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**Organización de las Naciones Unidas para la Agricultura y la Alimentación y el Instituto Internacional de Recursos Fitogenéticos**

# Plant Genetic Resources Newsletter

## Aims and scope

The *Plant Genetic Resources Newsletter* publishes papers in English, French or Spanish, dealing with the genetic resources of useful plants, resulting from new work, historical study, review and criticism in genetic diversity, ethnobotanical and ecogeographical surveying, herbarium studies, collecting, characterization and evaluation, documentation, conservation, and genebank practice.

## Management

The *Plant Genetic Resources Newsletter* is published under the joint auspices of the International Plant Genetic Resources Institute (IPGRI) and the Plant Production and Protection Division of the Food and Agriculture Organization of the United Nations (FAO).

## Availability

The *Plant Genetic Resources Newsletter* appears as one volume per year, made up of four issues, published in March, June, September and December. *Plant Genetic Resources Newsletter* is available free of charge to interested libraries of genebanks, university and government departments, research institutions, etc. The periodical may also be made available to individuals who can show that they have a need for a personal copy of the publication.

## Types of paper

### Articles

An article will publish the results of new and original work that makes a significant contribution to the knowledge of the subject area that the article deals with. Articles, which should be of a reasonable length, will be considered by the Editorial Committee for scope and suitability, then assessed by an expert referee for scientific content and validity.

### Short communications

A short communication will report results, in an abbreviated form, of work of interest to the plant genetic resources community. Short communications in particular will contain accounts of germplasm acquisition missions.

### Other papers

The *Plant Genetic Resources Newsletter* will publish other forms of reports such as discussion papers, critical reviews, and papers discussing current issues within plant genetic resources.

Book reviews will be printed, as well as a News and Notes section. Suggestions for books to review are invited, as are contributions to News and Notes.

### Submission

In the first instance papers may be submitted in typescript form or as an Email message. The final version may be submitted as an Email file or as an MS-DOS-readable file on diskette.

### Editorial office

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# Plant Genetic Resources Newsletter

## Editorial

The present publication is a manifestation of a title that first appeared in November 1957, No. 1 of the *Plant Introduction Newsletter* that was published by the Crop Production and Protection Branch of the Plant Production and Protection Division (AGP) of FAO.

From the December 1970 issue, No. 24, the *Newsletter* was published under the auspices of the newly created Crop Ecology and Genetic Resources Unit of AGP. The purpose and scope of the *Newsletter* were enlarged as plant introduction had by then become only one aspect of a broader field that included plant exploration and collecting, and evaluation and utilization of the genetic resources of cultivated plants and related wild species. The title of the publication was changed in the next issue, No. 25, to the *Plant Genetic Resources Newsletter*, which appeared in January 1971, as a reflection of the intensification of effort in the field of genetic conservation.

In February 1978, issue No. 33 appeared as the *FAO/IBPGR Plant Genetic Resources Newsletter*, published jointly by the Crop Ecology and Genetic Resources Unit and IBPGR, which had emerged as a CGIAR Centre in 1974, operating as a field project of FAO. The *Newsletter* sought to publish review articles, notes and news on current activities in plant genetic resources. Issue No. 39, September 1979, appeared under the auspices of AGP and IBPGR.

Issue No. 41 of March 1980 established a basic policy and format that persisted up to No. 79, June 1987, with increased emphasis on publishing reviews and new work that reflected developments in all aspects of plant genetic resources.

In September 1987, issue No. 71 marked the beginning of efforts to upgrade the standard of the content and presentation of the material. The scope of activity

internationally was such that IBPGR was able in 1989-90 to start producing a series of regional newsletters. Some of the material that had previously appeared in the *FAO/IBPGR Newsletter* was more suitable for publication in one of the regional newsletters. At the same time, the requirement was increasing for a publication to disseminate the results of the sophisticated research being done in all areas of plant genetic resources, together with accounts of the proliferating number of collecting missions being undertaken throughout the world. In addition, some of the articles that the *Newsletter* was receiving were becoming so specialized that expert refereeing was called for if the *Newsletter* was to fulfill its responsibilities to its readers. The aspect of peer review was discussed in the 1989 IBPGR In-house Review on Publications, and the *Newsletter's* management agreed to institute such a system.

On 1 January 1994, IBPGR ceased to exist and a new independent CGIAR Centre, the International Plant Genetic Resources Institute (IPGRI), came into being. A Memorandum of Understanding was developed between IPGRI and FAO to continue to publish the *Newsletter*, reverting to the title *Plant Genetic Resources Newsletter*. The Memorandum was signed in September and made a convenient point to relaunch and redesign the publication, continuing the tradition of publishing research and collecting articles, but expanding its coverage to include more informal material of immediate interest to the international genetic resources community. IPGRI and FAO share responsibility for the production of the publication. An Advisory Committee consisting of representatives from IPGRI and FAO meets four times a year. IPGRI nominates a Managing Editor who is responsible for the day-to-day operation of the *Newsletter*.



## REVIEW

# The use of biotechnology for conservation and utilization of plant genetic resources<sup>1</sup>

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### Summary

Plant genetic resources constitute genotypes or populations of cultivars (landraces, advanced/improved cultivars), genetic stocks, wild and weedy species, which are maintained in the form of plants, seeds, tissues, etc. The great wealth of genetic diversity currently available holds vast potential. However, the genetic resources are non-renewable and are among the most essential of the world's natural resources. It is essential that these be well conserved, be it at species, genepool or ecosystem level, for the use of present and future generations. The term biotechnology has been used to include a wide variety of biological manipulations such as cell, tissue and organ culture, embryo rescue, genetic engineering, including the transfer of DNA across sexual barriers, vaccine production, etc. In the context of plant genetic resources, it generally connotes an array of techniques providing new ways to manipulate germplasm. In this field, biotechnology has potential in improving germplasm acquisition and safe exchange, conservation, evaluation and genetic enhancement. International Plant Genetic Resources Institute (IPGRI) is promoting the use of biotechnological tools for more efficient conservation of and use of plant germplasm. This paper reviews the use of biotechnology in these respects and discusses the progress and prospects.

### Introduction

Crop germplasm collections are assemblies of genotypes or populations representative of cultivars, genetic stocks, wild species, etc., which are maintained in forms such as plants, seeds and tissue cultures (Frankel and Soulé, 1981). Functionally, plant genetic resources (PGR) can include landraces, advanced/improved cultivars and wild and weedy relatives of crop plants (either domesticated, semi-domesticated or non-domesticated). Landraces are distinct local types, adapted to the many variants and interactions of natural and cultural environments to which crop species were gradually exposed (Harlan, 1975) and are probably the most important of the PGR. Advanced cultivars, especially those of recent origin, may have been incorporated with most coadapted complexes which had been assembled in their precursors in the earlier stages of plant selection. These, along with genetic stocks (natural or induced mutants, breeding lines with specific characteristics, accessions with resistances), also have a part to play in the future improvement of economically important plant species and will need to be preserved (Frankel, 1990a; Frankel and Soulé, 1981).

The work of Nikolai Vavilov from 1920 to 1940 was a milestone in the field of PGR. Vavilov identified what

were first called the 'centers of origin of domesticated plants and animals' (Vavilov, 1951). Later these came to be regarded as 'centers of genetic diversity' and this, with some additional centres, is basically the current view (Zohary, 1970). This work led to the identification of gene pools of plants (in terms of taxonomic relatedness), which are essential to human life and survival. These are frequently used by breeders for crop improvement and this has offered new opportunities for agricultural development (Harlan, 1975; Hawkes, 1983). The genetic variation in plants was considered to be an unlimited resource. But it has now been realised that much of the genetic variation available in the centres of diversity will soon become extinct if care is not taken of it. The problem became serious with the wave of agricultural development demanded by an ever-increasing population, and this had a profound impact on traditional agriculture, including traditional cultivars. Many factors such as the extension of and changes in land use; the introduction of modern techniques of agriculture and the use of fertilizers, pesticides and fungicides made traditional cultivars obsolete and highlighted the need for their rapid replacement with improved cultivars (Frankel, 1975; Frankel, 1990b). The great wealth of genetic diversity existing in the genepools holds vast potential for current and future uses for the benefit of humankind. PGR are irreplaceable and it is essential that we should be concerned with their conservation, at species level, genepool level or at the ecosystem level. The potential could be exploited either through conventional means or the use of biotechnologies. In either case, PGR are the raw material, without which no

<sup>1</sup> An abridged version of this paper was presented at the 'International Conference on Agrotechnology in the Commonwealth: Focus for the 21st Century', 24-26 May 1994, held at the National University of Singapore, Singapore

progress can be made. So it is imperative that we conserve PGR, study them and make appropriate use of them.

Activities that relate to the conservation and use of PGR include: exploration and collecting; characterization and evaluation; conservation, assessment of variation and identification of useful genes, and exchange and genetic enhancement. Exploration is the preliminary survey of an area where germplasm collecting is to be carried out. However, due to resource constraints, exploration and collecting are often carried out simultaneously. Collecting refers to gathering seed or propagules of landraces, wild species, etc. from the field. It can also refer to the assembly of material through correspondence and exchange. The collected germplasm has to be studied in order to understand its genetic composition and identify useful traits. This is done through the systematic characterization and evaluation of material. Conservation includes the management and preservation of known genetic resources. Conservation of PGR can be divided into two approaches, *ex situ* and *in situ*. *Ex situ* conservation maintains germplasm outside its original habitats, in facilities that have been specifically created such as the seed, field and *in vitro* genebanks or botanical gardens (Frankel and Soulé, 1981). PGR can also be conserved as pollen, DNA libraries, etc. though at present the access time for such diversity is longer. The other approach, *in situ* conservation, has been defined as the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings or, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties (Article 2, Convention on Biological Diversity). In both cases, the use made of PGR, conserved for the betterment of human life, becomes critical. To make the best use of the conserved material, it has to be exchanged freely and this involves the movement of germplasm not only within a country but between countries. Finally, the germplasm that has been collected, studied and conserved has to be used for the improvement of present-day varieties of crops. This could be through simple selection from the material that has been assembled or may involve highly complex processes of hybridization, testing and selection, depending on the genetic distances between the materials being used for the purpose of improvement.

### **IBPGR/IPGRI**

The International Board for Plant Genetic Resources (IBPGR) was established in 1974 to promote and coordinate the work on plant genetic resources globally and was linked to the Food and Agriculture Organization of the United Nations (FAO) for administrative purposes. During the last 20 years, along with many achievements to its credit, IBPGR has now grown into a fully independent institute, the International Plant Genetic Resources Insti-

tute (IPGRI) and is one of the 19 institutes in the Consultative Group on International Agricultural Research (CGIAR) system.

IPGRI's mandate is to advance the conservation and use of PGR for the benefit of present and future generations. IPGRI does not have genebanks or laboratories. It works by collaborating with different partners who have an interest in conserving and using PGR. These partners include national programmes, universities, nongovernmental agencies and other international and regional organizations. IPGRI encourages, supports and engages in activities aimed at strengthening the conservation and use of PGR worldwide, with special emphasis on developing countries. To help achieve these aims, we have four main objectives: 1) to assist countries, particularly developing nations, in assessing and meeting their needs for the conservation of PGR and to strengthen links to users, 2) to promote and build international collaboration in the conservation and use of PGR, 3) to develop and promote improved strategies and technologies for PGR and complementary methods of conservation and 4) to provide an information service to inform the world genetic resources community of both practical and scientific developments in the field (IBPGR, 1993b). The basis for achieving these objectives is a set of multidisciplinary projects, comprising a series of activities. These form a network or matrix between the five regional offices and the headquarters in Rome. The Regional Office for Asia, the Pacific and Oceania is located in Singapore.

IPGRI recognizes that biotechnology provides some important tools for the effective conservation and use of PGR. These tools include tissue, cell or organ culture, molecular genetic techniques, serology, embryo rescue techniques and DNA extraction and sequencing. Some of the developments in biotechnology, such as the combination of *in vitro* conservation technology, genetics and molecular biology, are changing the way we collect, manage and use a wide range of diversity available to us (Withers, 1991a). The potential for crop improvement is also significant, but the discussion here will be limited to issues related to PGR conservation and their use. In order to make appropriate use of biotechnological tools to improve PGR conservation, there has to be a greater level of awareness among biotechnologists of the needs of PGR work and, conversely, conservationists have to be informed of the opportunities offered by biotechnology. It is foreseen that, as we move into the next century, increasing use will be made of biotechnological tools for better conservation and use of PGR.

### **The role of biotechnology**

So far, we have seen that the subject area for germplasm comprises the exploration and collecting, conservation, characterization and evaluation, exchange of germplasm, and genetic enhancement. In functional terms, these mainly constitute *in vitro* technology and molecular tech-

niques. The former, involving tissue culture, preservation, and regeneration of plant material, is useful for collecting, conserving, germplasm health and exchange. We have also looked briefly at the IPGRI and its role in the conservation and use of PGR globally. Now we will attempt to review some of the available biotechnologies and the ways in which these can assist in carrying out PGR-related activities effectively and efficiently. Some of the problems and needs for further research will also be discussed.

### **Exploration and collecting**

Plant genetic resources available to humans are being eroded rapidly. The reasons for this are many and include the introduction of new and uniform cultivars, deforestation, developmental activities (such as irrigation, hydroelectric projects and road building), urbanization and changes in agricultural practices. Under these circumstances, there is an urgent need to try and assemble whatever genetic diversity is still available, either from farmers' fields or from nature. Biotechnology can help reduce practical impediments to efficient collecting by 1) providing information on the available genetic diversity in a given area and 2) developing *in vitro* methods for application in the field in order to provide new ways of collecting, especially the problem species such as vegetatively propagated species and those species with recalcitrant seeds (Withers, 1994).

Germplasm collecting generally involves collecting samples of seed from populations of species. However, collecting clonally propagated plants and species with recalcitrant seeds is often problematic. This is because the material collected is often bulky, can deteriorate quickly and may be infested with pests. Advances in tissue culture have made available a range of techniques which can be used for *in vitro* collecting of germplasm of problem species (Withers, 1991b; Withers, 1994). An example of such developments was the inoculation of cacao budwood, collected and sterilized with non-toxic sterilant, on to a medium containing fungicides, prepared in advance in the laboratory (Yidana *et al.*, 1987). This method has been used successfully under difficult tropical conditions. In the case of coconuts, successful field inoculation of zygotic embryos on to medium has been achieved. The extracted embryos can be kept in salt solution for transport to a laboratory (Assy-Bah *et al.*, 1989). Another example is the development of a simple *in vitro* technique for collecting *Digitaria eriantha* ssp. *pentzii* and *Cynodon dactylon* to overcome the deterioration, quarantine and bulk problems of conventional methods of collecting vegetative material (Ruredzo, 1991). Attempts are being made to develop a nondestructive technique for collecting immature coconut inflorescence for tissue culture (Rillo, 1989). Most of the above studies have been supported by IPGRI/IBPGR.

An understanding of the extent and distribution of

diversity within a population is essential for effective sampling. The use of molecular techniques in studying genetic diversity (see next section for details) in recent years has contributed to a better understanding of the genetic diversity of some species (Hodgkin and Debouck, 1992). Ecogeographic surveys provide information on species distribution as well as infraspecific diversity. Biochemical as well as molecular techniques can be applied during such surveys for a proper assessment of the genetic diversity patterns which would then permit effective sampling of a particular region. For example, using restriction fragment length polymorphisms (RFLPs) it was demonstrated that the probability of adding new genes to a tomato collection would be about 20 times higher by adding one accession of *Lycopersicon peruvianum*, a wild relative of tomato (Miller and Tanksley, 1990). More recent developments in the area of extraction of DNA from dried specimens, such as herbarium material and fossils, will help us to better understand the patterns of genetic diversity and phylogenetic relationships (Engeln, 1993; Pääbo and Wilson, 1991). Efforts are also underway to develop methods that can be used in the field to assess population diversity, thereby facilitating sampling of maximum diversity. Developments in molecular biology may lead to the development of a practical field protocol for DNA collecting (Adams *et al.*, 1992), an additional option for germplasm collectors.

For successful exploration and collecting, a well-coordinated effort with appropriate financial and manpower resources is required. The methods followed must be based on sound scientific principles to recover maximum genetic diversity through sampling of different farms or geographic sites. IPGRI is presently preparing a comprehensive manual on germplasm collecting which describes the various requirements for a successful collecting programme (Guarino *et al.*, in press), including the use of available biotechnological methods to make collecting more efficient.

### **Characterization and evaluation**

So far we have seen the need and urgency for PGR collecting. Historically, germplasm collecting and conserving have had significance in elucidating taxonomic status and evolutionary relationships between and within species. Although this is an important role for germplasm collections, the main justification for genetic resource conservation is for its use in crop improvement. To use successfully the variability from broad gene pools, we need to broaden genetic attributes as well as the desirable traits available in the germplasm. This is only possible after systematic characterization and evaluation of germplasm has been carried out (Rao, 1980). Characterization and evaluation may serve two functions. Firstly, many of the characters that are recorded on individual accessions have diagnostic value and will help genebank curators to keep track of an accession and check its genetic integrity over

years. The second function is related to the use of the material. Both characterization and evaluation result in a recording of a number of traits and this will help the user to identify accessions with desirable traits for use in crop improvement. Usually, highly heritable, frequently morphological characters are employed for the purposes of characterization. Characterization is normally carried out by germplasm curators. For crop improvement purposes, evaluation for agronomic and economic characters is carried out mainly by multidisciplinary teams. Such traits are usually under polygenic control and are strongly influenced by environment. Recording of such traits may need complex techniques and adequate facilities. IPGRI has published a number of descriptor lists for a number of major crops to facilitate better characterization and evaluation of PGR.

Until recently, most of the characterization and evaluation of PGR has been based on recording of either qualitative and/or quantitative morphological characters. During the past decade or so, more and more emphasis has been placed on biochemical characterization and more recently on the use of molecular techniques. The use of morphological phenotypes for genotype characterization has advantages and disadvantages. The multilocus nature of most of these characters provides information that is extremely useful to breeders. However, complex inheritance makes prediction of breeding difficult. The use of gene products (proteins, peptides) or metabolites (terpenes, flavonoids etc.) has partly solved this problem. Mendelian inheritance of isozymes makes genetic analysis easier. However, variation in isozymes is often low. Molecular genetic characterization has several advantages, such as 1) no environmental influence, 2) any plant part from any growth stage can be used, 3) there is no limit on numbers for analysis, 4) it requires only small amounts of material, 5) DNA is highly stable, even dry samples can be used (Beese, 1992). But there are some practical disadvantages: it is not very suitable for large-scale screening; experimental data on nucleotide sequence variation usually characterise only very small parts of the whole genome and are often not related to economically interesting traits. More work is needed on repeatability, and on the use of nonradioactive isotopes.

Four areas of germplasm "characterization" in which biotechnology can be used have been identified: 1) identification of genotypes, including duplicate accessions; 2) "fingerprinting" of genotypes; 3) analysing genetic diversity in collections or in natural stands; and 4) assembling a core collection (Dodds and Watanabe, 1990). Although identification of accessions is possible, it has yet to be used on a large scale for identification of duplicates in collections, though some efforts are in progress at the International Potato Center. The value of fingerprinting is more in the area of varietal identification. As noted earlier, all these studies require the analysis of fairly large samples. So most of the work to date analysing the ge-

netic diversity in collections has been carried out using small collections. Such analysis of genetic diversity could be used to assemble a core collection, which would represent most of the diversity in a large collection with a minimum amount of redundancy. Information on genetic diversity in a collection would also assist in its maintenance as well as making use of the genetic diversity for crop improvement. Information on these aspects will follow in later sections.

The determination of the extent of genetic diversity and its maintenance in collections can be assisted by analysis of isozyme variation and molecular genetic variation (Clegg, 1990; Hubby and Lewontin, 1966; Miller and Tanksley, 1990; Simpson and Withers, 1986). Variation in DNA sequence has been used to examine single copy genes, multigenes and organelle genomes, but relatively few studies of variation in populations have been carried out. Although there is potential here, the available technology is slow and expensive. However, there has been much progress in the last couple of years. RFLP maps have been produced in some crops, though their use in studying genetic diversity has been limited. RFLP linkage maps have been constructed for several crop species including maize, tomato, rice and potato (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1989; Helentjaris, 1987; Helentjaris *et al.*, 1985; Landry *et al.*, 1987; McCough *et al.*, 1988; Zamir and Tanksley, 1988).

Rapid progress in recent years has resulted in the development of a number of methods for detecting variation in nucleotide sequences. These include: 1) Restriction fragment length polymorphism (RFLP): its resolution is at genomic or chromosomal level and it is principally a marker technique, mainly useful in gene mapping and marker assisted selection. Technically complex, it requires the use of short-lived radio isotopes (although some research on the use of non-radioactive labels is underway). So routine application of RFLPs in large-scale genetic diversity analyses may be difficult. 2) Random amplified polymorphic DNA (RAPD): this is a type of marker which uses polymerase chain reaction (PCR) technology. It is basically simple and quick, requires only small amounts of DNA, involves no radioactivity and is suitable for large samples. Resolution is at genomic level and it is a typical marker technology. RAPDs segregate in Mendelian fashion and are useful in population genetic studies, for locating and manipulating genes of interest, for identifying somatic hybrids at an early stage, for monitoring the levels of genetic diversity between and within populations, and for 'fingerprinting' individual accessions. 3) Variable number of tandem repeats (VNTRs): these are serial repeats of a core DNA sequence, and, depending on the size, can be micro- or minisatellites. In a single-locus situation, VNTRs are useful for identification of individuals and paternity analysis. In multilocus situations, they are useful for marker-assisted selection, and for studying relatedness of individuals and genetic variation. 4) Poly-

merase Chain Reaction (PCR/sequencing): this studies the base sequence of known genes. The gene is amplified and sequenced, revealing the composition. PCR has the highest level of resolution, as the differences are measured at base level. Automation of the technique is possible to a significant extent and this assists large-scale application. It is useful in population studies, taxonomy and/or phylogenetics. PCR is a powerful technique for the identification of infraspecific genetic variation (He *et al.*, 1992; Roy *et al.*, 1992; Welsh *et al.*, 1990). VNTR and PCR techniques offer distinct advantages over RFLP, isozyme or morphological analysis (Swennen, 1990). 5. Allele-specific polymerase chain reaction (ASPCR): This technique uses allele-specific oligonucleotide primers in PCR amplifications. It is a fast, accurate, sensitive technique but the main difficulty is the development of allele-specific reagents. This technique has been used for the identification of genotypes (Shattuck-Eidens *et al.*, 1992). 6) Denaturing/temperature gradient electrophoresis (DGGE/TGGE): this technique detects differences down to single-base substitutions so it is useful in studying mutations. It is used mainly in population studies.

Following are some examples of applications of the above techniques, either individually or in combination to characterize PGR.

The genetic diversity of sorghums, compared with maize, is less well characterised at the genetic and molecular level, despite its worldwide economic importance. Vierling *et al.* (1994) investigated the genetic diversity in sorghum for RFLPs and RAPDs. Using oligonucleotide probes for the fingerprinting of plant DNA may have several applications in PGR conservation and use and some of these have been highlighted (Kaemmer *et al.*, 1992; Weising *et al.*, 1990, 1991). They include characterising the extent of genetic variability within races; assessing the "purity" of inbred lines; selecting the recurrent parents in back-cross breeding programmes; identifying crop cultivars; characterising fusion hybrids; and evaluating the extent of somaclonal variation at molecular level. The usefulness of some of these applications has been demonstrated in apple (Nybom and Schaal, 1990), in avocado (Lavi *et al.*, 1991) and in *Brassica oleracea* (Nienhuis *et al.*, 1993). The use of molecular markers to study genetic stability of material conserved in genebanks, especially that conserved *in vitro*, has been attempted. Isozyme analysis, RFLPs and RAPDs are being successfully employed in monitoring somaclonal variation in banana and plantain (IBPGR, 1993c; Reuveni and Israeli, 1990; Vuylsteke and Swennen, 1990).

Identification of genotypes, fingerprinting and the study of genetic diversity have been carried out using isozyme markers (Asiedu, 1992; Balagtas and Ramirez, 1991; Bhat *et al.*, 1992; Glaszmann *et al.*, 1988; Jarret and Litz, 1986; Lebot *et al.*, 1993; Nevo, 1990; Nevo *et al.*, 1984; Ocampo *et al.*, 1993; Vaillancourt *et al.*, 1993). However, in most cases relatively few loci and alleles have been used

in the analysis. Since any method looks at a small part of the genome, a variety of methods should be used (Anderson and Fairbanks, 1990). Any drawbacks of isozyme analysis may be overcome with the development of molecular techniques. In order to obtain a complete picture, morphological and agronomic evaluation of germplasm should be combined with biochemical and molecular analysis (Singh *et al.*, 1991), since these studies provide complementary information. A fairly good example of such a complementary study was reported by Zhang *et al.* (1993) in comparing isozyme and RFLP analysis in wild barley (*Hordeum vulgare* ssp. *spontaneum*). In this study isozymes better demonstrated a larger amount within population diversity, whereas RFLPs resolved a higher proportion between population differentiation and detected more heterozygosity than did isozyme analysis. So by using both methods, it was possible to determine that the particular set populations of *H. vulgare* were not only divergent, but also that each population was highly heterozygous. Similar examples of complementarity of morphological and molecular analyses are yet to be noted.

### **Conservation of germplasm**

As mentioned above, there are two approaches to the conservation of PGR: *ex situ* and *in situ*. It is important to emphasize that these two approaches are not mutually exclusive, but are indeed complementary. The work involved in conserving a gene pool should employ a combination of methods, from nature reserves to genebanks. Appropriate strategies and the balance to be used depend on factors such as the biological characteristics of the gene pool, infrastructure and human resources, number of accessions in a given collection and its geographic site. For any given gene pool, the extent a particular method is used may differ from another gene pool (IPGRI, 1993a). There is a need to strike a balance between methods used and it is in this area that biotechnology can play a major role, especially for the so-called difficult crops (clonally propagated species, plants with recalcitrant seeds and plants with severe seed production problems).

### **Ex situ conservation of seeds**

It is well known that, under cool and dry conditions, orthodox seeds are viable for long periods since seed longevity is, to some extent, directly proportional to the storage temperature, humidity and seed moisture content. If seeds are maintained under such conditions, the life processes in seeds are minimized so that they can be stored for a number of years, depending on species, with little loss in genetic diversity, genetic integrity and viability. So it will not be necessary to regenerate them at frequent intervals (Ellis *et al.*, 1985a, 1985b). Nevertheless, due to conditions under which most genebanks operate, there is a need for periodic regeneration of accessions and restocking of seed in cold store. This is either

due to loss in seed viability or to depletion of seed stocks as a result of use and distribution. Critical levels for both viability and quantity are established for each genebank or species and when that level is reached seed samples of such accessions are planted under optimum agronomic conditions and regenerated. At every regeneration, there will be some change in the genetic structure of the accession. But it is important to take all the necessary steps to minimize any change in genetic structure that may be caused by genetic drift, genetic shift, selection, outcrossing or through simple mechanical mixture due to human error. Good overviews of these problems encountered during multiplication of germplasm are given by Breese (1989) and Ramanatha Rao (1991).

It has long been known that prolonged storage can cause genetic damage, the extent of the damage being highly variable. Recent studies using molecular probes to investigate the extent and nature of damage at DNA level have shown that RFLP and other techniques can be used with advantage (Engels, 1993). Work is in progress in different laboratories on the use of biochemical and/or molecular techniques to study the loss of genetic diversity in germplasm stored and regenerated several times. Some of these studies in Germany and China are being supported by IPGRI.

Work is also in progress on alternative methods of seed storage for the so-called recalcitrant seed. As opposed to seeds of most common species, generally called 'orthodox' seeds that can be dried to very low levels of seed moisture content (below 7%), there are a number of species whose seeds cannot be dried to low levels for optimum storage. Such seeds have been referred to as 'recalcitrant' (Chin and Pritchard, 1988; Roberts and King, 1986). For long-term storage of such seeds, imbibed storage (seeds maintained at higher levels of seed moisture contents) may be of considerable importance. Efforts are also being made to understand the genetic basis of recalcitrance in order to deal with this problem (Pers. Com. Dr. Shirata, 1994). Very low temperature storage using liquid nitrogen, cryopreservation, also appears to be promising, giving a much extended life span. Cryopreservation in liquid nitrogen of isolated zygotic embryos and whole seeds of cassava has been studied by Marin *et al.* (1990). They obtained a very high percentage of survival rates (ca. 97%) of zygotic embryos and whole seeds by both slow and rapid cooling methods followed by slow thawing. Another area in which much progress has been made is in the storage of ultra-dry seeds (dried to seed moisture content of 2-5%) at room temperature conditions. Much is expected from the efforts that are being made on *in vitro* conservation, as discussed later in this paper.

#### **Ex situ conservation of vegetatively propagated material**

There are a number of important plant species, including staple food crops and fruits such as cassava,

potato, sweet potato, taro, yam, apple, banana and citrus, which are difficult or impossible to propagate by seed and cannot be conserved as seeds. Generally, these are conserved in field genebanks. Though the field genebanks provide easy and ready access to conserved material for research as well as for use, they run a greater risk of being destroyed by natural calamities and virus infections. Field genebanks also require more space and labour and are expensive to maintain (Jarret and Florkowski, 1990). Another group of plant species that are also conserved in field genebanks are those with recalcitrant seeds, as mentioned earlier. Many tropical fruit species such as avocado, cacao, coconut, jack fruit and mango and a number of forest species produce recalcitrant seeds, presenting tremendous problems for the conservation of genetic diversity in these species. Several techniques for conserving such vegetatively propagated species have recently been developed and some of these are undergoing rigorous testing. Some promising approaches for solving the problem are discussed here.

#### *Tissue culture*

Possibilities now exist for conserving PGR as tissue cultures (Withers and Engels, 1990). For some species, *in vitro* conservation may be the only option available. Although tissue culture offers great potential for the conservation of germplasm of vegetatively propagated material and species with recalcitrant seeds, there are two major technical problems. Firstly, the genetic instability of the material conserved as tissue culture due to somaclonal variation at the time of regeneration of the tissue into seedlings. Secondly, the length of storage as tissue has been limited. Significant work is being done on both aspects and for some species, tissue culture maintenance is relevant due to improved techniques resulting in low levels of somaclonal variation. Work on cryopreservation of tissue culture, so that these could be preserved for long periods, is also making rapid progress. It appears that the morphogenetic potential is not affected by freeze preservation of tissue cultures and normal plants could be reproduced. Once these techniques are refined through further research and development, large-scale adoption will be possible and conservation of plant genetic resources could become very cost-effective (Engels, 1993; Ramanatha Rao, 1992; Simpson and Withers, 1986; Withers, 1978, 1980).

For the purpose of conservation of PGR, the growth of cultures should be kept to the minimum, if not completely arrested. This is essential to avoid frequent transfer to fresh media, which would require a high level of inputs and would make *in vitro* conservation expensive. There are several ways of achieving slow growth such as: 1) use of immature zygotic embryos, 2) modification of culture medium by adding osmotic or hormonal inhibitors or other growth retardants, 3) reduction of storage temperature (4-10° C for temperate species and 15-25° C

for tropical species), 4) mineral oil overlay, 5) reduced oxygen tension and 6) defoliation of shoots (Charrier *et al.*, 1991; Withers, 1991a). Although there is a wealth of information on the tissue culture of plants, the most important aspect of *in vitro* conservation using this technique is the capacity of plants to regenerate *in vitro*. The physiological and biochemical basis of this process is still poorly understood. A recent study in tomatoes indicates that some progress is being made in this area (Koorneef *et al.*, 1993). In this study, RFLP linkage analysis of the gene that controls the regeneration ability in tomato was combined with morphological and physiological analyses. This analysis showed that the genetic component associated with regeneration determines the morphogenetic competence and not the sensitivity to hormones as previously assumed. A locus that exerts a major effect on the tissue culture behaviour of tomato has been mapped. The eventual cloning of a tomato regeneration gene may help towards a better understanding of the regeneration process. However, it may not solve all problems encountered in regenerating other genotypes or species. Different steps controlled by different genes may have limited effects on others.

Regeneration and successful propagation of genetically stable seedlings from cultures is a prerequisite in any *in vitro* conservation effort. So far, extensive work has been done and protocols for clonal multiplication are well established for several species (Engels, 1993). Nevertheless, more work needs to be done for species like coconut, for which protocols are not yet available. Similarly, the somaclonal variation, which results in genetic instability as well as loss of genetic integrity of material conserved, for example *Musa* spp., methods of propagation have to be carefully devised so as to minimize such variation (Withers, 1994). Care should be taken while establishing large-scale production areas using rapid multiplication and clonal propagation by distributing sufficiently diverse material to the users to overcome the danger of genetic vulnerability. The work on rare medicinal plants (Sudha and Seeni, 1993) will have an impact not only on *ex situ* conservation but also on the implementation of effective *in situ* conservation. Through micropropagation and the establishment of cultures that produce the chemical needed or the establishment of fields of rare medicinal plants, the stress on material in the wild can be effectively reduced and uncontrolled extraction from the wild can be avoided.

It is important that any such method developed or adapted does not interfere with the genetic stability of the material conserved. Usually, organized cultures, such as shoots, are used for slow growth storage since unorganized tissues, such as callus, are more vulnerable to somaclonal variation. With some root and tuber crops, temperate fruits, ornamental and horticultural species and a few forestry species, apparently routine slow growth storage for 1-15 years (what could be called a medium-

term storage) is possible using cycles of up to 2 years before subculturing (Withers, 1991b). However, there is not enough data on long-term effects of slow growth. So more research on *in vitro* conservation is in progress so that this biotechnological tool can be used effectively for conserving genetic diversity. IPGRI has been supporting studies being carried out on the above. For example, though the medium-term *in vitro* conservation of *Musa* by slow growth is now routine, for long-term preservation somaclonal variation is a major problem (IBPGR, 1993a). IPGRI is supporting research for developing early markers to detect variants using RAPDs. The long collaboration with CIAT has resulted in the development of *in vitro* conservation techniques and management procedures for cassava germplasm. These successes indicate bright prospects for using *in vitro* conservation technology for many other vegetatively propagated species.

#### Cryopreservation

Theoretically, cryopreservation, i.e. using liquid nitrogen (either by immersion or in vapour phase) as a storage medium, is ideal for long-term storage since it virtually suspends all the metabolic activities in any living tissue, be it seed, cell suspensions, callus, cultured tissue, pollen or a shoot tip. It is a relatively new conservation method and research on the development of protocols for cryopreservation of *in vitro* plant material started in the early 1970s (Withers, 1990), almost at the same time as experiments for cryopreservation of seed were initiated (Bass and Stanwood, 1978; Stanwood, 1980). Some of the advantages of cryopreservation of *in vitro* material are: 1) physical and genetic stability for a long period of time, at least theoretically, 2) it is relatively economical (Charrier *et al.*, 1991) and 3) conserved material is easily accessible (Jones, 1991). However, as indicated earlier, there is need to gather data on a long-term basis to confirm these conclusions. IPGRI is supporting research on potato in Germany to refine cryopreservation method for potato (IBPGR, 1993a). We are also supporting research in Costa Rica on developing a cryopreservation method for somatic embryos of *Musa*.

The various stages in cryopreservation of *in vitro* cultured material are: selection, excision of plant tissues or organs and culture of source material; selection of healthy cultures; pregrowth related treatments; application of cryoprotectants; cooling/freezing; storage; warming/thawing; post-thaw treatment; viability testing and recovery growth (Charrier *et al.*, 1991; Escobar *et al.*, 1993; Withers, 1991a). Cryopreservation is most successful for cell cultures since differentiated cultures may be damaged by cryopreservation due to structural injury. One promising method that counters such effects is "vitrification". This method involves immersion of the culture in a very high concentration of cryoprotectant solution, followed by rapid cooling. The remaining water in the tissue vitrifies, forming a non-crystalline solid (Withers, 1994). Cultured cells

and somatic embryos derived from the mesophyll tissues of asparagus are cryopreserved by vitrification by Uragami *et al.* (Uragami *et al.*, 1993) and the survival rate, as determined by shoot formation, was 63%.

Cryopreservation of somatic, pollinic and zygotic embryos has also been successful in some 12 species (Charrier *et al.*, 1991). Work on cryopreservation of nucellar and somatic embryos of mango are in progress in the University of Florida, with IPGRI's support. RFLPs and RAPDs may be used to monitor genetic stability of cryopreserved material (Tanksley *et al.*, 1989). All the analyses performed so far using phenotypic, isozyme and molecular techniques have not indicated any genetic changes in cryopreserved material in comparison with the control (Engels, 1993); however, the number of tests and the length of time are not yet sufficient to conclude and recommend this method on a large scale. This is another area which requires further research.

#### Synthetic seeds

Another promising method for the conservation of species that are clonally propagated or species with recalcitrant seed is the possibility of producing the so-called 'synthetic' or 'artificial' seeds and conserving them as true seeds (Engels, 1993). This involves encapsulation of shoot-tips and somatic embryos in semi-solid material which serves as an artificial seed coat and endosperm, producing 'beads'. Beads may also contain nutrients and pesticides. Somatic embryos and shoot tips are active and lack desiccation tolerance. So a developmental switch to induce tolerance can be provided through the inclusion of a hormone "signal" in the medium. This has been achieved in somatic embryos of alfalfa where 100% of the embryos survived drying to 10% moisture content (Senaratna and McKersey, 1989; Dereuddre *et al.*, 1990; Schulthies *et al.*, 1990; Withers, 1994). If the current pace of development in this technology continues and reproducible and widely adaptable results can be achieved, production and storage of artificial seeds could become an extremely important technology in PGR conservation and use (Withers, 1994). Redenbaugh (1993) has reviewed the latest developments with respect to synthetic seeds, including new methods for encapsulation of somatic embryos and the creation of synthetic endosperm.

Case studies of the application of synthetic seeds are presented for crops such as alfalfa, carrot, celery, grape, lettuce, mango, mulberry, orchard grass, sandalwood, soybean and spruce. Encapsulation of somatic embryos can be an effective method for handling otherwise fragile somatic embryos. Encapsulation of somatic embryos using sodium alginate and calcium chloride solutions and growth of plantlets of encapsulated carrot embryos was successfully achieved (Tsuji *et al.*, 1993). In the work being carried out in collaboration with the University of Florida on mango, there are prospects for developing artificial seeds for conservation purposes (IBPGR, 1993a).

#### In vitro genebanks

So far we have discussed different components of an entirely *in vitro*-based conservation scheme for clonally propagated and other problem crops. There is need to put all these components together - protocols for tissue culture, successful regeneration, transfer to soil (the protocols which differ from species to species, sometimes there are genotypic differences), genetic stability and cryopreservation of cultured material either by vitrification or by encapsulation. When this is achieved, we will have a viable long-term conservation strategy for PGR for an *in vitro* genebank. To date, only for strawberry have all the stages of such a scheme been well researched and all major problems resolved (Withers, 1991b), though significant progress has been made in the case of potato and cassava (Engels, 1993). The production of potato and cassava plants capable of undergoing normal tuberization and rooting from meristems cryopreserved for 4 years with no significant changes in viability and genetic composition has been reported (Bajaj, 1990). Day-to-day management of *in vitro* genebanks has also been studied to some extent (Chavez *et al.*, 1987). The day-to-day operation of such a genebank would consist of receiving vegetative material from either a field genebank or a collecting mission followed by processing of the material for disease indexing, therapy and quarantine, as required. Healthy and clean material will go into the next stage of *in vitro* culturing. From this stage, the material could either go into the cryopreserved base genebank (long term) and/or into the *in vitro* active genebank in slow growth conditions. In the latter case, subculturing and transferring to a new medium may have to be carried out at regular intervals of 1-2 years, depending on the material. Active banks will be used for supplying material to users and/or to establish field genebanks (Engels, 1993). Successful implementation of an active *in vitro* gene bank for 14 yam species, as proposed by IBPGR (1985), has been recently reported (Malaurie *et al.*, 1993). Similar successes, though partial, have been reported by Dodds *et al.* (1991), Kuo (1991), Lizarraga *et al.* (1989) and Novak (1990).

#### DNA or genebanks

Progress in genetic engineering has resulted in breaking down the barrier of species or genera for transferring genes. The first successful plant-to-plant gene transfer was in 1983. Since then, transgenic plants have been produced with genes transferred from viruses, bacteria, fungi and even mice (Engels, 1993). Progress in this field has led to the establishment of DNA libraries containing single useful genes for breeding programmes. In addition, it has been suggested that total genomic information of germplasm could be stored in the form of DNA libraries (Benford, 1992; Ford-Lloyd and Jackson, 1986; Jung *et al.*, 1992; Matlick *et al.*, 1992; Peacock, 1989). Despite the current problems with this approach, rapid progress in this

subject area may make the storage of DNA an additional option for the conservation of PGR (Engels, 1993). Such readily available genetic resources (without going through the process of collecting, extracting, etc.) for scientists working at the molecular level will be an added advantage. The technique may also allow the recovery of genes from apparently extinct taxa by using herbarium and other non-viable materials as demonstrated by the sequencing of DNA of fossils (Engeln, 1993; Golenberg, 1991).

### **In situ conservation**

*In situ* conservation involves the conservation of diversity in natural habitats where the plant species have evolved or occur. *In situ* conservation can be carried out either in nature or on farm, depending on the material under consideration. This type of conservation is dynamic as opposed to the semi-static nature of *ex situ* conservation and provides the species or populations with an opportunity to evolve under natural conditions. For some forms of biodiversity *in situ* conservation is the only option. One of the main reasons given for choosing *in situ* conservation over *ex situ* is the need to maintain the evolutionary potential of species and populations (Frankel, 1970; Frankel and Soulé, 1981; Ledig, 1988). This view is not only from the plant breeder's perspective but also, of late, encompasses the conservation biologist's concern for maintaining the variability in small populations and endangered species. Three general research strategies that are needed for *in situ* conservation are: 1) assay genetic variation represented in specific areas to document its relationship to overall patterns of geographic variation (e.g. studies of isozyme- or DNA-restriction site variation) within and among populations, 2) genecological studies (reciprocal transplant studies or progeny tests) to compare performance of native-site-derived material within and among seed zones and 3) further study of special features of genetic variation that have been revealed either by previous research or experience with managed populations or plantations (Riggs, 1990).

From the above it follows that for the successful operation of any *in situ* conservation programme we also need information on the following aspects: 1) studies on genetic erosion due to the introduction of new varieties, 2) identification of genetic diversity rich regions, 3) effects of land fragmentation on genetic diversity, 4) temporal and spatial changes in genetic structure of populations, 5) biogeographic studies, especially when introgression is involved, 6) minimum viable population sizes and areas and 7) effects of inbreeding and seed banks. In the case of agrobiodiversity, the effects of farmers' practices, cultural preferences and environmental factors are the most important ones. In almost all the studies that provide information on the above, it should be possible to use biotechnological tools to allow precise assessment of extant diversity as well as changes in it over space and

time. These tools can also be used to monitor changes that occur in gene frequencies (Helentjaris *et al.*, 1985; Hodges, 1991; Hodgkin and Debouck, 1992; Moore and Moran, 1989; Templeton *et al.*, 1990; von Vornam, 1993), which are the core of any programme of conservation.

### **Safe movement of germplasm**

It is important that all accessions in the genebank should be available to all those who wish to use them, either in crop improvement or for other studies. No country is self sufficient in the PGR required for crop improvement programmes of the country. So there is significant exchange of germplasm worldwide. Though such free exchange of PGR is highly desirable, it may pose problems from a germplasm health point of view. This is because pests can move along with the seed/propagules of any plant material and can cause serious problems in the new environment into which they have been introduced. So, one of the most important requirements for expedition and safe transfer is that the propagules distributed be pathogen-free. This is especially important in these days of increased international exchange of PGR. In-country and international transfer of vegetatively propagated plant material plays an important role in modern agriculture (Spiegel *et al.*, 1993). IBPGR has played a significant role in developing guidelines for the safe movement of a number of important crops. IBPGR has collaborated with FAO and other organizations and has published technical guidelines for the safe movement of germplasm for cacao, cassava, citrus, edible aroids, grapevine, legumes, *Musa*, vanilla and sweet potato. Guidelines for coconut, potato, small fruits and sugar cane are under development. These incorporate a number of biotechnological tools that assist the genebank curator in distributing disease-free and viable germplasm. It is also essential to have information on the effect of infected germplasm on seed viability and longevity (Frison, 1991; IBPGR, 1988).

In the exchange of vegetatively propagated germplasm disease, indexing and therapy are the two most critical activities (Frison, 1988; IBPGR, 1988). The procedures that need to be followed in indexing and therapy of PGR are found in the recommendations of the IBPGR Advisory Committee on *In Vitro* Storage (IBPGR, 1988).

Biotechnology has played an important role in assisting the safe distribution of PGR through the exchange of germplasm as disease-free cultures (Delgado and Rojas, 1993; Dodds and Watanabe, 1990; Frison, 1981; IBPGR, 1988; Ng, 1988). Reliable virus detection methods are important. For some viruses, especially poorly characterized or unknown viruses, there are as yet no methods for detecting their presence. This is particularly a problem when dealing with many plant species about which very little is known or with wild species. For a recent review of diagnostic methods see Mathews (1993). The enzyme-linked immunosorbent assay (ELISA) test is commonly used to detect viruses in plant material. Development of

monoclonal antibodies has enabled the specificity of serological tests to be increased, especially for the detection of well-characterised viruses. Use of double-stranded RNA (dsRNA) analysis, along with bioassay, can provide some information on the presence of poorly characterised or unknown viruses. The need to develop broad-spectrum tests is urgent since, to date, a separate test needs to be conducted for each virus, using a specific antiserum or DNA probes (Frison, 1991). Application of broad-spectrum serological methods, such as those recently developed for potyviruses (Shukla and Ward, 1989), may be important tools as they are fairly simple to apply and require less sophisticated equipment than nucleic acid hybridization (Frison, 1993).

Nucleic acid hybridization methods using  $^{32}\text{P}$  have been successfully employed for the detection of small quantities of viruses in plants (Spiegel *et al.*, 1993). Some initial results on the use of DNA probes which would recognise coat protein genes or other genes common to a number of different viruses and, possibly, bacteria have been reported (Faquet *et al.*, 1993). However, various problems, including the availability of probes, mean that this technique cannot be used on a routine basis as yet (Mathews, 1993). Detection methods for bacteria and fungi, including serological methods, immunofluorescence colony-staining and molecular hybridisation, have been described (Hampton *et al.*, 1990; Singleton *et al.*, 1993). By using polymerase chain reaction (PCR), it is possible to detect even the smallest amounts of pathogens but it is not very suitable for use in routine germplasm testing. An example of the use of molecular techniques for the detection of specific viruses is the development of a molecular probe for banana bunchy-top disease, based on research into the causative agent (Harding *et al.*, 1991). They found that the virus-like particles associated with banana bunchy-top disease contained small single stranded DNA. Hanold and Randles (1991) describe the use of RNA hybridization techniques for the detection of "cadang cadang" viroid in coconut.

Detection of pathogens has to be followed up by elimination if a safe exchange or conservation of a particular germplasm is to take place. Some of the widely used methods for the elimination of viruses are heat therapy, meristem tip culture alone or combined with chemotherapy (IBPGR, 1988; Ng *et al.*, 1992; Spiegel *et al.*, 1993; Waithaka, 1992). Ng *et al.* (1992) described tissue culture techniques used for both disease elimination and micropropagation and discussed the advantages and disadvantages of different techniques. There is further need to develop rapid screening, testing and therapy programmes to make the movement of germplasm across the world safer.

### **Biosystematics and evolution**

Research on PGR, integrating many fields of study, greatly helps in understanding the evolution of cultivated plants

and their wild relatives (Hanelt, 1988). A good understanding of the evolution of a given species, its relationship to other species belonging to the same genepool, including the traditionally distinguished primary, secondary or tertiary genepools, as well as to species more distantly related, is of great importance to PGR conservation and use (Engels, 1993). Historically, it was mainly morphological information that was used to study and understand systematics, species relationships and evolution. This was followed by the use of biochemical markers and isozymes to study interspecific relationships. Some examples are the studies on common beans (Gepts, 1990), on maize (Doebley, 1989), on sorghum (Morden *et al.*, 1989), on tomatoes (Rick and Holle, 1990), on potato (Spooner *et al.*, 1992) and on *Musa* (Jarret and Litz, 1986a, 1986b) (for general review see Buth, 1984). Over the last 10 years or so, there has been a rapid increase in studies that use biotechnological tools to better understand biosystematics and the evolution of plant species.

Biosystematics at both interspecific and infraspecific levels is essential for the proper classification of the material conserved, to determine breeding behaviours such as incompatibility as for effective conservation and use of PGR. Although the advent of genetic engineering has meant that genes can be moved much more easily, even between completely unrelated taxa, we should not forget that recombination DNA technology is not going to free the geneticist from the need to make crosses and screen segregating populations, etc. Additionally, it is in the plant species most amenable to genetic analysis and manipulation through classical Mendelian methods that molecular technologies are being most avidly applied, for example *Arabidopsis*, tomato, potato and maize (Hansche, 1990). A good knowledge of species relationships as well as genomic homologies will facilitate the transfer of genes or even of substitutions of chromosomes or chromosome segments from one species to another (Tanksley *et al.*, 1989). A major strength of molecular genetic analysis is that it provides numerous independent molecular characters that can often rigorously define monophyletic lineages. The use of molecular markers can involve two separate steps. The first one deals with addressing questions about phylogenetic relationships. The next step is to address questions about character evolution (where, when and how the character states arose). It is through this second application that molecular phylogenetics has a major impact on many aspects of systematics, evolution, genetics and ecology (Sytsma *et al.*, 1991). Keeping this background in mind, we will now examine a few cases in which biotechnology has helped in the study of biosystematics and the evolution of plants.

At a conceptual level, several authors believe that two plant species isolated by a chromosomal barrier can, via hybridization, give rise to new fertile diploid species that are partially reproductively isolated from both parents. This mode of hybrid speciation has been termed recombi-

national speciation (Grant, 1981; Stebbins, 1957). This hypothesis has been tested by the experimental synthesis of new 'hybrid' species (Stebbins, 1957 and others). The actual extent of this mode of speciation in nature is unclear. For this, the detailed genetic information necessary in order to confirm or reject the hypothesized hybrid origin is lacking because most studies have relied on evidence from morphology, secondary chemistry, ecological and geographic data and/or the production of synthetic hybrids. All these approaches have one limitation - genetic additivity in the putative hybrid species generally cannot be demonstrated, especially for the quantitative morphological traits. Even isozyme evidence (Gallez and Gottlieb, 1982) cannot be conclusive as it is also a biparentally inherited character. One solution is to use genetic markers representing both the biparentally inherited nuclear genome and a uniparentally or clonally inherited cytoplasmic genome such as chloroplast or mitochondrial genome. For proof of recent hybrid origin, the putative hybrid taxon should combine the alleles of its two proposed parents and have a cpDNA like that of at least one of its parents. In this way, both the questions of genetic additivity and evolutionary polarity can generally be resolved. Rieseberg *et al.* (1990) combined enzyme electrophoresis and RFLP analysis of cpDNA and nuclear ribosomal DNA (rDNA) were used to test the hypothesis that both *Helianthus neglectus* and *H. paradoxus* are stabilized hybrid derivatives of *H. annuus* and *H. petiolaris*. Their study indicated, and partially confirmed, an earlier assumption that *H. paradoxus*, in contrast to *H. neglectus*, was derived via hybridization. Morphological, geographical and archaeological evidence has led to the hypothesis that the domesticated sunflower was derived from a wild/weedy form of *H. annuus*. A high degree of enzymatic and cpDNA sequence similarity was observed between wild and domesticated sunflower, and domesticated sunflower contained a subset of the alleles and cpDNAs found in wild *H. annuus*. The extensive polymorphism in the wild plants and the virtual monomorphism in the cultivated for both isozyme and molecular markers further suggest a single origin for domesticated sunflower from a limited genepool (Rieseberg and Seiler, 1990; Skoric, 1993).

Miller and Tanksley (1990) used RFLP analysis to study the phylogenetic relationships and genetic variation in the genus *Lycopersicon* and drew interesting conclusions, including that the amount of genetic variation in the self-incompatible species far exceeded that found in self-compatible species, more than tenfold on an individual accession basis. From the same study, it was also concluded that the traditionally used fruit colour as the major characteristic in the taxonomy of the genepool should be replaced by compatibility of species. The RFLP-based dendrogram for *Lycopersicon* genus was largely consistent with much of the previous classification based on morphology and crossability. CpDNA analysis, in combination with morphological data analysis, has been used to

investigate relationships between genera, subgenera and species within the Solanaceae family, revealing that *Lycopersicon* and *Solanum* are congeneric and both belong to the subgenus *Potatoe* (Spooner *et al.*, 1990, 1993).

Sugarcane taxonomy, genetics and breeding are made difficult by its extreme genetic complexity (Simmonds, 1976). Modern sugarcane varieties are aneuploids often having more than 100 chromosomes. More than 80 of these were derived from the noble canes, *Saccharum officinarum*, and most others contributed by a few *S. spontaneum* (Walker, 1987)). *S. officinarum* is thought to have *S. robustum* as its closest wild relative. Two other groups which might have contributed are *S. barberi* and *S. sinense*, which encompass old varieties grown for centuries in India and China. These groups have an uncertain taxonomic status and may have been derived from natural hybridization between *S. officinarum* and *S. spontaneum* (Price, 1968). Based on the DNA data, Glaszmann *et al.* (1990) concluded that rDNA variation agrees with the existing taxonomy. The wild species surveyed are generally differentiated, with the exception of five *S. robustum* clones and one *S. spontaneum* clone which displayed one variant. It was also shown that the diversity in *S. officinarum* is not fully encompassed by that of *S. robustum*. In their study, *S. barberi* displayed rDNA variants very characteristic of *S. officinarum* and of the Indian forms of *S. officinarum* - this supports its origin from *S. officinarum* x *S. spontaneum* hybridization (Daniels and Roach, 1987). Sobral *et al.* (1993) describe novel approaches to molecular mapping and fingerprinting and reported the use of DNA fragment polymorphisms in a study of phylogenetic relationships in *Saccharum* species. Several of these applications have also been reported, using VNTRs, in which a core sequence of only a few base pairs is called a "micro satellite" locus and, if larger, as a "mini satellite" (Hodges, 1991). Armour and Jeffreys (1992) give a good overview of this technique and its applications.

Other examples include lentils (Mayer and Soltis, 1994), potatoes (Spooner and Sytsma, 1992), rice (Dallas, 1988; Dally and Second, 1990), maize (Doebley, 1990a, 1990b), *Triticum* (Talbert *et al.*, 1991), Guinea yams (Terauchi *et al.*, 1992), cucurbits (Wilson *et al.*, 1992), *Musa* species (Gawel *et al.*, 1992; Jarret *et al.*, 1992), *Glycine* species (Menancio *et al.*, 1990), barley (Saghai-Marooof *et al.*, 1990; Zhang *et al.*, 1993), *Papaver* species (Srivastava and Lavania, 1991) and *Allium* species (Wilkie *et al.*, 1993).

There are tremendous problems with the biosystematics of many forest tree species. Some of these problems, as well as the use of biochemical and molecular genetic markers, have been reviewed by Strauss *et al.* (1992). They conclude that, although expensive, by providing huge numbers of polymorphisms for infraspecific analysis, and highly conserved gene sequences for analysis at interspecific level, molecular techniques promise to provide huge opportunities for understanding biosystematic relationships at all levels of biological organization.

These examples demonstrate the use of molecular methods in solving some of the problems of taxonomy, phylogenetics and evolution which will assist in better conservation use of PGR. Though molecular techniques offer unique possibilities, it is important to be cautious. Classical taxonomic treatments are based on the comparison of many morphological characters. Chromosome pairing in polyploid hybrids also represents a comparison of synaptic ability of many, if not all, Mendelian loci on the genome. Other techniques such as measurements of DNA content, DNA hybridization, arm ratios, differential staining, electrophoresis, immunochemical reactions and RFLP studies all allow comparisons involving smaller amounts of total DNA present. All the above techniques, at least at the present, represent measurements of the similarity of the DNA of the species being compared. The reliability of the technique is directly related to the proportion of the DNA compared (Kimber and Yen, 1990). Additionally, uniparental inheritance of character, such as cpDNA restriction analysis, makes molecular techniques very precise in comparing the similarities of DNA. However, uniparental inheritance of cpDNA is not fully substantiated (Harris and Ingram, 1991). Harris and Ingram (1991) reviewed the assumption of low levels of intraspecific cpDNA variation and concluded that far from being rare, intraspecific cpDNA variation was relatively common. Paternal inheritance of cpDNA may be common in pines and some other plant species (Dong and Wagner, 1994); recent studies in rice indicate occasional paternal inheritance and recombination of cpDNA (Dally and Second, 1990). This may, therefore, be a more widespread phenomenon than previously suspected. So cpDNA should be used along with a wider consideration of the degree of intra-individual and intraspecific variation and the mode of plastid transmission. Consequently, it is important to know more about the degree of intra-individual and intraspecific cpDNA variation in natural populations; the effect and influence of a slow 'leakage' of parental plastid DNA on an essentially maternal cpDNA phylogeny. We also need to know the effects of plastid dynamics, within and between populations, on cpDNA variability and the extent of recombination between cpDNA in wild plants. Information along these lines will make the use of molecular techniques for studies on biosystematics and the evolution of plant species much more precise.

### **Use of germplasm**

One of the major objectives of the conservation of PGR is to make genetic diversity available for immediate or future use. Abundant evidence exists to show that it is necessary to preserve a wide range of diversity in order to meet current crop improvement needs. However, it is also evident that the widest possible range of genetic diversity has to be conserved in order to meet future, as yet unknown, needs (Hodgkin and Debouck, 1992). PGR

programmes are expected to promote and facilitate the use of germplasm through the maintenance of sufficient healthy, readily-accessible and adequately characterised/evaluated material as well as through the proper documentation of relevant information.

Besides being useful for analysing genetic diversity, molecular techniques may play a major role in utilising this diversity for plant improvement programmes in the future. RFLPs may not have any adverse effect on the overall fitness of the populations (neutral). Consequently, the transfer of a particular RFLP marker into an adapted cultivar is not expected to cause any damage or to reduce the overall fitness of the recipient. However, a substantial proportion of RFLP variation may be the result of insertional events (e.g. Apuya *et al.*, 1988; Johns *et al.*, 1983) and a possibility does exist that the insertional mutations may be either slightly deleterious or unstable. In either case, it is important to conduct careful population surveys of insertional variation as a first step (Clegg, 1990).

Despite the problem mentioned above, the application of modern methods appears to contribute to rapid progress in breeding, using wide hybridization. Kräuter and Friedt (1990) used embryo culture *in vitro* in a crossing programme and identified hybrids using different techniques. Following this, RFLP analysis allows rapid and safe characterization in the early developmental stages of hybrids (Kräuter *et al.*, 1990) and selection for true hybrids.

RFLPs have been used for fingerprinting, to generate genetic maps (West *et al.*, 1989) and to enable the identification of specific genotypes and agronomic traits (Dodds and Watanabe, 1990; Rao and Grandillo, 1992). High-density RFLP maps provide an opportunity to resolve complex traits into their individual genetic components. It might then be possible to treat these characters as single gene traits. This technique is known as quantitative trait loci (QTLs) and a description can be found in Tanksley *et al.* (1989). The use of the polymerase chain reaction (PCR) has further improved the application of biotechnology by increasing our understanding of DNA sequences through selective amplification of a specific DNA segment. This facilitates the specific transfer of the desired segments. RAPD marker analysis allows genetic diversity studies in species for which no molecular information exists and permits a much more rapid data collection than RFLP. Because of this fast and cost-effective method, analysis of large numbers of accessions is possible. This will contribute to a better exploitation of PGR collections (Anderson and Fairbanks, 1990).

Gene mapping using RFLPs, etc. will promote better use of available diversity. However, the ease with which a genetic map can be developed and applied to a target crop depends on the genetic complexity of the species and the extent of DNA polymorphism present in the species. Genetic mapping in genetically monomorphic species has usually been achieved by using wide crosses

between highly divergent parental genotypes and, sometimes, even using different species. The low frequency of DNA polymorphism within a species can also limit the utilization of mapped DNA markers in crosses that are of agronomic importance, but which involve genetically more monomorphic parents (Tao *et al.*, 1993).

From the above, it is obvious that the application of modern molecular techniques will help germplasm curators enormously to increase significantly the knowledge base of individual accessions, of an entire crop species, and of its gene pool. Gene identification and its transfer into an existing genetic background using recombinant DNA technology and other applications of these new technologies will further increase the importance of *ex situ* collections and, thus, enhance their value.

### Concluding remarks

The world's population in 1850 was about 1.1 thousand million and increased to about two billion by 1930. However, it has been estimated that the population will be around 7 billion in the year 2010. As the world's population continues to increase, it demands a higher and higher yield from agriculture. Recent research in cell biology, molecular genetics, recombinant DNA, tissue culture and related fields is opening up new possibilities for progress in agriculture. Developments in biotechnology allow scientists to transfer genes for crop improvement in a relatively short time. But the genes for such engineering manipulations have to be provided from genetic resources.

The world's agriculture has always been confronted by numerous problems. For example, we do not know what new diseases, insects or other pests, and soil and atmospheric problems we will have to face in the future. New strains of pests continue to develop and attack those crop cultivars or landraces that were originally resistant to these pests. We do not know what physiological and morphological characters will be needed for crops to perform well in a possible post-nuclear-war era, although the chance for a nuclear war is less than before. We have been warned repeatedly that a "greenhouse effect" may cause temperature changes through a higher content of carbon dioxide and other gases in the atmosphere. If this happens, new varieties which can adapt well in the new environment will be required. But as the state of our future environment is largely unknown, even specialists do not know what genes will be required in the future. Therefore, genetic resources have to be collected, studied and conserved for future use before they disappear for ever.

We should be equally concerned about the conservation of the genetic diversity that has accumulated within a species during the long evolutionary processes. Most of such species have been useful to humans since the beginning of agriculture. People depend on them for food fibre and, to survive and to enhance the quality of life. The

current situation is very dynamic - changing pest problems, changing needs in human nutrition, growth in population, the need to extend agriculture to more environmentally stressed and marginal conditions and the increased use of plants as biomass for energy- and all of this makes it impossible to predict the genetic diversity that will be needed to meet these changing demands. This necessitates a large assemblage of plant germplasm and conservation of diverse genetic resources. To conserve and use such complex gene pools, biotechnological tools can be used to advantage. The rapid progress in *in vitro* culture technology and molecular biology is expected to assist both conservation and use, as shown in the above discussion. Some of the most important help will be in the areas of PGR conservation *in vitro* (use of growth retardants, growth regulation by reduced incubation temperature, vitrification, encapsulation and cryopreservation) and micropropagation; production of disease-free material and safe exchange; better understanding of genetic diversity, both its extent and structure; gene identification using molecular techniques and the transfer of desirable genes from the useful PGR accessions.

However, despite all the advantages of modern biotechnological tools, there is currently a great amount of discussion on their use, especially for developing countries in terms of access, property protection, etc. In the First Meeting of the Inter-Governmental Working Group on Global Forests, it was pointed out that countries with advanced biotechnology have exploited the biodiversity and range of genes from developing countries, converting them into billions of dollars of profits, without sharing them with the sovereign owners of the PGR (Khan, 1994). These issues are not discussed here but it is important to remember that most of the progress in biotechnology, unlike the Green Revolution, is financed largely by the private sector. Additionally, if proper care is not taken, development may be hindered since biotechnology may prove too expensive for developing countries, and the use of biotechnology may increase the gap between developing countries and industrialized countries (Lepoivre and Semal, 1989). We should also think in terms of linking commercial benefits from the exploitation of PGR through biotechnology and the conservation of PGR. This will assist the long-term sustainability of our conservation efforts which will, in turn, benefit biotechnologists by making the raw material accessible. Additionally, a spirit of understanding and sharing of ideas as well as technologies will help us all do a better job of conserving and using the most important natural resources available to man, i.e. PGR, both for the present and for many generations to come. Only then will it be possible to realise the most obvious point that can be made about plants, animals, fungi and microorganisms, that they are the means by which we will attain sustainability.

## References

- Adams, R.P., Do, N. and Ge-lin, C. 1992. Preservation of DNA in plant specimens from tropical species by desiccation. in Conservation of Plant Genes. DNA Banking and *In Vitro* Biotechnology. Adams, R.P. and Adams, J.E. (eds.) San Diego, USA, Academic Press Inc. 153-181.
- Anderson, W.R. and Fairbanks, D.J. 1990. Molecular markers: important tools for plant genetic resources characterization. *Diversity*. 6: 51-53.
- Apuya, N.R., Frazier, B.L., Keim, P., Roth, E.J. and Clark, K.G. 1988. Restriction fragment length polymorphisms as genetic markers in soybean, *glycine max* (L.) Merrill. *Theor. Appl. Genet.* 75: 889-901.
- Armour, J.A.L. and Jeffreys, A.J. 1992. Biology and applications of human minisatellite loci. *Current Options in Genetics and Development*. 2: 850-856.
- Asiedu, R. 1992. Isozyme analysis and its application in plant breeding. in *Biotechnology: enhancing research on tropical crops in Africa*. Tottapilly, G., Monti, L.M., Mohan Raj, D.R. and Moore, A.W. (eds.) Ibadan, Nigeria, CTA/IITA. 261-265.
- Assy-Bah, B., Durand-Gasselín, T., Englemann, F. and Pannetier, C. 1989. Culture *in vitro* d'embryos zygotiques de cocotier *cocos nucifera* L.. Methode, révisée et simplifiée, d'obtention de plants de cocotier transférables au champ. *Oléagineux*. 44: 515-523.
- Bajaj, Y.P.S. 1990. Cryopreservation of germplasm of vegetatively propagated crops. *Bull. Soc. Bot. Fr.* 137: 99-114.
- Balagtas, G.E. and Ramirez, D.A. 1991. Genetic variation in Philippine collections of mungbean (*Vigna radiata* (L.) Wilczek), rice bean (*Vigna umbellata* (L.) Thumb and Ohwi) (*Vigna unguiculata* (L.) Walp.). *Philipp. Agriculturist*. 74: 103-119.
- Bass, L.N. and Stanwood, P.C. 1978. Long-term preservation of sorghum seed as affected by seed moisture, temperature, and atmospheric environment. *Crop Sci.* 18: 575-577.
- Beese, K. 1992. Development and application of molecular genetic tools for the assessment and evaluation of genetic diversity. in United States - Commission of the European Communities Workshop - Biotechnology and Genetic Resources, October 21-22, 1992. Airlie, Virginia, USA. 10-16.
- Benford, F. 1992. Saving the "library of life". *Proc. Natl. Acad. Sci. USA*. 89: 11098-11101.
- Bhat, K.V., Bhat, S.R. and Chandel, K.P.S. 1992. Survey of isozyme polymorphism for clonal identification in *Musa*. I. Esterase, acid phosphatase and catalase. *J. Hort. Sci.* 67: 00-00.
- Bonierbale, M.W., Plaisted, R.L. and Tanksley, S.D. 1988. RFLP maps based on common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics*. 120: 1095-1103.
- Breese, E.L. 1989. Regeneration and Multiplication of Germplasm Resources in Seed Genebanks: The Scientific Background. Rome, IBPGR.
- Buth, D.G. 1984. The application of electrophoretic data in systematic studies. *An. Rev. Écol. Syst.* 15: 501-522.
- Charrier, A., Dereuddre, J. and Englemann, F. 1991. The implications of biotechnology in germplasm conservation and utilization. in *Crop Genetic Resources of Africa*. Vol. II. Proceedings of an International Conference on Crop Genetic Resources of Africa. Ng, N.Q., Perrino, P., Attere, F. and Zedan, H. (eds.) Ibadan, Nigeria, IITA/IBPGR/UNEP/CNR. 279-286.
- Chavez, R., Roca, W.M. and Williams, J.T. 1987. IBPGR-CIAT collaborative project on a pilot *in vitro* active genebank. *FAO/IBPGR PGR NL*. 71: 11-13.
- Chin, H.F. and Pritchard, H.W. 1988. Recalcitrant seeds, a status report. Rome, IBPGR. Pages.
- Clegg, M.T. 1990. Molecular diversity in plant populations. in *Plant Population Genetics, Breeding, and Genetic Resources*. Brown, A.H.D., Clegg, M.T., Kahler, A.L. and Weir, B.S. (eds.) Sunderland, Mass., Sinauer Associates. 99-116.
- Dallas, J.F. 1988. Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite DNA probe. *Proc. Natl. Acad. Sci. USA*. 83: 6831-6835.
- Dally, A.M. and Second, G. 1990. Chloroplast DNA diversity in wild and cultivated species of rice (genus *Oryza*, section *Oryza*). *Caldistic-mutation and genetic distance analysis*. *Theor. Appl. Genet.* 80: 209-222.
- Daniels, J. and Roach, B.T. 1987. Taxonomy and evolution. in *Sugarcane Improvement through Breeding*. Heinz, J. (eds.) Amsterdam, Elsevier. 7-84.
- Delgado, G.E. and Rojas, C. 1993. Cassava "seed" production program by meristem culture in UNPGR-Lambayeque (Peru). in *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network*, Cartagena, Colombia, 25-28 August 1992. Roca, W.M. and Thro, A.M. (eds.) Cali, Colombia, CIAT. 146-148.
- Dereuddre, J., Scottez, C., Arnaud, Y. and Duron, M. 1990. Resistance of alginate-coated axillary shoot tips of pear tree (*Pyrus communis* L. cv Beurré Hardy) *in vitro* plantlets to dehydration and subsequent freezing in liquid nitrogen: effects of previous cold hardening. *C.R. Acad. Sci. Paris*. 310: 317-323.
- Dodds, J.H., Huaman, Z. and Lizarraga, R. 1991. Potato germplasm conservation. in *In vitro Methods for Conservation of Plant Genetic Resources*. Dodds, J.H. (ed.) London, Chapman and Hall. 93-109.
- Dodds, J.H. and Watanabe, K. 1990. Biotechnical tools for plant genetic resources management. *Diversity*. 6: 26-28.
- Doebley, J. 1989. Isozymic evidence and the evolution of crop plants. in *Isozymes in Plant Biology*. Soltis, D.E. and Soltis, P.S. (eds.) Portland, USA, Diocorides Press. 165-191.
- Doebley, J. 1990a. Molecular evidence and the evolution of maize. *Econ. Bot.* 44: 6-27.
- Doebley, J. 1990b. Molecular systematics of *Zea* (Gramineae). *Maydica*. 35: 143-150.
- Dong, J. and Wagner, D.B. 1994. Paternally inherited chloroplast polymorphism in *Pinus*: estimation of diversity and population subdivision, and tests of disequilibrium with a maternally inherited mitochondrial polymorphism. *Genetics*. 36: 1187-1194.
- Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985a. Handbook of Seed Technology for Genebanks Vol. I. Principles and Methodology. Rome, IBPGR.
- Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985b. Handbook of Seed Technology for Genebanks Vol. II. Compendium of Specific Germination Information and Test Recommendations. Rome, IBPGR.
- Engeln, H. 1993. Mumien; monster, molekuele. *Geo*. 9: 36-50.
- Engels, J.M.M. 1993. How can biotechnology be exploited in the conservation and use of biological diversity? in *GTZ Workshop on Plant Biotechnology in Technical Cooperation Programmes*, 6-11 October 1993. Legaspi, Philippines.
- Escobar, R.H., Roca, W.M. and Mafla, G. 1993. Cryopreservation of cassava root tips. in *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network*, Cartagena, Colombia, 25-28 August 1992. Roca, W.M. and Thro, A.M. (eds.), CIAT, Cali, Colombia. CIAT. 116-121.
- Faquet, C.M., Schöpke, C., Chavarriaga, P., Sangare, A. and Beachy, E.N. 1993. Genetic engineering technologies to control viruses and their application to cassava viruses. in *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network*, Cartagena, Colombia, 25-28 August 1992. Roca, W.M. and Thro, A.M. (eds.), CIAT, Cali, Colombia. CIAT. 190-207.
- Ford-Lloyd, B. and Jackson, M. 1986. *Plant Genetic Resources: An Introduction to their Conservation and Use*. London, Edwin Arnold. Pages.
- Frankel, O.H. 1970. Genetic conservation in perspective. in *Genetic Resources in Plants*. Frankel, O.H. and Bennett, E. (eds.) Oxford, Blackwell. 469-489.
- Frankel, O.H. 1975. Genetic conservation - Why and how. in *Symposium on South East Asian Plant Genetic Resources*. Williams, J.T., Lamoureux, C.H. and Wulijarni-Soetjipto, N. (eds.), Kopo, Cisarua, Bogor, Indonesia. IBPGR/SEAMEO/BIOTROP/BPP/LIPI. 16-32.
- Frankel, O.H. 1990a. The future of the Global Genetic Resources Network: Activation or dissolution? *Diversity*. 6: 59-60.
- Frankel, O.H. 1990b. Germplasm conservation and utilization in horticulture. in *Horticultural Biotechnology*. (eds.) Wiley-Liss. 5-17.
- Frankel, O.H. and Soule, M.E. 1981. *Conservation and Evolution*. Cambridge, Cambridge University Press.
- Frison, E.A. 1981. Tissue culture: A tool for improvement and international exchange of tropical root and tuber crops. *IITA Res. Briefs*. 2: 1-4.
- Frison, E.A. 1988. Improving the efficiency and safety of germplasm movement. *Diversity*. 6: 7.
- Frison, E., A. 1991. Phytosanitary aspects of genebank management. in *Proceedings of Inter-Centre Meeting on Germplasm Health and Movement*, 23-25 October, 1990. IBPGR, Rome. IBPGR. 53-59.
- Frison, E.A. 1993. Production of cross-reacting and heterospecific mono-

- clonal antibodies for plant virus detection. Faculté des Sciences Agronomiques des Gembloux, Belgium. Ph.D Thesis.
- Gallez, G.P. and Gottlieb, L.D. 1982. Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. *Evolution*. 36: 158-1167.
- Gawel, N.J., Jarret, R.L. and Whittemore, A. 1992. Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. *Theor. Appl. Genet.* 84: 286-290.
- Gebhardt, C.E., Ritter, T., Debener, U., Schachtschabel, B., Wlkeneir, B., Uhrig, H. and Salamani, F. 1989. RFLP-analysis and linkage mapping in *Solanum tuberosum*. *Theor. Appl. Genet.* 78: 65-75.
- Gepts, P. 1990. Biochemical evidence bearing on the domestication of Phaseolus (Fabaceae) beans. *Econ. Bot.* 44: 28-38.
- Glaszmann, J.C., De los Reyes, B.G. and Khush, G.S. 1988. Electrophoretic variation of isozymes in plummules of rice (*Oryza sativa* L.) - a key to the identification of 76 alleles at 24 loci. International Rice Research Institute. November 1988. Research Paper Series. Number 134.
- Glaszmann, J.C., Lu, Y.H. and Lanaud, C. 1990. Variation of nuclear ribosomal DNA in sugarcane. *J. Genet. & Breed.* 44: 191-198.
- Golenberg, E.M. 1991. Amplification and analysis of Miocene plant fossil DNA. *Phil. Trans. Royal Soc. Lond.* 333: 419-437.
- Grant, V. 1981. *Plant Speciation*. New York, Columbia University Press.
- Guarino, L., Ramanatha Rao, V. and Reid, R. (eds.), in press. *Germplasm Collecting Manual*. Rome, IPGRI.
- Hampton, R., Ball, E. and De Boer, S. (eds.), 1990. *Serological methods of detection and identification of viral and bacterial plant pathogens: a laboratory manual*. St. Paul, USA, APS Press.
- Hanelt, P. 1988. Taxonomy as a tool for studying plant genetic resources. *Kulturpflanze*. 36: 169-187.
- Hanold, D. and Randles, J.W. 1991. Detection of coconut cadang-cadang viroid like sequences in oil and coconut palm and other monocotyledons in the south-west Pacific. *Ann. Appl. Biol.* 113: 139-151.
- Hansche, P.E. 1990. Plant size and number affect genetic analysis and the improvement of fruit and nut tree cultivars. *HortScience*. 25: 389-393.
- Harding, K., Burns, T.M. and Dale, J., L. 1991. Virus-like particles associated with banana bunchy top disease contain small single stranded DNA. *J. Gen. Virol.* 72: 225-230.
- Harlan, J.R. 1975. Our vanishing genetic resources. *Science*. 188: 618-621.
- Harris, S.A. and Ingram, R. 1991. Chloroplast DNA and biosystematics: The effect of intraspecific diversity and plastid transmission. *Taxon*. 40: 393-412.
- Hawkes, J.G. 1983. *The diversity of crop plants*. Cambridge, MA, Cambridge University Press.
- He, S., Ohm, H. and MacKenzie, S. 1992. Detection of DNA sequence polymorphisms among the wheat varieties. *Theor. Appl. Genet.* 84: 573-578.
- Helentjaris, T. 1987. A genetic linkage map for maize based on RFLPs. *Trends in Genetics*. 3: 217-221.
- Helentjaris, T., King, G., Slocum, M., Siedenstrang, C. and Wegman, S. 1985. Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. *Plant Mol. Biol.* 5: 109-118.
- Hodges, J. (ed.), 1991. *European Skills in Biotechnology and Genetic Resources*. Report on a meeting of the Commission of the European Communities, Innsbruck, Austria, 21-22 November 1991. Brussels, Belgium, EEC.
- Hodgkin, T. and Debouck, D.G. 1992. Some possible applications of molecular genetics in the conservation of wild species for crop improvement. In *Conservation of Plant Genes. DNA Banking and in vitro Biotechnology*. Adams, R.P. and Adams, J.E. (eds.) San Diego, USA, Academic Press. 153-181.
- Hubby, J.L. and Lewontin, R.C. 1966. A molecular approach to study of genetic heterozygosity in natural populations. *Genetics*. 59: 577-594.
- IBPGR 1985. IBPGR Advisory Committee on *in vitro* Storage. Report of subcommittee on design, planning and operation of *in vitro* genebanks. Rome, IBPGR.
- IBPGR 1988. IBPGR Advisory Committee on *in vitro* Storage. Report of subcommittee on conservation and movement of vegetatively propagated germplasm *in vitro* culture and disease aspects. Rome, IBPGR.
- IBPGR 1993a. Annual report 1992. Rome, IBPGR.
- IBPGR 1993b. Diversity for Development - The strategy of the International Plant Genetic Resources Institute. Rome, IBPGR.
- IBPGR 1993c. The use of RAPD to detect off-types of banana and plantain generated *in vitro*. Project proposal for collaboration between IBPGR, the University of Birmingham, INIBAP Transit Centre, Catholic University of Leuven and IITA. Rome, IBPGR.
- Jarret, R.L. and Florkowski, W.J. 1990. In vitro active vs. field genebank maintenance of sweet potato germplasm: major costs and considerations. *HortScience*. 25: 141-146.
- Jarret, R.L., Gawel, N., Whittemore, A. and Sharrock, S. 1992. (RFLP)-based phylogeny of *Musa* species in Papua New Guinea. *Theor. Appl. Genet.* 84: 579-584.
- Jarret, R.L. and Litz, R.E. 1986a. Enzyme polymorphism in *Musa acuminata* Colla. *J. Heredity*. 77: 183-186.
- Jarret, R.L. and Litz, R.E. 1986b. Isozymes as genetic markers in bananas and plantains. *Euphytica*. 35: 539-549.
- Johns, M.A., Strommer, J.N. and Freeling, M. 1983. Exceptionally high levels of restriction site polymorphism in DNA near *Adh 1* gene. *Genetics*. 105: 733-743.
- Jones, L. (eds.), 1991. *Biotechnological innovations in crop improvement*. Open University, Heerlen, Netherlands and Thames Polytechnic, London. Oxford, UK, Butterworth-Heinemann.
- Jung, C., Claussen, U., Horsthemke, B., Fischer, F. and Hermann, R.G. 1992. A DNA library from an individual *Beta patellaris* chromosome conferring nematode resistance obtained by microdissection of meiotic metaphase chromosomes. *Plant Mol. Biol.* 20: 503-511.
- Kaemmer, D., Afza, R., Weising, K., Kahl, G. and Novak, F.J. 1992. Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* spp.). *Bio/Tech.* 10: 1030-1035.
- Khan, F. 1994. Biodiversity: need ensure fair deal. *New Straits Times*. Kuala Lumpur. 19 April 1994. 3.
- Kimber, G. and Yen, Y. 1990. Genomic analysis of diploid plants. *Proc. Natl. Acad. Sci., USA*. 87: 3205-3209.
- Koornneef, M., Bade, J., Hanhart, C., Horsman, K., Schel, J., Soppe, W., Verkerk, R. and Zabel, P. 1993. Characterization and mapping of a gene controlling shoot regeneration in tomato. *Plant J.* 3: 131-141.
- Kräuter, R. and Freidt, W. 1990. Efficient interspecific hybridization via 'embryo rescue' for application in sunflower breeding. *Helia*. 13: 17-20.
- Kräuter, R., Steinmetz, A. and Freidt, W. 1990. Efficient interspecific hybridization in the genus *Helianthus* via 'embryo rescue' and characterization of the hybrids. in *Sunflower Biotechnology in Europe*, 3-6 September. (eds.), Mittlehir, France. 19.
- Kuo, C.G. 1991. Conservation and distribution of sweet potato germplasm. in *In vitro Methods for Conservation of Plant Genetic Resources*. Dodds, J.H. (eds.) London, Chapman and Hall. 123-148.
- Landry, B.S., Kesseli, R.V., Farrara, B. and Michelmore, R.W. 1987. A genetic linkage map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. *Genetics*. 116: 331-337.
- Lavi, U., Hillel, J., Vainstein, A., Lahav, E. and Sharon, D. 1991. Application of DNA fingerprints for identification and genetic analysis of Avocado. *J. Am. Soc. Hort. Sci.* 116: 1078-1081.
- Lebot, V., Aradhya, K.M., Manshardt, R. and Meilleur, B. 1993. Genetic relationships among cultivated bananas and plantains from Asia and the Pacific. *Euphytica*. 67: 163-175.
- Ledig, F.T. 1988. The conservation of diversity in forest trees. *BioScience*. 38: 471-479.
- Lepoivre, P. and Semal, J. 1989. Biotechnology applied to plants: upstream and downstream constraints. *Annales de Gembloux*. 95: 151-161.
- Lizarraga, R., Huaman, Z. and Dodds, J.H. 1989. *In vitro* conservation of potato germplasm at the International Potato Center. *Am. Potato J.* 66: 253-269.
- Malaurie, B., Pungu, O., Dumont, R. and Trouslot, M.-F. 1993. The creation of an *in vitro* germplasm collection of yam (*Dioscorea* spp.) for genetic resources preservation. *Euphytica*. 65: 113-122.
- Marin, M.L., Mafla, G., Roca, W.M. and Withers, L.A. 1990. Cryopreservation of cassava zygotic embryos and whole seeds in liquid nitrogen. *Cryo-Letters*. 11: 257-264.
- Mathews, R.E.F. (ed.), 1993. *Diagnosis of Plant Virus Diseases*. London, CRC Press.
- Matlick, J.S., Ablett, E.M. and Edmonson, D.L. 1992. The gene library - preservation and analysis of genetic diversity in Australasia. in

- Conservation of Plant Genes. DNA Banking and *in vitro* biotechnology. Adams, R.P. and Adams, J.E. (eds.) San Diego, USA, Academic Press. 15-35.
- Mayer, M.S. and Soltis, P.S. 1994. Chloroplast DNA phylogeny of *Lens* (Leguminosae): origin and diversity of the cultivated lentil. *Theor. Appl. Genet.* 87: 773-781.
- McCough, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y., Khush, G.S., Coffman, W.R. and Tanksley, S.D. 1988. Molecular mapping of rice chromosomes. *Theor. Appl. Genet.* 76: 815-829.
- Menancio, D.I., Hepburn, A.G. and Hymowitz, T. 1990. Restriction fragment length polymorphism (RFLP) of wild perennial relatives of soybean. *Theor. Appl. Genet.* 79: 235-240.
- Miller, J.C. and Tanksley, S.D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor. Appl. Genet.* 80: 437-448.
- Moore, N.J. and Moran, G.F. 1989. Microgeographical patterns of allozyme variation in *Casuarina cunninghamiana* Mig. within and between Murrumbidgee and coastal drainage systems. *Aust. J. Bot.* 37: 181-192.
- Morden, C.W., Doebley, J.F. and Schertz, K.F. 1989. Allozyme variation in old world races of *sorghum bicolor* (Poaceae). *Am. J. Bot.* 76: 247-255.
- Nevo, E. 1990. Molecular evolutionary genetics of isozymes: Pattern, theory, and application. In *Isozymes: Structure, Function, and Use in Biology and Medicine* (Progress in Clinical and Biological Research Vol 344). Ogita, Z.I. and Markert, C.L. (eds.) Wiley-Liss Inc. 701-742.
- Nevo, E., Beiles, A. and Ben-Shlomo, R. 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *Lect. Notes Biomath.* 53: 13-213.
- Ng, N.Q., Thottapilly, G. and Rossel, H.W. 1992. Tissue culture in disease elimination and micropropagation. In *Biotechnology: enhancing research on tropical crops in Africa*. Thottapilly, G., Monti, L.M., Mohan Raj, D.R. and Moore, A.W. (eds.) Ibadan, CTA/IITA. 171-182.
- Ng, S.Y.C. 1988. *In vitro* conservation and distribution of root and tuber crop germplasm. In *Crop Genetic Resources of Africa, Vol II: Proceedings of an International Conference Organized by the International Institute of Tropical Agriculture (IITA) and the National Research Council of Italy (CNR), in association with the International Board for Plant Genetic Resources (IBPGR) and the United Nations Environment Programme (UNEP) 17-20 October 1988*. Ng, N.Q., Perrino, P., Attore, F. and Zedan, H. (eds.), Ibadan, Nigeria. IITA/IBPGR/UNEP/CNR. II: 95-106.
- Nienhuis, J., Slocum, M.K., DeVos, D.A. and Mureu, R. 1993. Genetic similarity among *Brassica oleracea* L. genotypes as measured by restriction fragment polymorphisms. *J. Am. Soc. Hort. Sci.* 11: 298-303.
- Novak, F.J. 1990. *Allium* tissue cultures. In *Onion and Allied Crops*. Rabinowitch, J.L. and Brewster, J.L. (eds.) Boca Raton, Florida, CRC Press Inc. 233-250.
- Nybom, H. and Schaal, B.A. 1990. DNA 'fingerprints' applied to paternity analysis in apples (*Malus x domestica*). *Theor. Appl. Genet.* 79: 763-768.
- Ocampo, C., Hershey, C., Inglesias, C. and Iwanaga, M. 1993. Esterase isozyme fingerprinting of the cassava germplasm collection held at CIAT. In *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, 25-28 August 1992*. Roca, W.M. and Thro, A.M. (eds.), CIAT, Cali, Colombia. CIAT. 81-92.
- Pääbo, S. and Wilson, A.C. 1991. Miocene DNA sequences - a dream come true? *Curr. Biol.* 1: 45-46.
- Peacock, W.J. 1989. Molecular biology and genetic resources. In *The Use of Plant Genetic Resources*. Brown, A.H.D., Frankel, O.H., Marshall, D.R. and Williams, J.T. (eds.) Cambridge, Cambridge University Press. 363-376.
- Price, S. 1968. Cytology of Chinese and North Indian sugarcane. *Econ. Bot.* 22: 155-164.
- Ramanatha Rao, V. 1991. Problems and methodologies for management and retention of genetic diversity in germplasm collections. In *Proceedings of ATSAF/IBPGR Workshop on Conservation of Plant Genetic Resources*. Becker, B. (ed.), Bonn. ATSAF/IBPGR. 61-68.
- Ramanatha Rao, V. 1992. Genetic diversity and conservation and utilization of plant genetic resources. Keynote address presented at the Gregor Johann Mendel Birthday Lecture Series and Symposium. Cochin
- Rao, R. and Grandillo, S. 1992. Genotype identification and gen isolation. In *Biotechnology: enhancing research on tropical crops in Africa*. Thottapilly, G., Monti, L.M., Mohan Raj, D.R. and Moore, A.W. (eds.) Ibadan, CTA/IITA. 261-265.
- Rao, V.R. 1980. Groundnut Genetic Resources at ICRISAT. In *Proceedings of the International Workshop on Groundnuts, 13-17 October, 1980*. ICRISAT, Patancheru, 502324, A.P. India. ICRISAT. 47-57.
- Redenbaugh, K. (ed.), 1993. *Synseeds: Applications of Synthetic Seeds to Crop Improvement*. London, UK, CRC Press.
- Reuveni, O. and Israeli, Y. 1990. Measures to reduce somaclonal variation in *in vitro* propagated bananas. *Acta Horticulturae*. 275: 307-313.
- Rick, C.M. and Holle, M. 1990. Andean *Lycopersicon esculentum var cerasiforme*: genetic variation and its evolutionary significance. *Econ. Bot.* 44: 69-78.
- Rieseberg, L.H., Carter, R. and Zona, S. 1990. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). *Evolution*. 44: 1498-1511.
- Rieseberg, L.H. and Seiler, G.J. 1990. Molecular evidence and the origin and development of the domesticated sunflower (*Helianthus annuus*, Asteraceae). *Econ. Bot.* 44: 79-91.
- Riggs, L.A. 1990. Conserving genetic resources on-site in forest ecosystems. *Forest Ecology and Management*. 35: 45-68.
- Rillo, E.P. 1989. A nondestructive technique for collecting immature coconut inflorescence for tissue culture. *Philippine J. Coconut Studies*. 14: 16-17.
- Roberts, E.H. and King, M.W. 1986. Storage of recalcitrant seeds. In *Crop Genetic Resources - The Conservation of Difficult Material*. Withers, L.A. and Williams, J.T. (eds.) Paris, IUBS/IGF/IBPGR. 39-48.
- Roy, A., Frascaria, N., MacKay, J. and Bousquet, J. 1992. Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. *Theor. Appl. Genet.* 85: 173-180.
- Ruredzo, T.J. 1991. A minimum facility method for *in vitro* collection of *Digitaria eriantha* ssp. *pentzii* and *Cynodon dactylon*. *Tropical Grasslands*. 25: 56-63.
- Saghai-Marouf, M.A., Allard, R.W. and Qiang, Q. 1990. Genetic diversity and ecogeographical differentiation among ribosomal DNA alleles in wild and cultivated barley. *Proc. Natl. Acad. Sci. USA*. 87: 8486-8490.
- Schulthies, J.R., Cantliffe, D.J. and Chee, R.P. 1990. Optimizing sweet potato (*Ipomoea batatas* L.) root and plantlet formation by selection and proper embryo developmental stage and size, and gel type for fluidized slowing. *Plant Cell Reports*. 9: 356-359.
- Senaratna, T. and McKersey, B.D. 1989. Artificial seeds for germplasm preservation, exchange and crop improvement. *Diversity*. 2/3: 44.
- Shattuck-Eidens, D.M., Bell, R.N., Mitchell, J.T. and McWhorther, V.C. 1992. Rapid detection of maize DNA sequence variation. *GATA*. 8: 240-245.
- Shukla, D.D. and Ward, C. 1989. Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. *Adv. Virus Res.* 36: 273-314.
- Simmonds, N.W. 1976. Sugarcane. In *Evolution of Crop Plants*. Simmonds, N.W. (ed.) London, Longman. 104-108.
- Simpson, M.J.A. and Withers, L.A. 1986. Characterization of Plant Genetic Resources Using Isozyme Electrophoresis: A Guide to the Literature. Rome, IBPGR.
- Singh, S.P., Gutierrez, J.A., Molina, A. and Gepts, P. 1991. Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. *Crop Sci.* 31: 23-29.
- Singleton, L.L., Mihail, J.D. and Rush, C.M. (eds.), 1993. *Methods for Research on Soilborne Phytopathogenic fungi*. St. Paul, USA, APS Press.
- Skoric, D. 1993. Wild species use in sunflower breeding - results and future directions. *FAO/IBPGR PGR NL*. 93: 17-23.
- Sobral, B.W.S., Al-Janabi, S.M., McClelland, M. and Honeycutt, R.J. 1993. Novel approaches to molecular mapping and fingerprinting. In *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Roca, W.M. and Thro, A.M. (eds.) Cartagena, Colombia, 25-28 August 1992*. Cali, Colombia, CIAT. 31-46.
- Spiegel, S., Frison, E.A. and Converse, R.H. 1993. Recent developments in therapy and virus detection procedures for international

- movement of clonal plant germplasm. *Plant Dis.* 77: 1176-1180.
- Spooner, D.M., Anderson, G.J. and Jansen, R.K. 1990. Chloroplast DNA phylogeny of tomatoes, potatoes and pepinos (*Solanum* subgenus *Potatoe*). *Am. J. Bot.* 77: 156(ab).
- Spooner, D.M., Anderson, G.J. and Jansen, R.K. 1993. Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). *Am. J. Bot.* 80: 676-688.
- Spooner, D.M., Douches, D.S. and Andrés Contreras, M. 1992. Allozyme variation within *Solanum* Sect. *Petota*, Ser. *Etuberosa* (Solanaceae). *Am. J. Bot.* 79: 467-471.
- Spooner, D.M. and Sytsma, K.J. 1992. Reexamination of series relationships of Mexican and Central American wild potatoes (*Solanum* sec. *Petota*): Evidence from chloroplast DNA Restriction site variation. *Systematic Bot.* 17: 432-448.
- Srivastava, S. and Lavania, U.C. 1991. Evolutionary DNA variation in *Papaver*. *Genome.* 37: 763-768.
- Stanwood, P.C. 1980. Tolerance of crop seeds to cooling and storage in liquid nitrogen (-196 C). *J. Seed Technol.* 5: 26-31.
- Stebbins, G.L. 1957. The hybrid origin of microspecies in the *Elymus glaucus* complex. *Cytologia. Suppl. Vol.:* 336-340.
- Strauss, S.H., Bousquet, J., Hiplins, V.D. and Hong, Y.-P. 1992. Biochemical and molecular genetic markers in biosystematic studies of forest trees. *New Forests.* 6: 125-1258.
- Sudha, C.G. and Seeni, S. 1993. In vitro multiplication and field establishment of *Adhatoda beddomei* C.B. Clarke, a rare medicinal plant. *Plant Cell Reports.* 13: 203-207.
- Swennen, R. 1990. Limits of morphotaxonomy: Names and synonyms of plantain in Africa and elsewhere. in Identification of genetic diversity in the genus *Musa*. Jarret, R.L. (ed.) Montpellier, INIBAP. 172-210.
- Sytsma, K.J., Smith, J.F. and Berry, P.E. 1991. The use of chloroplast DNA to assess biogeography and evolution of morphology, breeding systems, and flavonoids in *Fuchsia* sect. *Skinnera* (Onagraceae). *Systematic Bot.* 16: 257-269.
- Talbert, L.E., Magyar, G.M., Lavin, M., Blake, T.K. and Moylan, S.L. 1991. Molecular evidence for the origin of the S-derived genomes of polyploid *Triticum* species. *Am. J. Bot.* 78: 340-349.
- Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: new tools for an old science. *Bio/Technol.* 7: 257-264.
- Tao, Y., Manners, J.M., Ludlow, M.M. and Henzell, R.G. 1993. DNA polymorphism in grain sorghum (*Sorghum bicolor* (L.) Moench). *Theor. Appl. Genet.* 86: 679-688.
- Templeton, A.R., Shaaw, K., Routman, E. and Davis, S.K. 1990. The genetic consequences of habitat fragmentation. *Ann. Missouri Bot. Gard.* 77: 13-27.
- Terauchi, R., Chikaleke, V.A., Thottapilly, G. and Hahn, S.K. 1992. Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear ribosomal DNA. *Theor. Appl. Genet.* 83: 743-751.
- Tsuji, K., Nagaoka, M. and Oda, M. 1993. Encapsulation of somatic embryos of carrot and promotive effects of the growth of plantlets *in vitro*. *JARQ.* 27: 116-121.
- Uragami, A., Skai, A. and Nagai, M. 1993. Cryopreservation of asparagus (*Asparagus officinalis* L.) cultured *in vitro*. *JARQ.* 27: 112-115.
- Vaillancourt, R.E., Weeden, N.F. and Barnard, J. 1993. Isozyme diversity in the cowpea species complex. *Crop Sci.* 33: 606-613.
- Vavilov, N.I. 1951. The origin, variation, immunity and breeding of cultivated plants (Translated by K. Starr Chester). New York, The Ronald Press Co.
- Vierling, R.A., Xiang, Z., Joshi, C.P., Gilbert, M.L. and Nguyen, H.T. 1994. Genetic diversity among elite *Sorghum* lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theor. Appl. Genet.* 87: 816-820.
- von Vornam, R. 1993. Genmarker als Entscheidungshilfen für die genkonservierung II. RFLPs als genmarker für die ausweisung forstlicher genressourcen. *Allg. Forst-u. J.* 165: 186-190.
- Vuylsteke, D. and Swennen, R. 1990. Somaclonal variation in African plantains. *IITA Research.* 1: 4-10.
- Waithaka, K. 1992. Micropropagation techniques and the production of pathogen-free plants. in *Biotechnology: enhancing research on tropical crops in Africa*. Thottapilly, G., Monti, L.M., Mohan Raj, D.R. and Moore, A.W. (eds.) Ibadan, CTA/IITA. 183-188.
- Walker, D.I.T. 1987. Manipulating the genetic base in sugarcane. in International Sugarcane Breeding Workshop. Sao Paulo, Brazil. Copersucar. 321-334.
- Weising, K., Fiala, B., Ramloch, K., Kahl, G. and Epplen, J.T. 1990. Oligonucleotide fingerprinting in angiosperms. *Fingerprint News.* 2: 5-8.
- Weising, K., Ramser, D., Kaemmer, D., Kahl, G. and Epplen, J.T. 1991. Oligonucleotide fingerprinting in plants and fungi. in *DNA Fingerprinting: Approaches and Applications*. Burke, T., Dolf, G., Jeffreys, A.J. and Wolff, R. (eds.) Basel, Switzerland, Birkhauser Verlag.
- Welsh, J., Peterson, C. and McClelland, M. 1990. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. *Nucl. Acids Res.* 19: 303-306.
- West, D.P., Cramer, J.M., Romer-Sverson, J., Ma, Y. and Murray, M. 1989. Application of restriction fragment length polymorphism to plant breeding. in *Strengthening Collaboration in Biotechnology*. Cohen, J.L. (ed.) Washington D.C., BST-AID. 175-184.
- Wilkie, S.E., Issac, P.G. and Slater, R.J. 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. *Theor. Appl. Genet.* 86: 497-504.
- Wilson, H.D., Doebley, J. and Duvall, M. 1992. Chloroplast DNA diversity among wild and cultivated members of *Cucurbita* (Cucurbitaceae). *Theor. Appl. Genet.* 84: 859-865.
- Withers, L.A. 1978. Freeze-preservation of cultured cells and tissues. in *Frontiers of Plant Tissue Culture 1978*, Proceedings of the 4th International Congress of Plant Tissue and Cell Culture. Thorpe, T.A. (ed.), University of Calgary, Alberta, Canada 20-25 August 1978. International Association for Plant Tissue Culture. 297-306.
- Withers, L.A. 1980. Low temperature storage of plant tissue cultures. in *Advances in Biochemical Engineering: Plant Cell Cultures II*. Fiechter, A. (ed.) Berlin, Springer-Verlag. 101-150.
- Withers, L.A. 1990. Cryopreservation of plant cells. *Biol. J. Linnean Society.* 43: 31-42.
- Withers, L.A. 1991a. Biotechnology and plant genetic resources conservation. in *Plant Genetic Resources Conservation and Management - Concepts and Approaches*. Paroda, R.S. and Arora, R.K. (eds.) New Delhi, IBPGR-ROSSEA. 273-297.
- Withers, L.A. 1991b. Crop strategies for roots and tubers: Potato - A model for refinement, Yam - a problem for development. in *ATSAF/IBPGR Workshop on Conservation of Plant Genetic Resources*. Becker, B. (ed.), Bonn. ATSAF/IBPGR. 11-14.
- Withers, L.A. 1994. New Technologies for the conservation of plant genetic resources. in *Proceedings of the International Crop Science Congress*. Ames, USA. Crop Science Society of America. 429-435.
- Withers, L.A. and Engels, J.M.M. 1990. The test tube genebank - a safe alternative to field conservation. *IBPGR NL for Asia and the Pacific.* 3: 1-2.
- Yidana, J.A., Withers, L.A. and Ivins, J.D. 1987. Development of a simple method for collecting and propagating cocoa germplasm *in vitro*. *Acta Horticulturae.* 212: 95-98.
- Zamir, D. and Tanksley, S.D. 1988. Tomato genome is comprised largely of fast-evolving, low copy-number sequences. *Mol. Gen. Genet.* 213: 254-261.
- Zhang, Q., Saghai-Marooof, M.A. and Kleinbaf, A. 1993. Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hodeum vulgare* spp. *spontaneum*. *Genetics.* 134: 909-916.
- Zohary, D. 1970. Centres of diversity and centres of origin. in *Genetic Resources of Plants*. Frankel, O.H. and Bennet, E. (eds.) Oxford, Blackwell. 33-42.

### Résumé

*Le rôle des biotechnologies pour la conservation et l'utilisation des ressources génétiques végétales*

Les ressources génétiques végétales incluent des génotypes et des populations de cultivars (races locales, cultivars avancés/améliorés), des stocks génétiques, des espèces sauvages et des mauvaises herbes, qui sont maintenus sous la forme de plantes, semences, tissus, etc. La grande abondance de la diversité génétique disponible actuellement renferme un vaste potentiel. Cependant, les ressources génétiques ne sont pas renouvelables et sont parmi les plus essentielles des ressources naturelles mondiales. Il est essentiel qu'elles soient bien conservées, que ce soit au niveau de l'espèce, du pool génique ou de l'écosystème, pour leur utilisation par les générations présentes et futures. Le terme biotechnologie a été utilisé pour inclure une grande variété de manipulations biologiques telles que la culture de cellules, tissus et organes, le sauvetage d'embryons, le génie génétique incluant le transfert d'ADN à travers les barrières reproductives, la production de vaccins, etc. Dans le contexte des ressources génétiques végétales, ce terme sous-entend une panoplie de techniques permettant de manipuler le germplasm. Dans ce domaine, la biotechnologie a le potentiel d'améliorer l'acquisition et l'échange du germplasm, sa conservation, son évaluation et son amélioration génétique. L'Institut International des Ressources Phytogénétiques (IPGRI) encourage l'utilisation des outils biotechnologiques en vue d'une conservation et d'une utilisation plus efficaces des ressources génétiques végétales. Cet article passe en revue l'utilisation de la biotechnologie dans ce contexte et en présente les progrès et perspectives.

### Resumen

*El empleo de la biotecnología para la conservación y utilización de los recursos fitogenéticos*

Los recursos fitogenéticos constituyen los genotipos o poblaciones de cultivares (cultivos criollos, cultivares superiores/mejorados), estirpes genéticas, especies silvestres y herbáceas, que se mantienen en forma de plantas, semillas, tejidos, etc. La grande riqueza de diversidad genética actualmente disponible encierra potencialidades inmensas. Sin embargo, los recursos genéticos no son renovables y figuran entre los recursos naturales más importantes del mundo. Es esencial que éstos se conserven, sea a nivel de especies, acervo génico o ecosistema, para el uso de las generaciones actuales y futuras. El término biotecnología se ha empleado para incluir una amplia variedad de manipulaciones biológicas como los cultivos de células, tejidos y órganos, el salvamento de embriones, la ingeniería genética, incluida la transferencia de DNA a través de barreras sexuales, la producción de vacunas, etc. En el contexto de los recursos fitogenéticos, la biotecnología entraña por lo general una serie de técnicas que proporcionan nuevas formas de manipulación del germoplasma. En este terreno, la biotecnología puede contribuir a perfeccionar la recolección y el intercambio seguro de germoplasma, la conservación, la evaluación y el mejoramiento genético. El Instituto Internacional de Recursos Fitogenéticos (IPGRI) promueve el empleo de herramientas biotecnológicas para una conservación y utilización más eficaz del germoplasma. En este artículo se analiza el empleo de la biotecnología en este terreno y se examinan los progresos y perspectivas.

## Viability of cereal seeds stored as germplasm in the Polish Genebank

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### Summary

Germinability of cereal seeds, a ratio of ungerminated seeds and percentage of abnormal seedlings were studied on 6395 accessions stored in the Polish Genebank. Some estimated accessions were freshly introduced into the storage and others were stored for 1-10 years. The results include average germinability, ungerminated seed ratio and abnormal seedling percentage for particular species, frequency of different ranges of germination after any time of storage and correlation between germinability or ungerminating seed ratio or abnormal seedling percentage and time of storage. It was found that the system of seed preparation and seed storage used by the Polish Genebank results in a suitable viability of stored seeds for up to 5 or 6 years of storing. After that period, a decreasing level of germinability and an increasing level of ungerminated seeds and abnormal seedlings was found. The best storing was found to be for spring and winter barley, winter common wheat and rye. Studied parameters can be used to estimate the condition of stored seeds and to foresee the trends of viability changes.

### Introduction

The maintenance of seed viability is a basic condition for successful preservation of seeds stored as germplasm. There are two kinds of problems in germplasm conservation which have to be solved. The first kind refers to engineering aspects and these problems will not be discussed here. This study has focused on the second group of problems, which is related to seed nature.

The storage of seeds, treated as a sowing material, has always accompanied agricultural activity and soon it started to address the question of seed storability. Among the many works related to the behaviour of seeds during storage and papers commenting on the preparation of seeds for storage, are some studies undertaken by scientists grouped around the unit in Wrocław (Litynski *et al.*, 1960).

Three well-known factors that have a significant influence on the longevity of stored seeds are: the preliminary germinability of seeds destined for storage, their moisture content, and the relationship between the moisture content of seeds and the relative humidity of air and temperature in the storage area. Some of them can be regulated relatively easily. It is also possible to influence the germinability of stored seeds by selecting the best-quality seeds at the beginning. From this point, it is recommended that the germination level of seeds put into storage should not be lower than 90%. Moreover, all seeds should be of a similar size, the same maturation degree and free from pests, fungi and organic residues. Widely accepted limits, recommended by IPGRI for genebanks, specify the germination level of stored materials as more than 80% (Cromarty *et al.*, 1982).

All those indicating lower results should be regenerated. A separate problem is how to preserve the seeds against humidity absorption and the selection of container materials which are neutral for seeds (Cromarty *et al.*, 1982).

The preservation of seeds in the Polish Genebank was started in 1981 in cooperation with IBPGR. In the years 1981-1990, 29 384 accessions belonging to about 80 species have been stored. Cereals are the main group and 18 446 accessions belong to this group - 62.5% of all collected taxons (Górski, 1991). The evaluation of their viability before storage and the monitoring of them during storage (especially after some years) are very important if we are going to maintain viable seeds. First, it is important to collect information about the quality of seeds destined for storage - high level of germinability - before putting them into the chambers. Second, it is necessary to monitor the stored seed viability in order to receive information about seed behaviour during storage. Even those seeds stored under controlled conditions that are suitable for prolonging their life period, may decrease their viability as a result of deterioration processes. There have been a number of studies showing that these deterioration processes include such phenomena as: an increase of the free radical content, changes in protein structure, depletion of food reserves, development of fat acidity, changes in enzymatic activity, membrane damage, chromosomal changes and an increase of respiration (Justice and Bass, 1979). The different degrees of power in these processes can manifest themselves in many ways; from the changes registered only by using very sensitive biochemical and physiological

tests, through the decreasing of germinability and production of numerous abnormal seedlings, up to complete loss of viability. By checking viability indicators such as germination capacity, the ratio of abnormal seedlings and ungerminated seeds, the genebank can obtain knowledge about the viability of stored materials and the deterioration processes appearing in seeds. This enables decisions to be made about the genebank strategy.

The aim of this study is to present a characterization of the behaviour of cereal seeds stored in the Polish Genebank and to examine the changes in their viability. An important factor is knowing after how long there is an increased risk that the viability level decreases below the recommended values.

## Materials

All seeds studied were supplied by the seed storage of the Polish Genebank. Seed samples were stored within the years 1981-1990. They belong to the different cultivars of cereals, newly registered as well as older, which are valuable characters for breeders in their work (*Avena* Catalogue, Catalogue of *Secale* spp., European Barley Catalogue, Catalogue of Wheat). The following seeds were studied:

- winter common wheat	-	2321	accessions
- spring common wheat	-	942	
- hard wheat	-	464	
- rye	-	100	
- winter barley	-	738	
- spring barley	-	309	
- oat	-	142	
- winter triticale	-	844	
- spring triticale	-	535	

The results of the evaluations of 6395 accessions have been used as the basis for this study.

## Methods

### *Preparation of seeds for storage*

After harvesting, the seeds are dried in a stream of air at a temperature of 25°C until their moisture content is 5-7%

(usually 1 or 2 weeks). The whole drying process occurs in specially prepared dryers. Next, the moisture content of each sample is estimated and a small part of the sample is separated in order to evaluate the germination of seeds. The rest of the sample is packed into containers (glass jars) which are vacuum sealed. Next, the containers are moved into the storage chamber at a temperature of -18°C. Closing the containers under vacuum protects the seeds against humidity.

### *Evaluation of seed viability*

The seed samples were evaluated in the laboratory according to the recommendations described in the Polish Rules PN-79/R-65950 and the ISTA Rules. As a medium for germination, a filter paper (RB) was used with prechilling (3 days at a temperature of 10°C) as the preliminary treatment. Seeds were germinated at a temperature of 20°C for 8 days. The germination capacity, ratio of dead and ungerminated seeds and abnormal seedlings were evaluated. Some of the accessions studied had been evaluated before they were introduced into storage and others were monitored after storage for various lengths of time. Because the differences between varieties were not the subject of interest to the authors, in the range of each plant different accessions were taken to study each year. The main task of seed germinability estimation has been the evaluation of their ability to be stored and their behaviour during long-term storage so the number of accessions studied varied. Not only the number of accessions for the plant which was used to study differed in each case, but in the range of the plant the different number of accessions was studied in the different years.

### *The result elaboration*

The results were registered with a computer using DBIII Plus. All results were ordered, checked and prepared for preliminary statistical counting using programmes based on DBIII Plus. These programmes were created by the second author. All statistical calculations (basic statistics, frequencies and correlations) were done with the MSTAT packet. with lower levels of germinability is not too high. The

Table 1. Germinability, ungerminated seed ratio and abnormal seedling ratio for each group of plants

Group of plants	Germinability		Ungerminated seed ratio		Abnormal seedling ratio	
	Average	S.D.	Average	S.D.	Average	S.D.
Winter common wheat	90.96	13.31	6.58	11.19	2.36	4.00
Spring common wheat	83.19	13.90	12.76	12.54	4.09	3.94
Hard wheat	77.32	12.09	16.00	11.84	7.11	5.33
Rye	87.66	13.30	9.18	10.33	3.09	4.48
Winter barley	98.07	5.38	1.37	4.31	0.57	1.64
Spring barley	93.03	8.66	4.81	5.22	2.13	5.40
Oat	88.25	7.93	9.87	7.33	1.88	2.19
Winter triticale	87.27	7.49	9.27	5.99	3.39	2.88
Spring triticale	84.13	10.64	13.10	9.81	2.77	2.17

Table 2. Number of the accessions (frequency) in each group of plants for the germination ranges after storage for various times

## a) Winter common wheat

Germination range [%]	Time of storage [years]										
	0	1	2	3	4	5	6	7	8	9	10
0	2	0	0	-	-	-	-	0	0	0	0
1 - 10	2	0	0	-	-	-	-	0	0	2	0
11 - 20	6	0	0	-	-	-	-	0	1	2	0
21 - 30	12	0	0	-	-	-	-	0	0	9	0
31 - 40	7	0	0	-	-	-	-	0	2	6	0
41 - 50	10	0	0	-	-	-	-	0	3	4	0
51 - 60	10	0	0	-	-	-	-	2	4	7	0
61 - 70	26	0	0	-	-	-	-	11	7	11	0
71 - 80	55	3	0	-	-	-	-	6	13	23	0
81 - 90	236	1	0	-	-	-	-	44	12	44	1
91 - 100	1656	6	1	-	-	-	-	27	17	27	3
Number of accessions	2022	10	1	-	-	-	-	90	59	135	4

## b) Spring common wheat

Germination range [%]	Time of storage [years]										
	0	1	2	3	4	5	6	7	8	9	10
0	-	-	-	-	-	-	-	-	-	-	-
1 - 10	2	0	0	0	-	-	-	0	0	-	0
11 - 20	6	0	0	0	-	-	-	0	0	-	0
21 - 30	8	0	0	0	-	-	-	0	0	-	0
31 - 40	4	0	0	0	-	-	-	0	0	-	0
41 - 50	6	0	0	0	-	-	-	0	0	-	0
51 - 60	22	0	0	2	-	-	-	0	0	-	0
61 - 70	78	0	0	0	-	-	-	2	0	-	0
71 - 80	162	0	0	2	-	-	-	1	0	-	0
81 - 90	306	3	0	0	-	-	-	2	0	-	3
91 - 100	314	11	1	1	-	-	-	2	2	-	6
Number of accessions	908	14	1	5	-	-	-	7	2	-	9

## c) Hard wheat

Germination range [%]	Time of storage [years]										
	0	1	2	3	4	5	6	7	8	9	10
0	-	-	-	-	-	-	-	-	-	-	-
1 - 10	-	-	-	-	-	-	-	-	-	-	-
11 - 20	-	-	-	-	-	-	-	-	-	-	-
21 - 30	-	-	-	-	-	-	-	-	-	-	-
31 - 40	0	1	-	0	0	-	-	-	-	-	-
41 - 50	10	0	-	0	1	-	-	-	-	-	-
51 - 60	30	0	-	0	0	-	-	-	-	-	-
61 - 70	82	0	-	0	6	-	-	-	-	-	-
71 - 80	132	8	-	0	2	-	-	-	-	-	-
81 - 90	89	16	-	2	13	-	-	-	-	-	-
91 - 100	27	17	-	0	28	-	-	-	-	-	-
Number of accessions	370	42	-	2	50	-	-	-	-	-	-

## d) Rye

Germination range [%]	Time of storage [years]										
	0	1	2	3	4	5	6	7	8	9	10
0	-	-	-	-	-	-	-	-	-	-	-
1 - 10	-	-	-	-	-	-	-	-	-	-	-
11 - 20	-	-	-	-	-	-	-	-	-	-	-
21 - 30	0	0	0	0	0	0	0	1	0	1	-
31 - 40	0	0	0	0	0	0	0	2	0	0	-
41 - 50	0	0	0	0	0	0	0	0	0	1	-
51 - 60	-	-	-	-	-	-	-	-	-	-	-
61 - 70	1	0	0	0	0	0	0	0	0	1	-
71 - 80	4	0	0	3	0	0	0	0	0	6	-
81 - 90	16	2	0	7	0	2	0	1	0	10	-
91 - 100	13	6	3	6	2	2	1	1	6	22	-
Number of accessions	34	8	3	16	2	4	1	5	6	41	-

## e) Winter barley

Germination range [%]	Time of storage [years]										
	0	1	2	3	4	5	6	7	8	9	10
0	-	-	-	-	-	-	-	-	-	-	-
1 - 10	-	-	-	-	-	-	-	-	-	-	-
11 - 20	-	-	-	-	-	-	-	-	-	-	-
21 - 30	-	-	-	-	-	-	-	-	-	-	-
31 - 40	0	0	0	0	0	0	-	0	0	0	1
41 - 50	0	0	0	0	0	0	-	0	0	0	2
51 - 60	0	0	0	0	0	0	-	0	2	0	0
61 - 70	0	0	0	0	0	0	-	0	0	0	2
71 - 80	0	0	0	0	0	0	-	0	2	1	2
81 - 90	0	2	2	0	0	0	-	3	1	0	5
91 - 100	86	362	216	36	1	1	-	5	2	0	4
Number of accessions	86	364	218	36	1	1	-	8	7	1	16

## f) Spring barley

Germination range [%]	Time of storage [years]										
	0	1	2	3	4	5	6	7	8	9	10
0	-	-	-	-	-	-	-	-	-	-	-
1 - 10	-	-	-	-	-	-	-	-	-	-	-
11 - 20	-	-	-	-	-	-	-	-	-	-	-
21 - 30	0	0	0	-	0	0	0	0	2	0	0
31 - 40	-	-	-	-	-	-	-	-	-	-	-
41 - 50	-	-	-	-	-	-	-	-	-	-	-
51 - 60	-	-	-	-	-	-	-	-	-	-	-
61 - 70	0	0	0	-	0	0	0	0	0	0	2
71 - 80	2	0	0	-	0	0	0	4	1	1	11
81 - 90	2	1	0	-	0	0	0	20	2	6	18
91 - 100	18	16	1	-	1	2	36	43	9	60	51
Number of accessions	22	17	1	-	1	2	36	67	14	67	82



Table 3. Correlation between years of storage and germinability, years of storage and ratio of ungerminated seeds and years of storage and ratio of abnormal seedlings (Prob. - probability of the rejection of hypothesis that a correlation between variables exists there)

Group of plants	Germinability		Ungerminated seed ratio		Abnormal seedling ratio	
	Correlation	Probability	Correlation	Probability	Correlation	Probability
Winter common wheat	** -0.383	0.000	** 0.339	0.000	** 0.331	0.000
Spring common wheat	* 0.059	0.068	* -0.066	0.043	^ -0.006	1.000
Hard wheat	** 0.330	0.000	** -0.220	0.000	** -0.297	0.000
Rye	* -0.115	0.211	* 0.133	0.148	^ 0.026	1.000
Winter barley	** -0.650	0.000	** 0.628	0.000	** 0.504	0.000
Spring barley	* -0.143	0.012	* 0.184	0.001	^ 0.047	1.000
Oat	^ 0.053	1.000	^ -0.053	1.000	^ -0.024	1.000
Winter triticale	* -0.089	0.009	* 0.074	0.031	* 0.088	0.010
Spring triticale	** -0.144	0.000	** 0.180	0.000	* -0.135	0.001

\*\* =high correlation, \* =low correlation, ^ =no correlation

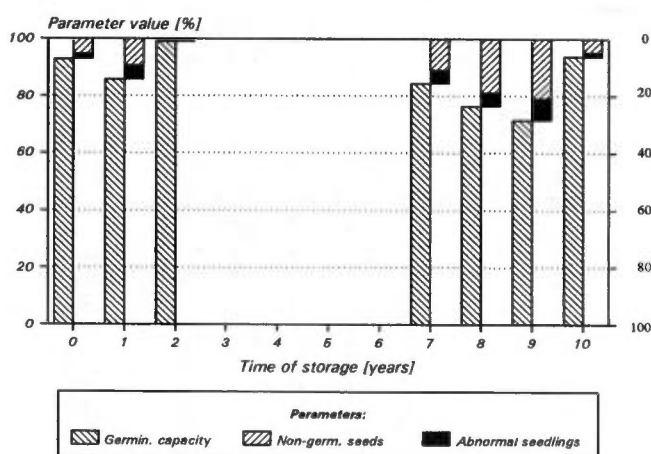


Fig. 1. Winter wheat collection

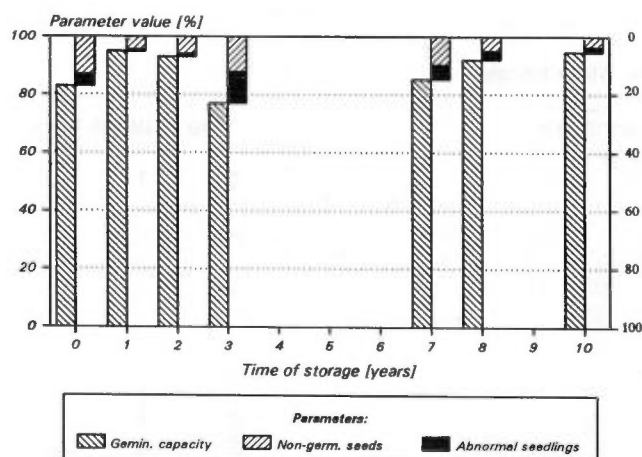


Fig. 2. Spring wheat collection

## Results and discussion

The results of the present studies are given in Tables 1, 2 and 3 and Figures 1 to 9. Data presented were computed as the average of accessions studied in the year. For all studied materials we have found: average germinability: 88.6% (s.d. = 12.6%), average ratio of ungerminated seeds: 8.5% (s.d. = 10.7%) and average ratio of abnormal seedlings: 2.9% (s.d. = 4.0%).

For winter common wheat, average germinability is 90.96%, ungerminated seeds ratio is 6.58% and abnormal seedling ratio is 2.36% (Table 1). An increase was observed in the number of accessions with a germination level lower than 80% for a time of storage longer than 7 years and among the seeds freshly introduced into storage (Table 2).

For spring common wheat, average germinability is 83.19%, ungerminated seed ratio is 12.76% and abnormal seedling ratio is 4.09% (Table 1). It was found for seeds freshly introduced into storage and those stored for 7 years that more than 30% of them show a germination below 80% (Table 2).

For hard wheat, average germinability is 77.32%, ungerminated seed ratio is 16.00% and abnormal seedling ratio is 7.11% (Table 1). More than 60% of the accessions freshly introduced into storage have a germination level lower than 80%. For seeds stored longer than 1 year, this participation decreases radically (Table 2).

For rye, average germinability is 87.66%, ungerminated seed ratio is 9.18% and abnormal seedling ratio is 3.09% (Table 1). Only the seeds stored for 7 and 9 years show an increased ratio of accessions with a germination level lower than 80% (Table 2).

For winter barley, average germinability is 98.07%, ungerminated seed ratio is 1.37% and abnormal seedling ratio is 0.57% (Table 1). A level of germination lower than 80% was found only among the accessions stored longer than 6 years (Table 2).

For spring barley, average germinability is 93.03%, ungerminated seed ratio is 4.81% and abnormal seedling ratio is 2.13% (Table 1). Generally only a few accessions show a germination lower than 80% and such cases are found for almost all periods of storage (Table 2).

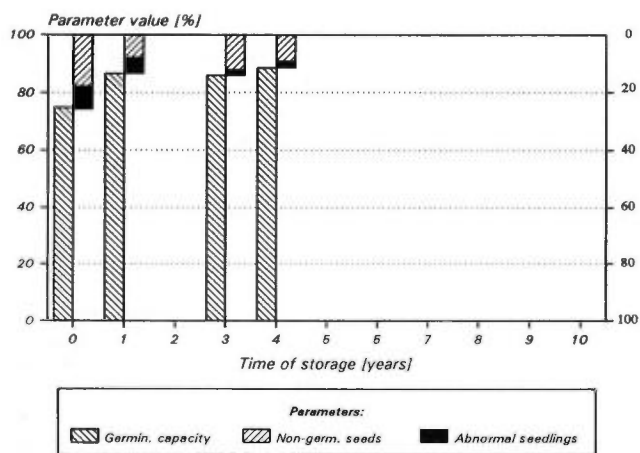


Fig. 3. *Triticum durum* collection

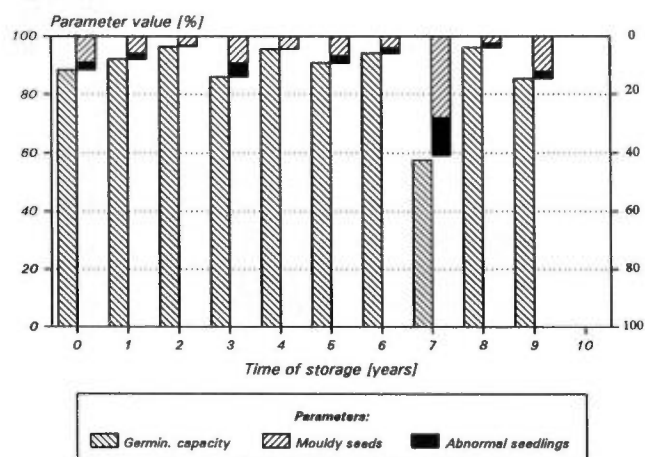


Fig. 4. Rye collection

For oats, average germinability is 88.25%, ungerminated seed ratio is 9.87% and abnormal seedling ratio is 1.88% (Table 1). There is similar situation as in spring barley with the participation of lower germinating seeds. Only after six years of storage do more than 30% of accessions have lower germination (Table 2).

For winter triticale, average germinability is 87.27%, ungerminated seed ratio is 9.27% and abnormal seedling ratio is 3.39% (Table 1). A percentage of accessions with lower germination is not too high (Table 2).

For spring triticale, average germinability is 84.13%, ungerminated seed ratio is 13.10% and abnormal seedling ratio is 2.77% (Table 1). A participation of lower germinating seeds is significant for accessions freshly introduced into storage and stored for one year (Table 2).

These results give a general view of the condition of stored seeds of each group of plants and permit us to compare them with the values recommended for seeds conserved as a germplasm (Cromarty, Ellis, Roberts, 1982). All materials, except hard wheat seeds, store well in the Polish Genebank seed storage conditions. Germinability

of materials such as spring common wheat and spring triticale reached an approximate limit of 80%. Standard deviation values inform us about the possible range of germinability indicating in some events, the need to pay special attention to these seeds. A decreasing germinability level is accompanied by an increase in the ungerminated seed ratio and, from time to time, an increase in the abnormal seedling ratio.

Analysis of the frequency of appearance, calculated as a number of accessions for any range of germinability and any time of storage, gives a wider view of the changes occurring in stored materials (Table 2). Most of the results belong to materials that have been stored for one year - a representation of seeds stored longer is a little weaker. The results illustrate the behaviour of seeds during storage. Their condition is correlated with the larger number of accessions belonging to the highest ranges of germinability. Generally the seeds of all plant groups studied were stored for up to 5 years and were in good condition while the participation of materials

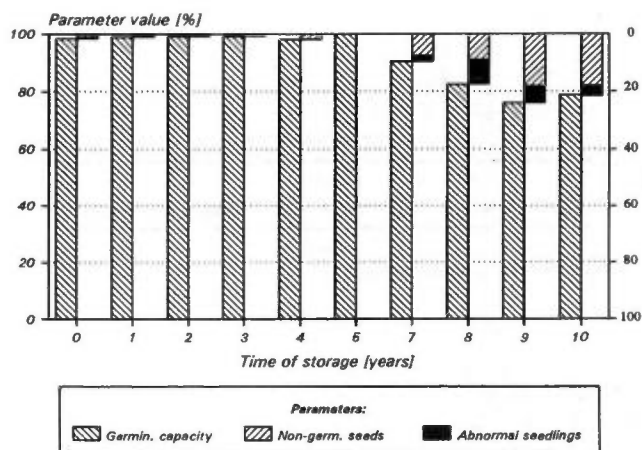


Fig. 5. Winter barley collection

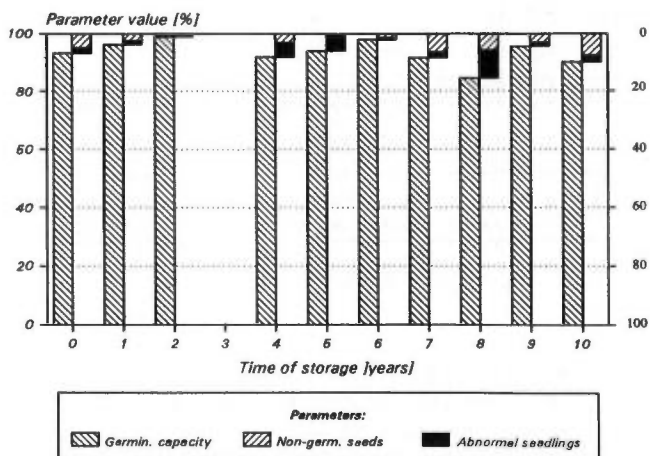


Fig. 6. Spring barley collection

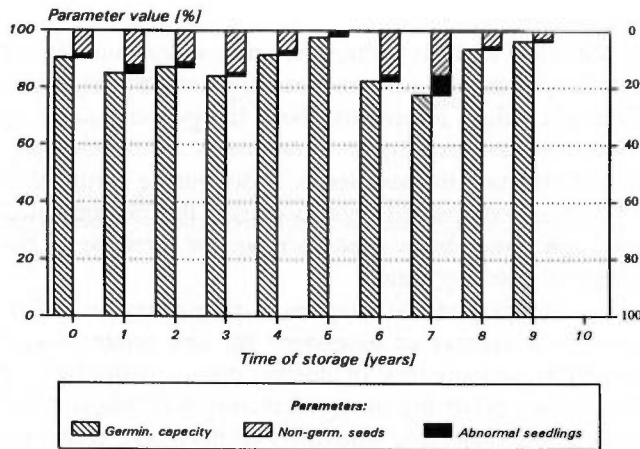


Fig. 7. Oat collection

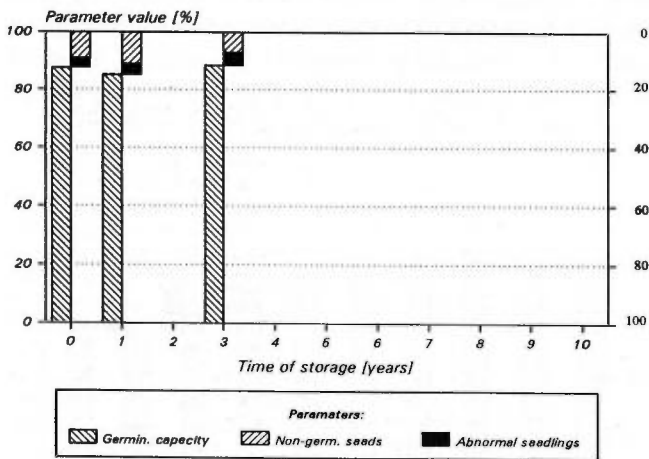


Fig. 8. Winter triticale collection

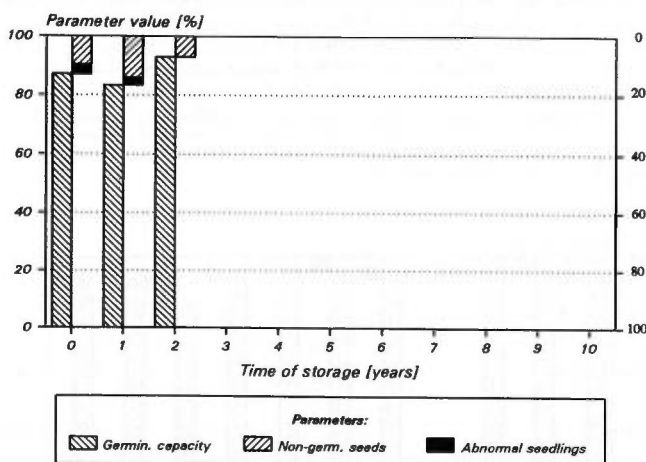


Fig. 9. Spring triticale collection

accessions starting the storage and ones maintained more than 5 years appeared whenever lower levels of germinability but it was not observed in each group of plants. An appearance of accessions with low ranges of germinability decides about the lower average germinability of a whole

group of plants. Of course, an increasing number of accessions for higher ranges of ungerminated seed ratio and abnormal seedling ratio were found in these cases. These results are supported by other ones (Fig. 1 to Fig. 9). There is some information about the viability: average germinability, ungerminated seed ratio and abnormal seedling ratio, after any time of storage. The graphs presented show that the germination level varies depending on the time of storage. Respecting the limit of 80% of germination, it seems that winter and spring barley show the best storability. Generally seeds of accessions stored less than 5-6 years have a satisfactory level of germination.

To make sure that it is possible to treat the changes of germinability, ungerminated seed ratio and abnormal seedling ratio as the indicators of deterioration processes we have counted the correlations between the years of storage and each of the factors given above (Table 3). Generally there is a negative correlation between the time of storage and germinability and a positive correlation between the time of storage and the ungerminated seed ratio. For winter common wheat, spring common wheat, hard wheat, rye, winter and spring barley and winter and spring triticale correlation value is respectively: -0.383, 0.059, 0.330, -0.115, -0.650, -0.143, -0.089, -0.144. The correlation between the time of storage and abnormal seedling ratio is generally lower than the other two correlations.

Present results can be treated as preliminary. We have observed that the studied correlations were lower for those cases where the number of observations was small. The sharpest results have been obtained for winter common wheat and winter barley where the number of observations was the highest. The number of observations for each year of storage differs very much (Table 2). Because of this, results should be treated as preliminary and the study should continue and be extended.

Earlier studies led by Tulo (Annual Reports 1986-1990) show similar results and tendencies as the present study. The best storage was found to be for winter common wheat and barley as well as for oats. The germinability of the accessions put into storage was different depending on the year of introduction. The changes in seed germination dynamics seem to be influenced by the season in which the seeds have been put into storage. As is well known, the germinability of seeds selected for storage is one of the most important factors in determining their lifespan. For this reason, the time the seeds are introduced into storage seems to influence a condition of stored seeds. According to the other studies carried out on field beans (Górecki *et al.*, Annual Reports 1987-1990) the time of the introduction of seeds into storage and the period of storage can influence seed vigour.

A lower level of germinability of seeds freshly introduced into storage seems to be after harvest dormancy, which is broken after a time of storage (generally the number of accessions for lower ranges of germinability has been lower after 1 or 2 years of storage).

## Conclusions

It can be concluded that the method of seed preparation for storage and the conditions met in the Polish Genebank are suitable for seed storage and that seeds can be expected to fulfill IBPGR recommendations for at least a 5-6 year period. After that period, a decrease in the germinability, accompanied by an increasing number of ungerminated seeds and abnormal seedlings, was observed. A lower level of germinability observed in the case of seeds freshly introduced into storage seems to be due to the dormancy.

It seems that factors studied such as germinability, ungerminated seed ratio and abnormal seedlings ratio can be used as good indicators of deterioration processes which take place in stored seeds. It would also be reasonable to organize a system for determining which seeds to test, which would include the careful monitoring of stored accessions and an early reaction to decreases in their viability. The number of tested accessions should be proportional to the number of accessions introduced into storage in any year but not lower than 30 accessions. Tested materials should be chosen randomly but perhaps it would be necessary to direct attention towards materials whose germinability was estimated before being put into storage, to be close to the recommended limit.

Systematic organization of monitoring of stored seeds could not only permit a good reaction to viability loss by seeds, but also it would give an opportunity for estimating such tendencies. Based on the results of the first and following tests, one could estimate the approximated deterioration curves for studied species dependent on the preliminary level of germinability and moisture content.

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## References

- Anonymus. Avena Catalogue of Polish Genebank, 1988. Catalogue of *Secale* spp., 1983. European Barley Catalogue, 1987 Eastern Europe Wheat Catalogue, 1992.
- Cromarty A.S., Ellis R.H., Roberts E.H. 1982. The Design of Seed Storage Facilities for Genetic Conservation, IBPGR Rome 1982.
- Górecki E. *et al.* Annual Reports 1987-1990 of CPBP 05.04
- Górski M., 1991. Plant Genetic Resources Conservation, Polish Genebank Bank Reports 1986-1990, Radzików 1991.
- Justice O.L., Bass L.N. 1979. Principles and Practices of Seed Storage, Castle House Publications Ltd., London 1979.
- Lityski M. *et al.* 1960. Bulletin IHAR 4 (37): 3-42
- Tulo M. Annual Reports 1986-1990 of CPBP 05.04.

## Résumé

*Viabilité de semences de céréales conservées dans la banque de gènes polonaise.*

Le pouvoir germinatif de semences de céréales, la proportion de semences non germées et le pourcentage de plantules anormales ont été étudiés sur 635 accessions stockées dans la banque de gènes polonaise. Certaines accessions évaluées avaient été introduites récemment, et d'autres conservées pendant 1 à 10 ans. Les résultats incluent le pouvoir germinatif moyen, la proportion de semences non germées et le pourcentage de plantules anormales pour des espèces particulières, la fréquence des différentes gammes de germination quelle que soit la durée de stockage, ainsi que la corrélation entre le pouvoir germinatif, la proportion de semences non germées ou le pourcentage de plantules anormales et la durée de stockage. Il a été trouvé que le système de préparation et de stockage des semences utilisé par la banque de gènes polonaise permet une viabilité satisfaisante des semences pour des durées de stockage de 5 à 6 ans. Au delà, une baisse du pouvoir germinatif et une augmentation du niveau de semences non germées et de plantules anormales ont été observées. Le meilleur stockage a été noté dans le cas de l'orge de printemps et d'hiver, du blé commun d'été et du seigle. Les paramètres étudiés peuvent être utilisés pour estimer l'état des semences stockées et prévoir l'évolution de la viabilité.

## Resumen

*El poder germinativo de las semillas de cereales almacenadas como germoplasma en el Banco de genes de Polonia*

Se estudiaron 6395 muestras almacenadas en el Banco de genes de Polonia para determinar la capacidad germinativa de las semillas de cereales, el índice de semillas no germinadas y el porcentaje anormal de plántulas. Algunas muestras analizadas habían sido almacenadas recientemente y otras tenían de uno a 10 años de almacenamiento. Los resultados incluyen la capacidad germinativa media, el índice de semillas no germinadas y el porcentaje de plántulas anormales de especies particulares, la frecuencia de diferentes rangos de germinación según el tiempo de almacenamiento, y la correlación entre la capacidad germinativa o el índice de semillas no germinadas o el porcentaje de plántulas anormales y el tiempo de almacenamiento. Se demostró que con el sistema de preparación y almacenamiento de semillas empleado por el Banco de genes polaco, se obtenía un poder germinativo adecuado en las semillas almacenadas por un período de hasta cinco o seis años. Después de este período, se observó una disminución de la capacidad germinativa y un aumento del nivel de semillas no germinadas y de plántulas anormales. Se comprobó que el mejor almacenamiento correspondía a la cebada de primavera e invierno, al centeno y al trigo común de invierno. Los parámetros estudiados se pueden emplear para estimar el estado de las semillas almacenadas y prever las tendencias a la modificación del poder germinativo.



## Pomegranate (*Punica granatum*) plant genetic resources in Turkmenistan

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### Summary

During the period 1934-1994, a world *Punica granatum* collection was formed in the Turkmenian Experimental Plant Genetic Resources Station (Garrygala, Western Kopetdagh). The collection includes 1117 cultivars and forms. It is replenished in several ways including expeditional studies of wild pomegranate coenopopulations, from centres of old-culture sites in the CIS countries and abroad, and through exchange and ordering from scientific institutions. It has been proved that it is possible to store pomegranate genetic resources by cryopreservation of inbred seeds. The *P. granatum* collection is used to form and replenish analogous collections in the scientific institutions of other countries. The best samples are sent to various nurseries and farms and used in selection works. Concentration of scientific investigation on *P. granatum* in this station and the creation of a Pomegranate International Centre and Genebank as a main point in the crop network are quite realizable.

### Introduction

The Punicaceae family includes two monotypical genera of *Punica* L. and *Socotria* G. Levin. *Socotria protopunica* G. Levin is an endemic of Sokotra island and is represented by a few specimens (Levin, 1980). Wild *punica granatum* is spread from the Balkan Peninsula to northwestern India. The coenopopulations of this species found in western Europe (Switzerland, Italy, Spain, etc.) and China (Yunnan) are usually considered as having run wild, although this requires a special study.

Pomegranate was domesticated 500 to 700 years ago. There are written sources testifying to its cultivation since the Sumerian culture in Mesopotamia. During this period, many cultural and decorative forms have been selected in different countries. *Punica granatum* was widely used among oriental peoples for medicinal purposes. In antique myths and oriental religions, it was mentioned as a sacred plant.

At present, *P. granatum* is grown as a fruit plant in Central Asian and Near and Middle East countries, and to a lesser degree in other subtropical and tropical countries (Table 1). *Punica granatum* takes the 18th place (Levin, 1979) among the main world fruit cultures.

The *P. granatum* genetic resources collection is in the Turkmenian Experimental Station of Plant Genetic Resources of the Academy of Agricultural Sciences of Turkmenistan (TES PGR of the AAST). From 1930 to 1991, it was known as the Turkmenian Experimental Station of the N.I. Vavilov Institute of Plant Industry (TES IPI). It is located in Garrygala (formerly Karakala). The station was initiated in 1934 when the first 16 accessions were admitted. This was expanded to 47 accessions in 1940 and in 1964 to 65, and at present there are 1117 sorts and forms held, i.e. the world collection of pomegranate genetic

Table 1. The natural and cultural pomegranate areas

Floristic regions <sup>1</sup>	Total	No. of regions where the pomegranate grows	
		in nature	in culture
Kingdoms	6	1	6
Regions	34	4	33
Provinces	147	14	114

<sup>1</sup> According to Takhtajan (1978).

resources has increased by 70 times the original number and it has samples from 26 countries on four continents. The number of popular selected cultivars from the CIS countries is 371, from overseas 158, selected cultivars and seedlings 251, soft-seeded 117, dwarf and low-growing 19, decorative 15, and samples from coenopopulations in different parts of the habitat amount to 97. But a large collection in itself is of little importance unless the accessions are studied from the genetic point of view and classified (Swaminathan, 1989). The genetic study of the collection is a vital future requirement, and as far as pomegranate intraspecific classification is concerned, despite multiple attempts, only a few steps have so far been made to our knowledge (Levin, 1991).

The peculiarities of the *P. granatum* collection of the TES PGR are as follows:

1. Of all the collections held by CIS countries, the TES pomegranate collection is situated furthest south. It is

located in a region (southwestern Kopetdagh) where the average annual air temperature is equal to that of the earth surface (16.1°C) and where the relatively subtropical climate creates an effective stimulatory environment, providing an opportunity to study the interaction and resistance shown by the samples held in response to the extreme abiotic factor during field trials.

2. The collection is situated on the northern border of the natural area of wild pomegranate in which marginal coenopopulations in west Kopetdagh (the largest wild population in the central Asia) are monitored on specimens of the *P. granatum* collection and on model plants of wild pomegranate.
3. Our collection contains endangered isolated varieties along with varieties of coenopopulation specimens from different countries.
4. Methods of pomegranate plant breeding are studied using material from the collection
5. Close proximity to the main territory of southwest Turkmenistan where, in the near future, a vast, new region of subtropical plant industry will be created of which 25% will be given over to pomegranate.

### The genepool origin

We have singled out primary, secondary and tertiary megacentres and, within them, the macrocentres of origin

and genetic diversity of the cultivated pomegranate (Levin, 1982). These mega- and macrocentres include all the regions where pomegranate has been cultivated both since prehistoric times and more recently (Table 2).

### Formation of the genepool collection

The main sources of the collection are expeditional studies of the wild (and wild-growing) pomegranate coenopopulations and of the points of its old-culture sites in the CIS countries and abroad, germplasm exchange and ordering from scientific institutes, and buying from commercial firms (Figs. 1 and 2). The individual impact of these differs, but undoubtedly the expeditions provide the main source required for the forming and replenishing of the genepool collection.

During 1962-1992 the author took part in trips to the countries of central Asia and Transcaucasus, and practically all, with little exception, resulted in specimens of pomegranate being returned to the TES PGR collection. Additional collections were obtained from scientific institutes of the Ukraine, Azerbaidjan, Georgia, Armenia, Russia, Turkmenistan, Uzbekistan, Tadjikistan, Kyrgyzstan and Kazakhstan by exchange. The author had the thankless task of witnessing old orchards and pomegranate plants disappear in the different regions during the span of the expedition studies. The wild pomegranate thicket declined and its separate coenopopulations disappeared: an obvious case of erosion of genetic resources.

As far as the mega- and macrocentres of origin and genetic diversity of the pomegranate cultivars are concerned, the heterogeneity shown in these areas is reflected

Table 2. The TES PGR AAST pomegranate genepool collection classification according to the centres of origin and diversity

Megacentres		No. of samples		Macrocentres		No. of samples	
Origin	Name	Quantity	Percent		Quantity	Percent	
Primary	Middle Eastern	1046	93.64	Minor Asian	5	0.45	
				Irano-Afghanian	98	8.77	
				Transcaucasian	291	26.05	
				Soviet Central Asian	650	58.19	
				Indo-Pakistanian	2	0.18	
Secondary	Mediterranean	32	2.87	Near Eastern	2	0.18	
				North African	7	0.63	
				Pyrenean	14	1.25	
				Balkanian	9	0.81	
	East Asian	2	0.18	Chinese	2	0.18	
Tertiary	American	35	3.13	North American	33	2.95	
				Central & South American	2	0.18	
	South African	2	0.18	-	2	0.18	

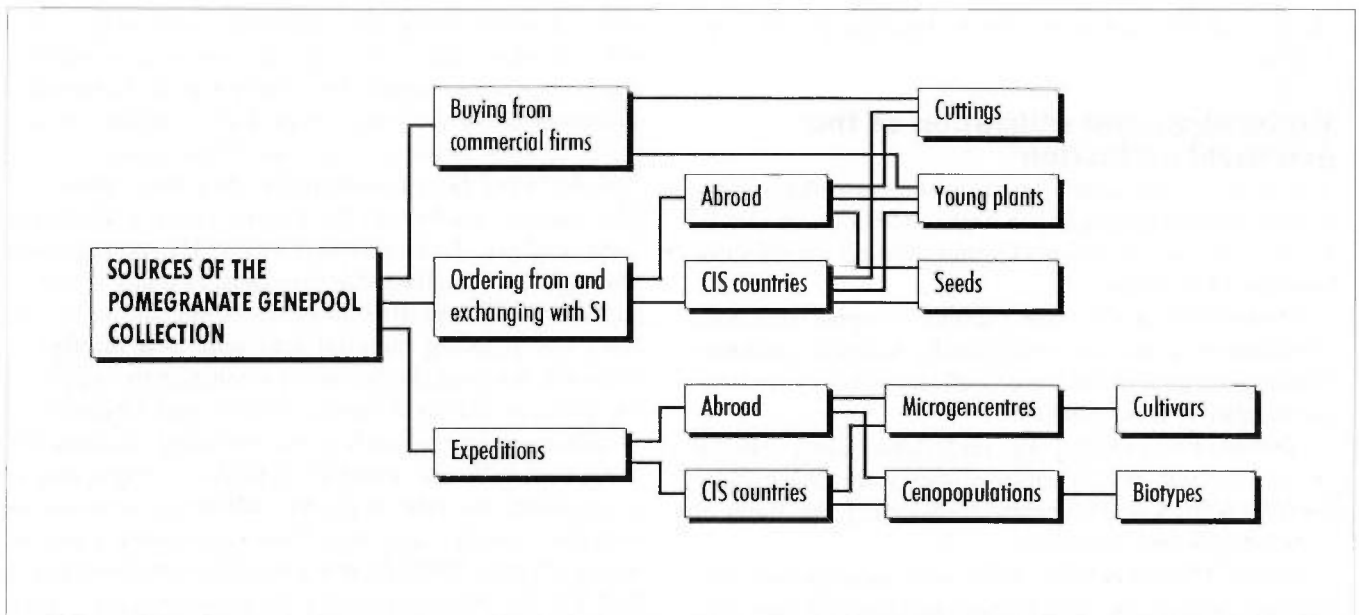


Fig. 1. Sources of the pomegranate gene pool collection

in the TES PGR collection (Table 2). Most of the secondary and tertiary macrocentres such as the Near East, China, Japan, and Central and South America are only symbolically represented by a small number of specimens. There is a need for new expeditions covering the main regions of the present pomegranate culture in these centres to enrich the collection with specimens. A plan of expeditions (TES PGR AAST) has been drawn up for the years 1993-2000 covering countries from the Middle East to China.

### The gene pool classification

We consider the collection of the gene pool of the pomegranate to be a specially assembled experimental population representative of the limits of species variability. To date, there are several systems for classifying these cultivated plant genetic resources (Konarev, 1991). When classifying the genetic resources of the pomegranate as a monotypic genus, indexes such as the level of culturing and selection are used for distinguishing the levels of the gene pool (Fig. 3). Proceeding from the specimen origin, we have singled out wild (autochthonous) populations, wild-growing-autochthonous (run-wild) and allochthonous ones.

Within the scheme the cultivated specimens are represented by fruit (subdivided into domesticated primitive forms, varieties of popular selection and contemporary selection ones) and decorative forms.

### The gene pool conservation

The main point in the classification of the conserved pomegranate gene pool is the division within it in response to the presence or absence of growth stoppage (Fig. 4). The *in situ* conservation of the wild pomegranate gene pool assures its preservation in coenopopulations (and small

groups of plants) within protected territories. There are about 60 reserves within the natural pomegranate area in the CIS countries. Available information shows that this wild species is preserved *in situ* in eight reserves of the Transcaucasus (Basutchaysky, Vashlovansky, Hyrkansky, Dylzhansky, Kyzyl-Agachsky, Pitzunda-Myussersky, Turyanchaisky, Shikikokhsy) and in 3 ones of the Central Asia (Dashtikjumsky [Tadjikistan], Razansaisky [Kyrgyzstan] and Syunt-Khasardaghsky [Turkemenistan]). Single specimens represent the pomegranate in 10 out of 15 botanical gardens of this region. It is necessary to preserve the genetic diversity of the declining pomegranate cultivars in the places of its old culture - the microcentres, which are quite numerous in the countries of the CIS, Middle East and central Asia.

Our experiments have shown the cryopreservation technique to be suitable for preserving pomegranate seeds and pollen. The suitability of pomegranate meristems conserved in this way and *in vitro* conservation of its gene pool as mericlones, callus tissues and cells has yet to be investigated. There has been success (Wilkins *et al.*, 1988) in storing pomegranate shoots at the temperature of +10°C. In our experiments of 1987-1988 pomegranate seedlings were preserved at +5-6°C for a year with a 100% viability (which gives an opportunity to investigate sapling preservation in similar conditions). Our experiments of 1976-1987 have shown that seedlings from pomegranate inbred seeds ( $F_1$ - $F_2$ ) are identical to the original varieties and forms (Levin, 1988). Consequently the inbred pomegranate seeds can also be conserved by the developed cryopreservation technique. Conversely, samples of 20 cultivars of pomegranate seeds obtained from self-pollination have been stored, since 1990, in the cryobank of

the Biological Centre of the RAS in Pushino (the Moscow region).

### Exploration and utilization of the genepool collection

A long-term study of the genetic resources collection has enabled us to distinguish prospective varieties for further variety tests in a commercial variety test on a number of Turkmenistan farms.

Presently over 6% of the collected samples have been identified as sources of selectionally valuable qualities. Further serious study of the collection will help to enlarge the number of these sources.

In 1986-1987 crossing was undertaken using material of the collection and hybrid seedlings were grown. Now we also have found cultivars transferring the traits of soft-seedness and dwarfness.

In the 1970s and 1980s inbreeding, mutagenesis and hybridization of the pomegranate were undertaken, and this will allow us to assemble genetic collections as well as working ones in respect of each selection trait (genetic collections).

For 30 years (1964-1994) the TES PGR collection of the pomegranate genepool has been a resource for found-

ing and replenishing the analogous collections in the CIS and other states, and over 3000 samples of superior and prospective varieties have been sent to 44 scientific institutes of 16 countries (nine CIS countries among them) to form working collections. The cuttings of these varieties have been transferred to five state farms and nine nursery gardens in the Central Asian countries to form original plantations and so on. The pomegranate plant and seed material has been sent to many botanical gardens in CIS countries to be tested for hot-house culture. The planting material was sent to a number of amateurs north of the border of pomegranate culture in the Ukraine, Moldova, Russia, Belarus and Khazakhstan to undergo some research work. Founding working collections in different scientific institutes is also a way of determining the rate of genetic difference between the superior samples acquired from contrasting environments (Popov, 1982). In fact I would be inclined to say that it is the only opportunity for evaluating a considerable number of cultivars.

### The future of the genepool collection

The fortunate siting of the TES PGR pomegranate genepool collection makes it possible to study the collected samples

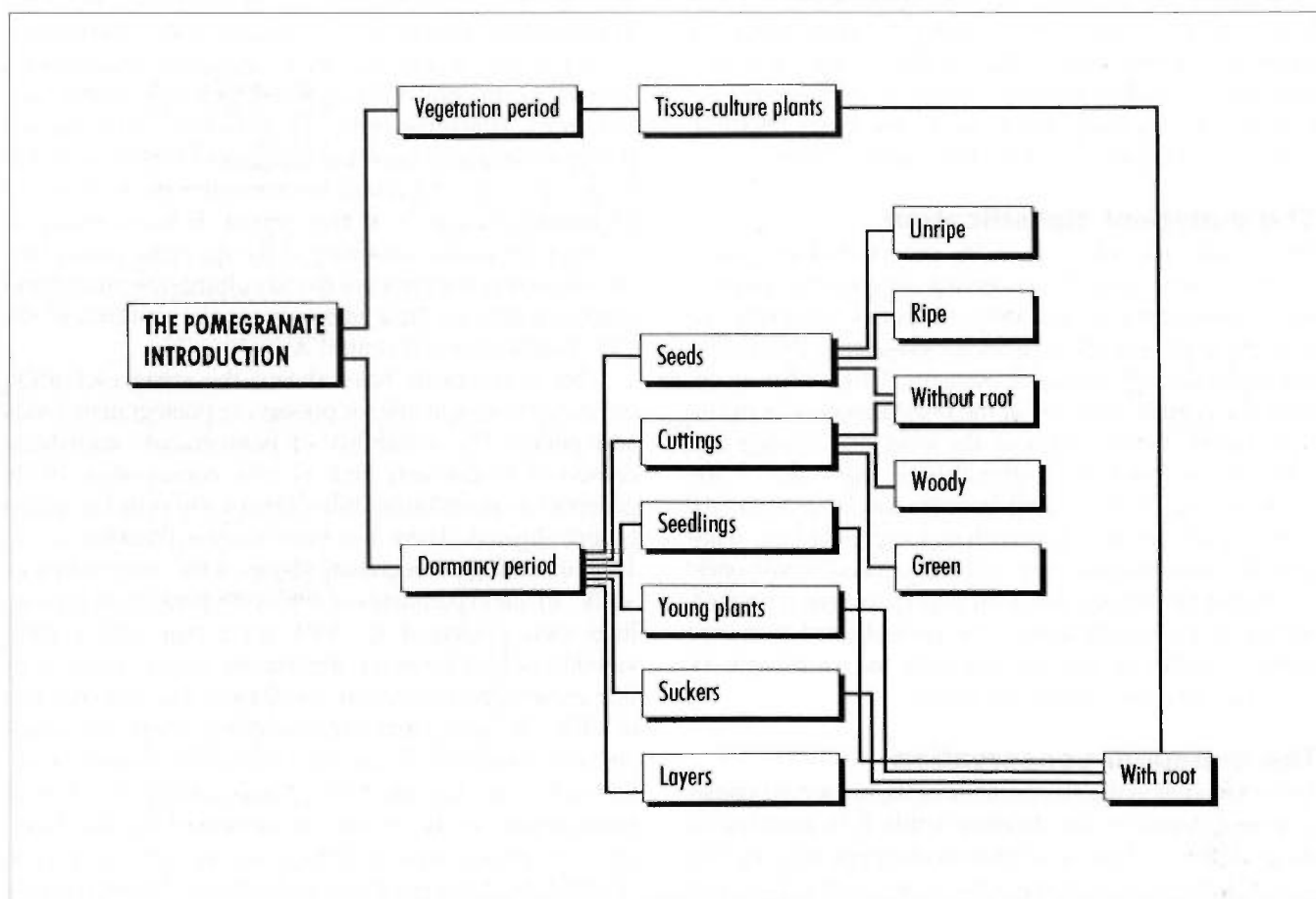


Fig. 2. Methods of pomegranate introduction

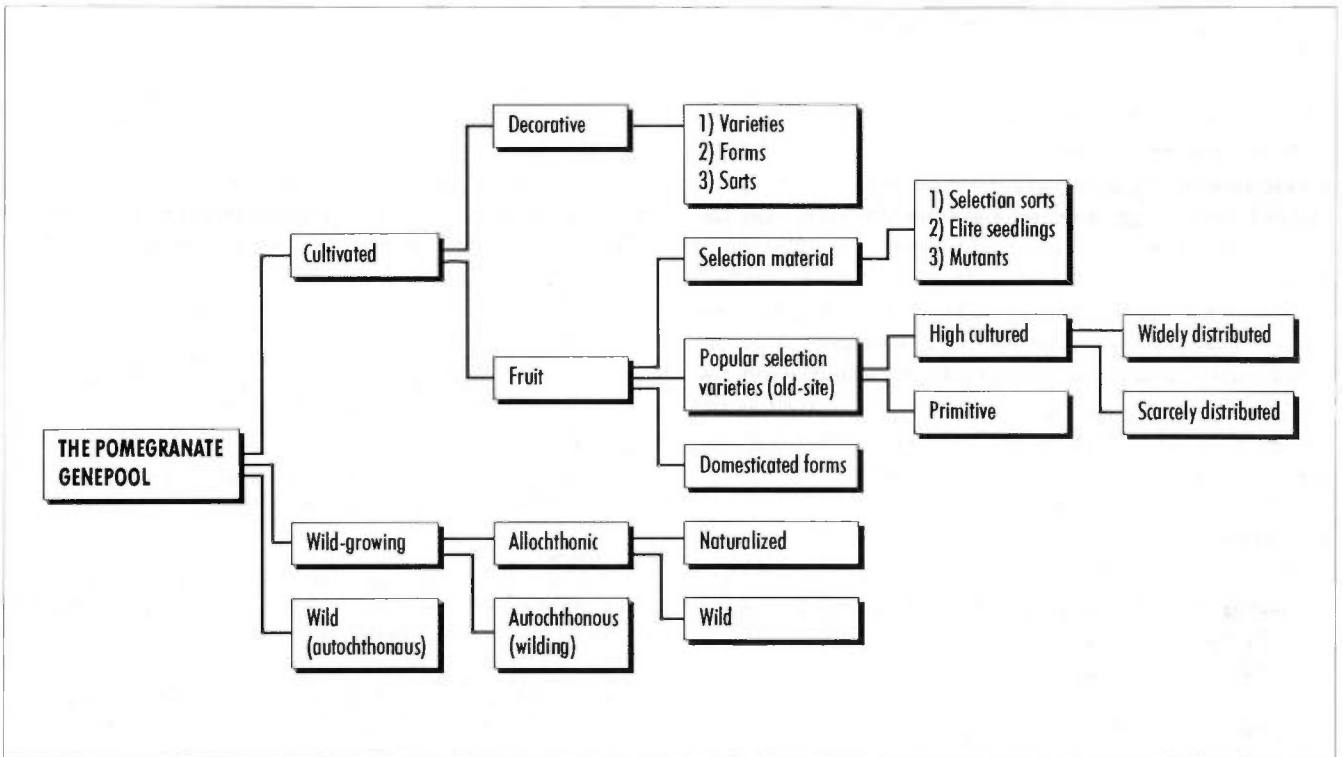


Fig. 3. The pomegranate genepool classification

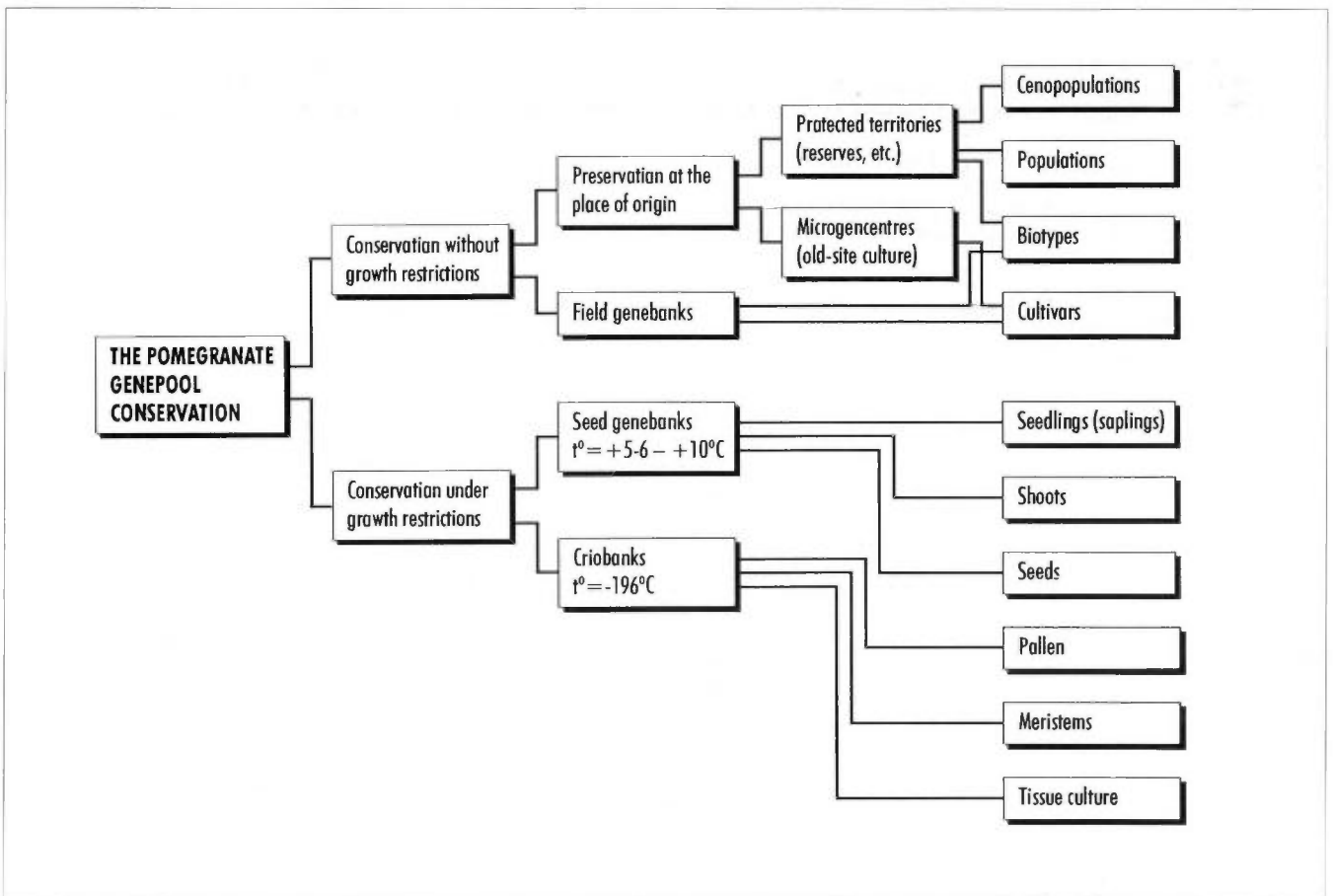


Fig. 4. The pomegranate genepool conservation

in their natural background. A combination of favourable factors such as a vast genepool collection, background and nearby regions of open pomegranate culture industry, provide for the industrial testing of new prospective varieties (the illegal analogue of the state variety test). This combination of factors also highlights the prospects for potential in concentrating the research work here and founding the pomegranate selection centre; this latter point is emphasized by the reduction in pomegranate selection in most of the CIS countries, due to the loss of pomegranate selection expertise. The prerequisites exist for the International Pomegranate Centre to be founded on the basis of the world pomegranate collection, as well as the genebank which will play a fundamental part in the network of specialized institutes of this profile.

## References

- Konarev, V.G. 1991. Genetic resources of selection: modern strategy of their exploration (rus). Mobilizatsia, izuchenye y ispolzovanye geneticheskikh resursov rasteniy. Sb. nauch. tr. po prik1. bot., gen y selektzii, v. 140. Leningrad.
- Levin, G.M. 1979. The overseas pomegranate culture (rus.). Subtropicheskiye kultury, No. 6.
- Levin, G.M. 1980. Materials for better knowledge of the Punicaceae family (rus.). Botanichesky zhurnal. v. 65, No. 3.
- Levin, G.M. 1982. The Pomegranate genetic diversity centres (rus.). Mirovye rastitelnye resursy v Sredney Asii. Vyp. 9. Tashkent.
- Levin, G.M. 1988. Seed reproduction of the pomegranate (rus.). Agropromyshlenniy kompleks Turkmenistana, No. 11.
- Levin, G.M. 1991. The pomegranate intraspecific classification (rus.). Izvestiya AN TSSR, ser. biol. nauk, No. 5.
- Popov, A.S. 1982. Deep freezing preservation of higher plants seeds and meristems (rus.). Pushino.
- Saminathan, M.S. Genepool preservation: from microbes to man (rus.). Vavilovskoye nasledie v sovremennoy biologii. Moscow, Nauka, 1989.
- Takhtajan, A.L. 1978. Floristic regions of the world (rus.). Nauka, Leningrad.
- Wilkins, C.P., Newbury, N.J., Dodds, J.H. 1988. Tissue culture conservation of fruit trees. Plant Genetic Resources Newsletter. Rome No. 73/74.

## Résumé

*Les ressources génétiques du grenadier (Punica granatum) au Turkmenistan*

Pendant la période 1934-1994, une collection mondiale de *Punica granatum* a été mise en place à la Station Expérimentale de Ressources Génétiques Végétales Turkmène (Garrygala, Kopetdagh occidental). La collection comprend 1117 cultivars et formes. Elle est réapprovisionnée de différentes façons, à partir de collectes de coenopopulations de grenadiers sauvages, à partir de centres d'anciens sites de culture des pays de la CEI et de l'étranger, et au moyen d'échanges et demandes à des institutions scientifiques. Il a été prouvé qu'il est possible de stocker les ressources génétiques du grenadier en cryoconservant des semences provenant d'autofécondations. La collection de *Punica granatum* est utilisée pour installer et réapprovisionner des collections analogues dans les institutions scientifiques d'autres pays. Les meilleurs échantillons sont envoyés à des pépinières et des fermes, et utilisés dans des travaux de sélection. La concentration de la recherche scientifique sur *Punica granatum* dans cette station et la création d'un Centre et d'une Banque de Gènes Internationale du Grenadier qui serviraient de centre pour le réseau existant pour cette espèce sont un objectif tout à fait réalisable.

## Resumen

*Recursos fitogenéticos del granado (Punica granatum) en Turkmenistán.*

Durante el período 1934-1994, se formó una colección mundial de *Punica granatum* en la Estación Experimental Turcomana de Recursos Fitogenéticos (Garrygala, Kopetdagh occidental). La colección comprende 1117 cultivares y formas. Se abastece de varias maneras, entre ellas con expediciones de estudio a las cenopoblaciones de granados silvestres y a los antiguos centros de cultivo en los países de la CEI y en el exterior, y mediante pedidos a las instituciones científicas e intercambios con ellas. Se ha demostrado que es posible almacenar los recursos genéticos del granado por criopreservación y semillas endogámicas. La colección de *Punica granatum* se emplea para formar y abastecer colecciones semejantes en las instituciones científicas de otros países. Las mejores muestras se envían a diversos viveros y fincas y se utilizan en obras de selección. Es totalmente factible concentrar la investigación sobre la *Punica granatum* en esta estación y crear un centro y un banco de genes internacionales del granado como punto principal de la red del cultivo.

## Tree seeds from the Sultanate of Oman in the Kew Seed Bank

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### Introduction

Of the 1200 or so wild species listed by Ghazanfar (1992) as native to Oman, I estimate some 55 to be trees. Few are common and many are rare; few are large and many are more usually shrubs than trees. After four expeditions to Oman (including one reported by Prendergast, 1993a), the Kew Seed Bank has seed collections from 15 species which are any combination of ecologically dominant, biogeographically interesting, and already, or potentially, important to man and livestock. Given that Oman is undergoing rapid economic change, and that trees will inevitably be affected in both predictable and unpredictable ways, conservation is a further factor spurring these collections.

Two expeditions have been to the southern province of Dhofar and two have been to the area around Muscat, the capital, and the nearby limestone mountain ranges of Jebel Akhdar and the Eastern Hajar. The whole of Oman

has either an arid or semi-arid climate: in the north most rain falls in the winter months, and increases with altitude; in the Dhofar mountains most rain (and precipitation from mist) is associated with the *khareef* (monsoon) from June to September. Some available rainfall figures (with altitude; data from FAO, 1987 and Ghazanfar, 1992) are: Capital Area (15 m), 95 mm; Saiq, Jebel Akhdar (1755 m), 307 mm; Salalah, Dhofar (22 m), 94 mm; Qairoon Hariti, Dhofar (878 m), 247 mm (see Fig. 1).

The forms of authors' names and the listing of genera in families follow, respectively, Brummitt and Powell (1992) and Brummitt (1992); collection numbers follow family names; coordinates were measured with a Global Positioning System (see Prendergast, 1993b). Seed numbers, where available, are calculated from the proportion of apparently whole seeds in an X-rayed sample of each cleaned collection (\* denotes collections which have not yet been tested for viability).

### Collections

*Acacia ehrenbergiana* Hayne

LEGUMINOSAE-MIMOSOIDEAE. HDVP 590

Widespread in Africa, this is a common species in the lowlands of northern Oman but is apparently absent from Dhofar (see Miller and Morris, 1988; Ghazanfar, 1992). It is one of the country's main rangeland browse species (Lawton, 1985; see Table 1). This collection of 10 710 seeds (\*) was made on 20/06/93 from drainage lines in the limestone foothills of the Capital Area between the suburbs of Qurum and Medinat Qaboos (23°35'38"N, 058°28'11"E; altitude 34 m). Fifty trees were sampled. Associated species were *Acacia tortilis* and *Maerua crassifolia* (see below).

*A. nilotica* (L.) Delile subsp. *indica* (Benth.) Brenan

LEGUMINOSAE-MIMOSOIDEAE. HDVP 592

This species is widely distributed in Africa, Arabia and the Indo-Pakistan subcontinent; throughout its range it is highly exploited (e.g. for the Sahel see von Maydell, 1990). In Oman subspecies *indica* occurs only in the north. The one site known to me was a group of five trees in the Capital Area suburb of Ruwi which had somehow escaped development activities (23°36'43"N, 058°32'25"E; altitude ca. 50 m). 12 211 seeds (\*) were collected, from the ground, on 20/06/93.

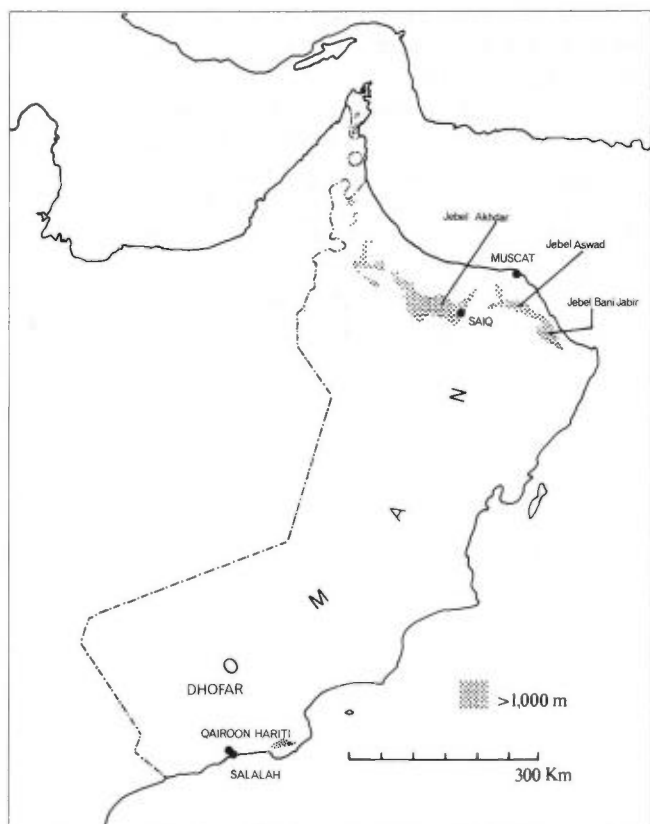


Fig. 1. Map of Oman showing mountain ranges and localities with climatic data.

*A. tortilis* (Forssk.) Hayne subsp. *tortilis*

LEGUMINOSAE-MIMOSOIDEAE. HDVP 588 and 589  
 "Probably the most widespread and in many areas the most common tree in Oman, occurring from the Musandam Peninsula south to Dhofar" (Miller and Morris, 1988). Subspecies *tortilis* extends also to Egypt and Sudan whilst other subspecies are found over much of arid western, eastern and southern Africa. Throughout its range, this species is highly esteemed for the nutritional value of its pods and leaves (see Table 1); in Oman it is one of the main sources of rangeland browse (Lawton, 1985). Even in the middle of the Capital Area, where livestock are forbidden, I saw herders collecting pods by beating them down from trees with sticks. Two seed collections (\*) of different age were made on 18/06/93 from 50 trees in 2.5 ha of a dense patch of *A. tortilis* woodland in a wadi bed in Al Khuwair suburb (23°35'33"N, 058°24'50"E; altitude 21 m). HDVP 588 (23 140 seeds) consisted of pods collected directly from the trees (many were still green) whilst HDVP 589 (37 237 seeds) consisted of pods collected from the ground below the trees (many pods were brown). The only associated woody species was the New World introduction *Prosopis juliflora* (Sw.) DC.

*Anogeissus dhofarica* A.J. Scott  
 COMBRETACEAE. HDVP 538

This tree, endemic to Dhofar (Fig. 2) and a small part of adjacent Yemen, grows to at least 10 m high although is often about half this. Despite being one of the most abundant species of the province, often forming pure stands, it remained undescribed until 1979 (Scott, 1979). It is also one of the most important species in several ways (Miller and Morris, 1988), especially as browse for livestock at the end of the dry season when it comes into leaf and when alternative fodder is scarce (see also Table 1 data from Lawton, 1985). The wood makes excellent building material and charcoal. In certain areas of Dhofar trees are virtually leafless due to severe overbrowsing. Seeds were collected in about 3 ha of *Anogeissus*-dominated mon-

soonal escarpment woodland on 19/09/92 above Ayn Razat (17°09'07"N, 054°13'05"E; altitude 420 m). Twenty-two trees were sampled (heavy browsing by camels meant that fruits on most trees were well out of reach) and 2540 green fruits (often turning brown) collected. Since fruits contain up to about 40 seeds, it was surprising that the end result was only 369 viable seeds. The most common associated species was *Blepharispermum hirtum* (see below).

*Blepharispermum hirtum* Oliv  
 COMPOSITAE. HDVP 545

This tall shrub or sometimes small tree is endemic to Dhofar (Fig. 2) (and a small part of adjacent Yemen) where it used to dominate the belt of vegetation along the foothills of the mountains receiving monsoonal moisture (Miller and Morris, 1988). It was previously heavily exploited for firewood. In 1991, I made a collection of this species which was rather small (4635 viable seeds) for Kew Seed Bank purposes (see Prendergast, 1993a) so a further collection was made on 27/09/92 from about 1.5 ha of the same site as *Anogeissus dhofarica* (above). 2663 capitula from ca. 150 plants produced 67 298 seeds.

*Ceratonia oreothauma* subsp. *oreothauma* Hillc., G.P. Lewis & Verd.

## LEGUMINOSAE-CAESALPINOIDEAE. HDVP 597

This species, the only known relative of the carob tree *C. siliqua* L., was described as recently as 1980 (Hillcoat *et al.*, 1980). It has two subspecies, one in the Eastern Hajar Mountains of northern Oman and in southern Yemen (where it has only recently been found; see Thulin, 1993; M. Thulin, pers. comm.), and the other in northern Somalia (Fig. 2). Its known range in Oman has recently been extended from the type locality, Jebel Aswad, to the area where this collection was made, Jebel Bani Jabir (22°49'N, 059°02'E on 26/06/93; altitude ca. 1700 m). The mountains are limestone and the climate very arid, much more so than that of Jebel Akhdar to the west. The tallest trees I

Table 1. Analyses of common browse species in Oman (from Lawton, 1985)

Species	Material	Ash %	C.P. % (N x 6.25)	P %	K %	DOMD <sup>1</sup> %
<i>Acacia ehrenbergiana</i>	Young shoots & leaves	7.34	10.62	1.03	0.54	58.2
<i>A. tortilis</i>	Old leaves	8.4	17.50	0.14	0.5	-
	Young leaves	7.71	7.06	0.07	0.66	41.2
<i>Anogeissus dhofarica</i>	Old leaves	8.84	11.81	0.14	1.10	54.4
<i>Maerua crassifolia</i>	Old leaves	23.4	21.25	0.07	2.8	-

<sup>1</sup> DOMD: % digestibility of organic matter in dry matter.

have seen are about 8 m high. The most conspicuous associated species were *Prunus arabica* (see below), *Dodonaea viscosa* Jacq. (Sapindaceae) and *Olea europaea* L. (Oleaceae). All trees were bearing pods. 2 600 seeds (\*) were collected from about 100 trees spread over about 10 km<sup>2</sup>. I saw herders knocking down the highly nutritious pods for their goats whose heavy browsing on every tree was all too apparent. I also saw no undamaged tree less than 2 m high (and very few less than 3 m), suggesting that there is no regeneration. The long-term security of the species may warrant attention. Kew's Micropropagation Unit has

successfully propagated plants *in vitro* (Woods, 1985). It is surprising that, as far as I have discovered, there has been no information published on the commercial potential of *C. oreoethauma*.

*Commiphora habessinica* (O. Berg.) Engl. in A.DC  
BURSERACEAE. HDVP 518

This low tree (only about 1.5 m high at the collection site and about 2 m across) occurs throughout south west Arabia (including both northern and southern Oman) and in Ethiopia and Sudan (Miller and Morris, 1988).



Fig. 2. Distributions for selected species (based on Browicz, 1969; Farjon, 1992; Friis, 1978; Hillcoat *et al.* 1980; Meikle, 1966; Miller and Morris, 1988; Thulin, 1993; M. Thulin, pers. comm.).

The resin used to be used medicinally in Dhofar (Miller and Morris, 1988). About 21 000 fruits were collected from ca. 120 trees on 21/05/92 on the lower slopes of arid limestone hills near Muscat (23°34'N, 058° 34'E; altitude ca. 100 m). They contained, however, only 2000 seeds (\*). Associated species were *Euphorbia larica* Boiss. and *Grewia erythraea* Schweing.

*Ficus cordata* Thunb. subsp. *salicifolia* (Vahl.) C.C. Berg.  
MORACEAE. HDVP 544

Widespread in Africa and Arabia, this fig is one of five wild species known from Dhofar; it is also the most drought resistant (Miller and Morris, 1988; there known as *F. salicifolia*). I made this collection on 25/09/92 from monsoonal woodland beside the perennial stream of Wadi Tobruk where *F. sycamorus* L. and *F. vasta* Forssk. were also growing in abundance (17°05'47"N, 054° 19'37"E; altitude ca. 100 m). It comprises 10 915 seeds (\*), sampled from 35 trees.

*Juniperus excelsa* M. Bieb. subsp. *polycarpus* Takht.  
CUPRESSACEAE. HDVP 539

No tree grows at a higher altitude in Oman than the juniper: from a lower limit of ca. 1450 m to the summits of Jebel Akhdar at 3000 m. Oman has the most southerly outpost of the species (Fig. 2). Size and shape are very variable; the largest specimen I have seen was about 17 m high and with a basal circumference of 14 m. This collection of 22 482 seeds was made from ca. 12 000 fruits from 50 trees spread over some 75 ha of Jebel Akhdar on 23/06/93 (23°18'N, 057°06'E; altitude 2250 m). The only other tree present was *Olea europaea* L.

*Lawsonia inermis* L.  
LYTHRACEAE. HDVP 546 & 547

*Lawsonia* is a monotypic genus whose sole species is widely distributed in Asia and Africa (the Kew Seed Bank has made collections from Madagascar and Somalia as well as Oman). The entry for the species in Ghazanfar (1992) says nothing about its status in Oman other than that it is cultivated; Miller and Morris (1988) say nothing at all. Given that from ancient times its leaves have been used as the source of dye, it seems appropriate that these collections of henna came from Wadi Hinna (or Henna), the only locality where I have seen it in Dhofar. Both collections were made on 29/09/92 and from the same 26 trees (maximum height about 5 m) along about 200 m of a well wooded wadi bed at the foot of the monsoonal woodland belt (17°02'32"N, 054°36'46"E; altitude 43 m). They differed, however, in age. HDVP 546 comprised completely dry fruits which had either formed during the previous *khareef* (the annual monsoon between June and September) or as a result of heavy rains in April 1992; it produced 110 498 seeds. HDVP 547 comprised fresh seed (i.e. from the 1992 *khareef*) and produced 20 635 seeds. Associated species included *Delonix elata* (L.) Gamble

(Leguminosae) and *Ziziphus spina-christi* (L.) Willd. (Rhamnaceae).

*Maerua crassifolia* Forssk.  
CAPPARACEAE. HDVP 591

This tree occurs from Senegal, through East Africa and eastwards to Pakistan, and in both northern and southern Oman. It is one of the country's main rangeland browse species (Lawton, 1985; see his data in Table 1). Von Maydell (1990) lists numerous uses in the Sahel zone of West Africa. This collection of 21 893 seeds (\*) came mainly from about 30 trees, the tallest about 6m high, on 20/06/93 in otherwise almost plantless foothills south west of Qurayat (23°04'51"N, 058°55'37"E; altitude 230 m). Many fruits were picked from the ground below the trees suggesting that they were not eaten by the only large animals seen in the area during a whole day, feral donkeys. Associated species were *Acacia ehrenbergiana* and *A. tortilis*.

*Moringa peregrina* (Forssk.) Fiori  
MORINGACEAE. HDVP 587

This species occurs from Sudan and Somalia northwards to Israel and eastwards as far as Oman (Verdcourt, 1985). It is uncommon in Dhofar but in the north of Oman is frequent on steep mountain slopes above about 700m where it reaches about 7 m in height. As with the better known *M. oleifera* Lam., its seeds are very effective water purifiers (see Jahn, 1981). In Oman a cooking oil used to be obtained from the seeds (Lawton, 1985) and some medicinal uses are recorded (Ghazanfar and Al-Sabahi, 1993). The bulk of this collection of 16 290 seeds came from about 80 trees near the approach road to the Saiq Plateau on Jebel Akhdar (22°55'N, 057°42'E; altitude 700-1300 m) but others were also taken from a further 70 trees in and near Wadi Ghul up to about 1500 m. Associated species were *Acacia tortilis* and the shrub *Acridocarpus orientalis* A. Juss. (Malpighiaceae).

*Prunus arabica* (Oliv.) Meikle.  
ROSACEAE. HDVP 596.

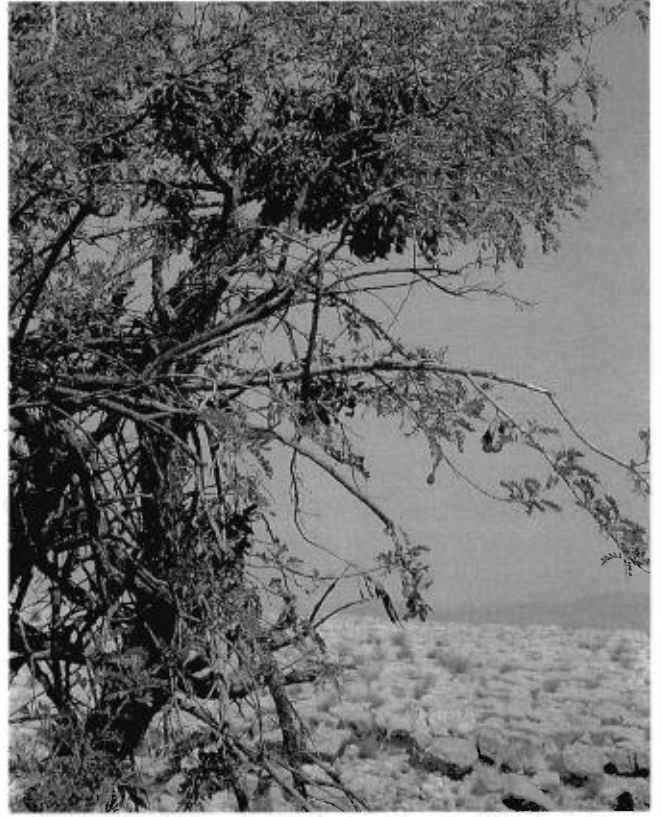
One of only two members of the rose family in Oman, this species grows alongside *Ceratonia oreothauma* on Jebel Bani Jabir, its most southerly outpost (Fig. 2). The only other Omani locality is the Musandam Peninsula (i.e. it does not occur in the intervening Jebel Akhdar). The tallest trees on Jebel Bani Jabir are about 6 m high; unlike *Ceratonia*, its foliage does not appear to be eaten by goats. About 30% of the trees were bearing fruit. 10 776 seeds (\*) were collected from about 100 trees scattered over about 10 km<sup>2</sup>.

*Rhus somalensis* Engel  
ANACARDIACEAE. HDVP 553

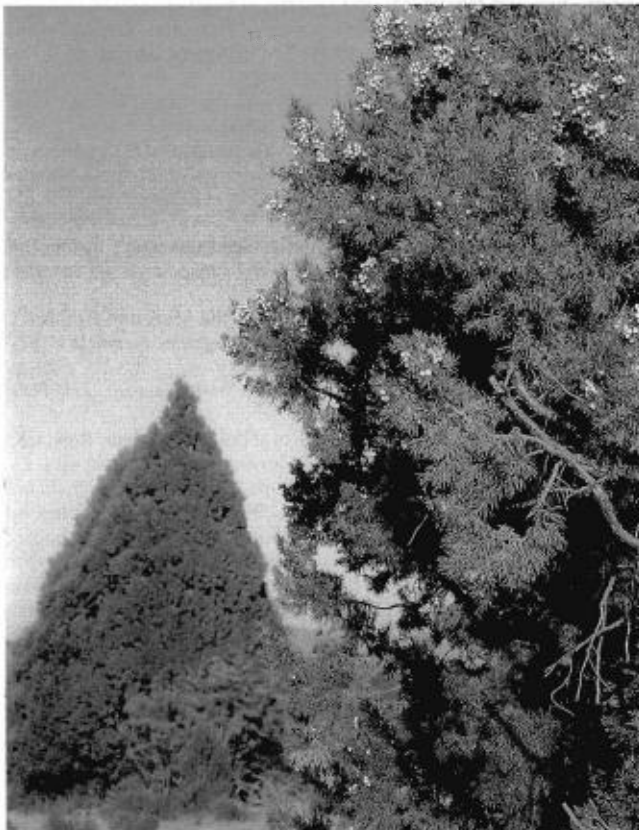
This shrub or small tree grows to about 6 m high. It has been found only in Dhofar and northern Somalia (Fig. 2). Its berries, when red, are delicious and used to be consumed in large quantities (Miller and Morris, 1988). This collection of 7618 seeds (\*) came from ca. 40 plants in a



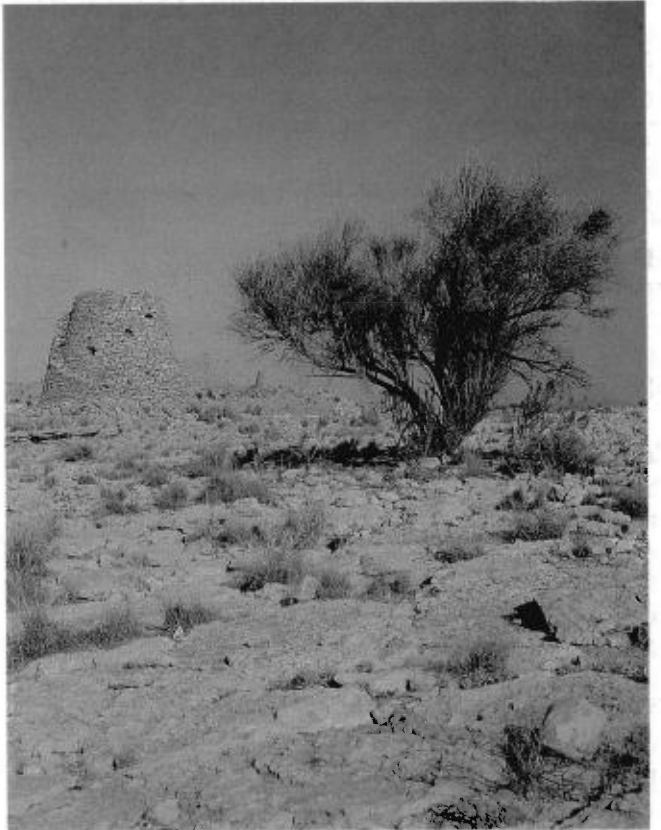
*Blepharisperrum hirtum* in foreground in monsoonal woodland; coastal desert and sea in background



*Ceratonia oreothauma*



*Juniperus excelsa*



*Prunus arabica*

monospecific stand in plateau monsoonal grassland near Nashib (17°08'25"N, 054°18'32"E; altitude 700 m). Nearby associated species were *Anogeissus dhofarica* and *Euphorbia smithii* S. Carter.

*Sideroxylon mascatense* (A.DC.) T.D. Penn.

SAPOTACEAE. HDVP 586

(*Monotheca buxifolia* (Falc.) A.D.C.; see Pennington, 1991). This tree, the only member of the family in Oman, grows to about 6 m high and is common on Jebel Akhdar above 1000 m; it also occurs in Dhofar (Miller and Morris, 1988). Oman lies in the middle of its range from northern Pakistan to southern Ethiopia (Fig. 2; see also Friis, 1983). It has a delicious one-seeded berry up to some 14 mm across which has for long been highly regarded and exploited (Mandaville, 1978). The species is surely worth investigating for cultivation. Large fleshy fruits are often indicative of recalcitrant seed storage behaviour but, as with the very closely related and even larger-fruited *Argania spinosa* (L.) Skeels (a north-west African species that also occurs in an arid climate - which is unusual for the Sapotaceae; see Prendergast, 1991), orthodox seed behaviour has been shown for *S. mascatense* at Wakehurst Place. On 09/06/93 I collected 23 250 seeds (\*) from ca. 100 trees by a tree- and shrub-lined wadi on Jebel Akhdar (23°14'14"N, 057°09'00"E; altitude 1450 m).

## Results

Seed collections of the following tree species have been made in Oman by the Kew Seed Bank from where a seed list is available: *Acacia ehrenbergiana*, *A. nilotica* subsp. *indica*, *A. tortilis* subsp. *tortilis*, *Anogeissus dhofarica*, *Blepharisperrum hirtum*, *Ceratonia oreoethauma* subsp. *oreoethauma*, *Commiphora habessinica*, *Ficus cordata* subsp. *salicifolia*, *Juniperus excelsa* subsp. *polycarpus*, *Lawsonia inermis*, *Maerua crassifolia*, *Moringa peregrina*, *Prunus arabica*, *Rhus somalensis* and *Sideroxylon mascatense*.

## Note added in press

In March 1994 further tree seed collections were made, of *Acacia gerrardii* Benth., *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl.) C.C. Berg, *F. johannis* Boiss., *Prosopis juliflora* (Sw.) DC, *Ziziphus spina-christi* (L.) Willd. and *Ziziphus* sp. nov.

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## References

- Browicz, K. 1969. Rosaceae-Prunoideae. In Rechinger, K.H. (ed.). *Flora Iranica*. Rosaceae 1. Akademische Druck - u. Verlagsanstalt, Graz, Austria, pp. 161-203.
- Brummit, R.K. (comp.) 1992. *Vascular plant families and genera*. Royal Botanic Gardens, Kew.
- Brummit, R.K. and Powell, C.E. (eds) 1992. *Authors of plant names*. Royal Botanic Gardens, Kew.
- FAO. 1987. *Agroclimatological data for Asia*. Volume 2. K-Z. FAO, Rome.
- Farjon, A. 1992. The taxonomy of multiseed junipers (*Juniperus* Sect. *Sabina*) in Southwest Asia and East Africa. *Edinb. J. Bot.* 49:251-283.
- Friis, I. 1978. A reconsideration of the genera *Monotheca* and *Spiniluma* (Sapotaceae). *Kew Bull.* 33:91-98.
- Friis, I. 1983. Phytogeography of the tropical north-east African mountains. *Bothalia* 14:525-532.
- Ghazanfar, S.A. 1992. *An annotated catalogue of the vascular plants of Oman and their vernacular names*. Scripta Botanica Belgica, Vol. 2. National Botanic Garden of Belgium, Meise.
- Ghazanfar, S.A. and Al-Sabahi, A.M.A. 1993. Medicinal plants of northern and central Oman (Arabia). *Econ. Bot.* 47:89-98.
- Hillcoat, D., Lewis, G., and Verdcourt, B. 1980. A new species of *Ceratonia* (Leguminosae-Caesalpinioideae) from Arabia and the Somali Republic. *Kew Bull.* 35:261-271.
- Jahn, S.A.A. 1981. *Traditional water purification in tropical developing countries. Existing methods and potential application*. Deutsche Gesellschaft für technische Zusammenarbeit, Eschborn.
- Lawton, R.M. 1985. Some indigenous economic plants of the Sultanate of Oman. In Wickens, G.E. Goodin, J.R. and Field, D.V. (eds). *Plants for arid lands*. Proc. Kew Int. Conf. on Economic Plants for Arid Lands, Royal Botanic Gardens, Kew, UK, July 1984. Allen and Unwin, London, pp. 267-275.
- Mandaville, J.P. 1978. *Wild flowers of northern Oman*. Bartholomew Books, London, in collaboration with The Office of the Government Adviser for the Conservation and Development of the Environment, Sultanate of Oman.
- Meikle, R.D. 1966. Rosaceae. In Townsend, C.C. and Guest, E. (eds). *Flora of Iraq*. Volume II. Ministry of Agriculture, Iraq, pp. 102-171.
- Miller, A.G. and Morris, M. 1988. *Plants of Dhofar. The southern region of Oman: traditional, economic and medicinal uses*. Office of the Adviser for Conservation of the Environment, Diwan of Royal Court, Sultanate of Oman.
- Pennington, T.D. 1991. *The genera of Sapotaceae*. Royal Botanic Gardens, Kew & New York Botanical Garden, Bronx.
- Prendergast, H.D.V. 1991. Conservation à long terme des semences de l'arganier *Argania spinosa*. Poster presentation at the Colloque International sur l'Arganier - Recherches et Perspectives, Faculté des Sciences, Agadir, March 1991.
- Prendergast, H.D.V. 1993a. Wild species seed collecting in Dhofar, Sultanate of Oman. *FAO/IBPGR Plant Genetic Resources Newsletter* 91/92:39-44.
- Prendergast, H.D.V. 1993b. Product review: the Magellan GPS NAV 5000 PRO. *FAO/IBPGR Plant Genetic Resources Newsletter* 91/92:60-61.
- Scott, A.J. 1979. A revision of *Anogeissus* (Combretaceae). *Kew Bull.* 33:555-566.
- Thulin, M. 1993. *Flora of Somalia*. Vol. I. Royal Botanic Gardens, Kew, UK.
- Verdcourt, B. 1985. A synopsis of the Moringaceae. *Kew Bull.* 40:1-23.
- Von Maydell, H.-J. 1990. *Arbres et arbustes du Sahel. Leurs caractéristiques et leurs utilisations*. Deutsche Gesellschaft für technische Zusammenarbeit, Eschborn; Verlag Josef Margraf, Weikersheim.
- Woods, A. 1985. The potential for the *in vitro* propagation of a number of economically important plants for arid areas. In Wickens, G.E., Goodin, J.R. and Field, D.V. (eds). *Plants for arid lands*. Proc. Kew Int. Conf. on Economic Plants for Arid Lands, Royal Botanic Gardens, Kew, UK, July 1984. Allen and Unwin, London, pp. 333-342.

## A tetraploid *Hordeum bulbosum* with high self-fertility

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*Hordeum bulbosum* is known as an allogamous species. Lein (1948) reported on pronounced self-sterility in diploid and tetraploid *H. bulbosum*, but in the tetraploid form self-sterility was sometimes broken. The selection of a plant with high self-fertility is described below.

A collection of tetraploid *H. bulbosum* consisting of about 200 samples was analyzed at Gülzow (Wustrack, Szigat 1985). The original aim of this investigation was to find out whether the self-sterility of *H. bulbosum* can be used for reducing labour of crossing in connection with the species hybridization between *H. bulbosum* and *H. vulgare*. Two first-developing ears of each plant were covered separately with bags in the greenhouse. Altogether 199 plants were isolated.

### Results in the *H. bulbosum* collection

For the most part, the isolated plants were highly self-sterile and without any seeds (Fig. 1). In 55 plants (= 27.6%) seed set was observed; for the most part, there were only a few kernels per spike: 39 plants had less than five kernels/spike, and only three plants had more than 10 kernels/spike.

A small seed set on some plants seems typical for self-sterile species. However, one plant (Gülzow BU 60) which was collected in Bulgaria stood out for its remarkably high seed set (30.5 kernels per isolated ear). Clones of this plant growing in different environmental conditions (in

the open field) also gave a high seed set. Seed set after isolation was just the same as with open pollination. This plant had normal vitality which is typical for tetraploid *H. bulbosum*.

The results show that self-sterility in this tetraploid *H. bulbosum* collection is not certain enough to wholly neglect emasculation as a step in crossing. On the other hand, the self-fertile plant may be useful not only for species hybridization but also for research on this wild species; it can be used to stabilize desirable characters in the progenies. Therefore, a part of the self-fertile plant (Gülzow BU 60) is to be transferred to the Genebank of the Institute of Plant Genetics and Crop Plant Research Gatersleben.

To obtain knowledge on the progeny of the self-fertile plant  $S_1$ , plants grown from selfed seeds were tested for their self-fertility. Two or three of the first developing spikes of each plant were covered with bags in the open field. Spikes of 128 plants were analyzed from isolation and from open pollination.

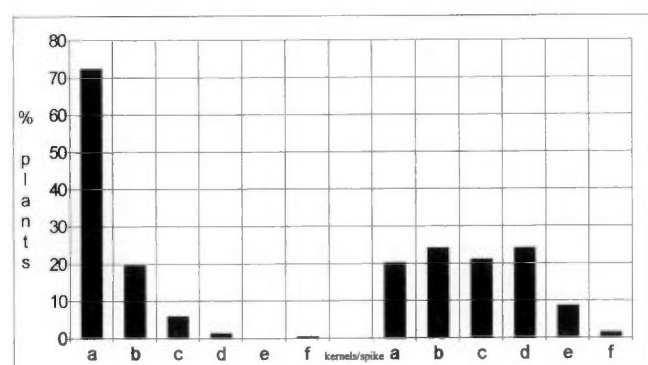
### Results in the progeny of the self-fertile plant

Comparing the distribution of the plants of the collection and of the  $S_1$  progeny of the self-fertile plant, there is a distinct accumulation of the self-fertile plants in the  $S_1$ . Only 20.3% (=26 plants) of the  $S_1$  were without seeds in comparison with 72.4% in the collection. Moreover, of the 26 sterile plants four were sterile when open pollinated; two of these had degenerated, small sterile anthers and the other two were plants with low vitality. Perhaps there is an influence of inbreeding. On the other hand, plants with high self-fertility had good fertility after open pollination. Further investigation is necessary in order to characterize this material.

### References

- Lein, A. Natürliche Standorte von diploidem und tetraploidem *Hordeum bulbosum* L. Der Züchter 19, 1948, 1/3 S.6-9.  
Wustrack, H., G. Szigat. Study of various *Hordeum bulbosum* accessions in relation with their fitness for inter-specific hybridization. Genetics and breeding 18, 6 Sofia 1985, S. 518-524.

*H. bulbosum* 4x Collection Gülzow      Progeny of one self-fertile plant (*H. bulb* 4x Gülzow BU 60)



a 0,5-5,0    b 5,5-10,0    c 10,5-20,0    d 20,5-30,0    e 30,5-40,0

Fig. 1. Distribution of the plants with different degrees of self-fertility after bag isolation

*Leishmania* spp. are unicellular flagellated protozoans that cause a wide range of diseases in humans and animals. The most common forms of leishmaniasis are visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). VL is a systemic disease that affects the reticuloendothelial system, while CL is a localized disease that affects the skin.

The most common species of *Leishmania* that cause VL in humans are *L. donovani*, *L. infantum* and *L. tropica*. CL is caused by several species, including *L. major*, *L. tropica*, *L. braziliensis* and *L. mexicana*. *Leishmania* spp. are transmitted to humans and animals by blood-sucking insects, primarily sandflies. The life cycle of *Leishmania* spp. involves asexual reproduction in the vertebrate host and sexual reproduction in the sandfly vector.

One of the most interesting features of *Leishmania* spp. is their ability to evade the host's immune system. They do this by residing in macrophages, where they can avoid being killed by the host's immune cells. Additionally, they can modulate the host's immune response, allowing them to survive and multiply within the host.

#### Results in the response of the self

The results of the study show that the *Leishmania* spp. with high self-toxicity are able to evade the host's immune system more effectively than those with low self-toxicity. This is evident from the higher survival rates of the high self-toxicity strains in macrophages and the lower levels of host immune response. The study also shows that the high self-toxicity strains are able to modulate the host's immune response, leading to a more prolonged and severe disease.

#### References

- 1. Sisti G, et al. (2006) High self-toxicity in *Leishmania* spp. *Journal of Parasitology*, **136**, 105–111.
- 2. Sisti G, et al. (2007) Self-toxicity in *Leishmania* spp. *Parasitology*, **137**, 105–111.
- 3. Sisti G, et al. (2008) Self-toxicity in *Leishmania* spp. *Parasitology*, **138**, 105–111.

The study also shows that the high self-toxicity strains are able to evade the host's immune system more effectively than those with low self-toxicity. This is evident from the higher survival rates of the high self-toxicity strains in macrophages and the lower levels of host immune response.

The study also shows that the high self-toxicity strains are able to modulate the host's immune response, leading to a more prolonged and severe disease. This is evident from the higher levels of host immune response in the low self-toxicity strains compared to the high self-toxicity strains.

#### Results in the *L. donovani* collection

The results of the study show that the *L. donovani* collection with high self-toxicity are able to evade the host's immune system more effectively than those with low self-toxicity. This is evident from the higher survival rates of the high self-toxicity strains in macrophages and the lower levels of host immune response.

The study also shows that the high self-toxicity strains are able to modulate the host's immune response, leading to a more prolonged and severe disease. This is evident from the higher levels of host immune response in the low self-toxicity strains compared to the high self-toxicity strains.

The study also shows that the high self-toxicity strains are able to evade the host's immune system more effectively than those with low self-toxicity. This is evident from the higher survival rates of the high self-toxicity strains in macrophages and the lower levels of host immune response.



Fig. 1. Survival percentage of *L. donovani* in macrophages for different self-toxicity levels.

## Seed-borne fungi of flax (*Linum usitatissimum* L.)

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### Introduction

Flax (*Linum usitatissimum* L.) is a traditional plant in Hungary used for fibres and seed oils (Hortobágyi, 1980). Mandy (1970) published the results of phenoecological examinations of oil flaxes; Mandy *et al.* (1971) investigated the cardinal point of germination and also presented data on storability. Later, Szabó and Virányi (1971) tested the seed samples stored in unconditioned environments. However, the pathogens which occurred were not documented (Simay, 1990), although some were reported from Hungary (Bánhegyi *et al.*, 1985). The aim of this report is to document the fungi observed on flax seeds during the seed tests.

### Material and Methods

The seed samples investigated were selected, during laboratory germination tests, by the high rate of mould-affected seeds. The seeds were germinated without surface sterilization, according to ISTA (1985) suggestions accepted mainly for genebanks (Ellis *et al.*, 1985). The fungi from the seed surface were studied and identifications were based on sporulation developed here and pure cultures. Representative pure cultures were made in the case of fungi which were potentially pathogenic especially on flax. The fungi were identified by Arx (1981), Bánhegyi *et al.* (1985), Booth (1971), Domsch *et al.* (1980), Gerlach and Nirenberg (1982) and Rádulescu and Negru (1971).

### Results and Discussion

In the course of seed pathological studies made during 1986-1990, 44 seed samples were tested for fungi occurring on the seeds. Species of 16 genera were identified (Table 1) and the percentage of contaminated samples was calculated. Because of the fast spreading of some fungi in the moist blotter used for germination, the correct values of contamination within the samples were impossible to determine.

The predominant fungi belonged to the genera of *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus*. The pathogens identified were *Alternaria linicola*, *Aureobasidium lini*, *Colletotrichum lini*, *Fusarium avenaceum*, *F. graminearum*, *F. oxysporum*, *F. verticillioides* and *Stemphylium botryosum*. Among these pathogens *A. lini*, *C. lini* and *A. linicola* are known from *Linum* spp. only, while *Fusarium* spp. and *S. botryosum* have a wide seed host-range (Malone and Muskett, 1964; Neergaard, 1979). Vörös and Husz

(1965) reported *A. linicola*, *A. lini* and *C. lini* causing seed and seedling diseases from Hungary earlier, but we have no previous data about seed transmission of *Fusarium* and *Stemphylium* on this host in Hungary.

On the other hand, *Fusaria* and *Stemphylium* are widespread on different seeds, even on flaxes (DeTempe, 1963; Malone and Muskett, 1964; Muskett and Malone, 1941; Neergaard, 1979, Rádulescu and Negru, 1971), and seed transmission of *F. oxysporum* also has an epidemiological role on several plants (Gambogi, 1983). Some other fungi, such as *Alternaria alternata*, *Bipolaris* sp. and *Trichotecium roseum* cause seed or seedling rot on flax. The contamination of seeds with *T. roseum* also inhibited the germination of the surrounding seeds.

Among saprophytes, *Cladosporium* is usually a field fungus, but also the frequently observed *Penicillium* and *Rhizopus* or *Aspergillus* are known storage fungi which occur on different plant seeds (Christensen, 1972). These storage fungi could cause deterioration of seeds during storage (Halooin, 1986). Fungi which can inhibit some pathogens were also observed (Vajna, 1987), but the identification of the role of saprophytes on flax in the Hungarian genebank needs further investigation.

### References

- Arx, J.A. von 1981. The genera of fungi sporulated in pure culture. (3rd edition) J. Cramer, Vaduz.
- Bánhegyi, J., Tóth, S., Ubrizsy, G., Vörös, J. 1985. Magyarország mikroszkópikus gombáinak határozókönyve. Handbook for identification of microscopic fungi of Hungary. Akadémiai Kiadó, Budapest.
- Booth, C. 1971. The genus *Fusarium*. C.M.I., Kew.
- Christensen, C.M. 1972. Microflora and seed deterioration. In Viability of seeds. Editor Roberts, E.H. pp. 59-93. Chapman and Hall Ltd., London.
- DeTempe, J. 1963. Health testing of flax seed. Proceedings of the International Seed Testing Association. 28:107-131.
- Domsch, K.H., Gams, W., Anderson, T.H. 1980. Compendium of soil fungi. Academic Press, London.
- Ellis, R.H., Hong, T.D., Roberts, E.H. 1985. Handbook of seed technology for genebanks. FAO/IBPGR, Rome.
- Gambogi, P. 1983. Seed transmission of *Fusarium oxysporum*: epidemiology and control. Seed Science and Technology, 11:815-827.
- Gerlach, W. and Nirenberg, H. 1982. The genus *Fusarium* - A pictorial atlas. Mittl. Biol. Bundesanst. Land- und Forstwirtschaft. Berlin-Dahlem, 209:1-406.
- Halooin, J.M. 1986. Microorganisms and seed deterioration. Physiology of Seed Deterioration, CSSA Spec. Publ. 11:89-99.
- Hortobágyi, T. 1980. Lenfélék-Linaceae (Flaxes-Linaceae). In *Agrobotanika*. Editor Hortobágyi, T. p. 376. Mezőgazdasági Kiadó, Budapest.
- ISTA 1985. International rules for seed testing. Rules 1985. International Seed Testing Association, Zürich.

L. pp. 189-291. Mezőgazdasági Kiadó, Budapest.  
 Vörös, J. és Husz, B. 1965. Deuteromycetes. In Növénykórtan (Phytopathology). Editor Ubrizsy, G. pp. 579-809. Akadémiai Kiadó, Budapest.

Table 1. Fungi observed on flax (*Linum usitatissimum* L.) seeds

Fungi	Contaminated seed samples	
	Number	Percentage
<i>Acremonium</i> Link ex Fries sp.	6	13.64
<i>Alternaria alternata</i> (Fr.) Keissler	2	4.55
<i>Alternaria linicola</i> Groves et Skolko	1	2.27
<i>Alternaria</i> Nees ex Wallroth sp.	40	90.91
<i>Aspergillus</i> Micheli ex Fries sp.	1	2.27
<i>Aureobasidium lini</i> (Lafferty) Hermanides-Nijhof	1	2.27
<i>Bipolaris</i> Shoemaker sp.	1	2.27
<i>Chaetomium</i> Kunze ex Fries sp.	2	4.55
<i>Clara elegans</i> Nag Raj et Kendrick	1	2.27
<i>Cladosporium</i> Link ex Fries sp.	37	84.09
<i>Colletotrichum lini</i> Manns. et Bolley	1	2.27
<i>Epicoccum purourascens</i> Ehrenberg ex Schlecht.	2	4.55
<i>Fusarium avenaceum</i> (Fr.) Saccardo	1	2.27
<i>Fusarium graminearum</i> Schwabe	1	2.27
<i>Fusarium oxysporum</i> Schlechtendahl	2	4.55
<i>Fusarium verticillioides</i> (Sacc.) Nirenberg	1	2.27
<i>Fusarium</i> Link ex Fries sp.	25	56.82
<i>Gliocladium</i> Corda sp.	2	4.55
<i>Penicillium</i> Link ex Fries sp.	35	79.55
<i>Rhizopus stolonifer</i> (Ehrenb. ex Link) Lind	15	34.09
<i>Stemphylium botryosum</i> Wallroth	2	4.55

Malone, J.P. and Muskett, A.E. 1964. Seed-borne fungi. Descriptions of 77 fungus species. Proceedings of the International Seed Testing Association. 29:179-384.

Mándy, Gy. 1970. Olajlenek fenoökológiai vizsgálata. Phenoecological examination of oil flaxes. Agrobotanika, X:175-191.

Mándy, Gy., Szabó, L., Papp, E. 1971. A csírázás kardinális pontjainak vizsgálata mák- és lenfajtáknál. Examination of cardinal points of germination in poppy and flax varieties. Agrobotanika, XI:169-174.

Muskett, A.E. and Malone, J.P. 1941. The Ulster method for examination of flax seed for the presence of seed-borne parasites. Annals of Applied Biology, 28:8-13.

Neergard, P. 1979. Seed pathology. 2nd edition. MacMillan Press, London.

Rádulescu, E. and Negru, A. 1971. Magkártevők és betegségek határozója. Handbook for identification of pests and diseases of seeds. Mezőgazdasági Kiadó, Budapest.

Simay, E.I. 1990. Archiv adatok Tápiószele környékének mikroszkópikus gombáiról. Mycoflora of Tápiószele. Bibliographic survey from 1959 until 1981 and rusts collected before 1963. Mikológiai Közlemények, 1990/1-3:121-128.

Szabó, L. and Virányi, S. 1971. Változó raktári körülmények között tárolt kultúrnövénymagok csírázási vizsgálata. Germination data of seeds of different crops, after storage in unconditioned environment. Agrobotanika, XII:15-20.

Vajna, L. 1987. A biológiai védekezés. Biological control. In Növénypatogén gombák (Phytopathogenic fungi). Editor Vajna,

## Screening of wild *Triticum* and *Aegilops* species for resistance to Karnal bunt disease of wheat

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### Introduction

Karnal bunt disease of wheat, caused by *Neovossia indica* (Mitra) Mundkur (Syn *Tilletia indica*), has become a serious disease of wheat in the Indian subcontinent and in the Yaqui valley of Mexico due to the introduction of susceptible high-yielding varieties coupled with gradual change in agronomic and cultural practices (Bedi *et al.*, 1992). This disease reduces the quality of wheat in addition to causing considerable yield losses. Control through fungicides is not completely effective as the disease is soil-, air- and seed-borne. Development of disease-resistant varieties is the only economic and effective method of control. There is very limited variability for Karnal bunt resistance in bread wheat (Aujla *et al.*, 1992; Royer and Rytter, 1988). Thus, there is a need to search for new sources of resistance to this disease among wild relatives of wheat. This paper reports the results of screening 90 accessions belonging to four wild *Triticum* and five *Aegilops* species under artificial inoculation conditions.

### Materials and Methods

The study was conducted at the Biotechnology Centre, Punjab Agricultural University, Ludhiana, India, during two crop seasons: 1991-92 and 1992-93. In 1991-92 54 accessions were evaluated and in 1992-93, 53 accessions; 17 accessions were common to both years. The materials were transplanted to the field following vernalization of seedlings of each accession at 6°C in a growth chamber for 6 weeks with an 8-hour daylength. Lines of *Ae. bicornis*, *T. araraticum* and *T. dicoccoides* were sown directly in the field. Flowering of wild *Triticum* and *Aegilops* species is delayed under normal daylength conditions of northern India. Therefore, a longer photoperiod was provided artificially to all the materials in both the growing seasons, to induce flowering in the middle of March for inoculation at the boot-leaf stage (Stage 10 on Feeke's scale). Two to five ears of each accession were inoculated with a mixture of isolates, including one recently obtained isolate that had shown virulence on triticale line TL1210. A suspension containing 10 000 secondary sporidia per ml was injected into the boot of the plant at awn emergence stage using a hypodermic syringe (Aujla *et al.*, 1982). At maturity, the inoculated heads were harvested and threshed by hand to record the percentage of infected grains for each accession. The accessions having no Karnal bunt- infected grains

were classified as resistant whereas the accessions having any infected grain in any of the two seasons were considered as susceptible.

Table 1. Reactions of different accessions of wild *Triticum* and *Aegilops* species to *Neovossia indica* under boot inoculation

Species with genome(s)	Number of accessions		Percentage of resistant acc.
	Resistant	Susceptible	
<i>Triticum boeoticum</i> (A <sup>b</sup> )	19	2	90.5
<i>T. urartu</i> (A <sup>b</sup> )	5	0	100.0
<i>Aegilops speltoides</i> (S)	6	2	75.0
<i>Ae. longissima</i> (S <sup>1</sup> )	2	4	33.3
<i>Ae. bicornis</i> (S <sup>b</sup> )	1	1	50.0
<i>Ae. squarrosa</i> (D)	4	1	80.0
<i>Ae. caudata</i> (C)	1	0	100.0
<i>T. araraticum</i> (AG)	4	6	40.0
<i>T. dicoccoides</i> (AB)	22	10	68.8
Total	64	26	

### Results and Discussion

The results of the artificial screening of various accessions of wild *Triticum* and *Aegilops* species are given in Table 1. The diploid species carrying the A genome, i.e., *Triticum urartu* (A<sup>b</sup>) and *T. boeoticum* (A<sup>b</sup>) had more than 90% accessions resistant to Karnal bunt. The diploid species of *sitopsis* section i.e., *Ae. speltoides*, *Ae. longissima* and *Ae. bicornis*, had a lower proportion of lines resistant to Karnal bunt than that of the species with A genome and *Ae. squarrosa* (D). The lower proportion of resistant lines of tetraploid wild *Triticum* species, *T. araraticum* (AG) and *T. dicoccoides* (AB), may also be attributed to the presence of G and B genomes, respectively, which are expected to be close to S genome. The only accessions of *Ae. caudata* were resistant. In general, these observations are in agreement with the earlier screening tests conducted under artificial conditions at three centres, viz., the United States Department of Agriculture (Royer and Rytter, 1988), the International Maize and Wheat Improvement Centre (CIMMYT) (Warham *et al.*, 1986) and the Punjab Agricultural University (Dhaliwal *et al.*, 1986). Interestingly, neither in the present study nor in previous studies by Dhaliwal *et al.* (1986) and Royer and Rytter (1988) has any *T. urartu* line with susceptibility to Karnal bunt been observed. The absence of disease in *T.*

*urartu* and high proportion of resistant *T. boeoticum* lines in the present study as well as in the previous study by Dhaliwal *et al.* (1986) indicated that the two A genome species are a good source for transfer of Karnal bunt resistance into cultivated wheats. However, *T. durum* - *Ae. squarrosa* amphiploids developed at the Punjab Agricultural University by using Karnal bunt resistant accessions of *Ae. squarrosa* have maintained resistance over years (H.S. Dhaliwal, pers. commun.). This indicated that *Ae. squarrosa* could also be a good source of resistance to this disease.

### Conclusion

The observations presented here showed that the diploid progenitor species, *Triticum urartu* (T<sup>u</sup>), *T. boeoticum* (A<sup>b</sup>) and *Ae. squarrosa* (D), are valuable sources for transfer of Karnal bunt resistance into cultivated wheats.

### Acknowledgements

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### References

- Aujla, S.S., A.S.Grewal, K.S.Gill and I.Sharma (1982). Artificial creation of Karnal bunt disease of wheat. *Cereal Res.Comm.* 10: 171-176.
- Aujla, S.S., I.Sharma and K.S.Gill (1992). Stable resistance in wheat (*Triticum aestivum*) to Karnal bunt (*Tilletia indica*). *Ind.J.agric.Sci.* 62: 171-172.
- Bedi, P.S., Sukhwinder Kaur and Pushpinder Paul Singh (1992). Recent developments in epidemiological research on Karnal bunt of wheat. In: Sokhi, S.S., Chahal, S.S. and Singh, P.P. (eds.), Progress of Plant Pathological Research. Ludhiana, India: *The Indian Society of Plant Pathologists*, pp.29-48.
- Dhaliwal, H.S., K.S. Gill, Paramjit Singh, D.S.Multani and Beant Bir Singh (1986). Evaluation of germplasm of wild wheats, *Aegilops* and *Agropyron* for resistance to various diseases. *Crop Improvement* 13: 107-112.
- Royer, M.H. and J.Rytter (1988). Comparison of host ranges of *Tilletia indica* and *T.barclayana*. *Plant Disease* 72: 133-134.
- Warham, E.J., A.Mujeeb-Kazi and V.Rosas (1986). Karnal bunt (*Tilletia indica*) resistance screening of *Aegilops* species and practical utilization for *Triticum aestivum* improvement. *Can.J.Plant Path.* 8: 65-70.

# The legume collection in Malang, Indonesia

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## Introduction

Soyabean is the dominant legume crop in Indonesia, followed by groundnut, mungbean and other minor legumes (cowpea, pigeon pea and lab lab bean). In the past, only local varieties were grown but recently many high-yielding improved (HYI) varieties were released (i.e. variety Orba, Wilis, Tidas, etc. for soyabean; Gajah, Rusa, Tapir, etc. for groundnut; and Walet, Manyar, Sriti, etc. for mungbean). These HYI varieties increased the national average of grain yield per hectare as well as national production overall. The release of HYI varieties was related to the activity of the germplasm section.

In 1982, a germplasm unit was established in MARIF (Malang Research Institute for Food Crops) with the objective of acquiring, maintaining, evaluating and documenting germplasm, in order to create an optimal choice of progenitors for breeders. From the start, collecting focused on varieties grown by farmers which usually constitute a rich source of adaptation to local conditions. Germplasm of soyabean, groundnut, mungbean and minor legumes is stored as seeds. MARIF's germplasm is coded MLG followed by a number.

There is great climatic variation, which produces a richness of legume crop. This is due to the influence of factors such as rainfall, altitude, and other important geographical features. This great diversity of legume crops should be collected.

The purpose of the present work was to collect local and introduced progenies of legumes and evaluate them for traits useful to breeders.

## Materials collected

Collecting activities were focused in the major legume areas such as Central Java, Bali, South Sulawesi and west Nusa Tenggara. In these areas, most farmers grow yellow-seed soyabeans, but some still grow black-seed soyabeans.

The mungbean varieties collected were small-seeded and differed in crop duration, leaf size and seed colour. The groundnut varieties differed in seed colour with either two or three seeds per pod.

To date, the legume germplasm collection holds a total of 2312 accessions (Table 1). Most of the accessions are local varieties collected from the major legume-growing areas of Indonesia. The other accessions are Indonesian improved varieties, breeding lines and introductions.

Table 1. Composition of the MARIF legume crops germplasm collection as of April 1992

Crop	Indonesian accessions			Foreign accessions	Total
	Local	Improved	Total		
Soyabean	550	284	834	207	1041
Groundnut	165	7	172	95	267
Mungbean	507	105	611	393	1004
Total	1222	396	1617	695	2312

## Evaluation of collections

Germplasm evaluation at MARIF is divided into characterization, standard evaluation and evaluation for specific traits. Characterization is the evaluation for traits easily observed. Standard evaluation is the preliminary evaluation of traits of agronomic importance. The most important traits of legume crops are maturity period, plant height, seed size, number of seeds per plant, shape and size of leaves and branching pattern. Evaluation for specific traits includes screening for resistance or tolerance to biotic and abiotic constraints, as well as investigating the quality aspects.

## Disease resistance

### Soyabean

Several soyabean genotypes were screened for major diseases such as peanut stripe virus (PStv), cowpea mild mottle virus (CMMV) and bacterial pustule. These diseases attack soyabean in farmers' fields and cause serious crop losses. The accessions most consistently showing low PStv indexes and most promising are all introduced varieties poorly adapted to Indonesian field conditions (Table 2) (Rulkens, 1992). Soehendi *et al.* (1989) found that 12 accessions were rated resistant to PStc and 23 moderately resistant.

Bacterial pustule, caused by *Xanthomonas compestris* pv. *phaseoli* (Smith) Dye, is a very common disease of soyabean which can cause early defoliation and yield reduction. Seven local varieties and 10 exotic cultivars showed resistance to this disease (Rulkens, 1992; Hardaningsih *et al.*, 1989).

Table 2. PStv index of tested accessions

Accessions	PStv index	Origin	Yield/plant % of Willis
Willis	100	Indonesia	100
MLG 2851	17	Brazil	15
MLG 2959	33	AVRDC	-
MLG 2870	36	Brazil	23

### Groundnut

Rust, caused by the fungus *Puccinia arachis* Speg, and late leaf spot, caused by *Cercosporidium personatum* (Berk and Curt.) Deighton, are fungal leaf diseases that seriously affect groundnut yield throughout Indonesia. The yield loss by both diseases is approximately 70% (Hardaningsih and Neering, 1989). Out of 267 groundnut accession available, three introduced varieties (MLG 7601, 7622, 7624) and one local variety (MLG 7646), gave the lowest score to rust. For late leaf spot disease, three varieties (MLG 7624, 7622, 7593) gave the lowest score (Rulkens, 1992). One introduced genotype from ICRISAT, India, ICGV 87165, was observed to be resistant and stable for rust and late leaf spot diseases (Soekarno and Sharma, 1989).

### Mungbean

Powdery mildew, caused by *Erysiphe polygoni*, and cercospora leaf spot, caused by *Cercospora canescen*, are two major leaf diseases that attack mungbean throughout Indonesia. Among 1004 accessions available at MARIF, three AVRDC lines were resistant to powdery mildew, while all local varieties showed a susceptible reaction. These resistant introduced lines can be used to improve the susceptibility of local varieties already adapted to the Indonesian area.

### References

- Hardaningsih, S., dan K.E. Neering. 1989. Pengendalian kimiawi penyakit daun Cercospora dan karat kacang tanah. Riset Seminar Hasil Pen. Tan. Pangan, Baliitan Malant. pp 15-18.
- Hardaningsih, S., A.A. Cook, and Yusmani. 1989. Evaluation of Soybean Cultivars and accessions for resistance to bacterial pustule in Penelitian Palawija. pp. 4(1):50-57.
- Rulkens, T. 1992. Genetic Resources of Palawija Crops at the MARIF March 1988-April 1992. MARIF/ATA-272. pp.56.
- Soekarno, B. and D. Sharma. 1989. Evaluasi ketahanan genotipe kacang tanah terhadap penyakit karat dan bercak daun, pp. 4(2):93-101.
- Soehendi, R., M. Anwari, and Y. Baliadi. 1989. Screening MARIF's soybean germplasm collection for resistance to peanut stripe virus. pp. 4(2):135-141 in Penelitian Palawija.

## Collecting wild relatives of yam (*Dioscorea* spp.) in the Espinhaço range, Brazil

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### Introduction

Genetic variability of the native yam (*Dioscorea* spp.) in Brazil is under threat from genetic erosion due to habitat destruction by deforestation and mining, and the indiscriminate use of fire to expand agriculture and cattle ranching, especially in the area of the Espinhaço range.

In this area, in the states of Minas Gerais and Bahia, campos rupestres prevail. These comprise vegetation with a grass and/or herbaceous strata composed of species of the Gramineae (=Poaceae), Cyperaceae, Eriocaulaceae and Xyridaceae, and spread shrubs from the Leguminosae, Lauraceae, Compositae, Melastomataceae and Velloziaceae families. A great number of endemic species are found growing over rocky and sandy soils. In addition to this vegetation, cerrados, mesophytic and gallery forests are also present.

### Materials and Methods

During 1992/1993, we collected the germplasm of threatened wild relatives and cultivated species of yam in the Espinhaço range (Fig. 1), including the national reserves of Serra do Cipó and Chapada Diamantina. The itinerary and data for field collections were selected on the basis of data obtained from the national herbaria vouchers as well as on previous knowledge about cultivation of these materials by small farmers. Also taken into consideration was the lack of collections of wild species from the east slope of the

Espinhaço range. The accessions were collected in the most preserved environments of the cerrados, mesophytic and gallery forests, along 4000 km of roads and in small farms.

The material collected during ten expeditions is kept in the Technological Centre Foundation of Minas Gerais State and in the National Centre of Genetic Resources and Biotechnology (Brasília, DF) as field collections. After collection, the herbarium specimens were dried and voucher specimens stored at Herbarium of Section of Earth Resources Technological Centre Foundation of Minas Gerais State, Herbarium of Department of Botany, University of São Paulo and Herbarium of National Centre of Genetic Resources and Biotechnology herbaria.

### Results and Discussion

Eighty-one wild and cultivated germplasm accessions were collected. Morphological analysis of plant material such as underground organs (mainly tubers and rhizomes) and seedling and reproductive parts (flowers, fruits and seeds) carried out since February 1993, in the laboratories of the above institutions show that *Dioscorea multiflora*, *D. regnellii*, *D. sincorensis* and *D. sinuata* var. *sinuata* were the most polymorphic of the wild species, both in morphology and flesh colour organs. These species have great potential for starch production and later utilization as human food. The most polymorphic species, mainly in tuber morphology, among the cultivated species, were *D. dodecaneura* and *D. alata*. The missions proved successful in collecting the rich diversity of native and cultivated species along the Espinhaço range. As a result of the floristic inventory, *D. kunthiana* and *D. microbotrya* were discovered for the first time in the national reserve of Serra do Cipó.

Results obtained, as well as the poor collections from the neighbouring and central areas of the Espinhaço range, especially in the physiognomic mosaic of the campos rupestres, showed that further collections of wild species of yam are required.

### Acknowledgements

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### References

- Pedralli, G. 1988. O. Inhame, Esse Desconhecido, Ciência Hoje, Rio de Janeiro, 8(46):58-62.
- Pedralli, G. 1991. Collecting Yam Germplasm in Brazil. Plant Genetic Resources Newsletter, Rome, (83/84):22.
- Pedralli, G. 1991. A. Biodiversidade dos Campos Rupestres. Biodiversidade em Notícias, Belo Horizonte, (3):3.



Fig. 1. Map showing collecting area (cross-hatched), 1992/93.



## Collecting plant genetic resources in Portugal

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In 1992, scientists from the N.I. Vavilov Institute of Plant Industry (Russia) and the National Institute of Agricultural Research (Portugal) collected plant genetic resources in Portugal. This country is of particular interest since it is situated on the lower west extremity of the Pyrenees peninsula and has abundant subtropical Mediterranean vegetation. Here one can find many wild relatives of grain legumes, fodder, grain, and industrial and fruit crops. Portugal is also one of the most ancient agricultural countries. It has rich experience in local breeding. But now in Portugal, local forms are being intensively replaced by commercial varieties and hybrids. This emphasizes the importance of collecting and maintaining local forms of different agricultural crops. In Portugal the soils are poor and acid, summer is hot and dry, and winter is wet. The lack of low winter temperatures does not permit the growth of real winter crops. Many spring and semi-winter crops such as lupin, fodder beans, peas and grains are sown in autumn. This practice presents special demands for breeders. The plants must accumulate great vegetative mass if they are to survive the wet winter. So, in Portugal, the basic material available for breeding may be assumed to contain resistance to abiotic factors, diseases and vermin. In this paper, we describe the genetic diversity and agronomy of major crop species and their wild relatives.

### Grain crops

Wheat is the main grain crop in Portugal. It occupies 630 000 ha. The centre of wheat breeding is the station in Elvash. Special attention is paid to resistance to abiotic factors and to grain quality. Work with rye, oats and barley is also carried out. Great success has been obtained in the breeding of triticale, such as producing new varieties of triticale without lax ear. Maize is second in importance in grain production after wheat. Local forms of maize are now very intensively replaced by commercial hybrids. Farmers presented us with some interesting forms both on the continent and on Madeira island, where rare samples with two rows of grains on the corn cob were found.

The main work involved in breeding, collecting and maintaining the genetic resources of maize is carried out by the experimental station in Braga (Pego, 1982).

### Lupine

One of the aims of the collecting mission was to collect wild and cultivated forms of *Lupinus albus* L., *Lupinus luteus*

L. and *Lupinus angustifolius* L. According to the results of our previous investigations (Kurlovich, 1991a, 1991b), the Iberian peninsula is the centre of origin of *L. luteus* and *L. angustifolius*. This supports the need for collecting this species in Portugal. Many scientists (Gladstones, 1974; Kazimierski and Nowacki, 1961; Mota, 1984; Simpson and Martins, 1984) consider Portugal to possess the greatest variety of lupin in the world, including very effective sources of resistance to such diseases as fusarial wilt and anthracnose. Iberian lupins contributed to the breeding of *L. luteus* for resistance to fusarium wilt and *L. angustifolius* for resistance to gray leaf spot and low temperature. Among Iberian lupins there is good initial material for the breeding of small-seeded varieties, with a large green mass. The local name for lupin is 'tremoco'.

### *Lupinus albus* L.

Peasants in Portugal grow this species of lupin for its high protein obtained after the extraction of alkaloids by means of boiling and soaking in flowing water. One can buy boiled lupin in markets, shops and restaurants. In the regions where lupin used to be grown, wild forms can be found on the roadside, in fields and at the foot of mountains. This form has great variety. Here one can find large-seeded and small-seeded and spring and winter ecotypes. From the south to the northwest of the country, the size of lupin beans and seeds decreases, but the duration of the vegetative period and florescence, number and size of leaf increases (Simpson and Martins, 1984). The greatest variety of forms was revealed in southern Portugal.

### *Lupinus luteus* L.

Alkaloid yellow lupin is traditionally grown in the south of Portugal (in the provinces Alentezhu and Algarvi). It is used as green fertilizer and forage for sheep. Here one can come across wild and savage forms of yellow lupine.

### *Lupinus angustifolius* L.

This species of lupin in Portugal can be found mainly as wild forms. Its local name is 'tremoco bravo'. Despite a wide variety of forms, its main forms are small-seeded ecotypes, which have grown for the longest time in the same place. They have primitive, dominant signs: small leaves and seeds, hard seeds and monopodial branching. Large-seeded and wild-leaved forms grow mainly on the

sides of roads and fields. This is evidence that Portugal is the centre of origin of wild *L. angustifolius* (Kurlovich, 1991a).

### Other grain legumes

Haricot, pea, chickpea, fodder beans, lentil and cowpea are widespread in Portugal. Mainly local varieties of this crop are cultivated. Haricot occupies annually 60 000-65 000 ha. Total production is 55 000-60 000 t, with an average yield of 1.5-1.8 tonnes/ha. Haricot is used both in ripe form and green blade. Climber and shrub ecotypes are grown. Green peas are cultivated mainly for long-term conservation. In addition, soaked green peas are very popular. Peas have a variety of seed and flower colour. Local forms also differ in the length of the vegetative period, weight of seeds and yield.

### Industrial crops

The main industrial crop is sunflower. Portugal has no endemic varieties, so farmers buy seed for sowing from Spain. New varieties are required to be tested by breeding institutions for adaptation to the local conditions, particularly for summer drought. These local conditions indicate the main demands placed upon the new varieties for sowing. One advantage of such unfavourable conditions is that they do not permit the majority of parasitic fungus to live. Another important crop is flax, cultivated in the northern part of the country and on Madeira island. This is a traditional crop for Portugal, but now the sowing area is greatly reduced. The main reason for this is that growing flax is very labour intensive. The majority of the remaining sown area is occupied by flax that is used for fibre. Linseed for oil is cultivated in mountainous regions as a winter culture.

The combination of low winter temperatures and reduced photoperiod delays growth and development until spring, when budding occurs. Absence of strong frosts permits linseed to survive in spite of the fact that it lacks real winter forms. Peasants mainly use local forms of flax and linseed, which makes a very valuable contribution to the conservation of plant genetic resources. Fibre of flax is used for handicrafts, and with the help of old wooden looms, is used to weave decorative items such as napkins and tablecloths. An initiative is being taken by the Ministry of Agriculture to revive the flax culture in Portugal. Flax varieties of foreign breeding such as Belinka, Natastha, Hera from Holland and Ariane from France are being imported for this purpose. These varieties, in the reduced photoperiod conditions found in the mountains in the north of Portugal, grow to a height of more than one metre, although this height is typical for long photoperiod conditions.

Other industrial crops are also cultivated in Portugal. These include rapeseed and safflower for oil production and groundnut for confectionery. Kenaf (*Hibiscus cannabinus*) is now being grown for paper production.

### Conservation in collections

The creation of plant genetic resources collections was initiated in Portugal in the late 1970s. Prior to this, there

were some uncoordinated collections of cultivated crops and wild relatives held in the country, but unfortunately these were lost in the mid-1970s. The National Institute of Agricultural Research (INIA) with stations in Elvash, Ueyrash and Braga holds collections of some major crops. Different methods are used for making collections. Collecting missions are organized annually. Scientists obtain seeds of local varieties from farms. International collaboration in collecting missions, especially with Spain, is also used, as well as the exchange of germplasm. Low temperatures of -18°C are used for storage of the collections. Firstly, seeds are dried in special dryers to a humidity of 5% and then packed in aluminium foil or plastic. Although a special storehouse has been built in Braga, in other stations domestic freezers are used, for economic reasons. Unfortunately, because of lack of money, specimens are not always studied and multiplied.

### Conclusion

Portugal is very rich in the plant genetic resources of cultivated crops and their wild relatives. All collected specimens will be evaluated, multiplied and used in breeding now or maintained for breeding in the future or for genetic investigations. For this purposes not only commercial crops but also their wild forms are necessary.

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### References

- Kurlovich B.S., 1991a, *Lupinus* L. (geography, classification, breeding). Dissertation for the Degree of Dr. Sci. (biol). St.-Petersburg. 48 p.
- Kurlovich B.S. Ecological-geographical classification of lupin and its use in breeding. St.-Petersburg, 1991b. 89 p.
- Gladstones J.S. *Lupinus* of the Mediterranean region and Africa. Bull. West. Aust. Dep. Agr. 1974. N 26. 48 p.
- Kazimierski T., Nowacki E. *Lupinus* of the Old World. Gen. Polonica. 1961. V.2. N 8. P.115.
- Mota M. *Lupinus* germplasm resources in Portugal. Abs. III rd. Inter. Lupine Conf. La Rochelle (France). 1984. P.8.
- Pego S.E. Genetic potential of Portuguese maize germplasm with abnormal ear shape. Dissertation for the Degree of Ph.D. Ames, Iowa. 1982. 249 p.
- Simpson M.J.A., Martins J.M.N. Distribution of plant types in *Lupinus albus* L. Abs. III- Inter. Lupine Conf. La Rochelle (France). 1984. P.7.

# Collecting okra, eggplant and their wild relatives in Nepal

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## Introduction

Under the IBPGR-funded okra and eggplant collecting programme in South Asia, with the cooperation of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, a survey and collecting mission was undertaken in Nepal from 1 to 14 October 1991, to assemble the germplasm of cultivated and wild *Abelmoschus* and *Solanum* spp. Part of the collected germplasm is maintained at NBPGR, New Delhi and part at the Central Institute of Plant Breeding and Biotechnology, Khamalter, Kathmandu, Nepal. Follow-up studies of the herbarium specimens have been undertaken at NBPGR Regional Station, Vellanikkara, Trichur, India.

## Cultivated okra (*Abelmoschus esculentus*)

Okra is a common vegetable in Nepal. Frequent as a backyard subsistence crop, it is seldom commercially cultivated. Due to close contact with neighbouring India, two important varieties released, namely 'Pusa sawani' and 'Parbhani kranti' are commonly cultivated in the Therai region of the country. In the hilly areas of the central and central-western regions and the Kathmandu plateau, local cultivars also occur. Seeds of improved types and local cultivars are available in all the weekly village markets visited by the authors. A total of 59 samples of cultivated okra and 12 samples of other species was collected (Table 1). A route map showing the distribution of okra and related wild species is also given in Fig 1.

Okra is usually grown under rainfed conditions during May to October, although, occasionally, a summer crop is also grown under irrigation. Most of the collections were made from farmers' fields. Local cultivars were sometimes found in hills up to an elevation of 1400 m asl. Although morphological variation was visible among the samples, the local cultivars appeared, from a subjective assessment, to have a common genetic base. The population of the crop had a number of common features which enabled intuitive classification into a group

Table 2. General variability noticed in okra

1. Sides on fruits:	5 sided	20
	6-7 sided	37
	Rounded	1
2. Colour of fruits:	Whitish green	13
	Light green	23
	Green	14
	Dark green	7
	Purple	1
	Light purple	1
3. Length of fruits:	Short	20
	Medium long	23
	Long	15
4. Thickness of fruits:	Thin	4
	Medium thick	39
	Thick	15
5. Plant height:	Very tall	9
	Tall	10
	Medium tall	27
	Dwarf	12

in which some prominent qualitative characters such as leaf shape, petiole colour, fruit shape and colour are very closely continuous in nature. This kind of intuitive methodology is sometimes used in conventional taxonomy and it helps workers to delimit races, subspecies and varieties. However, variation in plant height (dwarf to very tall), leaf lobing (very shallow to very deeply lobed), length of fruit (short to long), thickness of fruit (thin to thick), colour of fruit (whitish green to purple) (Table 2) and field tolerance to various pests and diseases were noticed. Among the two very distinct and rare types collected, the first had smooth, round fruits and this probably has an unknown exotic origin. The second type had a tall stature and purple pigmentation on all plant parts. It is probably related to the purple fruit type of the southern region of India. A similar type, namely CO1, has been released in India. An extremely tall type, reaching as high as 3.5 to 4.0m, was noticed in the central parts.

Table 1. *Abelmoschus* species collected in Nepal

<i>Abelmoschus esculentus</i>	cultivated	59
<i>A. manihot</i> ssp. <i>tetraphyllus</i> var. <i>pungens</i>	wild	8
<i>A. moschatus</i> var. <i>moschatus</i>	semi-wild	2
<i>Abelmoschus</i> sp.	wild	1
<i>A. crinitus</i>	wild	1

## Wild species of the genus *Abelmoschus*

According to the literature, four species of the genus *Abelmoschus* including the cultivated *A. esculentus* are known to occur in Nepal. Masters (1892), in the Flora of

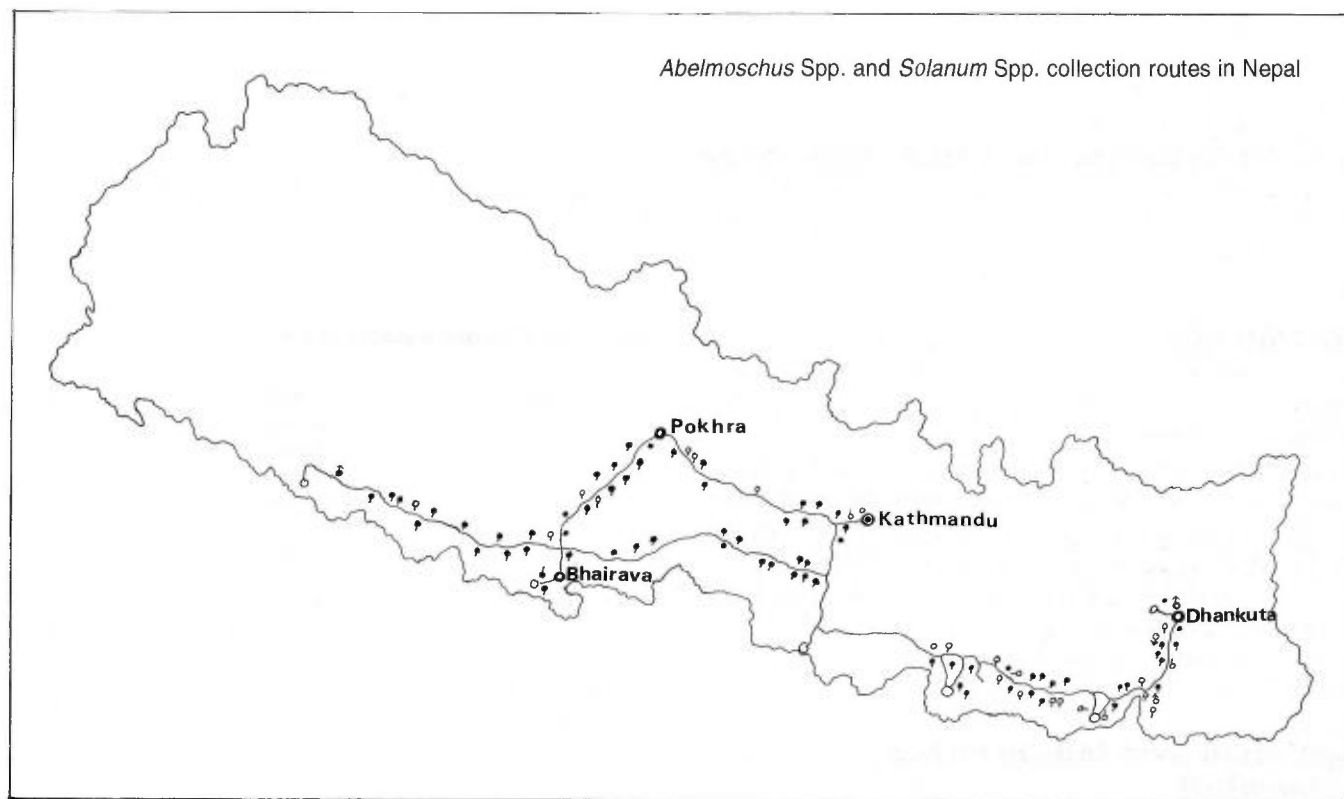


Fig. 1. Distribution of okra and related wild species. Routes in Nepal

British India, reported two species, *H. cancellatus* syn. *A. crinitus* Wall. and *H. pungens* syn. *A. manihot* subsp. *tetraphyllum* var. *pungens* from this region. *A. moschatus* var. *moschatus* is one that is cultivated all over the tropics in Asia and Africa, based on the classification adopted by the International Okra Workshop (IBPGR, 1991).

1. *Abelmoschus esculentus* (L.) Moench
2. *A. crinitus* Wall.
3. *A. tetraphyllum* (roxb. ex horneum.) R. Graham var. *pungens* (Roxb.) Hochr.
4. *A. moschatus* Medicus Subsp. *moschatus* var. *moschatus*

Among these, *A. tetraphyllum* var. *pungens* has a wider distribution in hills from 500 to 1800 m asl. This species is typically bush-like with sharp hairs on stem and petiole. *A. crinitus*, on the other hand, is very rare and has been collected only once from the forest grasslands of the central region at an elevation of 250 m asl. *A. moschatus* var. *moschatus* has been collected from two locations. Although observed at other sites, samples were not collected due to the absence of any apparent difference. It usually occurs in and around the backyards in villages and is probably semiwild. Another sample which closely resembled *A. tetraphyllum* var. *pungens* was collected near a village at about 250 m asl. It appears that this type is adapted to lower elevation. In Dhankutta, at about 1 800 m elevation, the farmers grow a species as a hedge plant and use its

leaves and flowers as a medicine. It is closely related to *A. tetraphyllum* and is probably a high-elevation ecotype of the same species.

### Eggplant (*Solanum melongena*) and its wild relatives

In general, the areas surveyed by the authors exhibited less variability in eggplant, mainly under two distinct agroclimatic situations. In the first situation, eggplant is mainly cultivated as a commercial crop in small holdings in the 'Terai' plains along the southern borders lying closer to India. Here water sources and rainfall are good and a few local types, along with some improved cultivars (probably originating from India), are found. Fruits

Table 3. List of *Solanum* species collected in Nepal

Species name	No. of collections
1. <i>Solanum melongena</i> var. <i>melongena</i>	20
2. <i>S. verbascifolium</i>	2
3. <i>S. surattense</i>	2
4. <i>S. indicum</i>	1
5. <i>S. viarum</i>	1
6. <i>S. torvum</i>	1
7. <i>S. sisymbriifolium</i>	1
8. <i>S. nigrum</i>	1
9. <i>Solanum</i> sp.	1

vary from medium to large, spherical and oblong with purple and green colours. The second largest area surveyed averaged 400 m asl in certain villages situated along the highway from Nepalganj to Pokhra. This region is hilly and highly undulating. The crop is mostly rain-fed and is grown in backyards and in kitchen gardens. In hills lying above 800 m elevation, it was very rare. Mainly small purple fruit type was observed. A single observation was made of a very long fruit type (35 cm) in a kitchen garden in Pokhra. Generally, the frequency of eggplant cultivation was much less in Nepal.

Seven taxonomic relatives of the eggplant were collected, namely *S. verbascifolium*, *S. nigrum*, *S. torvum*, *S. indicum*, *S. viarum*, *S. surattense* and *S. sisymbriifolium*. One unidentified species was also collected. Their distribution was very sporadic. *S. indicum* was collected at only one site. Most common among these collected species was *S. viarum*. Most of these taxa are either not genetically related or are distantly related to the cultivated eggplant. A total of 30 collections belonging to eight distinct species of *Solanum* as given in Table 3 have been made.

## References

- IBPGR. (1991). Report of an International Workshop on Okra Genetic Resources. 8-12 October, 1990. IBPGR, Rome pp 2-3.  
Masters, M.T. (1892). Malvaceae in Hooker's Flora of British India. Vol.1:317-353.

The Board of Directors has the honor to present to you the 1990-1991 Annual Report of the Board of Directors. This report provides a summary of the activities of the Board and the Corporation during the year. The Board has been pleased to continue its commitment to the community and to the advancement of the Corporation's goals. The Corporation has achieved significant milestones during the year, and we are proud of the progress we have made. We look forward to continuing our efforts in the future.

The Board of Directors consists of the following members: [List of names]

The Corporation has achieved the following milestones during the year: [List of milestones]

We are grateful to our staff and to the community for their support and cooperation. We look forward to continuing our efforts in the future.

**References**

The following references are provided for your information: [List of references]

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## Emergency collecting missions to Albania, 1993

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### Introduction

In 1993, a collaboration project was set up between IBPGR, the National Research Council (CNR) of Italy, the Germplasm Institute, Bari (Italy), the IPK (Institut für Pflanzengenetik und Kulturpflanzenforschung), Gatersleben (Germany) and the Agricultural University of Tirana (Albania) with the main aim of collecting, preserving and evaluating the plant genetic resources of Albania. The recent breakdown of the old Albanian political system, characterized by economic self-sufficiency with limited foreign trade, led the scientific community to promote the organization of expeditions to collect autochthonous plant germplasm in the country. In fact, due to foreign investments, opening of the borders and large imports of seeds, there is a great risk of local landraces being replaced by introduced varieties. In addition, the fact that many Albanian farms are in a state of neglect since they are no longer organized in agricultural cooperatives or state farms, considerably increases genetic erosion.

The first two IBPGR collecting missions were carried out in Albania in 1993, and these were largely organized by the Germplasm Institute, Bari. The collecting teams consisted of two researchers from IPK, two from Germplasm Institute and an Albanian local expert of agriculture and ecology. Italy has a long tradition of interest in cooperation with Albania. There has been a long and fruitful collaboration between Bari and Gatersleben in exploring the Mediterranean area (see e.g. Hammer *et al.* 1992). The Gatersleben genebank is specifically connected with plant genetic resources of Albania through early collections (1941/42) of H. Stubbe (see Stubbe 1982, p. 208) carried out in this country.

The highest priority of the recent missions was for the collection of Albanian landraces but, when the opportunity arose, wild relatives were also gathered (Fig. 1).

### Geography and sampling strategies

Albania has tremendous geographical variability, comparable to that of the whole of Italy but compressed into an area of 28 748 km<sup>2</sup> (ca. 340 km from north to south and ca. 148 km from east to west). Lower Albania in the western part of the country can be clearly distinguished from the rugged mountainous northern, central and southern parts, which cover nearly three-quarters of the territory. Climatic conditions are submediterranean in the west (warm summers with a relatively short drought



**Fig. 1.** Calcareous slopes with *Pinus heldreichii* at the Albanian Riviera (1025 m a.s.l.). Collecting relatives of cultivated plants from natural sites has been a part of exploration work

period and mild wet winters) but continentally influenced in the east (tempered summers and cold winters) (Berxholi and Qiriazi 1986). Wild vegetation varies from dominating anthropogenic shrub formations (garique, maquis, Sibljak) and oak forests in the lower belts, to vast mountainous woods of beech and pines and alpine grasslands (compare Horvat *et al.* 1974, Markgraf 1932).

The two collecting missions were organized for July and September during the main harvest time of many vegetables, cucurbits and pulses in the lowlands and mountainous regions. Because of the relatively good accessibility of the villages and farms in the plain and hilly parts of western Albania, it was possible to explore a large area (Fig. 2). Swamps and marshes along the coast have been drained, mainly during the socialist period. On the large fields of former cooperatives modern uniform varieties of winter wheat, maize and alfalfa predominate. The hills, often recently terraced, are covered by plantations of fruits, olives and citrus (only in the south) or vineyards. Because genetic erosion has increased considerably in the field crops of lower Albania, collecting activities have been concentrated in house-gardens and small fields around the villages (Fig. 3). The more promising mountainous regions have been visited relatively infrequently until now, because many villages and farms there can only be reached by landrover or on foot (Fig. 4).

In accordance with the different agroclimatic situations of the collecting sites, the samples were collected either directly from the fields or from seed storages. During the second mission some samples, still immature during the first expedition, were collected and delivered to the exploration team by some local agrarian experts in charge of communal farms. In other cases interesting germplasm was found and taken from small local markets (Fig. 5). At all sites, a larger bulk sample was gathered.

## Results

A total of 220 samples, belonging to 67 different species, were collected from 36 sites, mainly representing vegetables, pulses and cereals. Additionally, herbarium material (72 numbers in 104 sheets) was taken of wild and cultivated plants. Several of the species, listed in Table 1, and observed during the missions, are not yet reported in

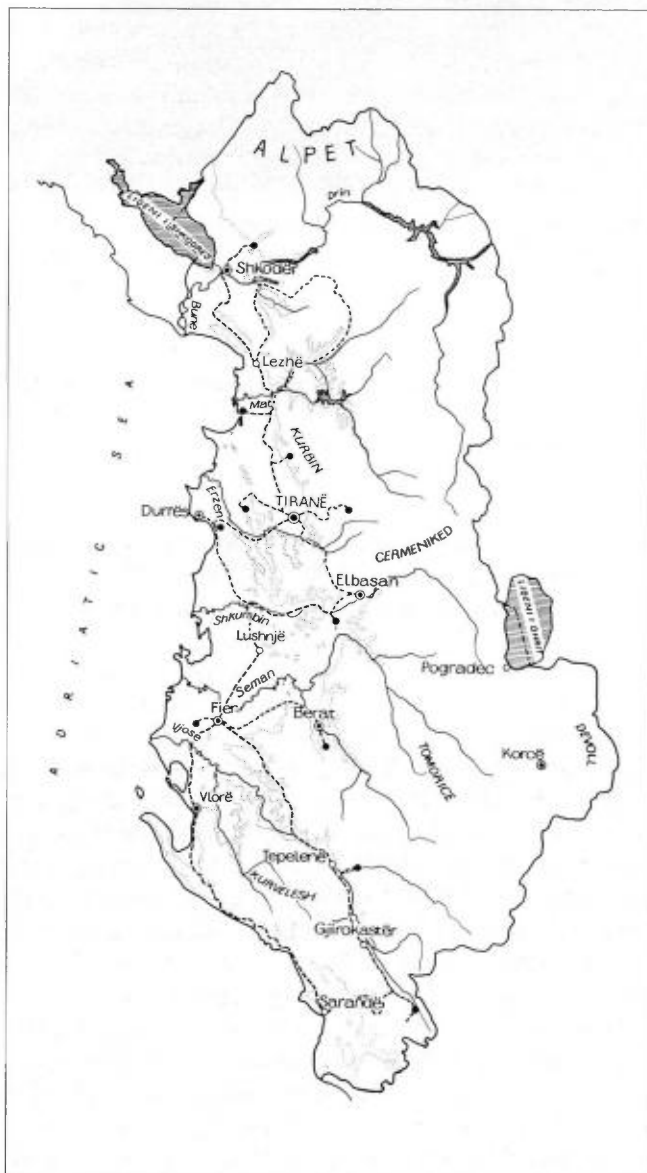


Fig. 2. Route of the collecting missions in July and September 1993

Table 1. Material collected during the missions in the western parts of Albania, 1993

Crop	Number of accessions		Total
	first mission	second mission	
<i>Abelmoschus esculentus</i>	2	1	
<i>Allium ampeloprasum</i>	1	3	
<i>Allium cepa</i>	7	3	
<i>Allium guttatum</i>	2		
<i>Allium sativum</i>	3	1	
<i>Atriplex hortensis</i>		2	
<i>Brassica oleracea</i>	3	3	
<i>Capsicum annuum</i>	6	3	
<i>Citrullus lanatus</i>	3	1	
<i>Cucumis melo</i>	6	5	
<i>Cucumis sativus</i>	2	1	
<i>Cucurbita maxima</i>	1	1	
<i>Cucurbita pepo</i>	3	1	
<i>Daucus carota</i>	2	1	
<i>Lactuca sativa</i>	4	2	
<i>Lagenaria siceraria</i>		1	
<i>Lycopersicon esculentum</i>	1	2	
<i>Petroselinum crispum</i>		3	
<i>Raphanus sativus</i>		1	
<i>Spinacia oleracea</i>		5	
Vegetables and cucurbits	46	40	86
<i>Lathyrus</i> spp.		7	
<i>Lens culinaris</i>		4	
<i>Phaseolus vulgaris</i>	19	14	
<i>Pisum sativum</i>	2	1	
<i>Vicia faba</i>	2	3	
<i>Vicia</i> sp.	1	1	
<i>Vigna unguiculata</i>		1	
Pulses	24	31	55
<i>Aegilops</i> spp.	4		
<i>Avena sativa</i>	1	5	
<i>Dasyphyrum villosum</i>	4		
<i>Hordeum bulbosum</i>	1		
<i>Hordeum marinum</i>	1		
<i>Hordeum vulgare</i>		3	
<i>Secale cereale</i>		2	
<i>Sorghum bicolor</i>	1	2	
<i>Triticum monococcum</i>		1	
<i>Zea mays</i>	2	18	
Other grasses	4		
Cereals and grasses	18	31	49
<i>Anethum graveolens</i>		1	
<i>Carthamus lanatus</i>	1		
<i>Linum usitatissimum</i>		1	
<i>Mentha pulegium</i>	1		
<i>Mentha spicata</i>	1		
<i>Nicotiana tabacum</i>	1	2	
<i>Origanum vulgare</i>	4	1	
Other wild species	9	8	
Other crops and wild relatives	17	13	30
Total	105	115	220



Fig. 3. Terraced hills with small house-gardens, olive plantations and larger fields for cereal and maize cultivation



Fig. 4. Mountainous villages situated on often hardly accessible steep slopes (Kudhësi, Albanian Epirus)

the local floras (e.g. *Vigna unguiculata* from Vrisera, south-east of Gjirokastrë; cp. Paparisto *et al.* 1988, Demiri 1979, Piperi and Kajno 1990).

### Cereals

*Triticum monococcum* as a relic crop was found at Progonat southwest of Tepelenë, with the grain being used as feed for animals and the straw for making roofs. No spring wheat varieties are present in Albania while for barley both winter and spring types exist. The governmental Instituti i Misrit dhe i Orizit at Shkodër, devoted to maize and rice research, stores ca. 1600 and 400 accessions respectively. These came from specific collecting missions carried out since 1956. Most of the maize samples are Albanian landraces, while those of rice are derived from Bulgarian, French and Italian (cv. Lido) varieties.

### Pulses

A large genetic variability of *Phaseolus vulgaris*, was recorded for seed size, shape and colouring patterns as well as growing period, drought resistance and growth habit. An interesting landrace of chickling vetch (*Lathyrus sativus*) could be collected in the southern Albanian Epirus. This extensive crop has reportedly also become rare in other European mountains (Hammer *et al.* 1992, Kühn *et al.* 1984). Here it is used for both animal and human consumption (e.g. for the local dish *pai*, prepared from spinach, pumpkin or vegetable marrow). When the testa is removed, farmers report that it can be utilised like common bean without problems of lathyrism. Cowpea (*Vigna unguiculata* subsp. *unguiculata* cv.-gr. *unguiculata*) could be observed and collected in southern Albania.

### Vegetables

Local onions are cultivated on a large scale and show remarkable variability. Mild-tasting types with large red and white bulbs are preferred for fresh consumption. Primitive types of kale (*Brassica oleracea* var. *viridis*) can be found frequently in the gardens of the Northwest. Okra



Fig. 5. Local market in Tirane



**Fig. 6.** Local germplasm of melons (*Cucumis melo*) is endangered by geneflow from newly introduced varieties

(*Abelmoschus esculentus*), known locally as *bamia*, is a very popular vegetable in Albania; variability was observed for plant height (even more than 2 m), cultivation time (usually from April to September/October) and fruit length. In the southern part of Albania (Gjirokastrë area), several pure local landraces of watermelon and melon were grown up until 10-20 years ago; today modern foreign varieties (e.g. Bulgarian and American) predominate and the old germplasm has nearly disappeared and/or hybridized with imported material (Fig. 6). According to information gathered during the mission, it might be possible to find some of these old cultivars in a few Greek villages close to the Albanian border.

#### Other crops

Cultivation of the famous Albanian tobacco has greatly decreased over recent years. Nevertheless, even a local variety was collected in the traditional growing centre at Drisht, northeast of Shkodër. In the small gardens around Albanian farm houses, as well as maize, vegetables, cucurbits, pulses and spice plants, a lot of fruit trees such as fig, plum, apricot, cherry, pear, quince, apple and persimmon are grown, including some interesting local fruit trees. Frequently imposing century-old olive trees can be found and these are reported to have been introduced by Venetians (C.T.I. 1939). Spice and medicinal plants are widely used in the country from both cultivated (Ahmataj and Eku 1984) and natural sites, such as the typical Albanian tea of *Sideritis syriaca*. Trees of *Populus*, *Platanus* and *Quercus* spp. are often pollarded for feed and fodder purposes. Other information on plant genetic resources will be prepared in a checklist of Albanian cultivated plants, which is developed and used as a working basis for the missions. This method is described by Hammer (1991). The material collected was divided into three parts and stored at Tirana, Gatersleben and Bari. The accessions will be included in the specific multiplication, evaluation and characterization programmes of the above institutes.

#### Conclusions

The first results of the 1993 missions to western Albania underline the urgent need for further activities to save the tremendous variability of old landraces of cultivated plants which are thought still to be found in the isolated mountainous regions of northern, central and southern Albania. It is important to constitute and safeguard a collection representative of the Albanian germplasm (Xhuveli, 1989, Xhuveli *et al.* 1992). In the near future, the following activities are scheduled in order to carry on the project successfully: i. well-prepared expeditions to the remote localities not yet visited by collecting teams (particularly parts of the Albanian Alps, the area close to the Macedonian border, remaining regions of the Albanian Epirus and specific agroecological niches not investigated during the first two expeditions); ii. evaluation of some already existing Albanian collections (e.g. maize, rice, *Triticum* spp.); iii. compilation of a catalogue of Albanian plant germplasm.

The participants of the missions owe local administrations and the Albanian farmers a great debt of gratitude for kindly supporting their activities.

#### References

- Ahmataj, H. and Eku, K., 1984. *Bimat aromatike*. Tirana, 135 pp.
- Berxholi, A. and P. Qiriazi, 1986. Albania. A geographical view. 8 Nantori, Tirana, 135 pp.
- C.T.I. (Consociazione Turistica Italiana), 1939. Albania. Ministero degli Affari Esteri Sottosegretariato di Stato per gli Affari Albanesi, Milan, p. 95.
- Demiri, M., 1979. *Përscaftues Bimësh (Flora e Vogël e Shqipërisë)*. Tiranë, 898 pp.
- Hammer, K., 1991. Checklists and germplasm collecting. *FAO/IBPGR Plant Genetic Resources Newsl.* 85, 15-17.
- Hammer, K., H. Knüpfner, G. Laghetti and P. Perrino, 1992. *Seeds from the Past. A Catalogue of Crop Germplasm in South Italy and Sicily*.
- Horvat, I., V. Glavac and H. Ellenberg, 1974. *Vegetation Südosteuropas*. G. Fischer, Jena, XXXII + 768 pp.
- Kühn, F., H. Ohle and K. Pistrick, 1984. *Katalog der 1981 in der CSSR gesammelten indigenen Kulturpflanzenarten*. Kulturpflanze 32: 153-196.
- Markgraf, F., 1932. *Pflanzengeographie von Albanien*. E. Schweizerbart, Stuttgart, VI + 130 pp.
- Paparisto, K., M. Demiri, J. Mitrushi and X. Qosja (eds.), 1988. *Flora e Shqipërisë*, vol. I. Tirana, 457 pp.
- Piperi, R. and Kajno, K., 1990. *Flora Mjekësore e Korçës*. Tirana, 408 pp.
- Stubbe, H., 1982. *Geschichte des Instituts für Kulturpflanzenforschung Gatersleben der Deutschen Akademie der Wissenschaften zu Berlin (1943 - 1968)*. Akademie-Verlag, Berlin, 428 pp.
- Xhuveli, L., 1989. *Bankat Gjentike*. *Shkenca dhe Jeta* 2, 5-10. Tiranë.
- Xhuveli, L., H. Cobani, A. Sallilari, V. Malo, A. Osja, P. Sotiri and F. Daci, 1992. *Resurset Gjentike të Bimëve, Koleksionimi, ruajtja dhe perdorimi*. *Agronomia* 1, 71-74.

## Collecting crop germplasm in Nigeria

**S. Appa Rao<sup>1</sup>, M.H. Mengesha<sup>1</sup>, C. Nwasike<sup>2</sup>, O. Ajayi<sup>3</sup>, O.G. Olabanji<sup>4</sup> and D. Aba<sup>2</sup>**

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Pearl millet is the most important crop in the Sahelian zone and is second to sorghum in the Guinean and Sudanian zones of northern Nigeria. Pearl millet is usually intercropped with a variety of crops; around Zaria, 24 crops were observed in 174 combinations. However, groundnut, sorghum and cowpea are the most common intercrops (Nwasike *et al.*, 1982). Pearl millet cultivars in Nigeria differ so much that Stapf and Hubbard (1934) classified the short-duration Gero millet as *Pennisetum typhoides* (Stapf and Hubb.), and the long-duration Maiwa as *P. maiwa* (Stapf and Hubb.). As these two forms cross easily they were grouped into a single species *P. americanum* (Brunken, 1977). Pearl millet from Nigeria was found to be a good source of genes for early maturity, high head volume and large grain size (Mengesha and Appa Rao, 1982).

Although pearl millet is grown over five million hectares and several forms were found in Nigeria, only the early maturing forms were collected (Appa Rao *et al.*, 1988). Hence, a mission to collect the late-maturing Maiwa and Dauro millets was jointly launched by ICRISAT, the Ahmedu Bello University (ABU), Samaru, Zaria and the Lake Chad Research Institute (LCRI), Maiduguri during November-December 1992.

### Areas explored and germplasm collected

The collecting team, consisting of S. Appa Rao (ICRISAT), C. Nwasike, D. Aba (ABU) and O.G. Olabanji (LCRI), covered most of the pearl millet growing areas, keeping in mind the distribution of Dauro and Maiwa forms and complementing the 1982 route. Although the mission was mainly for collecting late-maturing millet, we also collected early maturing millet and other ICRISAT crops. For collecting, the coarse grind sampling method was followed. Most of the late-maturing Dauro and Maiwa, and pigeonpea samples were obtained from farmers' fields, while most of the early maturing millet, sorghum and groundnut were obtained from farmers' stores or local markets. During this mission, we collected a total of 1050 samples, consisting of 748 pearl millet, 201 sorghum, 79 groundnut, 16 pigeonpea and 7 finger millet samples. All the samples collected were divided into three sets; one set was brought to the ICRISAT Center, one set was stored in the ICRISAT Sahelian Center, Niamey, Niger, and the

third set was for use by Nigerian scientists; sorghum with ABU and millet with LCRI.

### Diversity observed

#### Pearl millet

Three distinct forms of millet were collected: the early maturing form called Gero; the late-maturing transplanted form called Dauro, and the late-maturing and directly planted form called Maiwa.

#### Gero

This is the most extensively grown form of millet in Nigeria. It is commonly grown in the low-rainfall Sahel zone; Sokoto, Borno, and Katsina provinces. With the first rains, the field is made, often manually, into broad beds and furrows at 50 to 100 cm apart. About 10 to 25 seeds are dibbled in each hill spaced at 50 to 100 cm apart. After 3-5 weeks, the crop is thinned to 3-7 seedlings in each hill. Gero millet is planted in June and harvested in September. Depending on the shape and size of head, Gero millet is called 'Wutsiya Biri' which literally means monkey tail, or Wuya Bajini meaning cow neck; the former produces thin, very long (80-100 cm long) spikes which resemble a monkey tail; while the latter produces very thick, short (30-50 cm long) compact spikes. The most common grain colour is grey, but light grey and ivory white are occasionally found. We did not come across any field grown exclusively for the long bristled form, although a few heads with long bristles were found in a low proportion. Two distinct forms were recognized based on endosperm texture. Mar gero has soft endosperm and the farmers often eat them raw; Gayamba gero has partly corneous endosperm. Different forms of Gero were collected.

#### Zongo

In areas bordering Niger, a form of millet which produces very long (up to 100 cm long) spikes is grown. Though the head is long, seed set towards the base is very poor and the grain is very soft and large in size.

#### Boudouma

This is an early maturing millet extensively grown around Lake Chad in low-rainfall areas of the Sahel zone. It

grows up to 2 m tall and produces 3-5 heads, which are 20-30 cm long, thick and very compact. It was reported to be the earliest maturing in Nigeria. The grain is of medium size and almost starchy.

#### *Gwa gwa*

This is another early maturing millet grown in Borno province. It matures later than and produces heads which are larger than Boudouma.

#### *Mori*

The Kunari people bordering Cameroon call millet Mori. It grows to about 2 m tall and produces 2-5 tillers with small heads. The grain is medium in size and partly corneous.

#### **Maiwa**

This strongly photoperiod-sensitive, late-maturing form which produces long heads, is often grown mixed with sorghum (Fig. 1). It is grown in relatively high rainfall areas in heavy soils. Maiwa is directly planted in the field where 5-20 seeds are dibbled in each hill. Often gap filling is done after removing the excess seedlings from adjacent hills. In some areas, an early maturing maize is planted with the first rains and after harvesting maize, Maiwa and cowpea are planted in August. The Maiwa form matures



Fig. 1. In northern Nigeria, late-maturing pearl millet called Maiwa which produces more than 1-m-long heads is often intercropped with sorghum

late and plants grow to over 3 m tall. It has several long leaves, and dense long hairs on the leaf blades. Around Tegina, the heads are short with a conical shape.

#### **Dauro**

Usually grown at high altitudes, dauro is mostly transplanted and is strongly photoperiod sensitive. It is concentrated around Kafanchan Tegina and other high altitudes with relatively high rainfall. In general, a nursery is raised in June or July. After the first rains, broad beds and

furrows are made 1.0-1.5 m apart. Usually 3-5 week old seedlings are transplanted onto the top of the ridges. Sometimes 2-4 rows are also planted. In some areas farmers directly plant the Dauro millet in July and uproot the excess seedlings to transplant to another field. Normally 3-7 seedlings are planted in each hill. Dauro millet produces thin, medium-sized heads with partly corneous grains which are attractive and shiny. Dauro millet has very long (10-30 cm) peduncles. Mature heads of Dauro



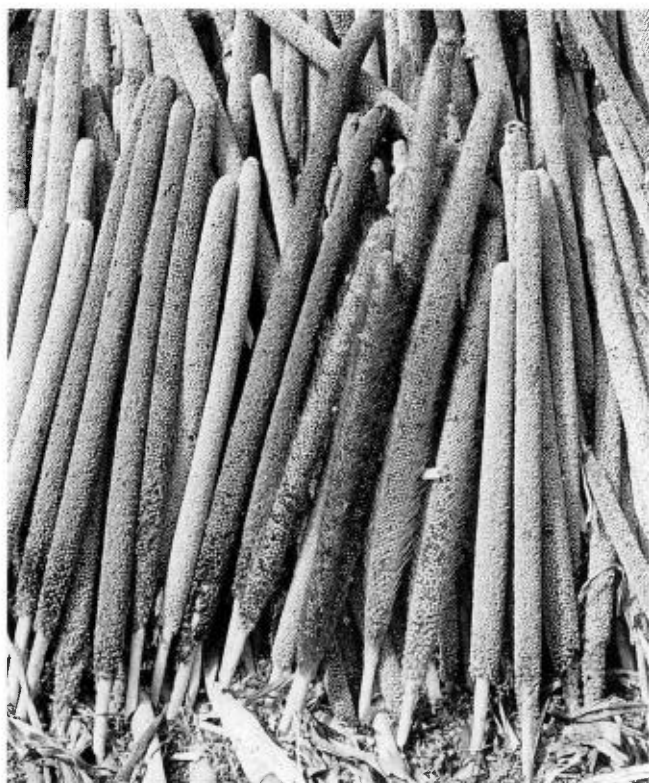
Fig. 2. A family of Hausa farmers carrying dauro millet from farm to threshing floor

are snapped with the long peduncles, tied into bundles and carried by women and children (Fig. 2) on their heads.

#### **Variation within a field**

Considerable variation was found within a field for plant height, flowering and maturity. However, there is relatively more variation for spike shape. A number of spike forms were found which vary for spike length, thickness, shape, bristle length and colour, head compactness, grain colour, shape and size (Fig. 3). Fields with predominantly short bristles have a few with long bristles. Dauro millet (see Fig. 2) appears to be more uniform than Gero, while Maiwa (Fig. 4) is intermediate in variability. Farmers often select long, thick, compact heads for seed purposes on the threshing floor, which resulted in the evolution of long heads in Nigeria. Some of the prominent millet accessions are AON 20, a gero with short, thick, compact heads, AON 21, a gero with thin long heads resembling a monkey tail, AON 81, 516, 517 and 619 with yellow grain, AON 522 which produces purple grain, AON 527 which produces heads with drooping peduncles, and AON 514 with 2-m-long spikes.

*Pennisetum* species grow wild almost throughout Nigeria. *Pennisetum polystachyon* and *P. pedicellatum* grow wild on the roadside and in millet fields at low altitudes. *P. purpureum* was found on the banks of ephemeral streams around Saminaka, Kassa, Wamba and in high-rainfall



**Fig. 3.** Enormous variation for head and grain characters was found in a single farmer's field



**Fig. 4.** Scientists from Ahmedu Bello University, Lake Chad Research Institute and ICRISAT gathering information and samples of long heads of Maiwa from a farmer

areas at high altitudes. *P. ramosum* was found in heavy black soils around Biu in Borno state. The weedy forms which shatter their grain at maturity are not common in Dauro and Maiwa millets though they are more frequent in Gero millet.

### **Sorghum**

In northern Nigeria, sorghum is the second most important crop after millet. It is grown in relatively high-rainfall areas and in heavy soils. Because of their panicle

shape, grain shape and grain colour, farmers call them Kaura, Fara-fara or Guineense. In general, sorghum grows to more than 3 m tall, stems are very thick, and it produces a small head. Panicle shapes vary from compact, oval and durra to very loose drooping guinea forms. Panicle length varies considerably from 5 to 70 cm. Grain shape, size, colour and endosperm texture also vary considerably. Different types of sorghum grown in Nigeria have been described by Curtis (1967) and Prasada Rao *et al.* (1985).

Kaura sorghums have large grains with yellow endosperm and show a wide range of variation in panicle shape and compactness, glume color, size and shape. These sorghums, with their characteristically elongated panicles, are considered by farmers to be high yielding and of excellent grain quality. Fara-fara is the common name given to large-seeded, white-grained sorghums grown in northern Nigeria. They display a range of variation for panicle compactness and shape, glume and testa colour. Guineense sorghums are tall and mature later than Kauras or Fara-faras. They have semi-loose, pendulous panicles with long branches. At maturity, the flattened grains are obliquely twisted and exposed between the long, widely gaping glumes. Guineense sorghums belong to subrace Guineense of race Guinea whose grains disarticulate readily from the spikelets



**Fig. 5.** Sorghum heads carried on camel back from farm to threshing floor

and are of good quality. Pericarp colour varies from red to yellow or white. The mesocarp is often thin, giving the grain a translucent appearance. In general, the testa is white with corneous endosperm. Guinea sorghums of West Africa are known to be weather resistant (Prasada Rao *et al.*, 1985). The other races found are Chad, Sokoto, Umbellate and Tunicate (Curtis, 1967). Farmers also call sorghum Badari (yellow grain), Goronyo (gooseneck), Rosuba (chalky white grain) or Mauri (white grain) (see Fig. 5).

### Groundnut

This is grown as a sole crop or as a mixed crop with millet or sorghum. Bunch types are commonly grown. Most of the samples were obtained from the farmers' stores or local markets. Variation was observed for pod size, number of kernels per pod and kernel colour and shape.

### Pigeonpea

This is commonly grown as a hedge crop around houses, in the backyard and on strips. It is grown extensively on a commercial scale around Kafanchan, Zonkwa, Wamba, Kukum Taji, Kagoro and Kachia. Those grown in backyards are the perennial type, while those grown on field scale are annuals. Pods are green or with a splash of purple tinge at the base. The number of seeds varied from four to eight and were usually white in color.

### Finger millet

So far, finger millet from Nigeria has not been described. It is called Tamba and is grown in small areas around Bukuru, Shere, Fuskam and Kachia in plateau state. Very primitive forms with shattering grain are grown in small areas. Variation was observed for shape, size and number of fingers per head.

### Pests and diseases

On pearl millet, downy mildew was found almost throughout the millet-growing areas. Downy mildew incidence was higher around Bukuru, Zonkwe, Gwantu, Kagoro and in isolated areas. Downy mildew incidence is relatively less on Dauro than on Maiwa as the farmers discard the downy mildew affected plants while transplanting. Smut was observed around Zonkwe, Gwantu, and in some areas sporadically. Ergot incidence was observed around Wushishi Bukuru, though we did not find considerable damage. Head caterpillar, which tunnels the spike spirally, was found in several places. Stem borer incidence was higher on Maiwa than on Dauro. Stalk borer (*Busseola fusca*), long smut and leaf diseases on sorghum were observed.

### Grain processing and utilization

Pearl millet and sorghum are the staple food crops which are used to prepare a variety of traditional food preparations. They are used to prepare a porridge known as Kunu, Tuwo, Fura or Koko. Fried cake called Marsa and a thick cake called Kamzu are prepared around Maiduguri.

### Possible use of millet germplasm

The Gero and Zongo forms are a good source of high head volume. Boudouma and Gwa Gwa are good sources of early maturity, high tillering and large grain size. Dauro is a good source for cold tolerance. Maiwa and Dauro are good sources for good grain quality and high biomass production.

### Genetic erosion

Improved cultivars are becoming popular, replacing the traditional cultivars. Early maturing maize is becoming popular in traditional sorghum and millet growing areas. However, as no improved cultivar is yet available in Dauro and Maiwa forms, farmers grow traditional cultivars only.

### References

- Appa Rao, S. Nwasike, C. and Mengesha, M.H. 1988. Collecting pearl millet germplasm in northern Nigeria. Genetic resource progress report 56, Patancheru, ICRISAT.
- Brunken, J.N. 1977. A systematic study of *Pennisetum* sect. *Pennisetum* (Gramineae). American J. Bot., 64:161-176.
- Curtis, D.L. 1967. The races of sorghum in Nigeria, their distribution and relative importance. Expl. Agric. 3:275-286.
- Mengesha, M.H. and Appa Rao, S. 1982. Evaluation of pearl millet germplasm from Nigeria. MILWAI Newsletter 1:2.
- Nwasike, C., Baker, E.F.I. and Egharevba, P.N. 1982. The potential for improving millet in farming systems of the semi arid tropics of Nigeria. Agriculture and Environment 7(1): 15-21.
- Prasada Rao, K.E., Obilana, A.T. and Mengesha, M.H. 1985. Collection of Kaura, Fara-fara and Guineense sorghums in Northern Nigeria. Journ. d' Agric. Trad. et de Bota. Appl. 32:73-81.
- Stapf, O. and Hubbard, C.E. 1934. Gramineae. Pages 954-1070. in Flora of Tropical Africa (Prain, D., ed. Vol. 9, London, UK: L. Reeve & Co.).

## Millet germplasm resources in China\*

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Bountiful genetic resources of millet have been collected and preserved in the National Genebank of China. To date, a total of 34 214 millet accessions have been documented and stored, including 26 222 accessions of foxtail millet, 7421 proso millet, 121 finger millet, 103 pearl millet, 116 barnyard millet, 3 teff, 2 kodo millet, 2 little millet and 224 *Setaria* spp. (except foxtail millet).

Foxtail millet (*Setaria italica* (L.) Beauv.) originated in China and is one of the most important crops, especially in northern China. Foxtail millet accessions (26 222, including 23 368 landraces and 2 854 breeding lines/cultivars) are distributed over the whole country. However, they are concentrated in northeast and north China, and the Loess plateau, i.e. Shanxi, Hebei, Shandong, Henan, Liaoning, Heilongjiang and Jilin provinces. From abroad, 461 accessions were introduced, mainly from east, south and west Asia, and Europe. In addition, *Setaria vidiris*, *S. pumila* and *S. faberii* are widely distributed over the temperate zone and the subtropics of China and 130, 30 and 26 samples were collected, respectively. One sample each of *S. forbasiana*, *S. palmifolia* and *S. magna* came from Shaanxi, Fujian and Xinjiang provinces, respectively.

Proso millet or broomcorn millet (*Panicum miliaceum* L.) is also one of the oldest and most important crops in northern China. The 7421 accessions of proso millet germplasm (including a few wild proso millet) are mainly distributed over the Loess plateau and the Mongolia plateau, i.e. Shaanxi, Shanxi, Neimenggu (Inner Mongolia), Gansu and Hebei provinces. As with foxtail millet, the distribution of proso millet landraces has close relations with its ecological and physiological characteristics. Both crops have high drought and sterile tolerance and adapt well to the arid and semi-arid climatic conditions in northern China.

The 121 accessions of finger millet (*Eleusine coracana* (L.) Gaertn.) landraces collected are mainly from southwest and south China i.e. Hunan, Guizhou, Yunnan, Xizang (Tibet) and Guangxi provinces. The 116 accessions of barnyard millet (*Echinochloa frumentacea* (Roxb.) Link) landraces are mainly from northeast China, i.e. Heilongjiang and Liaoning provinces. Most pearl millet

(*Pennisetum glaucum* (L.) R. Br.) samples preserved are from abroad and 14 pearl millet accessions from China are also indirectly from other countries. Additionally, seven accessions of teff (*Eragrostis tef* (Zucc.)), kodo millet (*Paspalum scrobiculatum* L.) and little millet (*Panicum sumatrense* L.) were introduced from the International Crops Institute for the Semi-arid Tropics (ICRISAT).

A total of 770 millet accessions from abroad has been collected and preserved in China. They are mainly distributed by ICRISAT. Unfortunately, countries of origin have not always been clearly known because of lack of background materials and frequent international germplasm exchange.

\*Tabulated data on accessions are available from IPGRI HQ citing ref. No. 94/14



## News and Notes

### From Australia...

The Australian Tropical Forages Genetic Resource Centre (ATFGRC) was founded in 1982, although the task of assembling a major tropical forage genetic resource started as far back as the 1960s. Initially, the emphasis was on developing a genetic resource for pasture improvement in the tropics and subtropics of Australia, following on from the successes in the south of the Continent. Being virtually the first on the scene, an international role soon developed, and seed samples are now distributed for research and pasture development throughout the world's tropics. Currently, the seed collection stands at about 25 000 accessions, with a major emphasis on legumes, although grasses are also well-represented. A sophisticated database enables ready access to the extensive data stored. ATFGRC staff are also actively involved in research into the genetic resource and into improving the effectiveness of its utilization through germplasm characterization.

The administrative centre of the ATFGRC is at St Lucia, a suburb of Brisbane, although the genebank and associated laboratories are at Samford, on the outskirts of Brisbane. An associated laboratory at Townsville, in North Queensland, shares responsibility for quarantine and seed production. Experimental studies are undertaken at a range of sites in the Australian tropics and subtropics.

Nowadays, one of the key words is 'communication', and this newsletter is one of the ways in which the ATFGRC plans to provide better communication with its clients and potential clients. The plan is to produce two newsletters a year, providing updates on the group's activities. We would welcome any feedback from clients as to whether they appreciate yet another item of unsolicited mail in their in-trays!

### ATFGRC now enlarged

This year, our GRC has grown. We welcome Dr Bryan Hacker as co-project leader. Bryan brings a new project which aims to select plants for revegetation of mine spoils, and an ecophysiologicalist will be appointed later this year to address this problem. Dr Dick Date brings his collection of legume root nodule bacteria and insights into rhizobiology. Dr Chunji Liu is soon to join us from the Cambridge Laboratory at Norwich, England, to apply molecular marker technology to assess genetic variability, identify centres of diversity and develop core collections. Bruce Pengelly commences new studies to assess diversity in drought tolerance in key species. Shannon Burns joins us to provide a range of research support activities. All staff are accepting new responsibilities.

ATFGRC will balance its role as a national centre with international networking. It will provide for the priority needs of plant improvement for tropical animal production systems while also developing genetic resources for the future.

*(Reprinted from The Australian Tropical Forages Genetic Resources Centre Newsletter Issue 1. 1993, c/o CSIRO Cunningham Lab, 306 Carmody Road, St Lucia, Queensland 4067, Australia)*

### ...and from Holland...

#### **A summary of the Dutch government's policy paper on tropical rainforests, 1992, The Hague, Netherlands**

##### **1. Introduction**

The natural environment is currently suffering very serious and widespread damage, and developments in and around tropical rainforests are a cause for particular concern. Tropical rainforests are among the most severely threatened ecosystems in the world and their disappearance has led to the very serious degradation of many tropical regions.

The government feels that it shares responsibility for finding solutions to this worldwide problem as developments in industrial and tropical countries are interrelated. The government is aware that the problems concerning the tropical rainforests are of a very complex nature, yet it is precisely for this reason that a coordinated and cohesive policy is needed.

##### **2. Tropical rainforests**

An estimated 1.2 billion hectares of tropical rainforest still remain, concentrated in three regions.

Tropical rainforests comprise very complex ecological communities with an incomparable variety of plant and animal species, many of which remain unidentified. The ecosystem of the tropical rainforest functions mainly as a closed cycle. An enormous biomass is contained within such regions.

In addition to their great natural value, the tropical rainforests possess many material and non-material functions. Where such functions entail human intervention, the aim is to tailor use to the natural ecosystem, leaving it intact and preserving the variety of species. Those forests that have already suffered damage may have lost some of their natural value, but, if managed properly, may still be of significance to CO<sub>2</sub> management and to protection of

the environment in the area concerned, as well as a source of raw materials and income for the local population.

#### *Natural value*

The tropical rainforest is the natural habitat of an estimated 50 to 90 per cent of the earth's living species. These form an ecological community in which relationships are very functional and often delicately balanced. Ecosystems are still evolving.

#### *Regulatory function*

Tropical rainforests regulate the microclimate, the local climate and play a role in worldwide circulation cycles. They prevent erosion, retain water and ensure the availability of fresh water.

#### *Genetic pool (preservative function)*

Conserving a diversity of genes is in the interests of such fields as pharmacy and - in the context of crop improvement - agriculture, horticulture and forestry.

#### *Tourism*

Tropical rainforests provide opportunities for tourism.

#### *CO<sub>2</sub>*

Tropical rainforests fix great quantities of atmospheric CO<sub>2</sub>. Degradation of the tropical rainforest interferes with the atmospheric CO<sub>2</sub> balance.

#### *Living areas (support function)*

Many millions of indigenous tribal people are dependent on the complex ecosystem.

#### *Forest products (production function)*

Local peoples exploit forest products such as timber, latex, cane, bamboo, resins, oils, spices and meat, which are also exploited on a commercial basis. Commercial exploitation often leads to the degradation of the forests and, indeed, to their destruction, certainly where current practices are concerned. Long-term timber production and forest management systems are virtually nonexistent.

As a consequence of many factors, such as poverty, unemployment, rapid population growth, and the unequal distribution of land, production means and prosperity, the long-term social relevance of the tropical rainforests and the direct and indirect consequences of their disappearance or degradation have come to assume a low priority in the present activities of the countries in which the tropical rainforests occur.

Official FAO (Food and Agricultural Organisation of the United Nations) figures for 1980 show that an estimated 9 million hectares of rainforest are disappearing each year. Provisional FAO Forest Resources Assessment figures for 1990 present a much graver picture. Annual deforestation in the tropics, i.e. including the rainforests, is now estimated at 17 million hectares out of a total of 1.2

billion remaining hectares. In addition, large areas of forest are becoming degraded.

The consequences of deforestation and degradation are far-reaching and to a great extent irreparable. Species of plant and animal life and complete ecosystems are disappearing. The future of indigenous peoples is directly threatened. In those places where forests disappear, serious erosion occurs and the climate is adversely affected. Further consequences include deterioration of landscapes, floods and a reduction in the fertility of the soil. Erosion and changes in local water regimes make the prospects for future generations especially bleak. Sources of tropical hardwood and other forest products are becoming exhausted.

Degradation and deforestation are the result of present practices. The most serious degradation of the tropical rainforest is caused by land reclamation for short-term agricultural purposes. The method generally used is to burn down tracts of forest, whereby large quantities of CO<sub>2</sub> are released. Approximately 80% of deforestation occurs in this way. The factors underlying such land reclamation include poverty, overpopulation and unemployment.

Other immediate causes of the degradation of the forests include the construction of infrastructural facilities such as roads and power plants, mining and the commercial exploitation of timber. In the current situation, the timber industry takes little or no account of the need for sustainability of supply or of the other functions of the forest. Exploitation of timber is generally followed by land reclamation for lack of long-term forest management based on balanced land-use planning.

### **3. Current policy**

The National Long-Term Forestry Plan represents policy geared to increased self-sufficiency as far as timber is concerned and to an expansion of forest acreage in the Netherlands. Tropical forests are given particular attention in the Nature Policy Plan, and policy will continue to address itself to the protection of tropical rainforests.

The National Environmental Policy Plan mentions targets involving the achievement in the short term of a worldwide balance between afforestation and deforestation and expansion of forest acreage from the year 2000. These targets were made more specific during the international conference for environment ministers in November 1989, where it was agreed that efforts would be made to expand forest areas worldwide by 12 million hectares annually by the beginning of the next century.

Forestry policy within the framework of development cooperation is mainly geared to the prevention of deforestation and soil degradation, to the encouragement of rational sustainable management of natural resources, to meeting the needs of local populations - including the urban poor - for forest products, and to preserving biological variety.

The Tropenbos Programme is intended to promote research into tropical forests. Forestry education specially geared to participants from developing countries is provided at Wageningen Agricultural University (LUW), the International Institute for Aerospace Survey and Earth Sciences (ITC) and the International Agricultural Centre (IAC).

The Netherlands is a member of many international organizations and is signatory to a number of conventions which have a bearing on the preservation of the forests in the tropics.

The Tropical Forestry Action Plan (TFAP), which is coordinated by the FAO, is regarded as the operational framework within which worldwide activities may be conducted in the field of tropical forests and afforestation. The Plan was established at the instigation of FAO, UNDP, the World Resource Institute and the World Bank. IUCN developed the World Conservation Strategy (WCS) in cooperation with UNEP, WWF, UNESCO and FAO) in 1980. As far as the tropical rainforests are concerned, the focus has been placed on sustainable usage in ecological terms. The International Tropical Timber Agreement (ITTA) provides scope for producing and consuming countries to reach agreements on guidelines for sustainable timber production.

UNESCO'S Man and Biosphere (MAB) programme is geared towards the preservation of natural resources.

The World Heritage Convention, adopted by the general assembly of UNESCO, aims to conserve the cultural and natural heritage of the world. The Netherlands is not a party to the Convention as yet.

Species of trees growing in tropical rainforests may be listed under the Convention of International Trade of Endangered Species (CITES), thus combating their use for trade purposes.

The World Bank and the Regional Development banks play a very significant role in the funding projects in and around tropical rainforests.

The Special Programme for Developing Countries (SPDC) of the International Union for Forest Research Organizations (IUFRO) acts as the coordinating mechanism for research in the tropics.

The Consultative Group on International Agricultural Research (CGIAR) recently added forestry research to its mandate.

#### 4. Policy considerations

The government regards the following aspects as vital in addressing the problems.

##### *Political and social backing in the tropics*

Governments in the countries involved are frequently poorly informed as to the actual significance of tropical rainforests and are therefore unprepared to initiate and implement political change.

##### *Sufficiently powerful authorities and sufficient motivation among the population*

National governments are formally responsible for the tropical rainforests. In practice, however, the actual exploitation is in the hands of a limited number of groups, such as businesses, landowners and, to a certain extent, farmers not in the possession of land. An essential prerequisite to altering the existing state of affairs is an administrative structure with both the power and the will to guide procedures, to break undesired power and ownership structures and to eradicate undesired intermingling of interests. In addition, the cooperation of the local population is needed.

##### *Legislation and law enforcement*

Legislation and law enforcement are both prerequisites to and significant instruments in a more conscientious policy on tropical rainforests.

##### *Alternative scope to meet the needs of the local population and the government in the short term*

Altering the present state of affairs, i.e. the exploitation of the forest and subsequent land reclamation, is only possible if alternative means can be found in the short term to meet the needs now provided for by exploitation and land reclamation. The main requirements in this respect are the availability of alternative land, sustainable agriculture, employment, income and hard currency.

The above processes all in fact take place in the developing countries themselves. North-South relations are equally relevant to the problems of deforestation and degradation.

##### *Global political and economic relations*

The preservation of the tropical rainforest depends on the creation of employment and the establishment of sustainable agriculture in developing nations. Yet on balance, the competitive strength of the industrially developed world - with or without the protection of trade barriers - only serves to delay the economic progress of the south.

We need to lay far more emphasis on economic development in our relations with these nations. And it is fully consistent with this aim that we focus on the interactions between their debt burdens, development potential, and problems of deforestation and environmental degradation.

Good political and economic relations between industrially developed and developing nations will be basic necessities if we are to solve problems through dialogue and material aid. We will, however, have to start looking more critically at both the consequences of our existing trade relations and the financial aid we give to development projects, since they both affect the future of the tropical rainforest.

##### *Awareness of the importance of the tropical rainforest*

A greater global awareness of the importance of the tropical rainforest is essential. More knowledge about ecosys-

tems, the diversity of animal and plant life, hydrological cycles, and the climatological influence of the tropical rainforest will all help enhance global awareness of the need to preserve these forests and hence speed up the implementation of measures to meet this need.

### 5. Policy objective

The Netherlands Government is aware of the complexity of the problems and of its own limited ability to help solve them. In shaping its policy objective, it has examined five key issues:

- the right of sovereign states to autonomous control and use of the rainforest within their territory
- the responsibility and commitment that all nations share in the face of global problems
- the existence of international agreements, treaties and organizations
- the fact that the tropical rainforest's destruction will exacerbate the already fragile position of indigenous peoples dependent on it for their existence
- the varying ecological and socioeconomic situations among regions and even nations where tropical rainforest is situated, along with differences in their relations with the Netherlands.

With all this in mind, the government has adopted the following central policy objective as a frame of reference in determining its stance on individual cases:

to encourage the preservation of the tropical rainforest through balanced and sustainable land and forest use, with a view to halting the current rapid process of deforestation along with other environmental damage and degradation.

### 6. Policy strategies

The Netherlands Government's policy towards the world's virgin forest - including its tropical rainforest - is aimed at its protection, and furthermore at managing and exploiting its natural resources in order to safeguard its preservation.

Nationally and internationally, the government aims to implement this policy by means of nine basic strategies. The first five are linked directly to the tropical rainforest and its restoration, and the last four are back-up strategies aimed at creating conditions conducive to implementation.

#### I. Active protection of surviving virgin rainforest

In view of the rapid pace at which the destruction of and damage to the tropical rainforest are proceeding, the gov-

ernment will encourage its protection wherever it survives, especially in a virgin state. The Government will do likewise in areas that are well placed for restoration. To these ends, it will back existing and initiate new projects.

#### II. In principle, no collaboration with projects and developments that are harmful or potentially harmful to the rainforest

The Government will not take part in or support projects or developments that are harmful or potentially harmful - either directly or indirectly - to the tropical rainforest. Indeed, government policy will be actively aimed at preventing such damage.

#### III. Encouraging planned land use and land management along with sustainable agriculture and forestry

As part of its contribution towards sustainable rural development, the Government will encourage planned land use and land management, with the accompanying aim of preserving and managing the tropical rainforest. It will back individual projects aimed at easing the pressure on the tropical rainforest.

#### IV. The tropical timber trade: controlled harvesting; encouraging the formulation and implementation of long-term planned timber production

The Government will encourage early measures to bring the harvesting of timber into line with sustainable forest management and put a complete halt to timber trade activities in virgin forest. These measures are intended to ensure a long-term future for the production and export of tropical hardwood. The Government will also call for an early prohibition on the harvesting of timber from endangered tree species.

#### V. National and international encouragement for afforestation and re-afforestation projects

With a view to preserving the tropical rainforest, safeguarding the world's timber supply, and limiting the rise in the atmosphere's dioxide content, the Government will call for a vigorous policy of afforestation and re-afforestation in the Netherlands, the EC, and indeed throughout the world. This is in line with the Noordwijk conference's objective (November 1989) of an annual net growth rate for forested land of 12 million hectares by the year 2000.

#### VI. Strengthening institutions and legislation; empowering local populations

The government will call for the empowerment of individual nations to take all structural measures necessary - including the development of competent institutions - for the preservation of the tropical rainforest and its sustainable use.

#### VII. Strengthening the political and social base in tropical nations

In the nations concerned, the Government will encourage the strengthening of political and popular recognition of

the usefulness and necessity of preserving the tropical rainforest for sustainable use.

#### VIII. *Improving economic relations and relieving the debt burden*

The Government will focus its economic relations with the nations concerned on creating better conditions for development. This will include the formulation of constructive proposals for the relief of these nations' debt burdens insofar as such proposals are consistent with Government policy as a whole. These measures will help improve conditions for sustainable development and hence for the preservation of the tropical rainforest.

#### IX. *Increasing scope for national and international tropical rainforest policy by strengthening research and institutions*

The Government will promote more efficient targeting among national and international research bodies, international organizations and central government on the problems of deforestation and environmental degradation in tropical nations. Such improvements will involve modifications at management, procedural and funding levels.

### 7. Financial and staffing overview

The Government is making both funds and staff available for policy implementation. Experts are being hired for various purposes: to support policy development in the nations concerned; to formulate, monitor, evaluate and implement projects; and for education and research purposes.

Funding made available in the framework of development cooperation will be spent in accordance with the basic principles and priorities of development cooperation policy. Funding for support to policy development and forest projects is also intended for tropical forests other than rainforests.

An effort is being made to spend at least one-third of these amounts in a way which will benefit the tropical rainforest either directly or indirectly.

Experts will be recruited for policy development, projects and research from a number of ministries, universities and other institutions. Their numbers will be determined by the funds available. 1990 staffing was estimated at 50 to 70 persons; by 1994, this number will have risen to about 100.

### ...and from Norway...

#### **Biological Biodiversity in Norway - A country study**

*DN-report 1992 - 5b, Odd Terje Sandlund (secretary/editor), 1992, Directorate for Nature Management, Tungasletta 2, N-7005 Trondheim*

### Summary

This report presents an overview of the biological diversity found in Norway. The factors determining biological diversity are discussed first, describing how climate, topography, biogeography and the history of immigration have shaped Norwegian nature. A description follows of the species diversity on land, in fresh water and in the sea, as well as among domesticated plants and animals, and the most important types of habitat in undisturbed, little disturbed and anthropogenic environments are also described. The most important developmental trends in Norwegian biological diversity, and the work done to protect and manage nature in a sustainable manner, are described. The value of biological diversity in economic terms is then analyzed, and the report concludes by listing the most urgent research and analysis requirements relating to Norwegian biological diversity.

#### 1. Factors determining the biological diversity

The biological diversity of Norway is characterized by a number of important factors:

- Norwegian ecosystems are young; it is only about 18 000 years since the ice began withdrawing, exposing land for colonization
- there are great variations in climate because of the large south-north extent of the country, the mainland stretching from about 58° N to more than 71° N, and when Svalbard is included, Norway covers 23 degrees of latitude
- habitats vary greatly as a result of a large altitudinal gradient and broken topography
- the populations of many species in Norway are marginal in relation to their main ranges; Norway lies on the southern fringe of the boreal zone and the northern fringe of the boreonemoral zone.

#### 2. Taxonomic survey

Norwegian species diversity embraces about 33 000 species of plants and animals (excluding viruses, bacteria and algae). The number of insect species is estimated to be around 22 000, whereas there are thought to be approximately 5000 species of fungi. A few large taxonomic groups, such as fungi and crustose lichens, as well as a number of insect families, are poorly known. The same applies to the benthic marine fauna. Of vertebrates, approximately 450 species of birds, 57 terrestrial mammals, 25 marine mammals, about 150 marine fish, 40 freshwater fish, 5 amphibians and 5 reptiles have been observed. Only a few endemic species are known in the flora and fauna, one of these being the Norway

lemming (*Lemmus lemmus*), which is only found in Fennoscandia.

Among the domesticated plants, it is largely certain species of grass that have their origin in Norwegian flora. Production of cereals, oil plants, potatoes, vegetables and fruit is largely based on imported species, even though Norwegian cultivars have been developed in some cases.

All species of domestic animals have been imported into Norway, except for those fish species which have gradually been domesticated and brought into use as table fish. However, Norwegian breeds of some domestic animals have also been developed.

### 3. Habitat diversity

Because of the great distance from south to north, the large altitudinal gradient (0 - 2500 m a.s.l.) and substantial variations in geology and geomorphology, the habitat diversity in Norwegian nature is huge. The most important types of terrestrial habitat in mainland Norway are forest (about 37%), areas with sparse or no vegetation (including rock and coastal heath, about 47%) and mires, wetlands and fresh water (about 12%; there are approximately 211 000 lakes in Norway). The long coastline (57 000 km) also results in the seashore being an important habitat. Characteristically, the vegetation in many of the habitats is dominated by only a few species; the boreal forest, for example, is dominated by the conifers, pine and spruce, along with three or four deciduous trees.

### 4. Developmental trends

The development of industry, roads and railways, and of operational methods in forestry has led to a great reduction in large, continuous areas of undisturbed forests and mountains in the course of the last century.

Otherwise, the developmental trends in Norwegian biological diversity have been dominated in recent years by the problems created by long-distance airborne pollution. This pollution has led to damage to freshwater ecosystems in southern Norway and eastern Finnmark, and a large number of fish stocks have become extinct. The terrestrial ecosystems have also been hit. Many local populations of freshwater fish and invertebrates have also been damaged or made extinct by the introduction of disease-bearing organisms and constructional disturbances in the river systems.

Most birds of prey and all the large predators have been greatly reduced during this century, and species such as the wolf and peregrine falcon are now seriously threatened. Several species which depend upon the agricultural landscape that was shaped by old methods of farming are also threatened, for example the corncrake. Other species have reacted positively to protection measures. Populations of polar bear, white-tailed eagle and beaver have increased following their protection. Other

species, such as elk, red deer and roe deer, have increased in numbers as a result of stock management and favourable changes in forest vegetation. This shows that measures to conserve species can be successful as long as the habitats of the species are protected.

Many local breeds and cultivars of domestic animals and cultivated plants have ceased to be used during the last fifty years. Many of the old forms have been preserved with the help of protected stocks and gene banks.

### 5. Measures for conservation and sustainable use

Existing Norwegian legislation provides a good basis for the conservation and sustainable management of biological diversity. However, many of the most serious threats to the diversity are of a regional or international character, and can only be reduced through international agreements and measures taken in other countries. Norway is therefore actively participating in international efforts for environmental conservation. Various monitoring programmes have been initiated to trace the development in long-distance airborne pollution and selected biological parameters.

Norwegian nature enjoying various forms of protection status comprises just over 6% of the mainland area, and more than 50% of Svalbard is also protected. Plans exist for protecting an additional 6% of the mainland. Even when these plans have been implemented, there will still be certain types of habitat that are greatly under-represented in the protected areas. This applies, for example, to productive boreal forest and other productive types of habitat.

*Ex situ* conservation of wild species is largely confined to salmon, but a comprehensive genebank system has been built up for domestic animals and cultivated plants, partly through cooperation on a Nordic level.

### 6. Economic value of biological diversity

Traditional harvesting of biological production through hunting, fisheries, forestry and agriculture has an annual gross value of nearly 34 billion NOK in Norway. The public costs of nature conservation add up to nearly 10 billion NOK. The wild species which are harvested also have a value beyond that of the pure meat value, in the same way as the non-harvested species have a value. This non-use value must be calculated to obtain a measure of the social benefits and costs of preserving biological diversity. It will be especially important to map and evaluate the benefits, so that these may be directly compared with the cost of species conservation. In cases where this has not been done, there has been a tendency to underestimate the benefits of preserving biological diversity when concrete decisions have been made.

Attempts to estimate the benefits to be derived from the wild species seek to take into account both the use value and the non-use value. There is considerable uncertainty about these values. In the three studies of wild species and habitats (large predators, salmon and stationary freshwater fish, and coniferous forest that resembles virgin forest) from which data on these values have been published, the conclusion has, nonetheless, been reached that it is highly profitable from a social viewpoint to preserve these species and habitats.

### 7. Need for research and analysis

When areas are to be managed with a view to conserving their biological diversity, relatively detailed knowledge is required about:

- the habitat demands of the various species and which elements in their surroundings are decisive for maintaining strong populations
- variations in types of habitat within the areas, to secure habitat diversity
- which species are found in the various types of habitat.

In addition to providing the homes which the organisms need, many habitats have intrinsic cultural-historical and aesthetic values. Many areas that have already been protected in Norway have been very superficially described or mapped, and most lack a satisfactory basis for implementing management; this applies to both national parks, nature reserves and landscape reserves.

Monitoring programmes have been started during the last few years that place greater emphasis on biological parameters in addition to environmental parameters of a chemical and physical nature. The extent of such monitoring should be further expanded, with a view to providing quantitative documentation of possible changes in the fauna and flora over time. Such programmes must include selected types of vegetation (vascular plants, bryophytes and lichens), invertebrates (insects, marine invertebrates) and also vertebrates (birds, freshwater fish, mammals).

The following research tasks should be given priority in research concerned with biological diversity: 1) loss of species and populations (what is the real scale of the loss, what ecological role does genetic diversity play, what are the ecological effects of the reduction in diversity?). 2) Knowledge of landscape ecology to enable an assessment of the consequences for animals and plants of changes in their surroundings, including information about the importance of habitat fragmentation. 3) Identification of key species and their role in the ecosystems. 4) Increased systematic and ecological knowledge about less well-known groups of plants and animals as a basis for assessing the environmentally related changes in biodiversity and im-

proving the basis for consequence evaluations. 5) Increased knowledge about the social benefits and costs of preserving biological resources and biological diversity.

### 8. Norwegian versus global biodiversity

At present, approximately 1.4 million of the world's species have been described, but the majority are still unknown to science. Estimates of the total number of species on the Earth vary greatly, from 5 to 30 million. However, scientists agree that the largest number of species is to be found in tropical areas, particularly in tropical rain forests. Tropical rain forests constitute barely 7% of the Earth's surface, but contain approximately 50% of the total number of plant and animal species.

Compared to tropical biodiversity, the total number of organisms in the northern temperate and arctic zones, where Norway is situated, is small. Estimates suggest that the total number of species (excluding viruses, bacteria and algae) in Norwegian ecosystems is approximately 33 000.

Within any small area in the tropics there are numerous unique, or endemic, species, which are not found anywhere else in the world. Destruction of even a relatively small area of tropical rain forest will therefore cause the disappearance of many unique plant and animal species. Extinction of species is a one-way process; we have no way of recreating the extinct species, even in the few cases when we know what they were.

In the temperate zone, most species have a wide distribution; most Norwegian species are also found in other parts of Europe, Siberia, or even North America. Can it then be so important to preserve the biodiversity of Norway which, in comparison with that of tropical areas, may appear relatively uninteresting. Norwegian biodiversity is, nonetheless, synonymous with Norwegian nature; it forms the basis for human settlement in this country. Ecosystems in Norway are relatively young, but an immense range of habitats has been able to evolve owing to the great variation in topography and the large latitude and altitude gradients. The natural environment is also the reason why many foreigners consider Norway an attractive place to visit. One characteristic of Norwegian nature is the interplay between agricultural land and other anthropogenic habitats, and relatively undisturbed ecosystems. Norway is a marginal area for many species, and their populations may therefore differ genetically from those in the main range of the species. The importance of conserving the biodiversity found in Norway should therefore be obvious.

Although we do not fully understand the consequences of reducing natural biodiversity over large areas, it is safe to say that great changes in biological diversity will lead to dramatic changes in those environments which we like to consider as typically Norwegian: natural scenery, forms of agricultural landscape and various urban landscapes. The economic output of agriculture, forestry, fisheries and hunting may also be reduced.

### ...and the Philippines...

The government of Switzerland and IRRI have signed an agreement to further the safeguarding and preservation of the genetic diversity of rice. It was signed by His Excellency, Dr Hanspeter Strauch, Ambassador of Switzerland to the Philippines, and Dr Klaus Lampe, Director General of IRRI.

Under the agreement, the Swiss Development Cooperation, a government aid agency, will provide IRRI with a grant of US\$3 286 000 for a 5-year project to improve the long-term preservation of rice genetic resources.

The project will enable IRRI to continue collecting and conserving wild and cultivated rices. In the past, collections have been made from easily accessible areas. The Swiss support will allow scientists to go on collecting expeditions in the less accessible highlands of mainland Southeast Asia and the lowland swamps of Central Kalimantan, Indonesia.

Environmental degradation, which leads to the loss of biodiversity, has put pressure on scientists to intensify the collection of rice seeds. Adverse climate conditions, such as the 1992 drought in southern Africa that possibly destroyed wild rice plants yet to be studied by scientists, serve as warnings of how biodiversity can be threatened.

A large part of the world's genetic resources of rice are conserved at IRRI in the Genetic Resources Center. About 80 000 accessions are stored in the centre's genebank. These are made freely available to rice scientists everywhere. IRRI, with a global mandate for rice and rice-related research, is an autonomous, nonprofit, international agricultural research and training center located in Los Banos, about 60 km south of Manila.

The world's wild rices remain under-represented in IRRI's germplasm collection. Most of Irian Jaya, Indonesia and parts of southern Africa, for example, are regions rich in wild rices that have yet to be explored. Wild rices may look like weeds but they are a rich reservoirs of genes, some of which control natural resistance to major rice pests and diseases. For example, *Oryza nivara*, a wild rice from India, is the only known source of genes for resistance to grassy stunt virus disease.

Germplasm conservation and evaluation are internationally recognized global responsibilities of IRRI. The Institute will continue to collaborate in conserving rice germplasm with IPGRI, national rice research institutes, nongovernment organizations, and community and farmer groups.

An important component of the Swiss-IRRI project will be the development of appropriate methods for farmers themselves to conserve rice varieties. In Asia, organizations such as the Southeast Asia Regional Institute for Community Education (SEARICE), TREE (Technology for Rural and Ecological Enrichment) in Thailand and MASIPAG (Farmer-Scientist Participation for Development), and the Sustainable Agriculture Coalition in the Philippines have been working with farmer and commu-

nity groups to collect and conserve traditional rice varieties. Community seed banks have been set up in some locations.

IRRI researchers will be evaluating these local conservation practices in order to develop appropriate methods for farm-based conservation of rice. They will also develop efficient, low-cost methods for storing rice seeds so that viability can be retained for at least 2 years.

IRRI is already providing training at the national level in developing countries on the principles and practices of genetic conservation. Under the project, IRRI will develop new training courses in germplasm conservation and database management as well as organize regional workshops on rice genetic resource conservation.

### ...and the USA

#### Crop potentials are higher than expected

Can the world grow significantly more food to keep up with population growth? The results of a new study challenge recent reports that agricultural crop yields have reached their maximum biological potential and are now decreasing. Donald L. Plucknett in the Science and Agricultural Transformation Lecture presented 9 September 1993 in Washington, DC reported on an International Food Policy Research Institute study which claims that gains in agricultural productivity show no sign of slowing in the future, as long as investments in international agricultural research are maintained as food needs go up. One might think that, according to the principle of diminishing returns, crop yields would begin to level off. However, although there has been some slowing at times, there is no indication that maximal yield potentials have been reached. And all crops except sorghum show a positive yield gain.

Yield potential is a concept developed by Dutch scientists, based on the upper limit of what can be grown on all suitable agricultural land. The resulting calculations are based on a scale of 'grain equivalents', on which the croplands of the world are ranked by their potential. The range is from 'extremely high' (more than 25 000 kg per ha per year) to 'very low' (5000 or less). Yield levels of 800 to 1500 seem to represent agriculture that has not yet enjoyed much gain from scientific improvements.

The central point is that significant productivity gains are possible provided suitable technologies are available for farmers. The author believes that yield improvements are the best hope for bettering the economic conditions of those living in the rural areas of the developing world. Farmers can increase their crop production whether by bringing more lands under cultivation or by increasing crop yields. Now that the world's new land frontiers are scarce, farmers must increase yields.

Many nations of the developing world are experiencing yield gains that are equal to and sometimes surpass

the yield gains in developed nations. This happens because these countries have been able to draw on the world's past investments in agricultural research. Each country, developing or developed, should have its strategy to carry out research toward increasing its own potential.

The developing world shows high rates of gain because these countries were able to draw on international pools of knowledge. The international agricultural institutions and mechanisms that began to emerge within the last two or three decades have made it possible for agricultural research to become a truly international enterprise.

The study asserts that higher yields are essential, because agriculture is destined to become even more intensive with regard to land use, requiring higher production levels, management skills and knowledge. Crop yields will improve only if we increase our investment in agricultural research. World population will double over the next few decades and food production must keep up. Furthermore, as agriculture becomes more 'environmentally safe', the agricultural enterprise becomes much more complex. Less use of fertilizers and pesticides requires better research in the laboratory and in the field, and better tailoring of crops to climates and soils.

*(Edited from an article in DevelopNet News, an electronic newsletter published monthly by Volunteers in Technical Assistance (VITA), a private, nonprofit, international development organization located in Arlington, Virginia).*

## Upcoming Events

**Seminar on 'Modern approaches to the study of plant viruses': 12 - 24 March 1995, Scottish Crop Research Institute, Dundee, Scotland.** Organizers are: Dr A Teifion Jones, Senior Principal Scientist, Virology Department, SCRI; Professor Bryan D Harrison, Department of Biological Sciences, University of Dundee; Professor T Michael A Wilson, Head of Virology Department, SCRI. Application deadline is 10 December 1994. Contact: International Seminars Department, The British Council, 10 Spring Gardens, London SW1A 2BN, UK. Tel. +44 (0) 71 389 4264/4252/4226; Fax. +44 (0) 71 389 4154; Telex. 8952201 BRICON G.

**Meeting on 'Viruses on the move: transport, translocation and transmission': 27 - 29 March 1995, Churchill College, Cambridge.** Organized by the Virology Group of the Association of Applied Biologists. Contact: Association of Applied Biologists, c/o Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK.

**IVth International Safflower Conference, 3 - 8 June 1997, Bari, Italy.** Organizers are: Agronomy Institute, Bari University; Agricultural Biology Department, Potenza University; Germplasm Institute, Bari-CNR. To receive the next announcement, contact the Organizing Committee

of the IVth International Safflower Conference, Istituto di Agronomia, Via Amendola 165/A, 70126 Bari, Italy. Tel. 0039-80-544 2953; Fax. 0039-80-544 2813.

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## Book Reviews

### Genes, Crops and the Environment

by John Holden, James Peacock and Trevor Williams  
1993. Paperback ISBN 0521437377. £12.95. 176 pages. Cambridge University Press, Cambridge, UK.

At first glance, this book may well be classified as yet another book on the story of plant genetic resources. However, at a further glance it is discovered to be more than this. Aimed at a wide audience, including 'interested taxpayers' as well as students of the discipline, this book is one of only several on the subject that I have had difficulty in putting down.

The preliminary chapters give a good, realistic and only mildly emotive coverage of the plant genetic resources story. The main defect found is the lack of simple vocabulary and explanations, particularly concerning the evolution of crop plants using four crop examples. The use of specialist genetics knowledge makes large assumptions about the abilities of both the 'lay' reader and the inexperienced student.

The intermediate chapters present good coverage of the need for, reasoning behind, and practical approaches to plant genetic conservation, which provides an overview of the discipline as it is at present. Recent advances and techniques still in early stages also are covered.

The final chapter adds to the framework of the previous chapters, and touches upon the associated moral, social, political and economic issues. Important, relevant issues are discussed, although not in great detail. An example of this incomplete picture is the political and social issues content; sovereignty of genetic resources is not touched upon at all, other than through a reference to the holistic conservation view that genetic resources belong to everyone.

The shortcomings and errors made in plant genetic resources to date are acknowledged, and it is difficult not to agree and empathize with the authors' practical 'hands-on' approach. Their approach to dealing with the 'gaps in the scientific base' is succinct and pragmatic, the keywords being collaboration and cooperation. This book, rather than provoking the emotions, provokes action. It focuses very much on application of knowledge, and is expressed so well that the reader passes beyond the point of merely thinking and on to the point of applying the ideas and strategies generated.

Overall, the book covers many essential aspects of plant genetic resources, although some important aspects are divided in their coverage (for example, data management) which detracts from their importance in the overall picture. The book is necessary reading for the student starting in plant genetic resources training. It could be further improved by the addition of a glossary, particularly aimed at the new student and the uninformed public. The resume at the start of each chapter provides an insight into the contents of the chapter and enables the reader to 'dip in' as desired. Plant genetic resources con-

servation is brought into focus in the much wider picture of 'biosphere' conservation through the holistic approach, which allows a relatively good snapshot view of genetic resources as they are at present.

Helen L. Ager (MSc student, University of Birmingham; now at IPGRI, Rome, Italy)

### Genes, Crops and the Environment

by John Holden, James Peacock and Trevor Williams  
1993. Paperback ISBN 0521437377. £12.95. 176 pages. Cambridge University Press, Cambridge, UK.

### Genetic Resources. A Practical Guide to their Conservation

by Daniel Querol  
1993. Hardback ISBN 1856492036. £32.95. 252 pages. Zed Books Ltd., London and Third World Network, Penang, Malaysia

How should plant genetic resources be conserved for the long-term benefit of humanity? Contrasting answers to this question emerge from these two books written by authors with very different perspectives on their subject.

Holden, Peacock and Williams are all academic scientists working in the industrialised North, whereas Querol has spent much of his career living in the rural South. The central theme which emerges on the first page of the book from the North is the need for "world-wide collaboration in conservation". Querol puts the opposing case, stressing that genetic resources are the property, not of the global community, but of local people. In the acquisition of genetic resources by the North over the past hundred or so years, Querol says, "we see the absurdity of the poor subsidising the rich".

**Genes, Crops and the Environment** takes on the huge responsibility of producing what the authors hope will be the first "overall picture of plant genetic resources conservation" to have been written with the aim of reaching "a wider audience than has been reached by most previous comprehensive treatments". There does seem to be a lack of such a book. For example, E.O. Wilson's popular **The Diversity of Life** (Harvard University Press, 1992) is curiously selective when dealing with agriculture. Wilson concentrates on highlighting novel potential food crops hidden in the rainforest, rather than the need to conserve the genetic diversity in crops, which is crucial for sustainable agriculture in both North and South.

Holden, Peacock and Williams describe how crop plants evolved into different landraces in parallel with human societies over the past 10 000 years, culminating in a maximum of crop genetic diversity about 100 years ago. Since then, they point out, the process has been reversed by plant breeders who discovered how to produce more uniform and higher-yielding varieties. These

new varieties replaced landraces and led to the loss of the natural diversity in our crop plants much more rapidly than the symbiosis between humans and plants that created them. The authors illustrate their argument with several detailed case studies such as the IR8 and related rice varieties.

My problem with **Genes, Crops and the Environment** is not with the basic biology or fieldwork methods they describe, but with the context, ecological and political, within which they frame their arguments. The authors call for the identification and use of "landraces and primitive varieties" as sources of genetic diversity to produce increased yields of crops to feed an increasing population. But there is increasing evidence suggesting that the very intervention of the global market, be it in distribution of new seeds or anything else, puts a community's fate beyond its control, draws it into debt and thus indirectly leads to population increase. The authors also suggest that habitat destruction faced by the "peasant farmer" is "partly because of his or her own activities" without criticising the economic system within which farmers are forced to work.

In **Genetic Resources**, Daniel Querol presents a different and in some ways opposing view. It began life as a manual for a genetic resources course in a Mexican university and was used as a discussion base for the creation of the Nicaraguan Genetic Resources Program. Querol first discusses the concepts of germplasm, centres and non-centres of variability, conventional and non-conventional species. Non-conventional species are defined by Querol as those "which have not entered the 'official' chain of breeding, production, and marketing", including many native species of the tropics which until now have been used locally without the support of modern research and development programmes. Querol describes how the Brazilian rubber tree was just such a non-conventional species until "English botanists illegally took germplasm for study at the Royal Botanic Gardens at Kew. A few years later, British colonies such as Sri Lanka, Singapore and Malaya had plantations which reduced the Brazilian industry to 5% of the market, with subsequent social crisis in Brazil".

For Querol, what was stolen was not just germplasm, but a people's resource. He sees the hope of worldwide collaboration as naive in that the global free market will always be driven by the needs of the economically powerful North at the expense of the economically weak South. He criticizes the CGIAR and the IPBGR, pointing out that the latter was set up by the transnational, Northern-based Ford and Rockefeller Foundations. The CGIAR, says Querol, was only set up after the realisation by North American corporations of the dangerously narrow genetic diversity of their crops and was, in its own words, devoted to "the exploration and [economic] evaluation of genetic resources", not their use by local people.

Querol argues that the priorities of the North centre around the study of endangered species, which if extinct would be a lost potential economic resource. Third World farmers have different priorities, to utilise their indigenous conventional and non-conventional species to feed themselves.

Having argued the reasons for having national genetic resource programmes and how countries can set up, finance and benefit from them, Querol explains the necessary collection, conservation storage and regeneration techniques.

While the biological processes and field methods described are similar, the contrast in world view between these two books could hardly be greater. **Genes, Crops and the Environment** concludes that "it is certain that if the rural poor are to be helped into new sustainable ways of improving their standard of living and if the developed world is to maintain that to which it has become accustomed, new types of crop varieties and management systems will be required." **Genetic Resources** disagrees, arguing that Third World farmers are already subsidising the lifestyles of the industrialised nations and the author sees increased intervention by the North as both undesirable and harmful.

Both books are valuable, but I find Querol's prescriptions particularly convincing and more likely, if implemented, to contribute to a fair and sustainable system of agriculture in the South. Perhaps his thesis also has important implications for how governments of the industrialised North perceive their role if they really are to engage in a dialogue of equals with communities of the South rather than exploit them.

*Tom Wakeford, Department of Biology, University of York, York, UK.*

### **How to Draw Plants, the Techniques of Botanical Illustration**

*by Keith West*

1993. 4th paperback edition. ISBN 0906969786. £9.95. 152 pages. Herbert Press/British Museum (Natural History), London, UK.

### **How to Draw and Paint Wild Flowers**

*by Keith West*

1993. Hardback ISBN 1871569567. £16.95. 128 pages. Herbert Press/Royal Botanic Gardens, Kew, London, UK.

Botany has always needed the services of skilled botanical illustrators to illustrate published work on plants, and many scientists and fieldworkers with some drawing ability find that their work is aided and enriched by their own drawings.

There are many books which purport to teach flower painting of a decorative kind, but these are of little help to the botanist who wants to learn the techniques of serious botanical illustration. However, Keith West has produced

the best introduction to botanical illustration available: **How to Draw Plants** is not just another 'how to do it' book, but a clear and well-illustrated guide to the use of various media in the accurate portrayal of plants.

The author is an experienced botanical illustrator who for 20 years was botanical artist for the Botany Division of The Department of Scientific and Industrial Research in New Zealand, and lectured for the Department of Extension Studies, University of Canterbury, Christchurch. He has also done work for the Missouri Botanic Garden and the British Museum (Natural History).

**How to Draw Plants** begins with a brief survey of the history of botanical illustration, which if nothing else serves to whet the appetite, followed by a short general chapter on basic equipment. (More detailed recommendations about materials are given in each of the later chapters on different media.) The short chapter on the handling and storage of live plants and herbarium material will already be familiar to readers of the "Newsletter", but the next, much longer one, entitled "Plants in detail" is of great value in that it looks at each part of the plant: leaves, hairs, roots, flowers, etc. and how they are arranged together. The author carefully describes the structures, their angles, positions and range of forms, and explains how to draw and construct each one in perspective on paper. This guide to thorough observation is important as it encourages the student to understand what he is looking at before attempting to portray the plant accurately in two dimensions.

The remaining chapters explain the use of the various media used in plant illustration: pencil, ink, scraper board, watercolour, gouache, acrylic and photography. Of course no book can really teach mastery of a particular technique, but Keith West's very readable explanations are an excellent introduction, clearly and interestingly presented, in lucid English.

Appropriately, the black and white illustrations throughout the book are numerous, well related to the text, and in themselves teach the student a lot about the variety of ways in which plants may be illustrated. Keith West's detailed pencil drawing of New Zealand forest interiors are particularly remarkable (pp. 75-76), although the reader may find the many simple ink sketches of leaf outlines and flower parts less intimidating!

Sadly, Keith West's fine colour illustrations have not been well reproduced in this book; there are only eight of them, and all these have been printed on grey-toned backgrounds. However, it should be borne in mind that had the publisher spent more on colour plates one of the other attractive features of this book might have been lost: namely its low price, which makes it more affordable for students.

I heartily recommend **How to Draw Plants** as a thorough, very readable and enjoyable book which will instruct anyone who wants to illustrate plants, whether for

scientific publication or for pleasure. For a number of years I have used this book when teaching illustration, and always recommend it to my students, including those who do not have English as their first language. There is no other book which does the job so well and its illustrations make it a book which "practices what it preaches"!

Perhaps in response to pleas for more colour illustrations, Keith West has subsequently written two books, the most recent of which is reviewed here. **How to Draw and Paint Wild Flowers**, like its predecessor, **Painting Plant Portraits** (1991), describes itself as "a step-by-step guide" using many illustrations of particular drawings and paintings at different stages in their making.

The book begins with 25 pages on Basic Equipment, Drawing Plant Structures and Mediums which repeat, in summarized form, much of the instruction to be found in **How to Draw Plants**, using a few of the same illustrations.

The bulk of the book is given over to the treatment of 20 individual plant species, all common British wild flowers such as Wood Anemone (*Anemone nemorosa*), Primrose (*Primula vulgaris*) and Honey Suckle (*Lonicera periclymenum*). For each plant we are given a clear step-by-step description of how the author drew or painted the subject, illustrated by a sequence of colour illustrations, many of them full page, showing how the drawing was executed and the colours gradually built up. At each stage detailed descriptions of the materials and colour mixes used are given, and the author has the courage to tell us on the few occasions when he wishes he had done things differently: "Two of the plants chosen are portrayed in pencil alone, one in ink, and one in gouache, but the remaining 17 are examples of watercolour painting over pencil."

This treatment comes as near as it is possible to come in a book to an actual demonstration of watercolour technique applied to plant illustration, and I am sure that many students will find it instructive. However, it must be emphasized that in this book Keith West demonstrates one particular way of painting in watercolour: that of painting the tones in first using a greyish "shadow mix" of watercolour, before any colour washes are applied (a technique used by the Old Masters). The results show that the author has mastered this technique, but he says himself that these are "watercolour procedures that have worked for me. But just as my own techniques and preferences have evolved, so no doubt will your own as your abilities grow." It is possible to apply shading at all stages of a painting, even at the end, and different illustrators favour slightly different approaches.

Sadly, Keith West has been let down by the printers, for many of the colour reproductions of his watercolours appear to be out of focus, with dulled colours and the usual grey-toned backgrounds.

This book is aimed at the student who wants to paint plants in watercolour, particularly attractive British wild

flowers to which many people will have access (we do not all have a botanical garden to hand!), but it does not aim to teach line drawing or dissection drawing for scientific publication. For that, one would do better to study **How to Draw Plants**.

Both these books are excellent, but **How to Draw Plants** covers more media in greater depth.

*J. Mark Fothergill (Freelance botanical illustrator, working at the Royal Botanic Gardens, Kew)*

### The Strangest Plants in the World

by Professor S. Talalaj and D. & J. Talalaj

1991. Paperback ISBN 0857722053. 166 pages. Hill of Content Publishing, Melbourne, Australia.

This book covers many aspects of 'strange', and includes the interesting and amusing as well as the more horrific applications of some plant species. Plants sharing anomalies are grouped together, and include the more obvious such as the oldest, largest, most peculiar seeds and fruits, as well as the less visible, hallucinogenic and gruesome uses of plants in black magic and human sacrifice.

The text is light and easy to read and is bursting with stories concerning the historical uses of these plants and how those uses have developed throughout history. This text is supported by colour photographs of these amazing plants, which emphasizes their eccentricity. Particular events and, in some cases, specific people in history are seen to have had immense effects on determining the future of certain species. In many cases, the original indigenous use has enabled the discovery of vital lifesaving products, which are now an essential component of modern medicine. For example, *Chondodendron tomentosum* was known as 'Flying death' by the Indian tribes living in the Amazon, who used this deadly tree extract to coat their arrows, as well as putting the poison beneath their fingernails to cause 'death by scratching'. Although a deadly poison in large doses, this is now an important medicine, used prior to surgery as a muscle relaxant.

This book provides a valuable insight into over 100 plants through stories based on ethnobotanical knowledge, emphasizing how the use of plants is an integral part of different human societies and cultures.

Of all the plants covered, some of the stranger ones appealing to me included the 'Sausage tree' (*Kigelia africana*), the 'Bottle Gourd' (*Lagenaria siceraria*) and the 'Boojum Tree' (*Idria columnaris*).

The more incredible and obvious are covered, with the aim of capturing the attention of the reader by the strange and incredible appearance, properties or behaviour of these plants. However, although the information presented is of great interest, it is not of great scientific depth, and so does not highlight the issue of conservation for continued and more effective utilization. The book is,

however, very informative and really captures the imagination of the reader with the various histories of the plants, including the amusing, the practical and the despicable.

This book is a very good read, with colourful, factual and light text. It appeals to a wide audience, since it is not restricted to a specific scientific audience, but to anyone with an interest in plants, or in planning a murder.

*Helen L. Ager, IPGRI, Rome, Italy.*





# Instructions to authors

Typescripts should be prepared in English, French or Spanish and submitted in duplicate to the Managing Editor. Typescripts should be double-spaced throughout, with generous (3-5 cm) margins. All pages (including tables, figures, legends and references) should be numbered consecutively.

## Title

The title should be as short as possible and should contain the common and full generic name of any species featured in the paper, as well as the main countries visited during, for example, collecting trips.

## Authors/addresses

Include the full names of all authors of the paper, together with the addresses of the authors at the time of the work reported in the paper. Indicate current or postal addresses as a footnote on the first page of the paper; indicate also the author nominated to receive correspondence and proofs.

## Abstracts

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Use simple clear language in the text. A native speaker of the language should preferably edit the paper before submission.

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These (also grants, support, etc. if any) should follow the text and precede the references.

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### Periodicals

Molina-Cano, J.L., P. Fra-Mon, G. Salcedo, C. Aragoncillo, F. Roca de Togores and F. Gardia-Olmedo. 1987. Morocco as a possible domestication center for barley: biochemical and agromorphological evidence. *Theor. Appl. Genet.* 73:531-536.

### Books (edited by someone other than the author of the article)

Hanelt, P. 1986. Cruciferae (Brassicaceae). Pp. 272-332 in Rudolf Mansfelds Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen), Vol. 2. (H. Schultze-Motel, ed.). Akademie-Verlag, Berlin, Germany.

### Books (identical author and editor)

Chapman, C. 1985. Genetic Resources of Wheat. A Survey and Strategy for Collecting. IBPGR, Rome, Italy.

## Nomenclature

*Taxonomical*: in line with *Index Kewensis*. *Genetic*: applications of the terms phenotype and genotype should be in accordance with Demerec *et al.* (*Genetics* 54:61-74, 1966); for summaries of genetic abbreviations, consult the *Journal of Bacteriology* Instructions to Authors.

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