × *Raphanobrassica* hybrids by embryo rescue - An attempt to introduce shattering resistance into *B. napus*. *Plant Breeding* **105** : 292-299.
- Agnihotri, A., J.P. Raney, N. Kaushik, N.K. Singh, R.K. Downey. 1995. Selection for better agronomical and nutritional characteristics in Indian rape-seed-mustard. In: Proceedings, IX GCIRC International Rapeseed Congress, Rapeseed Today and Tomorrow Vol. 2: 425-427.
- Anonymous, 1995. Annual Progress Report, All India Coordinated Research Project on Rapeseed-Mustard. Indian Council of Agricultural Research, Government of India, New Delhi.
- Downey, R.K., 1990. *Brassica* oilseed breeding-achievements and opportunities. *Plant Breeding Abstracts*. **60**(10): 1165-1170.
- Hayliss, M.W., 1980. Chromosomal variation in plant tissues in culture. *Intrn. Rev. Cytol. Suppl. IIA*: 113-144.
- Gopalan, C.D., D. Krishnamurthy, I.S. Shenolikar, K.A.V.R. Krishnamurthy. 1974. Myocardial changes in monkey fed on mustard oil. *Nutr. Metab* **16**: 352-365.
- Kaushik, N., A. Agnihotri, 1997. Evaluation of improved method for determination of rapeseed mustard FAMES by GC. *Chromatographia* **44**: 97-99
- Larkin, P.J., W.R. Scowcroft. 1981. Somaclonal variation - a novel source of variability from cell cultures for plant improvement. *Theor., Appl. Genet.* **60**: 197-214.
- Lee, M., R.L. Philips, 1987. Genetic variants in progeny of regenerated maize plants. *Genome*, **29**: 834-938.

- Malode, S.M., R.V. Swamy, A.S. Khalatkar. 1995. Introgression of '00' quality characters in *B. juncea* cv. Pusa Bold. In: Proceedings, IX GCIRC International Rapeseed Congress, Rapeseed Today and Tomorrow, Vol. 2: 431-433.
- Sauer, F.D., J.K.G. Kramer, 1983. The problems associated with the feeding of high erucic acid rapeseed oils and some fish oils to experimental animals. In: High and Low Erucic Acid Rapeseed Oils (Ed. by Kramer, J.K.G., F.D. Sauer, W.J. Figden), Toronto, Canada. Academic Press. p. 254-292
- Shepard, J.F. , D. Bidney, E. Shahin, 1980. Potato protoplasts in crop improvemen. *Science* **208**: 17-24.
- Thurling, N., R. Kaveeta, 1991. Yield improvement of *Brassica napus* through introgression of genes for earliness from primary and secondary gene pools. In: Proceedings, VIII GCIRC International Rapeseed Congress, p. 131-136
- Zehr, B.E., M.E. Williams, D.R. Duncan, J.M. Widholm. 1987. Somaclonal variation in the progeny of plants regenerated from callus cultures of seven inbred lines of maize. *Can. J. Bot.* **65** 491-499.

PLANT GERMPLASM REGISTRATION NOTIFICATION*

The Germplasm Registration committee of ICAR in its 3rd meeting held on 10th December, 1998 at NBPGR approved the registration of the following 34 germplasm lines/genetic stocks out of 57 proposals received.

INGR 98010 is a genetic male sterile line of cotton (*Gossypium hirsutum*), developed by G.V. Umalkar at Ankur Seeds Pvt. Ltd. Nagpur by crossing CA/H-148 × CA/H-144. The line being GMS system exhibits 50% sterile population and is designated as **CA/MH-133**.

INGR 98011 is fibre less, soft, necrosis free anola (*Embelica officinalis*) designated as **NA7**. It is a seedling selection from open pollinated strain of Francis, developed by R.K. Pathak, I.S. Singh, R. Dwivedi, A.K. Srivastava and H.K. Singh at NDUAT Faizabad.

INGR 98012 is a high yielding, drought hardy germplasm of water melon (*Citrullus lanatus*) designated as **Mateera - AHW19**. This sweet, juicy watermelon with longer shelf life and good transportability is selection made from local genetic diversity by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

INGR 98013 is a high yielding, drought hardy kachari (*Cucumis callosus*) designated as **Kachari AHK 119**. It is a pure line selection from local genetic diversity made by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

INGR 98014 is a high yielding, drought hardy germplasm of kachari (*Cucumis callosus*) designated as **Kachari AHK 200**. It is a pure line selection from local genetic diversity made by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

INGR 98015 is a high yielding, drought hardy germplasm of snap melon (*Cucumis melo var. momordica*) designated as **AHS10**. It is a pure line selection from local genetic diversity of arid region made by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

INGR 98016 is a high yielding, drought hardy germplasm of snap melon (*Cucumis melo var. momordica*) designated as **AHS 82**. It is a pure line selection from genetic diversity of arid region made by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

*Communicated by Dr. B.B. Singh, Member Secretary, ICAR Committee on Plant Germplasm Registration, NBPGR, New Delhi 110012.

INGR 98017 is high yielding, long fruited genetic stock of cucumber (*Cucumis spp*) designated as **AHC 2**. It is a promising selection made from variable material by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

INGR 98018 is a high yielding, unique, small fruited, drought hardy cucumber (*Cucumis spp.*) designated as **AHC 13**. It can bear fruits even under high temperature in arid region with limited irrigation. This genetic stock is developed by O.P. Pareek and D.K. Samadia at NRC for Arid Horticulture, Bikaner, Rajasthan.

INGR 98019 is a aromatic, thermo insensitive, photo period sensitive, extra long kernerled, stem rot resistant rice (*Oryza sativa indica* type) suitable for irrigated conditions. It is designated as **Taraori Basmati**. It is a pure line selection from HBC 19 made by D.V.S. Panwar, Ajmer Singh, K.R. Gupta, K.R. Battan, Rishi Pal and Rattan Singh at CCSHAU Rice Research Station, Kaul, Haryana.

INGR 98020 is a cytoplasmic male sterile mustard (*Brassica juncea*) carrying cytoplasm of *Brassica oxyrrhina* derived from the synthetic allopoloid *B. oxyrrhina* × *B. campestris*. This CMS line is developed at NRC on Plant Biotechnology, IARI New Delhi by Shyam Prakash and V.L. Chopra.

INGR 98021 is a Cytoplasmic male sterile mustard (*Brassica juncea*). This CMS line is developed through synthesis of somatic hybrid *Morandia arvensis* + *B. juncea* and its repeated backcrossing to *B. juncea* cv. Pusa bold at NRC on Plant Biotechnology, IARI New Delhi by Shyam Prakash, P.B. Kirti, V.L. Chopra and S.R. Bhat.

INGR 98022 is a fertility restorer for CMS (*Morandia*) *B. Juncea*. This fertility restorer CMS is developed by Shyam Prakash, P.B. Kirti, V.L. Chopra and S.R. Bhat at NRC on Plant Biotechnology IARI, New Delhi.

INGR 98023 is a cytoplasmic male sterile rape seed (*Brassica napus*). This CMS line is developed through synthesis of sexual allopoloid *Brassic oxyrrhina* × *B. campestris* and its repeated back crossing to *B. napus* by Shyam Prakash, P.B. Kirti and V.L. Chopra at NRC on Plant Biotechnology IARI, New Delhi.

INGR 98024 is a cytoplasmic male sterile mustard (*Brassica juncea*) developed through protoplast fusion between chlorotic CMS *B. juncea* carrying *Raphanus*/ogu cytoplasm with floral abnormalities and green fertile *B. juncea* and then backcrossing to *B. juncea*. This CMS is developed by P.B. Kirti, S.S. Banga, Shyam Prakash, and V.L. Chopra at NRC on Plant Biotechnology, IARI, New Delhi.

INGR 98025 is a chlorosis corrected, green cytoplasmic male sterile mustard (*B. juncea*) carrying recombinant mitochondria of *Morandia arvensis* and

chloroplasts of *B. jucea*. This CMS line is developed through protoplast fusion and selection at NRC on Plant Biotechnology, IARI, New Delhi by P.B. Kirti, Shyam Prakash and V.L. Chopra.

INGR 98026 is a cytoplasmic male sterile rape seed (*B. campestris* ssp. *oleifera* var. brown sarson) carrying cytoplasm of *B. oxyrrhina* derived from synthetic allopolyploid *B. oxyrrhina* × *B. campestris*. This CMS line is developed by Shyam Prakash and V.L. Chopra at NRC on Plant Biotechnology IARI, New Delhi.

INGR 98027 is a male sterile parental line of safflower (*Carthamus tinctorius*) hybrid DSH129 designated as **MS9 (O)**. This line is developed by M. Ramachandran and K. Anjani at DOR Hyderabad.

INGR 98028 is a high protein and high lysine content wheat (*Triticum aestivum*) designated as **Pusa 5-3**. It is developed from cross HW171 × CI 11721 at Genetics Division, IARI, New Delhi by N.C. Singhal and H.K. Jain.

INGR 98029 is a low ODAP and high yielding khesari (*Lathyrus sativus*) designated as **Bio-158**. This somaclone is developed from leaf explants of cultivar P-24 by S.L. Mehta, P.K. Roy, G.K. Barat, I.M. Santha and K. Ali at Biochemistry Division, IARI, New Delhi.

INGR 98030 is a garlic (*Allium sativum*) with compact bulbs, silvery white skin, cream coloured flesh and big cloves designated as **Agrifound White**. This is a local selection from Biharsharif (Nalanda) made by U.B. Pandey Lallan Singh and S.R. Bhonde at NHRDF, Nasik.

INGR 98031 is a garlic (*Allium sativum*) with compact bulbs, creamy white, with creamy flesh designated as **Yamuna Safed-3**. It is a local selection from Dindigul (T.N.) made by U.P. Pandey, Lallan Singh and R.P. Gupta at NHRDF, Nasik.

INGR 98032 is a fine grained, early maturing (115-120 days) dwarf rice (*Oryza sativa*) designated as **GAURAV Basmati A- 54**. It is a mutant of Basmati-370 developed by S.S. Malik at HAU, Kaul and further tested at NBPGR base center, Cuttack.

INGR 98033 is a castor (*Ricinus communis*) tolerant to *Fusarium oxysporum* sp. *ricini* wilt and moderately resistant to *Macrophomia phaseolina* root rot designated as **RG392**. It is a selection from Pothineni local - II from Tindivanam, Tamilnadu made by K. Anjani, M.A. Raoof and C. Hanumantha Rao at DOR, Hyderabad.

INGR98034 is a castor (*Ricinus communis*) tolerant to *Fusarium oxysporum* sp. *ricini* wilt and resistant to *Macrophomia phaseolina* root rot designated as **RG47**. It is a selection from SKI-6 collection from Dantiwada, Gujarat made by K. Anjani, M.S. Raoof and C. Hanumanta Rao at DOR, Hyderabad.

INGR 98035 is a pearl millet (*Pennisetum glaucum*) fertility restorter line and parent of very productive released hybrids HHB 45 and HHB 50, designated as **H90/4-5** resistant to drought and tolerant to high temperature stress. It is a selection from synthetic HS 1 developed by R.L. Kapoor, Sain Dass, I.S. Khairwal, H.P. Yadav, Prem Sagar, C.R. Bainiwal, D.C. Nijhawan and M.S. Narwal in collaboration with D.P. Thakur and M.S. Panwar. This line is developed at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98036 is a pearl millet (*Pennisetum glaucum*) restorter line and parent of very popular released hybrid HHB 67, HHB60 and HHB68. designated as **H 77/883-2** It is excellent combiner for productivity and gives early/extra early hybrids. It a selection from Haryana land race population and resistant to temperature, drought and salt stress, developed by R.L. Kapoor, Sain Dass, I.S. Khairwal, H.P. Yadav, Prem Sagar, C.R. Bainiwal, D.C. Nijhawan and M.S. Narwal in collaboration D.P. Thakur and M.S. Panwar. This line is developed at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98037 is a male sterile line of pearl millet (*Pennisetum glaucum*) designated as **HMS 1A** and **1B**. It is developed from DSA 134A × H 90/4-5. It is an A3 cytoplasm male sterile line and developed by Prem Sagar, D. C. Nijhawan, R.L. Kapoor, C.R. Bainiwal, I.S. Khairwal and H.P. Yadav in collaboration with D.P. Thakur at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98038 is a male sterile line pearl millet (*Pennisetum glaucum*) designated as **HMS 2A** and **2B** developed by converting H77/833-2 into male sterile line developed by Prem Sagar, D.C Nijhawan, R.L. Kapoor, C.R. Bainiwal, I.S. Khairwal and H.P. Yadav in collaboration with D.P. Thakur at Bajara Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98039 is a male sterile line of pearl millet (*Pennisetum glaucum*) designated as **HMS 3A** and **3B** developed by DSA 134A × HC 715-1. It is high tillering line developed by Prem Sagar, D.C. Nijhawan, R.L. Kapoor, C.R. Bainiwal, I.S. Khairwal and H.P. Yadav in collaboration with D.P. Thakur at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98040 is a male sterile, good tillering, early flowering pearl millet (*Pennisetum glaucum*) designated as **HMS 4A** and **4B**. It is a result of a cross DSA 134 A × HC 715-2 made by Prem Sagar, D.C. Nijhawan, R.L. Kapoor, C.R. Bainiwal, I.S. Khairwal and H.P. Yadav in collaboration with D.P. Thakur at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98041 is a male sterile pearl millet (*Pennisetum glaucum*) designated as **HMS 7A** and **7B**. It is a result of cross 81A × 35 made by D.C. Nijhawan, R.L. Kapoor, H.P. Yadav, C.R. Bainiwal, Prem Sagar and M.S. Narwal in collaboration with M.S. Panwar at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR98042 is a male sterile pearl millet (*Pennisetum glaucum*) designated as **HMS 8A** and **8B**. This line has experimental potential and several hybrids have HMS 8A as female parent. It is a result of 81A × 52 -37-1 developed by D.C. Nijhawan, R.L. Kapoor, H.P. Yadav, C.R. Baniwal, Prem Sagar and M.S. Narwal in collaboration with M.S. Panwar at Bajra Section, Department of Plant Breeding, CCS, HAU, Hisar.

INGR 98043 is a male sterile pearl millet (*Pennisetum glaucum*) designated as **HMS 9A** and **9B** this line is very promising female parent of hybrids is a result of 843A × 44-10 developed by D.C. Nijhawan, R.L. Kapoor, H.P. Yadav, C.R. Baniwal, Prem Sagar and M.S. Narwal in collaboration with M.S. Panwar at Bajra Section, Department of Plant Breeding, CCS HAU Hisar.

- A colorimetric method for estimation of capsaicin in chilli fruits
S. Mandal, Poonam Suneja and D.K. Hore ... 213
- Influence of storage relative humidity on viability of *Amaranthus hypochondriacus* *Brassica oleracea* and *Glycine max*
Sanjeev Saxena, Kalyani Srinivasan, R.K. Tyagi and B.B. Singh ... 219
- Conservation of bottlegourd germplasm seeds
S.D. Doijode ... 223
- Midstorage treatments for improving viability of mustard seeds
Kalyani Srinivasan, Sanjeev Saxena and B. B. Singh ... 229

Short Communications

- Evaluation of chillies germplasm
S.K. Verma, K.C. Pant and K.C. Muneem ... 237
- Collecting safflower germplasm from West Bengal
N. Dikshit, P.S. Mehtre and W.S. Yadav ... 241
- Genetic divergence in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.)
Rajiv K. Sharma ... 245
- Wild gingers of Andaman and Nicobar Islands
D.B. Singh, A. Subramaniam, P.V. Sreekumar and T.V.R.S. Sharma ... 249
- Transgressive segregation and selection of zero erucic acid strains from intergeneric crosses of *Brassica*
Abha Agnihotri and Nutan Kaushik ... 251
- Plant germplasm registration notification ... 275