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IMPLICATIONS OF THE CONVENTION ON BIOLOGICAL DIVERSITY : INDIAN APPROACH*

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The International Convention on Biological Diversity, which was adopted during the Earth Summit (UNCED) held in Brazil in 1992, came into force in December, 1993. Having ratified the Convention in February 1994, India is now obligated to undertake actions to implement its various provisions. This paper describes the genesis, objectives and salient features of the Convention. It also highlights various approaches to be taken into consideration by India for achieving the objectives and implementing its various provisions.

Key words : Convention, biological diversity, implication, Indian approach.

The Convention on Biological Diversity (CBD) was adopted by 171 countries during the historic United Nations Convention for Environment and Development (UNCED), known as the Earth Summit, at Rio de Janeiro, Brazil, in June, 1992 (UNEP, 1992), and came into force from 29th December, 1993. India ratified the Convention on 18th February, 1994. As per the provision, the Convention then became operative in the country from 19th May, 1994, that is 90 days after ratification.

GENESIS

The concern raised by conservationists during the past several decades over the rapid loss of biological diversity was given a sharp focus by the World Conservation Union (IUCN). Subsequently, this was politically and legally recognised at the international level during the Stockholm Conference on the Human Environment in 1972. These concerns were brought into the public domain by the acclaimed report *Our Common Future* (WCED, 1987). The Governing Council of the United Nations Environment Programme (UNEP), through its decision 14/26 of June, 1987 established an *ad hoc* working Group of Experts on Biological Diversity. Based on inputs provided by the Working

*The views expressed in this paper not necessarily that of the Government of India

Group during 1988-90, the Governing Council made this group the Inter-governmental negotiating Committee for the Convention on Biological Diversity. The actual negotiations took place during 1991-92. India contributed significantly to the draft of the Convention.

As the negotiations progressed through five normal sessions, the elements of the proposed Convention went through a sea change, becoming more complex. Sanchez (1994), who was the Chairperson of the Negotiating Committee, listed the following issues, which became the core of the negotiations:

- the cost of taking measures to conserve biological diversity *versus* the cost of not taking any measures;
- access to genetic resources and different possibilities of regulating it;
- whether the focus should be on wild species or should include both wild and domesticated species;
- access to and transfer of technology, including biotechnology, which must be considered for conservation and rational use of the components of biological diversity;
- the eventual source and method of funding the costs of the measures that would be agreed upon; and
- the consequence and impact of biodiversity conservation on trade and development.

SALIENT FEATURES OF THE CONVENTION

Preamble and objectives

The preamble of the CBD recognises and reaffirms (UNEP, 1992):

- the intrinsic value of biological diversity;
- the sovereign rights of States over their biological resources;
- the fundamental requirements of *in-situ* conservation of ecosystems and natural habitats;
- the supporting role of *ex-situ* conservation;
- the vital role of local communities and women in the conservation and sustainable use of biological diversity;
- the desirability of sharing equitably the benefits arising from the use of traditional knowledge, skills, innovations and practices;
- the importance of and need to promote regional and global cooperation for conservation; and
- the requirement of substantial investments to conserve biological diversity.

The objectives of the Convention are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilisation of genetic resources and by appropriate transfer of technologies, taking into account all rights over those resources and to technologies, and by appropriate funding (UNEP, 1992).

Provisions

Through its 42 Articles, the Convention (UNEP, 1992) recognises national sovereignty over biological resources (Art. 3); calls for taking general measures for conservation and sustainable use (Art.6); identification and monitoring (Art. 7); *in-situ* and *ex-situ* conservation (Art. 8, Art.9); sustainable use of components of biological diversity (Art.10); incentive measures (Art.11); research and training (Art.12); public education and awareness (Art.13); and impact assessment and minimising adverse impacts (Art. 14). The Convention's provisions facilitate access to genetic resources on 'mutually agreed terms' and with the 'prior informed consent' of the country providing the resources, with the recipient country being committed to share the accruing benefits (Art. 15). It also provides for the transfer of technologies, including biotechnologies, on 'fair and most favorable' terms, from the developed to the developing nations, which are the main providers of genetic resources (Art.16). Moreover, the Convention calls on the private sector to facilitate access to and transfer of such technologies developed by them (Art.14.4). The Contracting Parties are to cooperate in this regard to ensure that patents and other intellectual property rights are supportive of, and do not run counter to, the objectives of the Convention (Art.16.5). It commits the parties to consider the need for, and modalities of, a protocol in the field of safe transfer, handling and use of any living modified organism resulting from biotechnology (Art. 19.3). The Parties are also to take measures to facilitate access on a fair and equitable basis, and on mutually agreed terms, to the results and benefits arising from biotechnologies (Art. 19.2). The developed Country Parties are committed to contribute to a fund to enable developing Country Parties to meet the 'agreed full incremental costs' for implementing the provisions of the Convention (Art.20.2). This financial mechanism is to 'operate within a democratic and transparent system of governace' and 'function under the authority' of the Conference of Parties (Art. 21).

INDIAN APPROACH TOWARDS IMPLEMENTATION

Principle

From the national point of view, the Convention (Art. 3) establishes the most important principle of States having sovereign rights to exploit their own resources, while ensuring that their activities do not cause cross-boundary damage to the environment of other States.

General measures

Art.6 establishes a set of norms for developing national strategies, plans and programmes for the conservation and sustainable use of biological diversity and their integration into sectorial or cross-sectorial policies, plans and programmes. The Ministry of Environment and Forests (MOEF), which is the National Focal Point/Nodal Agency for this purpose, had (prior to UNCED) already finalised the *National Conservation Strategy and Policy Statement on Environment and Development* (GOI, 1992). This document incorporates a broad strategy for promoting conservation of biological diversity in the country, which is supported by earlier policy instruments including the National Forest Policy (GOI, 1988) and the National Wildlife Action Plan. MOEF has also undertaken a Country Status Report, which is in the final stages of preparation.

Identification and monitoring

Conservation and sustainable use of biological diversity requires actions at several levels. In order to (a) identify critical components i.e. ecosystems, species and genomes (Art. 7a); (b) monitor such components to determine priorities (Art. 7b); (c) identify activities and monitor their adverse impacts (Art. 7c); (d) improve data acquisition and management (Art. 7d), India has established a system of protected areas including special conservation areas like wetlands, mangroves and biosphere reserves. The multifarious tasks are shared by a network of official organisations, including the Botanical, Zoological and Forest Surveys of India (BSI, ZSI and FSI), the Indian Council of Agricultural Research (ICAR) and the Council of Scientific and Industrial Research (CSIR) systems, the Wildlife Institute of India (WII), the Indian Council of Forestry Research and Education (ICFRE), and the Central and State Pollution Control Boards. Also involved are the universities and various non- government organisations (NGOs) such as the World Wide Fund for Nature (WWF), the M.S. Swaminathan Research Foundation (MSSRF), the Bombay Natural History Society (BNHS), Centre for Science and Environment (CSE) etc. The gaps identified would help to strengthen the activities.

In-situ conservation

Article 8 of the Convention relates to *in-situ* conservation; it calls for establishing a national system of protected areas (PAs); development of guidelines for selection, establishment and management of PAs; regulating access to biological resources within or outside the PAs; and promoting protection in natural surroundings and conservation areas adjacent to PAs. India already has 80 National Parks and 441 Sanctuaries covering 1,48,700 sq km area which is about 4.5 per cent of the total geographical area (GOI, 1996). Further expansion of the PAs is envisaged on the basis of a systematic study

carried out by Rodgers and Panwar (1988). This would reduce the gaps in various biogeographic units and biomes. In addition, special conservation areas including 16 wetlands, 15 mangroves, 4 coral reefs and 8 biosphere reserves have been particularly designated by MOEF for scientific management and conservation. The National Afforestation and Ecodevelopment Board (NAEB) has been given specific mandate for conserving areas adjacent to PAs and overall ecodevelopment activities for restoring degraded ecosystems. Recovery of endangered and threatened species (Art. 8f) is covered through *ex-situ* preservation areas (GOI, 1994).

Of particular concern is to control the use of living organisms modified through biotechnologies and to guard against the associated risks (Art. 8g). In India, in 1989 MOEF in consultation with the Department of Biotechnology (DBT) had already framed rules to govern the manufacture, use, import, export and storage of hazardous microorganisms/cells. These rules regulate aspects of safety in research and production and provide for granting approval to proposals for use of hazardous microorganisms and recombinants in research and industrial production, including their release into the environment. MOEF has proposed to expand the scope of these rules through further guidelines and introducing risk-assessment procedures for the release of living modified organisms in contained and open environments and also for granting environmental impact clearance.

The obligation to control threatening alien or exotic species (Art. 8h) necessitates extensive review (in consultation with the concerned government agencies) of the scope of existing quarantine measures for screening biomaterials entering the country, with reference to fungal, bacterial and viral diseases and pests. Similarly, the aspects relating to the need of maintaining compatibility of physical components especially land and water, with the conservation of biological diversity shall have to be deliberated upon.

Article 8 (j) stresses the need to respect, preserve and maintain the knowledge, innovations, skills and practices of indigeneous/local communities, and to promote their wider application. The National Forest Policy and the National Conservation Strategy endorse this. MOEF, the Indian Council of Medical Research (ICMR) and the Anthropological Survey of India (ASI) are engaged in collecting information towards this end. In the light of the Convention, the existing arrangements need further review, for which MOEF has already initiated appropriate action.

MOEF has already done a comprehensive review of existing legislations and is in the process of formulating specific legal proposals for the proposed Biological Diversity (Conservation) Act. The details of legal requirements are dealt separately (Chauhan, 1996). Both financial and other kinds of support for *in-situ* conservation (Art.8m) is being provided under existing national

policy, legislation and institutional mechanism. However, it is desirable that the existing mechanisms be further strengthened through internal and external support.

Ex-situ conservation

To complement the *in-situ* measures (Art. 8), the Convention (Art. 9) also covers measures to be adopted for *ex-situ* conservation. These include establishing facilities for research, recovery and rehabilitation of threatened species; regulating and managing the collection of biological resources from natural habitats for *ex-situ* conservation purposes; and adequate financial and other support. India has already made concerted efforts in this direction. For example, under the aegis of ICAR, the three National Bureaus of Plant, Animal and Fish Genetic resources (NBPGR, NBAGR and NBFGR), have been established for *ex-situ* conservation and for undertaking research on domesticated biological diversity. Wild biological diversity has been conserved through 275 *ex-situ* areas and 66 botanic gardens. The research component is pursued through universities, and the institutions of ICAR, CSIR, BSI, ZSI, WII, ICFRE, the Salim Ali Centre of Ornithology (SACON) and the G.B. Pant Institute of Himalayan Environment and Development etc. The research on biotechnological aspects is supported by DBT through the national facility for Microbial Type Culture, Blue-green Algae, Marine Cyno-bacteria, Plant Tissue Culture Repository, Animal Tissue and Cell Cultures, Animal House, Seed Banks, Medicinal Plants, Genetic Engineering Units, Biochemical Engineering, Immunology and Biosafety etc. However, the financial and technical/scientific inputs need to be augmented, for which the cooperation of other Contracting Parties to the Convention is essential (GOI, 1994).

Sustainable use of components of biological diversity

Article 10 emphasises that sustainable usage must be integrated into national decision-making processes, through measures to control adverse impacts, protecting customary and traditional use, providing support to local populations in remedial action in degraded areas and encouraging cooperation between government and private sectors.

Through the various policy instruments relating to wildlife and forests, India has tried to integrate sustainable usage into decision making. However, the lack of organic linkages between various agencies both at the national and state levels has resulted in a rather unsatisfactory situation. Conservation of marine biological diversity and characterisation of micro-organisms have not been adequately addressed. On-farm conservation of agri-biological diversity is also rather poor. As a result, a significant number of traditional varieties

of important crops have been lost forever. The guidelines for imapct assessments of projects for industry and mining, thermal power, river valley development, rail/road highways, ports and harbours, airports, townships etc. lack focus on biological diversity aspects. An exercise is being undertaken to review these guidelines. For the purpose of sustainable use of ecosystems, criteria for determining ecological fragility have been developed and are being implemented.

Incentive measures

The Convention states that States should adopt economically and socially sound incentives (Art.11) for conservation and sustainable use of biological diversity. In this direction, the Government of India has launched several programmes. In view of the provisions of the Convention, further incentives need to be evolved for on and off farm conservation of agri-biological diversity. Similarly, incentives will have to be given to local communities for conserving and sustainably using rich freshwater and marine biological diversity.

Research and training

Education, training and research concerning certain aspects of biological diversity (Art. 12) are currently being promoted through (a) the universities; (b) government agencies such as MOEF, Ministry of Science and Technology (MST), DBT, CSIR, ICAR, ICFRE, WII, SACON, G, B. Pant Institute of Himalayan Environment and Development, and other associated institutes/organisations and (c) NGOS such as WWF (India), BNHS, Centre of Environmental Education (CEE) etc.

Public education and awareness

The National Environmental Awarenessess Campaign has had 'Biological Diversity' as its theme in current years. Communication media including television, radio, the national and regional Press have also been used to generate awareness about the Convention. NGOs are being actively involved throughout the country to spread information and understanding of the importance of conservation and sustainable use of biological diversity, and the measures required for this (Art. 13a). The University Grants Commission (UGC) and the National Council of Educational Research and Training (NCERT) have also initiated action to develop syllabi for undergraduate and postgraduate courses. The cooperation of other Contracting Parties has also been sought, including nations in the South Asian region. Collaboration with international agencies such as FAO, UNDP, UNEP, IUCN and the World Resources Institute is also being explored.

Impact assessment and minimising adverse impacts

Article 14 of the Convention deals with (a) introducing procedures requiring Environmental Impact Assessment (EIA); (b) arrangements to account for the environmental consequences of programmes and policies; (c) and to account for the adverse impact beyond national jurisdictions; (d) notification of adverse effects upon other States as well as initiation of action to prevent damage; and (e) promoting national arrangements for emergency responses to activities threatening biological diversity and establishing joint contingency plans with other Contracting Parties.

India's existing arrangements, under the Environment (Protection) Act, provide for impact assessment of development projects in the public sector alone, such as projects for river valleys and irrigation, industry and mining, thermal power, ports and harbours, railways, highways, airports, townships etc. It is proposed to extend the scope of EIA to private sector development projects as well, and to provide statutory backing for this.

Access to genetic resources

Access to genetic resources is covered by Art. 15 which provides for (a) recognition of the rights of sovereign states; (b) creating conditions to facilitate access to genetic resources by other countries; (c) and d) access on mutually agreed terms with prior informed consent; and (e) developing and carrying out research with full participation of the Contracting Parties.

Access to genetic resources is facilitated through bilateral arrangements under FAO which regulates the import/export of seeds, and the country's export-import policy under the Foreign (Trade) Act. However, arrangements to govern access to genetic resources in the future are yet to be worked out in accordance with the provisions of Articles 15, 16 and 19 of the Convention.

Access to and transfer of technology

The Convention recognises the importance of access to and transfer of technology and advocates that countries take facilitating, legislative, administrative and policy measures, under fair and most favorable terms to both government and private sectors. Granting access to and transfer of technology must take into account the issues relating to patents and intellectual property rights.

Since this is closely linked to access to genetic resources and will be crucial for successfully implementing the provisions of the Convention, India will have to carefully assess and review the existing protocols/treaties such as provisions of GATT and TRIPS along with our own Patent Law and other related legal regimes. The cooperation of other Contracting Parties may be

sought, as and when it is necessary. India will have to evolve a legal regime in a manner that it protects the knowledge, innovations, skills and practices of the local communities relating to the use of genetic resources, while taking into account the implications arising from the intellectual property regime.

Exchange of information and technical and scientific cooperation

Regarding the exchange of information (Art.17) and technical and scientific cooperation (Art.18). India has been engaged in collecting relevant information and development of technologies through independent or joint programmes/ventures undertaken by both government and private agencies. In the light of these two Articles, ongoing programmes could emphasise and integrate aspects related to biological diversity.

Handling of biotechnology and distribution of its benefits

As mentioned earlier, India already has rules for the manufacture, use, import, export and storage of genetically engineered organisms/cells which were adopted in 1889 under the Environment (Protection) Act. Besides, DBT has been actively promoting research activities related to biotechnologies through bilateral joint ventures in the frontier areas. India is also participating in developing the modalities for biosafety protocol and other related matters (Art. 19).

Financial resources

For effective implementation of the provisions of the Convention, India is already providing financial support for national activities in accordance with its capacity. However, the gaps identified in ongoing conservation measures and activities towards sustainable utilisation of biological diversity, indicate that India would require substantial support from the financial mechanism set up under the Convention. For this purpose, a National Action Plan is being drawn up to identify the areas for formulation of specific project proposals for bilateral and multilateral funding.

CONCLUSIONS

It is evident that India has already taken significant steps towards implementing the International Convention on Biological Diversity, and the four principles on which it is based: conservation of biological diversity; regulated access to genetic resources; access to and transfer of technology; and international equity in sustainable utilisation of the components of biological diversity. However, there are some challenging issues for which the country will have to take new policy initiatives. These issues are :

— developing effective data bases for monitoring the causes of loss of biological diversity;

— strengthening *ex-situ* and *in-situ* conservation measures;

— developing legal procedures for providing access to genetic resources without affecting the rights of local communities, their knowledge, innovations, skills and practices;

— developing regulatory mechanism dealing with technology/transfer, biosafety, rights of local communities, regional cooperation and augmentation of financial resources; and

— enhancing capacity in the institutional mechanism, human resources and technology.

These issues need to be comprehensively addressed by policy makers, administrators, scientists, and government agencies and NGOs through a consultative process, considering both short and long term requirements. The recommendations evolved through such a process would enable the Government of India to evolve a comprehensive 'National Programme on Conservation of Biological Diversity' (MSSRF, 1993).

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FIELD EVALUATION AND UTILIZATION OF COLLECTIONS OF CEREAL GENETIC RESOURCES: THE CURRENT STATUS

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Large collections of cereals harbouring a sea of variations have been assembled in gene banks around the world. These collections need to be utilized in crop improvement to justify high amount of effort and time expended and the financial cost involved in maintaining them at sub-zero temperatures. Evaluation is the essential link between conservation and use. Various approaches to evaluation of germplasm have been described but the optimal is one that not only satisfies the criteria for current breeding goals but also takes into consideration the needs of the future since in-depth evaluation of large collections cannot be repeated at frequent intervals. The use of modern techniques, such as tissue culture, embryo rescue, and genetic engineering have resulted in greater use of wild and alien germplasm from the secondary and even tertiary gene pool for crop improvement. The rich genetic resources of such gene pools are increasingly tapped to overcome the constraints to food production in moisture limiting and other stress prone environments.

Key words : Breeding strategies, crop improvement, documentation, *Hordeum* spp., landraces, obsolete/primitive forms, *Triticum* spp., wild progenitors.

As a result of considerable increase in collection and conservation activity of crop plant genetic resources during the last two decades a very large quantity of germplasm accessions of cultivated and obsolete/primitive forms of crop plants have been assembled at various genetic resources conservation centers around the world. However, genetic resources merely kept safely in storage can be of little value to plant breeders for utilization in their programmes unless these are evaluated and the data is made available through communication and exchange. Evaluation is essentially the link between conservation and use. Keeping this in mind it would be conceptually much more fruitful to develop a working collection of germplasm for specific traits in order to

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conserve germplsm in a more usable form. The information on a large number of stored genetic resources is often limited to an accession number, a seed source and sometimes, the country of origin.

Systematic evaluation of the biodiversity in large collections requires a multi-disciplinary team and an inter-disciplinary approach (Chang, 1985 a; Frankel, 1970). Interaction between members of the evaluation team should be at optimal level for more rewarding results and greater utilization of germplasm.

The aspects of evaluation and documentation of cereal genetic resources has been reviewed recently by Damania (1990). Problems arising during evaluation of large collection and suggestions to solve them have been given by Pecetti et al. (1991). The utilization of wheat genetic resources to meet diverse needs has been documented for cultivated types by Srivastava and Damania (1990) and for wild forms by Damania (1993). This paper will endeavor to stimulate discussion regarding correct handling of cereal germplasm in order to optimize its utilization for current breeding goals as well as for future contingencies.

GERMPLASM EVALUATION

The evaluation process in seed crops may involve some of the following steps (Chang, 1985a):

1. Seed multiplication and preliminary evaluation: During the initial cycle of seed multiplication the evaluator or germplasm curator should note some of the morpho-agronomic features and other observations of interest such as incidents of diseases and pests. Obvious duplicates are eliminated and promising selected accessions are re-planted for intensive further evaluation. Plant quarantine regulations, if applicable, must be observed.

2. Systematic characterization: For base collection maintained by conservation centers extensive observations are recorded. For instance, durum wheat (*Triticum durum* Desf.) germplasm evaluation at ICARDA records information on 29 different characters (Srivastava and Damania, 1989). However, handling such voluminous amount of data requires dedicated attention to detail and is manageable only if executed by a competent team of scientists.

3. Further evaluation of selected accessions: Mass screening of accessions from a large collection must be subjected to refined testing procedures at different locations as many economically desirable traits are controlled by polygenes which behave differently in diverse environments. After this exercise the number of accessions will have already shrunk to manageable size for the breeder to handle effectively.

Germplasm which survives detailed evaluation and testing is normally suitable as potential crossing material for one or two specific traits, hence it could be utilized as a donor of these traits rather than as lines for release as commercial cultivars.

At the present time several evaluation projects have been undertaken in response to current selection criteria of the breeders. This approach may not be optimal because the probability of a sample being examined for a given attribute is solely dependent on present needs. However, a more comprehensive approach can provide information suitable for a more wide use. It is not implied here that it may be possible to always ascertain breeding aims of the future. For example, at the turn of the century, short stature (dwarfness) was not considered a desirable trait as it was calculated to be linked to small grain size and hence low yields.

For the purpose of utilization, systematic analysis and description of samples is useful both in distinguishing between populations, identifying duplicates as well as providing information on the extent of variation within a given germplasm collection. It is axiomatic that the more documentation on a collection, the greater the chance of its rational utilization. Information from the site where a particular sample was collected may be extremely important.

Inadequate passport data very often inhibit effective utilization of collected germplasm. It must be emphasized to collectors and gene bank managers that passport data supply extremely valuable, in many cases the only available, information on the ecological adaptation of an accession and hence efforts should be taken to fill this important gap in documentation of germplasm (Frankel, 1987).

Information on the genetic variability of a sample is extremely useful and the eventual objective of every evaluator should be to describe the variation on the basis of a list of differences between and within samples in the sequence of nucleotides in the deoxyribonucleic acid (Erskine and Williams, 1980). At present, the study of storage protein (prolamins) variants by electrophoresis is the most convenient and rapid method available for detecting genetic differences close to the DNA in a cereal collection (Damania et al., 1983). However, it may be argued that the heterogeneity in storage proteins alone is of little value to the breeders or genetic conservationists because its correlation with any single agronomic character is obscure. Nevertheless, these markers can monitor the relative genetic diversity with a greater degree of accuracy than by other methods used in the past (Brown, 1978; Damania, 1983). A pre-screening procedure for identification of the ploidy levels and chromosomal aberrations such as deletions in wheat, with the use of electrophoresis has been described by Damania (1985). Electrophoretic technique can also be used

as a tool for elimination of duplicate stocks in gene banks (Damania and Somaroo, 1988).

In any survey of the distribution of genetic variability within a crop species of economic value or its wild relatives, the most obvious pattern which emerges is the variability associated with broad geographical regions (Allard, 1970; Damania et al., 1996). It is important to sample not only the broadest geographical variability but also to have samples from the extremity of distribution of the species, i.e. the marginal areas. According to the hypothesis of 'peripheral diversity' put forward by Yamashita (1980) it has been suggested that there is considerable accumulation of diversity where a species has reached its geographical limits due to physical or climatic barriers which it cannot traverse.

Evaluation of cross-pollinated and self-pollinated plants differs when it comes to describing variability within a population. Some have recommended considering 50 individual plants per cereal populations which are largely self-pollinated. Others have suggested taking at least 100 seeds from 50 individual plants of a cross-pollinating species. Unfortunately there can be no universal answer to this question because each species sampled from a particular geographic area may represent a unique problem in evaluation. A precise solution would require information on variates of each individual ecological system, observable 'marker' characteristics associated with and indicating variability and ways in which individual variates interact with climatic features of the area to produce the overall variation pattern.

Systematic description of samples for discrete traits has been limited to curtailing the phenotypic variation because of constraints in relating genotype with phenotype. Quantitative morpho-agronomic traits are also currently used in characterization. These traits are controlled by many genes which have a small effect which is quite often blurred by the environment. Consequently the correlation between genotype and phenotype is obscured.

Many evaluation studies have used ranking as a method of describing results. This ranking may change from one site to another for some quantitative characters such as plant height and days to heading (Damania, 1983). Such unstable characters cannot be adequately described when studied at a single location. Thus the concept of multi-location testing becomes imperative.

And lastly, there are those traits, such as resistance to diseases and tolerance to certain types of soils (such as saline) for which variability can only be observed at particular sites. Such traits are extremely important economically and every effort must be made to record them by carrying out the evaluation at particular sites where the incidence of that particular disease or pest is the greatest, the so called "hot spots". For example, for screening against resistance to *Septoria tritici* (leaf blotch) ICARDA use a humid and

high rainfall site located near Lattakia on the Mediterranean coast in Syria. For experiments on tolerance to salinity and drought affected site on the shores of salt lake Jabboul in northern Syria is used. Jana et al. (1983) first used this site to evaluate 3000 durum wheat accessions from various countries and ten lines were found to be highly tolerant to combined stresses of salinity and drought.

Wherever the purpose of evaluation is clearly known at the start the task is relatively simple. However, in the case of most wild and obsolete/primitive forms, evaluation aims to reveal potentially useful variability for direct use in the breeding programmes. This may necessitate initial characterization nurseries (Damania et al., 1995) and cataloguing of passport information followed by a more detailed field study in collaboration with the end-users of the germplasm.

Although improving yield and yield stability are the ultimate goals in most plant breeding programmes the most sought after characters in exotic landrace germplasm are disease and pest resistance, tolerance to stresses such as temperature extremes, drought, salinity, etc. and nutritive values. For example, in the case of wheats, these characters are: stable resistance to various rusts, and *Septoria tritici*; new sources of genes for semi-dwarfness; greater tolerance to stresses such as droughts, salinity and temperature extremes; earliness; cytoplasmic male sterility; as well as other factors related to wide adaptability. Some of these traits are highly heritable, others are tightly linked to undesirable traits.

METHODOLOGY FOR EVALUATION

Evaluation process begins when a sample is collected because it is at the site itself that the collector takes certain decisions regarding the sampling procedures he applies to the population which determines the efficiency of subsequent evaluation. Therefore, when collected germplasm samples arrive at a genetic resources centre the process of their evaluation and documentation has already commenced. For a review of sampling variation in genetic resources of seed crops see Porceddu and Damania (1992).

In order to save time and effort, multiplication of the seed material is normally carried out at the same time it is evaluated. But sometimes, especially in the case of wild germplasm, this is not possible due to paucity of seed in the original collection. In this case the multiplication has to be carried out in the first season (or in the glass house for precious wild material) and later evaluated in the second season.

A population structure of a species is defined as the totality of ecological and genetical relationships among individual members which may co-evolve as a result of gene exchange but may also diverge under localized forces of

evolutionary change (Jain, 1975). Landraces and obsolete/primitive cultivars are as a rule products of several years of crop evolution and it is vital to preserve their genetic composition during and after evaluation. Instances have been reported where polymorphic cereal populations have undergone radical changes in their genetic composition in one growing cycle (Shevchuk, 1973). However, in the case of samples collected from village markets or those which are subjected to biased sampling methods, it is sufficient to safeguard and maintain their genes and not necessarily their gene frequencies within populations.

It is ideal to evaluate accessions of germplasm at suitable sites in countries of origin but this is not always practical for several reasons. Alternatively, the ecological environment of growth for the purposes of preliminary evaluation could be made as identical as possible to that of the collection area. However, this is not always possible for in a world collection samples originate from all corners of the globe where cereals are grown and from different ecological zones. Therefore, no single location can be entirely suitable for all samples. ICARDA is fortunate in being located within the centre of diversity for wheat (*Triticum* spp.) and barley (*Hordeum* spp.) and, as such, is as near to an ideal site for evaluation of these two genera (Srivastava and Damania, 1989). Evaluation carried out at such almost ideal site not only minimize the effect of natural selection on the samples' genetic make-up but also provide an adequate harvest of seed quantity sufficient enough to make further multiplication redundant.

Several International Agricultural Research Centers (IARCs) have more than one location for the evaluation of their germplasm. For instance, the International Centre for Maize and Wheat Improvement (CIMMYT), evaluates samples of maize that originate from an altitude below 1500m asl at Ttaltizapan, Morelos (940m asl) whereas those samples from above 1500 m are grown at El Batan (2249 m asl).

The relationship between active collections of national programmes and base, long-term collection located at an IARC is an important link in the chain of evaluation work and the flow of data and other information. For example, in the preliminary evaluation and subsequent regeneration of germplasm ill-adapted to the environment of the centre cooperative arrangements with the national programmes play a vital role. These arrangements also help in the eventual flow of selected germplasm and data (through international nurseries) into the national breeding programmes.

All IARCs possess their own base collections of the mandate crops and these usually operate through a genetic resources unit. A base collection may be duplicated at a suitable national gene bank to avoid total loss due to natural or man-made calamities. The centres with common mandate crops

such as CIMMYT and ICARDA could exchange and meticulously compare accessions to minimize the maintenance of obvious duplicates and to ensure that no distinct ecostrain is over-looked in the inventorial process. Original names and accession numbers should be maintained as far as possible when germplasm is received at the genetic resources units in order to cross reference them later to avoid duplication. Therefore, major germplasm collections like the ones maintained at the IARCs should be encouraged to standardize their data systems and achieve sufficient compatibility for easy accessibility and exchange of germplasm data.

Systematic evaluation is an expensive and time consuming process as any worker in this discipline will agree. Therefore, it is imperative to carefully chose the traits which one wishes to evaluate. Priority-wise, the inclusion of traits in an evaluation programme will result in optimal use of physical facilities, manpower and financial resources.

UTILIZATION OF GERMPLASM COLLECTIONS

The criterion for assessing the success of a crop-oriented germplasm evaluation project is the use to which the conserved genetic resources are put. Varietal improvement and the incorporation of yield stability in the improved cultivars through the use of landraces has also been impressive in the cereals. For example, Duwayri *et al.* (1987) crossed "Stork", a semi-dwarf high yielding cultivar under optimum conditions, with "Haurani" the local well adapted landrace in Jordan and Syria which produces reasonable yields under stress conditions. A number of lines which resulted from these crosses appeared promising in low as well as moderate rainfall zones.

There are three ways in which obsolete/primitive forms and wild relatives of our cultivated cereal crops can be utilized (Frankel, 1970):1. Introductions for direct use as crops.2. Introductions which can confer particular traits to the adapted cultivars such as, disease resistance, protein content, etc. This type of utilization is the most prominent way in which obsolete/primitive forms can be utilized and the only way for the wild relatives.3. Introductions to increase yield *per se*, irrespective of resistance to physical or biotic stresses present in the environment.

However, the wild species remain the least collected, conserved and exploited category of germplasm, especially from the secondary gene pool. The extent of evaluation and initial usage (some times called "enhancement") among the three categories of germplasms are almost proportional to the degree of utilization.

Plant breeders in wheat producing regions of the world mostly use breeding material with known agronomic data and background. They are reluctant to cross their commercial varieties, even after these have begun to

deteriorate in performance, with an obsolete/primitive landrace or wild species simply because they do not want to introduce undesirable genes into their breeding programme (Srivastava and Damania, 1989). Lack of information and the unavailability of substantial seed quantities are additional impediments in the use of these germplasms.

Spagnoletti and Qualset (1992) make a strong case for the maintenance of a "core collection" in order that genetic resources in gene banks are utilized in crop improvement programmes by breeders. The concept essentially advocates separating a few hundred samples from a much larger collection consisting of thousands of samples. This core collection may contain a wide range of variability for traits which are considered desirable by current breeders of the crop. The composition of the core collection may change from time to time as newer needs for particular traits become evident or as material which was not previously available joins the main collection as new acquisition.

It becomes obvious that if greater use of obsolete/primitive and wild material has to be made it is essential to remove (or at least suppress) the close linkage between desirable traits and unfavourable alleles. This may be done through transporting the germplasm to areas similar to the native habitats where selection can be carried out under favourable conditions of soil, photoperiods and temperatures. For wild species, particularly the putative progenitors, either a naturally introgressed population or an artificially directed back-crossing programme would improve their chances of inclusion in a breeding programme (Chang, 1985b). This preparatory activity is often referred to as germplasm enhancement or pre-breeding.

Sears (1956) gives a good example of pre-breeding efforts involving a wild relative of wheat. *Aegilops umbellulata* was initially crossed with *T. aestivum* cultivar but the F1 was male sterile and had to be back-crossed to *aestivum* twice. The progenies of this back-cross were tested for leaf-rust resistance which was present in the wild species. A resistant plant was isolated carrying 21 bivalents. This plant was then crossed with the then high yielding cultivar "Chinese Spring" to produce "Transfer" which was widely used in North America as a rust resistant cultivar. Since then other wild species of wheat have been utilized by Canadian and U.S. breeders as a source for improving winter-hardiness, short stature and cytoplasmic male sterility (Stalker, 1980).

The current situation in cereal breeding demands continual evolutionary studies and mobilization of new and different sources of germplasm. Most of these potential sources incorporate the true landraces, obsolete/primitive cultivars and the wild relatives from the primary gene pool.

At the International Rice Research Institute (IRRI) the utilization of these forms has already yielded promising results in producing lines with disease resistance as well as tolerance to drought and salinity. A strain with an

apparently desirable trait, e.g. disease resistance, may not be truly resistant; not being infected by chance or a result of genotype × environment interaction under the prevailing test conditions. Therefore, any resistant accession found in the preliminary evaluations of large collections must generally be retested, often with several replicates and in more than one season by refined techniques to ascertain the true nature of the resistance. The same applies to abiotic traits such as tolerance to temperature extremities, drought and salinity.

At ICARDA selected germplasm for low rainfall areas is tested at Breda and Bouider for wheat and barley accessions, respectively. This is done over several seasons with the weakest accession being eliminated after each selection process and only the truly drought tolerant lines are retained for crosses to incorporate this attribute in the cultivars.

Invariably in all situations the early generations derived from wheat × alien species hybridizations tended to be poor agronomic plant types. However, by employing appropriate breeding methodologies, plant types can be significantly improved phenotypically up to the state that alien disomic chromosome additions or derivatives with subtle alien introgression are produced.

The utilization of species even from the primary gene pool or wild progenitors presents some problems as several undesirable traits from the wild show up in the progenies of crosses and these need to be eliminated by repeated selection process. For example, in a simple *Triticum durum* × *T. dicoccoides* cross characters from the latter species, such as brittle rachis, glume hairiness, profuse unsynchronized tillering, hybrid necrosis, grass clumping and loose crown, persist in subsequent generations but rapid progress can be made by making a top cross of this material with durum wheat. The *Triticum durum* × *T. dicoccoides* cross also transfers some disease resistance qualities from the wild species to the cultivated. These crosses have also shown that selection for high protein content as well as yield can be transferred to the cultivated form. Whereas the durums have a relatively low percentage of protein and the *dicoccoides* a higher figure, in the resulting progenies high percentage protein is maintained. Similarly, the 1000-kernel weight of the durum varieties is much higher than that of the *dicoccoides* and in most of the progenies high 1000-kernel weight is retained (Srivastava and Damania, 1989).

The wild progenitor of barley, *Hordeum spontaneum*, was probably domesticated within the 'fertile crescent' region. This region is, therefore, in a unique position to assess its potential for improvement of barley yields in the dry areas. In recent years the barley project has commenced evaluation of *H. spontaneum* collected from the region where it can be found growing under

diverse ecological conditions. Because it also occurs in very harsh environments it is considered to be a probable source of resistance to drought.

The utilization strategies of *Horedum spontaneum* are as follows : a) screen for tolerance to drought; b) evaluate the extent of diversity within the species for agronomic traits; c) select a number of accessions to initiate a crossing project with cultivated barley and d) evaluate a number of early generation families between the wild progenitor and cultivated barley plants with non-brittle rachis. Evaluations carried out so far have revealed considerable variability in the collections for growth habit, cold resistance and days to heading. Accessions with a relatively good potential for crosses with improved cultivated barleys were identified (Ceccarelli *et al.*, 1987). Crosses will be made with selected cultivated lines (used as maternal parents) adapted to arid environments. Early generations of the hybrid material will be grown at several sites representing the actual barley growing environments.

CONCLUSIONS

The assessment of use of germplasm collections in crop improvement for the major cereals has revealed the following:

1. The use of exotic germplasm: The successful use of landraces and wild species in cereals has been more extensive in the developed countries, which lack original indigeneous germplasm, than in the developing countries. This has been partially due to the fact that exploitation of these germplasms was undertaken earlier by the former. After the creation of the IARCs the benefits of such germplasms have improved crop production on a global basis. Both Sudan and Syria have become self-sufficient in wheat production for the first time this century largely due to the adoption of improved germplasm developed by the IARCs.

2. Constraints to the use of exotic germplasm: Many plant breeders have been reluctant to devote a greater part of their resources for the exploitation of landraces and wild species in the past. This was because the potential value of these germplasms was not fully known. Insufficient germplasm pre-breeding, lack of communication between gene banks and users and little or no feedback from users are some of the other reasons often cited for poor utilization of the exotic genetic resources.

3. Support for plant genetic resources programmes: Extensive use of landraces, obsolete/primitive forms and wild species appears more tenable when the process of conservation, evaluation and exchange of germplasm is strengthened and adequately funded. Improved communication channels and maintenance of core collections can also augment breeders' use of exotic genetic resources.

4. Use of computers and software packages: Software packages designed for analyzing large quantity of evaluation data have greatly reduced time and effort needed for arriving at tangible conclusions and the selection of genotypes with superior performance. This in turn has led to the publication of germplasm catalogues which have greatly facilitated dissemination of information on germplasm collections to actual users, thus encouraging greater utilization of services rendered by gene banks.

5. Applications of bio-technology: Modern techniques such as chromosome engineering, anther culture and haploid breeding in cereals, could serve as a powerful tool towards reducing research time and can expand considerably the horizon of plant improvement.

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DEVELOPING A CORE SET OR SUBSETS OF LARGE GERMPLASM COLLECTIONS : AN OVERVIEW

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The concept of developing core germplasm set as a minimal yet diverse set, representing diversity of the germplasm collection has attracted wide attention in recent years with the expectation that it will provide an effective window to access the crucial genetic diversity. Procedures for selection of core entries have been considered in view of the available information on core collections for *Glycine* spp., wheat, barley, okra and others. An example of constituting a subset in okra and its proposed extension in *Sesamum* is given.

Key Words : Core collection, germplasm

Huge germplasm collections representing diverse genetic variability of crop species and their wild relatives have been accumulated by several International Agricultural Research Centres (IARCs) and many national programmes through continuing explorations and exchanges. The volume of these collections has outgrown the management resources. Scientific community is increasingly interested in assessing potential of the existing genetic diversity contained in these collections enabling promotion and use of plant genetic resources. Suggestions for ways of managing large, diverse germplasm collections to facilitate their efficient use despite their size have been proposed (Chang, 1991). Conventionally, the accessions were short listed after characterization and evaluation only by few morpho-agronomic attributes, identifying as the promising accession(s), for single or multiple traits, for promoting their utilization. Further, the idea of developing core collections has been proposed and debated for a long time leading to suggestions whether breeders need one core or many of them depending upon their requirements.

Frankel (1984) first proposed that a huge population could be condensed to a 'core collection' which would contain, with a minimum of repetitiveness, the genetic diversity of crop species and its wild relatives in the whole collection. The core subsets should include as much as possible of its genetic diversity. Accessions not included in the core would not be jettisoned, according to the proposal, but retained as the back up 'reserve collection'. Alternatively

defined, a core collection is a diverse, representative yet severely limited sample (Peacock, 1989; Palmer, 1989). The definition was modified by Frankel and Brown (1984), Brown (1989a, b) and elaborated by CENARGEN (1992) to state that the core collection is a part of the existing collection and does not replace it. According to IBPGR, core collection is to denote the part of a gene bank collection freely available on request without restriction (IBPGR, 1985). The alternative part, or 'reserve', is the one unavailable for general distribution because of restrictions by plant breeders rights, narcotic regulations, etc. This usage, however, has neither precedence nor semantics in its favour.

CONCEPT OF A CORE COLLECTION

Genesis

Collection and collation of characterization and evaluation data describing the extent of variation in the germplasm and a better understanding of distribution of the genetic diversity, in the base collection is required for effective management and utilization of PGRs (Hodgkin, 1991; Crossa *et al.*; 1992; Gawley, 1992; Jaradat, 1992; Kresovich *et al.*, 1992 and Rana, 1992 b). The 'core' concept revolves round the investigations on the extent and distribution of genetic diversity in a cultivated species and its relatives followed by the delineation of accessions that optimally represent this available genetic diversity.

For setting up of a core collection, a few assumptions should be fulfilled such as: the base collection includes (i) accessions from the entire range of adaptability of the species, (ii) all the congeners, to ensure representative inclusion of alleles, (iii) above 3000 individuals, to ensure inclusion of upto 70 per cent of selectively neutral alleles, (iv) related wild species, (iv) passport data, to ensure description of core entries with respect to geographical origin, status of the germplasm i.e. landrace, old or obsolete cultivar, breeders stock etc., and homogeneous or heterogeneous nature of core entries.

The word 'core' means the most central, the innermost part or the heart. Brown (1989a) opined that core collection was most important component of the Plant Genetic Resources (PGR) dogma. The term 'core' has been frequently used in scientific literature and fiction in different contexts, but its proposal in PGR was a radical departure (Frankel, 1984). Until then, efforts were concentrated on an open ended task, irrespective of continuing cost and use, for collecting as many samples as possible and securing them in gene banks. Elimination of duplicates was suggested to reduce the maintenance costs yet it was unlikely to have a significant impact due to already huge size of the collections and a high number of unique types (Chang, 1991). Besides, Frankel and Brown (1984) introduced the notion of adequacy of sampling of the species range. This implied stratification of accessions into non-overlapping groups

based on analysis of the passport information on eco-geographical parameters. The proposal included selecting a proportion of the germplasm accessions from these groups of base collection, on the basis of well defined sampling technique(s) to represent major kind of diversity. However, setting up a core in smaller collections, particularly in cleanly propagated crops, by eliminating genetical redundancies, was also recommended to promote resource utilization and effective management (CENARGEN, 1992). Two modifications to the core concepts have been proposed. Due emphasis was given by national PGR programmes to develop situation specific subsets of the base germplasm in order to promote utilization. Thus instead of developing a single core the germplasm would be categorised into 'usable' sets of collection which adequately represented specific traits like disease and pest resistance, tolerance to stress conditions and adaptability to specific ecogeographic range. Secondly, core subsets, alteration of the core sets, is normally used for a set of designated accessions within an existing collection. This approach is more suitable for situations where population size is no consideration and entries of the core need not be physically separable from the entire collection (Brown, 1992).

The needs of molecular plant breeders have been given added impetus to the designation of core collection. Identification of single genes using recombinant DNA technology have, of late, focussed the attention of plant molecular markers may be used for documentation of germplasm and detection of variability in collections. Efforts are also being made to develop trait-specific DNA probes for screening of germplasm accessions to detect genes for resistance to biotic and abiotic stresses as well as for desirable agronomic traits.

APPLICABILITY

Germplasm collections are reservoirs of biological diversity but search for desirable breeding stocks from these collections often takes very long time. Therefore, breeders depend heavily on limited strains evolved in various breeding programmes and tested in multilocational evaluation trials under different ecosystems. The core germplasm would assist in searching for desired traits more efficiently and authentically from the available core set and related passport data.

Designation of a core is needed for testing general combining ability of diverse representative germplasm with locally adapted strains for yield contributing traits (Frankel and Brown, 1984). Mating of a constant parent with core entries in a test cross can also provide fair estimates of combining ability with delineated set of germplasm (Zeuli, 1992). The combining ability of the standby subsets or reserve collections may be tested by 'Line x Tester' crossing with known lines out of the designated core subset. The in-depth studies

carried on various basic and applied aspects on accessions in the core would streamline their effective utilization.

Most of the germplasm related activities require making choice or set priorities among accessions. Difficulties encountered in decision making whether new additions in germplasm would add new information or they would be redundant, selecting a particular set of accessions for evaluation, testing for viability or regeneration or responding to breeders' requests are of great concern (Brown, 1989a). A set of well documented core collections will greatly assist in indexing new accessions into various categories and also provide a guide for conservation and exploration activities (Strauss *et al.*, 1988).

Ways and means for improving management or utilization of germplasm collections, the reservoir of candidate genes, through the application of powerful tools of biotechnology, which deserve sharp focus due to recent advances in techniques for rapid screening and gene manipulation, can be studied efficiently on a representative set of core collections than on a random lot out of base collection (Spagnoletti Zeuli, 1992; Vaughan and Jackson, 1992; Tohme *et al.*, 1992).

CRITERIA FOR ESTABLISHING A CORE COLLECTION

Genetic diversity in plant populations is structured in a way that reflects the biological characteristics, distribution and ecology of the species examined (Hamrick and Godt, 1990; Nevo *et al.* 1988; Nevo and Beiles, 1989). A core subset should contain the breadth of such genetic diversity. The mode of reproduction alongwith the geographic origin of collections is significant in determining the observed distribution of quantitative characteristics and allozymes (Spagnoletti Zeuli and Qualset, 1987; Kahler and Allard, 1981). Outbreeding (Allogamous) species often possess higher diversity and less genetically differentiated populations than inbreeding (Autogamous) ones. Further, grouping of unique accessions into different sets/subsets require elaborate passport data, but many accessions may lack such information. Variable migration rates of germplasm material in the historical past and/or well structured selections in defined regions might have lead to uneven differentiation among groups of accessions. Such irregular phenomena make it difficult to select representative and yet non-redundant set of accessions. Methods for selecting a core subset still continue to be a matter of scientific debate (Brown, 1983a, b; Chapman, 1987; Strauss *et al.*, 1988).

Various statistical approaches have been advocated for core identification based alternatively on the sampling or the population genetic theory. Aspects like size of base population, size of 'core' sample, adequacy of data including diverse representation of collections from across the globe, skewness of evaluation data, and probability of upward or downward bias in sampling

while developing a core subset are a few important considerations underlying the statistical approach for core delineation. The case for a designated 'core' would strengthen if the information loss is minimal.

The criteria for set up of an efficient, representative and unbiased core which revolves around the genotype (population genetic theory) or the phenotype (sampling theory) may be reviewed separately in the relative contexts.

POPULATION GENETICS THEORY

The germplasm accessions were broadly classifiable into four classes of alleles based upon the preponderance or rarity of different alleles into their genetic build up (Marshall and Brown, 1975, 1983). The accessions classified under Class I carry widespread and common allele, whereas those represented by Class II have widespread and rare allele; the Class III individuals may posses localized and common allele and class IV accessions have localized and rare allele in their genetic background. The priorities for inclusion of various classes of germplasm based on gene frequency may vary according to the preponderance and occurrence. Least priority is given to deliberate inclusion of class I as the widespread and common allele has a high probability of inclusion in any one of the indirect selection criteria. Class III variables may have a cross-adaptive value and thus be used sooner or later in breeding programmes and may be safely represented in the core by taking care of appropriate sample sizes. Inclusion of common-localized alleles poses certain problems for selecting neutral alleles. Brown (1989a) used the neutral allele model of Kimura and Crow (1964), and its sampling theory (Ewens, 1972; Nei, Maruyama and Chakravarty, 1975). They assumed random sampling in order to meet the baseline expectations. However, stratified random sampling based on ecological factors can strengthen the inclusion of widespread and rare alleles (class II) in core collections. Localized and rare allele in class IV is highly desirable for a 'core' due to the fear of its being lost during regeneration in routine. These localized rare alleles may also be new recurrent mutations which could be useful for mutation breeding programmes. This class has many a times been put forward in debates as a counter example to proposed strategies (Bogyo *et al.*, 1980). However, the accessions carrying localized rare alleles can be included in the flexible core accomplished on their subsequent detection during exhaustive statistical analysis of evaluation data followed by verification from rapid screening techniques.

Brown (1989a) showed that in large populations irrespective of different levels of polymorphism, a 10% sample (at least 3,000 accessions) of the entire population retained at least 70% of the 'rare -widespread' group of alleles with a frequency greater than 0.0001 and with a confidence limit of 95 per cent.

Schoen and Brown (1992) discussed assemblage of core collection of wild crop relatives in terms of maximisation of allelic diversity. Such issues are helpful for theoretical core collections but information about individual alleles in wild relatives is difficult to obtain due to limited availability of base primers to detect alternate alleles and also because alleles don't occur independently (Gawley, 1992). The representativeness of core collections in terms of available genetic diversity in the base population can be validated at different levels viz., allelic representation in terms of allozymes, RFLP analysis etc. (Brown, 1989b), phenotypic representation in terms of clustering diversity for quantitative traits (Mackay, 1992; Crossa *et al.* 1992) and then delineating core entries. Yet another level of representativeness ie. the genetic level which would be most useful for efficient PGR utilization and which may be expressed in terms of elaborate multilocational evaluation traits, combining ability analysis and even inheritance studies is difficult to attain due to extremely high level of input resources required to meet such objectives. On the other side, the definition of base population would also have to be viewed at two different planes depending upon existing strategies of core delineaion (Mackay, 1992). Accordingly, international genepool based core collection in terms of efficient management under networking of genebanks and situation specific subsets (Rana, 1992 b) or alternatively terms as 'specific attribute based subset' (Mackay, 1992) in terms of efficient utilization of PGR's strongly fulfil the criteria for core setup population genetic theory.

SAMPLING THEORY

Sampling, drawing of a representative sample from the population, is bound to result in some loss of information in terms of adequate representation and remainders. The sampling technique e.g. stratified sampling are found adequately efficient for grouping collections into distinct groups (Brown, 1989 b) and better choice over simple random sampling, a default option.

The hierarchy of grouping is based on elaborate pass port and characterization data including taxonomy of the crop. At first step the taxa may be subgrouped or split according to the origin of accessions, as major regions based upon political maps i.e., continents, countries, states, or ecogeographic regions. Further, the collections subgrouped within a region may be clustered into groups of similar accessions for important inherited characters like resistance to major pests and diseases, pathogen races or physiological stresses etc. through multivariate clustering analysis (Spagnoletti Zeuli and Qualset, 1987). The hierarchical cluster analysis could, however, be used as a tool to classify germplasm collections even when no passport data is available (Peeters and Martinelli, 1989). Ideally, the extent of diversity within each cluster should be same as far as possible otherwise the selection strategies need be suitably modified. Lack of authentic information on evaluation data

or missing data, thereby making uneven distribution among accessions, leads to problems in hierarchial cluster analysis which generally require a complete rectangular data matrix. Exclusion of accessions with incomplete data is likely to reduce diversity of the core collection to a considerable degree (Gawley, 1992). Information on genetic diversity, from cytological studies, marker loci, or quantitative characters, if available, can also be used to ensure that each group has a substantial level of diversity.

Sample selection of accessions within each group for constituting the core may be guided by different strategies, viz., (i) Constant strategy (C): selection of equal sample size from each group, (ii) Proportional strategy (P): number of accessions being proportional to the total number of accessions in the particular group, and (iii) Logarithmic strategy (L): sample size being proportional to logarithm of population size in each group, have been widely acclaimed (Groth and Roelfs, 1987, Brown, 1989a, b). In addition, a few more sampling strategies have been proposed by various workers *viz.*, (i) Genetic-multiplicity-dependent strategy (G): sample size proportional to the amount of genetic multiplicity available in groups (Yonezama and Nomura, 1992). (ii) H-strategy; takes into account Neis' genetic diversity index (H) or panmictic heterozygosity to estimate variation among geographical regions using marker locus data in effective population size (Ne) or indices correlated with Ne (Schoen and Brown, 1992). It assumes selective neutrality of the marker locus variation and also that the populations are isolated. (iii) M-strategy: also suggested by above authors uses non-linear programming methods to pinpoint; accessions for maximum diversity within each geographical region. (iv) R-strategy: involves random sampling of accessions irrespective of stratification. Further, Gawley (1992) used C, L and P strategies for evaluation of effectiveness of the Shannon index H′ to account for the diversity of a discrete descriptor based on proportion of accessions having the ith descriptor state (P_i) where $H^l = -\sum p_i \log p_i$. Thus, a situation specific selection of particular sampling strategy would help developing a more meaningful, representative and effective core subset.

A well elaborated, systematic order for hierarchical (stratified) sampling has been reported by Chang (1991) in preference to random sampling. According to author the prioritis for stratification (continents or regions); species (*sativa, glaberrima*); ecogeographical race (indica, japonica, javanica, hybrid); geo-political information (country of origin); cultural type and hydroedaphic regime (lowland, upland, deepwaer, tidal wetland etc.); maturity, plant stature, other morphoagronomic traits; grain type (dimensions and shape, pericarp colour, endosperm type); known economic attributes (pest and stress tolerances); special types with genetic information; isozymes, seed proteins, RFLP's data, if available. The same can be suitably adapted, in general, subjected to modifications as per specific requirements of more or different strata in other crops as well.

Marshall (1990), on the other side, advocated that random, stratified-random proportional to geographic frequencies and based on statistical analysis of evaluation data as most optimal sampling strategies to ensure alleic inclusion from the four classes of their prevalence.

For delineation of core sets in structured populations, sampling proceaures from reserve collections were investigated on the principal of maintenance of largest genetic multiplicity within a given amount of resources (time, labour, facilities etc.). Empirical studies indicate that the P-strategy would have the widest range of application over C or L, although strategy G is expected to be superior to others in situations with known degree of genetic multiplicity within groups whereas the strategies H or M were most useful in situation where genetic diversity among loci within accessions was positively correlated (Yonezawa and Nomura, 1992). Brown (1989 b), however, demonstrated that strategy L was better placed for sampling accessions in *Glycine tomentella* and USDA's Barley core collections

EXISTING CORE COLLECTIONS

A survey was initiated at the end of 1989 by IBPGR to look into the ongoing work on core collections by scientific community throughout the world. Based on the overall interest shown by various national and international organizations, over 20 projects involving cereals, legumes, forage crop species, fruits and vegetable crops were identified (T. Hodgkins, Pers. Comm.). But till date the number of core collections with published information is very limited. At the same time, the absence of a well defined approach in the published literature on core germplasm does not substantiate any definite criteria for future guidelines. A few representative projects related to core collections are reviewed hereunder.

GLYCINE Spp.

A core collection of 111 accessions of perennial *Glycine* spp was developed in Canberra, Australia (Brown *et al.*, 1987) from a collection of around 1400 accessions of 12 species. The criteria in choosing the accessions for developing a dynamic core were (i) more than one accession per *Glycine* spp. selected to provide replication for generalization of results from any basic or applied research carried out from these accessions at species level, (ii) geographical coverage in respect of political states as well as scatter and range of all possible habitats in Australia, (iii) inclusion of morphological, cytological and isozymes groups with known intraspecific variation and (iv) priority inclusion of accessions used for research in the past and the first hand collections. The core thus constituted was much different from a random set and achieved an overall target of about 10 per cent sampling. proportion from each category

i.e. species, geographical region or political state etc. varied drastically (Brown, 1989a).

Further, Brown (1989b) intensively demonstrated the effect of sampling strategy on specific inclusion of accessions in the core on *Glycine tomentella*, a species collected extensively from all across its natural range in Far East Asia and Oceania and which comprised 5 diploid (2n = 38,40) and tetraploid (2n = 78,80) groups. In this example the choice of alternate strategies (C, P or L) showed marked effects on the chance of including rare types. For the diploid strategy P gave the best recovery whereas for the tetraploids strategy C was the best option. The strategy L, however, emerged as a good compromise and was thus favoured by Brown (1989b) in cases where the representative set of germplasm showed skewed distribution for rarest variants, e.g. in diploids, occurring in the largest group and tetraploids, available in the smallest group, in *Glycine tomentella.*

OKRA

Hamon and van Sloten (1989) established a core collection on okra based on the variability in passport, characterization and evaluation data from a single trial. A core comprising 189 accessions out of 2,283 accessions was developed with specific inclusion of rare types. This core collection on core was set up with a slightly varied approach in order to have a manageable collection scaled down to the needs of the breeders and other users. In order to include widest possible range of variability it was constituted primarily on the basis of rigorous statistical analysis of the available data on agromorphological characters. It suffered major limitations in terms of missing passport data to the tune of 20 percent for collectors' number, 30 per cent for town/province, 33 per cent for latitude/longitude 60 per cent for vernacular names and 99 percent for altitude thereby making it difficult to follow a stratified random sampling based on population genetics approach.

The statistical analyses resorted to were (i) univariate analysis *viz.*, for quantitative descriptors and some basic statistics like mean, range, standard deviation, coefficient of variation and frequency distribution for taxonomical or qualitative morphological descriptors, (ii) bivariate analysis e.g. correlations among quantitative traits, (iii) multivariate analysis like factor analysis, principal component analysis and hierarchical clustering methods elucidating the euclidean distances among various quantitative characters. Three distinct groups were earmarked at the euclidian distance level of 0.80 and a choice at level 0.30, which authors availed in this situation, allowed elimination of four descriptors.

Some of the interesting observations for setting up core in okra included using the cultivation system as an important source of information wherein

the graphical representation of variability by means of factorial analysis corresponded closely to the farming systems used, long tradition of okra cultivation in a particular country/agroecological zone and the choice of morphological descriptors. Generalization of such an indirect approach is, however, difficult to make due to the non- availability of descriptors like Farming System in the routine data recorded by collectors.

Other significant features, emerged from the core collection were that the reduction in number of descriptors obtained through multivariate analysis emerged as another significant feature in the okra core collection. A stepwise approach consisted of running a principal component/factor analysis projecting the variability in limited dimensions. Then a clustering analysis was done which could be later tested by a discriminant analysis. In such an approach the choice made by the curator/database manager leads to a deliberate but controlled loss of information. Nonetheless, the base collection was effectively reduced in size to a manageable representative set for further use.

WINTER WHEAT

A 'core' on Australian winter wheat collection was developed by Mackay (1990) on the basis of passport, morphological and agronomic data. This core collection was built up as a specific attribute based subset rather than genebank based core collection. It puts considerable emphasis on the use of ecogeographic data for selecting accessions that were likely to include all the diversity for a particular desired character. This approach could be easily extended to developing situation specific subsets in the national PGR systems of the developing countries as proposed by Rana (1992a).

BARLEY

Since 1989, an approach to develop a barley core collection was under consideration by the barley working group of the European Cooperative Programme for Conservaion and Exchange of Genetic Resources (von Bothmer *et al.*, 1990). It is based on World's barley holdings, and comprises the whole genus *Hordeum*, including the secondary and tertiary genepools as well as genetic stocks (Kunpffer, 1992). This is an obvious alternative to the built up situation specific or specific attribute based subsets. It was further mentioned that the accessions in the core would be maintained as homozygous lines. To ensure the maintenance of core by genebanks, different phases in the establishment and operation of core collection including networking of international barley genetic resources were described. It was further proposed that genetic and cytogenetic marker stocks be included.

Brown (1989b) reviewed the core set up of worked barley collections of USDA (Kahler and Allard, 1981) for the effect of various sampling strategies

(strategies C, L and P and simple random sampling R) on esterase diversity found in the accessions using geometric probability for sampling with replacement (Emigh, 1983; Brown, 1989b). About 75 per cent of the alleles were likely to be included in a 10 per cent core, as predicted. The sampling variance was expected to be greater than that for independent loci although the total would not be affected by the intense linkage disequilibrium among the loci. There was a meagre effect of the different strategies on allele inclusion for any one of the 4 esterase loci, eventhough strategy L was best for polyallelic esterase 2 locus. This was ascribed to coarse level of stratification and the former exchange of barley germplasm around the world.

COMMON BEAN

A core collection of 1400 accessions in *Phaseolus vulgaris* (common bean) from about 24,000 accessions stored at CIAT using evolutionary, agromorphology and agroecological data was established (Tohme *et al.* 1992). The core set comprised of 1200 accessions from primary centres, 200 from secondary centres and about 25 a priori selected bean lines as reference. A weighted stratified model was used. First an arbitrary weight as assigned to geographical areas delineated by similar subjective ecological classification. In corollary to the length of adoption of landraces in different farming systems in case of okra, the germplasm from the primary centers, in common bean, was assigned a higher grade than from area of recent introduction. The second level of stratification was based on morphological data.

SORGHUM

A core collection of sorghum was planned by the Germplasm Resources Unit of ICRISAT which holds over 33,000 accessions from 88 countries. The size of core collection was worked out to be 3,475 i.e. nearly 10 per cent of the whole collection (Prasad Rao, 1992). For indentifying core accessions usage of three types of data was proposed (i) country of origin/geographical diversity, (ii) taxonomic group in numerical data of agronomic traits for cluster analysis. In this analysis wild sorghum were excluded as they need different descriptors.

COFFEE

Out of nearly 20,000 genotypes representing about 75 taxons and raised under live conditions, 88 diversity groups corresponding to 73 taxons were taken into account for the hierarchical scheme of future coffee core collection (Hamon *et al.*, 1992). The core entries were proposed to be selected using principal component analysis to remove multicollinearity between descriptors and euclidian distances were weighted by standard deviation for measuring the basic distance. Genotype most distant from centroid (higher relative

contribution) was selected as the first genotype which maximizes the selected variability. Genotypes x descriptors interaction was involved to guide retention of accessions for the core or selection of descriptors for the analysis.

ALFALFA (*MEDICAGO SATIVA* L. SENSU *LATO*)

A core set of 200 entries was extracted from about 1100 perennial *Medicago* P.I.s collections from 47 countries (Basigalup *et al*, 1995). The entire collection was classified into 18 geographical groups based on the available passport information on Germplasm Resource Information Network (GRIN) system. Within each group, entire were selected by eight methods (multivariate procedures, random and/or direct selection, and a totally random selected core) and comparison was made by sign test (a nonparametric statistical test). The method based on direct selection of entries within each group was the best one and was ultimately used for the selection of core entries. Also, the entries extracted by this method retained the greatest variability for 38 of 50 agronomic, forage quality, root and crown marphology, pest resistance and stress tolerance traits. Further, the methology uses incomplete data sets.

In another study, a core collection of 200 accessions (Diwan *et al*, 1994) was delinneated from a subset of 1240 germplasm accessions of 36 annual *medicago* species on the basis of 16 agronomic and morphological traits. Cluster analysis was followed to classify the accessions in each of the species separately. Within species, accessions were chosen proportionally to the country of the origin in the germplasm collection. Species with more clusters were considered more diverse than those with fewer clusters. One accession per cluster was selected. The core collection when grown for 2 years showed stability for the evaluated traits.

In addition to the above published evidences scattered information is available from various national programmes on the ongoing work in relation to core set up. In USA, a core of around 505 lines of chickpea has been delineated out of a germplasm collection of about 3491 accessions maintained at WRPIS (w-6), Pullman WA. The core germplasm was set up based on a few determinant characters in the descending priority order viz. 'country of origin > seed colour > seed size > seed shape > seed surface etc.' (Hannon, R.; Pers. Comm.*). This core collection was intensively screened for blight and other foliar diseases for effective utilization of representative germplasm. The pea core collection developed at Pullman, USA included 505 accessions (17.5%) in the core from a total of 2886 accessions. To efficiently represent genetic diversity, their first step was to include all *P. sativum* spp. *elatius* members based on high level isozyme variation in this group. In the second step, accessions were selected from the material of various countries based on number of accessions representing a particular country. Due to low seed

inventory, seed is only available for 84% of the core accessions (Hannon R., pers. Comm.). Loos and van Duin (1989) identified a set of 19 characterization traits and an additional set of 11 evaluation traits important for breeding in order to establish a core collection representing available genetic variation in tulip.

CROP NETWORKING IN RELATION TO CORE

In the development of the core set or subsets accessions are selected from the existing holdings of genebank/s. These holdings are primarily available in base and/or active collections. The conceptual core collection may be safely treated as a curator guided working collection for the particular crop species. The plant improvement programmes, on the other side, revolve round the selection criteria and selection response on deliberately set up base population 'also referred to as working collection' which fulfills the criteria to meet the breeding objectives. A single working collection, leading to the set up of a base population for a defined breeding programme after a thorough uni/bi/multivariate analyses of the available data on quantitative traits, may be derived from a single base collection or may be developed from more than one base collections from different genebanks. Herein, the bulk population may be kept separate as a conservation alternative rather than an alternative for core set up or for active use in breeding programmes. Further, the base population/s may have be set up for individual breeding objectives, for incorporation of single or multiple traits from one or more of the working collections, core collections, base collections or collections over different genebanks depending upon the degree of diversity available.

The degree of diversity captured in a core set will be a reflection of diversity among accessions available in the genebank whereas the narrowness in apparent diversity for the adaptability factors may be specifically attributed to missing passport data as pointed out by Hamon and van Stolen (1989) in ORSTOM/IBPGR Okra collection, yet the real test of diversity could be provided by the biochemical or the genetical markers. Diversity is also restricted due to the closeness among cultivars originated from single breeding programme, co-adaptiveness of the landraces, or co-evolution of primitive cultivars etc. Various PGR programmes have duly recognized the need of conserving duplicate sets of germplasm accessions across the genebanks. There is a need of international collaboration to devise appropriate strategies likely to be of importance in maximizing the benefits of investigations on establishing core collections. The set up of international networks for effective exchange of database alongwith duplicate sets would help in appropriate statistical analysis, reduce the chance of availability of missing data for representative geographical sections of accessions and increase the probability of inclusion

of fullest range of diversity of the crop species in its core. The research workers' interest lies in exploiting maximum diversity in the genebanks for the core set up and, if the need be, to go in for further explorations in specific regions either unaccounted for or sparsely represented in the 'core'. It is thus desirable to have collaboration between genebanks, users and researchers in order to meet the objectives of maximum availability as well as diversity among the accessions. In other words, the development of crop-networks is closely linked to an effective core collection.

The basic idea of establishing crop-networks in order to strengthen core collections could be viewed differently at the international and national levels. Various international agencies like CGIAR, IBPGR and IARCs share the global responsibilities for crop based PGR programmes. On the other side, the national programmes like USDA, NBPGR would tend to fulfil the requirements for farming systems' need based, agroecological situation specific subsets for their respective countries and also contribute to global reserves through collaborative exploration, exchange and duplicate sets etc.

The region specific smaller PGR units within the national set ups constitute significant and integrated part within the national PGR network (Rana, 1992b). Besides other factors, such a netowrking within a national PGR programme would be helpful in retrieving further evaluation data (FED) from the multi-locational tests at National Active Collection Sites (Rana *et al.*, 1993) which would ultimately provide desirable database for constituting core collections. The need based, situation specific subsets, of the developing countries, may be developed by following either of the population genetic approach under stratified sampling using C, P or L strategy as in Soybean (Brown, 1989 a, b), in case the passport data is complete, or biometrical approach using statistical analysis of the evaluation data on quantitative characters as done in Okra (Hamon and van Sloten, 1989).

DEVELOPING A CORE SUBSET PROGRAMME AT NBPGR

A flow diagram depicting the essential features in establishment of core subsets program is given in Fig. 1. Essential features of such a programme aimed at developing a minimal core subset representing the diversity of base collections, would be as follows :

At the onset, grouping of accessions in the base collection is carried out on the basis of passport data for ecological, geographical or climatic factors, plant types through appropriate multivariate statistical tools. In the next step, within group selection is carried out for accessions accounting for maximum variability in qualitative and quantitative descriptors. A group may invariably be split into sub-groups equaling total number of combinations for a few

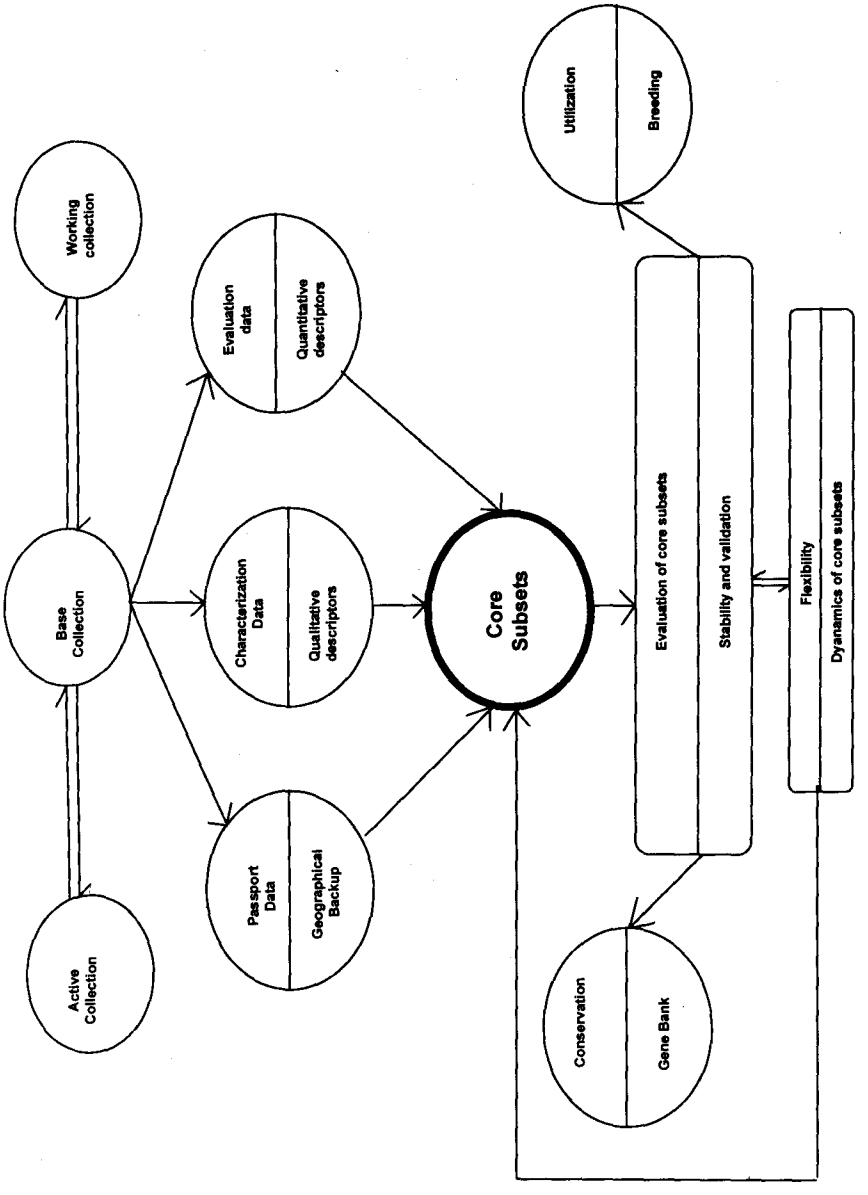

Fig. 1. Flow diagram for establishing core subsets

important qualitative descriptors at thistage, showing a fairly spread distribution order for accessions explaining maximum diversity within each subgroup through some appropriate multivariable technique (Mahajan *et al*, 1996). At the same time, Shannon Diversity Index (SDI) for these accessions is computed, for the important qualitative traits, on a cumulative scale. Accessions are retained upto a stage when the SDI of accessions becomes approximately equal to to greater than the corresponding value for the entire group/sub-group. The accessions thus selected from different sub-groups within a group would constitute a core subset. Situation specific subsets may be similarly obtained from other other groups.

A sample core set in okra has been worked out with limited size of base collection originated from series of interrelated exploration programmes and covering the variability from South East Asia, including some SAT (South African Taxons). There is ample variability for a species/taxa and the within species accessions in this set. However, this limited set was exercised at the initial stage to suit the available softwares of non- hierarchical cluster analysis which could accommodate 500 accessions and 10 quantitative descriptors and also to confirm the validity of the above approach. The 260 okra accessions produced 8 homogeneous unequal groups on the basis of quantitative characters (Bisht *et al.* 1995). In a later assessment of the database, these groups were ascribed to plant types. Each group was split into subgroups depending upon its size, frequency distribution of accessions on qualitative characters and variability observed within groups. Accessions were then selected from within each group/subgroup to account for maximum variability in quantitative characters, using Principal Component Technique, and qualitative characters using Shannons' Diversity Index (SDI). The selected accessions from various subgroups within a group consisted of a sample core set. This core set comprised of 50 accessions to which 3 rare types were deliberately incorporated making it a final set of 53 accessions.

Sesamum collection, more than 4,550 accessions is maintained at NBPGR. To provide effective germplasm utilization, improved genebank management and also to reduce the effective size of genebank holdings for different crops, a collaborative project with IPGRI for establishing a core collection has been initiated. The development of the methodology is in progress.

Identitficaton of Core Collections is likely to help in greater utilization of germplasm holdings in breeding programmes since researchers will find it to be a more practical way to reach ultimately the primary collection which is really the target for identifying desired traits and also for diversifying the sources of resistances to pests and pathogens among other goals.

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DIVERSITY IN PEARL MILLET GERMPLSM FROM SUDAN

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Out of 589 pearl millet (*Pennisetum glaucum* (L.) R. Br.) germplasm accessions from Sudan assembled at ICRISAT Asia Center, Patancheru, 581 were evaluated for morphological and agronomical characters during the rainy and postrainy seasons. The infloresences were small, thin with small corneous grain. Almost all defined spike shapes and grain shapes were found indicating considerable diversity in the germplasm from Sudan. The unique feature of 40 per cent yellow grain germplasm which is a rich source for carotene serves as a genetic base to develop improved cultivars with yellow grain. As most of the accessions produce several thin stems with high biomass, they are useful to breed forage types.

Key words : Diversity, pearl millet, germplasm, Sudan

Sudan lies between 22^0 and 39^0 E longitudes and 4° and 22° N latitudes (Anonymous, 1969). Climate of Sudan ranges widely from tropical type in the northern desert to wet southern part with more than 1000" rainfall in some areas. Most of the northern Sudan is dry sandy, while south of Khartoum between the White Nile and the Blue Nile rivers is wide clay plains. In Sudan, pearl millet is the most important crop next only to sorghum. It is grown on an estimated area of 1150 thousand hectares producing 221 thousand metric tones grain (FAO, 1994). The genebank at ICRISAT Asia Centre (IAC) had assembled 589 pearl millet germplasm accessions from Sudan. Since characterization of germplasm is a prerequisite to asses the diversity and to identify promising genetic stocks for crop improvement, all accessions were evaluated for morphological and agronomical characters during two contrasting seasons at IAC, which is described in this paper.

MATERIALS AND METHODS

Pearl millet germplasm assembled at IAC from Sudan include 6 accessions from the Rockefeller Foundation assembled in India, 140 collected by IBPGR

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and the rest collected by ICRISAT. They were evaluated at ICRISAT Asia Centre (IAC), Patancheru (18°N latitude and 78°E longitude) in alfisols during two contrasting seasons - the rainy and postrainy. Germplasm accessions were grown in an augmented block design with repeated checks for every 20 test accessions. For evaluation, each accession was planted in 2 rows of 4 m length spaced at 75 cm apart with 10 cm space between plants within the row. Life saving irrigations were provided in rainy season, but during the postrainy season irrigations were provided at regular intervals. Fertilizers were applied at the rate of 100 kg nitrogen and 40 kg phosphorus ha^{-1} during both rainy and postrainy seasons. Observations were recorded on selected morphoagronomic characters as per the descriptors for pearl millet (IBPGR and ICRISAT, 1993). Observations on time to 50% flowering, plant height, spike length and spike thickness were recorded both in rainy and postrainy seasons. Observations on other characters such as spike density, bristle length, endosperm texture, green fodder yield potential, grain yield potential, and the overall plant aspect, were scored visually on a 1-9 scale during rainy season, where 1 is the lowest or least desirable, while 9 is the highest or most desirable (IBPGR and ICRISAT, 1993). Grain characters were recorded in postrainy season only. The data were analyzed using GENSTAT statistical program.

RESULTS AND DISCUSSION

Considerable diversity was observed for several characters, such as time to 50% flowering, plant height, tiller number, spike exsertion, spike length and spike thickness (Table 1), and grain characters. In general, germplasm from Sudan flowered late with a mean flowering time of 86 days during rainy and 71 days in postrainy season. Time to flower ranged from 47 to 128 days during rainy and 38 to 120 days in postrainy season. The accessions which flower in less than 50 days such as IP 9866, IP 9894, IP 13326 in rainy season and IP 10831 in postrainy season are good sources for early maturity. The rainy season at IAC is characterized by long days and high temperature compared to the postrainy season. During the rainy season, the day length decreases from 13.93 hours in June to 12.47 hours in October. In the postrainy season it varies from 12.1 hours in November to 12.7 hours in March. Mean monthly temperature varies from 34.1°C in June to 20.2°C in October in rainy season and 10.6°C in November to 36.3°C in March. The reduction in flowering time during the postrainy season compared to the rainy season could be attributed to differences in day length and/or temperature sensitivity. On the other hand, IP 8631, IP 8658, IP 8661 and IP 13331 did not show any difference in flowering time due to season indicating their insensitivity to photoperiod and temperature.

Pearl millet germplasm from Sudan grew very tall with a mean height of 259 cm during the rainy and 131 cm during the postrainy season. The

range of variation for plant height is considerable and varied from 90 to 420 cm during the rainy and 60 to 285 cm in postrainy season (Fig. 1). All the

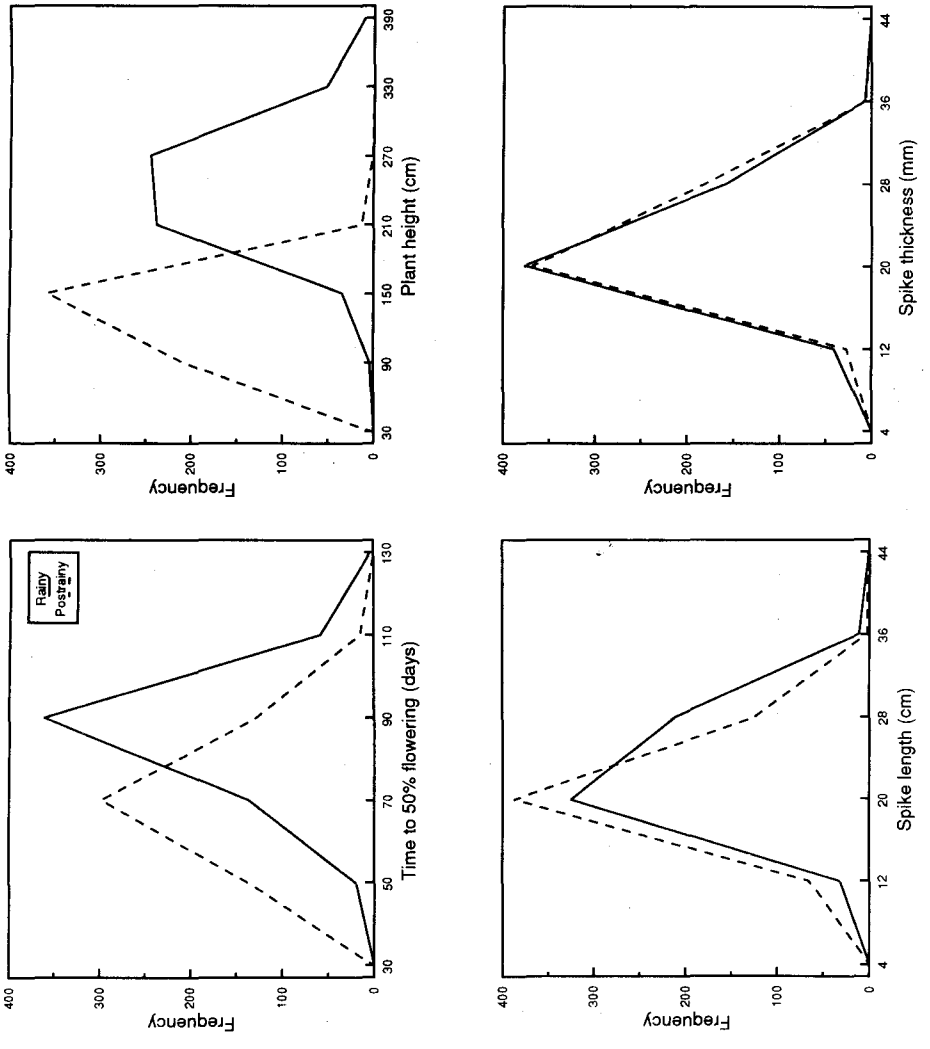

Fig. 1. Expression of pearl millet germplsm from Sudan when evaluated at IAC during rainy and postrainy seasons.

581 accessions, except 6 grew taller during rainy season when compared to those in postrainy season indicating the sensitivity of pearl millet for plant height. Out of six, only two accessions IP 8282 and IP 10932 were not affected by the planting time for plant height, and four accessions IP 8014, IP 11706, IP 11711 and 11714 grew taller in postrainy season. Plant height was reported to vary considerably depending on the growing season (Carberry and Campbell, 1985; Maiti and Sato, 1990). The reduction in plant height during the postrainy season was also associated with reduction in flowering time. Based on flowering time and plant height it could be considered that pearl millet from Sudan is both day length and photoperiod sensitive. IP 8645 which flowers in about 100 to 115 days grows upto 3m height in both rainy and postrainy seasons and IP 8652 which flowers in 100 days grows over 4 m are found to be good sources for forage.

In general, pearl millet germplasm from Sudan produces several basal tillers ranging from 1 to 16 with a mean of 3, but some of these tillers do not produce spike, so, the mean productive tillers was only 2 per plant (Table 1). Pearl millet from Sudan is more diverse and relatively a good source especially for productive tillers, when compared to the millet from other countries of Sahel region such as Central African Republic, Nigeria, Niger, Mali, Senegal etc. IP 8291 which produces more than 10 productive tillers per plant may be suitable for both grain as well as forage purpose.

Table 1. Diversity for agronomic characters of pearl millet germplasm from Sudan

Character	Range	Mean ± SE
Time to flower (days)-R*	47-128	86.0 ± 0.53
Time to flower (days)-PR*	38-120	71.0± 0.56
Plant height (cm-R	90-420	250.3 ± 1.94
Plant height (cm)-PR	60-285	131.6 ± 1.09
Total tillers (no.)	1-16	3.2 ± 0.08
Productive tillers (no.)	1-10	2.1 ± 0.04
Spike exsertion (cm)	-15-17	3.4 ± 0.23
Spike length (cm)-R	13-42	23.1 ± 0.19
Spike length (cm)-PR	9-46	21.4 ± 0.19
Spike thickness (mm)-R	13-37	22.0 ± 0.17
Spike thickness (mm)-PR	12-38	22.6 ± 0.17
Grain weight (g)	3-15	8.8 ± 0.06

*R = Rainy; PR = Postrainy season.

Spike length varies considerably (Fig. 2) and range from 13 to 42 cm in rainy and from 9 to 46 cm in postrainy season. Spike length is relatively stable over seasons as the mean spike length was 23 cm during the rainy and 21 cm during the postrainy season.

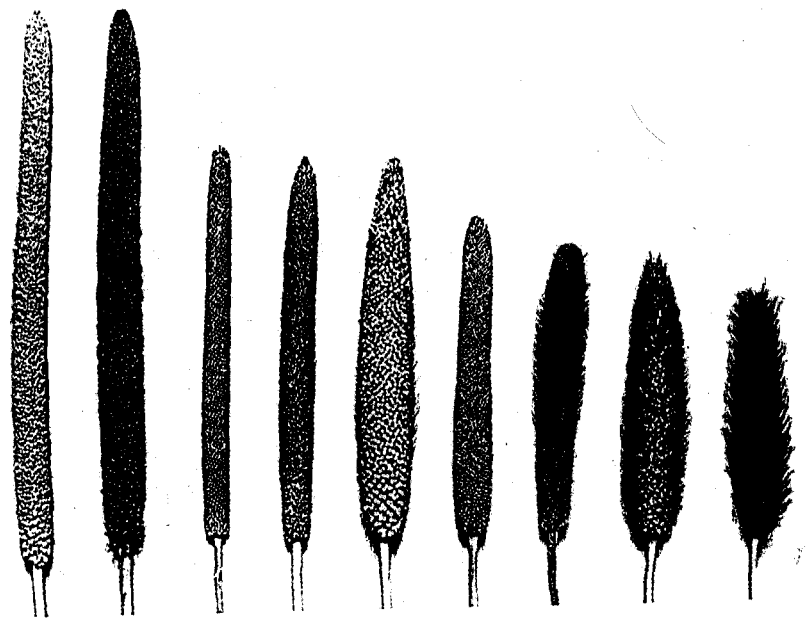

Fig. 2. Diversity for spike characters in pearl millet germplasm from Sudan

Variation for spike length among different plants within an accession is less compared to variation among accessions. Six accessions; IP 8736, IP 9867, IP 10772, IP 10775, IP 10893 and IP 13328 produced spikes more than 35 cm long in rainy season and IP 8655, IP 11716 and IP 11718 during postrainy season. Spike thickness, which is the diameter of the spike at maximum thickness, mainly depends on the length of the involurce, rachis thickness and grain size was most stable as the range and mean were almost the same during both the seasons and varied from 12 to 38 mm with a mean of 22 mm (Fig. 1, 2). Only two accessions; IP 9884 and IP 13337 in rainy and two accessions; IP 8010 and IP 13345 in postrainy season produced thick spikes (more than 35 mm). Variation for grain size was considerable as the grain weight ranged from 3 to 15 g per 1000 grains (Table 1). However, the mean grain weight is 8.8 g per 1000 grain, which is higher when compared to the mean of world collection (Mengesha and Appa Rao, 1986). As many as eight accessions namely IP 0847, IP 11678, IP 11679, IP 11682, IP 11683, IP 11684,

IP 11685 and IP 11689 produce large grains with grain weight more than 12 g per 1000 grains.

The relative length of the peduncle is one of the important characters for the production of quality grain. In accessions showing poor or negative exsertion, a part of the spike is held within the boot leaf leading to improper seed set, harboring of insects and diseases. Pearl millet germplasm from Sudan is highly diverse for spike exsertion ranging from -15 to 17 cm with a mean of 3.4 cm (Table 1). Accessions like IP 9840 and IP 10741 which showed considerable positive exsertion (more than 15 cm) are very useful in pearl millet improvement.

Table 2. Diversity for spike shape, grain shape and grain size in pearl millet germplasm accessions from Sudan

Character	Number of accessions	Frequency (%)
Spike shape		
Cylindrical	280	48.2
Conical	19	3.3
Club	1	0.2
Candle	187	32.2
Dumb-bell	2	0.3
Lanceolate	90	15.5
	5	0.8
Grain shape		
Oblong	110	18.9
Lanceolate	35	6.0
Elliptical	26	4.5
Hexagonal	117	20.1
Globular	293	50.4
Grain color		
Cream	33	5.7
Yellow	231	39.8
Gray	76	13.1
Gray brown	158	27.2
Brown	27	4.6
Purple	41	7.1
Other colors	15	2.6

No. of accessions studied = 581

Among the characters studied, spike shape was the most stable trait during both the seasons. Pearl millet germplasm from Sudan is highly diverse for spike shapes (Fig 2). The most common spike shape is cylindrical which accounts for 48 per cent of the accessions followed by 32.2 per cent of candle and 15.5 per cent of lanceolate spike shape (Table 2). Spikes of conical, club, dumbells and oblanceolate shapes are also found, but, they occur at very low frequency.

All the described grain shapes (IBPGR and ICRISAT, 1993) were found in Sudanese pearl millet germplasm, which vary from oblong to globular (Table 2). The most common grain shape is globular that account for 50 per cent of the total accessions. Globular grain shape is a character of the race globosum which is widely distributed in Western Africa (Brunken et al. , 1977). The occurrence of majority accessions with globular grain in Sudan suggests its similarity to the millet in Western Africa (Bono, 1973; Kumar and Appa Rao, 1986). The other major grain shapes to the world collection are 4.0 per cent for hexagonal, 3.4 per cent for oblong, 1.3 per cent for elliptical and one per cent for lanceolate shape.

The unique feature of pearl millet from Sudan is the occurrence of yellow grain,a rich source of carotene, the precursor of vitamin A, which is necessary for health of the eye. Hence, yellow grain is preferred by farmers over gray, brown, or purple. About 40 per cent of the pearl millet germplasm from Sudan produced yellow grains followed by gray-brown (27.2 per cent) and gray (13.1 per cent). However, other grain colors such as purple (7.1 per cent), purpish black (0.7 per cent) and ivory (0.2 per cent) were also found. Similarly, Pearl millet from Sudan is a rich source for purple and purplish black grain accounting for 26 and 15 per cent respectively in the world collection. Based on grain color, farmers in Sudan classify pearl millet in to three forms; *Dukhun Akhder* produces gray colored pericarp *Dukhun Abeit* has white and *Dukhun Ahmer* has red pericarp (Prasada Rao and Mengesha, 1980).

Considerable variation was also found for characters scored visually on a 1-9 scale (Table 3). More than 50 per cent of accessions showed synchrony for maturity with semicompact to compact spikes. As many as 23 accessions scored 8 for spike density which is an important yield contributing character. Almost all accessions produced spikes with very small bristles. However, few accessions IP 8009, IP 8708, IP 8713 and IP 9845 produced long bristles (Fig 2). More than 70 per cent germplasm produced partly starchy to corneous endosperm. Germplasm from Sudan is relatively a good source for grain yield potential with majority (77 per cent) accessions scoring 5 and 6 along with good score for green fodder yield potential (score 6 and 7), and overall plant aspect (score 5 and 6). However, IP 10833 with a score of 8 for grain yield and IP 9843 with a score of 9 for fodder yield were found as promising.

Table 3. Frequency distribution for different traits of pearl millet germplasm, visually scored on 1-9 scale

	Frequency (%)						
Visual Score	SSM	SPD	BL	ET	FYP	GYP	OPA
1	0.	0	61.3	0	0	0	0
2	0.7	0.7	27.4	0.7	0	0	0
3	3.1	1.4	6.5	2.9	0.9	1.2	1.0
4	5.9	6.5	1.6	5.7	4.1	14.6	25.0
5	19.3	32.2	1.4	17.0	11.7	48.4	50.0
6	30.6	36.5	0.7	22.6	36.0	29.4	21.2
7	34.1	18.8	0.5	25.8	44.9	6.2	2.9
8	6.4	4.0	0.5	22.7	2.6	0.2	0
9	0	0	0.2	2.6	0.2	0	0

**SM = Synchrony of Spike Maturity SPD = Spike Density
BL = Bristle Length
FYP = Folder Yield Potential
OPA = Overall Plant Aspect
ET = Endorsperm Texture
GYP = Grain Yield Potential

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GENETIC RESOURCES FOR DISEASE RESISTANCE IN WILD DIPLOID *TRITICUM* AND *AEGILOPS* SPECIES

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One hundred and twenty accessions of wild diploid species of *Triticum* and *Aegilops* were screened for resistance to stem rust (*Puccinia graminis tritici*), leaf rust (*Puccinia recondita*), stripe rust (*P. striiformis*) and powdery mildew (*Erysiphe graminis tritici*) under natural and artificial epiphytotic conditions in adult plant stage at Wellington and New Delhi. Twelve accessions belonging to *T. monococcum* and *Ae. squarrosa* were tested for resistance to karnal bunt (*Neovossia indica*). Einkon wheats, *T. boeoticum*, *T. monococcum* and *T. sinskajae* exhibited spectacular resistance to leaf rust and powdery mildew. Many accessions of *Ae. speltoides* showed resistance to stem rust also. Resistance to all the rusts and powdery mildew was observed among species such as *Ae. comosa*, *Ae. markgrafii*, *Ae. uniaristata*, *Ae. mutica* and *Ae. umbellulata*. Twelve diverse wheat stocks carrying specific genes derived from diploid wild species were also evaluated for rusts. The study revealed that the genes, Lr9, Lr28, Lr32 and Lr36 conferred a high level of resistance to leaf rust while Sr32 conditioned adult plant resistance to stem rust.

Key Words : *Triticum*, *Aegilops*, wild species, adult plant, stem, leaf & stripe rusts, specific genes.

The improvement of any crop lies in exploring and exploiting the rich gene pools available in plant's wild relatives. In India, wheat is attacked by several diseases, but rusts (*Puccinia* spp.), powdery mildew and karnal bunt are some of the important diseases of concern. Since the resistance in cultivars breaks down in a short span due to the evolution of new races in the pathogen, the need for additional sources of resistance against diseases arises to counter them. In the search of additional variation the progenitors of hexaploid wheat and other diploid *Triticum and Aegilops* species are becoming increasingly important. In the present study, evaluation of wild wheats was carried out to identify new and diverse genetic resources against rusts, powdery mildew and karnal bunt. A number of specific genes for rust resistance that have been transferred from diploid wheats are available in common wheat background. These genes conferring resistance to different rusts were also evaluated for rust resistance against Indian rust races flora and powdery mildew collected from the Nilgiris.

MATERIALS AND METHODS

The collection of wild diploid wheats available at the Indian Agricultural Research Institute, New Delhi was partly received from USDA and IPSR Cambridge, UK. The diploid wheats included the immediate progenitors of common wheat (*Triticum aestivum* L., 2n = 6x = 42, genome AABBDD), the species belonging to sitopsis group and other genomes such as U, M, C, T and Un. These species were evaluated for rusts and powdery mildew in adult plant stage at Wellington (South India), a'hot spot' for foliar diseases of wheat under natural epiphytotic conditions over a period of six seasons. The material was also screened under artificial epiphytotic conditions at Delhi for three seasons. All the accessions grown in pots were artificially inoculated with most virulent races, 40-1, 117-1 of stem rust (*Puccinia graminis* Pers f. sp. *tritici* Eriks & Henn.) and 77-1 77A, 77-2 and 104 B of leaf rust (*P. striiformis* West.) and powdery mildew (*Erysiphe graminis tritici* M. Marchal) was also recorded at Wellington. Rust reactions were recorded by combining the severity (percent infection) and response (type of infection) at adult plant stage while powdery mildew was scored on 0 to 4 scale. A few accessions belonging to A and D genomes were artificially inoculated in boot with Delhi isolate of karnal bunt (*Neovossia indica* (Mitra) Mundkar) using hypodermic syringe (Ajula et. al., 1982). The infection percentage among inoculated ear heads was calculated. The infected grains were also graded according to severity of karnal bunt infection on 0-4 scale. The data is not presented in the table because the species tested exhibited more or less similar infection type and pattern, but results are discussed in the text.

RESULTS AND DISCUSSION

Results are presented in Tables 1 and 2. Among the progenitors of the common wheat (*T. aestivum*), einkorn wheats, *T. boeoticum* ssp. *aegilopoides* exhibited spectacular resistance to leaf rust, stripe rust and powdery mildew at adult plant stage, while spp. *thoudar* was resistant to all the three rusts and powdery mildew. The accessions of *T. monococcum* also exhibited resistance to leaf rust, but resistance to stripe rust was of moderate magnitude. The accessions G1872, G2508, PI427446, PI427447 amd PI427481 of *T. boeticum* and SWAN 181 of *T. monococcum* were also resistant to stem rust. The strains of *T. urartu* in general were recorded susceptible to stem rust and stripe rust and moderately susceptible to leaf rust. However, the accession TMUO1 showed immunity to stem rust while G2035 and G3249 exhibited low reaction to stripe rust. Cultivated einkorn wheat *T. sinskajae* was found to carry resistance genes for all the three rusts and powdery midew. This species is morphologically similar to *T. monococcum* but free threshing. The accessions of *T. boeoticum* have been shown a high level of resistance in other geographical areas also (Zohary et al., 1969; Gill et. al., 1983).

Table 1 : Adult plant reaction to rusts (*Puccinia* spp.) and powdery mildew in wild diploid wheats

Species/accessions	Genome	Range of reaction to			
		stem rust	leaf rust	stripe rust	powdery mildew
1	2	3	4	5	6
Einkorn wheats					
Triticum boeoticum Bioss. ssp, *aegilopoides* (2n = 2x = 14), G2508, G1872, PI427446, PI427447, PI427481	AA	0	0	0	0
ssp. *thaudar* Revt.		0	0	0-TS	0
G2171		20S-50S	0-TR	0	0
102-1, G1848, G2398, G1372		30S-60S	0	0	0
T. urartu Tum. (2n= 2x=14)	AA				
SWAN171, SWAN172, 199-1, G1953		30S-60S	5MR-20MR	20S-70S	1
TMU 01		0	5MR-30MR	30S-50S	0
G2035, G3249		50S-70S	40S-50S	TS-5S	2
T. monococcum L. (2n=2x=14)	AA				
SWAN 181(IARI)		0	0	0	0
TMM 01, G1481, G2927, G683, G1372, G1471, ssp.*flavescemce, vulgare, nigraflavescence, hormanii*		20S-70S	0	10MS-15MS	0
G1483		30S-60S	0	TMS-5S	3
T. sinskajae A. Filat & Kurk.	AA	0	0	0	0
Sitopsis group					

1	2	3	4	5	6
Aegilops speltoides Tausch. (2n=2x=14)	SS				
K, TS 08, 2-3, ligustica, SWAN 471, PI369602, A, EC331772, EC331782		0	0	0	0
EC331782, EC331783, EC331784, EC331785, M, TS 01, TS 05, *typica*		0	5S-10S	0	0-1
EC331780, *ligustica tyoica* 3-1		TMX-20MX	0	0-TS	0
D, 2-1		10MS-40S	0	20S-40S	0
Ae. bicornis Forsk. (2n=2x=14)	S^bS^b				
SWAN 121, *typica* 4-1, *mutica*, *SWAN481 EC160080, EC160182*		0	40S-60S	30S-40S	0
EC162412		0	20S-30S	0	0-1
SWAN657, *typica* 5-1, TB 01		30S-50S	10S-40S	0	0
Ae. longissima Schw. et Musch.	S^1S^1				
SWAN 144, G130B, PI318697		0	0	0	0
G609, SWAN 143		5MR-60MS	50S-60S	40MR-50MR	0
Ae. aucheri Boiss.2n= 14, SWAN151	SS	0	0	0	0
SWAN 152		0	TR-10R	40S-60S	0
Ae. sharonensis Eig. (2n=2x=14)	S^sS^s				
G614, G1315, EC162416		0	0	50S-70S	2
SWAN 156, EC160079		0	40S-70S	60S-70S	0
Ae. searsii	S^sS^s				

1	2	3	4	5	6
TS 01, EC160086, TS 10		0	0	30S-50S	0
Ae. squarrosa L. (2n=2x=14)var. *typica*, EC331750, SWAN 475	DD				
SWAN 476, EC164801, EC331786		30S-50S	0	30S-60S	3
*strangulata**, EC 164800*, EC331751*		40S-60S	TR-TS	0	0
EC162403, EC331756, EC331757, EC331787, EC331788*		30S-50S	50S-80S	TMS-10MS	1-2
A, PI220641, *anthera*, typica 2-1, *meyeri*, *squarrosa*, SWAN 477*, SWAN478*, SWAN480*		30S-60S	30S-50S	60S-80S	3
Ae. umbellulata Zhuk. (2n=2x=14)	UU				
4033, 5901, PI 341797		5S-40S	0	0	0
Ae. comosa Sibth. and Sm. (2n=2x=14)	MM				
A, EC331776, EC331789, EC331790		0	0	0	0
EC162406, EC331753		0	15S-40S	0-TS	0
G		0	0	5S-20S	0
Ae. markgrafii (Zhuk.) Bowden	CC				
PI298888, PI369571, PI551126		0	0	0	0
PI542197		5S-10S	30S-40S	0	0
Ae. mutica Boiss. (2n=2x=14)	TT				
M&K		0	0	0	0
Ae. uniaristata vis. (2n=2x=14)	UnUn	0	30S-40S	0	0
PI276995					

*tested against karnal bunt

Three specific genes conferring resistance to stem rust, namely, Sr 21 (McIntosh unpublished), Sr 22 (Kerber and Dyck, 1973) and Sr 35 (McIntosh et. al., 1984) have been transferred from *T. monococcum* to common wheat. However the genes, Sr 21 and Sr 22 were found ineffective to stem rust at adult plant stage (Table 2). Overall the einkorn wheats offer an excellent source of genes for resistance to leaf rust and stripe tust. Most of the accessions belonging to A genome except G2035 and G3249 of *T. Urartu* and G1483 of *T. monococcum* have shown resistance to powdery mildew fungus occurring in the Nilgiris. Only six accessions viz., SWAN 181 and G1481 of *T. monococcum* TMU01 and G1953 of *T. urartu* and G2398 and PI427481 of *T. boeoticum* were inoculated with karnal bunt. All these accessions showed 0 to 5 per cent infection indicating that einkorn wheats are highly resistant to karnal bunt. A large number of accessions are to be tested to draw a more meaningful conclusions.

Table 2. Adult plant response of specific genes against stem rust, leaf rust and stripe rust

Stocks	Gene(s) present	Range of reaction to			Powdery mildew
		stem rust	leaf rust	stripe rust	
Transfer	Lr9	60S-90S	0	30S-40S	3
CS/Add. A 6U 7813-2	Lr9	50-S70S	0	30S-50S	3
RL5406/Thatcher*6	Lr21 Sr 33	20MS-30MS	20MR-30MS	60S-70S	4
RL5404/Thatcher*6	Lr22a	80S-90S	20MR-40MS	5MR-10MR	4
CS 2A/2M 3/8	Lr28	80S-90S	0	5MS-10MS	1
RL5497-1	Lr32	50S-70S	0	60S-70S	3
C 78.10/Condor	Lr 36	40S-60S	10R-20MR	20S-40S	3
Einkorn-*T.monococcum*	Sr21	30S-50S	10MS-30MS	TS-5S	1
Marquis/Stewart/ T. monococcum	Sr22	30S-40S	10MS-30MS	40S-60S	3
W3531	Sr32	10MR-20MR	TMS-5MS	10S-30S	2
W3725	Sr32	10MR-20MR	TS-5S	10S-20S	2
Compair	Sr34Yr8	70 S-90S	10MS-30X	10S-15S	2
Hexaploid derivative	Sr35*	-	-	-	-

*Seed not available: - not tested

Among the sitopsis group, *Ae. speltoides* showed a high level of resistance to stem rust, leaf rust and powdery mildew. Only a few strains exhibited resistance to stripe rust. The accessions of *Ae. bicornis* and *Ae. sharonesis* were found resistant to stem rust and powdery mildew except the accession EC162412

of *Ae. bicornis* which showed resistance to stripe rust also. An accession of *Ae. aucheri*, similar to *Ae. speltoides* showed immunity to all the three rusts under heavy natural infection. In general, the species belonging to sitopsis group possess genes for resistance to stem rust and leaf rust, but resistance to stripe rust is inadequate. The accessions of *Ae. searsii* were found highly susceptible to stripe rust only. Most of the accessions belonging to S genome showed resistance to powdery mildew fungus prevailing in the Nilgiris. These species were not tested for karnal bunt resistance. The gene Lr28 is highly effective against the Indian leaf rust races while Sr32 and Lr36 conferred moderate degree of resistance to stem rust and leaf rust respectively (Table 2). The major gene pool represented by the sitopsis section remains largely untapped for disease resistance. These results of screening of the genes alongwith the unidentified resistance indicated that the specific resistance (s) are very effective against Indian rust race flora. Dhaliwal et. al., (1986) evaluated a large collection of wild wheats and identified many accessions to have genes for resistance to different diseases. A high frequency of resistance to powdery mildew and leaf rust occured among the *Aegilops* species (Gill et. al., 1985).

Most of the *Ae. squarrosa* accessions tested at Wellington or at Delhi showed susceptibility to stem rust, but a few accessions have exhibited high level of resistance to leaf rust and only two accessions were found resistant to stripe rust. Kihara et. al. (1965) in a study concluded that *Ae. squarrosa* possess numerous forms with resistance to both stem and leaf rusts occuring in Japan. Kerber and Dyck (1978) determined a high frequency (44%) of forms resistant to leaf rust than to stem rust in a study of 85 accessions of *Ae. squarrosa*. The variant *strangulata* used by them for leaf rust resistance was found free to leaf rust and stripe rust races prevailing in the Nilgiris. Only three accessions of *Ae. squarrosa* var. *strangulata*: EC 164800, EC 331775 and EC162403 possess high to moderate degree of resistance to powdery mildew fungus. Eight accessions were inoculated with the Delhi isolate of karnal bunt disease and they were found to carry a high degree of resistance. Resistance to karnal bunt has earlier been identified among wild wheats and *Aegilops* species (Dhaliwal et. al., 1986; Warham et. al., 1986). Multani et. al. (1988) screened synthetic amphiploids involving susceptible *T. durum* with *T. monococcum, T. boeoticum* and *Ae. squarrosa*. In their study all the synthetic amphiploids except one were free from Karnal bunt disease indicating that wild progenitors of common wheat, *T. monococcum, T. boeoticum* and *Ae. squarrosa* carry genes for karnal bunt resistant and the resistance is expressed in the presence of *T. durum* compliment.

Only three accessions of *Ae. umbellulata* were tested for rusts and powdery mildew. All the three strains were found to carry resistance genes for leaf rust, stripe rust and powdery mildew. However, these accessions were observed to be susceptible to stem rust.

All the seven accessions of *Ae. comosa* showed adult plant resistance to stem rust while two of them were found susceptible to leaf rust. Low infection of stripe rust was recorded on *Ae. comosa* G and EC162406. Although both the genes, Sr34 and Yr8 deriving resistance from *Ae. comosa* are ineffective, the resistance available among other accessions may be very useful, if utilized.

Four accessions of *Ae. markgrafii* (syn. *Ae. caudata* L.) were screened at adult plant stage against four races of leaf rust and two races of stem rust for two seasons at Delhi only. Except the accession PI542197, all were found to possess resistance to stem rust and leaf rust. Only two accessions of *Ae. mutica* (syn. *T. tripsacoides* Jaub. & Spach.) showed a high degree of resistance to stem rust and leaf rust in adult plant stage at Delhi. The only accession of *Ae. uniaristata* tested, was observed susceptible to leaf rust, but possess a high degree of adult plant resistance to stem rust, stripe rust and powdery mildew.

Overall, the study revealed that the wild diploid wheats possess multiple resistance to diseases. Most of the species except *Ae. squarrosa* are potentially useful and may prove to be an excellent and diverse source of disease resistance. Interspecific and intergeneric hybridization in the *triticeae* (Muzeeb-Kazi, 1993) could be attempted for improvement of wheat and to enhance bio-diversity for searching new genetic recombinations. Work on the transfer of disease resistance and other desirable attributes from wild species into common wheat is under progress.

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LANDRACES OF RICE (*ORYZA SATIVA* L.) IN BIHAR

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The paper highlights the richness of genetic diversity in landraces in Bihar. Progress of work done on exploration, collection, maintenance, evaluation, utilization, seed supply and storage of rice landraces in the state and their current status have been presented.

Key words : Landrace, *Oryza*, diversity, ecosystem, genotype

Bihar is the reservoir of landraces of rice with rich genetic diversity. The landraces are spread all over the state, having three geo-morphological cum agro-climatic regions namely (i) North Bihar Gangetic Plain (ii) Central Bihar Gangetic Plain and (iii) Chotanagpur Plateau (Verma and Saran, 1974) (Fig. 1). Each of the regions has specific macro and micro environments with high level of variability and presence of genes for adaptation to different situations.

Systematic approach in respect of collection, evaluation and catologing of germplasm from various rice ecosystems of Bihar would help substantially in the rice improvement programme of the state and the country.

MATERIALS AND METHODS

During 1920's and 1930's 250 genotypes were collected from North Bihar and 5000 collections from all over Bihar and Orissa. In recent past 2000 landraces were collected from the plains that included 1250 from North Bihar and 750 from Central Bihar. More than 1000 collections were made from Chotanagpur.

During 80's and early 90's NBPGR undertook explorations in Chotanagpur and parts of north-east Bihar and collected 452 landraces (Chandel *et al.*, 1989, Gupta and Tomar, 1994).

Maintenance and evaluation has remained an important activity of the rice germplasm. Duplicates were discarded. Samples were maintained and

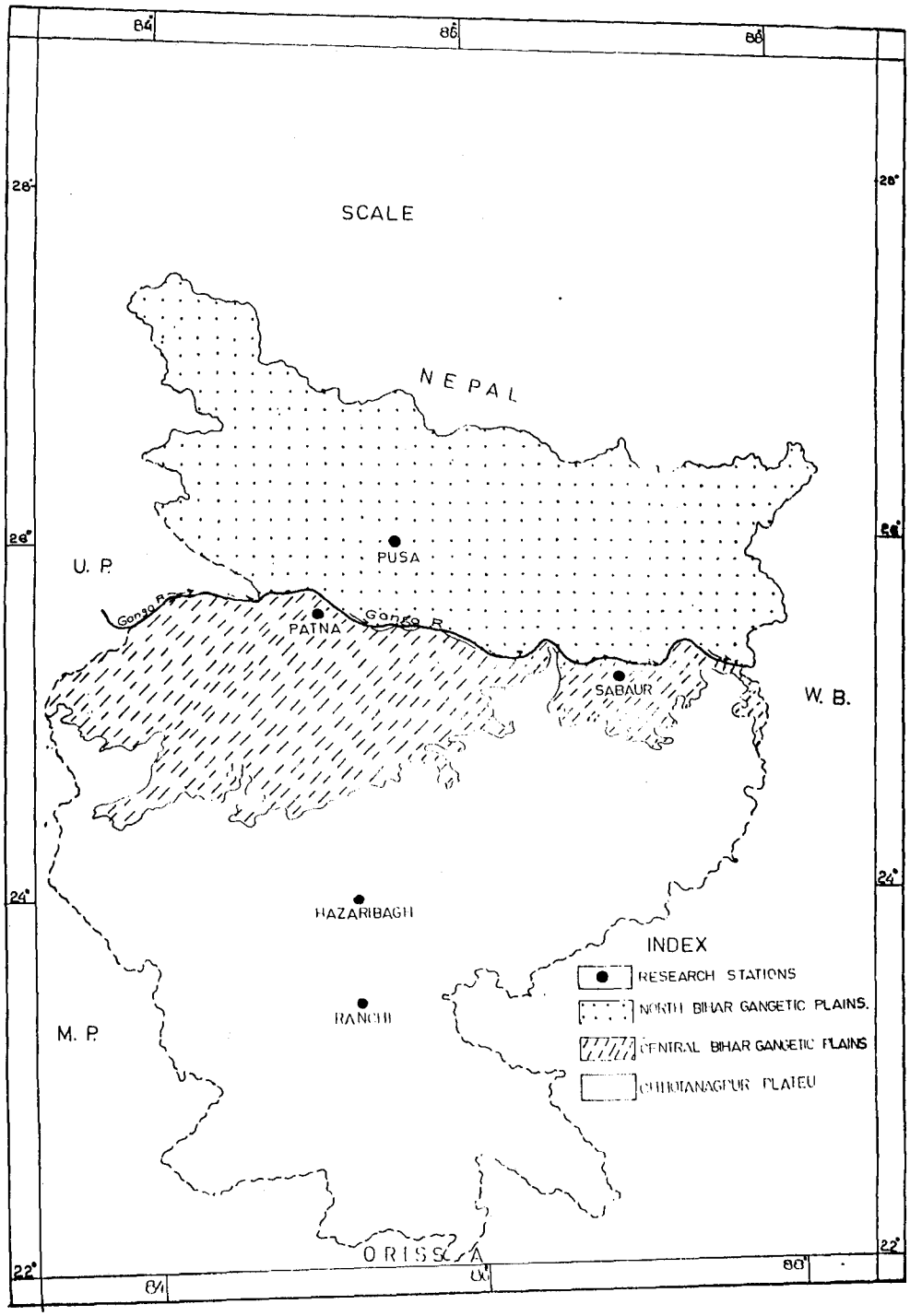

Fig. 1. Geomorphological divisions of Bihar and the research centres associated with the rice germplasm conservation

evaluated for their qualitative and quantitative characters and the accessions from Bihar were designated using prefixes BK, Br and RAU.

RESULTS AND DISCUSSION

The important landraces have been presented in Table 1 for central Bihar plains, and Table 2 for north Bihar plains.

Table 1. Important landraces of central Bihar Gangetic plains

District	Landrace	Area of adapability	Important characteristic
1	2	3	4
1. Patna	Laljori, Basmati 3, Rajmahali, Karibank-2, Dhura, Laldaiya, Mahin dhan, Sagarbhog, Lalka Bawag, Baranti, Baitarni, Sahdeyia, Gajpati	Rainfed medium lands/ lowlands	Tall, photoperiod sensitive, late duration.
2. Bhojpur	Sirhanti, Backoia, Jhulan, kalamdan, Mohan katiki, Basmati, Jhulanwa Gajmukta, Laldaiya, Kapsa, Karibank, Mohan, Bakoria, Moti, Pandey, Bir Bahadur, Bangla Katika, Kanakjeera, Ghilanwa, Badshahbhog, Sonachur, Ramoal.	Rainfed medium land/lowlands	Tall, photosensitive, late duration, Sonachur, Karibank, Basmati, Badshah bhog and Kanakjeera are aromatic rice with fine grain.
	Kamani, Sairha	Rainfed upland or medium lands.	Early maturing tall, photoperiod insensitive.
3. Rohtas	Lalkatika, Ghorbahra, Mohan katika, Madanwa, Korangi, Katarni, Awaria, *Katika, Sahdaiya,* Sahanhkatika.	Rainfed medium lowland	Tall, photoperiod sensitive, late duration.
	Kuari, Sairha and Ashbharan	Rainfed upland/ medium land.	Early maturing tall photoperiod insensitive.
	Sonachur, Shyamjeera, Basmati-3, Shahpasand.	Rainfed medium/ lowland.	Tall, late duration, fine grained, aromatic.

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1	2	3	4
4. Gaya	Kalamdan, Bajratwa, Olwa, Kanehonehur, basmati.	Rainfed medium/ lowland.	Tall, late duration, photoperiod sensitive. Kanehonehur and Basmati are aromatic with fine grain.
5. Aurangabad.	Batasi, Keshoria, Silhat, Shyamjeera, Poreya, Olawa, Dukh harni	Rainfed medium/ lowland.	Tall, late maturing photoperiod sensitive. Shyamjeera is fine grained aromatic.
6. Bhagalpur.	Kesore, Dahia, Kalamdan, Delongi, Tulsimanjari, Badshah bhog, katarni bhog.	Rainfed medium lowland.	Tall, photoperiod sensitive, late duration. Tulsimanjari, Katarnibhog are fine grained scented varieties.
7. Munger	Dolongi, Kalamda, Karibank, Tulsimanjari, Badshah bhog, Shyamjira, Kesore, Dahia.	Rainfed medium/lowland	Tall, Photoperiod sensitive, Tulsimanjari, Shyamjira, Karibank are fine grained aromatic.

Bihar has four mega rice growing environments namely (1) rainfed upland (ii) rainfed medium land, (iii) rainfed lowland and (iv) deepwater land. There is a lot of variability within each situations at macro and micro levels. The landraces of these environments possess genetic variability in respect of forms, quality characters and adaptation. However, the landraces belonging to rainfed lowlands/medium lands are spread all over Bihar; deepwater rices are confined to north Bihar while rices of rainfed uplands are concentrated in Chotanagpur plateau (Fig. 2).

Major part of rice cultivated area (70-80 per cent) in the central Bihar plains is covered by high yielding varieties which has pushed the landraces to the stage of extinction. Consequently priority should be given to the collection of the surviving traditional rices of this area.

North Bihar Gangetic plains having rainfed lowland and deepwater ecosystems, possesses enormous genetic variability which is the gift of the complex environments in which they have been growing for hundreds of years.

Table 2. Important landraces of north Bihar Gangetic plains

Landrace	Area of adaptability	Important characteristics
Gambhari, Gadari, Bachi gadari, Rani gadari, Punjabi gadari Jalli Bhadai, Asini, Ranghi, Satoria, Chalisa, Vadia Gadar, Baharni, Hathi Jhulan, Harnomia, Gajgaur, Sataria, Rangiva Gadar, Malbhog, Satari.	Rainfed upland/ medium land.	Tall, 120-125 days duration, photoperiod insensitive.
Bokol, Baster, Lakshman, Sugapankhi, Ammaghaur, Maldie, Ramjavairi, kalamkhora, Kalamsori, Chapis number, Jhulan sari, Katika, Dolongi, Samar, Jaiwa, Dudhia, Bangala, Deobhog, Lakhisar, Pakhari, Kasauri, Kesharbachi, Purbapankhi, Delan, Harinker, kamod, Tulsi, Marudangi, Bhat, Umtha, Punjhali, Ashwani, Kanyal, Meghnad, Bakai, Kahigaul, Morongia Pakhar, Aman, Laxman Dahia, Kakargar, Ramdulari, Parwa- Pankhi, Khora, Lalsar, Mujapur, Aman- Saded, Malbhogir, Sonbarsa, Jaswa, Picher, Kasounjh, Sidhat,Saribarsa Barogur, Malida, Ashwani, Kali Rai, Anuroopa, Ghurna, Motisair, Ghengule, Bijali Batti, Dhusari, Lal Kasaanjha, Satras, Baster Bakol, Ujla Kasaunjh, Harinkel, Bakol Raskushwa, Shai Kumar, Kusum-Katika, Duthari, Malida Sonbarsa, Sakaidhan, Sonasar, Parwa Pankh, Pakhar, Karuna, Soluka Saundh, Ratan Bakol, Karia Kamod.	Rainfed,medium lands/ lowlands.	Tall, photoperiod sensitive, late duration.
Gaipati, Bajra, Singhra, Darmi, Jagar, Desaria, Morgangia, Dorma, Desaria, Lodora, Dessaria, Jharmasdhan-dessari, Jessaria, Chenab.	Deepwater	Photoperiod sensitive, Tall, with elongation capacity, tolerance to flooding, drought and complete submergence.
Kanak jeera, Tulsiphool, Shyamjeera, Malbhog, Kesarbani, Badshahpasand	Rainfed medium	Photoperiod sensitive, tall, short fine grain with aroma.

The landraces were utilised for (i) breeding varieties (ii) genetical studies (iii) incorporating special traits e.g. disease, insect resistance, tolerance to physiological stress such as drought, flood etc. (Table 4). Some of the landraces having stable performance were recommended for cultivation as improved

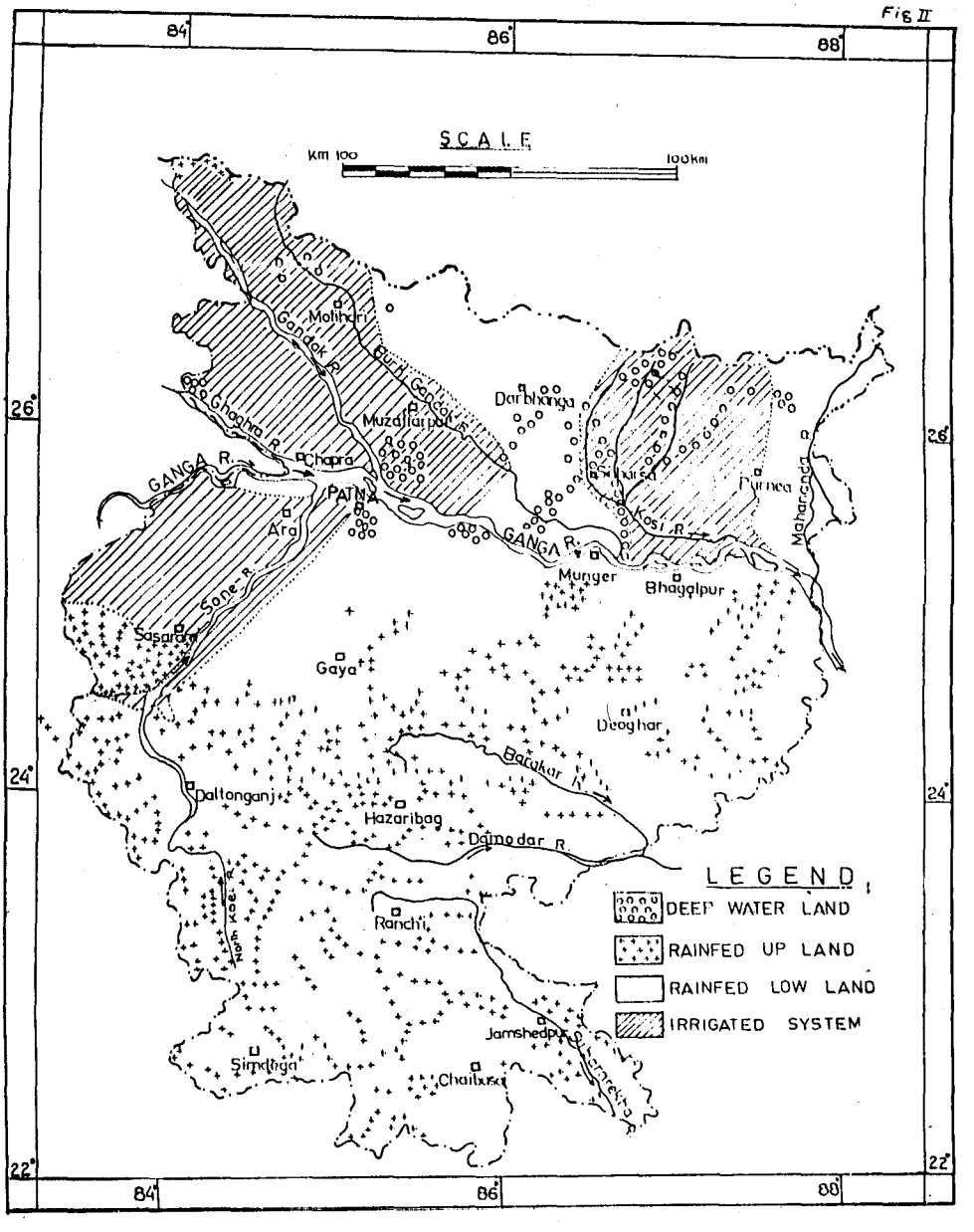

Fig. 2. Map showing major agro-eco-systems and distribution of land races in Bihar

variety particularly in adverse ecosystems such as rainfed upland, lowland or deep water rice ecosystem. The improved rice varieties recommended earlier have been developed, excercising pure line selection (Table 3). Some of these varieties like BR 34, BR 8, Brown Gora 23-19, *Janki* etc. are the very popular among the farmers.

Table 3. Landraces utilized as improved variety

Sl. No.	Landrace (local name)	Accession Number	Popular name	Traits for which utilized	Remarks
1	2	3	4	5	6
1.	Gora dhan	Brown gora 23-19	Gora dhan (Br 19)	Improved variety for the uplands of Chotanagpur.	It is still the best variety for rainfed uplands in Chotanagpur.
2.	Dahia	BK 115	BR 3	Improved variety for medium lands of Gangetic plains.	Common landrace of Bhagalpur district.
3.	Jhulansar	BK 114	BK 4	-do-	Common landrace of Munger district.
4.	Motisaul	BK 16	Br 5	Improved variety for medium land of Gangetic plains	Common landrace of old Shahabad district.
5.	Dahia	BK 88	Br 6	-do-	Common landrace of Bhagalpur district.
6.	Kessore	BK 36	Br 7	Improved variety for lowlands of Gangetic plains.	Common landrace of Bhagalpur district.
7.	Kessore	498-2A	Br 8	Improved variety for lowlands of Gangetic plains.	This variety is still popular in rainfed areas.

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1	2	3	4	5	6
8.	Tulsimanjari	818-3	Br 9	Fine grained aromatic rice for Pulao purpose.	-do-
9.	Badshahbhog	300-15	Br 10	Fine grained for Pulao purpose.	-do-
10.	Jessoria	-	Br 14	For cultivation in the deepwater areas of north Bihar.	The variety has the elongation ability & flood tolerance.
11.	Dolongi	2206 B	Br 34	Suitable for medium lands of Chotanagpur.	Collection from Munger district. It is still a very popular variety in the rainfed areas.
12.	Jessaria	-	Br 46	Suitable for deepwater lands of north Bihar.	Collection from Darbhanga district.
13.	Chenab	C-64-117	Janaki	Suitable for deepwater land.	Highly tolerant to flooding and complete submergence.
14.		TCA 48	Vaidehi	Suitable for deepwater land.	-
15.		TCA 72	Sudha	-do-	-
16.	Basmati dhan	-	Sugandha	Fine grained aromatic rice for Pulao purpose.	Introduction from Orissa.
17.	Katarni	RAU SBS-640	Kamini	-do-	Collection from Bhagalpur district.

Fig. 3. Map showing the area/region explored, route followed and collection sites south Bihar

FUTURE PROSPECTS

There is possibility for rice germplasm conservation in Bihar. The work done on the aspect so far has not been properly documented. Therefore, systematic and concerted efforts for exploitation and cataloging are the priorities

to conserve the valuable germplasm of Bihar. Non Govt. Organizations (NGO's) may be encouraged and involved in the rice germplasm conservation programme.

Table 4. Landraces utilized as donor parents in hybridization programme.

Sl. No.	Landrace	Popular name	Traits for which utilized
1.	Gora dhan	Brown gora 23-19	Drought tolerance, earliness.
2.	Kessore	Br 7	Photoperiod sensitivity, tolerance to water-logging. Sustainability under rainfed system.
3.	Kessore	Br 8	-do-
4.	Dolongi	Br 34	Photoperiod sensitivity, tolerance to BPH & sustainability under rainfed system.
5.	Chenab	Janaki	Tolerance to submergence, flooding, drought and adaptability under deepwater condition.
6.	TCA 48	Vaideshi	-do-
7.	TCA 71	Sudha.	-do-
8.	Tulsi Manjari	Br 9	Fine grain and aroma.
9.	Katarni	Kamini	-do-
10.	Jessaria	Br 14	Tolerance to flooding, drought, submergence, elongation ability.

The irrigated area need be explored on priority basis so that the remaining landraces of the region may be collected and saved from extinction.

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HEADING AND SPIKELET FERTILITY IN COLD TOLERANT RICE GENOTYPES AS INFLUENCED BY ENVIRONMENTS IN HIGH ALTITUDE AREA

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Eighty-eight cold tolerant rice germplasm consisting of *indica*, *japonica* and *javanica* types were evaluated in direct seeded, rainfed, upland condition at three sowing dates (SD) in high altitude area of Garhwal Himalayas. Heading time was not significantly altered by the first (24 April) or the Second (28 May) SD, but very few lines flowered in the third SD (28 June). Heading time was reduced by 2-37 days in the third SD. About 64% lines showed head emergence while, 28% lines bore fertile spikelets. *Japonica* and Javanica types showed increased head emergence and spikelet fertility. Average spikelet fertility was, in general, more in the cloudy conditions during anthesis, as compared to that of the first SD (3.64%) indicating not only the possible role of low temperature in influencing this character but also of other factors as well. High spikelet sterility in some early maturing lines vindicated this fact further.

Key words : Rice, germplasm, cold tolerant, environmental influence

Direct seeded upland rice is extensively cultivated in the hills of Uttar Pradesh, India. The crop is generally sown from end of March to April (Spring rice) and harvested in September-October depending on altitude. Transplanted rice is raised from May end to mid June, about a month earlier as compared to that of the plains. May-June sown direct seeded upland rice is also gaining importance in some sporadic pockets in recent years with the release of suitable improved varieties for this specific condition with a view to increase cropping intensity.

In high altitude areas cold temperature induced spikelet sterility is a common phenomenon. Early maturing cultivars are expected to perform better as they can escape cold temperature stress during the grain formation stage. However, it was observed that contrary to expectations, even early maturing lines do not always show high spikelet fertility. Therefore, the present

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investigation was carried out to find out the role of other environmental factors on heading time and spikelet sterility in a number of cold tolerant rice genotypes.

MATERIALS AND METHODS

Eighty eight cold tolerant rice lines received from the International Rice Research Institute, Manila, Philippines including three local checks were evaluated at three sowing dates (SD) viz., 24 April, 28 May and 28 June during 1991. The material consisted of 34 *indica* Early, 13 *indica* Medium, 39 *Japonica* and 2 *javanica* lines. The lines were raised at the experimental farm, G.B. Pant University of Agriculture and Technology, Hill Campus Ranichauri in Tehri Garhwal Himalayas situated between 30° - 15'N and 78° - 30' E at an altitude of 2150 m above msl in upland, dry seeded, rainfed condition. Seeds of the first sowing date germinated a few days later (1-5 May) due to insufficient soil moisture at the time of sowing. Therefore, observation on heading time was calculated from the date of germination of seeds. Plot size consisted of single 2 m long rows, spaced 20 cm apart, with inter-plant distance of 8-10 cm. Observations on heading time and spikelet fertility were recorded in three sowing dates while those on plant height and tillering ability could be recorded in the first sowing date only. For calculating spikelet fertility percentage, number of filled or semi-filled grains and empty grains were separated and counted in all the earheads in a line and expressed as percentage. Data on related important weather parameters were recorded during the crop growing period.

RESULTS AND DISCUSSION

A persual of the data (Table 1) revealed that only about 64 per cent lines (56) showed head emergence, while only 28 per cent lines (25) bore fertile spikelets pooled over all the sowing dates. *Japonica* and *javanica* groups showed increased head emergence and spikelet fertility as compared to *indica* lines showing better cold tolerance ability. In general, heading time was not significantly altered either by the first or the second SD as most of the cultures showed little difference in head emergence with respect to these two SDs. Although very few lines flowered in the third SD, heading time was reduced by 2-37 days in the third sowing date. The reduction was more pronounced in the *japonica* lines that are known to be photosensitive.

Spikelet fertility was, in general, more in the second SD (average 5.87%) as compared to that of the first SD (average 3.64%) indicating not only the role of low temperature but also of other factors in influencing this character. The details of important weather parameters recorded during the crop season

Table 1. Heading time and spikelet fertility of cold tolerant rice lines at three sowing dates at Hill Campus during 1991

Entry	Heading time (days)			Plant height (cm)	Spikelet fertility (%)			Tillering ability[a]
	I[b]	II	III	I	I	II	III	I
INDICA : EARLY								
CALIFORNIA BELLE (ACC 66836)	110	112	-[c]	75	0	0	-	7
CHINA 1039	112	108	-	54	0	0	-	7
DUBOVKSY 129	-	-	-	42	-	-	-	7
GASMAL 89 (ACC 29325)	-	-	-	89	-	-	-	7
IB 20	-	-	-	77	-	-	-	9
IB 42	180	-	-	42	0	-	-	9
IR 18502-PL P4 -3-3-3-1-1	116	118	-	62	0	0	-	5
IR 20654-R-R-R-15-1-B	-	-	-	55	-	-	-	3
IR 25884-94-3-2	-	-	-	58	-	-	-	7
KAEU N 101	95	99	-	86	0.5	0.5	-	9
KALIN	102	105	100	65	0	0	0	9
K 39-96-1-1-1-2	112	113	102	66	0	0	0	7
MILYANG 77 (CHILSEONGBYEO)	-	-	-	36	-	-	-	9
RP 1442-4-6-1-1-1	112	115	-	76	0	0	-	9
RP 1681-1701-4204	-	-	-	76	-	-	-	9
RP 1888-4259-1529-126	116	116	-	55	0	0	-	7
RP 2420-111-21-2-4	-	-	-	60	-	-	-	7
RP 2421-100-2-3-2	181	-	-	70	0	-	-	9
SPTLR 81346-PTG-B3-53-5-2-1	-	-	-	71	-	-	-	5
SPTLR 81366-PTG-16-2-3-3-1	-	-	-	68	-	-	-	9
SPTLR 81399-PTG-17-1-2-1-1	-	-	-	55	-	-	-	7
SPTLR 82078-PTG-B3-24-1-1	-	-	-	42	-	-	-	5
SUWEON 325 (JUNGWEONBYEO)	184	-	-	32	0	-	-	9
SUWEON 332 (YONGMOONBYEO)	-	-	-	47	-	-	-	7

(Cont. on next page)

Entry	Heading time (days)			Plant height (cm)	Spikelet fertility (%)			Tillering ability[a]
	I[b]	II	III	I	I	II	III	I
SUWEON 333 (YONGJUBYEO)	-	-	-	42	-	-	-	9
THIMPU MAAP	120	125	-	113	0	0	-	9
TOKAMBANO 669	135	135	-	82	0	0	-	5
T3	-	-	-	70	-	-	-	7
UGEY MAP (ACC 72530)	-	-	-	75	-	-	-	7
VL DHAN 16	-	-	-	74	-	-	-	9
VL DHAN 163	110	112	-	75	0	0	-	9
VL DHAN 206	115	110	-	85	0	0	-	5
WANGDI MAP (ACC 725291)	-	-	-	81	-	-	-	5
88-2165-7	115	118	-	75	-	-	-	9
INDICA : MEDIUM								
CN 880-1-2/1	-	-	-	60	-	-	-	7
CN 880-24-1/3	-	-	-	54	-	-	-	7
CN 881-5-1/1	-	-	-	54	-	-	-	9
HAO-HAI-HUAN (ACC 66984)	-	-	-	80	-	-	-	9
IB 15A	-	-	-	52	-	-	-	9
IB 23	-	-	-	75	-	-	-	9
IR 1552	-	-	-	48	-	-	-	9
IR 40094-1-4-3	-	-	-	66	-	-	-	9
SPTLR 81366-PTG-18-1-3-3-1	-	-	-	80	-	-	-	9
136 (ACC 66986)	118	120	-	79	0	0	-	9
RANICHAURI LOCAL	110	112	97	81	0	0	0	5
SAONLI LOCAL	113	115	-	91	0	0	-	5
JAGDHAR LOCAL	120	120	-	91	-	-	-	9
JAPONICA :								
BARKAT (K 78-13)	122	128	-	90	0	0.1	-	7
E WAN NO. 5	-	-	-	62	-	-	-	9
HOA VANG THAI BINH	-	-	-	56	-	-	-	7
HR 5824-B-3-2-3	112	115	93	77	0	1	0	7
HR 6419-B-42-2	110	110	-	74	0	0	-	7

(Cont. on next page)

Entry	Heading time (days)			Plant height (cm)	Spikelet fertility (%)			Tillering ability[a]
	I[b]	II	III	I	I	II	III	I
HR 7896-ACB7	115	115	-	62	0	0	-	9
HSA 255	110	112	95	86	2	30	0	9
HSC 19	112	110	95	66	2	20	0	9
HSC 236	108	115	-	81	0	2	-	9
HSC 55	110	112	97	65	1	1	0	9
HSC 6	112	110	97	86	0.5	10	0	7
HSL 303	108	108	97	80	5	2	0	7
HSL 447	105	105	-	80	0.5	5	-	9
H 247	120	120	-	56	0	1	-	7
H 433	102	110	-	94	10	2	-	9
H443	102	110	-	94	10	2	-	9
H 447	100	102	-	74	0	2	-	9
H 448	110	112	-	72	2	0.1	-	9
H 467	110	110	-	53	2	5	-	9
H 473	112	120	87	63	2	0	-	9
H 491	130	135	98	88	1	0	0	9
IRI 384	-	-	-	52	-	-	-	9
IR 47686-6-2-2-1	140	145	-	88	0	0	-	9
JINBU 8	-	-	-	80	-	-	-	9
JINBU 9	115	125	-	82	0	0	-	9
MENG-LI-WANG-GU (ACC 66985)	120	125	-	71	2	0.1	-	7
MULYANG 54 (GAYA BYEO)	125	115	-	69	2	0.1	-	7
MILYANG 93 (SANGNAMBATBYEO)	125	130	-	72	0	0	-	5
MOH TSIJU (ACC 01249)	-	-	-	87	-	-	-	7
SR 11451-T 204	130	135	-	72	0.1	0	-	9
SR 11842-91-4-3	132	135	-	81	0	0	-	7
STEJAREE 45	100	102	-	72	25	32	-	5
SUWEON 222	125	130	-	83	0	0	-	9
SUWEON 345	120	135	-	60	0	0	-	7
TK 5331-R-R-R-R-28-1	135	135	-	58	0	0	-	7

(Cont. on next page)

Entry	Heading time (days)			Plant height (cm)	Spikelet fertility (%)			Tillering ability[a]
	I[b]	II	III	I	I	II	III	I
YR 6488-ACP39	125	145	-	69	0	0	-	5
80007-TR210-14-1-1	130	135	-	105	2	0	-	1
80018-TR161-7-1-1	125	125	-	95	1	.5	-	5
80023-TR166-2-1-4	130	124	-	85	0	0	-	9
JAVANICA:								
NEP THAP	132	-	-	79	0.5	-	-	9
PLOVDIV	121	115	-	80	-	1	-	7

[a]Scored in 1-9 scale following Standard Evaluation System for Rice, IRRI at growth stage 8-9.
[b]I = 24 April, II = 28 May and III = 28 June sowing dates.
[c]No head emergence.

are given in Table 2. The average sunshine hours during August and September were only 3.1 and 5.8 hrs per day as against the maximum possible sunshine hours of 13.2 and 12.4, respectively. The number of sunny days was also the lowest (12 days) during August. The active period of anthesis in the first SD was from July end to late August while in the second SD, it was from August end to middle of September. Thus coincidence of uninterrupted rainy or overcast conditions with anthesis in the first SD could be an important factor contributing to reduced spikelet fertility. The lines in the second SD, though experienced comparatively lower temperature during anthesis, showed increased spikelet fertility as they escaped continuous rainy or cloudy conditions during

Table 2. Important weather parameters during the crop season 1991 at Ranichauri (UP)

Month	Temperature C		No. of rainy days	No. of sunny days*	Average sunshine hours	Maximum possible sunshine hours
	Max.	Min.				
April	21.0	8.5	5	23	8.9	12.9
May	26.1	13.3	6	19	10.1	13.7
June	24.5	14.4	12	15	8.7	14.1
July	24.0	16.4	7	17	5.9	13.9
August	22.3	15.7	19	12	3.1	13.2
September	22.0	14.9	8	15	5.8	12.4
October	20.8	10.3	0	16	8.8	11.5
November	15.4	5.7	1	21	6.7	10.6

*Number of days having sunshine hours equal to or more than the average monthly sunshine hours.

this critical period. This may also explain the reduced spikelet fertility observed in some early flowering lines like KAEU N 101 and also the differential spikelet fertility shown by some lines at different sowing dates.

Air temperatures, at later stages of plant growth, are known to affect percentages of unfertilized spikelets and percentages of ripened grains (Bhuiyan and Galag, 1989). Thus spikelet fertility or sterility has often been used as an indicator of cold tolerance or susceptibility (Satake *et. al.*, 1969; Kaw, 1991). But relying on spikelet fertility alone as a measure of cold tolerance may at times be misleading as revealed by this study. Spikelet sterility induced by low radiation caused by uninterrupted rainy or cloudy conditions particularly during reduction division and anthesis should be carefully separated from the induced by cold temperature in high altitude areas to arrive at clearcut conclusions. The same is corroborated by Yoshiba (1981) also who observed that unfavorable weather during reduction division and anthesis, cultural practices like high plant density and low radiation during post-flowering stage reduce the number of filled grains per panicle.

The cultivar Stejaree 45 showed high and almost uniform spikelet fertility in both the first (25%) and the second (32%) SD. Three local cultivars collected from nearby mid altitude (1200-1500 m above msl) areas showed complete spikelet sterility though in other seasons they had shown reasonable seed setting (data not presented). This suggests that a more critical analysis is required on the role of weather variables on spikelet sterility/fertility over the seasons encompassing different altitudes.

Plant height and tillering ability, the important parameters of plant growth, recorded only in the first SD showed wide variations. Plant height ranged from 32 cm for Suweon 325 to 113 cm for Thimpu Maap. Though most genotypes in general showed poor tillering ability (score 7-9), the *japonica* line 8007-TR 210-14-1-1 showed excellent performance (score 1) with respect to this character.

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COLLECTION OF GENETIC DIVERSITY OF MANGO (*MANGIFERA INDICA* LINN.) FROM UTTAR PRADESH

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Forty nine distinct genotypes of mango germplasm possessing wide range of diversity in agrobotanical and morphological traits of the fruits and maturity period were identified from 8 districts of western Uttar Pradesh. Positive and highly significant correlations were observed between average fruit weight with length and diameter; and between length and diameter of fruits. It provides ample scope for selection of donor germplasm for various traits. Most notable donor genotypes for various traits viz. maturity period (early, mid, late and extra late) sucking type, cluster bearing, pickle type, pleasant taste and aroma were collected.

Key words : Genotype, *Mangifera indica* Linn, genetic diversity, landraces of western Uttar Pradesh.

The mango a well-known fruit of Indian subcontinent for several centuries, was virtually unknown to any botanist until 1605 (Mukherjee, 1949). Besides the Indian subcontinent, mango is now grown in several countries of the tropical and sub-tropical world where it was introduced by Muslim rulers, missionaries, Spanish, voyagers and Portuguese explorers during the 15th to 18th century (Majumdar and Sharma, 1985). The genus *Mangifera* is reported to contain 41 species in all but almost all the edible cultivars of mango belong to single species *Mangifera indica* Linn. Most of the existing commercial varieties of mango in India have been developed through selection from the naturally cross-pollinated seedlings. Great variability exists throughout the country, however western Uttar Pradesh is extremely rich in mango germplasm. Efforts were made in the past to collect mango variability from western Uttar Pradesh which is being maintained at Horticulture Research Station, Saharanpur; IIHR Bangalore and Central Institute for Tropical and Sub-Tropical Horticulture, Lucknow. However, no systematic explorations were conducted to collect landraces/promising types. Hence during this exploration distinct genetic stocks of mango were identified and collected.

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MATERIALS AND METHODS

An exploration for identification of mango germplasm in western Uttar Pradesh was undertaken jointly by NBPGR, New Delhi and 'Central Institute for Tropical and Sub-Tropical Horticulture, Lucknow during June-July, 1991. Subsequently the bud sticks were collected and are being maintained at 'Central Institute for Tropical and Sub-tropical Horticulture, Lucknow. The area covered includes, Meerut, Muzaffarnagar, Saharanpur, Dehradun, Haridwar, Moradabad, Bijnor, and Ghaziabad district of Uttar Pradesh. State Government Officer's of Horticultural department of each district were contacted to get the first hand information about the genetic diversity and accordingly route was followed (Arora, 1981). The exploration was undertaken when fruits were physiologically matured and ripening initiated. Efforts were made to select the germplasm from seedling origin and also landraces/genotypes and primitive cultivars not yet collected by 'Central Institute for Tropical and Sub-Tropical Horticulture, Lucknow. Morphological observations of the fruits and pulp TSS were recorded from 5 randomly collected fruits in each genotypes. Based on the ripening period of the fruits, identified germplasm has been grouped in early, mid, late and very late (ripening in June, July, August and September respectively). Similarly the fruits weighing about 300g and above were grouped as extra large, between 201 g to 300g as large; 101 to 200g as medium and less than 100g as small. The identified germplasm has also been grouped in sucking type (highly juicy) 'table type' (fleshy with good taste and aroma), and pickle type (fleshy, acidic and less fibrous). Range, mean, S.E., Variance (Table 1) and character association analysis for quantitative characters (Table 2) were also studied.

RESULTS AND DISCUSSION

Forty nine distinct genotypes not existing in the collection of Lucknow were identified during June-July 1991 and bud sticks were collected during August-September 1992 from seven districts of western Uttar Pradesh. The range, mean and variance worked out for 4 quantitative characters is given in Table 1. Wide range of variation in fruit length (5.0-16.0 cm), diameter (4.5-11.0cm), average weight (50-509 g) and TSS (14.0-22.0%) was recorded.

Considerable variability were recorded in fruit morphological traits including fruits shape (round, oblong, ovate oblong, curved); base (roundish, medium, conical, narrow, broad); apex (narrowly round, roundish, sunken, conical round); colour at maturity (yellow, reddish, greenish, reddish yellow, greenish light-yellow etc.); taste and flesh character (Sour-sweet, acidic, sweet, Very sweet and aromatic. It was observed during the exploration that the ripening period of mango in western Uttar Pradesh differs with that of same varieties grown in the eastern Uttar Pradesh. In most of the commercial varieties, the ripening was observed to be late by 20-25 days in comparison

to those in eastern Uttar Pradesh. The Deshahari matures in mid-June around in east and central Uttar Pradesh as against with first fortnight of July in western Uttar Pradesh.

Table 1. Range, mean and variance for 4 characters in mango fruits, collected from western Uttar Pradesh

Collectors' No. (PNG/BL)	Fruit length (cm)	Fruit diameter (cm)	TSS (%)	Av. fruit weight (gms.)
1	5.0	5.0	14.5	50.0
2	6.0	5.0	14.0	80.0
3	6.0	7.0	16.0	125.0
4	6.5	5.0	20.0	60.0
5	7.0	5.5	18.5	135.0
6	8.0	6.0	19.0	75.0
7	6.0	5.0	18.0	105.0
8	8.0	5.0	21.0	80.0
9	7.0	5.0	15.5	75.0
10	10.0	8.5	15.0	310.0
11	10.0	7.0	19.0	220.0
12	12.0	9.0	17.5	340.0
13	7.0	6.0	18.0	160.0
14	9.0	7.0	16.0	175.0
15	15.0	11.0	17.5	500.0
16	11.0	8.0	20.0	350.0
17	16.0	7.0	16.5	300.0
18	10.0	7.0	19.5	175.0
19	10.0	8.0	18.5	190.0
20	9.0	7.0	19.0	150.0
21	8.0	5.5	19.0	80.0
22	8.5	9.0	19.5	180.0
23	11.0	7.0	19.0	150.0
24	12.0	8.0	18.5	190.0
25	12.0	7.0	18.5	160.0
26	6.5	6.0	18.0	100.0

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Collectors' No. (PNG/BL)	Fruit length (cm)	Fruit diameter (cm)	TSS (%)	Av. fruit weight (gms.)
27	9.5	4.5	18.5	80.0
28	9.0	6.0	21.0	145.0
29	10.0	7.5	22.0	190.0
30	9.0	6.5	20.5	150.0
31	10.0	8.0	19.0	225.0
32	7.0	5.5	21.0	80.0
33	12.0	6.5	20.5	230.0
34	10.0	8.0	20.0	330.0
35	9.0	8.0	17.5	210.0
36	8.0	7.0	19.5	175.0
37	9.5	7.0	20.5	190.0
38	11.0	9.5	19.50	260.0
39	9.5	6.0	18.0	105.0
40	7.5	7.5	19.5	150.0
41	11.0	7.5	19.0	275.0
42	9.5	7.5	16.5	200.0
43	7.0	6.0	20.5	130.0
44	9.0	6.0	19.5	150.0
45	11.0	7.0	21.5	200.0
46	9.7	7.2	160.0	295.0
47	12.9	7.8	17.0	370.0
48	12.4	9.2	17.0	509.0
49	8.0	5.5	14.5	160.0
Range	5.0	4.5	14.0	50.0
	16.0	11.0	22.0	509.0
Mean	9.35	6.89	18.46	190.29
SE±	0.39	0.20	0.28	14.90
Variance	5.29	1.90	3.75	10880.87

The difference in maturity period of fruits is mainly due to geographical and climatic variation of the region. However, wide range in ripening period and prolonged availability of fruits (mid-June to late September) was observed in the identified germplasm.

The outstanding landraces/germplasm identified have been grouped based on their notable characteristics viz. utility and maturity period. The name of the landrace alongwith collector's number are listed below :

A. Time/season of ripening

Early : PNG/BL-13 (*Heer*), PNG/BL-27 (*Jalmorni*);

Mid : PNG/BL-28 (*Dil Pasand*), PNG/BL-31 (*Kakran*); PNG/BL-37 (*Sharbati Bagarain*) PNG/BL-45 (*Sahi Pasand*)

Late : PNG/BL-34 (*Langra Behat*); PNG/BL-11 (*Meenakshi*);

Very late : PNG/BL-46 (*Prakash*); PNG/BL-49 (*Kanchan*) PNG/BL-47 (*Kilba Durgilal*), PNG-BL-48 (*Pathar*).

B. Utility purpose

Sucking type : PNG/BL-5 (*Anphas*), PNG/BL-7 (*Larankoo*) PNG/BL-8 (*Gur se Meetha*) and PNG/BL-2 (*Angoor Amin*).

Table type : PNG/BL-12 (*Washim bhog*); PNG/BL-15 (*Mota Gola*), PNG/BL-16 (*Shakar-chini*) and PNG/BL-34 (*Langra Behat*)

Pickle type : PNG/BL-10 (*Ram Kela large*), and PNG/BL-9 (*William*)

C. Highly scented/aromatic : PNG/BL-45 (*Sahib pasand*) and PNG/BL-11 (Meenakshi)

D. Bunch type : PNG/BL-2 (*Angoor Amin.*)

The character association analysis of 4 quantitative characters showed positive and highly significant correlation between average weight with length and diameter (Table 2). Correlation between length and diameter of the fruits

Table 2. Correlation-Coefficient for 4 characters of mango germplasm

	1	2	3	4
1. Fruit length (cm)	1.000			
2. Fruit diameter (cm)	0.667**	1.000		
3. TSS (%)	0.092	0.026	1.000	
4. Av. fruit weight (gm)	0.767**	0.826**	0.090	1.000

**Significant at 1% level.

was also observed to be positive and highly significant. However, the correlation between TSS with length and diameter of the fruit was positive but not

significant. Therefore the genotype having higher average weight can be taken for improvement. The diversity existing can be utilised in selection and improvement programme.

OUTSTANDING COLLECTIONS

PNG/BL-2 : It is seedling tree of about 50 years locally known as *'Bulbul'* which is renamed as *'Angoor Amin'* due to its appearance like a grape bunch. It is located at 'Kaidi' Village in Muzafarpur District. It bears 25-40 fruits in a single compact bunch with single fruit weight of 60-100g. A bunch with 40 fruits had the total weight of 3200g. On an average, the fruit size is 6 × 5 cm, roundish-oblong; base flat-broad, mild depressed; apex roundish, beak absent; fruit skin yellow, lenticells white, submerged; taste slightly acidic. Fruit ripens in the second week of July. It is moderately regular in bearing. About 80 bunches were counted during this year. However, in the previous year the tree fruited more than 300 such bunches. Such bunch type mangoes can successfully be used in breeding heavy bearing, ornamental looking trees, suited for pickle industry, garden and parks as well as for rootstock purposes.

PNG/BL-45 : It is a chance seedling locally known as *'Sahib Pasand'*, growing in the orchard of Sri Kalimuddin S/o Mohd. Nasiruddin of Amroha. The tree with a height of about 4m. is spreading. The overage fruit weight ranges between 180 to 210g. Shape ovate elliptical; base medium-narrow, tapering, peduncle medium sized; apex roundish, tapering; beak absent; skin yellow; lenticles very few; skin thin; flesh dark-yellow, very sweet, very tasty, aromatic. This variety can be directly adopted for large scale cultivation.

Among sucking type the notable selections are PNG/BL-5 (*Anphas*), PNG/BL-7 (*Larankoo*) and PNG/BL-2 (*Angoor Amin*). These can replace old seedling types of mangoes in the entire western Uttar Pradesh. Among pickle types PNG/BL-10 (*Ram Kela Large*) and PNG/BL-9 (*William*) are outstanding. Since, these have thick flesh, small stone and highly acidic in taste. These two can be adopted on commercial scale for pickle industry.

Among table type remarkable variation existed in shape, size, weight, colour, taste, presence of aroma and ripening time. Landraces such as PNG/BL-12 (*Washim bhog*), PNG/BL-15 (*MotaGola*), PNG/BL-16 (*Shakar-chini*) and PNG/BL-34 (*Langra Behat*), exhibited bigger size fruits, having thick aromatic flesh and small size stone suitable for table purpose. These may find place alongwith the popular landrace *Rataul*. However, those interested in large size fruit may adopt *Rajawala* which bear fruits upto 4.0 kg.

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COLLECTING GENETIC DIVERSITY OF EGG-PLANT AND ITS WILD RELATIVES FROM SOUTH INDIA

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From five exploration and collection trips undertaken in Southern Peninsular region, 216 accessions of *Solanum melongena* var. *melongena*, 1 of *S. macrocarpon* and 134 lines belonging to 25 wild species, exhibiting rich variability in plant and fruit morphology were sampled. This includes a number of rare genotypes/ land races of egg plant and its related species. A large number of indigenous and introduced naturalized species widely occur, particularly in pockets of Western Ghats.

Key words : Germplasm, *Solanum*, egg plant, wild relatives, land races, local cultivars, variability, diversity

In India, the genus *Solanum* is represented by 45 species including 22 indigenous (Deb, 1949). Three species such as *S. melongena, S. tuberosum,* and *S. macrocarpon* are cultivated and a total of 32 species are reported to be useful. The *S. melongena* has wide diversity and hence Vavilov considered it of Indian origin (Vavilov, 1951). A large number of local varieties/landraces occur in the country and adapted to different situations. The third species being an introduced one, is mainly cultivated in homesteads in Kerala as vegetable. The landraces/strains are being evolved due to influx of new varieties. Hence, collaborative explorations were undertaken by NBPGR and IBPGR for collection of eggplant and its wild relatives from Southern region during 1989-1991.

MATERIALS AND METHODS

Five explorations were undertaken in parts of Southern peninsular region comprising Kerala, Karnataka, Andhra Pradesh and Tamil Nadu during 1989-1991 for collection of egg-plant and its wild relatives. Few small trips were also made for collection of wild species in pockets of Western Ghats. Sampling sites included traditional egg-plant cultivation belt, tribal areas, places of ethnic importance, forest pockets and market places. Latest nomenclature and taxonomy of the genus were followed. In most of the cases mature/ripened fruits were collected for extraction of seeds except in certain cases where vegetative cuttings were obtained.

RESULTS AND DISCUSSION

From five exploration trips undertaken, a total of 351 samples belonging to 27 species were collected. Distribution of egg-plant is noticed from sea level to elevations as high as 1800 m in Western Ghats. The crop is commercially raised in all states except in Kerala, where homestead cultivation is practiced. In most of the areas, traditional landraces have been replaced by improved cultivars to a great extent. Statewise collection of *Solanum spp.* is given in Table 1.

Table 1. Statewise collection of *Solanum* spp.

S.No.	Species name	Kerala	Karnataka	TN	AP	Total
1.	*Solanum melongena* var. *melongena*	40	45	81	50	216
2.	*S.melongena* var. *insanum*	11	3	1	1	16
3.	*S.melongena* var.*incanum*	6	-	-	-	6
4.	*S.pubescens*	-	-	3	3	6
5.	*S.surattense*	-	1	3	3	7
6.	*S.trilobatum*	-	2	3	1	6
7.	*S.torvum*	10	2	2	2	16
8.	*S.nigrum*	1	2	1	2	6
9.	*S.anguivi* var. *anguivi*	13	4	6	-	23
10.	*S.anguivi* var. *multiflora*	5	-	3	-	8
11.	*S.myriacanthum*	4	5	3	1	13
12.	*S.seaforthianum*	1	1	2	-	4
13.	*S.aculeatissimum*	2	1	-	-	3
14.	*S.erianthum*	1	-	4	-	5
15.	*S. mammosum*	1	-	-	-	1
16.	*S.sisymbriifolium*	-	-	1	-	1
17.	*S.capsicoides*	-	-	1	-	1
18.	*S.elaeagnifolium*	-	-	1	-	1
19.	*S.mauritianum*	-	-	3	-	3
20.	*S.giganteum*	-	-	1	-	1
21.	*S.jasminoides*	-	-	1	-	1
22.	*S.stramoniifolium*	1	-	-	-	1
23.	*S.convolvulus*	1	-	1	-	2
24.	*S.barbisetum*	-	-	1	-	1
25.	*S.hispidum*	-	-	1	-	1
26.	*S.macrocarpon*	1	-	-	-	1
27.	*S.vagum*	-	-	1	-	1
Total		98	66	124	63	351

In Kerala, local land races of egg-plant are cultivated between the attitude 0-1770 m in red sandy and alluvial soils during the rainy season as a rainfed subsistant crop where as in Andhra Pradesh both local and improved cultivars are grown between 0-1000 m in clayey, sandy, red sandy loam and alluvial soils throughout the year. In Tamil Nadu, predominantly improved types are grown between 0-1700 m under irrigated condition. In Karnataka also cultivation of egg-plant has been noticed from coastal plains to plateau and in hills.

Variability in egg plant

Depending on certain morphological characters, the local varieties are known by different names. Based on fruit shape, varieties are called as 'gundu kathiri' for round fruited type in Tamil, 'jammu badanai' for very large round fruited type in Kannada and 'poonathalai kathiri' for fruits having the shape of a cat's head in Tamil. With respect to colour of the fruits, landraces are known as 'myla kathiri', for purplish blue types, 'vellai kathiri' for white fruited types and 'varikai' for scriped fruits in Tamil. 'Tella venkai' for white types, 'gulab venkai' for pinkish fruits and 'kappu venkai' for purplish blue types in Telugu, 'vella vazhuthina' in Malayalam, 'vellai badanai' for white types and 'kappu badanai' for blue types in Kannada. Plants with spiny stem, leaf or calyx, are known by the name 'mullu kathiri' in Tamil and 'mullu venkai' in Telugu. Small fruited type is known as 'cheru vazhuthina' in Malayalam. 'Puliyampoo kathiri' in Tamil denotes the type with purple and green stariated fruits. 'Nattu kathiri' in Tamil and 'sada venkai' in Telugu indicate the common local types. 'Nalla venkai' in Telugu is an antonym to wild brinjal. 'Guthi venkai' in Telugu stands for erect plant type.

In general variability in plant types, plant height and branching pattern was also observed. The plant types varied from tall erect branching to bushy, highly branching and dwarf spreading. A total of 34 spiny plant types mostly from Karnataka, Kerala an Andhra Pradesh were collected.

Frequency distribution of 195 collections of egg-plant in relation to their fruit shape include spherical (81), oval (34), oblong (80). Based on fruit size 203 accessions are grouped in large (56), medium (105) and small (42). Fruit colour varied considerably from white in 78 collections, purple tinge or shade in 27, purple in 35, light green with purple tinge in 13, green with purple shade in 14, dark purple in 10, purple with white lines in 5, green with light purple shade in 3, green with white shade in 6, light purple in 3, green in 8, light green in 1 and bluish purple in 1.

Geographic distribution of these types is presented in Fig. 1 which indicates that the distribution of these types is area specific. Large oblong types were common in Kerala. Spherical fruit types were common in Tamilnadu

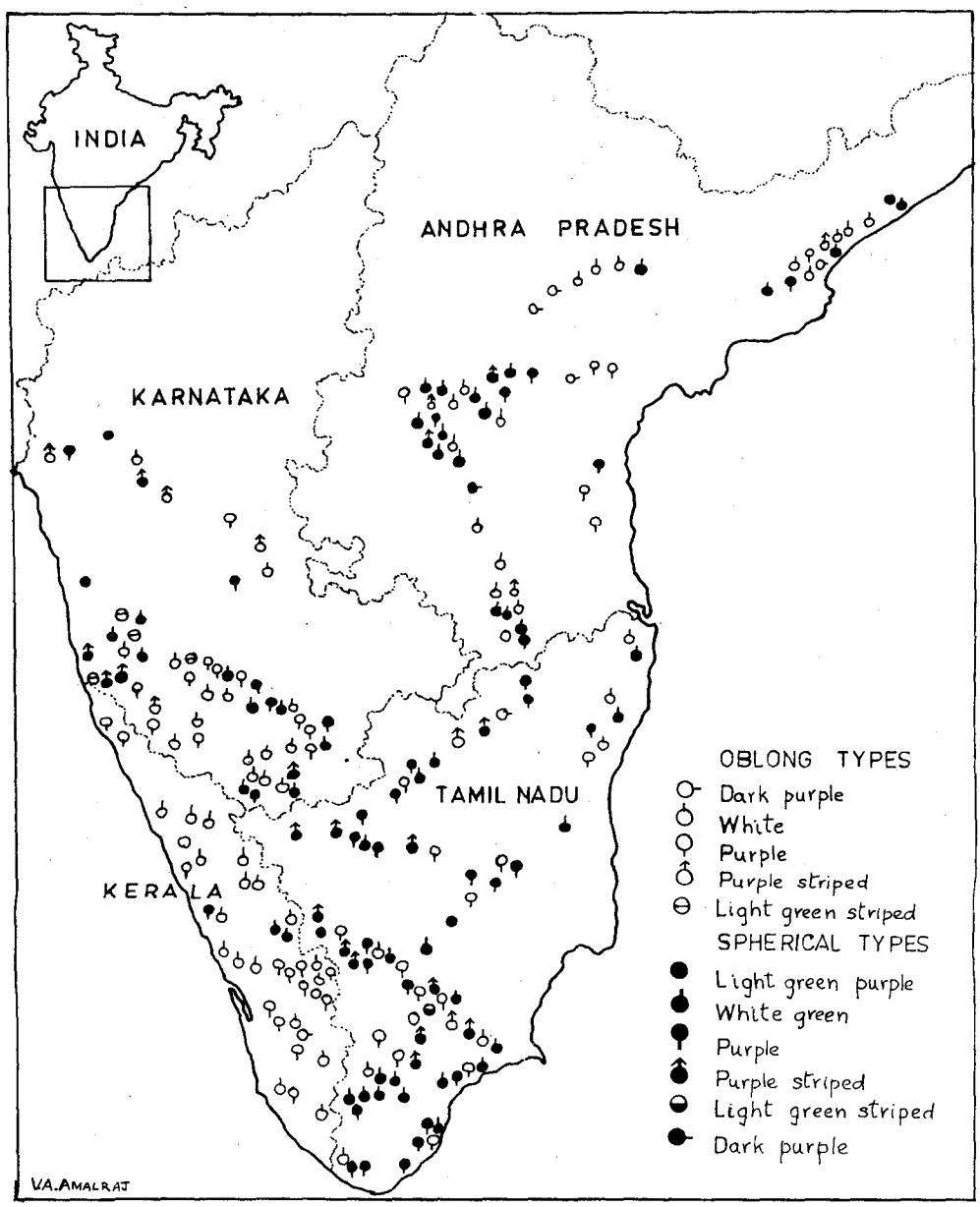

Fig. 1. Distribution of Brinjal types in southern India

and less common in other states. Large fruit type was very common in the coastal sandy plains of Tamil Nadu and Karnataka. Purple pigmentation on fruits was common in Tamil Nadu and Andhra Pradesh. In Kerala, purple fruited type were less common. However, a local type known as Wynad giant was more common in Wynad district.

Diversity in *Solanum* species

The review of literature on the genus *Solanum* in India indicates that species/taxa in the southern region can be broadly classified on the basis of their origin into indigenous and exotic with 17 and 20 taxa, respectively (Clarke, 1883; Rama Rao, 1914; Gamble, 1921; Saldanga & Nicolson, 1976; Sharma et al., 1977; Deb. 1980; Yoganarasimhan *et al*, 1981; Mathew, 1983; Sharma *et. al.*, 1984; Henry *et al.*, 1987; Subramanian *et al.*, 1987; Manilal, 1988; Ellis, 1990 and Mohanan & Henry, 1994. Out of the 37 taxa reported to occur in Southern Region, 27 taxa including 2 cultivated species viz. *S. melongena* and *S. macrocarpon* were collected.

From the distribution pattern of *Solanum* spp (Table 2), it is clear that maximum number of exotic and indigenous species are distributed in Western Ghats and its surrounding areas in the states of Tamil Nadu, Kerala and Karnataka. This is due to the fact that most of the introduced species have been naturalised inhabitants in hill stations. *S.myriacanthum, S.convolvulus, S.erianthum, S.capsicoides, S.seaforthianum* and *S. aculeatissimum* were found in these pockets. Many indigenous species were also found to occur under situations of high rain fall and cool weather of the hills. Species such as *S.pubescens* and *S.surattense* were confined to dry situations in plains. An exotic species *S.elaeagnifolium* has been naturalised in black soils in plains of Tamilnadu. The widest distribution was noticed in the case of *S.torvum,* which is very rarely cultivated for its fruits. *S.trilobatum* has been restricted distribution in black soils in all the three states except Kerala.

The closest wild relatives of cultivated brinjal, *S.melongena* var. *insanum* and *var. incanum* were found to occur around human inhabitations and near the egg-plant fields. These species also possesses considerable variability in fruit size, shape and colour. Taxonomically, these two varieties are disputed. However, *S.melongena* var. *incanum* was usually found to grow in interior isolated forest pockets or along the disturbed roadside vegetation in Tamilnadu and in Kerala. It has spherical fruits with withering and non-inflated calyx lobes at maturity as compared to highly inflated persistent calyx lobes on slightly oblong fruits in *S.melongena* var. *insanum.* Both varieties are being used for medicinal purposes. Among all the species collected *S.vagum* is the rarest, restricted to Tirunelveli hills in Tamil Nadu followed by *S.stramoniifolium,* a species that is adapted to the tropical evergreen forests in Kerala.

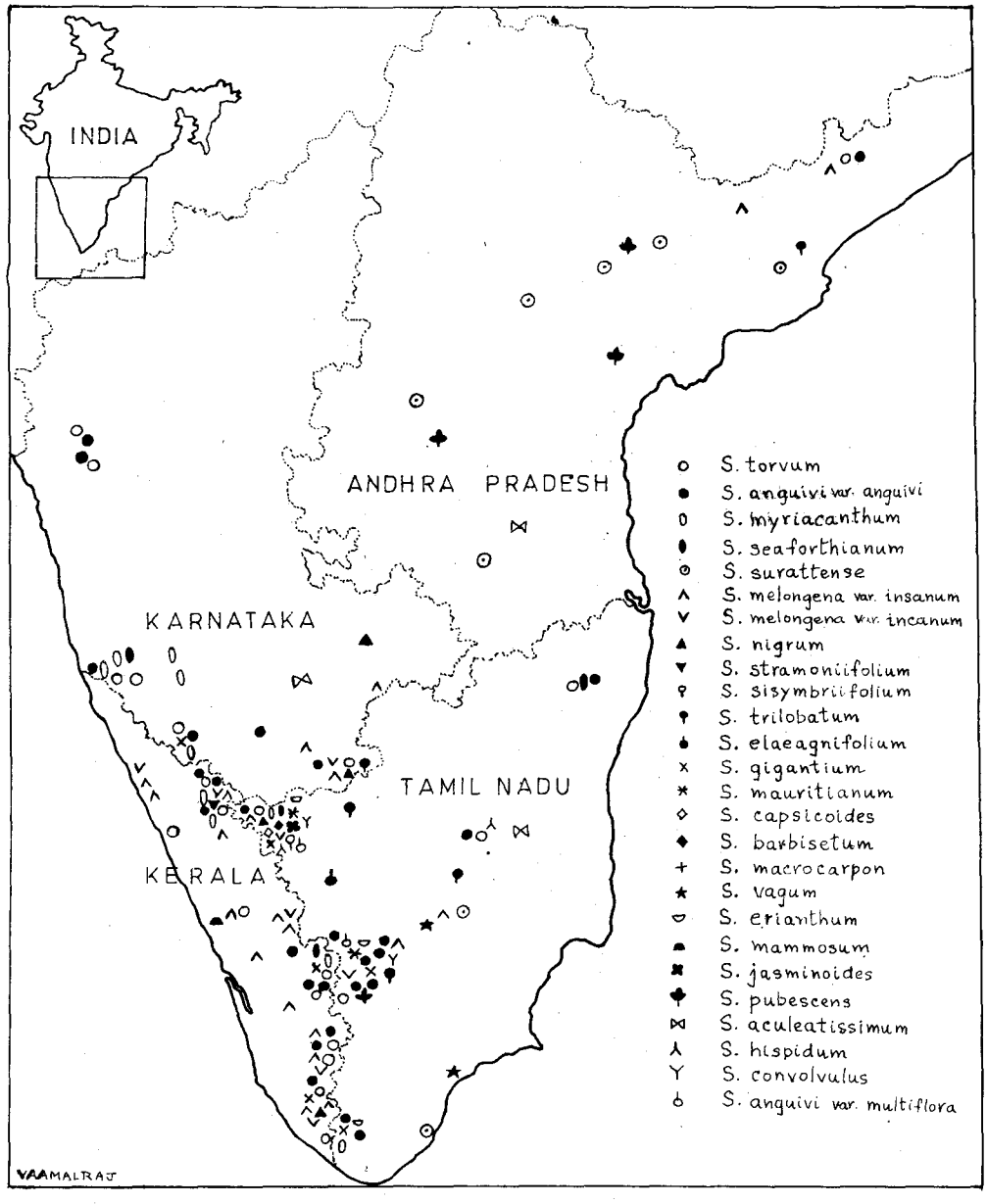

Fig. 2. Distribution of *Solanum* species in southern India

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GENETIC RESOURCES OF ASPARAGUS (*ASPARAGUS OFFICINALIS* L.)

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Thirty-nine introductions of asparagus varieties/strains from the collections of genetic stock were evaluated at Regional Research Laboratory farm Sanat Nagar, Srinagar. Four distinct complexes were recognised on the basis of number of spears/plant and weight of spear/plant. Majority of the introductions were in the complex IV. Marked differences were observed between complex I and complex II. The introductions SL 17, SL 21, SL 29 were among top ranking for weight of spear/plant and spear yield/ha.

Key words : Genetic resources, *Asparagus officinales* L.

The morphological characters offer valuable criteria for the systematic cataloguing of the available germplasm and have the clear understanding of various complex characters. Anderson (1957) proposed that the best way to analyse the complex variation is to reduce the measurements of different characters to a pattern or score and compare the latter. The present paper reports the use of metroglyph analysis suggested by him in analysing the morphological variations in a set of 39 introductions of Asparagus varieties/strains from the collection of the genetic stock.

MATERIALS AND METHODS

The seed of 39 introductions (11 from U.S.A., 22 from Newzealand and 6 from South Africa) of different varieties were sown in a nursery in May, 1986 at Regional Research Laboratory Farm Sanat Nagar, Srinagar and were transplanted in the main field with row to row and plant to plant distance of 1.5 × 0.5 m respectively in October, 1986. The plot size of each entry consists of two rows and each row consists of twenty plants. The lay out was randomised design with three replications. All the agricultural operations were carried out as discussed by Pandita *et al.* 1987. The spears were harvested after the establishment period. Data were recorded on 15 plants from each replicate on plant height, number of stalks/plant, stalk diameter, number of

marketable spears/plant, spear diameter and weight of spear/plant each year (1988, 1989, 1990). The data was pooled for all the three years and the statistical analysis were carried out as given in Steel and Torrie (1960). Metroglyph and index score analysis was carried out according to the method suggested by Anderson (1957). The class interval for various morphological and symbols used for different characters is represented in Table 2. The index score were obtained by alloting numerical values 1, 2, 3 of each expression recognised in respect of each character and finally summing up the score obtained by each accession for all characters under study.

RESULTS AND DISCUSSION

Performance of 39 introductions for different characters is presented in Table 1. The weight of spears ranged from 12.53 to 31.36g with a mean value fo 19.49 g, while the weight of spears/plant ranged from 29.05 to 171.44 with a mean value of 87.71 g. SL 21 gave the highest weight of spears/plant followed by SL 29 and SL 17.

The calculated yield for weight of spears/ha was maximum in the Strain SL 21 followed by SL 17 and SL 29 respectively. The number of spears/plant varied from, 2.07-9.40 with an average value of 4.61 spears/plant. The spear diameter ranged from 1.02-1.99 with an average value of 1.39 cm, heighest being in Rutgars Becans (SL3). Though SL 21 gave the maximum yield/ha, the weight of spears was less than the mean. In 'Marry Washington' (SL5) the weight of spears and the spear diameter was above the mean value but the total yield of spears/ha was less which was in confirmation with our earlier findings (Pandita and Bhan, 1984). Also SL16, SL4 and SL11 which gave yield of 2389.48, 2389.24 and 2345.49 kg of spears/ha, having the weight of spears 12.71, 22.54 and 19.71, g, with a spear diameter of 1.13, 1.56, 1.39 cm respectively thrive well in the Kashmir valley.

The results of metroglyph analysis are presented in Fig. 1. Each accession is represented by a semi circle, the co-ordinate of each circle being the number of spears/plant and the Y co-ordinate being the weight of spears/accession. Five other characters have been represented by rays at different positions on the glyph. The scatter diagram shows the following four complexes. Complex-I comprised three entries (SL 33, 37, 39). Its main characteristics were few number of stalks/plant, less spear diameter and least weight of spears/plant.These accessions were without a ray. Complex-II contains two accession (SL 16, 21). The accessions within this complex were characterized by medium plant height, number of stalks/plant, average weight of spears/plant, minimum stalk diameter. The accessions were represented by minimum of three rays.

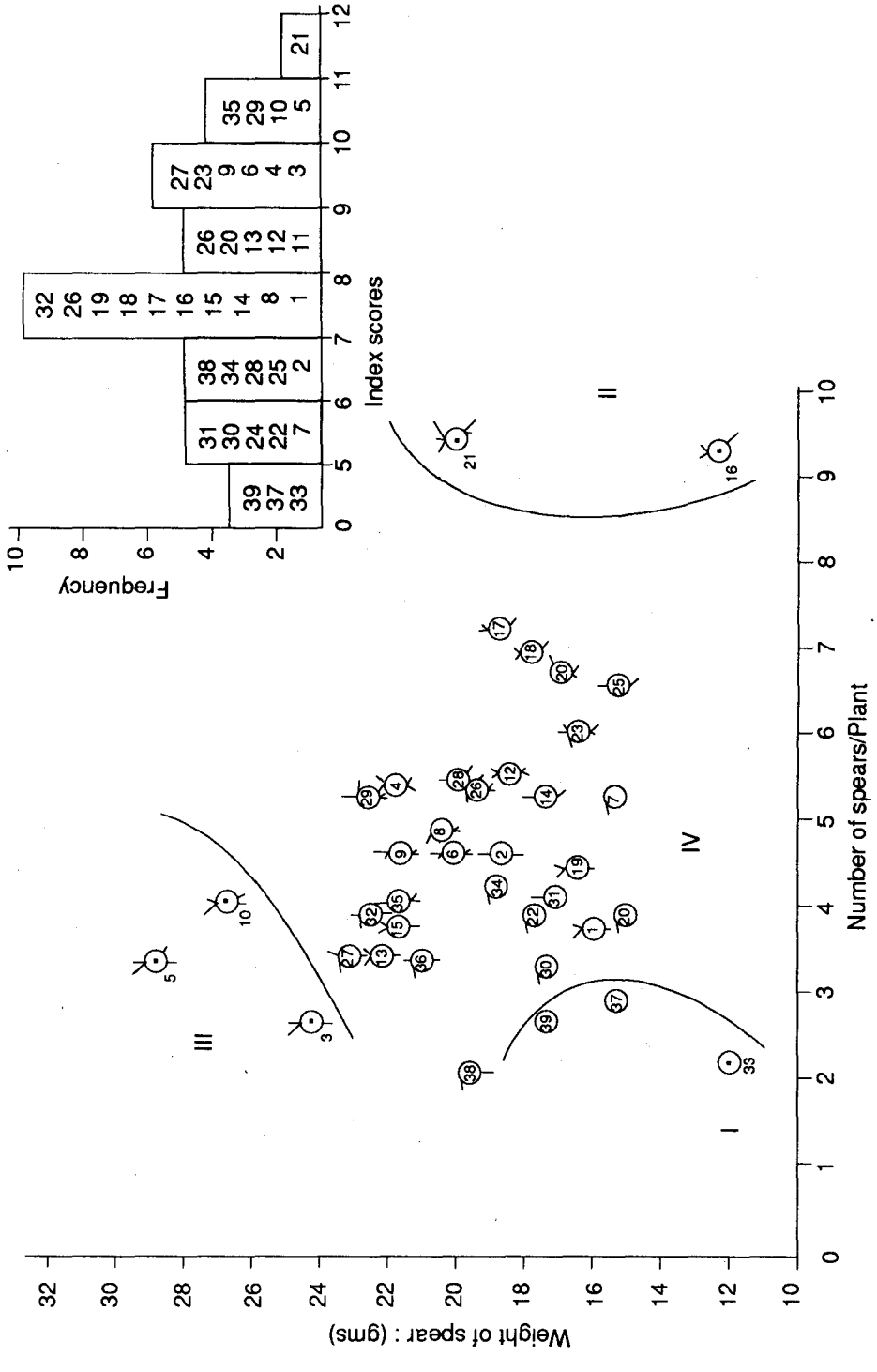

Fig. 1. Scatter diagram in metrogylph analysis

Table 1. Pooled performance of 39 introduction of *Asparagus officinalis*

Accession	Plant height (cm)	Number of stalks/plant	Stalk diameter (cm)	Number of spears/plant	Spear diameter (cm)	Wt. of spear (g)	Wt. of spears/plant (g)	Number of spears/ha.	Wt. of spears/ ha. (kg)
SL 1	199.83	3.46	1.44	3.87	1.36	15.57	59.76	77400	1205.118
SL 2	193.60	5.66	1.23	4.44	1.46	18.65	83.35	88800	1656.120
SL 3	202.08	3.46	1.54	2.83	1.99	26.20	74.03	56600	1482.920
SL 4	243.46	4.16	1.49	5.30	1.56	22.54	118.56	10600	2389.240
SL 5	226.06	2.80	1.60	3.05	1.71	31.36	90.18	61000	1912.960
SL 6	250.00	5.46	1.44	4.45	1.47	20.33	90.06	89000	1809.370
SL 7	182.00	4.40	1.37	5.15	1.19	15.21	78.74	103000	1566.630
SL 8	188.20	3.26	1.28	4.40	1.61	20.95	91.93	88000	18.43.600
SL 9	231.73	4.20	1.27	4.40	1.52	22.47	96.39	88000	1977.360
SL 10	229.60	3.60	1.62	3.90	1.40	27.34	106.53	78000	2132.520
SL 11	196.80	3.40	1.37	5.95	1.39	19.71	116.38	119000	2345.490
SL 12	198.88	4.91	1.40	5.20	1.37	18.59	96.88	104000	1933.360
SL 13	193.06	1.86	1.51	3.35	1.45	22.88	76.93	67000	1532.960
SL 14	199.46	3.26	1.21	5.10	1.29	17.38	87.87	102000	1772.760
SL 15	191.80	3.33	1.32	3.70	1.56	22.48	83.51	74000	1663.520
SL 16	202.15	8.28	1.05	9.40	1.13	12.71	118.82	188000	2389.480
SL 17	218.66	5.93	1.18	6.93	1.24	18.41	127.60	138600	2551.626

(Cont. on next page)

Accession	Plant height (cm)	Number of stalks/plant	Stalk diameter (cm)	Number of spears/plant	Spear diameter (cm)	Wt. of spear (g)	Wt. of spears/plant (g)	Number of spears/ha.	Wt. of spears/ ha. (kg)
SL 18	208.06	5.46	1.36	6.66	1.24	17.37	115.27	133200	2313.684
SL 19	211.73	6.40	1.20	4.45	1.26	16.93	75.34	89000	1506.770
SL 20	181.11	14.50	1.23	6.57	1.40	16.20	106.22	131400	2128.680
SL 21	191.31	11.50	1.34	9.15	1.37	18.65	171.44	183000	3412.950
SL 22	166.16	4.55	1.26	3.70	1.28	17.96	65.46	74000	1329.040
SL 23	192.73	6.40	1.26	5.95	1.40	15.89	94.96	119000	1890.910
SL 24	184.80	4.06	1.30	3.98	1.14	15.54	61.85	79600	1237.780
SL 25	194.00	5.33	1.06	6.32	1.29	14.18	89.60	126400	1792.352
SL 26	168.06	4.66	1.42	5.17	1.43	19.72	102.62	103400	2039.048
SL 27	212.60	2.86	1.47	3.28	1.64	23.95	77.33	65600	1571.120
SL 28	203.20	4.26	1.24	5.10	1.50	19.75	100.65	102000	2014.500
SL 29	219.81	3.60	1.39	5.38	1.67	23.67	127.97	107600	2546.892
SL 30	188.00	3.83	1.43	3.31	1.17	17.67	58.55	66200	1169.754
SL 31	202.23	4.86	1.23	4.06	1.31	17.64	71.50	81200	1432.368
SL 32	196.93	3.46	1.33	3.67	1.35	23.27	85.14	73400	1708.018
SL 33	177.93	2.66	1.22	2.31	1.05	12.53	29.05	46200	578.886
SL 34	187.40	3.10	1.65	4.15	1.02	18.65	77.35	83000	1547.950
SL 35	212.16	4.53	1.71	3.85	1.57	22.64	89.01	77000	1743.280

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Accession	Plant height (cm)	Number of stalks/plant	Stalk diameter (cm)	Number of spears/plant	Spear diameter (cm)	Wt. of spear (g)	Wt. of spears/plant (g)	Number of spears/ha.	Wt. of spears/ ha. (kg)
SL 36	193.78	2.36	1.48	3.43	1.43	21.13	72.58	68600	1449.518
SL 37	177.76	3.04	1.21	2.92	1.29	15.90	46.98	58400	928.560
SL 38	160.90	2.33	1.26	2.07	1.57	20.53	42.91	41400	849.942
SL 39	163.00	2.76	1.18	2.75	1.24	17.78	49.72	55000	979.900
Mean	198.69	4.56	1.35	4.61	1.39	19.49	87.41		
Range	160.0-243-46	1.86-14.50	1.05-1.71	2.07-9.40	1.02-1.99	12.53-31.36	29.05-171.44		
CD at 5%	6.630	0.784	0.050	0.530	0.063	1.284	8.761		

Table 2. Index range and symbols used for different characters in *Asparagus officinalis*

Characters	Range of mean	Score I. Index range (1)	Symbol	Score 11 Index range (2)	Symbol	Score 111 Index range (3)	Symbol
Plant height (cm)	160.90-243.46	160.90-190.90	0	190-91-210- 90		210.91	-
Number of Stalks/Plant	1.86-14.50	1.86-5.86		5.87-10.85	σ	10.87	σ
Stalk diameter (cm)	1.05-1.71	1.05-1.25	0	1.26-1.45	-	1.46	-
Number of spears/plant	2.07-9.40	2.07-4.67		4.68-7.27	-	2.28	
Spear diameter (cm)	1.02-1.99	1.02-1.34	0	1.35-1.66	-	1.67	-
Weight of spear (gm)	12.53-31.36	12.53-19.05	0	19.06-25.31	-	25.32	
Weight of spears/plant (gm)	29.05-171.44	29.05-86.15	0	86.16- 143.24	-	143.25	-

Complex III was represented by a minimum of three rays and contains three accession (SL 3, 5, 10). Their main characteristics were minimum number of stalks/plant, medium height, large spear diameter and maximum stalk diameter with an average weight of spear/plant. The rest of accessions fall into Complex IV. The group was represented by a minimum of two rays and maximum of 5 rays excepting the accession SL 37 and SL 39 which are not represented by any ray. The main features are few number of spears with a average spear diameter medium spear weight/plant and medium plant height. The maximum similarities were between Complex III and II, with a minimum number of stalks/plant, medium height and average weight of spears/plant. The Complex-I was totally different from other complexes as it was not represented by any ray. The complex IV was the intermediate between Complex-III and II. The frequency diagram (Fig. 1) clearly shows that accessions in Complex I come under 0-5, whereas the Complex III come under 10-11 and Complex II and IV come under 7-12. Complex I and complex II differ markedly in having extreme characters. The extreme score of 11 & 12 were obtained by the accession SL 21, SL15, SL 10, SL 29, Sl 35 respectively. These accessions could be selected for further breeding programme for various characters. Their actual worth could, however, be known through subsequent testing.

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EVALUATION OF GENETIC RESOURCES OF EGYPTIAN CLOVER (*TRIFOLIUM ALEXANDRINUM* L.)

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Five hundred and fifty three germplasm accessions of Egyptian clover, commonly known as *berseem (Trifolium alexandrinum* L.) collected from various parts of India were evaluated for quantitative attributes of green and dry matter yield. Limited amount of variability was observed for various characters. All the characters were significantly and positively correlated with green and dry fodder yield. Plant height had maximum direct effect and indirect effect via green fodder yield. A few lines namely HFB 163, 188 and 155 were better in both green fodder yield/plant and dry matter yield/plant, whereas genotypes HFB 158, 173 and 136 possessed desirable height and HFB 260, 214 and 209 possessed high number of tillers/plant. Their use in breeding programmes to develop ideal plant types in Egyptian clover has been discussed.

Key words : Egyptian clover, *Trifolium alexandrinum* biodiversity, germplasm evaluation

Evaluation of genetic resources for economically important attributes is one of the most essential and useful step for initiating breeding programme for bringing genetic improvement in any crop species. In Egyptian clover, an important fodder crop of northern India and other subtropical countries, systematic evaluation of germplasm has not been reported. Accordingly, the available germplasm of 553 accessions of Egyptian clover maintained at Haryana Agricultural University were evaluated and the results are presented in this paper.

MATERIALS AND METHODS

Five hundred and fifty three berseem accessions collected from different parts of major berseem growing states of India viz., Punjab, Haryana and Uttar Pradesh were grown during *rabi* 1991-92 at the experimental farm of Forage Research Section, Department of Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar in an augmented block design with one check namely *mescavi*. Each accession was raised in a single row of 3 m length. The distances between and within rows were kept at 30 and 10

cm, respectively. Every 44 accessions were followed by one check to constitute one block. Normal cultural practices were followed. Observations were recorded on three competitive and randomly selected plants from each accession including check on quantitative characters like plant height (cm), number of tillers per plant, green fodder yield per plant (g) and dry matter yield per plant (g). Mean, range of variation, coefficient of variation, correlation and path coefficient were worked out. The germplasm lines were also categorised as per their variation present for various characters.

Table 1. Mean, range and coefficient of variation for fodder characters in *berseem* germplasm

Character	Mean value		Range of variation		C.V. (%)	No. of accessions below/above the mean value of check variety		Superior germplasm lines
	Germplasm	Check	Minimum	Maximum		Below	Above	
Green Fodder yield/plant (g)	45.9± 0.62	51.6± 5.18	5.0 (HFB 278)	113.3 (HFB 163)	32.0	399	154	HFB 163, 188, 155, 187, 189, 233, 234, 40, 41, 154, 157, 162, 164, 191
Dry Matter yield/plant(g)	11.3 ± 0.16	11.9± 1.21	1.7 (HFB 278)	23.3 (HFB 163,188)	33.3	295	258	HFB 163, 188, 155, 426, 434, 445, 461
Plant height (cm)	46.3 ± 0.26	47.9 ± 1.69	21.0 (HFB 487)	62.0 (HFB 158)	13.0	312	241	HFB 158, 173, 135, 140, 40, 9, 188, 538, 25, 154, 153, 162
Tillers/plant	14.9 ± 0.21	17.3 ± 1.80	3.7 (HFB 21)	42.3 (HFB 260)	33.9	393	160	HFB 260, 214, 209, 252, 203, 208, 439, 440, 155, 305, 213, 441, 304, 308, 187, 193, 306, 41

RESULTS AND DISCUSSION

Mean, range and percent coefficient of variation and number of accessions below/above the check value are given in Table 1. The highest coefficient of variation was observed for number of tillers per plant (33.9%) followed by dry matter yield per plant (33.3%) and green fodder yield per plant (32.0%), whereas the lowest coefficient of variation was recorded for plant height (13.9%). The range of variation for green fodder yield per plant was from 5.0 g (HFB 278) to 113.3 g (HFB 163) and the average green fodder yield per plant was 45.9 ± 0.62 as compared to check having 51.6 ± 5.18 9 per cent. The dry matter yield which is the ultimate objective for improvement in any fodder crop was found to vary from 1.7 g (HFB 278) to 23.3 g (HFB 163 and 188) and the mean value of all the germplasm lines for this character was 11.3 ± 0.16 h as compared to check cultivar having mean value 11.9 ± 1.21 g. The range of variation for plant height and number of tillers per plant was from 21.0 cm (HFB 487) to 62.0 cm (HFB 158) and 3.7 (HFB 21) to 42.3 (HFB 260), respectively. The average plant height and number of tillers per plant was 46.3 ± 0.26 cm and 14.9 ± 0.21 as compared to check variety having mean value as 47.9 ± 1.69 cm and 17.3 ± 1.80, respectively. These results indicated narrow variability in the existing germplasm. Efforts should be made to enhance the genetic variability through mutation breeding as has been done and suggested by Jatasra *et al* (1987) and Shukla and Patil (1988) and through polycross progeny testing procedure (Singh *et al.*, 1988). Thus, the variability created through mutation could be exploited for further improvement of *berseem* whereas, gain from the base population for fodder and seed yield in *berseem* could be realized utilizing family selection (Bakheit, 1988).

Correlation analysis (Table 2, 3) revealed that all the characters showed

Table 2. Correlation coefficients in *berseem* germplasm

Characters	Tillers/plant	Green Fodder yield/plant	Dry Matter yield/plant
Plant height	0.182*	0.512*	0.838*
Tillers/plant		0.591*	0.548*
Green Fodder Yield/Plant			0.794*

*Significant at P = 0.05

significant and positive association with green and dry fodder yield and these results corroborate the findings of Sidhu and Mehndiratta (1976), Jatasra (1981) and Shukla and Patil (1988) in this crop. Therefore, selection for dry matter improvement in berseem should be based mainly on plant height and tiller number per plant. Some of the best and diverse genotype amongst those given in Table 1 could be utilized in studying genetics of fodder yield and other

Table 3. Direct (diagonal) and indirect effects of dry matter yield components in *berseem* germplasm.

Characters	Plant height	Tillers/plant	Green Fodder Yield/Plant	Correlations with dry matter yield
Plant height	0.623	0.043	0.172	0.838*
Tillers/Plant	0.113	0.237	0.198	0.548*
Green fodder yield/Plant	0.319	0.140	0.335	0.794*

Residual Effect : 0.286

related characters which is lacking in *berseem* and further to exploit this information and also to utilize the promising genotypes to evolve high fodder yielding genotypes in Egyptian clover.

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IN VITRO CONSERVATION OF *ORCHIS LATIFOLIA* : A THREATENED, MEDICINAL TERRESTRIAL ORCHID

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In vitro shoot multiplication of *Orchis latifolia*, a threatened medicinal orchid has been achieved on MS medium supplemented with BAP (1.0 mg/l) and NAA (0.1 mg/l) using sprouting buds as explants. Occasionally "protocorm" like structures were also produced on the same medium. The *in vitro* raised shoots could be successfully conserved for more than 10 months at 10°C without intermittent subculture.

Key words : Salam Panja, *Orchis latifolia*, orchid, *in vitro* multiplication, *in vitro* conservation

INTRODUCTION

Orchis latifolia L. (Orchidaceae), commonly known as "Salam panja" is a terrestrial orchid which yields "Salep" of commerce. It grows wild as herb in damp places in the Himalayas from Kashmir to Nepal at an altitude of 2,500-5,000 m. The tuberous roots of the plant are used as aphrodisiac and are extensively used in Ayurvedic and Unani Systems as nervine tonic (Anonymous, 1966). An infusion of the tubers is used to relieve hoarseness. Tubers are also used medicinally as astringent, expectorant and for bone fracture (Jain 1991). The tubers contain a bitter principle and a volatile oil and the leaves contain a glucoside, loroglossin.

The plant is facing extinction due to over-exploitation and lack of cultivation efforts (Hussain, 1982; Gupta, 1993). The loss of valuable germplasm may be mitigated by conservation of this species through *in vitro* methods. To our knowledge, there are no reports on micropropagation of this threatened species. The present paper is the first report on *in vitro* multiplication and short-term conservation of *O. latifolia*.

MATERIAL AND METHODS

Source and preparation of explants

Plants of *O. latifolia* collected from Lahaul Spiti region during 1992 were planted in soil to allow sprouting of buds. Rhizome pieces with sprouting buds were washed in 'Tween 80' or 15 min. at slow speed on a magnetic stirrer and later washed thoroughly under running tap water for 2 h and subsequently surface disinfected with 0.1% mercuric chloride (BDH) for 10, 15 or 20 min. and rinsed several times with sterile distilled water. The outer leaves were removed aseptically and explants were implanted vertically on nutrient medium.

In vitro multiplication

The basal medium comprised of the mineral salts and organic nutrients of Murashige and Skoog (1962) (hereafterward referred to as MS), 3% sucrose and 0.8% bacteriological agar (Hi-Media).

Different concentrations of 6-benzyladenine (BA) singly and in combination with α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) were added for initiation and further multiplication of cultures.

All the adjuvants were added to molten agar and the pH of the medium was adjusted to 5.8 before autoclaving at 106 kPa (121°C) for 15 min. in culture tubes (150 × 25 mm, rimless) closed with polypropylene caps. The cultures were maintained at 25° ± 3°C under 10 h photoperiod provided by cool white fluorescent tubes. Each treatment was replicated six times and all experiments were repeated twice.

Regenerated shoots were subcultured after 4-6 weeks. At each subculture, The individual shoots were separated and transferred to fresh medium.

Low temperature storage

Shoot cultures were transferred to either culture tubes or wide mouth culture vessel (Bioplast) containing 15 and 40 ml of culture medium, respectively. The tubes were closed with either cotton plugs or polypropylene caps. All the cultures were kept at culture room conditions for two weeks to detect and eliminate contamination. For cold storage the cultures were incubated at 10°C and monitored periodically.

RESULTS AND DISCUSSION

Sprouted buds and basal part of rhizome with bud obtained from *ex situ* were cultured on MS medium supplemented with 0.2-2.0 mg/1 BA alone

or in combination with either NAA, IAA or IBA (0.1-0.5 mg/1) to initiate the cultures. Responses of the explants to selected treatments are described briefly. Disinfection of explant with 0.1% mercuric chloride for 10 min. was sufficient to establish aseptic cultures. At low concentrations of BA (< 0.5 mg/1) no growth of explant was observed. In all other combinations of growth regulators tested, the explants remained green and leaves started expanding after two weeks from culture. Single bud sprouting was observed in most of the combinations tested after 4 weeks. Multiple shoot formation was observed only on MS medium supplemented with BA (1.0 mg/l) and NAA (0.1 mg/1). Higher concentrations of growth regulators lead to basal callus proliferation, hence its use was avoided in further experiments.

Data obtained after 4 and 8 weeks of culture revealed that the same medium i.e. MS + 1.0 mg/l BA + 0.1 mg/1 NAA gave an optimal response with regard to overall shoot development as assessed in terms of number of shoots/culture and shoot growth (Fig. 1A, B). Shoot emergence was observed between 2 - 3 weeks and 50% explants produced multiple shoots. On an average 3-4 shoots/culture were formed after 2 - 2.5 months and medium started browning. However, if on the same medium the number of shoots increased upto 10 (Fig. 1B). Inconsistent rooting was observed on the same medium after 10 weeks. Occasionally, small white "protocom like" structures were also observed on the same medium, which produced shoots on fresh medium of the same composition. This medium has supported the persistence of the regeneration for more than 3 years so far.

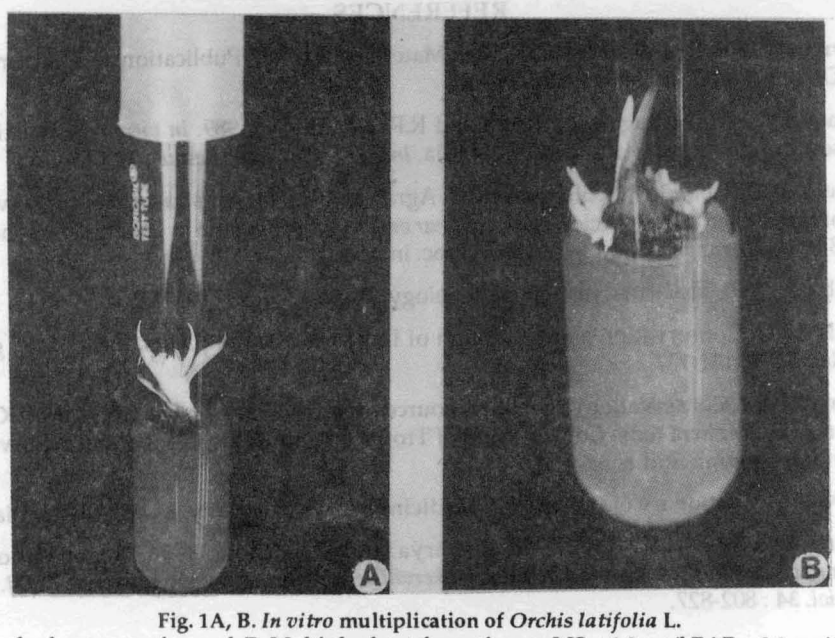

Fig. 1A, B. *In vitro* multiplication of *Orchis latifolia* L.
A. Single shoot sprouting and B. Multiple shoot formation on MS + 1.0 mg/l BAP + 0.1 mg/1 NAA after eight weeks

A preliminary study was undertaken on storage of available *Orchis* germplasm. The routine subculture period is 4-6 weeks as the medium turns brown the cultures can be maintained in viable condition at 25°C for 12 weeks with cotton plug as enclosure and for 20-22 weeks with polypropylene caps as enclosures. Maintenance of shoot cultures at low temperature (10°C) improved the survival and reduced the growth rate. The cultures could be successfully conserved for over 10 months.

Tissue culture studies are being increasingly exploited for *in vitro* multiplication and conservation of threatened plants of potential medicinal value including orchids (Chandel *et al.*, 1995; Constable, 1990; Malemnganba *et al.*, 1996; Sagawa and Kunisaki, 1984). Expectations are high about tissue culture methods providing sound strategy for conservation specially for the species in which roots or rhizome contain the active compound. In the present system, adventitious shoots regenerated without callus formation. Short-term conservation of shoot cultures for over 10 months was also achieved. The results compare favourably well with earlier published reports for medicinally important threatened plant species (Bhojwani *et al.*, 1989, Sharma and Chandel, 1992, Sharma *et al.*, 1995).

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INTRODUCTION, MASS MULTIPLICATION AND ESTABLISHMENT OF EDIBLE BAMBOO *DENDROCALAMUS ASPER* IN INDIA

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Bamboo is an economically important and multi-purpose crop. In India, 126 species of bamboos are reported out of which 25 exotic species are introduced from Myanmar, China, Japan, Malaysia and Thailand. *D. asper* is native to China and is valued economically for its edible tender shoots. A tissue culture protocol was developed for large scale multiplciation of this plant. Successful hardening and acclimatization along with field transfer at number of places in India was carried out for its establishment.

Key words : Tissue culture, micropropagation, *Dendrocalamus asper*, exotic species, Bamboo, germplasm.

Bamboos are long lived, woody evergreen grass members of the family Gramineae, tribe Bambuseae. Bamboo is a term used for more than 60 genera and 1500 species of the Gramineae (Soderstron, and Calderon 1979). India is rich in bamboo resources having 19 genera and around 126 species. This diversity is distributed all across from coastal areas in the south to around 10,000 feet above sea level in the Himalayan highlands (Kochhar and Rana 1993). A detailed account of 113 species of bamboos found in India alongwith their distribution has been given by Varmah and Bahadur (1980).

Dendrocalamus asper is one of economically and socially important bamboo species. it is native to China and is valued economically for its edible tender shoots which are consumed by local people as well as sold as canned food all over the world, earning valuable foreign exchange. Moreover, its mature culms are also used for pulp and paper manufacture. The food industry based on young shoots of *D. asper* is already well developed and expanding very fast.

The conventional sexual or vegetative methods for propagation of *D. asper*, pose numerous problems. The production of seeds takes 60 to 100 years. The seed have short viability, high seed sterility and low germinability, resulting in insufficient seedlings for plantation. The offset and rhizome cuttings are bulky and available in less numbers, involving colossal investment for prepara-

tion of planting stock as well as for transportation. Moreover, their survival rate is also very low. Thus, urgent need arose to search a non-conventional tissue culture technique for rapid and large scale micro propagation from survived *D. asper* plants through axillary bud and seed culture.

MATERIALS AND METHODS

In 1993 some (9-12) *D. asper* plants were raised from rhizome and root shoot cuttings were brought from Thailand. The cuttings were tried for establishment at different places in India *viz* - 2 plants each at Tropical Forest Research Institute (TFRI), Jabalpur, Forest Research Institute (FRI), Dehradun, Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Conservator Forest House (CFH), Chhindwara and 1 at State Forest Research Institute (SFRI), Jabalpur. Out of these only 3 plants (1 each at TFRI, FRI and CFH) survived. Also, 100 g of *D. asper* seeds were obtained from F.A.O. (Food and Agriculture Organization) consultant from Royal Forest Department, Chatuchak, Bangkok.

In vitro axillary bud culture

Nodal segments with axillary buds were collected from young juvenile shoots of mother plant grown at TFRI, Jabalpur and washed with 5% cetavelon (detergent) for 15 min. followed by surface sterlization with 0.1% mercuric chloride solution for 5-10 min. The sterilized buds were rinsed thrice with autoclaved distilled water before transfering them to liquid and semi-solid Murashige and Skoog's 1962 (MS) medium supplemented with different concentrations of benzyl aminopurine (BAP). Explant was placed vertically in medium (Fig. 1-A). The cultures were incubated in the culture room at 26 ± 1°C with 16 h photoperiod (3000 lux) provided by cool white fluorescent tubes.

In vitro seed culture

Seeds of *D. asper* stored at 4°C were disinfected with 90% ethanol for 1 min. and subsequently in 6% Sodium hypocholorite solution for 25 min. Seeds were rinsed thrice with autoclaved distilled water and cultured on MS medium supplemented with 1.0-10.0 mg/l BAP. For rooting, shoots (1-3 cm long) were cultured on MS medium supplemented with α-naphthalene acetic acid (NAA) or Indole butyric acid (IBA).

RESULTS AND DISCUSSION

Multiplication of shoots

Axillary bud break was achieved in all healthy cultures within two weeks (Fig. 1-B). The proliferated shoots from axillary buds were excised and

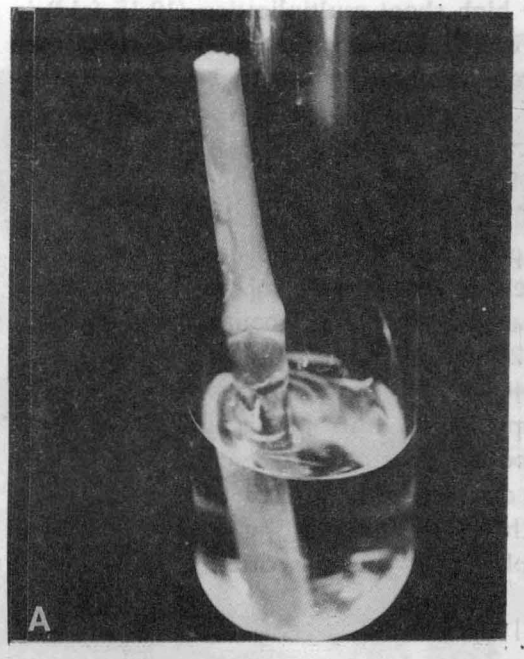

Fig. 1a. Axillary bud cultured on liquid MS + 5.0 mg/1 BAP

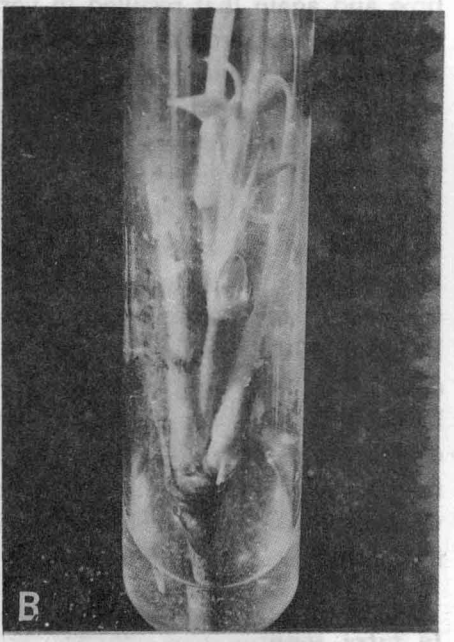

Fig. 1b. Axillary bud proliferation in 2 weeks

subcultured on MS medium supplemented with 2.0-5.0 mg/1 BAP for further shoot multiplication. Multiple shoots 1-20 were formed immediately after seed germination. Number of shoots developed from the single seed was directly correlated to BAP concentration used in the medium.

After 3-4 weeks of subculture, the *in vitro* shoots further multiplied (Table 1). The cluster of shoots were carefully excised (clusters of three shoots) and

Table 1. Micropropagation and survival of plants of *D. asper* through axillary bud and seed culture

Source	Shoot multiplication after				Survival (%) of plants after	
	I subculture	III subculture	X subculture	Rooting (%)	Hardening	Field transfer
Single Axillary bud (TFRI plant)	1-2	1-3 fold	15 fold	100	95	70-80
Seed (Ten lines)	1-20	5-7 fold	10-12 fold	100	95	80-90

again subcultured on shoot multiplication medium. The procedure was repeated time and again that resulted in very high shoot multiplication (10-15 fold) in every 3-4 week cycle of subculture (Fig. 1C). Similarly, shoots were maintained and multiplied by repeated subculture for large scale shoot production. Thorpe and Patel (1984) and Ahuja (1991) have reported *in vitro* asexual multiplication by axillary bud breaking and production of adventitious buds in tree species. The recommendations of the third International bamboo workshop held in India (Ramanuja Rao *et al.* 1990) on the conservation of bamboo resource gave emphasis on the collection of gene pools and the germplasm exchange. The FRI's Collections which include 25 exotic species of bamboos, introduced in the past from Myanmar, China, Japan, Malaysia and Thailand (Vermah and Bahadur, 1980) is now increased to 26 exotic species with the addition of *D. asper*. Thomas *et al* (1990) have reviewed the strategies for conservation of bamboos which also gave due emphasis on micropropagation. The major difficulty for active exchange of bamboo germplasm among countries/continents is the remote availability of seed due to long flowering cycles and the vulnerability of bulky rhizomes which get damaged during transportation but in comparison tissue culture-raised plants can be transported easily.

In vitro rooting

Rooting was achieved in shoots (3-5) cultured on MS medium supplemented with 3.0 mg/1 NAA or 10.0 mg/1 IBA in 98-100% culture. The root initiation was observed after 2 weeks from culture on rooting medium. Usually 8-10 roots developed from each shoot clumps when cultured on MS + 2.0-3.0 mg/1 NAA and 10-20 roots on MS + 8.0-10.0 mg/1 IBA medium (Fig. 1D).

Hardening and acclimatizaton of tissue culture raised plants

Four-week-old cultures on rooting medium developed healthy root and shoot system. These plantlets were first transferred to polybags containing soilrite: sand (2:1 v/v) mixture and were kept in the mist chamber under 80% RH and 30± 2°C temperature for hardening (Fig. 1E). The plants were irrigated with half strength macro- and micro-nutrients of MS salts. After 20-25 days, the hardened plants were transferred to polybags containing a mixture of soil: sand : organic manure (1:1:1) and kept under high density agronet shade house for acclimatization (Fig. 1F). Plants wrre ready for field transfer in one month. Till date, some 18,000 plants have been propagated from single axillary bud culture and around 5,000 plants through seed culture. Field transfer of tissue culture raised plants was carried out during June-December at Jabalpur, Baster and Chhindwara regions of Madhya Pradesh and other states of the country (Table 2). The field plants developed rhizomes within two months,

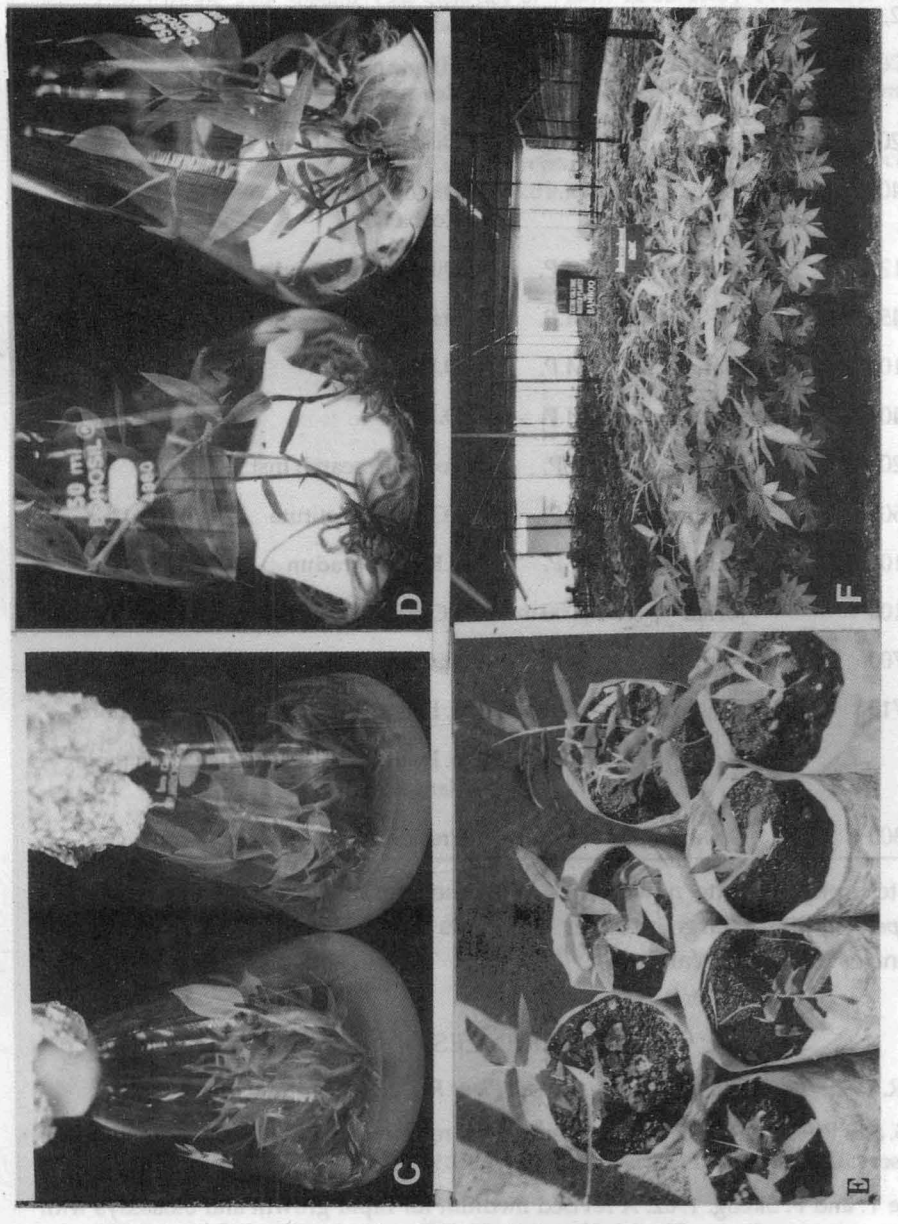

Fig. 1c. Shoot multiplication on MS + 3.0 mg/l BAP, d. *In vitro* rooting on MS + 3.0 mg/l NAA, e. Hardened plants in polybags and f. Acclimatization in shade house

Table 2. Distribution and field plantation of *D. asper* in 1995

S. No.	No. of Plants	Place	State	Address
1.	2,000	T.F.R.I.	M.P.	T.F.R.I., P.O. R.F.R.C., Jabalpur
2.	700	Chhindwara	M.P.	Human Resource Development Research Institute, Chhindwara
3.	200	Bastar	M.P.	Distributed among farmers
4.	400	Korba	M. P.	District Forest Officer (D.F.O) Korba Div.
5.	1200	Kanker	M.P.	Distributed to farmer
6.	450	SFRI, JBP	M.P.	State Forest Research Institute, Jabalpur
7.	100	Mandla	M.P.	D.F.O.
8.	40	Seonui	M.P.	D.F.O.
9.	20	Kanour	U.P.	Forest Research Inst., Kanpur
10.	500	Allahabad	U.P.	I.C.F.R.E. Institute
11.	10	Dehradun	U.P.	F.R.I., Dehradun
12.	100	Jorhat	Assam	I.C.F.R.E. Institute
13.	700	Nagpur	M.S.	Divisional Manager, F.D.C.M., Nagpur
14.	715	Banglore	Karnataka	a. Forest Research Centre, Bangalore
				b. Institute of Wood Science technology, Bangalore
15.	300	Delhi	Delhi	President House, Delhi

which later sprouted into new culms. With the present efforts of multiplication of *D. asper* plants, a large number of plants have been supplied to various user agencies including farmers.

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PRE-TREATMENTS TO IMPROVE GERMINATION IN *SOLANUM VIARUM* DUNAL

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Seeds of *Solanum viarum* exhibits dormancy/hardseededness and thus complicates the recording of exact germination which is prerequisite for long term storage in the gene bank. In the present study, the initial germination percentage of seeds varied from 22% to 30% in *Solanum viarum (var. Arka sanjeevani)*. Pronounced effects of seed pretreatment with sulphuric acid and gibberelic acid on germination have been reported by the authors. The best treatments worked out was over-night soaking in GA_3 (500 ppm) and acid scarification with sulphuric acid (25%) for one hour. These treatments not only increased the germination percentage but also reduced the mean germination time significantly under laboratory conditions.

Key words : Pre-treatments, seed, *Solanum viarum*, germination, dormancy.

Steroids bearing Solanum (*Solanum viarum* Dunal syn. *S. khasianum* CB Clark var. Sengupta), is an important medicinal plant belonging to the family Solanaceae. It is a much branched prickly under-shrub or shrub distributed in the Assam Khasi hills. Mature fruits are rich source of diosgenin/solasodine, an analogue of 16-DPA, which is commercially exploited as raw material for the manufacturing of sex hormones and contraceptives. Studies on germination behaviour under laboratory conditions have not been yet done in respect to *Solanum viarum*. Seeds show poor germination (upto 30%) at optimum temperature of 25°C in dark (standerdised earlier for this genus). This may be either due to impermeable seed coat which inhibits water imbibition and thus arresting the germination or due to dormant embryo which needs triggering of hydrolytic enzymes responsible for the germination (Bewley and Black, 1978). The experiments reported here are an attempt to enhance the germination percentage through various physical and chemical treatments in laboratory conditions.

MATERIALS AND METHODS

Physiologically matured seeds of *Solanum viarum* Var. Arka sanjeevani (1994 harvest) were procured from M&AP center Bangalore through Project-Co-ordinator, Medicinal and aromatic plants. The seeds were subjected to the following dormancy breaking treatments:

a) Hot water treatment- Seeds were dipped in hot water at following combinations of temperature and time periods :

i) 60°C ... 30 min.

ii) 65°C ... 15 min.

iii) 70°C ...15 min.

iv) 75°C ... 3 min.

v) 80°C ... 1 min.

vi) 100°C ... 1 min.

After the treatments seeds were removed and cooled to room temperature.

b) Acid treatment — Seeds were subjected to the following combinations of treatments:

i) 100% Sulphuric acid ... 1 min., 5 min., 10 min.

ii) 50% Sulphuric acid ... 30 min., 60 min.

iii) 25% Sulphuric acid ... 30 min., 60 min.

After this treatment seeds were washed in running water for 1 hour.

c) Gibberelic acid (GA_3) (Pre-application) — Seeds were soaked for over-night in the following concentration of the gibberelic acid-

i) 500 ppm

ii) 100 ppm

iii) 50 ppm

d) KNO_3 — seeds were soaked for over-night in 0.2% solution.

e) GA_3 and KNO_3 were also given as co-application.

f) Acid scarified seeds (25 % H_2SO_4 for one hour) were dried to moisture level of 5.5% and stored for a fortnight. Then germination was tested.

The germination test was carried out at 25°C in dark. Twenty five seeds were placed on double layered Whatman no. 1 filter paper moistened with 5 ml of distilled water in sterilized plastic petri-plates. The percentage of normal seedlings and hard seeds were calculated from the total number of seeds placed. Mean germination time was calculated as per the formula of Ellis and Roberts (1980). Germination index is calculated as the product of radical length and germination percentage.

Germination count was taken after every 3rd day taking the protrusion of the radical as indicative of germination. Root length and shoot length of the seedlings were taken on the final day. Vigour index was calculated as the product of shoot length and germination percentage.

Data were analysed as a factorial design with minimum of four replications in all cases. All the data were subjected to an analysis of variance and were tested for significance using MSTATC Software.

RESULTS AND DISCUSSION

Statistically analysed data showed significant effects of various seed pre-treatments on germination reported in Table 1. The initial percentage of

Table 1. Effect of various pre-treatments on germination and vigour of *Solanum viarum*.

Treatment	Normal Seed-ling	Hard seed	MGT days	Total time for max. germina-tion	Root Vigour (cm.)	Shoot Vigour (cm.)	Germi-nation Index (rlx%g)	Vigour Index (shlx %g)
SULPHURIC ACID TREATMENT								
1. 100% (1 min.)	31.00	67.00	3.17	36	0.47	1.47	13.80	41.65
2. 100% (5 min)	28.00	72.00	3.15	36	0.65	1.65	18.80	46.80
3. 100% (10 min)	51.00	49.00	3.29	36	0.47	2.00	19.75	102.10
4. 50% (30 min)	28.00	71.00	6.06	36	1.15	2.00	32.40	55.60
5. 50% (60 min.)	25.00	75.00	6.12	36	1.27	1.87	34.47	47.10
6. 25% (30 min.)	72.00	28.00	13.73	21	2.12	3.67	153.80	264.20
7. 25% (60 min.)	92.00	8.00	13.47	18	3.60	4.82	330.90	443.50
GIBBERELLIC ACID PRE APPLICATION								
8. GA3 (500 PPM)	89.00	7.00	13.18	21	2.87	4.15	258.00	367.70
9. GA3 (100 PPM)	53.00	50.00	12.21	36	2.30	3.32	122.40	177.10
10. GA3 (50 PPM)	49.00	51.00	15.12	36	1.42	2.67	69.10	133.60
GA-CO-APPLICATION								
11.	58.0	42.00	17.28	36	2.60	4.15	150.80	241.50
12. Pre-treat and stored	91.00	9.00	8.058	21	2.97	4.75	272.00	430.00
13. CONTROL	31.00	67.00	13.03	36	0.62	1.77	20.67	55.50
CD at 5% level	6.73	6.88	2.48	-	0.45	0.68	44.87	60.67

dormant seeds without any treatment ranged from 65%-70% in the tested accessions. Of the twenty two treatments tried only two treatments showed positive effects i.e. above 90% germination. Immersing seeds in hot water at various combinations of temperature and time did not result in improved germination. 100% sulphuric acid treatment when given for 10 minutes, increased germination to 51% while smaller durations (1 min & 5 min.) had no effect (Fig. 1). Similar effect were seen with 50% sulphuric acid. (Table 1).

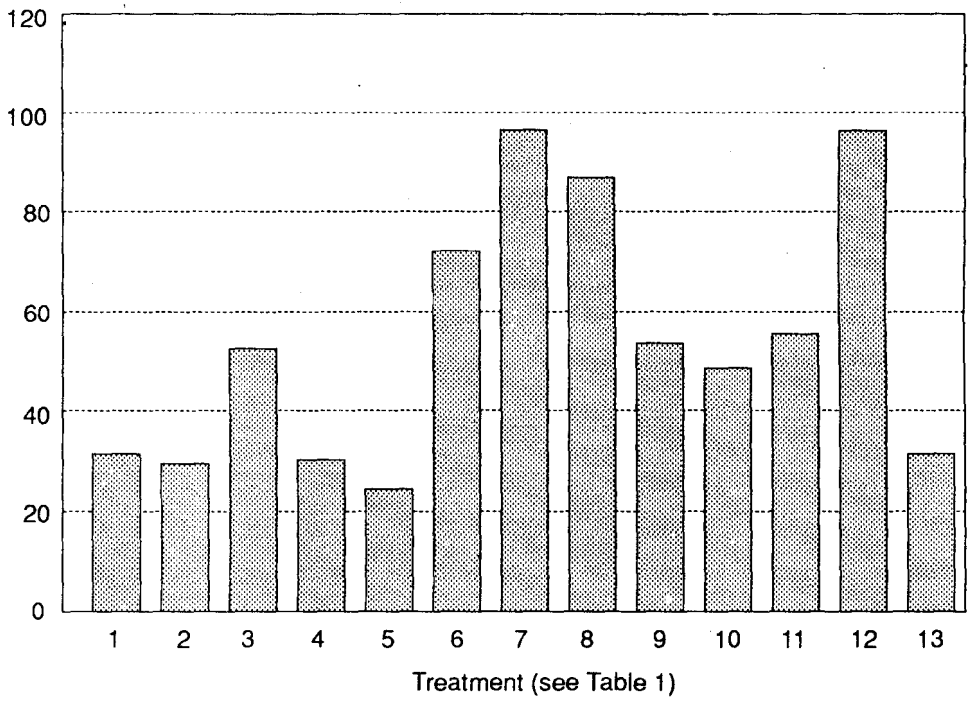

Fig. 1. Total Germination Vs. Treatments

The most successful treatment was found to be the exposure of seed to 25% sulphuric acid for one hour where germination was 100 percent. GA3 treatment for overnight in 100 ppm and 50 ppm also showed germination percentage of 50 and 53 percent respectively. Higher concentration at 500 ppm level significantly increased the germination (up to 90%). Presoaking treatment in 25% sulphuric acid for one hour where germination was 100 percent. GA3 treatment for overnight in 100 ppm and 50 ppm also showed germination percentage of 50 and 53 percent respectively. Higher concentration at 500 ppm level significantly increased the germination (up to 90%). Presoaking treatment in 25% acid for one hour and then storing seeds for a fortnight (after drying to moisture level of 5.5%), and then testing for germination was also beneficial in breaking the seed dormancy.

Rate of Germination

In the seeds without any pre-treatment (control) only 30% germination was recorded after 36 days. Various treatments resulted in reducing the total time for germination (Fig. 2). 25% Acid treatment as well as GA_3 showed

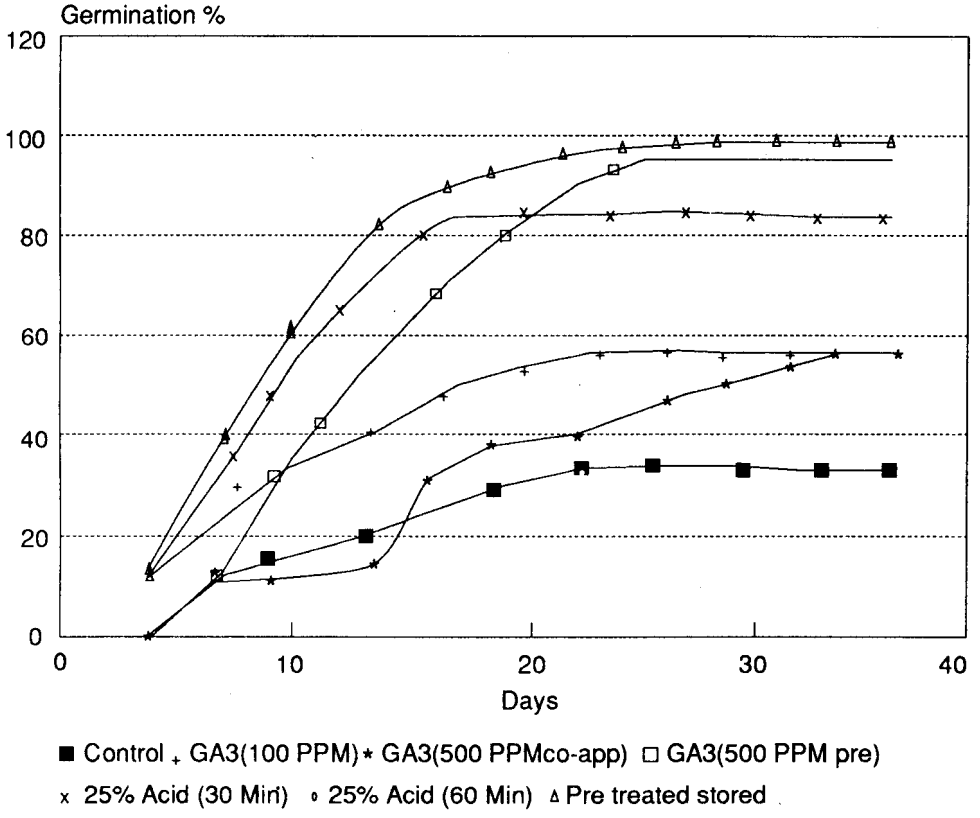

Fig. 2. Rate of Germination

100% germination and significantly reduced the mean germination time to 18-21 days. Stored pretreated seeds also took 21 days to complete the germination. Concentrated (100%) and 50% sulphuric acid treatments showed high initial germination but after 12th day it ceased. Similarly germination index was maximum in 25% acid treatment (258). The minimum germination index was recorded in 100% sulphuric acid (13.80%).

Vigour Parameter

Treatments with higher concentration of acid showed lower values for root length and shoot length and correspondingly low value of vigour index.

Maximum value of root (3.6 cm) and shoot (4.32 cm) vigour was recorded in 25% acid treatment (Table 1). Similarly vigour index was higher in 25% acid treatment (Table 1). It was followed by 25% pretreated stored seed (430.00%) and GA3 (500 ppm) with 367.7%), Interestingly all GA3 treatments were found favourable in radical and hypocotyl elongation (2.8 cm and 4.15 cm) over other treatments.

This enhancement in germination due to pre-treatments may be attributed to the easy imbition of water through scarified seed surface in acid treatment thereby facilitating the onset of germination due to mobilization of the food reserves whereas GA3 directly activates the embryo by stimulating the synthesis of proteins, m-RNA and the hydrolytic enzyme activity in the embryo (Bewlay and Black, 1978). This data is well supported by the significant improvement in the seedling vigour and rate of germination. Promotive effects of hot water treatment are reported by several authors (Padma et al 1993 in different *Acacia* spp), but in *Solanum viarum*, it had no effect in improving the germination. Sulphuric acid (100% & 50%) as well as GA3 (100 ppm & 50ppm) were less effective as compared to other successful treatments. Similar promotory effect of acid treatment and GA3 are reported by Carpenter and Boucher (1992) in different cultivars of *Catharanthus roseus*. In contrast to the findings of Rana and Nautiyal (1989) who reported that fresh acid sacrified seeds of *Acacia farnesiana* showed late onset and longer duration for completion of germination, in the present investigation acid scarification and GA3 treatment not only increased germination with an enhanced hypocotyl and radical vigour but also resulted in significant reduction in the total germination time over control.

Hence for the first time it has been worked out that seed pretreatment with 25% sulphuric acid (one hour) or with GA3 (500 ppm) resulted in 100% germination and also reduce the total germination time. This could be of great significance and of practical use in rapid multiplication of this economically important commercial medicinal plant.

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RESPIRATION AND SEEDLING VIGOUR OF *BRASSICA OLERACEA* UNDER NATURAL AND ARTIFICIAL AGEING CONDITIONS

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Respiration and seedling vigour of naturally and artificially aged seeds of *Brassica oleracea* were studied to elucidate the nature of deteriorative changes under these two types of conditions. Results indicate that the ageing conditions had a variable effect on oxygen uptake and seedling vigour and metabolic changes associated with artificial ageing may differ from those during natural ageing.

Key words : Respiration, seedling vigour, *Brassica oleracea*, ageing

The respiratory characteristics of stored seeds have received particular attention from investigators. Oxygen uptake of seeds have been found to have a bearing on the inherent storability characteristics of species (Vertucci and Leopold, 1987). Low rates of O_2 uptake have been observed during imbibition of aged seeds. This has usually been ascribed to mitochondrial deterioration.

Studies on seed ageing have often used techniques to accelerate the ageing process, thereby shortening the time necessary for study. Exposure to high temperature and high moisture conditions (typically 40-50°C and 100% RH) is a standard method used for accelerated ageing of seeds in the laboratory (Powell and Mathews, 1977). The effect of artificial ageing conditions of high temperature and low humidity on seed processes has not been studied. Similar conditions could be encountered in some situations in the drying room or in the seed store when power fails. Of particular interest are the differences between accelerated ageing at high temperature and high relative humidity and deterioration under less stressful conditions.

This investigation was conducted to evaluate changes in respiration and seedling vigour of *Brassica oleracea* seeds during deterioration under (1) controlled conditions of storage at –18°C and 6 per cent seed moisture content and (2)

artificial ageing conditions of high temperature, high relative humidity and high temperature, low relative humidity.

MATERIALS AND METHODS

Seeds of two lots of *B. oleracea* var. capitata cv. Early Round Dutch, stored for different durations under controlled conditions of –18°C were obtained from the seed bank at National Seed Storage Laboratory, Fort Collins, Colorado. Lot 1 was stored since 1961 and lot 2 was stored under same conditions since 1981. Germination test showed 36% and 92% viability respectively. These were considered naturally aged seeds. Another seed lot stored at 10°C for five months was used to get artificially aged material. The germination value of this lot was 93 percent. The experiment was started in Dec. 1989. Seeds were aged under two different environments. One lot was held at 45°C and near 100 per cent relative humidity (RH) for 3 days. Another lot was placed over concentrated sulphuric acid in an airtight container at 35°C for 12 weeks. These two treatments provided seeds aged under high humidity and desiccating conditions respectively. Oxygen uptake as a function of seed moisture was measured manometrically at 25°C in dark using Gilson differential respirometer. 1.5g seed weight was used per sample flask. The well contained 0.1 ml of 60% KOH solution. Different seed moisture contents were obtained by equilibrating seeds over saturated salt solutions, sulphuric acid solution or by adding calculated amounts of water in a sealed petridish and equilibriating the seeds overnight at room temperature. Moisture contents are expressed on a dry weight basis. The dry weights were determined immediately after O_2 uptake experiment by drying seeds at 95°C in a forced draught oven for 5 days. Measurement at each moisture content were replicated three times.

Standard germination test was conducted for all four seed lots. Four replicates of 25 seeds each were germinated at 25°C. Radicle lengths were measured after 72 hrs. Germination Index (GI) was calculated as a product of radicle length and germination percentage.

The rate of leakage of electrolytes from seeds within first hour of soaking was measured using an ASAC-100 conductivity meter. Three replicates of 5 seeds each were used. Seeds were prehumidified overnight at saturating humidity to avoid possible leakage due to imbibitional damage.

RESULTS AND DISCUSSION

Ageing conditions had a variable effect on the seedling vigour expressed as germination index. When seeds were accelerated aged at 45°C and near 100 per cent relative humidity for 3 days seedling vigour declined drastically as in the 29 years old "Naturally" aged seed (Table 1). In comparison the

decline in GI was not so drastic when the same seed was artificially aged over H_2SO_4, (1%RH) at 35°C for 12 weeks. The rate of leakage of electrolytes was 1.5 to 3.0 fold higher in the artificially aged seed lots (Table 1). Rate of leakage was more in the lot aged at 45°C and 100% RH which is a more stressful treatment. These results show deteriorative changes in the membrane integrity as measured by conductivity in response to artificial ageing treatments and that the damage to membrane integrity is greater as a result of artificial ageing treatment than due to "natural" or slow ageing of seeds. Therefore, impaired membrane semipermeability is suggested as a critical lesion caused by artificial ageing in seeds both under high humidity and desiccating conditions. Increase in solute leakage from seeds after accelerated ageing (45°C, 100% RH) were also documented in soybean. The amount of leachate collected was positively correlated with the degree of accelerated ageing. Solute leakage is notably increased even before reduced performance is detectable (Powell and Mathews, 1977). A similar trend was observed in seed aged over H_2SO_4 at 35°C, where the decline in GI was not concomitant to increase in conductivity indicating that leachate conductivity is not closely correlated with seedling vigour (Table 1).

Table 1. Germination index and rate of leakage of electrolytes in seed lots stored/aged in different environments.

Storage/ageing environment	Germination Index (Radicle length × Percent germination)	SE	Rate of leakage (μmhos $min^{-1} g^{-1}$)	SE
"Naturally" aged Seed: Stored at -18°C, 6% MC				
9 year old lot	38.54	1.53	463.89	11.72
29 year old lot	1.52	0.18	753.16	17.62
Artificially aged seed :				
45°C, 100% RH, 3 days	3.36	0.73	1471.12	563.53
35°C, 1% RH, 12 weeks	29.18	2.03	1113.25	226.83

These observations support previous suggesting that membrane dusruption is one of the factors responsible for drying damage (Herter and Burris, 1989). Studies have also shown that majority of cellular membranes remain physically intact following a lethal desiccation stress but their biophysical and biochemical properties are dramatically altered (Mckersie *et al.*, 1988). This might explain the increased rate of leakage of electrolytes in seeds aged over $H_2 SO_4$ with no concomitant change in the GI.

Seed deterioration is usually associated with one or more aspect of respiratory metabolism. Respiratory oxygen uptake as a fuction of seed moisture content was observed in naturally and artificially aged seeds at 25°C in dark. At specific moisture contents ranging from 0.10 to 0.20g H_2O /g dry weight, oxygen uptake increases sharply with increase in moisture contents (Fig. 1). Below these threshold moisture levels the O_2 consumption was lower. Oxygen uptake at low moisture contents (i.e. < 0.10 to 0.15 g/g dw) is higher in the artificially aged seeds, more so in the seeds aged at 35°C over H_2SO_4. At high MC (> 0.10 to 0.20 g/g dw) the trend is reversed i.e. uptake at higher MC is lower in the accelerated aged seeds than in the naturally aged (9 year old) seeds, however O_2 uptake was slowest in the 29 years old lot reflecting the high degree of deterioration.

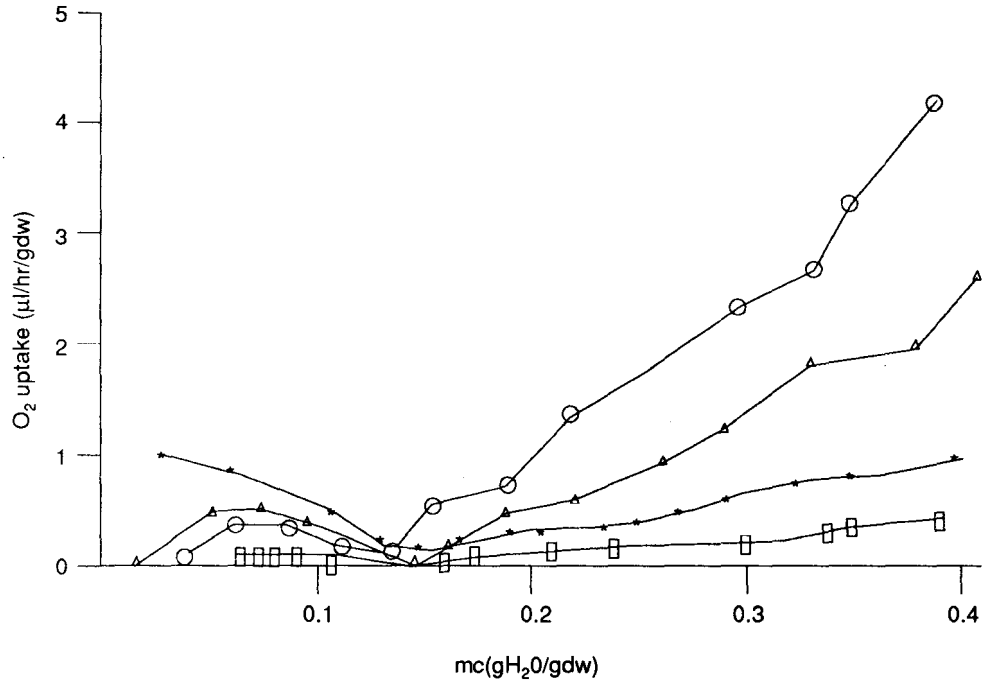

Fig. 1. The relationship between moisture content and oxygen uptake for the 29 year old "naturally aged" seed (□), 9 year old "naturally aged" seed (o), Artificially aged, 35°C, over H_2SO_4, seed (x) and artificially aged 45°C, 100% RH seed (Δ). Each point represents the mean of four replicates.

These trends suggest that oxidative reactions at low moisture level are predominant in the artificially aged seeds whereas the respiratory O_2 uptake at high moisture gets depressed. The decline in mitochondrial function begins early during seed deterioration and apparently can progress significantly before

changes in whole seed vigour are observed. Correlation between respiration and seedling vigour are therefore not universal. The nature of oxidative reactions responsible for the slow rates of O_2 uptake at low water contents is not clear but it is suggested that oxidative reaction at these low water contents have deleterious effects on seed viability (Ibrahim and Roberts 1983). The increased O_2 uptake at low water contents in artificially aged seeds indicates enhanced level of these oxidative reactions in response to the stressful conditions prevailing during the ageing treatment.

Among the accelerated ageing treatments, seeds aged at high humidity showed higher respiratory oxygen uptake than the seeds aged at low humidity (Fig. 1).

Studies have shown that the mechanism of ageing change with hydration level and that in very dry seeds autooxidations are largely responsible for ageing (Wilson and McDonald, 1986; Flood and Sinclair, 1981). Artificial ageing techniques introduce damage processes not present in naturally ageing seeds and/or alter the relative rates of damage processes responsible for ageing. Reports on whether the mechanism of natural and artificial ageing are similar or not are conflicting (Mathews, 1987; Priestley and Leopold, 1983). The results presented here, do however, support the reservation of Priestley and Leopold (1983) and Petruzzelli and Taranto (1984) that the metabolic changes associated with artificial ageing may differ from those during natural ageing.

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POLYMORPHISM AND GENIC EXPRESSION OF ESTERASE AND ACID-PHOSPHATASE ISOZYMES AT DIFFERENT DEVELOPMENTAL STAGES IN *LENS CULINARIS* MEDIKUS

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Esterase and acid-phosphatase isozyme patterns were studied at different developmental stages (0hr, 24 hrs, 48hrs, 96hrs, 120hrs, 144hrs) in four cultivars of *Lens culinaris* Medik). The results of this study show that the total enzyme repertory of an organism changes during development and differentiation which can be detected by the expression of their isozymes at the various developmental stages which are under genetic regulatory control mechanisms. In accordance with this a characteristic isozyme pattern was found in the different varieties of *Lens culinaris* at different developmental stages. Plant populations can also be differentiated genetically as was studied by observing the degree of polymorphism with respect to these two isozymes.

Key words : *Lens culinaris*, isozyme, PAGE, esterases, acid-phosphatases.

Lentils, *Lens culinaris* assume considerable importance due to their high protein content (24-25%) and as a means of improving soil fertility. The use of isozymes as genetic markers has increased dramatically over the last decade as it has a number of important advantages over more conventional morphological markers (Scandalios, 1975).

In order to gain evidence on the existence of polymorphic variation and assessing genetic variation in terms of isozymes, we initiated this investigation on four varieties of *L. culinaris* hypothesizing that genetic variation at molecular level would exist. The study was based on the changes in banding patterns of esterase and acid phosphatase isozymes at different stages of development of seed and seedling growth in four cultivars of *L. culinaris*. Comparisons of

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these isozyme patterns were made between and within the cultivars at different developmental stages in order to observe differential gene expression at different developmental stages as also to determine the extent of genetic variation expressed as polymorphic forms of the above isozymes existing at inter-varietal level.

MATERIALS AND METHODS

Experimental system :

The experiment comprised of the selected four varieties of *Lens culinaris* (Table 1) obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Table 1. Variety, parentage, type and their unique characteristics

Variety	Parentage	Type	Unique characteristics
L-4163	L-293 XL-830	Macrosperma	
LC-74-5	L-293XL-830	Macrosperma	
Precoz	A selection from ILL-4605 (Argentinian origin)	Macrosperma	Early maturing
L-4076	Pant L-234X Pant L-639	Microsperma	One of the oldest cultivar released

The anionic system of Davis (1964) was adopted for separation of isoenzymes by disc electrophoresis using polyacrylamide gels (PAGE).

Experimental method :

Preparation of protein extract :

The seeds of different varieties were kept for germination in wet sheets of filter paper after thorough washing of the seeds with 0.1% mercuric chloride. 0.5 gm of seeds were weighed and crushed in a pre-chilled pestle and mortar into a fine paste by adding 3.5 ml extracting buffer (0.2 M Tris-HCl). The ground paste was contrifuged for an hour in a refrigerated centrifuge at 12000 rpm using 10 × 15 ml 300 angle rotor head of IEC-25 high speed refrigerated centrifuge. The supernatant (extract) was drawn for each stage of seed and seedling growth. The protein extract thus drawn was stored in glass vials in the deep freeze and subsequently used for isozyme analysis for esterases and acid-phosphatases.

Electrophoresis and enzyme localization

The stacking and separating gels respectively were prepared according to Davis (1964). The gels were stained for esterases and acid phosphatases. Each isozyme band was characterized by its Rf value.

RESULTS AND DISCUSSION

The morphological variation which is a product of genotype and the environment is no doubt an important parameter but much diversity which remains unexpressed morphologically can be revealed by biochemical methods. Study of isozymic variation is one such important and powerful procedure which has often been employed for this purpose. Isozymic variation has been chosen here to reveal the diversity existing at molecular level in the genepools of *Lens culinaris*.

Isozyme patterns of esterases :

Esterase isozyme pattern obtained on polyacrylamide gels different developmental stages of seeds of four cultivars of *Lens culinaris* Medikus have been presented in the form of zymograms, (Fig. 1A).

It is evident that each variety has its own pattern of increase and decrease in number of bands at different stages of seed development. But in general, it was observed that there was an increase in the number of bands from 24 hours onwards till 72 hours of seed germination and seedling growth after which there was a slight decline in the number accompanied at times by appearance of some new band at a new locus. The only exception to the above mentioned observations was the LC-74-5 variety.

The appearance of isozymes at different stages of development in all varieties could be restricted to three zones, a fast moving zone in which isozymes reach the anodal end, a slow moving zone in which isozymes occupy the cathodal end and an intermediate zone occupying the central region of the gel.

Each zone is occupied by a particular isozyme in the form of a band and is representative of the expression of a particular gene locus coding for that isozyme. In certain stages, in a particular zone more than one distinct band is resolved. These bands could represent allelic isozymes ("allozymes", Prakash et al., 1969), coded by different alleles of the same gene at that locus and thus occupy that particular zone on the gel.

The esterases are a complex and heterogenous group of enzymes catalyzing the hydrolysis of the ester link. The isozyme forms of this enzyme being the primary gene products, gene homology can be deduced with precision by

comparing variation in their expression patterns in different cultivars of *L. culinaris* at different stages of development.

The general pattern of increase in number of zones of activity concomitant with an increase in number of isozymes in three varieties upto 72 hours of seed germination can be explained by the fact that dry seeds (0 hours) contain the nutrients for the initial plant growth and development but carry on all life processes at low metabolic rates. When seeds are kept in moisture for germination, the biological activity is initiated necessitating the production of more isozymes (Singh and Gupta, 1978)

Gradual shifts of isozymes' patterns in seeds of different varieties during the course of development demonstrated the appearance or disappearance, or both, of individual isozymes. The changing pattern during development may be interpreted as evidence for differential timing of gene expression correlated with the physiological changes during germination as observed in other plants (Presely and Fowdson, 1965; Johnson et al., 1973).

The isozymes confined to the anodal zone operational from 0 hours in all varieties and had remained expressed at all stages of development the other two zones. This increased intensity of isozyme bands may indicate either additive effects or enhanced synthesis of these isozymes.

The four varieties of *L. culinaris* under the investigation were also compared amongst each other for their electrophoretic variations in non-specific esterases at different stages of development, (i.e. 0, 24, 48, 72, 96, and 120 hours).

Each variety displayed a characteristic banding pattern in different stages of development which can be compared with other varieties at these particular stages for cultivar identification.

Figure 1A indicates the polymorphic variation of esterase isozymes in the different varieties of Lentils showing an extensive polymorphism existing at inter-varietal level with respect to these isozymes. With the exception of band (Rf-0.75) which is present in all varieties except in variety L-4076 at some stage of development, all other isozyme bands appear to be polymorphic in nature.

Band with Rf value 0.74 was observed in variety L-4163 only, at all stages of development and being an ubiquitous band could be used as a genetic marker for that variety.

Isozyme patterns of acid-phosphatases:

Electrophoretic banding patterns of acid phosphatases in four varieties of *L.culinaris* at different stages of seed germination (0, 24, 48, 72, 120 and 144 hours) are also displayed in the form of zymograms (Fig. 1B).

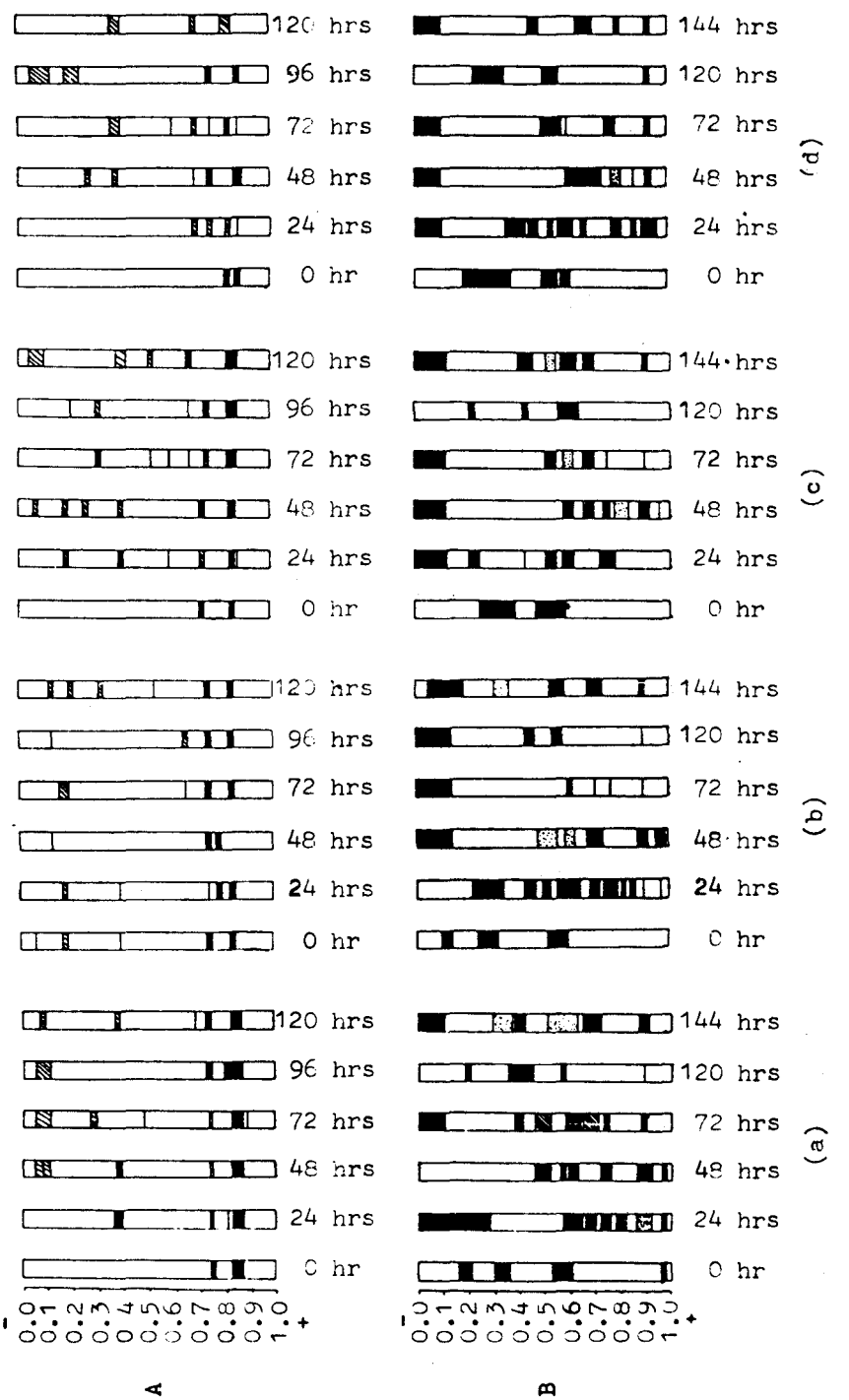

Fig. 1. Represents electrophorograms of isozymes (A) esterases, (B) acid phosphatases at different developmental stages of seed and seedling growth in four cultivars of *Lens culinaris* Medik.; (a) L-4163, (b) LC-74-5, (c) Precoz, (d) L-4076 separated using disc. polyacrylamide gel

The general pattern observed in all varieties was a sudden increase in number of bands from dry seeds stage to 4 hours seed germination stage followed by a slight reduction in the later hours. A further reduction in number of bands at 120 hours developmental stage followed by an increase in the number at 144 hours was also observed.

There are several plant species within which varying numbers of molecular forms of acid phosphatases (orthophosphoric monoester phosphohydrolase, E. C. 3.1.3.2) have been described (Dorn 1965; Brown and Allard, 1969; Cherry and Ory, 1973; Baker and Takeo, 1973) They function in various aspects of cell metabolism such as transport of sugars across membranes (Sauter, 1972), senescence in fruits (De Leo and Sacher, 1970) and in catabolic processes in seeds (Rychter et al, 1972) such as the hydrolysis of phosphomonoesters, important in a variety of biochemical reactions including the formation of sucrose in photosynthesis.

The sudden increase in number of isozymes at 24 hours seed germination can thus be explained on the basis of increase in metabolic activity when seeds are soaked for germination, thereby increasing the production of isozymes. A slight reduction in the later hours, followed by an increase in the number of bands at 144 hours seed germination stage is in consonance with the general belief that metabolic changes occur during seed development and germination (Janardhan et al., 1986).

The general pattern of appearance and disappearance of bands can be explained similarly on the basis of gradual shifts of isozyme patterns in samples taken in the course of development due to differential activation of genes involved in synthesis of these enzymes at the different stages of development (Scandalios, 1969). No unique band present in a particular variety at all stages of development could be located with respect to acid-phosphatase isozymes.

Since the predominant measure of genic polymorphism is currently electrophoretic analysis (Milkman, 1975), consequently the results obtained by determining the extent of genetic variation expressed as polymorphic forms of acid-phosphatases of inter-varietal level shows that extensive polymorphism exists in the different varieties for these isozymes.

The isozyme data obtained and analyzed here can thus express basic trends in the organization of the variations existing in the gene pool of *Lens culinaris* Medik.

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Short Communication

ALTERNATIVE SOURCE OF RESISTANCE FOR BACTERIAL LEAF BLIGHT DISEASE OF RICE

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Key words : Bacterial leaf blight, resistance

Bacterial Leaf Blight (BLB) of rice caused by *Xanthomonas campestris* pv. *oryzae* (Ishiyaa) Dye has been occurring in different levels of severity depending upon the degree of susceptibility of rice cultivar grown in different parts of the country. Varieties selected for disease resistance have often become susceptible due to pathogen adaptability. Resistance in cultivated rices has remained only for a shorter durations. Among the wild rice, *Oryza barthii* was earlier reported to be immune to the disease (Devadath, 1983). An attempt has been made in the present study to test number of accessions of different wild species of *Oryza* and to identify additional sources of resistance.

Forty-two accessions belonging to 17 species of wild rices maintained at Central Rice Research Institute (CRRI), Cuttack, India were used for this study. Two tillers in each of the species were removed from the original plants and planted in 12" diameter earthen pots filled with 10 kg of well puddled field soil. They were fertilized with urea @ 120 kg N/ha and irrigated regularly with tap water.

Culture of *Xanthomonas capestris* pv. *oryzae* used for this study was isolated from rice cultivar IR8 (CRXCO 28) grown in the farm of CRRI. Single colony isolates were maintained on the slants of potato-sucrose-agar medium. Forty-eight hour old bacterial culture was used for artificial inoculation. Bacterial suspension was made in sterile distilled water and its concentration was adjusted to @ 10^9 cells/ml.

Forty-five days after planting, fully developed top 2-3 leaves were inoculated through clipping method (Kauffman *et al.*, 1973). Observations were recorded on period of incubation for initial symptom expression and progress of the lesion was monitored at five day intervals until 25 days after inoculation.

Wild accessions of *Oryza* species have shown in general reduced lesion-length when compared to cultivated rice variety IR-8. Wide variations

Table 1. Reaction of different accessions of 17 wild species of *Oryza* against bacterial blight disease of rice

Oryza species	Accession No.	Incubation period for symptom expression (days)	Maximum lesion length (cm)	Reaction R = Resistant S = Susceptible
O. longistaminata	RN 1014	8	2.7	R
	RN 1026	10	1.1	R
	RN 1027	10	0.9	R
	RN 1059	10	5.0	S
O. officinalis	RN 1047	8	3.5	R
	RN 1049	6	10.6	S
	RN 1050	7	3.1	R
	RN 1052	8	3.5	R
	RN 1053	6	13.0	S
	RN 1055	8	2.1	R
	RN 1066	8	3.1	R
	RN 1068	6	10.2	S
O. eichingeri	RN 1040	9	1.2	R
	RN 1041	9	1.7	R
	RN 1042	10	0.9	R
	RN 1065	9	1.1	R
O. minuta	RN 1024	10	1.3	R
	RN 1025	10	1.5	R
	RN 1028	9	2.1	R
	RN 1963	8	4.1	R
O. latifolia	RN 1001	8	7.1	S
	RN 1002	8	6.8	S
	RN 1005	9	3.3	R
	RN 1007	9	3.6	
O. nivara	RN 205	9	2.3	R
	RN 210	7	15.5	S
	RN 211	9	10.0	S
O. malampuzhaenis	RN 1046	8	4.9	S
	RN 1048	(no symptom)	0.0	R
	RN 1061	10	4.9	S
O. grandiglumis	RN 1002	11	4.2	
	RN 1055	10	5.5	S

(Cont. on next page)

Oryza species	Accession No.	Incubation period for symptom expression (days)	Maximum lesion length (cm)	Reaction R = Resistant S = Susceptible
O. australiensis	RN 201	7	16.1	S
	RN 202	7	13.8	S
P. coarctata	RN 1152	12	0.0	R
O. rufipogon	RN 1154	6	5.1	S
O. cubensis	RN 1019	7	3.0	R
O. punctata	RN 1034	6	10.0	S
O. collina	RN 206	9	1.9	R
O. schwenfurthiana	RN 1062	10	1.5	R
O. alta	RN 203	7	11.1	S
O. granulata	RN 999	10	2.6	R
O. sativa (IR 8)	Control	4	24.8	S

in lesion length was observed among different accessions of any single species of wild rices. However, twenty three out of 42 accessions of different species were found to be resistant to bacterial blight disease. Accessions resistant to the disease were found mostly in 12 out of 17 species tested. Majority of accessions resistant to the disease belong to the species of *Oryza longistaminata, O. officinalis, O. eichingeri* and *O. minuta*. In addition, accessions resistant to the disease were also found among the species of *O. latifolia, O. nivara, O. cubensis, O. collina* and *O. granulata* (Table 1). Characteristically, initiation and progress of the lesion was very slow with brown necrotic lesions covering entire width of the leaf in resistant host pathogen combination. While typical lesions developed under susceptible pathosystem, one of the accession of *O. malampuzhaeis* and *P. coarctata* did not develop any symptoms until 25 days after inoculation.

Incubation period required for symptom initiation varied greatly in different species of wild rice. Symptoms were expressed after six to seven days of inoculation in susceptible pathosystem, whereas it was between 8-10 days in incompatible host pathogen combination. Symptoms were initiated 10 days after inoculation in some of the accessions belonging to *O. longistaminata* and *O. eichingeri*. Maximum lesion length recorded in compatible host-pathogen combination was 15.5 cm in *O. nivara*, 13.8 and 16.1 cm in *O. australiensis* followed by some of the accessions of *O. alta, O. officinalis* and *O. punctata*. On the other hand, minimum lesion length of 1.1 to 4.1 cm was recorded in different species of *Oryza* as a result of host pathogen incompatibility.

It is evident from the studies that wild rice germplasm forms a great repository of genes conferring resistance against bacterial blight disease of rice which can be effectively used in inter-specific hybridization.

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Short Communication

CHARACTERIZATION OF RICE (*ORYZA SATIVA* L.) ACCESSIONS USING PHENOL COLOUR STAINING

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Key words : *Oryza sativa* L., phenol reaction, rice genetic resources.

Oka (1958) used phenol colour staining of rice grains (browning or blackening of grains when soaked in aqueous solution of phenol) in the classification of cultivated rice. Since then, this test has been used to ascertain the genetic purity of rice cultivars and their identification. Because this method is rapid and cost effective, attempts were made to use this test in the characterization of rice accessions.

Four hundred ninetyseven rice accessions of diverse agro-ecology and origin were evaluated for phenol staining during 1991-93. Of 497 accessions 404 were indigenous and 93 exotic accessions (upland 69; medium land 24).

The rice grains (10 to 15) of each accession were placed on filter paper and soaked in freshly prepared aqueous solution of phenol (C_6H_5 OH, AR grade, freezing point: 40.5-41°C, molecular weight: 94.11 and purity: 99.9%) for 48 h at room temperature. The phenol staining was rated as no colour development, designated as (–) or colour development, designated as (+). The intensity of coloration was given by +, ++ and +++ using checks - Jaya (+++) and Type 3 (–).

Of the 497 rice accessions only 115 (23.1%) did not produce any colour and yielded negative reaction indicating the predominance of accessions showing positive phenol reaction. Nevertheless, there was variation in the intensity of coloration. (Fig. 1). Among the indigenous accessions, the pattern of phenol reaction was not influenced by the agroecology i.e. upland or medium/low land. The accessions showing phenol negative reaction were found in both agroecologies and the proportion of such accessions was 16.3 and 11.2%

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respectively, in upland and medium/low land collections. Most of the breeding lines tested gave positive phenol reaction. Oka (1958) suggested that varieties from *indica* group of cultivated rice were phenol positive. However, the results of the present study as well as some earlier reports (Chauhan and Nanda, 1984, Vannangmudi, 1988) have shown the existence of variability for phenol staining in *indica* group. Though, by and large, majority of varieties of this group exhibited positive reaction.

Among the exotic collections, phenol reaction of most of the breeding lines developed at IRRI, Philippines was positive (87.5%). However, the number of lines tested was less. This could be due to the fact that these breeding lines were mostiy from *indica* group. Fifty six (81.2%) accessions yielded phenol negative reaction in the exotic upland rice accessions. These accessions were originated mainly from IRAT-Africa (18), CNA and IAC, Brazil (20) Bangladesh and IITA, Nigeria (Table 1). This group might be representative of tropical *japonica* or their derivatives. Because these accessions did not react to phenol, thus, indicating the absence of gene(s) for this trait.

Table 1. List of rice accessions giving phenol negative reaction.

Agroecology/ Group	Accessions
Indigenous upland	Dular, Pankee, Akashi, N22, Surajmukhi, Bala, Sukhawel 20, Brown gora (298, 409), White gora (305, 312, 313, 320, 322, 323), Bangalore, Laloo 14(A), Laloo 14(B), Beali-26, ARC 11775, CR143-2-2, CR634-1, RR2-6, RR47-3, RR145-22, NDR1015, RP2307-7109-8, RP2346-2325-148-70, IET10387, Mettasannalu, GS529, ARC7046(A), ARC7046 (B), ARC10372.
Indigenous medium/ low land	Raria 9268), Jhulaka (578), Agnisal (699, 700), Jhillidhan (703), Kalamdani (706), Tulsimanjri (719), Nanhiya (721, 722), Bhojana (727), Jugadi (733), Baligoya (736), Jerra (739), Ladusar (Dumka 14), Tilasar (105470), Hathipanjar (105629), Bhogana (105681), Maghi (105747), Bad (105800), Jonga (105812), 4/323 and 4/335.
Exotic upland	Aus (257, 454), IRAT(1108, 112A, 133, 146, 147, 193, 208, 211, 216, 237, 260, 263, 267, 284, 291)
	IR47686-5-1-B, IR47686-5-10-8, IR47686-9-1-8-1, IR47701-99-B- 2, Arroz de Aldeia caulina, Arroz dos indios, Arroz de Productor, ITA257, Khaolo 33, Saita, Lakshmijata, Precoce Branco, Khao shino yao, Early Setterial, Rangpur, 63-83, AOB-253, AOB-391, AOB-394, A12-309, IAC25, IAC165, IAC1246, CNA(4097, 4121, 4124, 4125, 4127, 4130, 4136, 4164, 4173, 4744, 4745, 5160, 5164 A, 5164 B, 5166, 5366).
Exotic medium land	BG731-2, IR29725-21-1-3, IR32429-47-3-2-2

*Numbers in parentheses indicate source of the material.

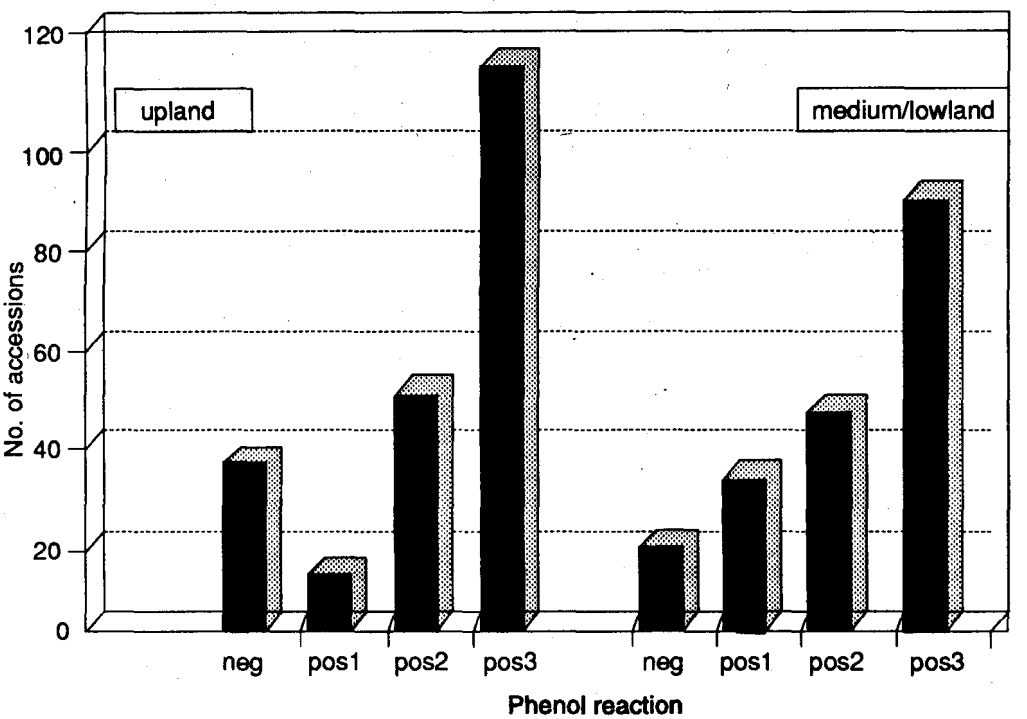

Fig. 1. Distribution of indigenous rice accessions based on phenol reaction

A few accessions exhibited mixed reaction (+/–). This could be either due to mixed population of the land races or these might be segregation for this trait because these accessions were not evaluated and selected for this character. The accessions giving phenol negative reaction are listed in Table 1. The findings of the present investigation revealed that this test in combination with other morpho-agronomic characters, chemical and biochemical tests could be used in the characterization of rice genetic resources. Further, majority of the traditional cultivars from Eastern Indian upland are *aus* where as most of the advanced breeding lines derived from crosses involving *aus* as parent(s) fell in the *indica* group of isozymic analysis. It seems that during selection process at research station the characters of *aus* types were lost. This might be due to selection against *aus* traits in the better managed research fields while in the farmers' conditions *aus* traits express to their optimum. Since a major proportion of varieties identified as *aus* also gave phenol negative reaction. It may, therefore, be possible to use this test in the segregating generations to select for *aus* types. This needs further investigation.

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Short Communication

CHARACTERIZATION OF CMS LINES AND MAINTAINERS IN RICE BY HPLC OF SEED PROTEINS

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Key words : Characterization, CMS lines, rice, HPLC, Seed proteins

The morphological and histochemical properties have been used to identify or distinguish CMS lines and maintainers of rice. Based on these two properties, it could be possible to differentiate CMS (A) lines from that of maintainer (B) lines when they are at flowering stage. It would be convenient to discriminate CMS (A) lines from that of maintainer (B) lines when they are at flowering stage. It would be convenient to discriminate CMS lines on the basis of analysis of seed protein rather than characters of growing plant or the histochemistry of pollen.

In recent years, extensive research has been done in the application of HPLC for the differentiation of rice cultivars (Hussain et. al. (1989), Huebner et al (1990) and Su-Hwa Chen (1991). However, HPLC technique has not been used so far for the differentiation of male steriles and fertiles of rice based on seed protein pattern. So an attempt has been made to differentiate A and B lines of rice by using HPLC analysis.

The seeds of six male steriles, IR 54752 A, V20 A (WA source) Mangala A, Pushpa A, ES 18 A, Intan mutant A (MS 577A source) and their maintainers were used for protein analysis using HPLC equipment. Pharmacia, Sweden. This is based on the principle of size-exclusion chromatography. The sample preparation was done according to Bhowmik et. al. (1990) and the samples were analysed as per standard procedure.

Molecular weight of unknown proteins in the sample was estimated by making use of molecular weight of standard proteins. The standard proteins used here in this study with their molecular weight (MW) are given below :

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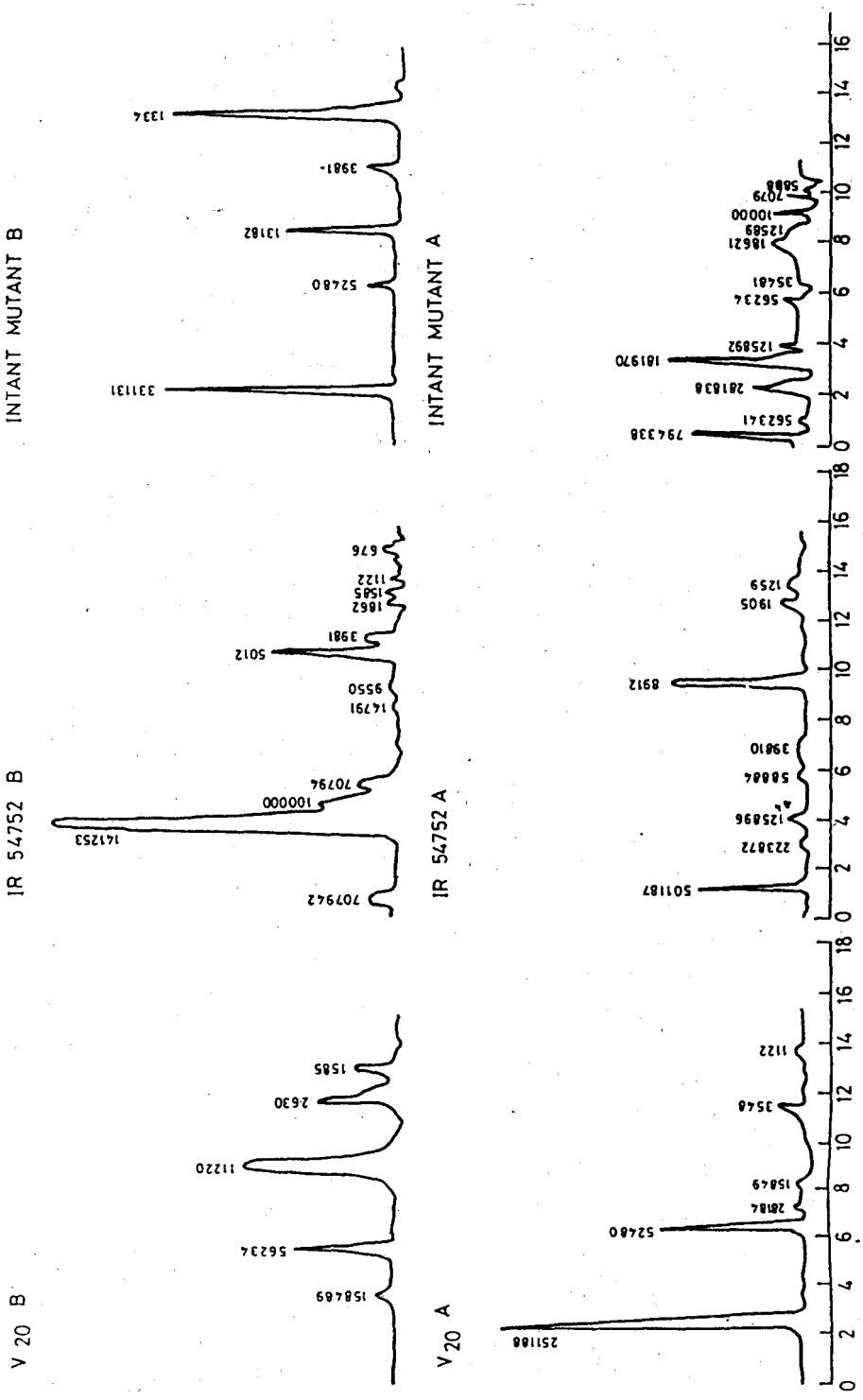

Fig. 1a. Chromatograms of seed proteins from three male steriles and their maintainers. Estimated molecular weights are indicated for major chromatographic peaks

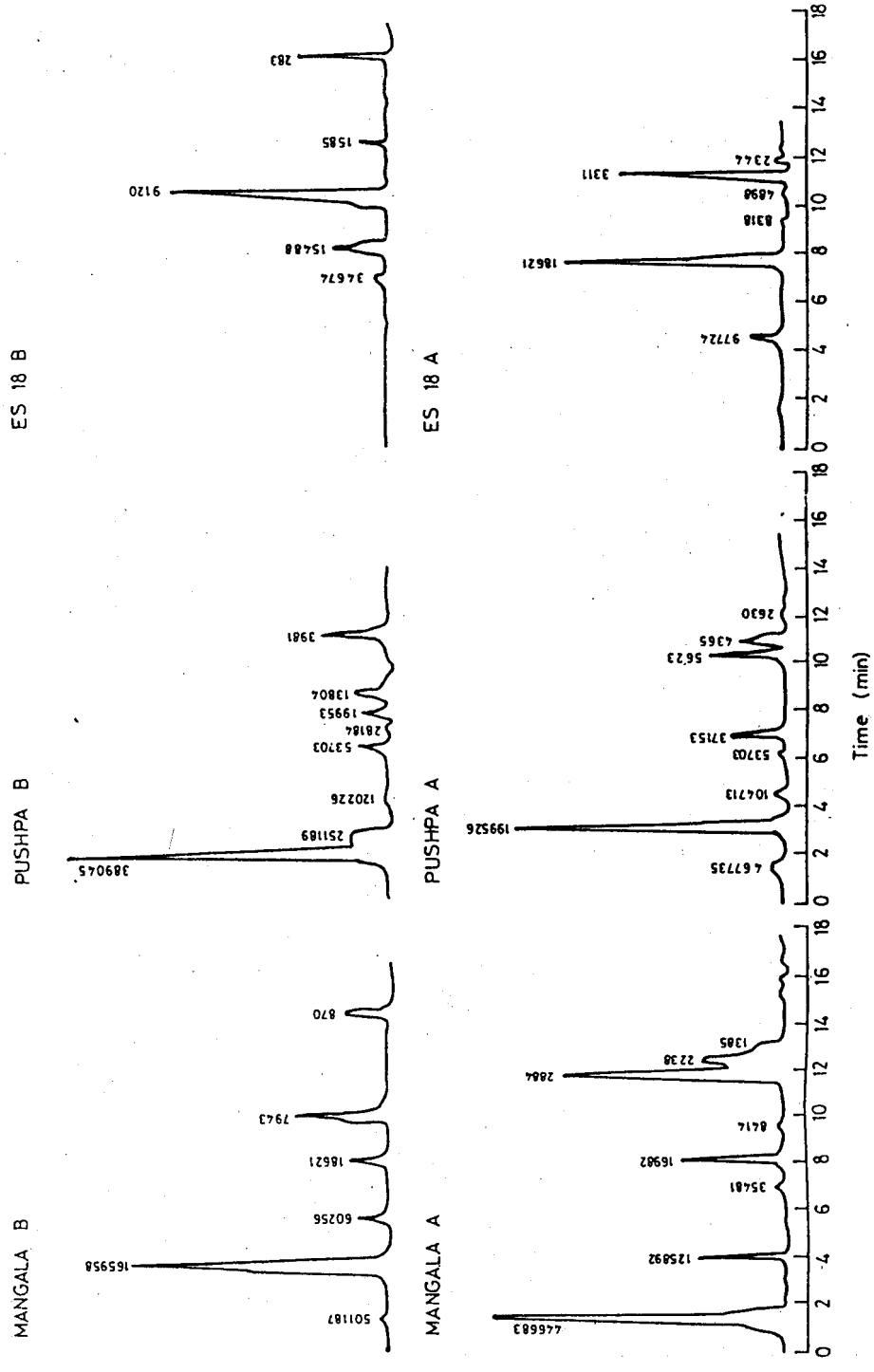

Fig. 1b. Chromatograms of seed proteins from three male steriles and their maintainers. Estimated molecular weights are indicated for major chromatographic peaks

Compound	MW (daltons)
Macroglobulin	340,000
Phosphorylase B	97,400
Glutamate dehydrogenase	55,400
Lactate dehydrogenase	34,500
Trypsin inhibitor	20,000

The chromatograms obtained by HPLC showed that each male sterile line had its own characteristic peak pattern and it varied with that of corresponding maintainer line. All the six cytoplasmic male sterile (A) lines and their maintainers (B lines) differed from each other qualitatively as well as quantitatively. Though the CMS lines IR 54752A and V20A originated from the same cytoplasmic source (WA), they differed from each other in number of protein peaks and also their MW. Even the isogenic lines i.e., V20A and V20B did not have similar kind of protein peaks (Fig. 1a and 1b).

Molecular weight of proteins estimated by using standard proteins with known MW also revealed both qualitative and quantitative differences in protein patterns of CMS lines and their maintainers (Fig. 1a and 1b). In A lines, the MW of proteins ranged from 794328 to 1122 daltons. Whereas in B lines, the protein peaks varied with a MW ranging from 707946 to 282 daltons.

In general, the number of protein peaks i.e., different protein fractions were more in B lines compared to A lines, except in IR 54752B and Mangala B. It was also observed that high MW proteins were more in A lines except IR 54752A and Mangala A, while low MW proteins were more in B lines. The differences in accumulation of proteins with different MW may be a cause of sterility in CMS lines. This can be confirmed by studying pollen protein pattern through HPLC analysis.

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Short Communication

COLLECTION AND CONSERVATION OF INDIGENOUS TEMPERATE FRUITS DIVERSITY IN HIMALAYA

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Key words : Collection, conservation, indigeneous temperate fruits, Himalaya

The Indian Himalaya occupies 4,18900 sq km. climatically the area offers a diversity of gradients, cool moist Arunachal Pradesh to cold desert ladakh and cold dry Tibetan plateu to subtropical Indo-gangetic plains. The Himalaya acts as barrier against monsoon winds of south and cool siberian winds of north, the rainfall pattern varies and decreases from east to west. Different floristic records on the Himalayan context are available (Pei 1992, Jain 1987). Occurrence of 9000 plant species (Myers, 1988) in Eastern Himalaya reflects the relatives richness compared to West Himalayan domain with 3054 species (Dhar and Kachroo, 1983). Wild relatives of crop plants in the region reveal the occurrence of 132 species in the north east, 82 in the east and 128 in the west (Arora and Nayer, 1984). This include the species of *Pyrus, Prunus, Malus, Citrus, Rubus, Ribes, Sorbus, Myrica, Corylus, Ficus, Cydonia, Docynia, Cotoneaster, Actinidia, Rosa, Cornus, Crataegus, Punica, Castenia, Viburnum, Vitis, Pinus and Juglans* etc. among temperate fruit plants. This rich diversity in temperate fruits and their wild relatives is eroding fast in the recent past due to increasing population pressure, various developmental activities like construction of roads, hydroelectric projects, industry and deforestation by local inhabitants for their livelihood.

The collection sites were deep forest, farmers orchards, marginal lands and the areas bordering the cultivated lands. The exploration route were followed according to the distribution, species richness and maturity of the crops. Local people were also contacted to know the exact location and their uses. The passport data were recorded at the site of collection. The material was collected in the form of scion wood (20), fruits (10), seeds and cuttings (20) and two suckers each from mother plant where it was available.

A total of 330 collections of temperate fruits was made (Table 1) from Uttar Pradesh (60), Himachal Pradesh (190), Sikkim (55), Meghalaya (15) and Arunachal Pradesh (10). Of these collections, only 170 could survive and established in the field genebank which include *Malus baccata, M. baccata var, himaliana, M. sikkimensis and M. dirangensis; Prunus prostata, P. wallichi, P. nepulensis, P. persica, P. cornuta, P. tomentosa, P. armeniaca, P. cerasoides and P. spp (Behmi); Pyrus jacumontiana, P. polycarpa, P. pyrifolia, P. pashia, P. pashia var. kumaoni, P. communis,* and *P. khasiana; Sorbus lanata, S. verrucosa, S. cuspidata; Cotoneaster frigida, C. accuminata, C. numularia; Cynodia oblonga, Docynia hookeriana, Crataegus crenulata, C. oxycantha; Vibernum cotnifolium, V. lanata, Rubus ellipticus, R. biflorus, Myrica nagi; Persea edulis, Corylus avellana, C. colurna; Cornus capitata; Actinidia callosa* and *A. strigosa, Elaegnus latifolia, Juglans regia* and two species of *Rosa* having edible hips. Elite material in walnut was collected from Chakarata area of Dehradun district having thin celled, bigger fruit size and very good percentage of kernel/nut ratio. Wild *Rosa spp.* and *Pinus gerardiana* collected from Spiti valley and Kinnaur could not survive. It appears that they have specific ecological requirements for conservation, therefore, *in situ* conservation for these species will be most appropriate approach. Rich diversity of temperate fruits and their wild relatives such as; *Prunus prostrata, Prunus tomentosa* are restricted to the Kinnaur district, *Prunus cornuta* occurs in the higher altitude of Shimla district (Narkanda, Khadrala). *Malus baccata* was also found in kinnaur and Rohru area of Himachla Pradesh, locally known as dhak which is commercially used as rootstocks for apple cv. Royal in Kinnaur. While *Malus sikkimensis* occurs in Lachun and Lachung area of North Sikkim. *Pyrus communis* occurs largely in Kashmir, *P. kumaoni* is localized in U.P. hills, *Pyrus jacumontiana* is confined to Shimla and Kinnaur (Kochli, Powari) whereas *P. pyrifolia, Pyrus khasiana* and *Pyrus thomsoni* are confined to NEH regions. The species of *Rubus* also exhibited special distribution pattern; *R. fruitcosus* is confined to Western Himalaya; *R moluccans, R. niveus and R. reticulatus* showed wide spread distribution in the Himalayas. *Rubus ellipticus* and *R. lasiocarpus* extends south to penninsular hills. In Ribes; *R. nigrus* occurs in Western Himalaya and *R. gracilis* and *R. accuminatum* in the eastern Himalaya. In *Corylus* very good variability and distribution was observed in Pangi valley, satlundi (Chamba) in Himachal Pradesh and Baragaon and Oli-peak in U.P. Hills. *Pinus grardiana* was confined to Kinnaur and Pangi valley only. Very good variability and distribution of *Actinidia* was observed in the forests and was confined to Bomdila In Arunachal Pradesh and Hilley in South Sikkim. Very good variability and distribution in *Persea edulis* was restricted in North Sikkim and Darjeeling district of West Bengal.

Table 1. Areas surveyed and diversity collected in indigenous temperate fruit germplasm

S.No.	Collection period (Month/year)	Diversity collected	Details of Diversity	States/districts surveyed
1.	March-April, 1993	49	Apricot(6), Walnut(15), Apple(2), Pear(7), Peach(6), Chestnut(2), Almond(1), Hazelnut(1), Bamora(2) Pomegranate(1), Miscellaneous(6)	Shimla and Kullu districts of H.P. and hill districts of UP.
2.	June-July, 1993	65	Apple(1), Sorbus(2), Hazelnut(2), Filbert(1), Walnut(34), Pear(10), Apricot(3), *Prunus* sp.(2), Roses (6), Elaegnus(1), Hipppohae(1), Cotoneaster(1), Chilgoza(1)	Sirmaur, Solan ditricts of Himachal Pradesh and Chamoli, Pithoragarh, Almora, Nainital, Uttarkashi district of U.P.Hills
3.	August-Sept, 1993	30	Walnut(12), Apricot(7), *Prunus* sp.(3), *Malus* sp.(1), Sorbus(1), Hazelnut(3), Elaegnus(1), Hippophae(1), Blackberry(1)	Chamba (Pangi Valley), Lahaul & Spiti, and Kinnaur district of H.P.
4.	December, 1993	30	Almond(8), Walnut(11), Plum(2), Apricot(3), *Prunus sp.*(3), *Malus sp.*(3)	Kinnaur district of H.P.
5.	January-Feb, 1994	81	*Malus sp.*(9)Sorbus (8), Pear(15), *Pyrus sp.*(8), *Prunus sp*(9), Walnut(3), Peach(7), Hazelnut(03), Filbert(2), Kivifruit(3), Elaegnus(3), Persea(2), Other fruits(7)	Sikkim, Meghalaya, Part of Assam and Arunachal Pradesh
6.	October, 1994	32	Walnut(9), Sorbus(3), Rubus (5), Peach(2), Apricot(2), Almond(1), *Viburnum*(1), Filbert(1), Hazelnut(1), *Prunus*(1), Plum(1), Pear(3), Apple(1)	Bharmaur, Hadsar, Satlundi, Tissa areas of Chamba district of H.P.
7.	August, 1994	50	Pomegrante (50)	Solan, Sirmaur, part of Bilaspur and Shimla districts of H.P.
8.	September, 1995	40	Pomegranate (40)	Kullu and Mandi districts of H.P.
9.	December, 1995	10	Walnut (10)	Chakrata area of Dehradun district of U.P. hills.

Some of the species like *Prunus rufa* is confined to Tonglu and Sandakpu in Darjeeling and not in N-W Himalaya. Like wise *Prunus prostrata, P. tomentosa, P. spp.* (Behmi) and *Prunus jacumontiana* are in Kinnaur and Ladakh. In case of *Sorbus* species; *Sorbus microphylla, S. ursina, S. insignis and S. foliolosa* are confined to Darjeeling hills whereas *S. acuparia* is confined to Koksar and *S. lanata* in Khadrala, Pangi valley and Munshiyari area of Pithoragarh in U.P. (Joshi and Rana, 1994). *Sorbus verrucosa and S. granulosa* and confined to Cherrapunji hills of Meghalaya.

The fast depletion of temperate fruit diversity in the Himalaya including their wild relatives in the forest need to be collected and conserved on priority basis. These resources will provide drought resistance, cold and frost resistance, and hailstorms resistance types with good adaptability genes for breeding programme or as rootstocks. The species with distribution in each genus available in temperate fruits and other wild minor fruits in the Himalayas are on the verge of extinction and need urgent collection priorities. These species rarely exist in the forest and have not been collected or did not establish at Shimla. These species do not have any patronage for protection by forest department and hence urgent action is required for the collection and conservation. Some of them need *in situ* conservation because of their specific ecological requirements (*Pinus gerardiana*). The other wild fruit species e.g. *Prunus prostrata, P. jacumontiana, P. wallichi, Pyrus pyryfolia, P. jaccumontii, Hippophae, Myrica, Corylus, Malus baccata* var. *himaliana, Holbelia latifolia, Sorbus microphylla, S. ursina, S. insignis, S. foliolosa, Persea edulis, Elaegnus spp. and Ascendra butrecia* need urgent attention for their conservation.

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Short Communication

MANAGEMENT OF PASTURE LEGUME GENETIC RESOURCES IN INDIA

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Key words : Pasture legumes, Genetic resources

Native pasture legumes as forage crops substantially improve quality and productivity of natural pastures and provide nutritious diets to grazing livestock. These legumes help improve soil productivity with addition or organic matter and atmoshperic N_2 to the soil and this largely obviate the need for costly nitrogeneous fertilizers. In mixed sward of grasses, these are generally more effective in controlling water runoff and soil erosion. These rangelegumes, therefore, constitute an important component of soil-plant-animal nutritional cycle (Walker, 1983)

Indian sub-continent is endowed with richness of genetic diversity in pasture legumes. Nearly 400 species belonging to over 60 genera spread over different agro-ecological zones are endemic to this area (Arora and Singh, 1988). However, the exponential increase in human and livestock population, land degradation, overgrazing and expansion of crop land into previously uncultivated areas threaten to extinct these valuable genetic resources of natural rangelands. This warrants for prudent management of pasture legume genetic resources in India.

Collection

The Indian Grassland and Fodder Research Institute (IGFRI), Jhansi, has been designated to take responsibility for holding collections of germplasm of pasture legumes under the IN-PGR system. The variety of genera and species within this group create a challenging task for genetic resources activities. A total number of 355 accessions of 12 species has been collected (Tabe 1). But very few of these are working collections for research. This collection also includes a number of exotic species. The number of accessions per species is,

however, very limited. Complementary collecting activities are therefore urgently needed to establish and maintain genetic variability.

IPGRI (International Plant Genetic Resources Institute) supported pasture collection in India was carried out in 1984 by the Queensland Department of Primary Industries, Australia in collaboration of National Bureau of Plant Genetic Resources (NBPGR). However, under IPGRI prgramme, genera and species of 13 tropical pastures legumes (*Aeschynomene, Centrosema, Desmenthus, Desmodium, Lablab, Leucaena, Lotononis, Macroptilium, Macrotyloma, Neonotonia, Pueuraria, Stylosanthes and Zonia*) merited a priority rating for collection on the basis of their potential as forages (IBPGR, 1983).

Since information available on the biology, taxonomy, species relationships, etc., is inadequate for many of these potentially useful species, the attention need to be drawn for further research. Ecological and geographical surveys need to be conducted to maximize the sampling of genetic diversity in areas of high diversity.

Rhizobium germplasm resources collections

For the success of the introduction programme, it is essential to collect *Rhizobium* in the case of new or exotic species and where there are known difficulties with nodulation. But in presenting the *Rhizobium* gene pool the major constraints are the extra resources needed in terms of time, facilities for storage and maintenance of cultures. Moreover, there are very few rhizobia cultures of pasture legumes and so it would be useful if the collection of rhizobia from pasture legumes were made standard practice. Details for the collection of nodules for the isolation of rhizobia and of the recording of site information have been described by Date and Halliday, (1979).

Characterization and Preliminary Evaluation

In pasture legumes the number of accessions to define, maintain and despatch is limited. But as soon as the number of accessions exceed the processing capacity of holder, a choice has to be made either to eliminate extra accessions or form a gene pool. But it is very difficult to preserve all samples collected for accession present in the gene bank. Following the IPGRI definitions, passport data, characterization and preliminary evaluation of the accession are referred. With passport data no special problem arises, since the data are not crop specific. But characterization and preliminary evaluation do present some difficulties. Morphological i.e. vegetative plant data, those on inflorescence, pod and seed are specific for each species or atleast cluster of related species. Seasonal type is an important physiological trait. Quite a number of species are biennial and mostly are perennial. Persistency and perenniality are two important aspects. Agronomical characters can be evaluated either in field experiments or, if necessary, in net houses.

Table 1. Genetic Resources of Pasture Legumes in India

	Species	Common name	Scope of collection	Accessions at IGFRI, Jhansi
1.	*Centrocema pubescens*	Centro	Central- eastern U.P., Madhya Pradesh, Southern Gujrat, Bihar, Assam, West Bengal, Orisa and Coastal Tamil Nadu	12
2.	*Clitoria ternatea*	Butterfly pea	Central Punjab, Rajasthan, Gujarat, Southern U.P., West M.P., A.P., Karnata and Northern Tamil Nadu.	96
3.	*Desmodium intortum*	Green leaf Desmodium	West Bengal, Assam and Orissa.	13
4.	*Desmodium uncinatum*	Silver Leaf Desmodium	Eastern M.P., Central Eastern M.P., and Rajastan	11
5.	*Desmenthus virgatus*	Hedge lucerne	Punjab, Haryana, U.P., Northern M.P., Gujrat and Maharashtra.	07
6.	*Lablab purpureus*	Field bean	Rajasthan, Punjab, Himachal Pradesh, Gujarat, Western U.P., Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, and Hills of Bihar/ West Bengal	170
7.	*Macroptilium atropurpureum*	Siratro	Southern U.P., Madhya Pradesh, Gujarat, Bihar, Andhra Pradesh, Karnataka and Coastal Tamil Nadu.	15
8.	*Stylosanthes guinanensis*	Stylo	Punjab, Himachal Pradesh, and U.P.	07
9.	*Stylosanthes humilis*	Townsville Stylo	Rajasthan & Northern Gujarat, Maharashtra, Western M.P. and Andhra Pradesh.	05
10.	*Stylosanthes hamata*	Carbian stylo	Tamil Nadu, Karnataka and down hills of West Bengal	11
11.	*Stylosanthes viscosa*	Viscosa stylo	-do-	03
12.	*Stylosanthes scabra*	Scabra stylo	-do-	05

Conservation

Pasture genetic resources can be conserved in gene banks (*ex situ*) or in nature (*in situ*). All the species of pasture legume can be stored in orthodox fashion in gene banks. Seeds are stored according to whether these are to form (1) an active collection or (2) a base collection. Until such time, as there are adequate national programmes the IGFRI serve as an active collection centre. A long term (–20°C) storage facility is available at NBPGR, New Delhi. However, this security deposit should also be complemented by Institutional seed stores (where adequate facilities are available) which will, of course, hold national material. For this a medium term (–5°C) storage facility is being developed at IGFRI to hold pasture genetic resources.

In situ methods are preferred for pasture legume species which have germplasm that at present time cannot be practically maintained in genebanks (Anon, 1984). But all efforts to date suffer from the lack of plant inventories of reserve areas. This requires to set up the task of making an inventory of existing natural reserves and other valuable areas, of their botanical compositions etc. The careful management of these reserve areas is very crucial as in pasture species the loss of germplasm is usually associated with overgrazing.

Therefore, both *in situ* and *ex situ* conservation are needed. These two approaches complement each other. It is not realistic to expect these species and their populations can all be covered by nature reserves, national parks and other protected areas. Nor are protected areas always inviolate they remain vulnerable to loss of destruction. Likewise *ex situ* seed conservation and field gene bank are vulnerable to human failings, natural disasters and technical problems such as power cuts, fires and floods. Also *ex situ* conservation disrupts the process of evolution in population. So both approaches are needed in varying mixed for different species.

Information Management

For the pasture legume genetic resources the quality and complexity of information involved as well as the diversity of the information to be provided, require comprehensive and efficient data management system. Data is documented using a computerized data bank based on a standardized system of descriptor and descriptor states of pasture legumes introduced by Anderson and Davies (1984).

A national programme has to be looked at also in the International context International action to collect, preserve and exploit pasture legumes has been taken by IBPGR in 1983. Cooperative ties have been enhanced for forage germplasm collections with ICARDA, CSIRO, CIAT and ILCA. The institutes like CSIRO, Australia; Fooder Crops and Pasture Institute, Greece; Belize Forage Legume and Pasture Research Programme, Belize; INIA, Spain

have been entrusted for characterization work. For security and long term storage the IBPGR designated INIA (Spain), Bari (Italy), Greek Genebank (Thessaloniki), ICARDA (Syria) and CSIRO (Australia). These security deposits are being complemented by collections made by different nations (Anon, 1985).

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Short Communication

GENETIC DIVERGENCE IN SESAME (*SESAMUM INDICUM* L.)

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Key Words : Sesame, genetic divergence

Genetic divergence among parents is essential for the expression of heterosis and for enlarging the variability in the subsequent segregating generatons. Generally, genotypes drawn from diverse geographical regions are preferred in the hybridization programmes, on the presumption of genetic diversity among such genotypes.

Fifty genotypes of sesame, both of indigeneous and exotic origin, were grown at the School of Genetics, Tamil Nadu Agricultural University, Coimbatore, during January 1987 in randomized block design with four replications. Selfed seeds of each genotype were sown with a spacing of 45 cm between rows and 30 cm between plants. Five adjacent plants per replication in the middle of the row were selected in each genotype and observations were recorded on individual plant for fifteen morphological and developmental characters.

The data were subjected to analysis of variance as well as multivariate analysis suggested by Mahalanobis (1936). Clusters were formed as suggested by Tocher (Rao, 1952) and canonical analysis was done as per the method suggested by Rao (1952).

The available divergence between genotypes for the characters studied was tested by Wilks lamda criterion (Wilks, 1932) and it was found to be significant (X 735 = 2683.78). The generalised D values ranged from 29.17 to 1813.69.

The fifty genotypes were grouped into eight clusters by the application of clustering techniques. Cluster I had as many as thirty two genotypes representing different geographical regions of the world. There were five genotypes in Cluster III, of which, one was indigeneous and others exotic.

Clusters II and IV had four genotypes each. Cluster II had three exotic genotypes from Chile, Japan and Greece, and one indigenous genotypes from Uttar Pradesh. Cluster IV had a single exotic genotype from Japan, while the other three genotypes were from Tamil Nadu. Two genotypes, one each from Greece and Palestine, constituted Cluster V. Clusters VI, VII and VIII had one genotype each. The clustering pattern failed to indicate any relationship between genetic divergence and geographical distribution. This is in agreement with the findings of earlier workers such as Trehan *et al.* (1975), Thangavelu and Rajasekharan (1983) and Dhamu *et al.* (1984).

Table 1. Inter and intra (diagonal) cluster average of D values and the extent of diversity among the clusters.

	I	II	III	IV	V	VI	VII	VIII
I	10.793	24.091	13.725	15.113	15.579	21.106	20.149	15.511
	-	M	C	C	C	C	C	C
II		10.953	23.923	29.172	24.886	37.314	30.074	27.672
		-	M	M	M	H	H	H
III			10.619	19.548	13.368	28.169	23.829	16.832
			-	C	C	M	M	C
IV				11.857	24.096	18.246	13.157	19.469
				-	M	C	C	C
V					10.834	30.142	30.407	18.758
					-	H	H	C
VI						-	20.637	23.232
							C	M
VII							-	21.504
								C
VIII								-

H : Highly divergent = Above 30; M : Moderately divergent = Between 21 and 30;
C : Closely related = 22 and below

The clustering pattern indicates the existence of wide genetic diversity among the genotypes chosen from the same geographic region. This is in agreement with earlier reports (Trehen *et al.*, 1974 and Dhamu *et al.*, 1974). They supposed that extremely variable environment within the same origin could have caused such diversity. Murty and Arunachalam (1966) explained that such a wide adaptation could be possible due to heterogeneity, genetic

Table 2. Cluster means for fifteen characters in *Sesamum*

Clusters	Plant height (cm)	No. of primaries	No. of secondaries	First capsule bearing node	Capsule on main stem	Capsule on branches	Capsule length (cm)	Seed per capsule	1000-seed weight (g)	Days to maturity	Oil content (%)	LAI	DMP (q)	Harvest index (%)	Seed yield (g)
I	69.51	4.11	2.51	3.93	18.14	35.09	2.39	62.17	3.41	85.85	42.25	1.61	23.71	31.16	6.32
II	55.61	2.98	0.92	3.45	12.85	10.38	2.18	111.48	3.48	88.80	42.62	1.04	15.74	31.04	4.05
III	57.14	2.66	1.54	2.42	15.56	13.31	2.23	56.18	3.14	84.45	42.59	0.67	15.39	28.43	3.32
IV	74.09	5.75	4.12	5.05	19.79	48.42	2.26	52.22	3.54	96.13	43.17	1.55	26.51	32.33	7.63
V	56.44	2.00	0.89	3.00	17.55	20.15	2.61	63.18	3.61	75.13	41.22	0.62	16.33	28.57	3.71
VI	93.10	6.15	4.85	5.35	19.94	70.05	2.77	53.37	3.32	91.00	41.92	3.58	43.77	37.43	16.14
VII	74.10	5.30	4.30	4.70	16.75	49.85	2.26	57.07	2.65	108.75	41.45	1.74	27.47	30.28	6.96
VIII	74.10	4.00	1.04	3.40	17.80	27.97	2.42	52.00	2.53	6.25	35.85	1.53	20.11	28.70	4.63

LAI - Leaf area index

DMP - Dry matter production

architecture of the population, past history of selection, developmental factors and the degree of general combining ability. Such situation is desirable for the breeder to select parents with similar adaptability.

Canonical analysis suggested that primary axis was differentiated mainly by plant height, capsules on branches, leaf area index, number of secondaries and days to maturity. First capsule bearing node, plant height, number of primaries and harvest index constituted the secondary axis of differentiation.

The intra and inter cluster distances among the eight clusters are presented in Table 1. There did not exist much difference in the intra cluster distances. The inter cluster distance ranged from 13.157 (between clusters IV and VIII) to 37.314 (between clusters II and VI). It was found that Cluster II was highly divergent from Clusters VI and VII. Similarly, Cluster V was highly divergent from Clusters VI and VII. Between themselves, Clusters VI and VII appeared to be closely related. This indicates that hybridization programme of genotypes belonging to highly divergent clusters can be used for exploitation of hybrid vigour and for getting good recombinants.

Among the eight clusters, Cluster VI recorded the maximum values of cluster mean (Table 2) for most of the characters studied except seed per capsule, 1000-seed weight and oil content. Cluster II registered the maximum values for seed number per capsule and oil content. The 1000-seed weight was the highest and days to maturity was lowest indicating earliness in Cluster V. This crossing programme involving genotypes from these clusters could help in obtaining wide spectrum of variability for the above mentioned yield components in the subsequent segregating generations.

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Short Communication

COLLECTING CASTOR (*RICINUS COMMUNIS* L.) AND *JATROPHA* GERMPLASM IN INDO-GANGETIC PLAINS

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Key words : *Ricinus communis*, *Jatropha*, exploration, germplasm

India is the secondary centre of diversity for castor and ranks first in acreage in the world. Castor is an important oilseed crop of India. Its oil has great industrial and medicinal value. The species is common throughout the country, particularly in the drier tropical climate.

For collection of diversity in castor from Indo-gangetic plains including Patna, Begusarai, Samastipur, Muzzaffarpur, Munger, Bhagalpur, Nalanda districts in Bihar, were explored in October, 1992. The parts of central Uttar Pradesh, Bundelkhand region and adjoining Madhya Pradesh were explored in October, 1994. The surveyed region lies between 75° to 87° East longitude and 20 to 27° North latitude. Coarse grid method of sampling at 10-15 km intervals and occassionally fine grids, in the areas of the concentration of diversity were followed (Jain, 1975 and Hawkes, 1976). The bulk, random and non-random selective methods of sampling was done to capture maximum diversity. Depending on the availability a few to 400 capsules/seeds were collected. The passport data and *in-situ* observations on highly heritable/ important characters were recorded during exploration and 100 seed weight, length and breadth of seed were also recorded in laboratory.

The Indo-gangetic plains are the centre of botanical variability in castor (Stuhlmann, 1909; Kulkarni and Ramanamurthy, 1977; Moskin, 1986) where it grows as escape near roadsides, back/court yards, temple compounds, wastelands and most preferably on garbages around. The organized cultivation of castor as a main crop is rarely seen in the targeted areas except in *diara* lands of river Yamuna near Kalpi and Ghatampur. Castor is grown, by and large, as guard/fence rows to protect the main crop from men/animals, frost,

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bright sun and as support to the viny crops like lablab bean, winged bean and cucurbits etc. It is frequently inter/ mixed cropped with chillies, groundnut, pigeonpea, cucurbits, beans and other vegetables.

Of 192 seed samples of castor, 83 were collected from 63 sites in Bihar. The maximum number of 21 samples were collected from Ganga river beds around Patna and followed by 16 collections from Begusarai, 10 from Samastipur, 9 from Muzzaffarpur, 7 from Munger, 5 each from Bhagalpur and Nalanda, 2 each from Dhumka, Khagaria and Deoghar and 1 each from Dhanbad, Ranchi, Hazaribagh, Nawada and Vaishali. One hundred and nine population samples representing local diversity in castor and 18 seed samples in *Jatropha* were collected from 90 sites in parts of central U.P., Bundelkhand region and adjoining areas of M.P. (Fig. 1). The germplasm was sampled from diverse habitats including roadsides, courtyards/backyards, waste lands/garbages around, cultivated fields, farm stores and local markets. In castor 18 samples represented the diversity from Hamirpur, 16 each from Banda and Jhansi, 14 from Jalaun, 13 from Kanpur, 12 from Firozabad, 10 from Etawah and 8 from Allahabad. The collections exhibited remarkable variation in seed colour (white, brownish white, brown, red and chocolate, black without and with consipicuous mottling), seed size (small, medium and bold), seed shape (elongated, square, oblong and triangular), appearance (shining and dull), plant/spike colour (green, sun red and golden), spike (short/long and compact/loose in conical, umbrella and irregular shapes), leaf size (small/large), leaf lobing (deep/shallow), growth habit (fast growing tall types and slow growing) branching (convergent/divergent), shattering and non shattering types and weedy forms. The extent of variability in 100 seed weight was very high from as low as 9.3 g (SDKA-1) to as high as 79.0 g (SDKA-39). Seed length varied from 0.82 cm (SDKA-15 and SDKA-94) to 1.85 cm (SDKA-102). Seed breadth exhibited variation from 0.57 cm (SDKA-1) to 1.47 cm (SDKA-48). Similar trends in variability have been reported in castor collections from Bihar (Anjani et. al., 1993)

The germplasm of *Jatropha* represented variability in two species, comprising 15 samples in physic nut (*Jatropha curcas*) and three samples in *J. gossypifolia*. *Jatropha gossypifolia*, vernacularly known as *jangli arandi/van arandi/safed arand or bagbheranda* was found distributed in the areas between Jhansi and Mahoba *via* Mauranipur, with high concentration of diversity around Barua Sagar. The diversity further intensifies south eastwards. Two distinct types i.e. green coloured and red coloured occurred simultaneously within the same populations as well as separately in different populations. Three population samples of small seeded, early maturing types were collected from roadsides. Physic nut (*Jatropha curcas*) is locally known as ratan jyoti and mainly distributed in Ratalam, Dewas, Khargaon, and Khandwa districts in semi-wild state.

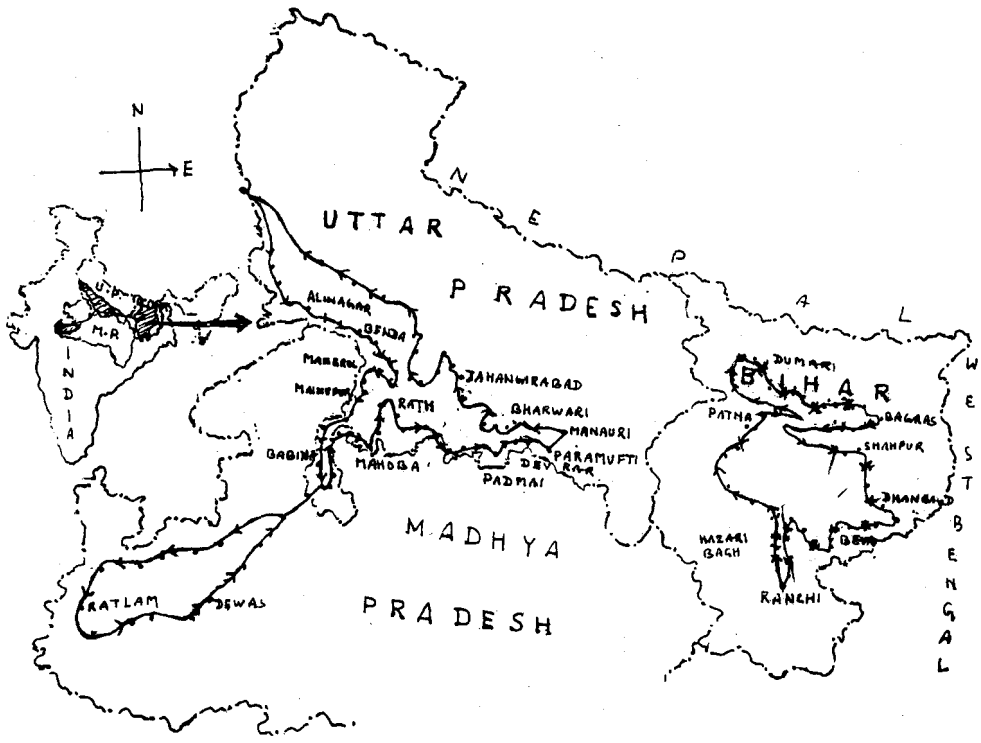

Fig. 1. Exploration routes for castor collection in Indo-Gangetic plains

Though the oil yielding potential of physic nut has been recognized recently, the people grew it as fence rows in the vicinity of villages to protect their fields/gardens from stray animals as the animals do not graze on it. Besides, physic nut is extremely useful in conservation of soil and water and also acts as repellent to rats in the crops prone to rodent attack. Population samples of physic nut were collected from one to seven years old plants/trees and represented the variability in plant height (1-5m), plant colour (green, dark green and sun red), capsule size, capsule length (20-30 mm), seed size (small to bold), seed shape and colour, leaf lobbing (3-5), bearing capacity, capsule (shedding/non-shedding types, shattering/non-shattering types) and early/late maturing types. The tribal people use the tender shoots of this tree to brush their teeth which, they believe, improves the eye sight and therefore the popular name *"ratan jyoti"* has been derived/originated for this plant.

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Short Communication

ARROW ROOT PRODUCING CURCUMA SPECIES IN INDIA

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Key words : Arrow root, *curcuma*, spp. *Maranta arundinacea*

The genus *Curcuma* (L.) of the family *Zingiberaceae* is Indo-Malayan in origin and is mainly tropical Asian in distribution. It includes several species of economic importance. Apart from turmeric being used as a condiment and medicines and few others as medicinal (Kirtikar & Basu, 1918), several others are used for preparation of arrowroot which is an ideal food for infants and invalids (Anon, 1948). The present report highlights the production of arrowroot from various turberising species of the genus *Curcuma* in comparison with *Maranta arundinacea.*

During the past, attempts have been made by the station to collect and conserve the genetic resources of turmeric and its related wild and cultivated species in India resulting in amassing over 700 accessions of 31 identified species of *Curcuma.* Out of these 13 identified and 4 unidentified turberising or sessile finger bearing ones with *Maranta arundinacea,* an introduced arrowroot producing plant have been subjected for the present studies. The local method of extracting arrowroot comprising grinding the fresh cleaned tubers to a fine paste, making a colloidal solution by mixing the paste with fresh water, filtering with a fine seive and subjecting it to sedimentation has been followed. The process of sedimentation has been repeated to obtain almost a colourless sediment of starch granules. The sediment is sun dried and powdered to obtain fine arrowroot. Taste, colour and odour of the starch have been observed and fresh weight and dry weight percentage have also been worked out.

Table 1 shows the origin, status, colour of finger, dry weight% of the underground rhizome and percentage of starch on dry weight and fresh weight basis of tubers from 18 species studied. The results show that *Curcuma malabarica* from Kerala gives the highest fresh weight of tubers (1750 gm/pt) followed by *C. zedoaria* (1250 gm/pt) as compared to

Table 1. Colour, odour, fresh rhizome wt., dry matter% and starch% in *Curcuma* sp.

Species	Rhizome		Rhizome		Starch Dry wt.%	
	Colour	Smell	Fresh wt. g/pt	Dry wt.%	on fresh wt. basis	on dry wt. basis of rhizome
Curcuma zedoaria	orange yellow	camphor	1250	22.73	2.46	3.10
C. caesia	Blue	camphor	313	33.73	0.51	1.52
C. aeruginosa	Virdigris green	"	583	15.69	8.50	17.00
C. malabarica	Light blue	"	1750	20.44	3.77	4.73
C. aromatica	Pale yellow/ wight	"	833	24.08	1.37	5.40
C. amada	"	Mango	416	10.67	0.72	6.72
C. frutescens	"	Camphor	266	18.63	1.48	7.92
C. sylvatica	"	"	183	23.49	2.51	10.70
C. raktakanta	"	"	300	13.77	1.23	6.68
C. harita	"	"	500	23.06	5.92	25.02
C. soloensis	Mustard yellow	"	400	23.54	1.55	6.56
C. latifolia	yellow	"	275	23.42	0.69	2.94
C. comosa	pale yellow	"	183	23.49	0.80	3.24
Curcuma sp. No. 207	Mustard yellow	-	283	18.92	3.40	18.22
No.204	"	-	400	22 .01	0.25	1.78
No. 181	Yellow	Fruity	370	26.27	4.54	17.29
No. 193	Light mustard yellow	-	100	14.31	0.25	1.78
Maranta arundinacea	White	Odourless	1750	26.38	22.00	30.00

1750 gm/pt in *M. arundinacea*. Maximum dry weight of percentage of starch (8.5%) based on fresh weight of tubers has been obtained in *C. aeruginosa* from North Eastern Region followed by 5.92% in *C. harita* from Kerala as against 22% in *M. arundinacea*. However, a maximum of 25% of arrowroot on

dry weight basis is obtained in *C. harita* followed by 18.22% in *Curcuma* sp. no. 207 as against 30% in *M. arundinacea*. The overall results show that considerable variation exists in arrowroot production ability of the 17 *Curcuma* spp. studied. Though *M. arundinacea* is far more superior to them in all aspects of arrowroot production, the importance of *Curcuma* spp. in this aspect cannot be underscored as these occur in plenty in the rain forests of India and provide very cheap raw-material for arrowroot preparation to tribals and locals.

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Short Communication

MICROPROPAGATION OF AN ENDANGERED SPECIES OF *PIPER BARBERI* GAMBLE AND ITS CONSERVATION

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Key words : *Piper barberi*, endangered species, micropropagation, conservation

The genus *Piper* is represented by about 13 species in South India of which *Piper barberi* is very. rare (Gamble, 1925). The other important species of *Piper* and *P. nigrum* (black pepper), *P. betle* (betel vine) and *P. longum* (Indian long pepper). *Piper barberi* is reported to be almost extinct and recorded in the red data book of Indian plants (Nayar and Sastry, 1988). After the type collection of C.A. Barber in 1901, it was thought to be extinct. Its occurance was again repoted six decades later by Dubramanyam and Henry (1970) and later by Nirmal Babu *et al.*, (1992) and Mathew and Mathew (1992). *Piper barberi* is a remarkable species and can be easily distinguished by its pinnately veined leaves and spikes borne on very long filiform peduncles. This species is a slender deiocious climber and has little resemblance with South Indian taxa of *Piper*. It is more alike to Central and Northern South American forms with its reticulately veined leaves, persistant prophylls and long peduncles (Fig. 1A), and at the same time differs from them in its climbing habit and dioecious nature. Probably *P. barberi* could be sole survivor of an ancestral type that reached India from Central American region (Nirmal Babu *et al.*, 1992). The present study was taken up to standardize protcols for micropropagation for rapid multiplication and conservation of this endangered species.

Shoot tips, approximately 2-3 cm long, were excised from field grown plants and were washed in detergent (teepol) for 15 minutes. They were surface sterilized with 0.1% $HgCL_2$ for 5 minutes and were rinsed in 3 times in sterile water and transferred to culture media in aseptic condition. Fully expanded, Woody Plant Medium (McCown and Amos, 1979) was used as basal medium with 2% sucrose and 0.6% agar supplemented with N^6 benzyladenine (BA; 0.5, 1.0, 2.0 and 3.0 mgl^{-1}) and kinetin (0.5, 1.0 mgl^{-1}) in

various combinations. The pH of the medium was adjusted to 5.8 before autoclaving at 1kg cm^{-2} pressure (121° C) for 20 minutes. All the cultures were incubated at 22 ± 2°C with 14 h photoperiod of 30 μ mol s^{-1} m^{-2} light intensity provided by cool fluorescent tubes.

Establishment of cultures from shoot explants taken from field grown plants were difficult due to contamination by endogenous bacteria. About 40% of the cultures could be established. Further explants were obtained from 3-4 months old *in vitro*-cultured shoots. Different explants *viz.*, shoot tips, nodal segments and leaves were cultured on WPM supplemented with various concentrations of BA and kinetin as mentioned above. The best response was obtained on WPM supplemented with 3 mgl^{-1} BA and 1 mg 1^{-1} kinetin for the production of multiple shoots while growth regulator-free medium was adjudged better for rooting. Hence, these two media (growth regulator free WPM and WPM with 3mgl^{-1} BA and 1mgl^{-1} kinetin) were used for further studies.

An initial swelling at the base of the shoots in 45% of the cultures in both shoot tip as well as nodal segment explants on WPM supplemented with 3 mgl^{-1} BA +1 mgl^{-1} kinetin was observed. Multiple shoots were observed from the base as well as from the nodal regions in over 90% of the cultures within 60 days of culture on above medium. The number of multiple shoots ranged from 10 to 20 (Fig. 1B). Most of the shoots developed to 3-5 cm in length in about 90 days of culture, however, they did not develop roots on the same medium.

When whole leaves, with a portion of the petiole, collected from *in vitro* grown plants were cultured on the WPM + 3 mgl^{-1} BA + 1mgl^{-1} kinetin direct regeneration of plantlets from the cut end of the petiole in over 75% of the cultures was observed. The number of plants regenerated ranged from 5 to 15 in 60 days of culture (Fig. 1C).

On growth regulator-free WPM, rooting was induced in over 90% of shoot cultures obtained from both shoot and leaf explants. Over 5 cm long roots developed ranging from 3 to 6 in about 40 days from culture on root inducing medium.

The *in-vitro*-raised rooted plantlets were transferred to potted soil mixture (garden soil : Perlite: sand; 1:1:1) and hardened in humid chamber (80-90% RH) for 20-30 days. Over 80% plants survived in pots (Fig. 1E).

Studies have shown that *P. barberi* cultures could be maintained in *in vitro* repositories (Fig. 1D) by yearly sub-culture on minimal growth medium i.e. on WPM supplemented with 25 gl^{-1} sucrose and 5 gl^{-1} mannitol in sealed culture tubes.

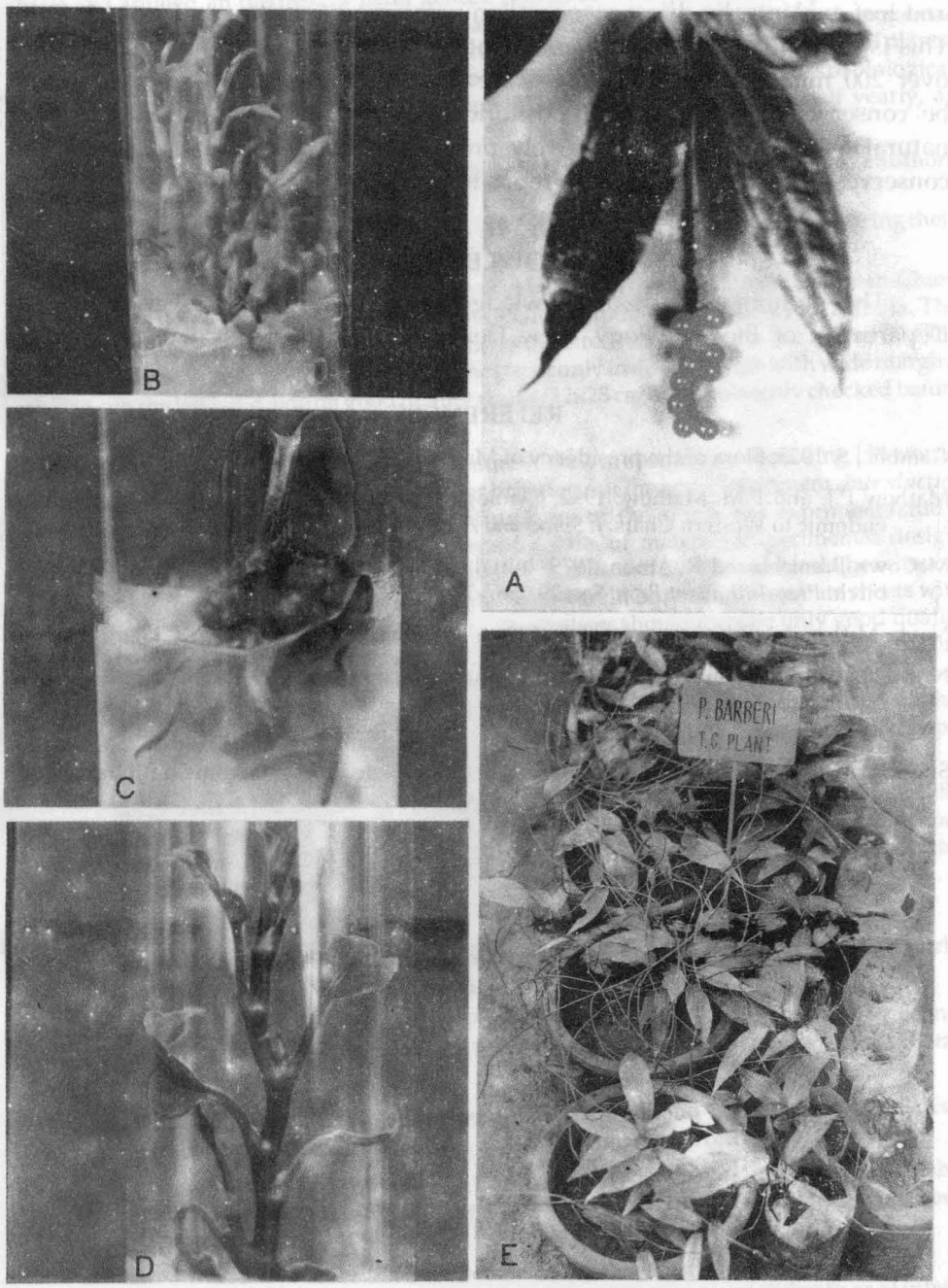

Fig. 1. Micropropagation of *Piper barberi* (A) A twig of *P. barberi* (B) Multiple shoots from shoot tip explant on WPM + 3mgl^{-1} kinetin (C) Plant regeneration from the base of the petiole on WPM + 3 mgl^{-1} BA + 1mgl^{-1} Kinetin (D) 1-Year-old *P. barberi* plantlet on minimal growth medium i.e. WPM + 25 gl^{-1} mannitol + 5 gl^{-1} Sucrose (E) Tissue culture raised plants in pots.

Thus, *P. barberi* could be successfully micropropagated from both shoot and leaf explants, by direct regeneration using modified Woody Plant Medium. This is the first report on micropropagation of *P. barberi*. By using this protocol over 200 micropropagated plants were planted in the pots. These plants can be conserved either in field repositories or may be re-introduced into their natural habitat. Being a vegetatively propagated species, it is also possible to conserve this species in *in vitro* repositories.

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