Regeneration of Seed Crops and their Wild Relatives

Proceedings of a Consultation Meeting
4–7 December 1995
ICRISAT, Hyderabad, India

J.M.M. Engels *and* **R. Ramanatha Rao,** *editors*

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International Plant Genetic Resources Institute
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IPGRI is an institute of the Consultative Group on International Agricultural Research (CGIAR)

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Preface

The *ex situ* conservation of plant genetic resources is of vital importance to ensure their long-term safety and continued availability for use by scientists and farmers in order to jointly contribute to long-term global food security. *Ex situ* conservation consists of a series of routine operations and activities, of which many are interlinked, and all of which need proper management. Regeneration of stored germplasm seed samples is one of the key activities as it has a direct bearing on the quality of the material conserved, requires specific knowledge and expertise, and is usually labour intensive. According to the Global Plan of Action, which lists the regeneration of threatened *ex situ* accessions as one of the priority activities, the "*capacity for regenerating accessions was often not considered when assembling collections and disseminating accessions, with the unintended consequence that much material collected in the past cannot now be properly maintained*". The Plan continues to state that "*an average of 50 percent of current national collections are in need of regenerating*" and that urgent action is needed to avoid much of the stored genetic diversity, as well as a large proportion of the public investment that has been made to establish the collections, being lost forever.

For the above reasons IPGRI, together with the CGIAR System-wide Genetic Resources Programme (SGRP), the Food and Agriculture Organization of the United Nations (FAO) and the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), organized a technical consultation meeting with scientists from National Plant Genetic Resources Programmes, the CGIAR and other international research centres, and the private sector. The meeting was held at the ICRISAT Asia Centre at Patancheru, India in December 1995, and details are presented in these proceedings. The deliberations resulted in a much better understanding of the complexity of the regeneration process, and generated suggestions and ideas on how to make the process more efficient and cost-effective. It also provided a basis for the preparation of a decision guide for genebank curators on regenerating accessions in seed collections which has been jointly published by SGRP, FAO and IPGRI. Unfortunately, due to the high priority accorded to the decision guide, the publication of these proceedings was delayed. IPGRI trusts that this delay will not have caused any inconvenience to those who prepared papers for this consultation meeting.

It is hoped that this publication will contribute to better planning and implementation of systematic regeneration procedures, thereby assisting genebank staff in promoting the overall efficiency and cost-effectiveness of genebank operations.

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The editors hereby acknowledge the significant contributions made by Ms Jane Toll to the planning and implementation of the Consultation Meeting. They also wish to thank FAO, SGRP and IPGRI for financial contributions for the publication of these proceedings.

Executive Summary: the Consultation on the Regeneration of Germplasm of Seed Crops and their Wild Relatives

The consultation was held from 4–7 December 1995, at ICRISAT Asia Centre, Patancheru, India, as part of the CGIAR System-wide Genetic Resources Programme (SGRP), in collaboration with FAO. It was organized by IPGRI, hosted by ICRISAT and funded through contributions from SGRP, FAO, ICRISAT and IPGRI. It was attended by 37 scientists from 12 national programmes in Europe, America, Africa and Asia, six CGIAR Centres (IPGRI, ICRISAT, ICARDA, CIMMYT, IITA, IRRI), AVRDC, Pioneer Hi-Bred and FAO. The objectives of the Consultation were:

- identifying criteria and options in curator / genebank manager decisions on managing and carrying-out regeneration, and formulating a curator decision framework;
- identifying topics requiring further information and / or research and opportunities to gather the information and / or carry out research;
- proposing strategies and mechanisms for addressing the regeneration needs of existing collections.

The practices and experiences in seed regeneration in 12 national and six international genebanks were presented, as well as overview papers on the scientific principles underlying regeneration. In addition, there were presentations on the experiences of the collaborative project in Latin America on the regeneration of maize landraces (LAMP), an analysis of information on practices obtained by questionnaire from 200 institutes, and evidence on the global regeneration need collated from Country Reports to the IVth Technical Conference. The principal steps in regeneration, major constraints and problems were identified, synthesized and further examined in Working Groups.

The meeting identified three key approaches to cost-effective regeneration of seed germplasm:

- minimize the regeneration requirement of the collection (by managing the size of the collection / minimizing redundancy);
- minimize the regeneration frequency of accessions in the collection (by maximizing initial viability and quantity, and optimizing the maintenance of viability and quantity in storage);
- conduct cost-effective regeneration of the accessions (minimize genetic change to accessions and costs during regeneration).

Working Groups were formed to examine each of these approaches and to identify the steps and criteria in decision-making to meet the objectives of each approach. The decision guides developed by each Group have been pulled together and are presented. This constitutes a framework for the management of genebank collections that emphasizes the importance of curator decision-making in controlling and carrying out regeneration. The exercise also identified the major information and research needs for better informed curator decision-making in regeneration, and the improvement of regeneration procedures.

The following points were agreed upon by the participants as critical aspects to be addressed and / or considered by genebank curators while regenerating germplasm as well as by international organizations as part of their research agenda.

- Importance of institutional / genebank policies on germplasm acquisition and conservation to ensure collections are built up in a rational manner.
- Need for greater attention to managing collections at the individual accession level to take account of important differences in origin and history of the accessions. The concept of an accession-specific “basic unit” (minimum plant number) for regeneration was explored as a means to take better account of the uniqueness of individual accessions in factors such as: number of plants constituting original sample, number of individuals remaining after quarantine, numbers used in regeneration, cycles of regeneration, etc.

4 REGENERATION OF SEED CROPS & WILD RELATIVES

- Importance of the base–active collection linkage and matching storage conditions to storage life, to reduce regeneration frequency and for improved cost-effectiveness.
- Many species lack basic information (or it needs collating) on cultivation, mating systems and patterns of genetic diversity, flowering biology and seed production, seed physiology including storage characteristics, dormancy and germination.

Follow-up activities were agreed upon, including the following.

- The decision framework will be used as the basis of developing general guidelines to genebank management and regeneration which will be put to the FAO Commission of Genetic Resources in 1997 for endorsement and publication as international guidelines. The recommendation of the meeting to develop crop-specific guidelines, similar to the crop descriptor lists, needs to be explored.
- Follow-up on recommendations of information and research needs is required.

Regeneration of tropical field crop germplasm in Australia

Peter Lawrence

Germplasm collections

Australian agriculture is based almost entirely on exotic species imported from other countries. Germplasm collections of field crops in Australia mostly contain 'breeding' accessions plus some landraces and wild species, whilst germplasm collections of forages are mostly landraces.

Post-entry quarantine

One of the major functions of a Genetic Resource Centre, as seen by our research clients in Australia, is to provide a service for importing new germplasm through post-entry quarantine.

All crop accessions and some pasture accessions entering Australia must pass through one generation in post-entry quarantine. This is the major bottleneck in maintaining an effective population size of germplasm accessions and preventing loss of genetic variability through genetic drift over generations. Usually four plants are grown for inbreds, whilst the number of plants grown for outbreeding populations depends on the size of the budget.

For maize populations 100 plants are grown, and full-sib pollination is used to maintain genetic variability. In terms of cost this is equivalent to growing 25 inbred accessions. The decision whether to import one population of an outbreeding species or 25 inbreds is dependent on the genetic variability available in the population versus the genetic variability in the 25 inbreds.

To overcome the post-entry quarantine bottleneck we are currently negotiating with Australian quarantine officials to allow germplasm seed of sorghum that has been grown under quarantine conditions in the US Virgin Islands to enter Australia directly, without having to be grown for one generation in a post-entry quarantine glasshouse in Australia. This is a test case which, if successful, could be extended to other crops from other countries.

This idea could be expanded to an international approach to quarantine growouts which could be a cooperative effort between a number of countries.

Regeneration of seed germplasm

10 years ago germplasm collections in Australia were maintained by individual plant breeders. There were no pre-storage drying facilities and most collections were stored at 5°C. Some collections were regenerated every 10 years, whilst others were left on the shelf. With the establishment of eight Genetic Resource Centres in Australia the first task has been to acquire samples of these collections, and to grow the collections for seed regeneration and long-term storage.

Most germplasm accessions of tropical field crops are regenerated in the field at Biloela Research Station (23° latitude) using irrigation. Wild species are regenerated in the glasshouse. Table 1 lists the initial germination of various crops after regeneration, and some comments are provided on the reasons for low viability of some accessions.

Good agronomic management, including control of insect and disease damage, especially in navybeans and soyabeans, is essential for the production of good quality seed. Staff with experience in growing the particular crops are the key to success.

Accessions with a wide range of genetic variability are often grown in the field under one agronomic regime. Consequently, the unusual genotypes (e.g. short-day accessions or early maturity accessions), which are often the most valuable ones, are not provided ideal conditions for the production of good quality seed. Often, limited information is available on these accessions before they are grown, and consequently we are unaware of the special conditions required to grow the accessions for seed regeneration.

Table 1. Details on initial germination of various crops after regeneration

Crop	Germination (%)		Comments on low germination
	Average	Range	
Arachis hypogaea	80–94	38–98	Some landraces susceptible
Glycine max	50–92	14–96	Late maturity
Gossypium hirsutum	80–94	64–96	Late maturity landraces
Helianthus annuus	80–100	30–100	Susceptible to *Sclerotium*
Nicotiana tabacum	86–98	60–98	Old varieties susceptible to leaf diseases
Wild *Nicotiana*	50–96	28–98	Some species require arid climate
Phaseolus vulgaris	90–100	36–100	Require good agronomic management
Sorghum bicolor	82–100	44–100	Late maturity landraces
Indigenous *Sorghum*	20–80	0–98	Original seed
Vigna	86–98	68–100	
Zea mays	92–100	62–100	Old varieties susceptible to cob rots

Therefore, it is suggested that when seed of an accession is sent to a genetic resource centre, information on the conditions required to grow the accession for seed regeneration should also be supplied.

Australia has a wide range of environments (e.g. frost-free environments with irrigation which are ideal for growing accessions during the winter) which could be used for the regeneration of plant genetic resources. However, it is often difficult to identify skilled staff at those locations who could spend, say, 10% of their time regenerating germplasm accessions.

Wild species

Australia has a range of indigenous species of genera such as *Cajanus, Glycine, Gossypium, Nicotiana, Oryza, Sorghum* and *Vigna.* These species may have useful genetic traits which could possibly be transferred to cultivated crop species using biotechnology techniques. Recently we have started making collections of these indigenous wild relatives of crops to supply to researchers in Australia and overseas.

Sampling strategies for collecting seed of *in situ* populations are mainly influenced by practical considerations. Often the wild populations have only a few plants at the right stage of maturity for harvesting; in other cases it is possible to sample 100 plants. The low germination rate for some indigenous *Sorghum* accessions (see Table 1) is due to the collection of immature seed. The interval between flowering and maturity when the seed drops is 10 days, and during that time the weather and road conditions make it difficult to collect seed from *in situ* populations. Therefore it is often necessary to regenerate accessions of indigenous wild species before placing seed in long-term storage.

Our experience in seed regeneration of wild species is limited to *Nicotiana* and *Sorghum*. Most wild species of *Nicotiana* can be grown successfully in the glasshouse, however at least 12 species indigenous to the Australian desert are susceptible to fungal diseases and are difficult to grow in glasshouses with evaporative coolers where the humidity is high.

Our limited experience with the regeneration of wild *Sorghum* is that it is difficult to obtain seed set in some species, especially if the heads are bagged to prevent cross-pollination. Obviously, further research is required into the floral biology of wild *Sorghum*. We plan to undertake this research in the next few years in association with graduate students from the university; however, we have not yet secured funding for this project.

Pre-storage processing and storage conditions

At Biloela, we have adequate facilities for pre-storage processing. Seed is dried to 6% moisture content in a drying room which operates at 15% relative humidity and 15°C. After drying, the seed is sealed in aluminium foil packets and placed in long-term storage at –20°C.

As our Centre has been operating for 7 years, we do not have data on the change in seed germination during storage. We assume our storage conditions are more than adequate and we plan to monitor the germination of all accessions every 10 years.

Base and active collections

We operate one seed store at −20°C which serves as an active/base collection. We aim to store 2000 seeds for inbred lines and 4000 seeds for heterogeneous accessions. In Australia, the number of requests for an accession is low, so these quantities of seed should meet requests for the next 100 years. We generally supply 50 seeds for inbreds and 100–200 seeds for populations. Plant breeders and other clients are encouraged to maintain their own individual collections for active use.

Brazilian genetic resources conservation system

Antonio Carlos Guedes

Introduction

Brazil, with an area of 8 511 965 km^2, is the largest country of the South American continent and is considered one of the countries with the greatest biological diversity in the world. Among the 250 000 species of higher plants, nearly 60 000 are native to Brazil. The Brazilian flora is important because it possesses a great number of domesticated species and/or wild relatives, including: guarana (*Paullinia cupana*), cocoa (*Theobroma cacao*), rubber (*Hevea brasiliensis*), cotton (*Gossypium* spp.), cashew (*Anacardium occidentale*), pineapple (*Ananas comosus*), peanut (*Arachis hypogaea*), cassava *(Manihot esculenta*), etc. The richness of species found in the different Brazilian ecological domains includes timber, fruits, palm trees, forages, medicinal, industrial and ornamental plants. In the Amazon (3.5 million km^2) alone, nearly 800 species were identified with potential for economic exploitation.

In situ conservation of genetic resources

The most important component for *in situ* conservation consists of the Indigenous Areas (554 reserves, an area of more than 94 million ha) whose communities are composed of 146 different ethnic groups located mainly in the northern region of the country. The Units of Conservation, under the responsibility of the Federal Government, State or Municipalities, add up to approximately 50 million ha of conservation area.

Ex situ conservation of genetic resources

Ex situ conservation in Brazil is carried out in the form of conservation of seeds in cold chambers, *in vitro* conservation, cryopreservation in liquid nitrogen and in the field. The decision of the Brazilian Corporation for Agriculture Research (EMBRAPA) to create the National Center for Genetic Resources (CENARGEN) for *ex situ* conservation was highly significant. In 1984, CENARGEN incorporated research activities using biotechnology aimed at conservation and use of genetic resources. At that time the name of the Center was slightly modified to the National Center for Genetic Resources and Biotechnology, but the acronym remained the same.

Before the establishment of CENARGEN, activities concerning genetic resources in Brazil were carried out (with rare exceptions) in a random and sporadic manner. Frequently there were gaps in some areas and duplications in other areas of research throughout the country. The creation of CENARGEN and the consolidation of the Cooperative System of Agriculture Research (SCPA), which is today known as the National System of Agriculture Research (SNPA), helped to organize and increase the efficiency of germplasm collection, exchange, quarantine, characterization, evaluation, documentation and, most importantly, conservation and utilization of germplasm.

Under the EMBRAPA System of Planning – SEP – all the activities related to genetic resources conservation and use are handled under Program 2, which carries this title. The general objective of this programme is to "enrich and conserve the exotic and native genetic resources of current socioeconomic importance and potential for the country and to promote and increase their utilization in breeding programmes, for the development of sustainable agriculture". The basic activities of the programme are developed through projects and sub-projects financed mainly by the Ministry of Agriculture with participation of the SNPA members. SNPA is composed of EMBRAPA with its 39 research centres, of its member research institutes supported by the states, and of universities carrying out agricultural research. With the participation of these different entities, a national network was formed for genetic resources conservation which presently comprises 84 Active Germplasm Banks (BAGs) distributed over 47 locations working in conjunction with CENARGEN. The

System's Base Collection (COLBASE) of plant germplasm is kept at CENARGEN and the active collections are maintained at the respective BAG.

A recent survey revealed nearly 194 000 accessions of plant germplasm, including duplicates, with 60 000 stored at the COLBASE and 134 000 in other banks or collections. Of the accessions conserved in the system, nearly 76% are exotic and 24% are native/local populations. The main food crops which are important to Brazil are dependent on the introduction of exotic germplasm, and almost 95% of the grain accessions conserved at the SNPA collections are from exotic species. The maintenance of quality, regeneration and continual enrichment of the genetic variability of those collections have been of constant concern.

The Brazilian system for conservation of genetic resources has adopted the international standards for seed quality established by IPGRI and FAO. Adjustments are made, when necessary, depending on the circumstances and the species being conserved. The multiplication and regeneration of each BAG collection is carried out at the active genebanks, where the active collections of germplasm are maintained. The multiplication and regeneration of materials from the Base Collection are also carried out at the Research Center and location where the respective BAG is located.

The linkage between the CENARGEN activities and the EMBRAPA Research Centers and other institutions is through the Curators of Germplasm, based at CENARGEN, and the Active Bank Curators. The Curatorship Germplasm System was officially established by EMBRAPA in 1993, and under this system activities at CENARGEN are related to enrichment, documentation, conservation and use of germplasm, and the Curators of the BAGs are responsible for maintaining, regenerating and distributing the germplasm.

Despite the fact that the Brazilian System is well structured, the activities of multiplication and regeneration are still the most difficult to handle. Contributing factors include lack of specific funds, infrastructure, trained human resources, specific strategies for the establishment of regeneration priorities, etc.

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Ex situ conservation and regeneration in the Bulgarian seed genebank

S.D. Stoyanova

Introduction

Germplasm as a resource of genetic material can be used both to reproduce organisms and, through selection, to change them. Genetic conservation in the form of crop germplasm is a means to protect the living materials now exploited by agriculture and industry. *Ex situ* conservation, the most convenient, safe and economic method, is an integral part of conservation strategy for biological diversity at national and international levels.

Long-term seed storage at the Institute for Plant Genetic Resources is operated according to the preferred standards of IBPGR. Over 36 000 seed accessions are currently in the base collection, and these accessions require periodic monitoring of seed viability and genetic integrity.

Monitoring seed viability during storage

Because the seed genebank consists of collections of living materials, it is our duty to maintain the seed viability of every accession within a collection. Seed accessions in long-term storage are monitored 10 years after the start of storage. In cases where initial seed germination is poor because some species are not able to produce high quality seeds, then seed viability monitoring is carried out after 5 years of storage. Heterogeneous seed accessions are a special case and their monitoring is described later.

The seed viability equations developed by Roberts and Ellis (1982) illustrate the survival curve during seed storage:

$$\nu = K_i - p/\sigma$$

where ν = probit viability after time of storage; K_i = seed lot constant; p = storage time in days; σ = standard deviation in days.

This equation is a practical tool in our work for seed viability monitoring. As K_i is a seed lot constant, which illustrates the storage potential of an accession and depends on pre-storage environment and seed processing, it may be estimated from the result of the initial germination test. The standard deviation, σ, describes the value of deaths over time and depends on the storage conditions. The decline of seed viability during storage is negatively proportional to σ:

$$\sigma = p/(K_i - \nu)$$

Thus we can predict the probable change in seed viability of stored accessions. For example, if the viability of an accession is reduced 95% after 10 years of storage from an initial value of 97%, σ will be 12 717.77 days. That means that seed viability will probably decline to lower than 85% after 29.54 years.

Genetic changes in *ex situ* germplasm collections may occur during both seed storage and regeneration.

Genetic changes induced during seed storage

Although under standard seed bank storage conditions seed deterioration is very slow, it still occurs (Roos 1982, 1984; Roberts 1988, 1992; Roos and Davidson 1993). Genetic changes in stored seeds may occur both via gene changes in individual seeds and by population changes created by irregular loss of viability in a seed lot (Harrington 1970; Roberts 1972, 1991).

Table 1. Seed survival of gliadin biotypes during ageing of wheat accessions

Wheat cultivar	Viability constant		Seed viability in the seed lot (%)		
	K_i	p_{50}	90–100 days	80 days	50 days
Jubileina 3	1.62	38.80	94.75	80.00	50.00
biotype A	1.38	33.90	91.60 (*0.60*)	72.50 (*0.57*)	40.50 (*0.52*)
biotype B	1.41	34.64	92.00 (*0.20*)	73.50 (*0.19*)	41.70 (*0.17*)
biotype C	3.09	75.92	99.90 (*0.10*)	98.50 (*0.13*)	92.90 (*0.20*)
biotype D	1.62	39.80	96.00 (*0.10*)	80.00 (*0.11*)	50.00 (*0.11*)
No 14	2.43	59.70	99.25	80.00	50.00
biotype A	1.98	48.64	97.60 (*0.40*)	65.20 (*0.44*)	32.60 (*0.26*)
biotype B	1.88	46.19	97.00 (*0.20*)	61.50 (*0.16*)	29.10 (*0.12*)
biotype C	3.09	75.92	99.90 (*0.40*)	93.70 (*0.40*)	74.50 (*0.62*)
BGR 9218	2.39	58.72	99.16	80.00	50.00
biotype A	2.33	57.24	99.00 (*0.78*)	78.20 (*0.73*)	47.60 (*0.68*)
biotype B	2.46	60.44	99.30 (*0.15*)	81.80 (*0.15*)	52.70 (*0.15*)
biotype C	3.08	75.67	99.90 (*0.07*)	93.70 (*0.12*)	74.50 (*0.17*)
BGR 5958	2.54	62.40	99.45	80.00	40.00
biotype A	2.51	61.67	99.40 (*0.81*)	79.10 (*0.79*)	49.00 (*0.77*)
biotype B	2.81	69.04	99.75 (*0.19*)	86.60 (*0.21*)	60.60 (*0.23*)

Genetic shifts due to seed survival

Genetic shift in heterogeneous accessions may be induced by differential seed survival of the constituent genotypes. The seed viability equation (Roberts and Ellis 1982) could be used to evaluate seed survival differences in a heterogeneous seed accession. The changes in the genetic composition of heterogeneous wheat accessions were determined using the above equation (Stoyanova 1996). Genetic variability of two old Bulgarian wheat cultivars and two wheat landraces was evaluated by gliadin electrophoresis. The biotypes as recognised by gliadin composition were designated A, B, C and D. Biotype A was the most common, with biotype B the next, C and D being rarer.

The constant K_i and p_{50} fitted by probit analysis for every biotype and every wheat accession illustrate that biotypes differ in their sensitivity to seed ageing (Table 1). Whatever the similarities or differences as indicated by their gliadin spectra, the biotypes within a wheat accession are genetically different. As a result they can be affected differently by the pre-storage conditions, through the influence of these conditions on seed viability. To predict the changes in composition of the accessions, the following relationship can be used:

$$k_p = \nu_p.k \;/\; \sum_{i=1}^{m} v_{(i)p}.k$$

where k_p = composition coefficient per biotype after time of storage (p); v_p = seed viability of the heterogeneous seed accession after time of storage (p); $v_{(i)p}$ = seed viability per biotype in the initial seed lot before storage; k = composition coefficient per biotype in the initial seed lot before storage; i=1...m = number of biotypes observed. From the practical point of view, the composition coefficient per biotype is its frequency in the population. The predicted composition of biotypes at the different levels of seed viability is shown in parentheses in Table 1.

Mutation

It is well known that there is a close correlation between loss of viability and accumulation of chromosomal aberrations in the surviving seeds (Abdala and Roberts 1969; Murata 1979;

Murata *et al*. 1984). Several reports indicate that heritable point mutations are induced in seeds during storage (Dourado and Roberts 1984; Rao *et al*. 1987).

In an investigation of genetic heterogeneity of wheat cv. Sadovo 1, a dominant mutation was observed (Stoyanova 1994). The mutant was established as a single plant in the first generation grown from aged seeds. Nevertheless it did not differ morphologically, the distinction being described by gliadin electrophoretic analysis.

It was not possible to definitely associate the appearance of one mutation to ageing – it may have been a spontaneous mutation. However, these results confirm that unforeseen changes in individual seeds during storage may occur. Mutations are not only rare, they are also undefined in their survival and adaptability. More effective methods for their discrimination should be used.

Genetic shift due to seed multiplication

Regeneration of a seed accession is an important process which is related to the life cycle and reproductive biology of individual crops and species. There are special problems with wild species, including seed dormancy, seed shattering and seed production. The population structure could be affected to a different degree because of different selection pressures during multiplication.

Our results from 3 years of investigations showed that biotypes recognised by their gliadin spectra in wheat accessions have different seed productivity. Evaluation by STATGRAF variance analysis described significant differences between the gliadin biotypes (Table 2). The differences in seed productivity per plant were related to hundred-kernel weight and seed productivity in the main ear. The calculated relative seed yield indicates the intra-cultivar variation as a result of biotype × environment interaction, a major cause of shifts during *ex situ* regeneration.

A measure of the heterogeneity remaining after n regenerations of seed samples of different viability could be calculated by the equation (Stoyanova 1996)

$$\mathrm{RSP} = k_p.N.(\mathrm{MSY})^n \;/\; \sum_{i=1}^{m} k_{(i)p}.N.(\mathrm{MSY})^n$$

where RSP = relative seed productivity per biotype; N = number of seeds in the sample for regeneration; MSY = mean seed yield per biotype; n = number of regeneration.

It is difficult to decide which is more dangerous for genetic integrity: seed ageing or seed multiplication. Nevertheless it is clear that genetic shift induced by multiplication of seed samples with reduced viability is more dramatic than that of samples with higher viability.

Table 2. Effect of genetic variability by gliadin spectra on the seed yield of wheat accessions following three successive regenerations: 1990, 1991 and 1992 (Stoyanova 1996)

Wheat accession	Gliadin biotype	Seed yield per plant (g)	No. seeds per plant	Relative seed yield per plant
Jubileina 3	A	7.13	171.76	0.88
	B	7.14	174.26	0.89
	C	8.14	193.35	0.99
	D	8.21	195.01	1.00
No 14	A	6.87	176.26	0.82
	B	8.62	214.42	1.00
	C	8.45	213.38	0.99
BGR 9218	A	7.56	236.69	0.76
	B	10.14	307.60	1.00
	C	8.62	286.38	0.93
BGR 5958	A	11.08	300.28	1.00
	B	8.85	213.26	0.71

Our results confirmed those of Gale and Lawrence (1984) that over four or five regeneration events for similar, constant population sizes, the rare alleles or biotypes may be lost. However, we observed significant changes in the genetic composition of the accessions consisting of more than two biotypes over five regenerations only (Stoyanova 1996).

Seed sample size

Since the random loss of alleles is directly related to effective population size, changes due to genetic shift can be limited by increasing the sample size for regeneration. If the fractional composition of a biotype is lower than one seed, it is in practice eliminated from the sample. The critical level of the biotype composition may be calculated by the formula (Stoyanova 1996):

$$k_{cr} = 1/N$$

where k_{cr} = critical value of the biotype composition; N = seed number in the sample for multiplication.

In order to avoid the genetic shifts resulting from ageing and multiplication, the fractional composition (k_p) of a biotype in the seed accession following storage has to be higher than the critical level. The effective size, N_e, of a heterogeneous seed sample may be designated as follows:

$$N_e > 1/k_{pr}$$

where k_{pr} is the rarest biotype coefficient after time of storage p.

From a practical point of view this means when the composition of the rarest biotype is reduced to 0.01 frequency, then more than 100 viable seeds will be required to maintain this biotype.

In conclusion, it is suggested that when biotypes of a seed accession are genetically different, they are differently affected by regeneration and storage environments. As a result, unpredictable and undesirable selection may occur. These genetic changes could be predicted only by monitoring the seed viability and characterization of the genetic heterogeneity.

Biochemical genetic markers

If genetic changes are considered from the population standpoint, where the variation is visible, then the effect of undesired selection may be estimated relatively easily by the phenotypic characters. However, genetic change also happens in cultivars in which the individual differences may not be so obvious, and may depend on environmental factors. Biochemical genetic markers represent only genetic variation and may be used to compare genetic diversity within and between accessions during maintenance and regeneration. In an investigation of 56 Bulgarian wheat cultivars, 38 were determined to be heterogeneous, because there had not been selection pressure for gliadin uniformity. Although the gliadin biotypes are similar, they survived differently under storage conditions (Stoyanova 1991).

Our experience shows that seed storage proteins as genetic markers are a useful tool to monitor both genetic variation in the seed accession and the occurrence of mutations (Stoyanova 1991, 1992, 1994).

Seed collections

The base collection in the Bulgarian seed genebank has more than 120 crops and wild species (Fig. 1). The seeds are preserved in hermetically closed glass containers or vacuum packages of laminated aluminium foil at –18°C. The seed desiccation is carried out according to IPGRI standards.

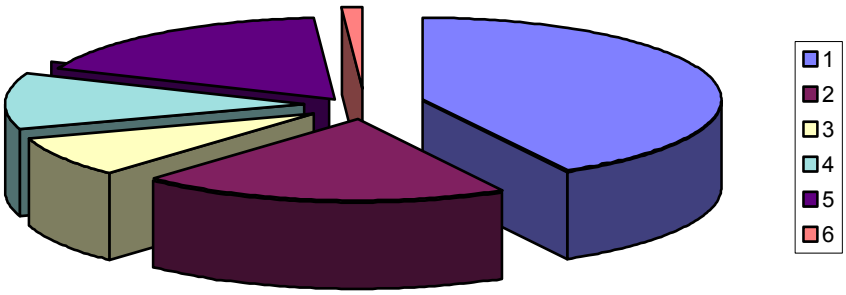

Fig. 1. Structure of the crop collections preserved in long-term storage. 1, cereal crops; 2, crop legumes; 3, grasses; 4, vegetables; 5, industrial crops; 6, flowers and rare plants.

The base collection materials are not used for distribution, except to replace materials from the active collection. As a rule seeds are passed to the base collection after the first or second multiplication. In this way, the negative effect of multiple regeneration is minimized.

Seed storage of the active collection is carried out at medium-term storage conditions at +6°C and about 50% RH. All small seed accessions that are either newly collected or received by free exchange are preserved in this way. The accessions intended for exchange are maintained under the same conditions.

The regeneration strategy is based on three predominant factors: seed viability, genetic heterogeneity and seed quantity (Fig. 2). The seed viability reduction and its vulnerability to genetic shift are the most important factors in regenerating a seed accession.

How many seeds per accession?

Seed regeneration is costly in terms of resources and time, while the risk of genetic drift and genetic shift are compounded over each regeneration cycle. The most cost-effective way of minimizing the loss of genetic integrity is to keep the frequency of regeneration to a minimum. Thus, as many seeds as possible should be maximized to provide adequate seeds for use in active collections and long-term storage.

In our genebank, we maintain about 3000 to 10 000 seeds per accession in the base collection. Since it may not be possible for some species to produce more seeds, a limited number of rare and threatened species are preserved in about 1000 seeds.

Further research

The practice in the seed genebank shows that three major factors are related to the viability of stored seeds:

- old seeds have poor vigour and are more sensitive to stress conditions;
- because of rapid rehumidification, imbibitional injury may occur when very dry seeds are set to germinate;
- hard-seededness, which confers seed coat impermeability, increases with reducing seed moisture content.

The genetic integrity may be affected because of damage to individual seeds or because of differences in germination rate. More information about maintenance of old seeds is needed to reduce changes in storage.

Although the regeneration events are predictable, the risk of selection pressure and human error are considerations which emphasize the need to minimize multiplication. Seed sample size is bound to vary with each regeneration, so several regenerations can result in serious genetic loss within an accession. When and how the seed accessions should be multiplied so that genetic shift and costs can be minimized is a question which needs more research.

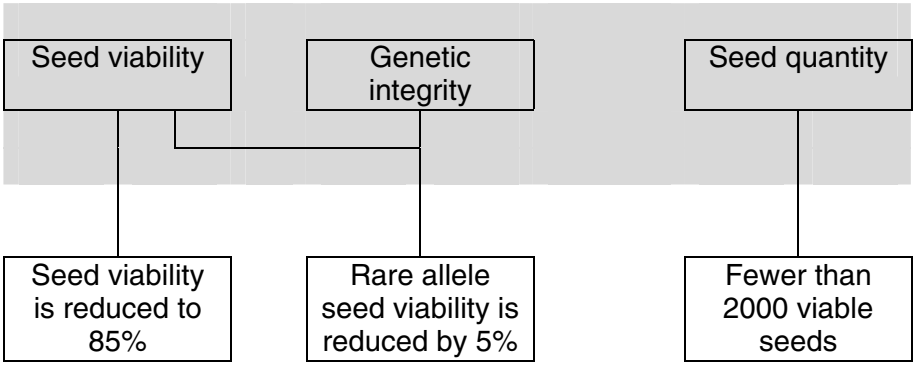

Fig. 2. Regeneration strategy in relation to the seed viability, genetic heterogeneity and seed quantity per accession.

The genetic variability in accessions is influenced by environmental factors. That is why biochemical markers may be used as a practical tool to monitor the genetic integrity. Investigation of applications of this method in seed genebank practice will help to predict the genetic shifts.

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Conservation and regeneration of crop germplasm resources in China

Fan Chuanzh, Ma Yuansheng, Tan Fujuan and Wang Shuming

Introduction

China is one of the major centres of origin for many crops. Although large-scale nationwide collecting was organized by the Chinese government in the 1950s, a systematic programme on collecting, multiplication/regeneration, conservation and evaluation began only in 1978. Since then, significant progress has been made in crop genetic resources work in China, especially in the areas of multiplication, regeneration and conservation.

Conservation

A two-tier conservation system has been established in China. It is a combination of the National Genebank and provincial or specialized genebanks. The National Genebank was established in the Institute of Crop Germplasm Resources (ICGR), the Chinese Academy of Agricultural Sciences (CAAS), Beijing. The National Genebank is responsible for long-term preservation of crop germplasm in the whole country, including the preservation of materials introduced from abroad, as well as for the international exchange of genetic resources. The National Genebank consists of two genebanks: Genebank No. 1 is used for medium-term storage of crop germplasm from abroad and for material meant for international exchange; Genebank No. 2 is equipped with long-term storage facilities with a temperature of −18±2°C and a relative humidity of 50±7%. To date, seeds of around 300 000 accessions (including 29 families, 164 genera and 473 species) have been stored in the latter genebank. A duplicate set of these collections is housed for security in Qinghai province, in a facility set up by the ICGR in collaboration with Qinghai Academy of Agricultural Sciences, which also has long-term storage facilities. Provincial or specialized genebanks are located in various provincial agricultural research institutions or/and technical institutes of CAAS. About 20 medium-term storage facilities have been constructed for the conservation of local or particular crop seed materials in the provinces.

Regarding vegetatively propagated crops such as wild rice, sweet potato (*Ipomoea batatas*), potato (*Solanum tuberosum*), perennial wild peanut (*Arachis* spp.), aquatic vegetables, ramie (*Boehmeria nivea*), tea (*Camellia sinensis*), mulberry (*Morus* spp.), as well as 16 fruits, including apple (*Malus* spp.), pear (*Pyrus* spp.), grape (*Vitis* spp.), peach (*Prunus persica*), orange (*Citrus* spp.), walnut (*Juglans* spp.), chestnut (*Castanea* spp.), strawberry (*Fragaria* spp.), persimmon (*Diospyros* spp.), jujube (*Ziziphus* spp.), sand pear (*Pyrus pyrifolia*), banana (*Musa* spp.), loquat (*Eriobotrya* spp.), plum (*Prunus salicina*), apricot (*Prunus armeniaca*) and temperate zone fruits, are conserved in the respective field genebanks, distributed in the provincial and agricultural institutions, or in technical institutes of CAAS.

Regeneration

In order to manage crop germplasm resources in the National Genebank in Beijing for long-term conservation, a national key programme has been undertaken during the past 10 years. The programme was organized and coordinated by ICGR, in collaboration with provincial institutes and other institutes of CAAS. The multiplied/regenerated seeds were sent to the National Genebank in Beijing for long-term storage. The main criteria for the acceptance of regenerated germplasm for long-term storage are acceptable seed germination rate and seed purity. Germination tests are made under controlled conditions. When the germination rate is above 90%, varying with different crops, the seed sample is qualified for long-term storage. If the germination percentage is less than the set standard, the responsible institute has to regenerate the seed sample again during the next growing period. The seed number of each accession varies with seed size, 10 000 grains for small-seeded species, 6000 for medium-sized and 2500 for large-seeded types. It is planned to regenerate an accession when the seed viability of that accession falls below 80–85%. As the long-term storage has been in operation for only 10

years, no accessions that are stored in the National Genebank have had to be regenerated to date.

As mentioned above, regeneration is mainly carried out in local or specialized institutes where the medium-term storages are located. The regeneration frequency varies for different crops in different regions and is carried out under natural conditions in northern China. The cereal crops need to be regenerated once every 3–5 years, maize 4–5, vegetables 2–3, cotton 5–6, flax 8–10, and oil crops 2–3 years.

The regeneration methods adopted in China are based on a breeding system. For self-pollinated species, no special care is taken during regeneration. Randomly selected seed from stock of the accession are grown out in the field with one accession per plot. Crops such as rice, wheat, millet and sesame are regenerated in this way. For cross-pollinated species, several isolation methods are adopted to prevent pollen contamination: (i) female inflorescence bagged, each female crossed with mixture of pollen from other plants, and seeds harvested from female plants, e.g. maize; (ii) inflorescence bagged, flowers within the inflorescence pollinated with each other and seeds harvested from these inflorescences, e.g. sorghum and sunflower; (iii) nylon net or gauze net used to cover a plot where an accession is regenerated, e.g. crucifers such as rape seed, Chinese cabbage and radish; (iv) where possible, spatial isolation is adopted for cross-pollinated crops.

Research on regeneration methods

Work is in progress at ICGR on optimum method of regeneration for five species, including four cross-pollinated crops: common buckwheat (*Fagopyrum esculentum*), Chinese cabbage (*Brassica pekinensis*), Job's tears (*Coix lachryma-jobi*), multiflora bean (*Phaseolus multiflorus*) and one self-pollinated crop, sesame (*Sesamum indicum*), in collaboration with the International Plant Genetic Resources Institute (IPGRI). It is well recognised that the population size, mating system and mode of pollination, isolation and harvest method are the major factors affecting genetic stability during the regeneration process. Therefore, we are working on the effects of different population sizes (200, 150, 100, 50 and 25 individuals per accession), isolation method (artificial barrier – gauze, nylon, plastic and bagging), crossing methods (chain-crossing, pair-crossing, controlled poly-crossing and poly-crossing) and harvest methods (individual harvesting and bulking a certain number seeds from each plant) on seed-set and morphological, agronomic characters.

Changes in genetic variation are being studied using variation in morphological and agronomic characters, and isozyme constitution between progeny and parents. The preliminary results of the 1994 and 1995 experiments showed that the seed-set of Chinese cabbage was above 70% either through chain-crossing or pair-crossing; making chain-crossing much easier than pair-crossing. The pod-set percentage was about 60% when poly-crossing was adopted in isolated plots. Seed-set was less than 20% in buckwheat when hand pollination was carried out. However, the seed-set improved when a brush was used for pollination and pollinators (bee, fly) were used. It was found that seed-set was highest (up to 77.7%) when flies were used as pollinators. Hand pollination was not suitable for multiflora bean as the seed-set was only 2.9%. It was found that bees were slightly better pollinators for multiflora bean and the seed-set increased to 16.8%. Moreover, natural insect pollination is favourable for seed-set of multiflora bean. In the case of Job's tears, the seed-set was about 40% with poly-crossing.

Pre-tests of isozyme analysis of peroxidase, expressed sequence tags, superoxide dismutase and malate dehydrogenase were made for the accessions tested. This study is continuing. Isozyme analysis and comparisons between progeny and parents of each variety in the five species will be carried out next year.

Constraints and problems of regeneration in China

Although it is understood by most germplasm workers and researchers that regeneration is one of the important activities in a genebank, insufficient attention has been given to genetic erosion caused by genetic drift and genetic shift during the process of regeneration. Not many studies

have been carried out on regeneration methods for different crops. Scientific knowledge on proper regeneration methods is urgently needed for genebank managers, especially for those who work with medium-term storage as there will be a need for more frequent regeneration. It is felt that further studies on regeneration methods are very important in order to maintain genetic integrity.

Financial support is needed to carry out regeneration properly in China. Due to a lack of funding, correct regeneration of some seed samples could not be done in some provincial genebanks, and they faced the problem of losing those materials as seed viability decreased very significantly.

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Regeneration of germplasm of seed crops at the Ecuadorian genebank

Raúl O. Castillo

Introduction

Seed regeneration in any genebank is one of the most important steps to be carried out in the process of germplasm conservation. Seeds eventually deteriorate, whatever the conservation conditions, and have to be regenerated. Ideal conditions of low temperature and seed moisture content can only delay seed deterioration, while storage of seeds in ambient conditions accelerates the decline of viability. Therefore, the frequency of regeneration is conditioned by the seed storage conditions and the availability of seeds to satisfy the requirements of plant breeders or researchers.

Along with the need for seed regeneration, the process itself has to be managed in such a way that the genetic integrity of accessions is maintained as far as possible. In outcrossing species, regeneration of germplasm should take into account the possibility of the transfer or mixing of genes from one accession to another. If controlled pollination is practised, all individual plants from an accession have to be included in the process of pollination to include all genes from the accession. The ultimate objective of a seed conservation programme is to preserve the genes with the same frequency as found when they were collected in the field.

Researchers and curators at the National Department of Plant Genetic Resources and Biotechnology (DENAREF) of Ecuador closely follow the recommendations of the International Plant Genetic Resources Institute (IPGRI) and other centres, and the principles of population genetics, in order to maintain the genetic composition of an accession during the regeneration process (FAO/IPGRI 1994). This short paper gives an overview of practical considerations and procedures carried out at DENAREF while regenerating germplasm of seed crops.

Considerations for regeneration

The decision on the number of accessions and priorities of seed regeneration at DENAREF depends on two factors (Nieto *et al*. 1984; INIAP-DENAREF 1995): (i) seed viability and (ii) seed availability.

Seed viability

The general recommendation for seed regeneration in any genebank is when seed viability is reduced to 85% germination (FAO/IPGRI 1994). However, there are many tropical species with germination percentages of only 90% even after harvest under the best of conditions. These seeds will have lower germination percentages after a period of storage under the same conditions as other species with initial germination percentage of close to 100%. Therefore one cannot expect to regenerate those seeds every year or two. Accessions are chosen based on a germination percentage range of 75–85% compared to the initial germination tests (INIAP-DENAREF 1995).

Accessions are taken at random within species collections and between species to determine the germination percentage. The number of accessions to be regenerated will depend on the availability of resources (financial and personnel) and the type of accession (wild, weedy or cultivated). Wild species are more difficult to regenerate than cultivated species. Most wild materials have variable seed dormancy and require specific environmental conditions to germinate. If these conditions are not met, seeds might die or only few will germinate, producing changes in gene frequencies or loss of rare genes/alleles.

DENAREF has only the base collection genebank. Therefore regeneration and multiplication are carried out to satisfy the conservation needs for a base collection and seed availability for distribution.

Availability of seeds

Although in general terms seed regeneration and seed multiplication have the same definition for most genebank curators, there is a difference between these two processes. Seed regeneration implies the maintenance of the same gene frequency as at the moment of collection, whereas seed multiplication allows for some changes in gene frequency due to a need to maximize the quantity of seeds available for distribution, stored at –0°C. At DENAREF, both activities are carried out together. The decision to regenerate an accession is made when the number of seeds of an accession has reached a level where it can no longer be distributed. At least 2000 seeds for each accession of small grains and at least 1500 seeds for accessions with larger seeds are conserved at –10°C. The lower number of seeds for larger grains is due to space availability in the storage room (INIAP-DENAREF 1995).

Each accession consists of four aluminium–plastic envelopes containing 1500 or 2000 seeds each. Seed distribution is carried out from one of the envelopes. If only two envelopes of an accession are left, then multiplication / regeneration has to be done even though the seed germination percentage may be high.

Regeneration process

Several factors can modify gene frequencies during the regeneration process. Most are related to selection (Petersen 1982), mutation, migration, and genetic drift in small samples (Sevilla and Holle 1995).

Most of the problems of changing gene frequencies observed at DENAREF were due to selection. If seeds are regenerated when germination percentage is below the range explained above (i.e. 75–85%), only those seeds with good germination will survive and maintain the allele frequency of certain genes, whereas seeds with weak viability might decrease the frequency of rare alleles. These weak seeds will produce seedlings with slow establishment compared to other, more vigorous seeds. These conditions may induce selection for specific genes.

Destruction of a few individuals by pests during regeneration can also result in selection. Permanent checks on the incidence of pests and their control are practised, because regeneration is not an evaluation for agronomic traits. Some post-harvest processes may also contribute to some degree of selection. Some individuals with a late maturity period might be damaged during harvest and cleaning. Workers can also select bigger or better-looking grains and therefore might cause a change in gene frequency (Sevilla and Holle 1995).

Natural mutations can be caused by and in the environment where the regeneration plot is planted. Plots planted in soils with herbicides from previous planting seasons, or exposed to ultra-violet light at high altitudes such as in the Andes, are susceptible to mutations. Selection can also result from the environmental conditions of the regeneration site. When accessions collected in different ecological niches are planted in one location, some may not be adapted, and only strong individuals with good adaptation survive and produce seed. To reduce the possibility of selection, regeneration plots are planted in sites with an average altitude taken from the passport data. DENAREF also has screenhouses where environmental conditions can be simulated, as similar as possible to those of the site where the samples were collected.

Contamination and mixture of accessions can be produced by pollen or seed mixtures. In outcrossing species, pollen contamination is a common problem. Thus, migration can be a factor changing allele and gene frequencies. Pollination control is practised at DENAREF for both outcrossing and self-pollinated species.

*An example of seed regeneration in quinoa (*Chenopodium quinoa*)*

Accessions are planted in the field surrounded by three rows of tall types of corn. Each accession consists of three rows of 10 plants each. Paper bags are used to cover the inflorescence. Pollen is collected from one plant to pollinate another, allowing a chain-crossing system (Nieto *et al.* 1984; INIAP-DENAREF 1995). However, a small amount of pollen contamination might occur due to the presence of some insects. Assuming that

accession A has a gene frequency of q_0 = 0.9, which is contaminated with pollen from accession B with a gene frequency of Q = 0.1, then 10% of grains from accession A will have genes from accession B. If one assumes a gene frequency of m = 0.05 of contamination from B into female gametes of accession A, the gene frequency of q_1 will be:

$$q_1 = q_0(1-m)+mQ$$
$$q_1 = 0.9(1-0.05)+0.05(0.1)$$
$$q_1 = 0.86$$

Therefore, in one generation accession A will be reduced to 0.86 from 0.9 which, in general terms, is not a big change. However, if contamination of pollen occurs in larger amounts, then the gene frequency will decrease significantly in just one generation.

To avoid genetic drift, regeneration should be done using an adequate number of individuals. The sample size depends on the less common alleles or genotypes. If a rare allele in an accession is present in 10%, only 20 individuals per accession will be required to maintain these alleles with a 90% probability, or 40 individuals for 99% probability. In general terms, if the probability of rare allele is less than 5%, at least 100 individuals are required to maintain it in the population with a probability of 95%. For multiple alleles in a locus the number of individuals will increase.

Constraints on proper regeneration

Facilities

Facilities for seed regeneration with special environmental requirements are lacking. Greenhouses and other facilities are required for accessions from localities with high temperatures, wild relatives of crops from special ecological niches, weedy species which require breaking of seed dormancy, etc.

Research on specific crops and wild relatives

Many seed samples have not been regenerated successfully due to the lack of basic information on germination procedures, pollination control, and floral biology in general. There are several lesser-known crop species awaiting research in order to recommend the best regeneration procedures.

International cooperation and networking

For many crops, seed regeneration has to be carried out in their original environmental conditions, or using specialized facilities available in other genebanks around the world. Cooperation at national and international levels may help to regenerate samples under the best conditions.

Resource allocation

Most genebanks lack adequate financial support for germplasm regeneration. Germplasm seed regeneration is costly and requires adequate financial support.

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Seed regeneration activities at the plant genetic resources centre of Ethiopia

Hirut Kebede

Introduction

Ethiopia, with its wide range of agro-climatic conditions, is one of the centres of diversity and domestication of many cultivated plants. In order to conserve this tremendous plant diversity, the Plant Genetic Resources Centre of Ethiopia (now the Biodiversity Institute) was established in 1976. The total holdings in the Genebank have now reached 54 000 accessions of about 100 species, of which over 70% are cereals.

Landraces make up the bulk of the collections conserved at the Genebank. They are mixtures or heterogeneous populations consisting of up to five types. Farmers maintain them as mixtures so that they are buffered against different environmental hazards in different seasons.

Active and base collections and source of seeds for regeneration

In the Genebank, accessions are stored in active and base collections (Table 1). The base collection is never touched as long as the viability of the seeds is adequate. The active collection is used for evaluation, utilization and exchange purposes. Samples for regeneration are taken from the active collection when the amount of seed in the active collection is depleted. If the viability of the seeds in the base collection drops below the set threshold, the seed sample for regeneration is also taken from the active collection and the old seeds in the base collection are replaced by the fresh seeds.

Priority is always given to accessions with low viability for regeneration. The Conservation Division of the Genebank determines the initial viability for each sample before its storage, and monitors viability on randomly chosen accessions of the stored seeds at regular intervals (5 or more years). Accessions with a viability <85% and also those with insufficient amount of seed are registered for regeneration. These lists are passed to the Multiplication, Evaluation and Utilization Division, which conducts the regeneration activity.

Procedures followed and difficulties encountered during seed regeneration

In order to preserve the genetic integrity of the original sample during seed regeneration we strictly follow certain procedures.

Regeneration sites

Genetic shift due to natural selection may occur because of differential viability, survival and fecundity of genotypes in a population. This effect can be minimized by selecting regen-

Table 1. Genetic composition, thousand grain weight (TGW) and corresponding seed number for active and base collections of germplasm accessions

	TGW (g)	
Genetic composition	**<200**	**>200**
	8000 seeds	**4000 seeds**
Heterogeneous material (populations, mixtures)	3000 base coll.	1500
	5000 active coll.	2500
	3200 seeds	**1600 seeds**
Homogeneous material (pure line, modern cultivars)	400 base coll.	200
	2800 active coll.	1400

eration sites similar to those of the areas of natural adaptation of the original samples. In order to fulfil this requirement the Genebank has about 14 regeneration sites with altitudes ranging from 450–2800 m above sea level, and varying temperature and moisture regimes. Out of these 14 sites, 12 are research sites of the Institute of Agricultural Research and Alemaya University of Agriculture. These two institutions give full support to our seed regeneration activities by providing the land and taking care of the field preparation at their cost. This collaborative work has cut down costs of machinery and its maintenance, labour costs for field preparation, guards, stores, etc. This collaborative work has also enhanced the utilization of our germplasm. As these sites are the respective breeding stations of the crops concerned, we work in close collaboration with the breeders, pathologists, entomologists and other scientists. Therefore these scientists have the opportunity to observe the germplasm in the field each year and to choose interesting accessions for their crop improvement programmes. Additionally, we consult them in elaborating and evaluating the descriptor lists to be used.

In order to decide in which site the samples should be regenerated, the accessions of a given crop are grouped according to the altitude obtained from their passport data and are planted accordingly at the sites in corresponding altitudes. For example, highland sorghum is regenerated at 1960 m altitude (a site called Arsi Negelle), the intermediate sorghum types at an altitude of 1580 m (Nazareth) and the lowland types are planted at 1320 m (Mieso). However, we face difficulty identifying appropriate regeneration sites for accessions with no passport data. They may be planted in an unfavourable environment and some or most of the plants could be lost. As most of the collections at the Genebank are landraces of mixed types the genetic shift is more serious for them than for a homogeneous sample under unfavourable conditions. Some genotypes in the mixture may not germinate to start with, or may germinate but may not survive to set seed.

Isolation

In predominantly outcrossing species, the use of strict isolation is important to avoid adulteration by foreign pollen. It is necessary to know the flower biology of the species (self- or cross-pollinated), and if cross-pollinated, whether it is wind-pollinated, insect-pollinated or both. Once this information is available, it can be decided which isolation technique should be used for each species. At present, the major outcrossing species we are handling are rape seed, faba bean (under ideal conditions), safflower, sunflower, sorghum, maize, hot pepper and noog. Some of the isolation techniques used are presented below.

*Noog (***Guizotia abyssinica***)*

This annual oil crop has been domesticated in Ethiopia and has developed a wide diversity. The species is predominantly pollinated by insects and is self-incompatible. This is mainly because the receptive part of the stigma rarely touches the pollen of the floret. Since selfing of this species would cause serious inbreeding depression and might expose the accessions to unwanted natural selection, a form of sibbing within each accession is applied. A sufficiently large number of plants are covered with cheesecloth bags and at regular intervals hand-pollination between plants within the accession is carried out. The use of pollinating insects within a bag could increase seed setting.

Brassica *species*

Brassica carinata, B. nigra and *B. oleracea* are species either indigenous to or widely grown in Ethiopia. All are predominantly cross-pollinated and, therefore, require special attention to avoid gene flow between accessions. In this case also, cheesecloth bags are used to isolate a group of plants of the same accession, and natural pollination within a bag is used to obtain the required seed amount.

*Faba bean (***Vicia faba***)*

This is generally a predominantly self-pollinating species, but under certain conditions up to

50% outcrossing is observed, mainly by bees. The isolation method presently in use is *Brassica* fencing around each accession. The assumption is that the pollinating bees stay mainly within a plot, and before approaching another plot they will be attracted by the *Brassica* flowers where the faba bean pollen will be brushed off. Although this method does not ensure the avoidance of any gene flow between accessions, it is relatively simple and cheap and thus large numbers of accessions can be regenerated.

Multiple regeneration cycles

Genetic drift due to random loss of alleles can happen when we have multiple regeneration cycles and when sample size taken for regeneration is too small to capture all the variation in the original sample. Regeneration cycles have been minimized as much as possible as long as the original sample is large enough to take the appropriate sample size for regeneration. However, even with the appropriate sample size, when adverse growing conditions occur insufficient seeds are produced and another regeneration cycle is needed. With those accessions where the original sample is small, mainly those exchanged, repatriated or donated from abroad, carrying out multiple regeneration cycles is indispensable.

Seed longevity

Growing conditions, post-harvest handling, pre-storage processing and storage conditions determine seed longevity. With regard to growing conditions, efforts are made to produce high quality and disease-free seeds. However, problems such as moisture stress, diseases and insect pest attacks are encountered. Examples of the major diseases encountered are leaf and stem rust on wheat; powdery mildew, rust and chocolate spot on faba bean. In order to deal with these problems, cultivation is avoided at sites where the respective diseases, insects and environmental stresses are prevalent. For example, the site that was used to grow the wheat accessions was found to be a hot spot for leaf and stem rust which resulted in the production of shrivelled seeds. So the wheat accessions are now regenerated at another site in order to avoid this problem. If these problems are a one-time experience and if they are thought to have a significant effect on the plants, where possible chemicals are used to control the insects and diseases. Supplemental irrigation is used where rainfall falls short. Otherwise, application of chemicals is not recommended during seed regeneration activities in the field.

Care is also taken in harvesting each accession at the right time, giving priority to the shattering types. However, since the majority of the accessions we handle are landraces, consisting of mixtures of populations, the harvesting time may not be the best for all the types and this may have an effect on the longevity of the seeds in storage and possibly result in unwanted natural selection.

Post-harvest handling, such as avoiding mechanical damage to the seeds during threshing, and fumigation of seeds with phosphine to prevent weevil attack (a major problem under our conditions), is carried out with care before the pre-storage processing activities begin. Before storage samples are hand-cleaned for impurities, seeds are counted, dried to 4–6% moisture content and hermetically sealed in aluminium foil bags for storage, and this process is carried out as fast as possible to shorten the life of the sample in the pre-storage condition. Both the active and base collections are then maintained at –10°C.

Since 1980, over 75 000 accessions have been regenerated for both seed multiplication and rejuvenation purposes. With regard to germplasm distribution, during the past 10 years we have distributed over 30 000 accessions of 25 crop species to various researchers in different institutions. With the increasing demands for germplasm by researchers, at present approximately 2500–3000 accessions distributed per year, we will have to continue multiplying seeds to have sufficient amounts for utilization. The germplasm characterization work is almost always combined with seed regeneration activities. So far over 70% of the accessions (38 000 of 54 000) conserved in the Genebank have been characterized for basic morpho-agronomic characters.

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Regeneration procedure in the Gatersleben genebank

C.-E. Specht, K. Hammer and E.R.J. Keller

The Gatersleben genebank consists of an integrated system which includes storage and regeneration under one roof. The roots of this system can be found in the early beginnings of the genebank. Initially, after genebank's foundation within the Institute of Crop Plant Research in 1943, the complete collection had to be regenerated each year (Hammer 1993; Hammer *et al.* 1994). At that time seeds were stored only at room temperature.

In 1976, the establishment of new cold storage facilities improved storage conditions. After that the regeneration rate declined permanently, and at present it amounts to 12%, meaning that about 12 000 accessions are regenerated annually – the whole collection of the Gatersleben genebank, including external branch stations, amounts to 100 000 accessions (IPK 1995). Fortunately all the necessary cultivation can be done in Gatersleben itself or at its four branches. Each year, 11 ha of land and about 0.5 ha under glass are used for planting germplasm for regeneration. To minimize the risk of disease infection, every field is used only once in a 10-year rotation system.

A good mixture of self- and cross-pollinating species is important, as well as the use of isolation cabins or different glasshouses. Self-pollinators can be used for spatial isolation of cross-pollinators: for example *Secale* spp. accessions, which need a distance of 100 m between each other, are isolated by surrounding self-pollinators.

For regenerating cross-pollinating species, insect species such as Hymenoptera and Diptera are used. Species of these families are useful to obtain an optimal seed set in crops, especially in genera such as *Allium*, *Apium*, *Brassica*, *Daucus* and *Raphanus*. Further investigations are necessary to find out which species are particularly useful and how to rear them (Gladis 1992).

At Gatersleben, the principle is followed to split morphologically variable populations within cross-pollinating species. This procedure is supposed to minimize the risk of losing genetic information caused by genetic shift and drift within a population during *ex situ* regeneration. At present 16 permanent employees and 10 seasonal workers are involved in the regeneration work, compared to a staff of five persons working in the seed laboratory. Labour force is the main problem in these two working groups.

For the regeneration of vegetatively propagated crops, *in vitro* culture has been established at Gatersleben (e.g. *Allium*) and in the northern branch of the genebank (potato).

In the accessions stored as seeds, the key factors that determine the frequency of the regeneration of a certain accession are:

- loss of viability
- too small quantity of seeds
- many requests for a given accession
- morphological characterization that is scheduled
- need to separate different lines of a given accession.

It is clear that the crucial factors for regeneration depend on the individual accession. The quantity of seeds stored per accession is determined by the prevailing circumstances. It is not possible to use more than one glass jar per accession, because in Gatersleben the storage capacity is nearly completely occupied. One glass jar has a volume of 1 litre, so in case of large-sized seeds (*Vicia faba*) about 4000 seeds are stored, whereas for small-sized seeds (cereals), the quantity amounts to about 14 000. But genetic losses, which might be caused by too small quantities of seeds, are reduced through the above-mentioned separation of lines.

All accessions of cereals and grasses are stored at 0°C in Gatersleben. The seeds of vegetables, medicinal plants, ornamentals, oil and dye plants, legumes, etc. are stored at –15°C. Inside every glass jar there is a bag of silica gel, since it is not possible to control the

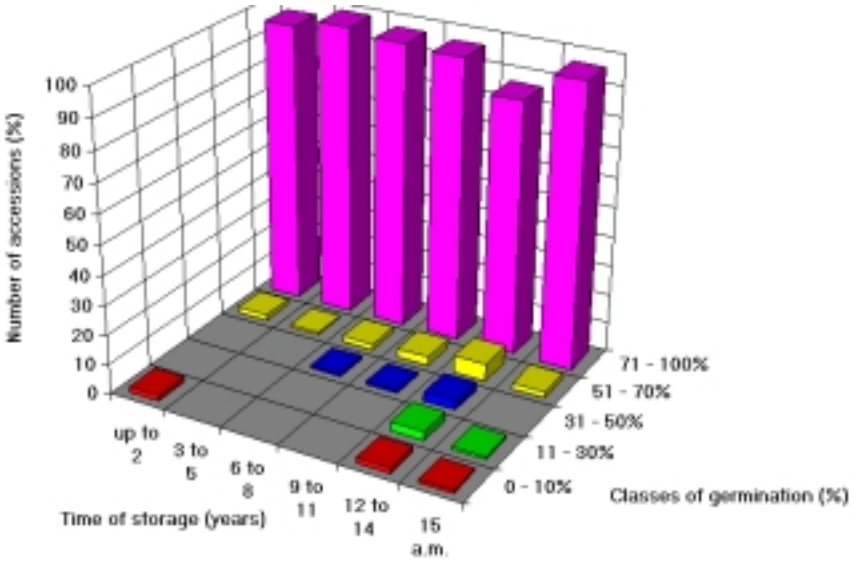

Fig. 1. *Hordeum* ssp. – seed germination after storage at 0°C.

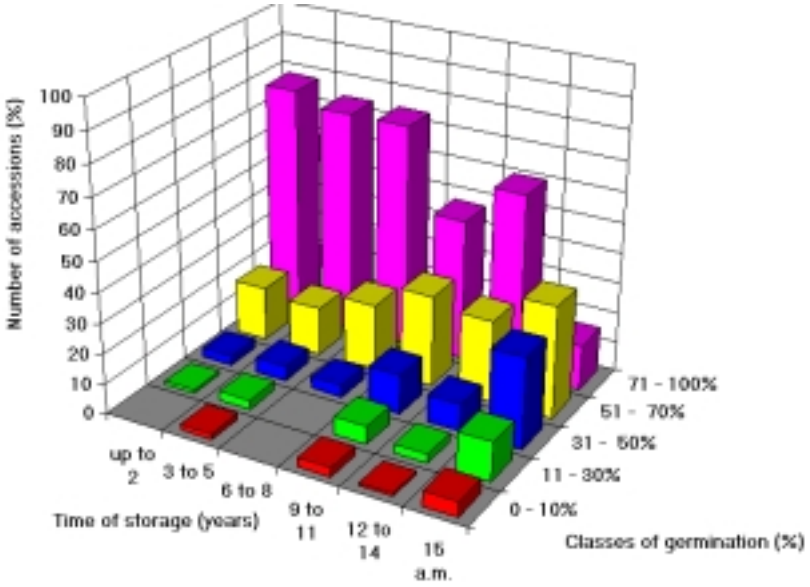

Fig. 2. *Avena* spp. – seed germination after storage at 0°C.

air humidity in the cold storage room. Due to lack of space the accessions are not differentiated into base and active collections.

A few diagrams give an impression about the influence of temperatures determining the viability of different genera after storage. (0°C: *Hordeum* spp., *Avena* spp.; –15°C: *Phaseolus vulgaris, Allium cepa*) (Figs 1–4). Fig. 5 shows five columns, each of which represents an average of germination results from different harvest years of *Allium cepa*. Two columns show a germination percentage of about 90% after a storage period of 15 and 17 years, respectively. Three others have an average of about 60% after 16, 14 and 14 years of storage, respectively. Compared to an average derived over the past 40 years, the weather in 1973 and 1976 was dry and hot. These years correlate with high germination results after long-

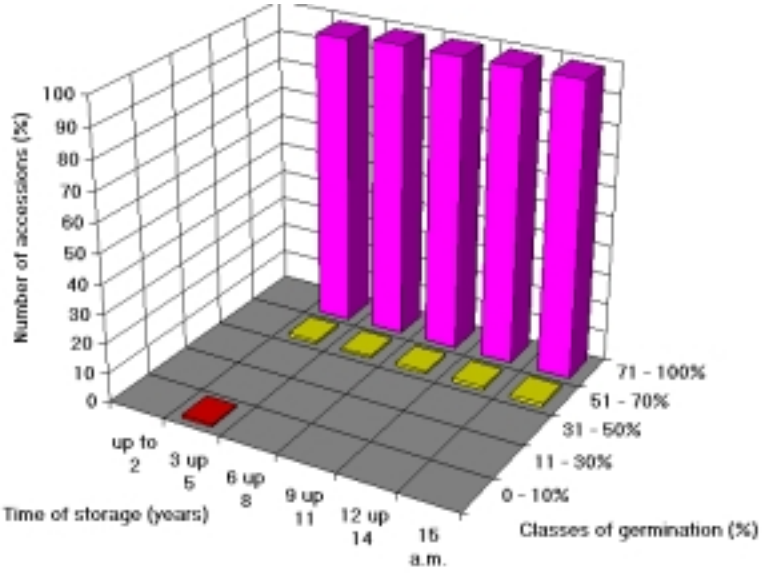

Fig. 3. *Phaseolus vulgaris* – seed germination after storage at –15°C.

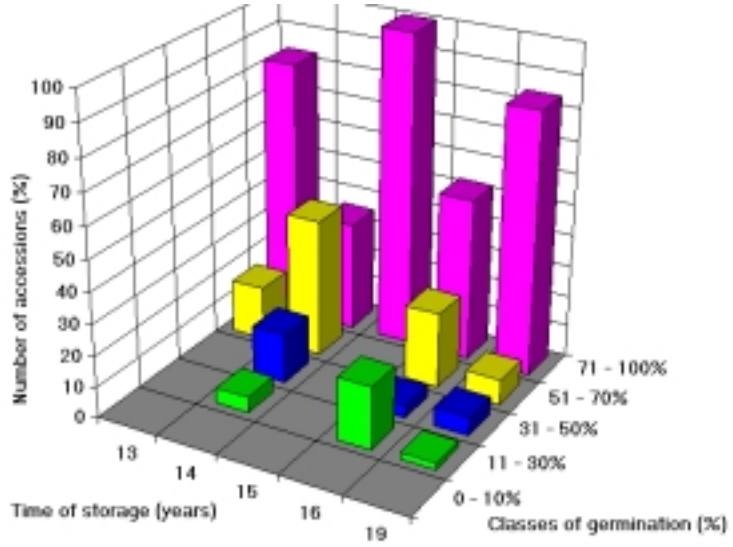

Fig. 4. *Allium cepa* – seed germination after storage at –15°C.

term storage. Although there was only a slight difference in viability at the beginning, now huge differences of about 30% appear. So it can be concluded that the weather conditions of the harvest year strongly influence the quality and viability of seeds during the time of storage (Keller and Specht 1994).

Another topic which has to be taken into account for the future is the elaboration of methods on how to regenerate certain species. Fig. 6 shows the influence of different temperatures on germination results in different subgenera of the genus *Allium*. Without specific information on the most suitable germination temperature, seed regeneration within this genus is extremely difficult (Specht and Keller 1995).

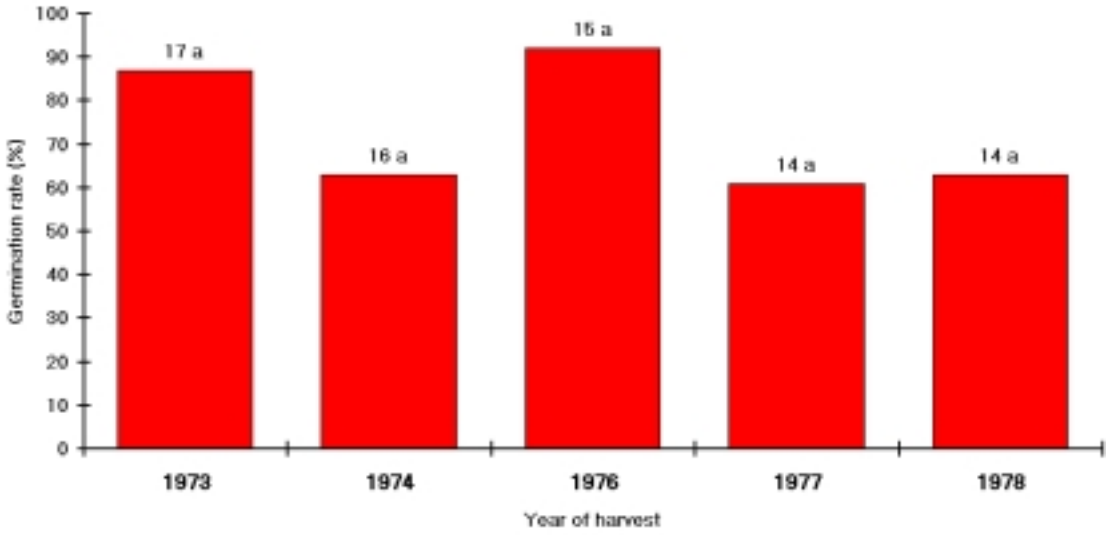

Fig. 5. *Allium cepa* – seed germination after storage at –15°C.

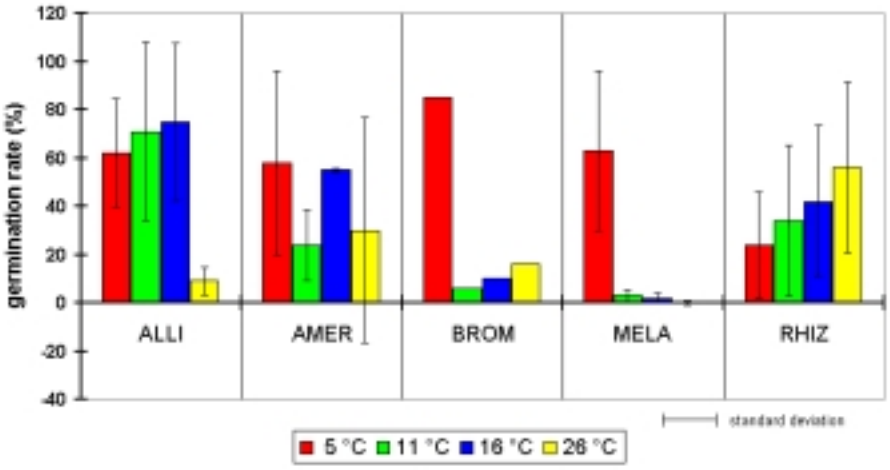

Fig. 6. Influence of temperature on germination in different subgenera of *Allium.*

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Germplasm regeneration under the Indian National Plant Genetic Resources System

B.B. Singh

Introduction

The origins of Indian agriculture date back to 2300–1750 BC and exhibit a great diversity of farming systems and crops, with a multiplicity of biotic and abiotic stresses which, in their long association with useful plant species, have resulted in the establishment of vast diversity. India is a primary centre of diversity of crops such as rice, black gram, moth bean, pigeon pea, smooth gourd, ridge gourd, pointed gourd, tree cotton, capsularis jute, jackfruit, banana, mango, jambolan (*Syzygium cumini*), large cardamom, black pepper, several minor millets and medicinal plants such as *Rauvolfia serpentina* and *Saussurea lappa*.

The introduction of germplasm in the distant past from the Mediterranean, African and American regions has also significantly enriched Indian wealth in plant genetic resources (PGR). The Indian gene centre developed as a secondary centre of diversity for African crops, such as finger millet, sorghum, cow pea, cluster bean, okra, sesame, niger and safflower, and for tropical American species such as maize, tomato, pumpkin, chayote or chou chou, chilli and amaranth. In addition, the linkages and contiguity with the other regions of diversity, i.e. the Indo-Chinese, Indonesian, Chinese–Japanese and the Central and West Asian regions, is largely responsible for regional diversity of crops such as maize, barley, amaranth, buckwheat, proso millet, foxtail millet, mung bean, chick pea, cucumber, bitter gourd, bottle gourd, snake gourd, brassicas, rice bean, tomato, citrus, small cardamom, *Saccharum*, ginger, turmeric and tuber crops, particularly yams, taros and bamboos.

To this day, India retains an extensive reservoir of ancient diversity in farmers' fields in many parts of the sub-continent, but especially in mountainous and tribal areas. However, there is a constant threat to these priceless resources because of their replacement with high-yielding modern varieties or destruction of their natural habitats.

Table 1. Active germplasm holdings at NBPGR centres

Station/ centre	Holdings	Major crops/crop groups
Delhi	38 772	Cereals, legumes, oilseed, vegetables, forages, fruits, medicinal and aromatic plants
Akola	28 552	Chickpea, pigeonpea, sorghum, groundnut, millets & small millets, soyabean, safflower, sesame, lentil, amaranth, horsegram, okra
Amravati	4839	Mungbean, rice bean, urad bean, sem bean, beans, sweet potato, chillies, onion, garlic, fruits (grapes, pomegranate, papaya, citrus)
Shimla	12 596	French bean, rice bean, soyabean, lentil, horse gram, minor millets, pseudocereals, oil seeds, temperate fruits, ornamentals
Jodhpur	11 622	Guar, moth bean, mungbean, sesame, pearl millet, cowpea, castor
Trichur	12 689	Paddy, horse gram, cowpea, finger millet, chillies, sesame, bitter gourd, ginger, *Curcuma*, taro, okra, brinjal, cassava, *Dioscorea*, *Amorphophallus*, *Musa*
Bhowali	5066	Wheat, maize, barley, lentil, beans, hill rices, alliums
Cuttack	1852	Paddy
Shillong	2006	Hill rices, maize, rice bean, root crops, fruits
Ranchi	2232	Paddy
Total	**120 226**	

Source : NBPGR 1995.

Table 2. Directory of National Active Germplasm Sites

Crop	NAG site	No. accns
Wheat	Directorate of Wheat Research, Carnal 132 001, Haryana	18 000
Rice	Central Rice Research Institute Cuttack 753 006, Orissa	42 000
Maize	Directorate of Maize Research, Indian Agricultural Research Institute, New Delhi 110 012	2000
Barley	Directorate of Wheat Research, Karnal 132 001, Haryana	–
Sorghum	National Research Centre for Sorghum, Rajendranagar, Hyderabad, Andhra Pradesh 500 030	5160
Pearl millet	All-India Coordinated Pearl Millet Improvement Project, College of Agriculture, Shivaji Nagar, Pune 411 005, Maharashtra	–
Small millets	All-India Coordinated Small Millets Improvement Project, University of Agricultural Sciences, Bangalore 500 065, Karnataka	8572
Pulses	Indian Institute of Pulses Research, ICAR, Kanpur 208 024, Uttar Pradesh	9310
Soyabean	National Research Centre for Soybean, Indore, Madhya Pradesh	2500
Oilseeds	Directorate of Oilseeds Research, Rajendranagar, Hyderabad 500 030, Andhra Pradesh	15 629
Rapeseed & mustard	National Research Centre on Rapeseed & Mustard, Bharatpur, Rajasthan	8082
Groundnut	National Research Centre for Groundnut, Timbawadi, PO Junagadh, 362 015 Gujarat	6432
Sugarcane	Sugarcane Breeding Institute, Coimbatore 641 007, Tamil Nadu	3979
Cotton	Central Institute for Cotton Research, PO 125, Nagpur 440 001, Maharashtra	6896
Jute & fibres	Central Institute of Jute & Allied Fibres, Barrackpore 743 101, West Bengal	3226
Vegetables	Directorate of Vegetable Research, Varanasi 221 005, Uttar Pradesh	16 139
Potato	Central Potato Research Institute, Shimla 171 001, Himachal Pradesh	2375
Forages	Indian Grassland & Fodder Research Institute, Jhansi 284 003, Uttar Pradesh	6267
Spices	National Research Centre for Spices, Marikunnu, Calicut 673 012, Kerala	2847
Tobacco	Central Tobacco Research Institute, Rajahmundry 533 105, Andhra Pradesh	1500
Plantation crops	Central Plantation Crops Research Institute, Kasargod 671 024, Kerala	307
Medicinal & aromatic plants	National Bureau of Plant Genetic Resources, New Delhi 110 012	375
Agro-forestry	National Research Centre for Agro-Forestry, Indian Grassland & Fodder Plants Research Institute, Jhansi 284 003, Uttar Pradesh	40
Fruits	National Research Centre on Arid (Semi-Arid) Horticulture, Bikaner, Rajasthan	541
Fruits (subtropical & temperate)	NBPGR Regional Station, Phagli Shimla 171 004, Himachal Pradesh	454
Fruits	Indian Institute of Horticultural Research, 255 Upper Palace Orchards, Bangalore 560 080, Karnataka	13 118
Citrus	National Research Centre for Citrus, Seminary Hills, Nagpur 440 006, Maharashtra	51
Fruits (Northern Plains)	Central Institute for Horticulture for Northern Plains, Lucknow 226 016, Uttar Pradesh	587
Tuber crops	Central Tuber Crops Research Institute, Sreekariyam, Trivandrum 695 017, Kerala	3586
Pseudo-cereals	NBPGR Regional Station, Phagli, Shimla 171 004, Himachal Pradesh	3682

The Indian PGR system

The Indian Council of Agricultural Research (ICAR) established the National Bureau of Plant Genetic Resources (NBPGR) in 1976 for executing and coordinating PGR-related activities in India. NBPGR has a network of 10 regional stations (Table 1) in the different agroclimatic zones of the country and an active partnership with over 30 ICAR Institutes, National Research Centers, All-India Coordinated Projects and State Agricultural Universities. These centres are designated as the National Active Germplasm Sites (NAGS) and are responsible for evaluating and maintaining the active collections of specific crops (Table 2). These NAGS form the major source for supply of the germplasm out of their collections to the National Gene Bank (NGB) under the Germplasm Conservation Division at NBPGR, for maintaining the base collections. NGB also obtains germplasm of more than a dozen crops from various countries because of the responsibility entrusted by IPGRI for maintenance of global and regional base collections of these crops. The active collaboration with the International Agricultural Research Centers (IARCs) also results in considerable exchange of germplasm, especially from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India and the International Center for Agricultural Research for the Dry Areas (ICARDA) in Syria. Bilateral agreements with countries have resulted in germplasm exchange with over 80 countries. These accessions eventually find a place in the NGB through the Indian system. NBPGR, in collaboration with crop-specific institutions, continues to collect germplasm in areas not previously explored. These collections are regularly added to NGB and NAGS.

Indian National Gene Bank

The National Gene Bank (NGB), established at NBPGR Headquarters, New Delhi, installed the first cold storage module of 100 m^3 capacity in 1983, comprising two compartments, one operating at 4°C and the other at –10°C. Since then, four more modules (two with 100 m^3 and two with 176 m^3 capacity) have been added. The total long-term storage capacity for over 200 000 accessions has thus been created. At present the NGB has in its base collections about 150 000 accessions (Table 3). Twelve new long-term storage modules with a capacity of 170 m^3 each, operating at –20°C, and one medium-term module operating at +4°C, 35% RH, are being procured under the INDO–US PGR project. By 1996, NGB will have a capacity for long-term storage of over 1 million accessions. NGB can also safety duplicate germplasm from the South-east Asian countries to ensure the long-term safety of the region's PGR.

Considerations for regeneration

The main factor determining the frequency of regeneration in the NBPGR genebank is the viability of the accessions. The NGB, which has the mandate of long-term conservation of base collections, stores for each accession a minimum of 3000 seeds of self-pollinated and 6000 seeds of cross-pollinated plant species with seed moisture content of 5±2%, in hermetically sealed aluminium foil pouches kept at –20°C. The distribution requirements of seed are met from NAGS where seed is stored at 5°C and 35% RH. At NAGS both factors, quantity of seed and viability, determine the frequency of regeneration. In both categories, the accessions where the quantity is reduced to 1/4 and viability drops to 75% are regenerated. The base collections are regenerated when their viability drops by 10–15%. The quantity of seed produced through regeneration is decided by the quantity required for distribution and to represent the genetic variability of the sample. The seed quantity of the more promising accessions is higher and reflects the anticipated greater seed requirement of these accessions.

Major constraints

The maintenance of a large number of collections at NAGS with limited resources and facilities, particularly in tropical and sub-tropical climates, is a difficult task. This is more complicated for the regeneration of cross-pollinated species where controlled pollination has to be practised. Cold storage facilities are being developed at various active germplasm sites.

Table 3. PGR conservation status in *ex situ* repository of National Gene Bank at 30/09/95

Crop groups	No. accns
Cereals and pseudocereals	63 822
Millets and minor millets	16 115
Oilseeds	21 062
Pulses	25 527
Fibre crops	4592
Vegetables	7813
Medicinal & aromatic plants	179
Spices	67
Other crops	778
Released varieties (reference samples)	930
Safety duplicates (IARCs)	6722
Total	**147 607**

Seed longevity in storage

Efforts are made to harvest germplasm for conservation at optimal maturity, dried in shade and gently processed to ensure maximum initial viability. The seed samples are dispatched to the genebank for long-term conservation with minimum delay after harvesting. On receipt, the seeds are kept in a short-term storage facility maintained at 20°C and 35% RH, and processed quickly for their transfer to long-term stores.

At NAGS the curators are advised to store germplasm seeds in rooms with both low temperature and RH. At sites where cold-room facilities are not available, the seeds need to be dried (or even ultra-dried) and sealed hermetically to enhance their longevity even when stored at ambient room temperatures. We have found the technique of ultra-dry seed to be effective, based on our data on pearl millet, soyabean and onion seeds.

Management of seed collections in the Indian system

The genetic variability available in landraces, obsolete cultivars, released high-yielding cultivars, parental inbred lines of improved hybrids, elite lines, donor lines with desirable traits, and wild relatives of cultivated crops and vegetables, are conserved as base collections in the NBPGR. Seed from recently collected germplasm or freshly regenerated seed with viability above 85% is dried to 5±2% seed moisture content by keeping the seed in muslin cloth bags and placing in a seed drier (15°C and 15% RH). When reaching the recommended moisture content, seeds are packed in hermetically sealed and labelled tri-layered aluminium foil pouches, arranged in plastic baskets and stored at –20°C at the NGB.

Research needs in regeneration

- For several plant species the number of accessions is enormous, and it is questioned whether such a large number of accessions is essential to represent total variability. Techniques need to be worked out to eliminate duplicates and to identify accessions with desirable traits.
- Methods for controlled pollination need to be standardized and practised while regenerating the accessions of cross-pollinated species. Possibilities of forming genepools of phenotypically similar germplasm of common geographic origin may be explored (Singh and Jika 1987).
- Frequent regeneration of all the active collections may not be essential, as it may result in genetic drift and shift. Active collections at NAGS may be maintained until thorough characterization and evaluation are completed. Seed of these accessions has to be deposited in long-term storage and the most promising accessions will be identified. Subsequently, only the accessions required for frequent distribution and exchange should be maintained at NAGS. Further research is needed to establish core collections.

- Simple and precise laboratory and field techniques need to be developed for characterization and evaluation of germplasm, particularly to assess their potential for desirable traits.

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Regeneration of seed crops and their wild relatives – the Kenyan experience

E.N. Seme, R.K. Wataaru and D.O. Nyamongo

Introduction

The need to conserve diverse genetic resources within a country and/or region has led to the establishment of genetic resources centres in a number of countries or regions. It has become increasingly evident, especially in the developing countries, that rapid changes in land use, modernization of agriculture, deforestation, etc. have led to the rapid disappearance of indigenous crops and landraces in these regions (Bouvarel 1970; Leon *et al*. 1979; Prescott-Allen and Prescott-Allen 1982). Together with these, some weedy and wild relatives of cultivated crops have also disappeared. Activities of humans, and natural disasters including desertification, drought, floods, volcanic activity etc., have led to the extinction of some species thought to be of low economic value.

Kenya has been no exception in the erosion and extinction of its diverse genetic resources. As it is within a centre of origin of some of the small grain cereals, the disappearance of these crops prompted the Ministry of Agriculture and the Government to seek funds for the establishment of a national genebank. The Federal Republic of Germany gave the Kenyan Government the technical and financial assistance to build this national genebank, which became operational in 1987.

Seed viability and monitoring during storage

The viability of stored seeds has to be monitored by periodic germination tests. It is recognised that the death of a proportion of the viable seeds in a population is inevitable during storage (Ellis and Roberts 1984). Regeneration should be carried out either when supplies are nearing exhaustion or when viability has dropped. The FAO panel recommends regeneration when germination has dropped below the initial value by 5% (preferred standard) or by 10% (accepted standard). Frankel and Soulé (1981) report that others propose a 20% drop from the initial value, hence reducing the risk of losing genetic integrity as the number of multiplication cycles is reduced.

At the Genebank of Kenya, the viability of accessions is determined upon receipt of material, which is then monitored at intervals during storage. The genebank uses the germination test to ascertain whether the entry is fit for storage. The 1975 FAO panel of experts on plant exploration and introduction recommended a minimum of 80% viability for storage acceptance. The genebank recommends a minimum viability of 85% for seeds to be stored long-term. During viability testing, recommendations of the International Plant Genetic Resources Institute (IPGRI) and International Seed Testing Association (ISTA) rules are adhered to.

Seed viability is the primary factor whenever a decision on germplasm regeneration is to be made. It is imperative that materials with very low viability are given first priority to avoid losing them altogether. When the genebank became operational, most of the accessions were received from research centres, and most were found to have very low viability. It was necessary that funds should be solicited to enable immediate regeneration. When the viability is so low that direct seeding may not guarantee a good stand, as was the case with most grasses that we had to regenerate, pre-germination is essential. The pre-germinated material is transferred to pots in a screenhouse or glasshouse before transplanting into the field.

At times, accessions donated to the genebank are not accompanied with the necessary information, such as viability status, genetic status, etc. A case in point is the global collection of sesame received from the Hebrew University for duplication at the Genebank of Kenya. The only information available was that the seed had been kept under ambient conditions for quite a long period and hence the viability could be low.

Furthermore, the sample sizes were not adequate to allow viability testing. During their regeneration, in collaboration with IPGRI and the University of Nairobi, it was realised that materials thought to be of low viability were actually highly viable. A lot of thinning had to be done which may have amounted to some kind of selection. It was also noted that their days to maturity were not uniform: some were early maturing whereas others were late. After harvesting the early maturing accessions, the late maturing ones were left scattered throughout the field that had to be maintained (irrigated) until they matured. Had there been adequate information on their maturation behaviour, the field could have been laid out more appropriately by grouping the late maturing together, to minimize costs.

Regeneration of plant genetic resources

Experience shows that it is not always possible to collect sufficient seeds per accession during germplasm collecting. There is often a need to increase the sample size both for conservation and distribution. In order to ensure an appropriate increase or rejuvenation, the problems of loss of germplasm and/or loss of its integrity can be minimized by the correct choice of sample size for multiplication or rejuvenation in the appropriate environment. Attention should also be given to the breeding system and other factors (Singh and Williams 1984).

Sample and plot size

The loss of genetic integrity of an accession should be controlled as much as possible. The sample size to be planted will determine the size of the plot. The right size of plot should be chosen where the seed sample size is not limiting.

When many multiplication cycles are required to obtain sufficient seed for conservation, each multiplication cycle may affect the genetic composition of the original accession, by altering frequencies from one generation to another or by the loss of alleles. The influence of random genetic drift and natural selection are the main factors for these losses or changes. Burton (1975) concluded that it is difficult to advance germplasm pools through several generations without losing genes or having gene shifts. A comparison of the last generation with the first in six different germplasm accessions of pearl millet showed that advancing germplasm of this species narrowed phenotypic variability, lost genes and obscured some characters, with the population then appearing to be more uniform. The bigger the plot size the better the representation of the original accession in the field that is obtained, and from a certain size onwards random genetic drift can be neglected. The lower the number of multiplication cycles the less is the cumulative effect of natural selection. Therefore, the frequency of multiplication should be kept as low as possible when the seed population size is small (Singh and Williams 1984).

Appropriate multiplication environment

Multiplication in the appropriate environment, i.e. selecting sites that are ecologically comparable to the original collection sites, will also minimize the effect of natural selection. When genotypes or populations of plants are grown in an area different from where they were collected, either in a country or worldwide, it is found that the scope of natural selection will be greatly increased (Frankel 1970; Esser 1976; Raven 1976; Frankel and Soulé 1981).

Breeding system

During the multiplication of cross-fertilizing crops, strict isolation measures are deemed necessary to avoid pollen contamination. Outcrossing might be negligible in self-pollinating crops. Apomictic species also present no problem of isolation. Examples include *Hyperrhenia* spp., *Brachiaria brizantha*; *Brachiaria decumbens*, *Cenchrus ciliaris*, etc. During regeneration, these are normally grown together without any fear of contamination through crossing. However, minimal isolation is necessary to avoid mixing of seeds in case the plots overlap.

Growing different accessions of the same species in close proximity without any isolation may facilitate hybridization and loss of genetic identity in the seed harvested from the accessions (Leon *et al*. 1979). Isolation is mainly achieved by selfing as many plants as possible, or sibbing as in the case of maize using paper bags. Temporal isolation can be practised on the perennial forage grasses. By cutting back, the plots of different accessions are maintained at different stages of development, hence assuring that where crossing is minimal only distant accessions are at the same phenological stage, say flowering or heading. Isolation plots, bagging or cages are some of the measures for controlling inter-accession pollination (Singh and Williams 1984), where bags cover one head or a branch and cages cover a whole plot.

Crop rotation

Contamination in the field can occur by planting germplasm of a given species where another crop of the same species was grown the previous season. This calls for rotation of different species over seasons in a given area of the field.

Other considerations

For forage grasses there could also be contamination by planting grass accessions in virgin land where the grass weeds could be of the same species as the accession planted. It will be difficult to distinguish between the planted accession and volunteers, leading to harvesting the wrong material and thus introducing new genetic variation into the known accession.

Preparation for planting normally starts in the office where the list of germplasm to be included in the multiplication programme is drawn up. Accessions are sorted depending on the species and the ecological sites where they will be sown. The samples are packed in suitable envelopes and packed according to the site. This eliminates confusion and the possibility of sending the wrong materials to a given site.

Sowing in the field generally follows the agronomic requirements of the crop. Proper land preparation, field / plot markings and sowing also depend on the crop. It is recommended that during sowing, where the seeds are drilled into the furrows, a low seed rate should be used. A high seed rate would bring about thinning which can be a type of selection, hence losing some of the genetic material in the accession. Fertilizer application and timely weeding are recommended. Chemical control of pests / diseases should be applied as a last resort. Roguing or cutting off the infected plant parts should be the first step before resorting to chemical control.

Accession size

Genebanks have set the required seed number for long-term storage. The seed amount of a new accession is checked by weighing all the seeds. Some genebanks store 2000 seeds for genetically homogeneous populations such as self-pollinating species, inbred lines and cultivars produced from crossing inbred lines. For the genetically heterogeneous material, 8000 seeds are required. The Genebank of Kenya stores 4000 seeds for the homogeneous populations, and 12 000 seeds for the genetically heterogeneous populations. Sub-samples for germination tests and exchange are required, and these are in addition to the accession size.

Maximizing seed longevity in storage

Growing conditions

To achieve high initial viability of the seed, it is important that infestation of the materials by pests and diseases during growth is minimized. This is ensured through spraying crops in the case of severe attacks. Early planting is also encouraged, not only to minimize the attacks but also to ensure a good crop by making maximum use of the rains. Irrigation facilities have been established in four sites where rains are most unreliable. It is also important to have materials harvested in time and delivered to the genebank for processing without delay.

Processing

If not properly conducted, seed processing can be very detrimental to the seed. To minimize damage to wet seed, accessions are normally pre-dried before threshing and/or cleaning. Machine threshing is used only for those species that can withstand the machine pressure, e.g. *Eleusine*, and when materials are in bulk. Otherwise, hand threshing and cleaning are encouraged particularly for delicate species such as *Phaseolus vulgaris* and *Ricinus communis*.

Seed moisture content and drying

Two types of moisture are involved in seed conditioning. One is moisture occurring on the outer surface, which the air readily absorbs under dry atmospheric conditions. The other is internal moisture which is distributed throughout the inner parts of the seed. Removal of internal moisture involves the diffusion to the surface of seed where evaporation can take place. A low seed moisture content of 5+1% and low temperatures are the two preferred or desirable conditions for storage. It has been pointed out by Roberts and Ellis (1984) that the viability period is increased by a given factor for a given decrease in temperature or moisture content.

The higher the seed moisture content, the lower the drying temperature must be, and consequently more time is needed to reach the desired condition. The genebank uses the cool drying method with a drying temperature of 20°C and a relative humidity of 15%. The size of the drying room is 19 m^3. The seeds are dried for between 2 weeks and 1 month depending on the initial moisture content of the seed sample and the species. This method is less damaging to seed with a high initial moisture content, because the enzyme systems within the seed are sensitive to damage when in a fluid state and can be destroyed rapidly by heat if drying is done under high temperatures (WPBS 1978). Seeds subjected to moisture or temperature extremes during processing may also exhibit reduced viability thereafter (Ford-Lloyd and Jackson 1986).

The genebank has set a standard for seed moisture content for long-term storage as 3–6% depending on the species. Seeds are put in cloth bags, placed on shelves in the drying room and allowed to dry to the desired moisture level. Moisture content is monitored frequently during drying until the correct percentage is reached.

Seed packaging for storage

One of the preferred or desired conditions for long-term storage of seeds other than low temperature and low moisture content is the storage of seeds in air-tight containers. These may be in the form of glass vials, metal cans or laminated aluminium foil packets.

The seed samples already dried to the required moisture content are placed within these containers and then sealed. The Genebank of Kenya uses laminated aluminium foil packets for the storage of seeds. The sealing operation is carried out in the seed pre-drying unit which has similar temperature and RH conditions to the drying room, thus the chances of the seeds re-absorbing moisture after drying are reduced.

Seed storage facilities in the Genebank of Kenya

The design and construction of seed storage facilities are important and have to take into account specific technical details. Financial constraints are the limiting factors to the amount of material that any genebank curator can manage, but it is important for the collection to be large enough to satisfy breeders' requirements or to represent adequately the variability of the crop species in question (Chapman 1984).

Apart from low moisture content, low temperature is preferred for conservation, and IPGRI has recommended that after seeds are placed in sealed containers they should be stored at −18°C or lower. For storage of seeds on a small scale, domestic deep freezers are quite adequate (Ford-Lloyd and Jackson 1986) and the seeds should be pre-dried and kept in any of the recommended sealed containers. Other forms of storage facilities are controlled temperature rooms or cold rooms. The Genebank of Kenya has two cold rooms of 75 m^3 kept at –20°C for long-term seed conservation.

The tasks of conserving base collections and active collections are divided between the genebank and the research stations: the genebank handles base collections while the stations handle active collections. It is the responsibility of the plant breeders to maintain and regenerate the latter. Breeders come to the genebank only to replenish their stock or for new germplasm. This is to reduce the number of regeneration cycles, as the base collection will only be touched when viability is reduced.

Constraints

Facilities and costs

The National Genebank lacks facilities such as a glasshouse, pollination cages, irrigation facilities and vehicles. Glasshouses are particularly essential when handling materials with very low viability that need to be nursed under a controlled environment. Pollination cages are important for outcrossing species such as cucurbits. The vehicles available are old and expensive to maintain and cannot withstand the harsh environments normally visited both for germplasm collecting and regeneration. There is no adequate finance to cater for the above requirements. There is also a problem of cash flow: funds may not always be available at the beginning of the season or when most required. The inability to pay for field labour on time tends to affect the output.

Trained personnel

There are not enough trained staff for all our regeneration sites. Cash flow problems and/or inadequate funds make it impossible to fulfil the need for regular visits by genebank staff to those sites with inadequate personnel.

Regeneration environments

The regeneration sites are well distributed and take care of all the agroecological zones needed. In some of the sites however, rainfall is not dependable and irrigation facilities are either lacking or not reliable due to their condition. As a result, crop failures are occasionally experienced.

Remedial options

In regenerating materials with very low viability, a screenhouse is used to provide a controlled environment. However, this needs urgent rehabilitation. As for contamination, bagging is used to avoid outcrossing. Spatial and temporal isolation are also used. Borrowed vehicles are occasionally used to cover some trips to the regeneration sites. Our collaborators such as IPGRI and other Kenyan Agricultural Research Institute (KARI) Research stations regularly come to our rescue in times of emergency.

In all genebank operations, the users of germplasm, i.e. the research community, are involved. The regeneration activities are therefore undertaken in collaboration with other KARI scientists, usually breeders. Where applicable, this collaboration tends to minimize the constraint of insufficient trained personnel.

Further research on germplasm regeneration

The following activities are identified as priority areas for the Genebank of Kenya:

- effects of sun drying on seed longevity in the tropics
- minimum quantities/sample sizes necessary to cater for all possible genotypes in populations
- the possible occurrence of genetic variation during storage
- the inability of stored materials to adapt to the ever-changing environment.

The funds available to run the cold rooms are inadequate, and other techniques which will not affect the variability and longevity of seeds need to be explored. The technique of ultra-drying seeds and how it could relate to storage of seeds at ambient temperature should

be investigated, especially for short- and medium-term storage. This way the funds for running the cold stores for medium-term storage could be used for other purposes.

The current 85% viability requirement for storage is ideal for most crops, especially for cereals, but is difficult to achieve for some species, including *Panicum maximum*, *Chloris gayana* and most grasses. More investigations need to be carried out in order to come up with lower but suitable storage viability requirements for these crops.

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Seed regeneration practices at the National Plant Genetic Resources Laboratory, Philippines

Nestor C. Altoveros

The National Plant Genetic Resources Laboratory (NPGRL), which operates the national genebank of the Philippines, is a component division of the Institute of Plant Breeding (IPB), University of the Philippines at Los Baños (UPLB). It maintains germplasm of 225 crop species as seed. Its collections were assembled through collecting expeditions in the Philippines, through germplasm exchange with genebanks, agricultural research centers and private seed companies, and through breeding lines and genetic stocks given by the plant breeders of the IPB.

Regeneration of seed germplasm is carried out in the experimental fields of the institute when initial seed stock is sufficient, or in pots when seed quantities are limited. The number of plants grown for regeneration depends primarily on the initial seed quantity. If the latter is not limiting, 48–96 plants are grown in the field, depending on the amount of regenerated seed needed and the fecundity of the species. In many instances, especially when the initial seed stock comes from seed requests, seed quantities are low and regeneration is then carried out in pots.

Pollination control is done on outcrossing and predominantly cross-pollinated species through any of the following methods: use of isolation nets, bagging and clipping. Pollination is done either by hand or by the use of insect pollinators.

The decision to regenerate germplasm accessions depends equally on the viability and quantity of seeds held in store. The decision criteria used are as follows:

- regenerate newly arrived collections with very few seeds immediately – testing for percentage germination is not an option;
- test for percentage germination of newly arrived collections with sufficient number of seeds, regenerate if germination is below 85%;
- for accessions already maintained in the genebank, regenerate if quantity falls below 1000 seeds or when germination falls below 85%.

The amount of seeds maintained is dictated by the following factors.

- Breeding system of the species – the target numbers of seeds are as follows. For base collections: at least 3000 for inbreeding species; at least 8000 for outbreeding or predominantly cross-pollinated species. For active collections: at least 6000 for inbreeding species; at least 16 000 for outbreeding or predominantly cross-pollinated species.
- Fecundity of the species/accession. It takes longer to make up the quantities of seeds desired for certain species (e.g. *Arachis*, *Psophocarpus*, *Momordica*) and certain accessions in a species due to the limited number of seeds produced. This problem is further aggravated by the smaller number of seeds produced when bagging and hand pollination are practised (e.g. in *Zea mays*, *Luffa*, *Momordica*). The problem is also acute in the case of accessions which suffer from inbred depression.
- The rate by which seed stock is depleted. Seeds of certain accessions are more frequently requested, so larger seed quantities are planned for often-requested accessions.

Initial viability of the accession is optimized by:

- practising pest and disease control to ensure a greater proportion of healthy seeds upon harvest;
- harvesting at the proper stage;
- proper drying of seeds – prior to this year, seeds were dried at 22°C using silica gel. Beginning this year, seeds are dried in a drying room at 15°C and 20% RH;
- manual processing of seeds at the proper moisture content to minimize damage.

Viability during storage is optimized by maintaining the seed moisture content at 3–6% depending on the species.

The national genebank maintains base and active collections of germplasm. Long-term and medium-term storage facilities are kept at –20 and 0°C, respectively. The main impact of maintaining two types of collection is on the sample size during regeneration. Procedures for optimizing initial viability are similar for materials to be stored under medium- and long-term storage.

Issues that may need further research when regenerating germplasm include:

- procedures for regeneration of predominantly cross-pollinated species, e.g. determination of the proper sample size, pollination controls, consequences of treating the materials either as inbreeders or as outbreeders;
- breeding behaviour of lesser-known species;
- variation in the breeding behaviour of accessions within the same species.

Genebank management of Turkey, with emphasis on regeneration and multiplication

Ayfer Tan

Introduction

In agricultural history, Turkey, a centre of origin and diversity of many crop species, is a cradle of crop domestication and agriculture. In more recent times, Turkey was selected to be home of the first internationally supported regional centre commissioned to work specifically on plant genetic resources (PGR). In 1964, the Crop Research and Introduction Centre–Izmir (CRIC), now the Aegean Agricultural Research Institute (AARI), became one of the first agricultural research institutes created by an international agreement between the Turkish Government and FAO. Its mandate was to collect, conserve and research PGR from the South-west Asian region. These activities were conducted at CRIC until the project was terminated in 1974. In 1976, the studies were reorganized within the framework of a National Plant Genetic Resources Research Project (NPGRRP). The objectives of the NPGRRP are exploration, collecting, conservation (both *ex situ* and *in situ*), and documentation and evaluation of existing PGR and diversity in Turkey. Since 1964, collecting has been carried out for *ex situ* conservation in seed and field genebanks. Building on its historic background in PGR, since 1993 Turkey has moved forward to the *in situ* conservation of genetic diversity.

Seed conservation

Turkey uses international standards for *ex situ* seed conservation. The national genebank is located at AARI. Duplicates of the base collection will be stored in Ankara at the storage facilities of the Central Field Crop Research Institute (CFCRI). At AARI Genebank–Izmir, there are three types of storage facilities: cold rooms maintained at –18 to –20°C for long-term and at 0°C for medium-term storage. There are also temporary storage facilities at 4°C. There are two types of collections: base and active.

The base collection is for long-term conservation where the seeds are stored at 3–6% moisture and at subzero temperatures (–18 to –20°C). The seed stored in the base collections is not used for seed distribution purposes.

The active collection is for medium-term conservation where seeds are stored at 0°C under controlled relative humidity conditions. These collections are used for regeneration, distribution and characterization / evaluation.

Temporary storage facilities are also available at the AARI Genebank for accessions which contain less than the required quantity of seeds for storage and need regeneration. Storage facilities have been constructed in Ankara for the safety duplication of base collection accessions.

The first task at the genebank is the preparation of material for storage. This process includes threshing, cleaning, health inspection, drying, viability testing, quantity measurement and packing. These activities are the same as at most genebanks. At the AARI Genebank, material is always processed as quickly as possible for immediate conservation, in order to minimize the loss of viability of accessions. In order to fulfil the simultaneous demands of conservation and distribution, several factors are taken into consideration when deciding on the quantity of seed needed per accession. The preferred standard is 4000 seeds for homogeneous accessions and 12 000 for heterogeneous accessions. Normally, a sample is held in long-term storage as part of the base collection and the remainder of the seed is kept as a safety duplicate in long-term, and as active collections in medium-term storage. The latter is for distribution and use. For the routine operation of the genebank, seed quantities are also considered for seed moisture testing, initial viability and viability monitoring tests.

A small amount of seed per accession is needed for moisture content determination, and 200–400 seeds are required for the initial viability test. Further samples of at least 200 seeds are required for each subsequent monitoring test during storage. Additionally, at least 400–

500 seeds would be retained for regeneration (more for genetically heterogeneous accessions). Additional seeds will be required to replenish losses from the active collection or for supply where no active collection exists, especially for the wild species for which small quantities of seeds are collected. Hence, larger sample sizes are suggested for heterogeneous accessions, even though 100 seeds for distribution are considered satisfactory. However, for different reasons the targeted amounts might not be achieved, especially for wild species.

Although there is no quantified evidence on a constant relationship between laboratory germination percentage and field emergence, some species, especially wild ones, show low emergence in the field. Therefore in the genebank this is taken into account in the size of seed sample to be sent to users.

The viability of all accessions needs to be determined on receipt, and then monitored at intervals during storage. International standards for testing the viability of accessions and moisture content are applied. This monitoring produces a considerable workload because of the large number of accessions stored in the genebank. At present, monitoring tests require a sub-sample from the accession in the genebank and its testing. Depending on the result obtained, a decision is made to either maintain the accession in store until the proposed date of the next monitoring test (5-year interval for active collection and 10-year interval for base collection) or to regenerate. The critical level of viability is 85% for initial viability. However, for some wild species a lower level is accepted, according to the uniqueness of the accession.

Seeds are dried before storage. The conditions of the drying room are set to work at 15% RH and 20°C.

Regeneration

Regeneration and multiplication are important tasks of the AARI Genebank. Therefore the viability and quantity of the stored materials are always being monitored. The regeneration / multiplication is done through collaboration with the breeding programme.

The viability determines when to regenerate an accession at the AARI Genebank. The highest priority is given to accessions with the lowest seed viability and low quantity. The second priority is given to accessions with low viability but with high seed quantity, and the third to accessions with high seed viability but little quantity. During monitoring, the number of regeneration cycles is taken into account since genetic changes are accumulative over cycles. Generally, accessions from the active collection are used for regeneration, unless the active collection itself needs replenishing. Then the seed sample is drawn from the

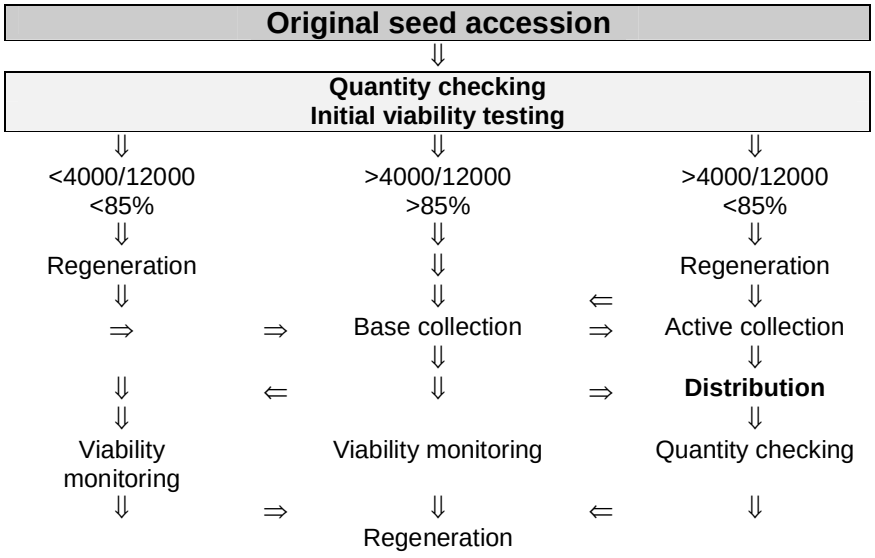

Fig. 1. Regeneration system of the AARI Genebank.

base collection. The regeneration system is shown in Fig. 1.

Loss of diversity from original accessions can be caused by various factors: wrong choice of sample size, regeneration in an unsuitable environment, lack of attention to the breeding system, and/or reproductive mode of the species, etc. To overcome these problems and to keep the original population or genetic structure of the accession, these factors as well as the required infrastructure plus growing and restoring costs are taken into account while planning AARI activities.

Since the Turkish genebank handles different types of genetic resources, i.e. cultivated, wild and weedy material, and a wide range of species, different strategies and methods of maintenance are applied. The policies on use of genetic resources material and distribution rely on the viability and quantity of material.

During regeneration the following considerations are taken into account:

- initial viability
- sample size
- population/genetic structure of accession
- breeding system of species
- life cycle
- similar environment to original collecting site
- pollination requirements
- number of regeneration cycles.

The following research needs have been identified: study of breeding systems and reproductive modes of some wild species; pollination requirements; and number of regeneration cycles per accession to maintain the genetic integrity of accessions.

The regeneration of temperate forage grass germplasm at IGER, UK

K.H. Chorlton

Introduction

The Plant Genetic Resources Unit (PGRU) at the Institute of Grassland and Environmental Research (IGER) was formed in 1964. One of the main objectives of the PGRU is to collect and characterize forage grasses to provide contrasting material for studies of population genetics, cytogenetics and plant physiology, in addition to providing 'elite' populations for variety production. Initially PGRU concentrated on the provision of material for breeding programmes, and the accessions collected and stored in the genebank in the first 3 decades of operation reflect this. The present remit of the PGRU takes a broader approach but still maintains a close working relationship with breeders at national and international levels. The remit is to assemble a collection that contains a full range of genes present in the species in the greatest feasible diversity of combinations, and to maximize the genebank's value for present and future uses.

Ecogeographical studies are carried out to predict where to collect novel genes, and a number of species are collected from a broad range of diverse habitats (Chorlton *et al.* 1994). The PGRU maintains a collection of over 10 000 documented accessions as seed in the genebank. The accessions consist of populations of forage grasses and legumes and their associated rhizobia, collected on expeditions throughout Europe and North Africa. As well as ecotypes, landraces and wild populations from expeditions, the genebank also contains advanced cultivars and breeding lines.

The major species in the genebank are *Lolium perenne* L., *L. multiflorum* Lam., *Festuca pratensis* Hudson, *F. gigantea* (L.) Vill., *F. arundinacea* Schuber, *Dactylis glomerata* L., *Trifolium repens* L. and *T. pratense* L. The PGRU is represented on the International Plant Genetic Resources Institute (IPGRI) European Cooperative Programme on Crop Genetic Resources Networks, Forage Grass Section. The PGRU has particular responsibility for the European databases on *L. perenne, L. multiflorum* and *T. repens,* and is taking a major role in establishing the *L. perenne* 'core' collection.

Breeding system of forage grasses

The wind-pollinated, perennial forage grasses are outbreeding. Populations contain large amounts of genetic variation, and selection and drift change their genetic composition. There is a vast array of adaptations to various combinations of climatic, edaphic and biotic factors.

Regeneration scheme

Regeneration of original collections of seed or plants, and of accessions already stored in the genebank as seed, is undertaken. An adequate supply of high quality seed is needed for the initial evaluation and characterization phase, breeding programmes, research, seed exchange (seed from medium-term storage) and future activities (seed from long-term storage). The regeneration scheme is summarized in Fig. 1.

Collecting (July–August)

The collecting technique influences the method used in the initial regeneration. The objective of collecting is to obtain as representative a sample as possible. Seed and vegetative sampling techniques are used.

Collecting seed

Seeds are collected where the reproductive phase has been allowed to develop unhindered (e.g. hay meadow, hedgerow, ungrazed areas, etc.). Approximately 100 heads, randomly distributed over the site, are collected, taking one head per point to ensure equal genotypic

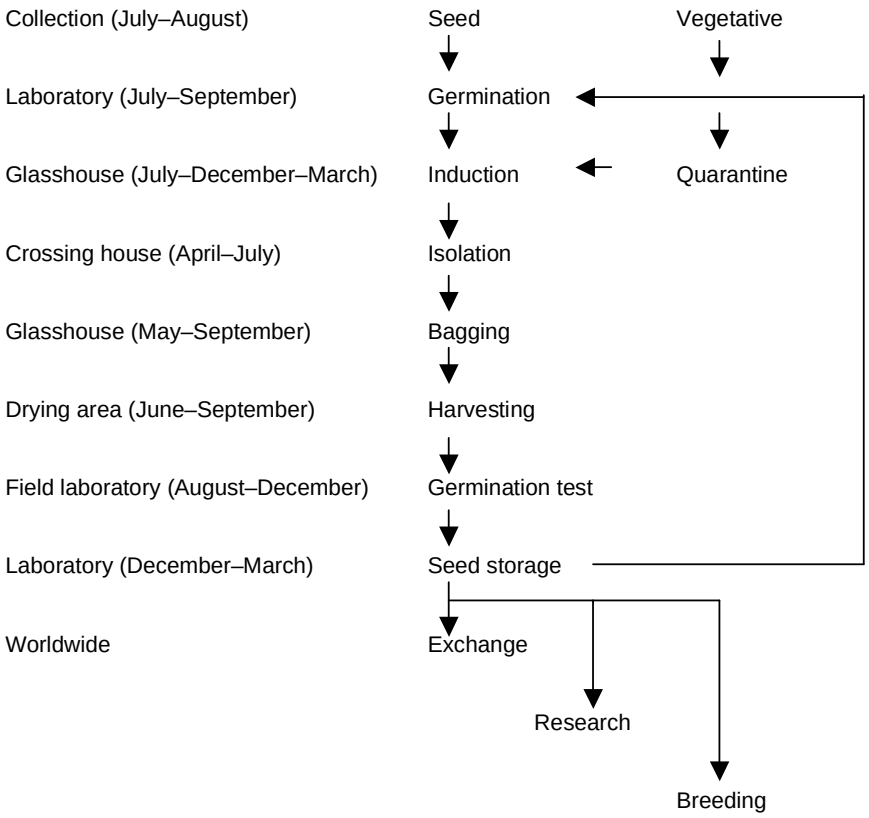

Fig. 1. Regeneration scheme.

representation and with at least 10 m between points to avoid duplication. The seed heads are bulked unless a mixture of ploidy levels is suspected (Chorlton *et al.* 1978). This a quick process, seed is easily maintained during the expedition, and the bulk is low. Seed collection is biased towards the selection of flowering genotypes, is impossible on grazed swards, and is limited by season. A seed collection does not require quarantine on return to IGER.

Collecting vegetative material

Vegetative material is collected when sampling perennial, mainly asexually reproducing, populations. Approximately 25–30 vegetative units (divots) are collected, the same considerations of random distribution and sampling being used while collecting seed. A vegetative collection avoids bias toward sexually reproducing genotypes and is not limited by season. This method is slow and samples are bulky and difficult to maintain during the expedition. Such a collection requires quarantine on return to IGER (Chorlton *et al.* 1978).

Laboratory and quarantine glasshouse (July–September)

The seed collection is threshed and cleaned. Approximately 100 seeds per population are sown either into compost in a glasshouse or onto damp filter paper in an incubator, depending on seed quality, species and previous experience. Thirty seedlings, taken at random, are potted on into progressively larger plastic pots up to 12 cm diameter.

The vegetative material collected is allowed to recover in quarantine. The quarantine house is an insect-proof, sectioned glasshouse ventilated with filtered air. The divots are monitored by an IGER pathologist and, where necessary, may be sprayed with insecticide and fungicide. The divots are cleaned and stripped to one tiller per divot and planted into plastic compartment trays of compost. As the tillers grow they are potted on into progressively larger pots up to 12 cm diameter. This ensures that the populations consist of a finite number of different genotypes.

Glasshouse and quarantine glasshouse (July–mid-December)

When collections are made between July and August from western Europe, germination of seed collections and subsequent growth of seedlings and tillers derived from divots occurs in September–November at IGER. There is a progressive shortening day-length intensity, and it is necessary to use supplementary light, heat and nutrients so that sufficient growth has occurred to enable the plants to be given conditions for floral induction. Optimal growing conditions must be provided to avoid loss of individual genotypes.

Glasshouse and quarantine glasshouse (mid-December–March)

Any supplementary heat and light is removed by mid-December to support the short day length and low temperatures required for floral induction. The aim is to have plants of sufficient size, hardened and placed outside under natural day-length and temperature conditions by mid-December. In practice this is impossible. A vegetative collection must remain in the quarantine glasshouse and plants derived from seed collections may need protection over winter, especially if they are from warmer regions, in order to avoid differential kill.

In March, the plants derived from seed and vegetative collections are repotted into 15 cm diameter plastic pots.

Crossing house (April–July)

The aim is to produce maximum yield of high-quality seed from each genotype in the population. Populations are placed in separate crossing houses just before the onset of anthesis and remain in there until 18 days after peak anthesis.

The isolation houses are approximately 1.4×1.2 m (floor area), adjacent units of conventional aluminium and glass construction. Each sealed unit is pollen-proof, air-tight and water-tight. Fan-blown, filtered air (to remove 'foreign' pollen) is ducted into the chamber across the plants and out of the chamber through a filter in the door. This maintains a positive air pressure in the chamber. The rate of air change is about 60 changes per hour, which is increased when a second fan cuts in at a thermostat setting between 15 and 20°C.

Water is supplied by capillary action using matting which draws water from a central channel containing a perforated plastic water pipe and a fibre-glass wick. The progressive repotting of the plants through to March encourages root growth through the drainage holes in the base of the plastic pots. This is essential for the uptake of water from matting.

The population is placed on the matting in a roughly square block with the pots touching each other. This gives a spacing of approximately 15 cm between plants. Tall, well-grown plants are supported by canes pushed into the sides of the pots and loosely tied to the canes by plastic-coated wire.

Glasshouse – bagging (May–September)

At IGER there are heavy demands for space in the crossing houses. The aim of bagging is to achieve a rapid throughput of populations. The populations are observed on a daily basis in the crossing houses so that the date of peak anthesis can be noted. At peak anthesis plus 18 days, each individual genotype has a labelled pollen-proof bag placed over the heads. The bag is secured around the stems to prevent ingress of foreign pollen and loss of seeds. The bag is of light-weight, permeable paper and is attached to the cane already supporting the plant. This operation is carried out in the crossing house. The bagged plants are then moved

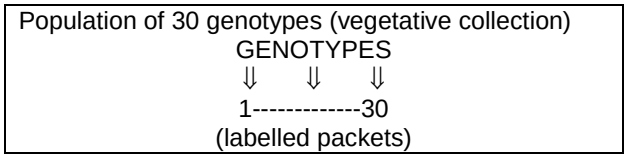

Fig. 2. Moisture content sub-sampling.

to a standard greenhouse or suitable holding area where they can be watered and protected from the weather and from damage caused by insects and birds.

Drying area – harvesting (June–September)

The aim is to harvest heads with fully set seed and then to dry the seed heads ready for threshing. The bagged heads are cut 10 days (minimum) after bagging and the bags of seed heads stored in dry atmosphere. Sufficient stem is left on the heads to allow for ease of handling in the threshing operation and the bags are opened to allow air to circulate. The labelled bags are kept together as population groups (usually 30 bags) and hung up in the greenhouse to dry under ambient conditions. A well ventilated greenhouse is used, rather than forced drying.

Field laboratory – threshing (August–December)

The aim is to obtain clean, high-quality seed from each plant and to maintain the identity of the individual genotype seed throughout the process. The seed heads are pounded by hand in order to shake most of the seed loose, and then mechanically threshed to remove the remainder of the seed. The sample is then hand-sieved to remove dust, debris and broken stems, and put through a column-blower at a predetermined setting to separate out light seed and chaff.

Laboratory/genebank – germination test and seed storage (December–March)

The aim is to store dried seed of known viability. Labelled seed packets (permeable manilla envelopes with a sealable flap) are used to store the seed from the threshing process. These packets are placed in drying cabinets (air-tight perspex boxes) containing silica gel straight after threshing, and remain in the cabinet until storage.

Each accession consists of approximately 30 genotypes, each as separate, labelled packet of seed. When seed is dried to 5% moisture content, sub-sampling is carried out as in Fig. 2. Each seed packet is sub-sampled:

- female plant seed (one seed up to a maximum of 0.50 g) – into long-term storage (30 packets)
- balanced bulk seed (1.0 g up to a variable maximum) – into medium-term storage (one bulk packet)
- unbalanced bulk seed (whatever is left over) – into medium-term storage (one bulk packet).

When possible, sub-sampling is done by seed weight after weighing each genotype seed lot to ascertain the lowest genotype seed weight and the range. When individual genotype seed lot weights are very low, it may be necessary to count female plant seed and accept very low weight balanced bulk based on the lowest seed weight genotype.

The balanced bulk seed lot is used for the germination test. If there is a very low seed weight in the balanced bulk then the unbalanced bulk seed lot is used for the test. The test consists of two replicates of 50 seeds per replicate, germinated at 20°C constant on damp filter paper in plastic repli-dishes in a laboratory incubator.

The dried, sorted seed of known germination is stored in moisture-proof foil-laminate packets in the genebank. The 30 individual packets of female plant seed are stored in one large foil-laminate packet in long-term storage at –25°C in commercial deep-freeze cabinets. The balanced bulk seed lot and the unbalanced bulk seed lot are stored in separate foil-laminate packets in medium-term storage at 0±2°C in the IGER main seed store.

Conclusions

The genebank originally developed within the plant breeding station, and earlier expeditions set out to collect material for utilization in plant breeding programmes. Expeditions went to

regions chosen on the basis of macro-climatic and agricultural management practices to collect semi-natural populations with specific adaptations and growth attributes (Tyler and Chorlton 1978).

Original regeneration scheme

The regeneration scheme evolved to produce (i) seed for initial evaluation and characterization, as well as supplying breeders and research workers; and (ii) seed for long-term characterization. All seed heads were harvested together and the seed was treated as a bulk. Some seed (10 g) was placed in long-term storage, and the rest of the bulk was placed in medium-term storage to supply all current needs. The requirements of evaluation (approximately 30 g for *L. perenne*), breeding, research and seed exchange of popular accessions required further regeneration and this was done from the bulk in medium-term storage.

Current regeneration scheme

The regeneration scheme described in this paper aims to satisfy the needs of breeding, associated research and seed exchange via its medium-term store, and the need to conserve as much genetic information as possible via its long-term store. This is achieved by separate bagging, threshing and storage of individual genotype seed lots and the production of balanced and unbalanced bulks. The original bulk method of regeneration could be undertaken by PGRU staff from collection in July/August to seed storage by November/December, whereas the present scheme occupies the period from July/August to February/March. If further regeneration is required then seed is used from the female plant seed lots in long-term storage.

Frequency of regeneration

The quantity of seed left in the genebank is the predominant factor governing the decision to regenerate accessions. If any accession falls below 5.00 g combined weight of balance plus unbalanced bulk in the medium-term storage, regeneration is required. In practice, the PGRU regenerates approximately 150–200 accessions per year, every other year including at least 100 'new' accessions obtained from expeditions. If there are more accessions of less than 5.00 g in the genebank than there is isolation space available, then priorities are assigned on the basis of species, demand (is it a popular line with genebank users?), and the current programme of research. For example, the establishment of a European *L. perenne* core collection has led to a major regeneration programme of the early collections of UK *L. perenne* accessions.

The viability of accessions in the genebank is tested only when material is used for regeneration or research. Time and staff are not available to run comprehensive viability tests as matter of course.

Quantity of seed per accession

The quantity of seed per accession is dependent on the number of genotypes collected per population. A population of one genotype is the lower limit and a population of 25–30 genotypes (in a vegetative collection) is the ideal number. The system of regeneration described will produce an average yield of 5.00 g of seed per genotype for *L. perenne*, giving yields per population of 5.00 g minimum to 150 g maximum.

Material collected on expeditions is evaluated and characterized, and this requires 25–36 g seed per accession depending on species or ploidy level. This requirement is met from the balanced bulk where possible, or from the unbalanced bulk if the weight of the balanced bulk is low (usually when the seed weight of the lowest-yielding genotype is below 2 g). Seed requirements for long-term storage are given priority and female plant seed is always separated from individual genotype seed packets before bulking is carried out.

Constraints to achieve desired stock levels

Any vegetative material brought into IGER from an overseas expedition is placed in a quarantine glasshouse. The number of vegetative units collected is limited by the logistics of the expedition and not by quarantine space at IGER.

The PGRU has direct control of quarantine glasshouse facilities, but the crossing houses are a station facility and are shared with other users. The PGRU is usually allocated two thirds of its requirement. Bagging after peak anthesis plus 18 days and manipulation of onset of anthesis are used to increase throughput.

It is unlikely that more crossing houses will be built because of the prohibitive costs. Polythene houses have been built and modified for use in regeneration, but there are problems of temperature control and condensation which affect seed yield. Low priority accessions, seed exchange material for example, are regenerated under polythene.

Seed longevity in store

Initial viability of the accessions is determined by growing conditions and post-harvest handling. Populations are collected as discrete groups of individual genotypes, and the aim in growing on the plants for seed production is to provide optimum conditions to avoid selective suppression of individual genotypes. In practice, this involves continual monitoring for pathogens, and the provision of adequate heat, light, nutrients, water and space throughout the regeneration process.

Post-harvest handling requires that seed heads are cut off at the correct time after bagging and then dried correctly prior to threshing. The drying process is carried out in glasshouses by hanging the bagged heads from the framework of the glasshouse. The process is not controlled and is subject to changes in local weather conditions. A drying room with controlled air circulation and temperature would be more satisfactory.

Sample viability in storage is determined by pre-storage processing and storage conditions. Pre-storage processing is carried out using standardized techniques and equipment at pre-fixed settings for the species. The number of individual samples involved makes this a lengthy process, with early heading populations being hung in the drying area in June and the last populations going into the store in March of the following year. This is due to staffing levels within the PGRU rather than lack of equipment and facilities to carry out post-harvest and pre-storage operations.

Storage conditions within the genebank follow internationally agreed guidelines. The medium-term store and long-term store are located in separate buildings, and a controlled-humidity seed-handling room has recently been built next to the medium-term store.

Base and active collections

The accessions in long-term storage are considered to be our base collection and the accessions in medium-term storage are considered to be the active collection. It is only possible to store material as individual female plant seed in long-term storage if original genotype identities are known. In practice, only vegetative collections from PGRU expeditions can be stored in this way. Material collected as seed or donated to the genebank as seed is put in long-term storage as balanced bulk samples, where possible.

Need for further research

The PGRU has developed an operational scheme for the regeneration of forage grass germplasm. The scheme is a compromise between the theoretically desirable and the practically possible, limited by staff numbers and facilities. All accessions are regenerated *ex situ* in an environment which is different from the point of origin. Regeneration is time-consuming, labour-intensive and costly. The aim is to regenerate accessions as infrequently as possible by maximizing the seed yield.

There are parts of the operation which need further research. Some conditions can be controlled in crossing houses but many cannot, e.g. day length and temperature. Matching controlled conditions during regeneration to conditions at the point of origin using growth

chambers could be a suitable line of investigation. Not every genotype in a population contributes equally to seed yield. In an operational scheme, with space at a premium, it is difficult to justify repeat regeneration cycles to investigate the phenomena, but this could be a suitable line of investigation.

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Regeneration of vegetable germplasm – the AVRDC experience

L.M. Engle

Introduction

The regeneration of vegetable germplasm poses several problems because this group covers a wide variety of species with different physiological behaviour, ecological and cultural requirements and breeding structures. In addition, there are very few studies that can serve as a guide on how genetic drift and/or shift can be avoided. If a germplasm holding runs into the thousands, then the problems are compounded by the wide range of diversity within each species. Furthermore, as most germplasm collections include the wild relatives of crop species, we can expect additional problems.

The Asian Vegetable Research and Development Center (AVRDC) holds one of the largest collections of vegetable germplasm: 38 000 accessions of its eight principal crops and 5000 of regionally important crops. Accessions that have been multiplied and characterized are stored as a base collection under long-term storage conditions (–20°C). An active collection, for distribution purposes, complements this base collection.

The Genetic Resources and Seed Unit (GRSU) of AVRDC regularly undertakes regeneration of its germplasm collection. This paper presents the procedures followed and the problems often encountered.

Factors considered during regeneration

There are two factors considered during regeneration of germplasm: quantity of seeds to be produced and preserving the genetic integrity of the accession.

Quantity of seeds to be produced

New introductions frequently arrive with insufficient quantity of seeds to be directly stored for preservation. At least one cycle of seed multiplication is needed to produce sufficient and viable seeds for preservation and distribution. The guiding principle is that it is best to produce large quantities of seeds sufficient for preservation and distribution in as few cycles as possible. Frequent regeneration of seeds is not only costly but may also result in questionable genetic fidelity, possibly due to mechanical errors, genetic drift when the sample size is too small, or genetic shift caused by loss of unadapted genotypes. The acceptable size of homogeneous material in base collections is 1000 viable seeds within the accession. The preferred size is 1500–2000 viable seeds (IBPGR 1994).

At AVRDC, the practice is to produce at least 20 000 seeds per accession. This quantity is divided into two groups: 8000–12 000 for the base collection under long-term storage conditions and the remainder for the active collection under medium-term preservation. The amount is enough to cover the need for long-term preservation, distribution for a period of 5–10 years, and safety duplication in at least two sites. To produce the target amount of seeds, 30 plants of each accession need to be established if the accession is uniform.

Preserving genetic integrity

Regeneration procedure should consider the need to preserve the genetic integrity of the original population.

To avoid problems posed by adaptability, AVRDC has taken the strategy of doing the initial multiplication of newly collected materials as far as possible in the country of collecting. This also helps to avoid problems of quarantine and permits national research staff to observe the materials while they are being grown out. However, it also means standardization of procedures and providing adequate training to the national staff who will be participating in the activity.

Among the Center's principal crops are three solanaceous crop species (tomato, pepper and eggplant), two brassicas (Chinese cabbage and common cabbage) and three bulb *Allium* spp. (onion, shallot and garlic). Species in the first group are mostly self compatible, and predominant self-pollination ensures that cultivars and landraces are quite uniform.

However, varying degrees of natural cross-pollination (1–91%) has been known to occur in pepper (Quagliotti 1979; Tanksley 1984). The degree of cross-pollination is influenced by distance of plants, wind direction and insect activity, with the last contributing the most to cross-pollination (Murthy and Murthy 1962; Franceschetti 1972). In eggplant, cross-pollination had been reported from 0.2 to 47% (Quagliotti 1979), with insects playing a major role. Variation in their floral structures may also affect the degree of outcrossing. Species in the second group are cross-pollinating with some degree of self-incompatibility. The last group consists of onions and shallots which are also cross-pollinating, and garlic and shallot are vegetatively propagated.

To preserve the genetic integrity of each population in the first group, cross-pollination between accessions is prevented. They are planted under insect-proof nylon net cages to prevent pollen contamination by insects. A row between plots is left vacant to separate accessions. Furthermore, plants are staked and pruned if necessary to prevent intermingling of branches. These procedures also minimize the possibility of mixing fruits harvested from adjacent plots. On the other hand, the cross-pollinating species need insect pollinators. Therefore each accession needs to be protected from being contaminated by pollen from other accessions.

To save on resources, one accession each of five different crop species (pepper, eggplant, tomato, brassicas, bulb onion or shallot) are planted in one net cage. Insect pollinators, usually bees, are released inside the net cage at flowering time to enhance pollination within each accession and thus increase yield. Still, in some cases supplementary hand-pollination may be necessary, e.g. wild species of tomato and eggplant. Insect pollinators may also show preference for one species.

However, the use of net cages poses several problems. Aside from being expensive and laborious to construct, the shading that results affects plant growth and may distort characterization data. The environment inside the net cage is also favourable to mites: severe infestation is frequent and often difficult to control even with repeated spraying of miticides. On the other hand, growing in net cages results in the exclusion of insects that damage plants and/or fruits, such as fruit borers, which can cause reduced seed yield as well as reduced seed vigour (Krishnasamy 1990).

Several workers reported that peppers normally show a large amount of phenotypic variation. Additionally, some accessions may look uniform based on morphoagronomic traits, but they may carry hidden heterogeneity, evidenced by hetemorphy for chromosomal markers (Quagliotti *et al*. 1972; Pickersgill 1986). Our data show that out of 2118 accessions of pepper characterized, 60% can be considered as homogeneous and only 6% as highly heterogeneous.

For heterogeneous populations the number of plants used for regeneration should be sufficient to preserve the genetic composition. If a sample shows distinct morphologic variants, e.g. red versus yellow mature fruits, it is separated into two sub-accessions and a second planting may be necessary to obtain the required amount of seeds. An inherently heterogeneous population is harvested as a bulk.

Accession identity

We begin systematic characterization using morphoagronomic characteristics along with the first seed increase. Such characterization data not only provide information on the potential utility of the accession, but serve as identifying information to check on the correct identity of the accession during regeneration.

When seeds show variation that can be visually detected, a seed reference file is prepared at the time of receipt and incoming materials are registered. This can also provide a check on accession identity. At AVRDC, reference slides on accessions are maintained, showing leaf, flower and fruit traits.

Other identifying information may be included in the passport data. If the material has already been characterized in another institution, such characterization data can be used to re-check the identity of the accession.

Succeeding cycles of regeneration

When enough seeds for the base and active collections are produced, regeneration may not be necessary until 10 years after the first regeneration cycle. The data used as a basis in deciding whether an accession needs to be regenerated are the number and viability of seeds in the cold store. AVRDC–GRSU maintains a seed inventory and determines the initial viability of accessions. For each crop species, there is a critical seed amount (1000 seeds). Whenever the critical amount is reached, a decision is made to regenerate the accession. The Expert Consultation on Genebank Standards (IBPGR 1994) recommends that regeneration be undertaken when viability falls to 85% of the initial value. This poses a problem in many vegetable crop species. The range for initial viability observed in many vegetable crop species is very wide (AVRDC 1992). In tomato, about 95% of accessions show more than 85% germination, in pepper only 70–78%. The cause of low germination is not conclusively known. When accessions that had less than 85% germination in tomato were retested after a year, about 81% showed increased germination (more than 85%). The improvement in germination a year after suggested that a certain amount of dormancy may be present in some of the accessions tested. In pepper, retesting of germination a year after showed an increase in germination in the majority of the accessions. However, unlike tomato, only 19% showed improved germination of more than 85% (AVRDC 1992).

To devise a more practical and efficient way of monitoring the viability of stored seeds, grouping of pepper and tomato accessions based on percentage germination was attempted using a clustering technique (AVRDC 1992). With the use of viability constants, storage conditions and nomographs, a prediction can be made as to when accessions in a particular cluster are expected to fall below 85% of the initial viability. The cluster then serves as the sampling unit in viability monitoring. Other procedures that are being tried are the use of frequency distribution and check varieties.

The need for collaborative efforts

The complex and stringent requirements in the regeneration of vegetable germplasm necessitate the availability of facilities and funds designed for long-term operation as well as trained and dedicated personnel. It is not a job for a single entity. Joint regeneration and collaborative efforts in many of the activities are necessary. AVRDC acknowledges the cooperation of several national programmes in its germplasm regeneration activities. Among them are the Lembang Horticultural Research Institute, Indonesia; the National Plant Genetic Resources Laboratory of the University of the Philippines, Los Baños, Philippines; the Tropical Vegetable Research Center, Kasetsart University, Thailand and the National Plant Genetic Resources Center, Taiwan Agricultural Research Institute, Taiwan.

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Regeneration of maize and wheat accessions at CIMMYT

J. Crossa, B. Skovmand and S. Taba

Introduction

The wheat genetic resources unit of the International Maize and Wheat Improvement Center (CIMMYT) handles 95 247 accessions of various types: bread wheat (52 839), durum wheat (13 443), triticale (13 268), barley (7991), rye (194), primitives (4523) and wild relatives (2984) (Skovmand *et al.* 1992). During the past 4 years the number of wheat accessions has increased by 25% with the same proportions for the various types of species. All are maintained as an active collection stored at –2°C, which conserves the viability of the accessions for 40–50 years.

The maize genetic resources unit of CIMMYT has more than 13 200 accessions, and new introductions are constantly added from a cooperative project to regenerate endangered accessions of landraces in Latin American maize collections (Taba 1995). The maize accessions stored in the genebank are classified into two collections, base and active. Base collection seed is kept in sealed containers at sub-zero temperatures and low seed moisture content, allowing it to remain viable for 50–100 years. Active collection seed is kept at just above freezing (0–2°C) and constitutes the working collection from which seed requests are filled.

An important activity of the maize and wheat genetic resources units of CIMMYT is to replenish seed samples when their germination falls below acceptable levels or their size is reduced by distribution. In regenerating accessions, genebank managers avoid contamination as far as possible via outcrossing (in outbreeding species such as maize), accidental mechanical mixture of seeds, other handling errors, and any loss of genetic diversity due to dramatic reductions in sample size (population bottlenecks). An optimum sample size for regenerating non-inbred accessions (such as maize open-pollinated cultivars) is determined by the frequencies of the rare alleles present in the accession (Crossa 1989). On the other hand, for self-pollinated species such as wheat, small sample sizes are needed if the accession regenerated is homogeneous.

The objective of this paper is to give a brief description of the practical aspects of the maize and wheat germplasm seed regeneration activities at CIMMYT.

Regeneration of wheat accessions

Regeneration of wheat accessions is one of the most important activities of the wheat genetic resources unit because long-term seed viability is highly dependent on the quality of the seed being placed in storage. CIMMYT multiplies and regenerates wheat seed in screenhouses rather than in the field, because this facilitates the production of high-quality seed for medium- and long-term storage and avoids accidental mechanical mixing and other possible handling errors (Skovmand *et al.* 1992). In addition, a limited number of accessions can be regenerated at any given time by planting weekly or bi-weekly, instead of strictly during the annual crop season. This procedure has been proven to be much better than the traditional, simultaneous harvesting of thousands of lines when a single planting is made in the field.

The number of seeds planted for regeneration depends on two factors: (i) the homogeneity (or heterogeneity) of the accession, and (ii) the size of the seed sample originally received. The wheat genetic resources unit of CIMMYT plants 'basic units' of 25 plants ('hill' plot). If the accession is judged to be homogeneous or the sample is small, only one basic unit is planted. As the heterogeneity of the accession increases, more than one basic unit can be included in the planting. More than four basic units are never required. Materials coming from the CIMMYT wheat-breeding programme are relatively homogeneous, whereas wheat landraces collected in the field are, in general, more heterogeneous.

Introduced material is judged for homogeneity when planted in the introduction blocks for quarantine inspection.

All materials collected by the wheat genetic resource unit in the field are single spikes and are subsequently managed as such. Regeneration, characterization and evaluation are difficult when an accession is heterogeneous.

Regeneration of maize accessions

For maize landraces or other panmictic populations, the maize genetic resources unit of CIMMYT plants 256 seeds per accession from a balanced seed bulk. The accession is sown in 16 rows (5 m rows) with two seeds per hill, so there are 16 plants per row and 256 plants in total.

Pollination control in the field is achieved by making plant-to-plant crosses (dioecious mode) or chain crosses (monoecious mode). Female gamete control is done by taking an equal number of seeds from each harvested ear. Controlling the number of seeds and pollen plants, the effective population size can be larger than the size of the original population. At harvest 50 good kernels are taken from each pollinated ear as one set and bulked for conservation in base collection (long-term storage). Then another set of 50–100 kernels are taken from each pollinated ear and bulked to form the active collection. If less than 100 ears are harvested, a new regeneration cycle is planted next season to make up the difference.

Over many regeneration cycles it is important to maintain more or less equal effective population sizes to avoid genetic drift, increased inbreeding, and a subsequent loss of alleles. Maize inbred lines can be maintained by selfing or sib-mating within lines.

When isolation field plots are not available, maize regeneration requires artificial pollination. In the case of older seed samples whose germination capacity has significantly diminished, it is difficult to establish enough plants for pollination; therefore two subsequent regeneration cycles are made. In some cases, accessions not adapted to the site fail to germinate, therefore two subsequent regeneration cycles might be needed to complete the original number of seeds planted (150–250). In other cases, specifically adapted maize populations need to be regenerated in the proper environments, requiring local cooperation.

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Multiplication and rejuvenation of genetic resources at ICARDA

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Introduction

The genetic diversity of crop plants and their wild relatives is a resource which needs to be not only adequately collected but also maintained properly to ensure the availability of seed, and to maintain as far as possible the original diversity collected. The principal aim of preserving genetic resources in genebanks is to prevent the loss of some of our traditional crop cultivars (landraces) and their wild relatives. The major objective of any genebank is to make these genetic resources available to researchers for crop improvement to meet needs for current and potential production constraints.

The International Center for Agricultural Research in the Dry Areas (ICARDA) Genetic Resources Unit (GRU) serves the world, and in particular the West Asia and North Africa (WANA) region, as a repository for global base collections of faba bean, chickpea, lentil, durum wheat and barley, and their wild relatives. ICARDA also maintains large, regionally important collections of pastures and forages (*Vicia* spp., *Lathyrus* spp., *Medicago* spp., *Trifolium* spp., and other genera and species) and of bread wheat. The activities on the kabuli chickpea and its wild relatives are carried out in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the activities on bread and durum wheat and their wild relatives in collaboration with the International Center for the Improvement of Maize and Wheat (CIMMYT).

The maintenance of these germplasm collections is crucial, as the WANA region is the centre of origin and primary diversity for barley, wheat and temperate cool-season legumes (Zohary and Hopf 1988). These crops were first domesticated in this region and then spread to the rest of the world (Vavilov 1992). According to Harlan and Zohary (1966), emmer wheat was probably domesticated in the upper Jordan watershed and einkorn was domesticated in south-east Turkey. Barley could have been domesticated anywhere in the arc bordering the Near East fertile crescent. The Near East arc displays the highest biodiversity of closely related wheat wild relatives, *Triticum* spp. and *Aegilops* spp. (Feldman 1977; van Slageren 1994).

Table 1. Status of base and active collections in the ICARDA genebank

Crop	Base collection (No.)	Base collection (%)	Total holdings in active collection
Barley	21 235	88	24 093
Durum wheat	15 707	87	18 036
Bread wheat	6318	81	7836
Wild wheat	3342	72	4659
Total cereals	**46 602**	**85**	**54 624**
Chickpea	8067	84	9586
Wild cicer	–	–	291
Lentil	6847	92	7407
Wild *Lens*	–	–	433
Faba bean ILB	–	–	4434
Faba bean BPL	–	–	5248
Total food legumes	**14 914**	**54**	**27 399**
Medicago	4446	57	7845
Vicia	3205	60	5349
Pisum	–	–	3553
Lathyrus	–	–	1590
Trifolium	–	–	3396
Alfalfa	–	–	960
Other genera	–	–	5129
Total forages	**7651**	**27**	**27 822**
Grand total	**69 167**	**63**	**109 845**

Table 2. Quantity of seed maintained in ICARDA base and active collections

Crop	Base collection, no. of seeds (g)	Active collection
Cereals		
Barley	2000	300
Durum wheat	2000	300
Bread wheat	2000	300
Wild wheat	150–1000	150–600
Food legumes		
Chickpea	1200	1000
Wild Cicer	–[1]	As available
Lentil	2800	280
Wild *Lens*	–[1]	As available
Faba bean ILB	–[1]	1000–2000[2]
Faba bean BPL	–[1]	1000–2000[2]
Pasture and forages		
Medicago	2500	100
Vicia	1000	300–600[2]
Pisum	–[1]	250
Lathyrus	–[1]	300–400[2]
Trifolium	–[1]	100
Alfalfa	–[1]	100
Other genera	–[1]	100

[1]Stocks are maintained separately for each multiplication phase.
[2]No base collection maintained at present.
[3]Dependent upon seed size.

The wild progenitors and relatives of lentil and chickpea (annual species) are found primarily in the West Asian region (Cubero 1981; van der Maesen 1987). *Lens orientalis* and *Cicer reticulatum* are endemic to this region (though *L. orientalis* has been distributed to some extent as a weed with the cultigen in the Asian Republics of the former Soviet Union). The collections of the *Vicia* and *Lathyrus* species are weedy forms, found mostly in cereal fields and orchards in WANA.

ICARDA placed its collections under the auspices of the Food and Agricultural Organization (FAO) in October, 1994 by formal agreement with FAO, and holds this germplasm in trust for the benefit of the world community, recognising the requirements of the Convention on Biological Diversity.

Status of ICARDA Collections

The ICARDA GRU holds a large collection of its mandated crops (110 000 seed samples). This large collection is heavily utilized by scientists from ICARDA and by national programmes, both from WANA and the rest of the world. The number of accessions of different crops in active and base collections held at ICARDA is given in Table 1. About two-thirds of this germplasm is from the WANA region (over 22 000 accessions) collected by ICARDA in 125 missions, jointly with national programmes.

Table 3. Viability tests for barley, durum wheat, chickpea and lentil germplasm in ICARDA collections

Viability (%)	Chickpea (no. accessions)	Lentil (no. accessions)	Barley (no. accessions)	Durum wheat (no. accessions)
<80	20	29	173	82
80–85	9	26	325	113
85–90	20	92	1083	828
90–95	266	379	3233	2982
95–100	8376	6746	16 816	6617
Total	**8691**	**7272**	**21 631**	**10 622**

Table 4. Viability test for germplasm (base collection) stored in the GRU long-term store (–22°C) for 5 years

Crop	IG No.	Storage year	Viability in 1990 (%)	Viability in 1995 (%)
Durum wheat	76300	1990	98	98
	76301	1990	98	98
	76302	1990	100	98
	76303	1990	100	95
	76304	1990	92	90
	76305	1990	100	100
	76317	1990	95	98
	76318	1990	92	100
	76319	1990	98	98
	76320	1990	98	98
Mean			97.1	97.3
Barley	16963	1990	98	95
	16946	1990	95	98
	16947	1990	98	95
	16948	1990	100	93
	16949	1990	100	98
	16950	1990	92	98
	16951	1990	98	100
	16952	1990	98	100
	16953	1990	98	98
	16954	1990	100	98
Mean			97.7	97.3

Table 5. Status of safety duplication of ICARDA collections.

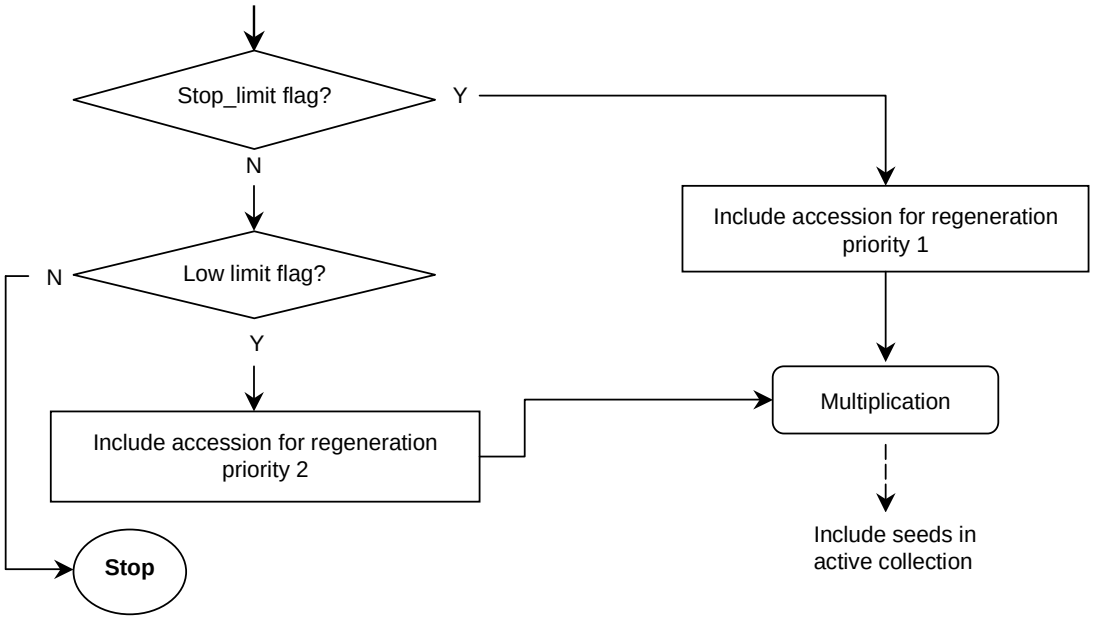
Crop	No. duplicated	Institution	Country
Barley	5326	CIMMYT	Mexico
Durum wheat	7435	CIMMYT	Mexico
Bread wheat	1238	CIMMYT	Mexico
Wild wheat	2749	CIMMYT	Mexico
Cereals	**16 748**		
Chickpea	4851	ICRISAT	India
Lentil	6771	NBPGR	India
Faba bean	1554	FIA	Austria
Food legumes	**13 176**		

Storage

Systematic and careful preservation of germplasm, stored as seed, coupled with a sensible seed distribution policy, eliminates frequent regeneration which is a time-consuming and expensive process. This also avoids the dangers of contamination, outcrossing, unwanted selection pressures and human error during regeneration, which can nullify the value of an accession.

We use controlled environmental storage with low temperature and low relative humidity for medium- and long-term conservation. After drying, seeds are packed in plastic containers and stored in the medium-term store (active collection), which operates at 2±2°C with a controlled relative humidity of 15–25%. In the long-term cold store, seeds are dried to 6–7% moisture content, hermetically vacuum-sealed in aluminium foil packets and stored at –22°C (base collection). The quantity of seed held for base and active collections for the various species in the ICARDA genebank is given in Table 2.

1. Seed quantity in active collection – performed to assist in planning multiplication



2. Seed quality in active and base collections – performed to assist in planning regeneration

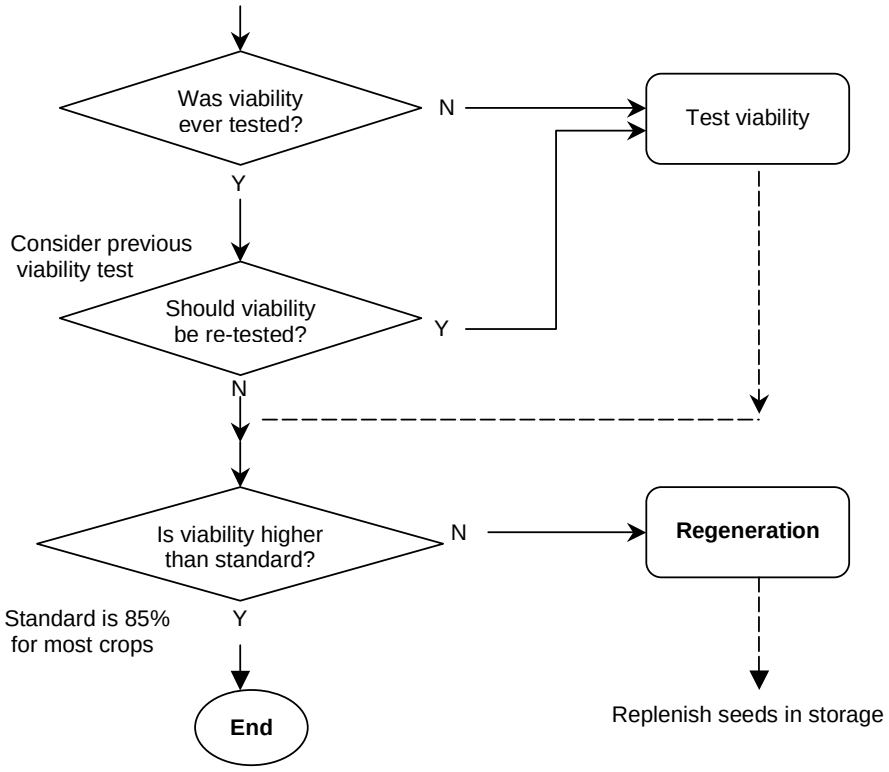


Fig.1. Flow diagram for monitoring seed quantity and seed quality in the ICARDA germplasm collections.

Table 6. Distribution of samples from ICARDA–GRU 1990–94, excluding safety duplication

Crop	Distribution	1990	1991	1992	1993	1994	Total
Barley	Center staff	2294	1749	1412	697	887	7039
Barley	Other IARCs	–	–	–	1599	–	1599
Barley	NARS in developing countries	277	1155	522	819	460	3233
Barley	NARS in developed countries	1201	5609	72	240	185	7307
Barley	GRU's own work	1305	1102	1069	841	563	4880
Barley	Safety duplication	–	–	–	–	–	–
Barley	**Total**	**5077**	**9615**	**3075**	**4196**	**2095**	**24 058**
Wheat	Center staff	467	490	6922	1649	3101	12 629
Wheat	Other IARCs	86	28	2	40	–	156
Wheat	NARS in developing countries	404	4385	1269	1451	4323	11 832
Wheat	NARS in developed countries	1012	229	3324	1822	1057	7444
Wheat	GRU's own work	464	1300	367	444	2090	4665
Wheat	Safety duplication	–	–	–	–	–	–
Wheat	**Total**	**2433**	**6432**	**11 884**	**5406**	**10 571**	**36 726**
Lentil	Center staff	1035	1811	1955	280	91	5172
Lentil	Other IARCs	–	–	52	–	–	52
Lentil	NARS in developing countries	40	366	797	1549	2830	5582
Lentil	NARS in developed countries	126	575	423	94	29	1247
Lentil	GRU's own work	314	1078	3400	826	2519	8137
Lentil	Safety duplication	–	–	–	–	–	–
Lentil	**Total**	**1515**	**3830**	**6627**	**2749**	**5469**	**20 190**
Chickpea	Center staff	914	318	1414	1459	431	4536
Chickpea	Other IARCs	–	–	–	–	–	–
Chickpea	NARS in developing countries	7	15	372	143	2660	3197
Chickpea	NARS in developed countries	455	68	411	22	685	1641
Chickpea	GRU's own work	1175	574	782	1576	1498	5605
Chickpea	Safety duplication	–	–	–	–	–	–
Chickpea	**Total**	**2551**	**975**	**2979**	**3200**	**5274**	**14 979**
Faba	Center staff	196	60	36	–	1	293
Faba	Other IARCs	–	–	–	–	–	–
Faba	NARS in developing countries	94	–	745	1660	397	2896
Faba	NARS in developed countries	3	–	497	1045	15	1560
Faba	GRU's own work	203	653	–	–	1	857
Faba	Safety duplication	–	–	–	–	–	–
Faba	**Total**	**496**	**713**	**1278**	**2705**	**414**	**5606**
Forages	Center staff	694	616	441	3075	1679	6505
Forages	Other IARCs	–	–	–	1	–	1
Forages	NARS in developing countries	296	–	847	1622	2186	4951
Forages	NARS in developed countries	66	891	440	806	1526	3729
Forages	GRU's own work	284	803	3496	1522	3542	9647
Forages	Safety duplication	–	–	–	–	–	–
Forages	**Total**	**1340**	**2310**	**5224**	**7026**	**8933**	**24 833**
Grand total		**13 412**	**23 875**	**31 067**	**25 282**	**32 756**	**126 392**

Viability monitoring

When viability for the material stored in the genebank falls below the critical standard of 85% germination, the accessions are regenerated for production of seeds with good viability for storage. Viability monitoring is an ongoing activity (Fig. 1) with several thousand accessions tested for viability each year (Tables 3 and 4). Accessions which show <85% germination are prepared for regeneration in the next season.

Safety duplication

At ICARDA, genetic resources are stored according to international standards including back-up generators and compressors. However, an important aspect of long-term preservation of germplasm is to provide for a duplicate set of samples in another genebank with good storage facilities. This provides safety from major man-made and natural disasters beyond the control of the centre. ICARDA has taken concrete steps to duplicate its base collection of unique samples at other locations and institutions. Safety duplication agreements have been signed for most crops and the process of implementation is under way (Table 5).

Distribution policy

Around 32 000 seed samples are distributed worldwide annually, upon request, from our collection (Table 6). This heavy demand affects seed stock in the genebank by reducing seed quantities, which necessitates further cycles of multiplication.

The GRU distributes seed samples of all available accessions. In the distribution process the seed stock is checked to determine that sufficient seed stock exists for the distribution of the accession (Fig. 2). ICARDA is in the process of implementing a policy that would require users to sign a seed order form which states that the recipient agrees (i) not to claim ownership over the material received nor to seek intellectual property rights over that germplasm or related information without prior negotiation and permission of the country of origin; and (ii) to ensure that all subsequent persons or institutions to whom they make the germplasm available are bound by the same provision.

We provide only small samples of seeds for research purposes. This allows the active collection seed stocks to be kept for a long time, thus reducing the regeneration frequency. Standard provision is 50–100 seeds per normal accession, 25–50 seeds per wild cereal accession, 25 seeds per wild *Lens* or *Cicer* accession, and 10–20 seeds per faba bean pure line (BPL). Users are advised that if more seed is required they are responsible for seed multiplication.

Seed health testing

A vigorous spraying programme against fungal pathogens and the vectors of insect-borne viruses is applied during the multiplication of the ICARDA germplasm. During the multiplication/regeneration of germplasm, plots are periodically inspected for seedborne pathogens by the Seed Health Laboratory and Virology Laboratory. Any suspect plants are rogued, any suspect plots are noted, and laboratory seed tests are conducted to confirm freedom from seedborne pathogens.

A programme has been started for virus testing of all seed stocks of barley, lentil and faba bean. This is a long-term process and is being implemented as resources allow. In the documentation system (see below), provision is made to record the status of virus and fungal seed stock tests.

Documentation

The seed stock is documented in the ICARDA genebank database. Seed, when being prepared for storage, is given to the genebank manager who is responsible for updating

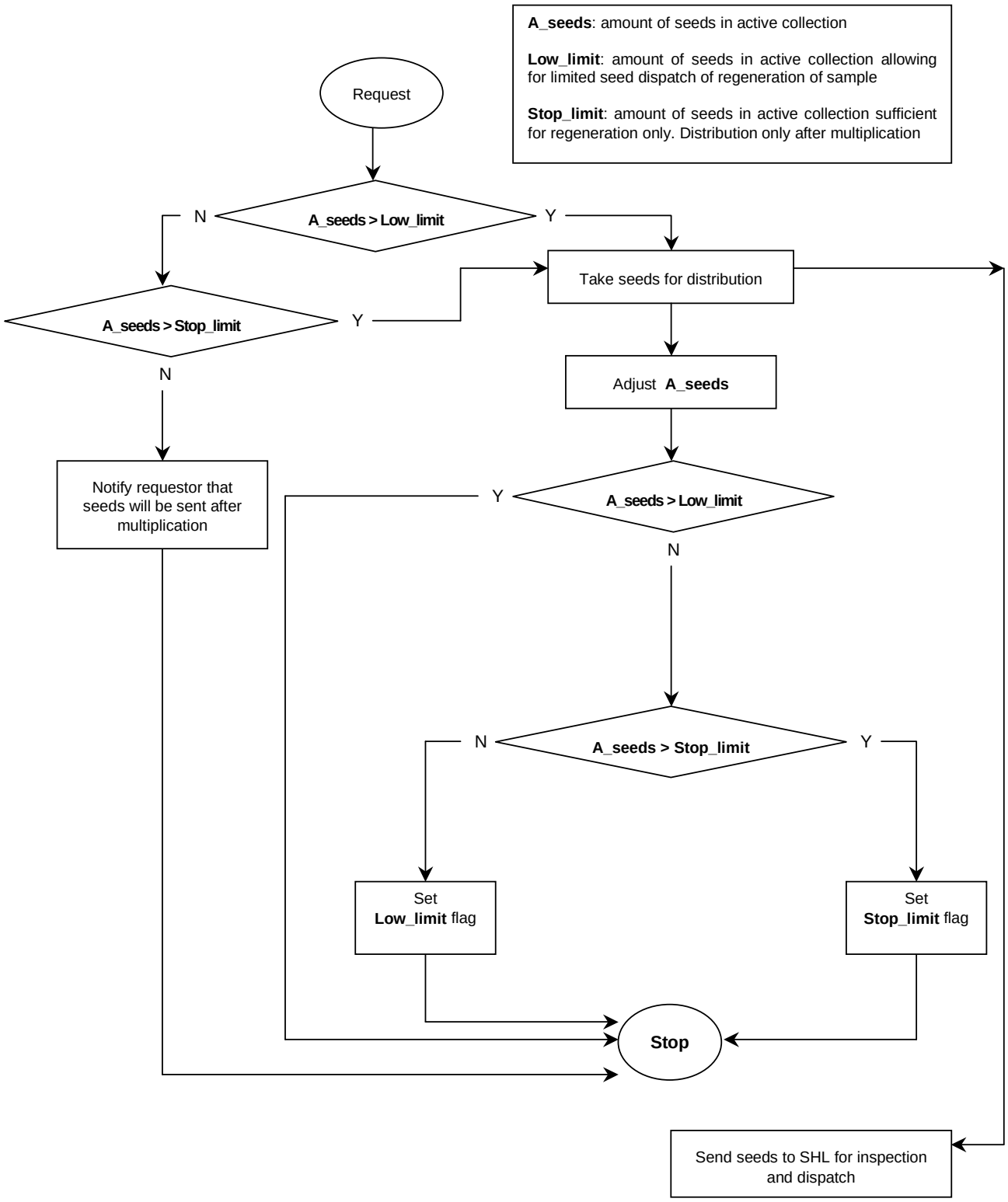


Fig.2. Flow diagram for monitoring and maintaining seed stock records while handling seed requests for germplasm from ICARDA collections.

stock data while storing fresh seed. Only the genebank manager attends to requests for germplasm seed and all requests are computerized, with selection files maintained for all requests and used to update the stock data at the time of despatching a request. This allows accurate data to be maintained on the status of seed stock on an ongoing basis for all collections.

These seed stock data are used to monitor the status of all germplasm collections at ICARDA. Critical seed stock levels have been established, and once the critical level is reached, the accessions are flagged for multiplication by the crop curators (Fig. 1). This level is usually high enough to meet all requests for seed during the period when the accession is being multiplied. There is a further level at which an accession is flagged for stopping distribution until seed stocks have been replenished. This level is sufficient to allow for several plantings for multiplication before a sample will have to be drawn from the base collection. Regeneration limits and distribution limits for the species in the ICARDA genebank are given in Table 7.

Regeneration

Population size

Once an accession, acquired either through centre-sponsored collecting missions or through donations, enters the genebank, it is assigned an ICARDA Genebank Number (IG number) and checked for the possibility of being a duplicate accession. It is represented in the initial multiplication phase by approximately 80% of the material received (Fig. 3). This sample size is usually sufficiently large to represent the accession and to reduce the danger of genetic drift. The percentage is adjusted according to the viability of the sample and the quantity of seed received. After initial multiplication, the viability of the seed is checked before the new accession is added to the active collection and, if the seed amount is sufficient, to the base collection.

Subsequent multiplications to produce full active and base collections of the accessions, and to replenish the seed stocks of the active collections as seed is distributed, are done with plots which allow sufficient seed numbers in the multiplication to reduce the danger of genetic drift (Table 8).

Table 7. Standard limits (g) for collections stored in ICARDA genebank

Crop	Stop limit	Low limit[1]	Available
Cereals			
Barley	±50	±100	>100
Wheat	±50	±100	>100
Wild cereals	±100 (seeds)	±200 (seeds)	>200 (seeds)
Food legumes			
Chickpea	±250	±500	>500
Wild *Cicer*	±100 (seeds)	±200 (seeds)	>200 (seeds)
Lentil	±25	±50	>50
Wild *Lens*	±100 (seeds)	±200 (seeds)	>200 (seeds)
Faba bean	±250	±500	>500
Forages			
Medicago	±20	±40	>40
Trifolium	±20	±40	>40
Alfalfa	±20	±40	>40
Vetch	±100	±150	>150
Lathyrus	±100	±150	>150
Pisum	±100	±150	>150
Others	±20	±40	>40

[1]Distribution with care, accession marked for multiplication.

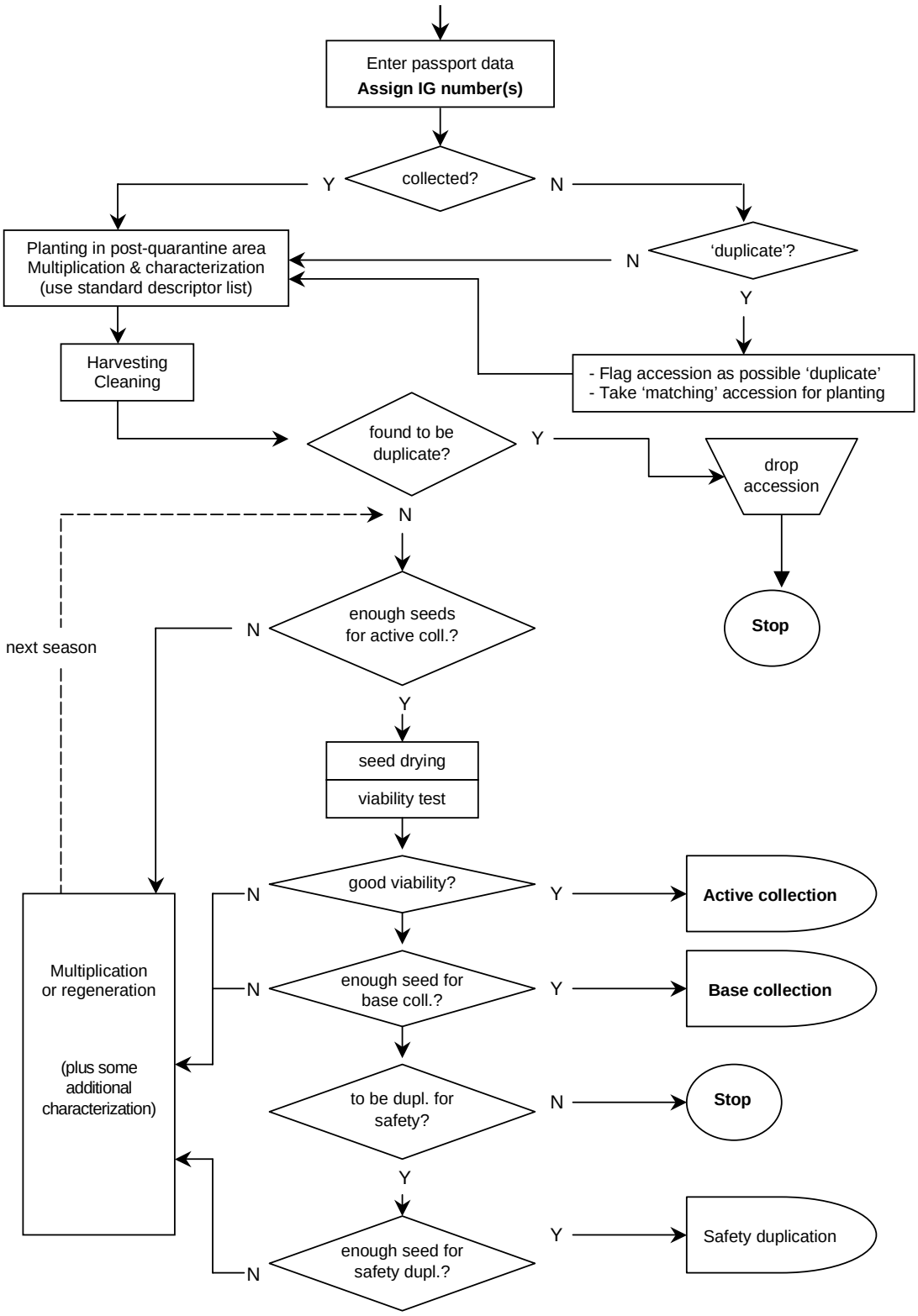


Fig.3. Flow diagram for introduction of new accessions into ICARDA germplasm collections.

Table 8. Regeneration standards at ICARDA for increase of seed supply for cereals and legumes

Crop	No. seeds	Remarks
Cereals (cultivated)	250–400	2 rows 2 m long
Cereals (wild)	20–50	2 rows 2 m long
Lentil	1200	4 rows 4 m long
Chickpea	240	4 rows 4 m long
Wild *Lens*	20	Plastic house
Wild *Cicer*	20	Plastic house
Faba bean	60–100	3 rows 3 m long
Medicago	600	3 rows 3 m long
Vicia	200	3 rows 3 m long
Lathyrus	200	3 rows 3 m long
Pisum	200	3 rows 3 m long
Trifolium	600	3 rows 3 m long
Other forage genera	600	3 rows 3 m long

Methods of regeneration

Self-pollinated crops

In the case of cereals (barley and wheat), of self-pollinated food legumes (chickpea and lentil) and of pasture and forage legumes, we follow a bulk method for multiplication and regeneration of the original samples and subsequent regeneration / multiplication using plot sizes which allow sufficient population samples to minimize the danger of genetic drift. This method allows us to manage a higher number of populations with limited facilities (labour and land). Isolation is used while regenerating the germplasm.

The plot-to-plot spacing adopted depends on the species (1–1.5 m). Accessions prepared for regeneration are labelled by plot and accession number. Both hand and machine planting are used with special care and close supervision during planting to avoid mechanical mixing and to minimize human error. All harvested material from the plot is threshed and cleaned, and the amount of seed required for storage is taken from the bulked sample after thorough mixing.

Cross-pollinated crops

In contrast with the other temperate legumes, faba bean is partially cross-pollinated by insects (Bond and Poulsen 1983). The traditional way of maintaining faba bean germplasm is as open-pollinated bulks. The first collection started at ICARDA was the open-pollinated international legume faba bean (ILB) collection which was maintained as populations. The major problem with this type of collection is the loss of genetic identity resulting from inter-crossing among different accessions. There are three ways to reduce the loss of identity of accessions:

- reduce the rate of inter-crossing among accessions during multiplication through increased distance between accessions or increasing plot size;
- reduce generation advance;
- do multiplicationss under conditions of selfing.

Witcombe (1982) proposed a system to reduce generation advance using a base collection, foundation seed and active collections. The ILB collection proved difficult to maintain for a large number of accessions, and several other types of collection were investigated.

A decision was made at ICARDA to derive a second, 'pure-line' collection from the original collection. A set of pure lines of faba bean has been derived from the usually heterogeneous and heterozygous ILB accessions (Robertson 1985). These were developed through a 'pre-breeding' process by taking randomly selected single plants to progeny rows

in a cyclic manner, using insect-proofed screenhouses to ensure selfing. The faba BPLs have the advantages of:

- ease of maintenance;
- repeatability and uniformity of evaluation;
- reduction in loss due to genetic drift;
- uncovering of recessive genes which otherwise might be hidden by heterozygosity.

Repeated inbreeding has not led to loss in vigour due to inbreeding depression. The BPL collection is multiplied using mesh-covered insect-proof screenhouses to ensure self-pollination.

Trait-specific genepools (TSGs) were proposed for faba bean by Witcombe (1982), where accessions similar for such traits as maturity, seed size, height and growth habit are bulked and maintained by growing in isolation to allow inter-crossing. TSGs allow the reduction of a large number of accessions to a few TSGs without loss of much genetic variability, as variability is not randomly distributed and much germplasm may be duplicated. This method has many advantages for the core collection. One limitation is the need for good evaluation data for the material to bulk. Multivariate techniques would be useful to group accessions to be selected for such TSGs.

Burton (1979) proposed that cross-pollinated germplasm accessions be maintained as self-pollinated bulks. This type of collection would contain accessions which are a bulk of homozygous lines, i.e. each accession is heterogeneous but all genotypes are homozygous. This should be better than producing a fixed number of pure lines from each open-pollinated accession, since it might better represent the full range of variability in each original accession. A more refined way to maintain this type of collection would be to self a large number of plants of each accession and take one seed or pod to bulk to produce the next cycle seed [the single seed descent method designed by Brim (1966)]. This type of collection of selfed composites would maintain maximum variability with minimum chance of loss of genes because of inter-crossing among accessions. Also, it would allow the detection of recessive genes in heterogeneous populations which are bulks of homozygous genotypes. The ILB collection at ICARDA is evolving into this type of collection, as the multiplication phase has been switched to insect-proof screenhouses because of the impracticality of growing even a small number of accessions in isolation.

Wild species

Wild cereals (*Triticum*, *Hordeum* and *Aegilops* species) are multiplied in the field if seed is sufficient, otherwise they are multiplied in the plastic house. Field multiplication is done in two-row plots as with the cultivated species. Harvesting starts as the first spikelets begin to mature and is done on a continuing basis as the spikelets mature. In the plastic house, the wild cereals are bagged at anthesis. The wild *Lens* and *Cicer* species are multiplied in the plastic house in pots, with plants bagged at the start of anthesis.

Summary

The WANA region is the centre of origin and primary diversity for the cereal and legume species in the ICARDA collections, and is a unique resource. ICARDA has the responsibility for its proper maintenance in active and base collections. This germplasm is maintained in the base and active germplasm stores as per the international standards set by the FAO and IPGRI. Steps have been taken to ensure safety duplication of unique germplasm accessions.

Seed stock levels and viability of the germplasm are constantly monitored at ICARDA using a computerized documentation system to ensure adequate levels of good quality seed. When germplasm is required to be regenerated, adequate samples are taken to ensure that multiplication does not result in significant genetic drift. Plot sizes used are large enough to ensure adequate samples are planted and that sufficient seed is produced for replenishment of the active collection. Special methods are used for pollination control in the multiplication

of the faba bean germplasm collections because faba bean is partially cross-pollinated by insects.

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Germplasm regeneration at ICRISAT

J. W. Stenhouse and N. Kameswara Rao

Introduction

The mandate of ICRISAT includes responsibility for large germplasm collections of several crops (Table 1). The collections are maintained as seed at ICRISAT Asia Center, Hyderabad, India, in both medium-term (4°C and 20% RH) and long-term (–18°C) storage conditions. The full collections are maintained in medium-term storage. Approximately one-quarter of the collection has been transferred to long-term storage, the facilities for which were developed in 1991.

The different crops have markedly different breeding systems and multiplication rates. Groundnut, chickpea and the minor millets are inbreeding. Sorghum is wind-pollinated, and pigeonpea is insect-pollinated; both are partially outcrossing (sorghum 0–30%; pigeonpea 0–40%). Pearl millet is wind-pollinated and predominantly outcrossing. Groundnut has a multiplication rate of approximately eight whereas for pearl millet it is in excess of 200. These differences require the crops to be treated very differently for seed multiplication and regeneration purposes.

Managing regeneration requirements

The active collections at ICRISAT are managed in ways that simplify regeneration and reduce its frequency. For example, mixed samples of some crops are separated out, particularly sorghum, pigeonpea and groundnut, and are maintained individually by selfing. This makes the regeneration procedure more reliable, and reduces the risk of loss of variability, although it increases the number of samples to be maintained. The initial quality of seed conserved at ICRISAT is optimized by appropriate management of seed multiplication and seed handling procedures, and by optimizing the storage conditions. The minimum quantity of seed required to represent an accession for distribution is also supplied by ICRISAT. This reduces the demand on seed quantity. Where it is known from experience that an accession is in demand, a larger bulk is maintained specifically for distribution.

The identification of core collections is being researched, particularly in sorghum, as an aid to genebank management. At present, however, core collections are not used as part of the germplasm management strategy at ICRISAT.

Regeneration criteria

The mandate crops of ICRISAT produce orthodox seed. There is normally little difficulty in producing seed for storage with 95–98% initial viability. The generally accepted standards are followed of monitoring viability of active collections at 5-year intervals and regenerating the accession when its viability falls below 85% (IPGRI 1994). Given the relatively recent establishment of the genebank, the good initial seed quality of most samples, and the favourable storage conditions, the number of accessions that need to be rejuvenated because of reduced viability is currently small (approximately 400 each year).

Table 1. Status of ICRISAT germplasm collections (number of accessions), Dec. 1995

Crop	Cumulative total	Number of countries
Sorghum	35 643	90
Pearl millet	21 191	49
Chickpea	17 244	44
Pigeonpea	12 885	72
Groundnut	14 716	91
Minor millets	9015	43
Total	**110 694**	**–**

Large numbers of samples of germplasm are also distributed by ICRISAT to users round the world. Approximately 50 000 samples are distributed each year to crop improvement scientists. This also drives the need to regenerate fresh seed, particularly for those accessions in greatest demand. Maintaining bulks of accessions known to be requested frequently helps to reduce the regeneration load. However, demands for seed to be used in collaborative evaluation of germplasm can place heavy demands on seed stocks, particularly where testing across locations is involved. The low seed-multiplication rate for groundnut results in a need for more frequent regeneration for that crop. The numbers of accessions that are regenerated because of low seed stocks are therefore quite substantial (approximately 1500 each year). Samples are routinely regenerated when seed stocks are reduced to one-quarter of their initial level.

The regeneration needs of ICRISAT are currently being driven predominantly by the need to multiply fresh seed for transfer to long-term storage, and for safety duplication of the collections at other locations. This is likely to remain the case for the next decade until both these needs are met. During that time, approximately 10 000 accessions will need to be regenerated each year.

Regeneration procedures

Accessions are normally regenerated under irrigation during the post-rainy season. At this time of year, conditions are favourable for crop growth and productivity, and low temperatures and humidity during the growing period result in less pest and disease pressure and generally better seed quality. Short days promote early flowering in many photoperiod-sensitive accessions of sorghum and pearl millet, but may delay flowering in chickpea.

For groundnut, chickpea and minor millets, all of which are inbreeding, no pollination control is practised during regeneration. Sorghum is selfed by enclosing heads in paper bags prior to anthesis. Heads from three to four adjacent pearl millet plants are enclosed together in a single paper bag. This method is referred to as cluster bagging, and is designed to encourage cross-pollination in this normally cross-pollinated crop. Special genetic stocks of pearl millet are maintained as inbred lines by selfing. Pigeonpea plants are selfed by enclosing individual plants within muslin bags.

To minimize genetic drift during regeneration, it is ensured that adequate numbers of plants are grown and sampled equally in constituting new seed stocks. The numbers used vary according to the crop: at least 20 in sorghum, 25 in pigeonpea, and 120 in pearl millet. The numbers of plants used for chickpea and groundnut are more variable, and tend to be driven by the quantities of seed required. But because of lower multiplication rates the numbers of plants tend to be high.

Harvesting is done by hand for all crops. Sorghum and millets are threshed by hand, and groundnut is shelled manually for long-term storage. Chickpea and pigeonpea are threshed by machines. Pre-storage deterioration in seed quality is minimized by harvesting promptly when maturity is reached. Cleaned and disease-free seeds or pods are maintained in short-term storage while they are prepared for medium- and long-term conservation. Moisture content at harvest time in the post-rainy season (February–April) is generally low, 7–8% in groundnut and 10–12% in other crops. These levels are acceptable for medium-term conservation. For long-term conservation, however, samples are dried further to 5–2% moisture content in special drying cabinets operated at 15–20°C and 10–15% RH.

For medium-term conservation, ICRISAT stores about 350 g of sorghum, pearl millet and chickpea, 450 g of pigeonpea, and 1 kg of groundnut pods. Sorghum, pearl millet, chickpea, pigeonpea and minor millets are stored in aluminium cans with screw tops and rubber gaskets. Groundnuts are stored in plastic bottles to accommodate the greater volume. For long-term conservation, about 12 000 seeds of pearl millet, 5000 seeds of sorghum, and 2000–3000 seeds of chickpea, pigeonpea and groundnut are stored. In all cases, samples for long-term conservation are stored in vacuum-sealed aluminium foil packets.

Plant health is monitored throughout the regeneration process. For chickpea and pigeonpea, care is taken to avoid fields infected with soilborne diseases to which much germplasm is susceptible. Particular attention is paid to viral diseases of groundnut. Any infected plants are rogued from the plots and destroyed. Similarly, diseased plants are removed from other crops before harvest to ensure that only healthy seed is collected.

Problems in regeneration

The experience of ICRISAT in regeneration has generally been positive, largely because of the relative ease with which these crops can be maintained and multiplied at ICRISAT Asia Center. However, ICRISAT is not without problems.

Past bottlenecks in plant numbers are poorly documented in the collections at ICRISAT. Some of these were systematically introduced as plants passed through quarantine. For example, the need to test groundnut plants individually for virus infection led to 10 plants or fewer being released from quarantine (this is no longer the case, as residual seed of accessions that prove to be free from infection are now released). Such systematic bottlenecks, and their implications for sample size to reproduce faithfully the original sample, can be readily accommodated in ICRISAT's regeneration practices. However, accession-to-accession variation in sample size at collection, or in subsequent grow-outs, are difficult to accommodate and undoubtedly lead to wasted resources because of over-large populations being maintained.

Low seed-multiplication rates for some samples cause an inability to achieve the target quantities of seed for conservation and distribution. This problem is particularly acute for groundnut where the multiplication rate is low for all accessions. For other crops, it is usually just a few accessions, often among the wild relatives, that present difficulties. Wild relatives that do not set seed can also present problems as they have to be maintained as living plants which can act as reservoirs for disease, particularly virus infections.

High-temperature damage can result when harvest is delayed beyond the optimum time. This is particularly true for groundnut and chickpea when harvest and crop processing is delayed into the hot months of April or May. Delays in crop maturity can be due to photoperiod reactions in some accessions. Similarly, undue delay in post-harvest handling of the crop, especially drying, can lead to seed being exposed to extreme temperatures and reduced viability.

Disease pressure can also pose problems for regeneration and multiplication of chickpea and pigeonpea accessions. A large proportion of the germplasm collection is susceptible to soilborne diseases such as fusarium wilt, which can lead to failure of certain accessions to produce seed. Disease pressure can also result in genetic shifts in variable accessions.

Research needs

The handling of wild relatives of the mandate crops of ICRISAT presents problems. In particular, lack of information on their breeding systems results in uncertainty about handling procedures. Studies of the breeding systems of the wild relatives would help in this respect.

Similarly, knowledge of the control of flowering in both the cultivated forms and their wild relatives is limited. Greater understanding of the physiology of flowering would help to manipulate environmental conditions or to choose appropriate locations to achieve more assured flowering, and enhanced multiplication rates.

The quantity and quality of seed available for conservation depends on how the regeneration crop is handled throughout its duration. Problems of plant stand, pest attack and disease infection can reduce seed quantity. Delayed harvest or inappropriate drying and crop handling procedures can severely prejudice seed quality. Further research is required into the optimization of yield and initial seed viability through crop management procedures.

For some crops, particularly the outbreeding ones, it is not clear whether the regeneration procedures at ICRISAT are resulting in accurate reconstitution of the original accessions.

Molecular marker technologies could help to determine whether sample sizes and pollination control strategies are effective in accurately reproducing variable germplasm accessions. The same molecular technologies could help to study variation within and between accessions, and to optimize conservation strategies generally.

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Theoretical and practical considerations in the regeneration of cowpea germplasm at IITA

N. Q. Ng and J. d'A. Hughes

Introduction

The Genetic Resources Unit (GRU) at the International Institute of Tropical Agriculture (IITA) conserves germplasm of many diverse crops which are important in sub-Saharan Africa. The IITA genebank contains about 40 000 accessions, conserved as vegetative propagules and/or as true seed. Conservation as true seed accounts for about 90% of the total collection, of which the largest proportion is cowpea (*Vigna unguiculata*) with over 15 200 accessions of cultivated and 450 of wild species. Various other crops (see Table 1) account for the remainder of the accessions conserved as true seeds. GRU collaborates closely within the framework of multi-disciplinary projects, both within IITA (particularly with respect to plant health and germplasm characterization) and outside, with international centres, national programmes and advanced laboratories (IITA 1988; Ng and Monti 1990).

Facilities for genetic resources at IITA

IITA's facilities for the conservation of genetic resources include temporary, short- and long-term collection seed stores as well as a laboratory and canning/packaging room (Table 2), all of which are contained within GRU. Many other facilities available at IITA, such as those in the Seed Health Unit, Biotechnology Research Unit and virology laboratories are also utilized, in order to maintain and improve the quality of the seed material in the genebank.

Considerations for conservation and regeneration of cowpea

The following aspects are considered to be of major importance for the conservation and regeneration of seed germplasm and will be treated briefly below:

- seed moisture levels and storage temperatures
- longevity in storage – monitoring of viability
- number of seeds to regenerate in order to maintain genetic diversity.

Seed moisture levels and storage temperature

Assuming an initial seed viability of 95% (K_i), the number of days (P) for the decline in cowpea seed viability to 85% (V) can be calculated for a range of seed moisture contents (m) and seed storage temperatures in degrees Celsius (t), as predicted by the model of Ellis and Roberts (1980):

$$K_i - V = P / 10 \exp(K_E - C_W \log m - C_H t - C_Q t^2)$$

where $K_E = 9.102$, $C_W = 4.967$, $C_H = 0.0295$ and $C_Q = 0.000491$ are viability constants for cowpea (Ellis 1988). For samples stored at ambient conditions (seed moisture 12%, storage temperature 25°C) the time for viability to decline to 85% is 1 year, thus requiring propagation each year. For seed stored at 5°C, the time for a similar decline to occur is 6.5 years at 12% seed moisture, compared with 493 years at 5% seed moisture content (Table 3). At –20°C, in long-term storage, cowpea seeds theoretically could remain in storage at a seed moisture content of 5% for 1761 years and still retain a viability of 85% (at the percentage seed germination when regeneration is required). However, because the seed moisture level may be in the range of 5–7% and the initial viability can be as low as 90%, we estimate that the seed viability will decline to 85% in about 100 years (assuming a seed moisture content of 7% and initial viability of 90%). Thus it is safer to assume that regeneration of cowpea is required after 100 years of storage in the long-term seed store where the base collection is maintained.

Table 1. Seed collections of crop species and their wild relatives stored in the Genetic Resources Unit at IITA

Species	Number of accessions	Storage conditions
Vigna unguiculata	15 200	Long & medium term
Vigna spp. (wild *Vigna* and wild cowpea)	1500	Long & medium term
V. radiata	79	Long & medium term
V. subterranea	2000	Long & medium term
V. umbellata	7	Medium term
Oryza sativa	9473	Long & medium term
O. glaberrima	2503	Long & medium term
Oryza spp. (wild rice)	147	Long & medium term
Manihot esculenta	1900	Medium term
Glycine max	2500	Long & medium term
Sphenostylis stenocarpa	123	Long & medium term
Psophocarpus tetragonolobus	45	Medium term
Cajanus cajan	13	Medium term
Phaseolus lunata	29	Medium term
Lablab pupurea	42	Long & medium term
Kirstingiella geocarpa	9	Long & medium term
Canavalia ensiformis	5	Medium term
C. gladiata	4	Medium term
Mucuna puriens	2	Medium term
Pachyrhizus tuberosus	1	Medium term

Table 2. Seed storage and testing facilities in the GRU at IITA

Facility	Temp. (°C)	Relative humidity (%)	Volume (m^3)
Temporary or withholding seed store also used for seed drying and packing	17	<10	68
Active collection seed store used for medium-term conservation	5	25–30	410
Germination/testing laboratory	26	50–60	–
Canning/packaging room	26	40	–
Dehumidified chambers for seed drying	20	<10	–
Base collection seed store used for long-term storage	–20	Seeds stored, sealed, at 5% seed moisture	132

Longevity in storage – monitoring of viability

Monitoring the viability of seed under storage is done in three different ways (Ng 1991). Five randomly selected accessions which have been stored over 5 years are tested annually. This characterizes the general trend of viability under long-term storage. Five control accessions are tested every 5 years to monitor accurately the effect of long-term storage on these particular accessions over time, and to ascertain their storage characteristics. Finally, all cowpea accessions are tested for viability at one-fifth of their predicted rejuvenation interval, i.e. 20 years after beginning long-term storage at –20°C. This may prevent loss of accessions due to genotypic differences or errors.

Number of seeds to regenerate in order to maintain the genetic diversity

The estimation of the retention of genetic variance in cowpea is based on several theoretical considerations. Although cowpeas are usually inbreeding, some outbreeding does occur, and consequently accessions of cowpea landraces collected from farmers' fields are usually very

Table 3. Time in years for cowpea seed viability to decline from initial viability of 95% to 85%

Storage	Seed moisture content				
temp. (°C)	5%	7%	8%	10%	12%
–20	1761	331	171	57	23
–10	1253	236	122	40	16
0	712	134	69	23	9
5	493	93	48	16	6.5
10	322	60	31	11	4
20	116	22	11	4	1.5
25	64	13	6	2	1

heterogeneous. In order to ensure maintenance of the genetic integrity of individual cowpea accessions, the effects of outbreeding and mixed populations must be taken into consideration. The estimation of the retention of genetic variability in an outbreeding population of constant size after *t* generations can be calculated as follows:

$$(1–1/2N)^t$$

where N = the effective number of individuals in the population and t = the number of generations (Table 4) (Oka 1983). Based on this theoretical information, as a guideline for sampling size, a minimum of 50 plants per accession are grown out for rejuvenation of cowpea to ensure the retention of the genetic integrity of the original accession.

The probability of success of maintaining a gene distributed randomly in a breeding population can be calculated using the probability formula:

$$P = 1–(1–p)^N$$

where P is the percentage certainty, p is the frequency of the alleles concerned and N is the effective number of plants that are necessary to restore in the next generation the alleles which are randomly distributed in the population. Therefore, to maintain an allele distributed randomly in a population with a 5% gene frequency, with a chance of 95% success, the minimum size of N must be 58.4. Thus we grow out a minimum of 50 plants and harvest seeds from all plants to ensure that genetic drift does not occur through rejuvenation.

Seedborne viruses of cowpea

Eight viruses (Table 5) are reported to be seedborne in cowpea, and transmission rates range from 0 to 90% depending on virus strain and cowpea variety. Some of the virus strains may exist as symptomless infection of some cowpea accessions. Many of the accessions in storage may not have been adequately screened for virus infection before storage. In addition, regeneration of accessions on the scale required cannot be completed in insect-proof conditions due to the scale of the regeneration that must be done. Where possible, up to 20

Table 4. Estimate of the retention of genetic variance in a small outbreeding population of constant size

Constant population size	Genetic variance remaining after *t* generations (%)			
(No. individuals)	1	5	10	100
10	95	77	60	<1
20	97.5	88	78	8
50	99	95	90	36
100	99.5	97.5	95	60

Table 5. Seedborne viruses infecting cowpea

Virus and type	Vector	Symptomless infection	Seed transmission (%)
Cowpea yellow mosaic comovirus	Beetle	?	0–5
Cowpea aphid-borne mosaic potyvirus	Aphid	?	0–40
Blackeye cowpea mosaic potyvirus	Aphid	?	0–40
Cowpea mottle (carmo)virus	Beetle	?	0–10
Cucumber mosaic cucumovirus	Aphid	Yes	4–26
Southern bean mosaic sobemovirus	Beetle	Yes	3–4
Cowpea mild mottle carlavirus	Whitefly	Yes	0–90
Sunnhemp mosaic tobamovirus	?	?	4–20

plants of each accession undergoing regeneration, or which have been requested, are planted in an insect-proof screenhouse and examined for virus infection. Plants with symptoms are tagged and no seeds are collected from these plants. Suspect plants are also tested by agar-gel diffusion, enzyme-linked immunosorbent assay and/or electron microscopy. Seeds from these virus-tested plants are bagged separately from the bulk seed and held in the active store for distribution. Regeneration of seed from virus-tested plants is not feasible with the present screenhouse facilities due to the large numbers of plants required for each accession. However, seeds from these virus-tested plants can be multiplied, as sub-samples, for international distribution.

Constraints

Several constraints to cowpea regeneration exist and require consideration.

- Some cowpea lines do not produce sufficient seed. In these cases more than the 50 individual plants are required. These utilize more resources and increase the cost of maintenance and regeneration.
- Wild cowpeas have a particularly low seed production rate. There are thus difficulties in obtaining sufficient seed for storage and, as above, in being able to regenerate sufficient seed.
- Environmental constraints exist in some accessions which are adapted to different environmental conditions than those found at IITA main campus at Ibadan, Nigeria. In some cases, the cowpeas can be grown but they may be less vigorous. In other cases, regeneration may have to be carried out at other sites.
- There are pest and disease constraints to the storage and regeneration of cowpea. Normal genebank procedures do not recommend fumigation of stored seeds. However, we do fumigate seeds with phosphine gas to control bruchids which infest seeds, to prevent large number of seeds being lost during post-harvest storage and processing, before long-term storage. Several insect vectors transmit seedborne viruses in cowpea that have plant quarantine implications (Table 5). When large numbers of accessions are regenerated in the field, many susceptible plants are infected by viruses. Application of insecticides has not been very effective in controlling the spread of viruses in the field. Roguing of virus-infected plants has been suggested as a way of controlling the amount of seedborne virus infection, but then the question arises whether roguing eliminates some of the genotypes. The susceptible individuals within the accessions may carry gene(s) which may predispose the plants to infection. The consequences of roguing of susceptible plants may

have selected genotypes which are less susceptible to virus infection. Ultimately this may lead to genetic drift.

Regeneration of germplasm accessions to produce virus-free seeds is difficult to achieve, unless the plants are immune to virus, resistant to insect vectors, or are planted in a field free of the insect vector or in insect-proof screenhouses.

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The multiplication and regeneration of rice germplasm at the International Rice Genebank, IRRI

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The genebank

The International Rice Genebank (IRG), formerly known as the International Rice Germplasm Center, is responsible for maintaining the large collection of rice landraces, wild species and some improved lines at the International Rice Research Institute (IRRI) in the Philippines. The collection comprises more than 80 000 accessions of which 95% are *Oryza sativa*, 2% *O. glaberrima*, and 3% cover the 20 wild species of *Oryza* and related genera.

In 1993 and 1994, the genebank underwent a major renovation, including the addition of a dedicated seed-drying room. After this refurbishment, the genebank now has the following facilities.

- Base Collection storage room (164 m^3) for long-term (50 to >100 years) conservation, maintained at −20°C, with a capacity for about 108 000 accessions, each with two aluminium cans, approximately 120 g.
- Active Collection storage room (927 m^3) for medium-term storage and distribution of samples, maintained at 4°C, with a capacity for about 110 000 accessions, approximately 500 g each.
- Seed drying room at 15°C / 15% RH, where seeds equilibrate to approximately 6% MC.
- Seed testing and germplasm characterization laboratory, containing five Conviron CMP 3244 germination cabinets.
- Two screenhouses with a combined area of >4000 m^2 – one is used to grow cultivated rice accessions with low viability or with low seed stocks, while the other is used to grow wild rices, in pots or special seedbeds.
- Data management laboratory, with four PCs (soon to be upgraded to 90 MHz Pentium processors), connected to the IRRI local area network, managing the International Rice Genebank Collection Information System (IRGCIS). The IRGCIS will soon be installed on its own server to be acquired through the SINGER project.
- Conservation support laboratory for tissue culture and embryo rescue of seeds of low viability, or for accessions with few seeds, and for cytogenetic and biosystematic studies of the wild rices.
- Laboratory for studies of isozymes, random amplified polymorphic DNA (RAPD) and other polymerase chain reaction (PCR)-based markers.
- Access to >10 ha of field space on the IRRI Central Research Farm – upland site with assured irrigation facilities for germplasm regeneration / multiplication and field characterization.

Along with these changes and / or additions, important changes to germplasm multiplication, regeneration and conservation procedures were also introduced.

Multiplication and regeneration procedures

Our prime objective through multiplication and regeneration is to produce about 1 kg of high quality, high viability rice seeds through the least number of cultivation cycles in the field, while maintaining the genetic integrity of the germplasm samples. For most accessions in the collection this can be accomplished in a single growing season. Two categories of germplasm for multiplication or regeneration are selected, according to the following criteria.

Incoming materials

- newly-acquired / introduced samples for which the seed quantity is not sufficient for immediate placement in the active and base collections;

- seeds with low viability that must be multiplied to produce a sufficient quantity of high quality seeds for long-term storage;
- incoming samples from an initial multiplication at Los Baños, without an accession number because the harvest was not sufficient to permit immediate placement in the collection.

Conserved germplasm

Materials are prioritized into:

- 'old' accessions not yet stored in the base collection;
- materials in base and active collections whose viability drops below 85% of the initial viability after monitoring (Table l);
- accessions in the active collection with seed quantity <60 g;
- accessions that are frequently requested.

Site, season and number of samples grown each year

Before 1993, an off-station area about 10 km from IRRI, Los Baños was used for germplasm multiplication and regeneration, due to the relatively low incidence of pests at that site. Since 1993, all seed production activities have been transferred to the IRRI research farm located at 121°15′ E, 14°13′ N, and 21 m above sea level, where rice can be grown all year round. The implementation of a close season on the IRRI farm in 1993 effectively brought under control some of the major diseases and pests. Now germplasm multiplication and regeneration are carried out only during the dry season between the end of October and March. About 7000–8000 samples are grown in the field annually.

The time of planting is critical for some materials. Photoperiod-sensitive accessions are planted in October, so that the maximum vegetative phase coincides with the short days at the end of December, thereby inducing flowering. Japonica accessions are planted in early November so that the reproductive and grain-filling stages coincide with the relatively cool nights in January to February under Los Baños conditions (Kameswara Rao and Jackson 1996a, b, 1997). These cultivation practices have significantly increased the quality of all germplasm grown at Los Baños, and our research has shown this environment to be suitable for all types of cultivated rice germplasm that is multiplied or regenerated in the field.

Seed preparation and seedling establishment

To complete an 8×5-m-row plot, 25 g (1000 seeds) are needed per accession. Materials with few seeds are seeded and transplanted in the IRG nursery screenhouse. Accessions difficult to germinate are cultured on agar, and seedlings are raised in culture solution in the phytotron and thereafter transferred to the nursery screenhouse. Samples sown in the field which produce few seedlings are also transplanted in the nursery screenhouse.

Field maintenance

The *O. sativa* accessions are grown under modified lowland conditions where the fields are irrigated intermittently, while *O. glaberrima* accessions are grown under upland conditions, with overhead irrigation. Currently we are exploring the possibility of growing *O. glaberrima* accessions in lowland conditions with minimum irrigation. Based on recommendations from

Table 1. Seed viability monitoring schedule for cultivated rices in active and base collections of the International Rice Genebank

Initial viability (%)	Interval (no. of years)			
	indica/javanica/ glaberrima		japonica	
	Active	Base	Active	Base
85–89	–	–	3	5
90–95	5	7	4	6
96–100	7	l0	5	7

the Central Research Farm, the fertilizer rate is computed for specific field plots. The nitrogen is applied in three to four split doses during the growing period. Pre-emergence herbicide is applied to suppress weed growth and this allows the rice seedlings to grow more vigorously. Hand weeding is done for subsequent weed control.

Pest management

Intensive pest control is practised, although we have begun to adopt many of the principles of integrated pest management, and the use of pesticides in germplasm plots has decreased significantly in the past year. There are many pests which attack the rice crop. Many insects damage stems, leaves and grains; others transmit virus diseases. Fortunately there are no known seedborne viruses that infect rice. However, infection by viruses such as tungro and grassy stunt cause stunting, lower productivity and reduced yields. Fungal and bacterial pathogens such as blast and bacterial leaf blight may kill rice plants. Some can be important for seed production since they can be carried on the seed surface, as for example the fungal pathogen *Tilletia barclayana*. There is always a routine check for this during seed health tests, since spores on the seed surface can be an important source of inoculum in seed beds.

Field inspection of seed multiplication plots is carried out on a regular basis. Seed Health Unit inspectors, together with representatives from the Philippine Plant Quarantine Office, inspect the incoming materials grown in a field quarantine area at the seedling and vegetative stages, and just before harvest.

Purification

Since rice is an inbreeding crop, germplasm accessions are essentially managed as pure lines. This not only reflects how farmers in Asia cultivate and select rice varieties, even in mixed cultures, but this method enhances the conservation, evaluation and utilization of the germplasm. Seedlings that germinate outside a row in the seedbeds and in the field are rogued out. At the reproductive stage, germplasm samples are re-identified. Any discrepancies are resolved using the 'seedfile' which is created using the original seeds of an accession at the time of acquisition. However, no roguing is done when materials are received as mixed samples or when they are known to be cultivated as mixed accessions.

Harvesting and post-harvest activities

Based on research done at IRRI by Kameswara Rao and Jackson (1996a, b, 1997), harvesting is carried out 28 days after anthesis. Harvesting is done by cutting panicles and placing them in clean cloth bags with proper labels. Samples are threshed using a Vogel-type self-cleaning thresher. Initial seed cleaning is done right after threshing to remove any unfilled grains and stubble before transfer to smaller cloth bags for drying. Within 3–4 h after harvesting, the materials are placed in the drying room for slow, passive drying at 15°C and 15% RH. After about 30 days the seeds dry to 6% MC. After drying, the materials are transferred to paper bags to facilitate seed processing. Verification and authentication of harvests are done prior to final seed cleaning.

Seed multiplication for wild species follows different protocols, as each species differs in its cultural requirements. All species are reared in the GRC screenhouse nursery. To collect seeds, shattering panicles of each plant are enclosed in net bags. Several studies are being conducted to improve conservation protocols for this special germplasm.

Seed processing and storage

Authentication and verification of harvests determine the success of the regeneration process. The seeds are compared with those in the seedfile for correct identity, and if the amount of seeds is sufficient, they are processed for packing and storage. After all the pre-cleaning activities, hand selection follows. Diseased grains, mechanical mixtures and inert materials are removed. About 120 g of seeds are needed for the base collection, and 500 g are stored in the active collection; 20 g are destined for duplicate 'black box' storage at the National Seed Storage Laboratory at Fort Collins in the USA. Two 10 g samples are also pre-

packed for seed distribution. Another 20 g sample of clean seeds is kept for viability tests and seed health inspection. All of the above cleaning operations are done inside a seed processing room at 40–50% RH to minimize absorption of moisture by the seeds.

Viability test and seed health inspection

The storage potential of seeds depends on the initial viability of seeds. Prior to 1991, a set of control accessions was employed to monitor annually the viability of stored germplasm. In 1991, we initiated viability tests of all stored germplasm, as well as determining the initial viability of samples for long-term storage. Germination tests in two replications are made for each accession following ISTA rules. A third set is prepared if the difference between two tests exceeds the maximum tolerable limits at a probability of 2.5%.

Standard routine seed health testing is carried out by IRRI's Seed Health Unit. Only materials that pass the limit set by the Plant Quarantine Officers are stored, otherwise these entries are included for the next multiplication cycle, taking the necessary steps to avoid disease recurrence .

Final drying and packing

While waiting for the results of the seed health test, the materials are placed again in the drying room for one more week, and we can calculate when the seeds have once again equilibrated more or less to 6% seed MC. MC determination is done following ISTA rules. The packing materials used are impermeable to water. Rust-proof aluminium cans with 60 g capacity are used for the base collection, while resealable, laminated aluminium foil bags, 240×l55 mm, have been used for the active collection since 1992. This facilitates handling during distribution. Only indica and *O. glaberrima* materials with viability >90% and japonica materials >85% are packed for long-term conservation. During storage, viability of materials is monitored on a scheduled time interval (Table 1).

Problems and constraints

- accessions that are not well adapted to Los Baños conditions;
- heterogeneous materials present problems for seed production and post-harvest management;
- insufficient seed sample size of incoming materials;
- possible outcrossing, even though a high degree of selfing is assumed for rice;
- high seed sterility of wild species.

Research areas

In order to improve our management of the large rice germplasm collection at IRRI, we have initiated research in several areas, including:

- determination of outcrossing in rice – this will be important to monitor the genetic integrity of germplasm samples;
- identification of duplicate samples – we are collaborating with the University of Birmingham using molecular markers, which has already led to several publications (Virk *et al*. 1995a, b, 1996);
- flower induction of highly photosensitive wild species, e.g. *O. schlechteri*;
- biosystematics of the rice genepool – knowledge of the diversity of rices species and increased knowledge about the biology of the wild species will enhance management of this important germplasm.

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Discussion paper on the global regeneration need: evidence collected from country reports prepared for the International Technical Conference on Plant Genetic Resources

Suzanne Sharrock, N. Murthi Anishetty and Cary Fowler

Introduction

Regeneration of seeds in storage is an important part of the work of any genebank. Even under optimal *ex situ* storage conditions, viability declines, and reduction in viability results in the loss of both genes and genotypes. In the case of unique material, such losses may be irreplaceable. Monitoring of viability and timely regeneration should be a priority activity of all genebanks.

The processes of multiplication and regeneration require the growing-out of part of the seed sample to produce fresh seed. Unfortunately, this is not always a straightforward task. A certain amount of genetic change is almost inevitably associated with the process, especially if it is performed under conditions markedly different from those at which the sample was originally collected. Agroecological situations, biotic and abiotic factors and selection pressure may result in the loss of genes from the sample, and repeated regenerations can result in genetic drift. In the case of open-pollinated species, great care must be taken to prevent contamination of the sample during pollination. Human error and accidental mixing of seed can affect the genetic character of the seed produced. The lack of facilities to handle the controlled pollination of cross-pollinated species may result in these species being neglected or receiving low priority in the regeneration schedule.

Regeneration requires resources – land, personnel, funds and facilities – and these may be lacking at the genebank. It is well known that worldwide there is a backlog of samples requiring regeneration. This has been recognised as an urgent problem by the international community and it is clear that a strategy to address the problem must be developed and implemented. Agenda 21, Chapter 14G on conservation and sustainable utilization of plant genetic resources for food and sustainable agriculture specifically mentions in its objectives "to complete the first regeneration and safe duplication of existing *ex situ* collections on a world wide basis as soon as possible".

At the present time, although regeneration is known to be a problem, the scale of the problem is not clearly defined. Despite the fact that low initial sample size and demand for samples from long-term facilities can shorten the regeneration cycle, proper long-term storage conditions should obviate the need for regeneration for decades and even centuries. Assuming the regeneration cycle to be 10 years or more, routine, on-going regeneration might be expected to amount to less than 10% of accessions. However, some 95% of countries responding with specific information on regeneration report a far higher level of need (information from the World Information and Early Warning System, WIEWS).

On a national basis, the average percentage of total accessions requiring regeneration as reported by countries is 48%. Although this figure of 48% is derived from data provided by only 44 countries worldwide, it does give some indication of the scale of the problem. In an attempt to further clarify the global situation with regard to regeneration backlogs, an analysis was made of information provided in the Country Reports submitted to FAO in preparation for the International Technical Conference for Plant Genetic Resources which will be convened by FAO in Leipzig, Germany in June 1996.

National regeneration capabilities

A total of 109 reports were reviewed and the information extracted is presented in Appendixes 1–7. Some supplementary information was also obtained from the WIEWS, which is an FAO database developed from information supplied by IBPGR and from country questionnaires. It must be emphasized that this report does not include information from

regional and international agricultural research centres, especially the International Agricultural Research Centres of the CGIAR. Nevertheless, it is recognised that these centres have large germplasm collections and they will have to play a major role in the development and implementation of a regeneration strategy.

Two factors have a major impact on regeneration capacity. These are (i) conditions of storage; and (ii) the capability to handle the regeneration of cross-pollinated species. Poor seed storage conditions result in the rapid loss of seed viability and consequently the need for frequent regeneration. Inadequate facilities for handling cross-pollinated species mean that their regeneration may be delayed, or carried out in such a way that the genetic integrity of the sample cannot be guaranteed. These factors were taken into account when analysing the Country Reports.

Major genebanks

The total number of accessions being stored in national genebanks worldwide, as recorded in the Country Reports and the WIEWS, is approximately 4 500 000. Of these accessions, more than 2 000 000 (49%) are stored in 15 major national genebanks. In addition, approximately 500 000 accessions are stored in the CGIAR centres. If good regeneration practices can be ensured in these national and international genebanks, especially those holding a large amount of diversity of the major crops, a part of the global regeneration problem may be resolved.

The situation regarding regeneration in the major national genebanks is presented in Table 1. It can be seen from the table that two large genebanks, Russia and Ukraine, do not have long-term storage facilities. Regeneration must be carried out every few years. This obviously puts a great strain on the resources of the genebank. In addition, frequent regenerations are likely to result in genetic drift and an increased chance of selective elimination of certain genes from the original accession, and in mechanical mixtures.

Only Japan, Ethiopia and Poland report less than 10% of the total accessions in the genebank requiring regeneration. Regeneration backlogs are reported by India with 63%, Korea 50%, and Brazil 64% of accessions requiring regeneration. The situation in the 15 major genebanks is summarized in Fig. 1.

Regeneration situation – global

On a global scale, reports from 109 countries were reviewed. These are summarized in Appendix 1. Twenty countries reported no major problems in the regeneration of the accessions in their genebanks. These genebanks hold 36% (1 609 025) of the total number of accessions under consideration. In 54 countries reliable long-term storage facilities are lacking and these are responsible for 28% of accessions (1 258 421). Twenty-three countries reported particular problems in handing the regeneration of cross-pollinated species. These countries hold 26% of accessions. This figure is, however, distorted by the inclusion of the USA with 550 000 accessions. Not including the USA, only 13% of accessions are held in countries with inadequate facilities to handle cross-pollinating species.

Regarding the main constraints to regeneration, 42 countries with 28% of accessions reported funding to be a major constraint. Forty two countries with 46% of accessions reported lack of infrastructure and facilities as a constraint, while lack of personnel (technical and non-technical) is a problem in 30 countries holding 35% of accessions. Again, these figures are somewhat distorted by the inclusion of the USA in the latter two cases. Without the USA, the figures are 34 and 22%, respectively. Many countries report the ability to manage the regeneration of self-pollinating species according to international guidelines. However, inadequate isolation facilities can result in the use of sample sizes smaller than recommended in the regeneration of cross-pollinating species. Information on the numbers of accessions requiring regeneration is rarely provided in the country reports, and data for this are available for only 44 countries from the WIEWS database.

Table 1. Number of accessions and regeneration requirements in different countries[1]

Country and Institute	No. accessions	% requiring regeneration	Regeneration situation
China			
Institute of Crop Germplasm	350 000		Not yet started. Genebank is only 8 years old
Russia			
N.I. Vavilov Research Institute	333 000		No long-term storage facility. Regeneration is required frequently. Main constraints are funds and facilities
USA			
National Seed Storage Laboratory	268 000	19% of active collection	Backlog of samples requiring regeneration. Main constraints are lack of staff and facilities for regenerating cross-pollinated crops
Japan			
National Institute of Agrobiological Research	229 048	4	No specific problems reported
India			
National Bureau of Plant Genetic Resources	144 109	63	No specific problems reported
Ukraine			
National Centre for Plant Genetic Resources Ukraine	136 398		No long-term storage
Korea			
Rural Development Administration	120 000	50	Problems with regenerating cross-pollinating species. Main constraints are land, facilities and staff
USA			
National Small Grain Collection	119 775		As for NSSL
Germany			
Institute of Plant Genetics and Crop Research, Gatersleben	103 000		No specific problems reported
Canada			
Plant Gene Resources of Canada	100 000		No major problems reported
Brazil			
National Research Centre of Genetic Resources & Biotech.	60 000	64	Main constraints are funds, infrastructure and trained staff
Germany			
Institute of Crop Sciences Braunschweig	57 000		Main constraint is staff. Breeding companies are involved in regeneration
Ethiopia			
Ethiopian Genebank	54 000	8	Main constraints are funds and staff
Poland			
Plant Breeding and Acclimitization Institute	45 000	3	No specific problems reported
Hungary			
Institute of Agrobotany	45 000	40	No specific problems reported. Landraces are regenerated in the area where they are collected

[1]Information abstracted from Country Reports and from WIEWS.

Some countries are involving private companies, NGOs and/or farmers in regeneration when they do not have sufficient facilities. This has, however, been reported by only four countries: Germany, Greece, The Netherlands and Canada. One genebank in the UK has developed a policy whereby regeneration is generally not carried out. A sufficiently large sample is collected so that it should not be necessary to further multiply the sample. Effort is made to ensure that long-term storage conditions are maintained, and if some time in future fresh seed is required, if at all possible, this is re-collected rather than regenerated from the original sample. This system may work well in the particular circumstances of this genebank, which is responsible for conserving wild relatives of crop plants, not landraces or cultivars.

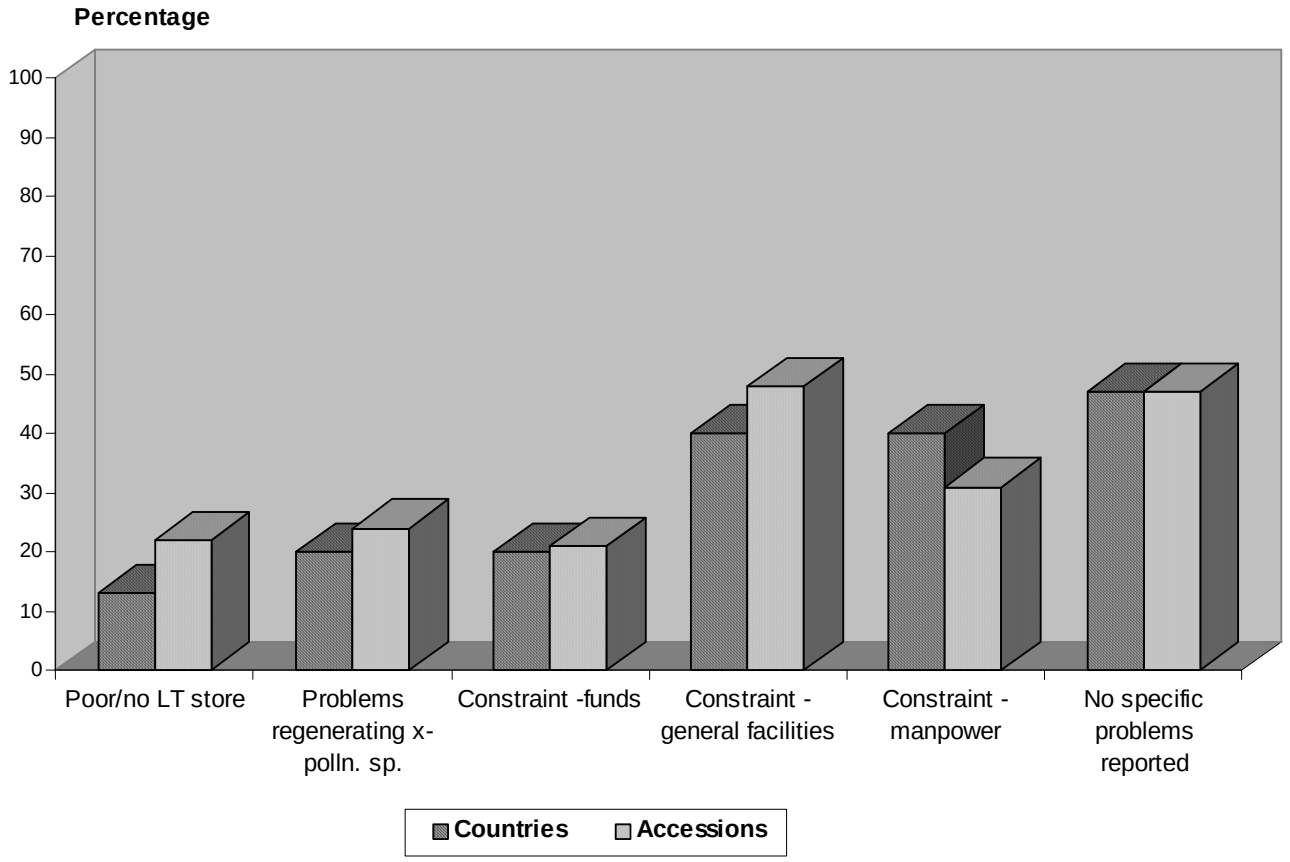

Fig. 1. Regeneration situation in major genebanks: 15 genebanks, 2 164 330 accessions.

Regeneration situation – Europe

Reports were reviewed from 33 countries holding 1 622 554 accessions, and details are given in Appendix 2. No major problems with regeneration were reported by 10 out of 33 countries. These countries are: Austria, Belgium, Czech Republic, France, Germany, Italy, Netherlands, Poland, Switzerland and UK. Together they hold 674 283 accessions or 42% of the European total. Poor or no long-term storage facilities are reported by 13 countries with 41% of accessions. However, 85% of these accessions are in three countries only – Romania, Russia and Ukraine. Problems with the regeneration of cross-pollinated species are reported by eight countries with 15% of accessions. One country, the UK, accounts for almost half (46%) of these. The major constraints to regeneration are reported to be availability of funds (13 countries) and inadequate facilities (11 countries). Three countries, Germany, The Netherlands and Greece, have agreements with private sector/farmers to assist in regeneration. Four countries, Austria, The Netherlands, Poland and Switzerland, report having 10% or fewer of accessions requiring regeneration.

Regeneration situation – Latin America and the Caribbean

Reports from 18 countries were reviewed, and the details are summarized in Appendix 3. Only one country, Argentina, reported no major problems with regeneration. Lack of long-term storage facilities was reported by 11 countries, holding 33% of accessions in this region. Of these, 76% of the accessions are in two countries – Columbia and Peru. The major constraint to regeneration was reported to be lack of funds, affecting nine countries with 57%

of accessions. Lack of staff is reported to be affecting eight countries with 65% of accessions. No countries report having fewer than 10% of accessions requiring regeneration.

Regeneration situation – Asia

Reports from 19 countries were reviewed. The details are summarized in Appendix 4. No major problems were reported by five countries – China, Japan, Malaysia, the Philippines and India. Together these hold 65% of Asian accessions. Less than 10% of accessions requiring regeneration are reported by only two countries – Japan and Thailand. Inadequate long-term storage facilities were reported by eight countries holding 18% of accessions, and problems in regenerating outcrossing species were reported by four countries holding 16% of accessions. The major constraint to regeneration was lack of facilities, reported by eight countries holding 28% of accessions.

Regeneration situation – Africa

Reports from 30 countries were reviewed, and the details are summarized in Appendix 5. Only three countries, Cameroon, Morocco and Namibia, reported having no major problems with regeneration. Ethiopia reports less than 10% of accessions requiring regeneration. Eighteen countries with 44% of accessions report having inadequate long-term storage facilities. Only four countries, with 8% of accessions, report problems handling cross-pollinated species.

Regeneration situation – Near East

Reports from seven countries were reviewed; details are summarized in Appendix 6. No country reported having no major problems with regeneration. Lack of adequate long-term storage facilities was reported by four countries with 51% of accessions.

Discussion

At the global level, only 20 countries do not report any specific problems in relation to regeneration, and half of these are in Europe. This indicates that 89 countries are experiencing some or major difficulties regarding regeneration, and these countries hold nearly 3 million (64%) of the total accessions in national collections.

Information provided in the majority of Country Reports is unfortunately not sufficiently detailed to allow an accurate estimation of the size of the regeneration backlog in national genebanks. However, on the basis of unquantified information that is provided, it would seem that the figure of 48%, derived from the WIEWS, is probably a reasonable estimate.

The total number of accessions being stored in *ex situ* genebanks is approximately 5 million. Assuming a level of redundancy of the order of 50%, and taking into account that the regeneration of one accession may suffice to replenish genebanks holding duplicates of that accession, approximately 25% of accessions remain to be regenerated. That is, the backlog can be estimated at around 1.25 million accessions. Some of these accessions, however, may already have lost their viability or genetic integrity, or they may be from populations where re-collecting might be more cost-effective than regeneration. Therefore an efficient, coordinated global effort with clear priorities might aim to regenerate a backlog of approximately 1 million accessions in order to save the most valuable material already in *ex situ* collections.

If it can be assumed that the cost of regenerating one accession (in a developing country) is $10.00, and regeneration would take place over a period of, say, 10 years, a budget of approximately $10 million would be required. This sum does not include funds for facilities, infrastructure development, training, etc. Provision of funding is not the only requirement, however. Priorities for regeneration must be agreed upon and some global coordinating mechanism developed. In this regard, lack of information on accessions constitutes a major constraint to rational regeneration strategies.

In addition to the costs of clearing the backlog of accessions, routine regeneration must continue and sufficient funds must be allocated for this activity. It must also be recognized

that funds are required not only to facilitate routine regeneration activities, but for other activities such as infrastructure development and research. Many developing and developed countries report as major problems to be overcome: a lack of long-term storage facilities, lack of facilities for handling cross-pollinated species, and lack of trained staff. It is especially necessary to develop appropriate methodologies for the regeneration of outcrossing species.

The issue of regeneration is included in the Global Plan of Action prepared for presentation at the International Technical Conference on Plant Genetic Resources in Leipzig, Germany in June 1996. The following points are some tentative proposals for inclusion in the Global Plan of Action.

Proposed Action Plan for Regeneration

Long-term objectives

To complete the first safe worldwide regeneration of accessions in *ex situ* storage, under conditions designed to preserve the genetic integrity of material.

Intermediate objectives

- formulate a strategy
- establish coordinating mechanisms
- identify locations for regeneration
- complete agreements needed to formalize cooperation among institutions
- improve capacity and infrastructure as necessary.

Policy/strategy

As appropriate and feasible, regeneration efforts should strive to maintain both allelic and genotypic diversity / adapted gene complexes of the original sample. Priority should be given to the following.

(i) Samples which meet the criteria of being:
   - globally unique, i.e. either unduplicated or the best / original sample of material which has been duplicated;
   - threatened, e.g. cultivars or species which were originally collected from changed sites or habitats under threat;
   - in good condition, i.e. usually not having been regenerated often and still representing the original genetic make-up of the sample;
   - in long-term storage or intended to be placed in long-term storage or for safety duplication.

(ii) Collections in the International Network of *Ex Situ* Collections under the FAO auspices, as a means of ensuring wide sharing of benefits from regeneration efforts.

(iii) Accessions which are of relevance to food and agriculture in a given region or sub-region.

Where possible, national resources should be directed to regeneration for plants of purely national importance. Identification of specific samples should be made in cooperation with national programme breeders and curators who often have intimate and detailed knowledge of crops and of the existing collections, as well as of the availability of materials from *in situ* locations. Efforts should take into account the need to reduce unnecessary and / or unwanted redundancies within and between collections as a means of improving efficiency and minimizing ongoing conservation costs. Regeneration should not be viewed as a means of maintaining collections on a long-term basis. In this regard, it is noted that minimizing the frequency of regeneration is an important goal and consequence of other activities under the Global Plan of Action.

Governments, institutions (including in particular the IARCs of the CGIAR) and NGOs should cooperate to make efficient use of existing capacity and to ensure that regeneration can take place, if scientifically, technically and administratively feasible, at sites approximating that of the original sample. Characterization activities should be undertaken

in conjunction with regeneration, as feasible, without compromising the effectiveness or scientific goals of the regeneration exercise.

Capacity

Training programmes should consider the need for personnel trained in the regeneration requirements of specific crop species. Specific training courses on regeneration should be developed, especially covering the particular requirements of cross-pollinated species, with both a scientific and a technical basis. As appropriate and cost-efficient, proper facilities, trained staff and equipment should be made available to national programmes and international institutes involved in regeneration activities undertaken as part of the Global Plan of Action.

Research and technology

IPGRI and FAO should develop guidelines for regeneration and, as appropriate, standards for regeneration for different species or groups of species. Guidelines should, *inter alia*, provide guidance on how accessions are chosen or prioritized for regeneration. They should take into account planning and management as well as applicability to different institutional situations and collecting purposes. IPGRI and FAO should oversee research in further developing scientific methodologies for prioritizing choices of accessions to be regenerated through national as well as global efforts.

Research should be undertaken to increase the effectiveness and efficiency of regeneration efforts, broadly defined as follows:

- identification of markers that are associated with seed longevity to assist in devising regeneration strategies;
- causes of mutations in conserved germplasm;
- negative effects of seedborne pests on genetic diversity in storage and the reduction of such effects;
- various questions regarding breeding systems and technical problems associated with regeneration practices.

Data on existing accessions in *ex situ* collections should be assembled and analysed in order to assist in planning and implementation.

Coordination and administrative issues

There is a need to develop an operational plan for a coordinated, global regeneration effort. IPGRI, in cooperation with FAO, could play a major role in coordinating and administering the implementation of this plan, including identification of institutes/locations, consistent with agreed goals and the need for cost-efficiency. Ongoing monitoring of the need for regeneration, including consideration of prevalence of adequate duplication, storage behaviour of the species, storage conditions and individual accession viability, should be undertaken.

Acknowledgements

Information on the costs of regeneration was provided by Ms J. Toll and Dr V. Ramanatha Rao of IPGRI. Some specific information was also provided directly by the genebanks during telephone conversations. The authors gratefully acknowledge this assistance. In addition, the authors acknowledge the many useful comments and suggestions which were provided by Drs D. Cooper, I. Kermali, J. Serwinski and C. Spillane during the preparation of this paper.

Appendix 1. Summary of information obtained from country reports (percentages in italics)

Region	No. of countries	No. of accessions	No/poor long-term storage facility		Lack of facilities for cross-pollinated species		Constraint – funds		Constraint – general facilities		Constraint – manpower		No major problem	
			Countries	Accns	Countries	Accns	Countries	Accns	Countries	Accns	Countries	Accns	Countries	Accns
Europe	33	1 622 554	13	663 050	8	246 626	13	655 881	11	803 839	6	294 412	10	674 283
			39%	*41%*	*24%*	*15%*	*39%*	*40%*	*33%*	*50%*	*18%*	*18%*	*30%*	*42%*
LAC	18	532 646	11	178 163	4	103 110	9	304 737	7	165 370	8	348 400	1	30 000
			61%	*33%*	*22%*	*19%*	*50%*	*57%*	*39%*	*31%*	*44%*	*65%*	*6%*	*6%*
Asia	19	1 204 362	8	219 620	4	192 734	4	91 748	8	333 331	4	161 748	5	780 843
			42%	*18%*	*21%*	*16%*	*21%*	*8%*	*42%*	*28%*	*21%*	*13%*	*26%*	*65%*
Africa	30	333 045	18	145 338	4	9 356	14	203 231	13	190 652	10	169 705	3	23 899
			60%	*44%*	*13%*	*3%*	*47%*	*61%*	*43%*	*57%*	*33%*	*51%*	*10%*	*7%*
Near East	7	102 238	4	52 250	2	46 400	2	14 400	2	14 400	1	8000	0	0
			57%	*51%*	*29%*	*45%*	*29%*	*14%*	*29%*	*14%*	*14%*	*8%*	*0%*	*0%*
North America	2	650 000	0	0	1	550 000	0	0	1	550 000	1	550 000	1	100 000
			0%	*0%*	*50%*	*85%*	*0%*	*0%*	*50%*	*85%*	*50%*	*85%*	*50%*	*15%*
Global	**109**	**4 444 846**	**54**	**1 258 421**	**23**	**598 226**	**33**	**1 269 997**	**42**	**2 057 592**	**30**	**1 532 265**	**20**	**1 609 025**
			50%	***28%***	***21%***	***13%***	***30%***	***29%***	***39%***	***46%***	***28%***	***34%***	***18%***	***36%***

Appendix 2. Europe – regeneration information

Country	No. of Accessions[1]		Type of storage[2]	Regen-eration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germina-tion % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
	CR	WIEWS								
Albania	20 000	16 760	ST	Yes					Yes	No LT storage Regen. required. every 2–3 years Problems with regen. outcrossing spp. Manpower, facilities, funds, information
Armenia	2000		ST	Yes		Yes				No information
Austria	na	7891	LT, ST	Yes	10	Yes				No information
Azerbaijan	25 000	na	na	na						No information
Byelarus	4000	na	MT, ST	Yes		Yes			Yes	Mainly field collections. No LT store. Problems regen. veg seed accessions Funding, facilities
Belgium	2036	11 189	LT	Yes		Yes				No information
Bulgaria	55 420	39 340	LT, MT, ST	Yes		Yes		85	No	Manpower, land, funds, isolation facilities
Cyprus	1155	12 342	ST	Yes	50	Yes		85	Yes	No LT store Some accns duplicated in IARCs Problems regen. cross-pollinating spp.
Czech Republic	42 792	54 632	LT, MT, ST	Yes	40	Yes			Yes	Viability monitoring since 1995
Denmark	2295[4]	3660	ST							Most accns stored in Nordic Gene Bank
Estonia	3000	1186	ST	Yes		Yes			No	No LT store. Considering agreement with Nordic Genebank. Intend to store PGR which cannot be regen. in store where regen. is possible.
France	150 000	214 080	LT, MT, ST	Yes		Yes				

Appendix 2. continued

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Germany	200 000	188 729	LT, MT, ST	Yes	33	Yes	Yes		Yes	Facilities sufficient at present, may become limiting if accn no. in store increases. Breeding cos involved when isolation facilities insufficient
Greece	na	17 556	LT, MT, ST	Yes	50	Yes	Yes		Yes	Manpower, funds Isolation facilities Problems with regen. of cross-pollinating spp.
Hungary	NR	75 487			40					
Ireland	na	2758	MT, ST	Yes						No LT storage Funds, facilities
Israel	50 540	52 159	LT, MT, ST	Yes		Yes				Regen. previously had low priority, now backlog of samples exists
Italy	80 000	78 841	LT, MT, ST	Yes						No information
Latvia	NR	9672			30					
Lithuania	na	18 737	ST	No	50					Funds No LT storage, regen. required frequently Intend to give preference to local PGR
Republic of Moldova	6000	na	MT, ST	Yes		Yes				No LT store, conditions poor Difficulties regen. cross-pollinating crops. May try to regen. difficult spp. elsewhere
Netherlands	15 000[5]	75 343	LT, MT, ST	Yes	<5	Yes	Yes	75–80		Funds Facilities limited for cross-pollinating spp.
Norway										All accns stored in Nordic Gene Bank
Poland	45 069[6]	90 729	LT, ST	Yes	3	Yes		Varies with species	Yes	Approx. 1000 samples/year regen.
Portugal	29 200	29 361	LT, MT, ST	Not yet started				70–80		Funds

Appendix 2. continued

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Romania	93 000[7]	43 270	ST	Yes	45			85		No information
Russian Fed.	333 000	247 243	ST	Yes		Yes			Yes	Regeneration previously carried out by regional stations; with disintegration of USSR, number of stations reduced Funds, equipment
Slovak Rep.	5000	14 547	MT, ST	Yes					Yes	No LT storage Regen. required frequently
Spain	na	78 174	LT, MT, ST	Yes		Yes				Funds, facilities
Sweden		81 605						75		All LT storage in Nordic Gene Bank
Switzerland	17 000	22 722	LT, MT, ST	Yes	10	Yes				Funding Isolation for cross-pollinating spp.
Turkey	na	27 036	LT, MT, ST	Yes	33	Yes		80	Yes	Manpower, funds, facilities Information on breeding behaviour of wild spp.
Ukraine	136 400	70 013	MT, ST	Yes		Yes			No	Some disease problems Contamination in field plots Manpower, facilities
United Kingdom	114 495	112 582	LT, MT, ST	Yes		Yes				In cross-pollinating spp., population size may be constrained by availability of isolation facilities
Yugoslavia	38 000	27 694	ST	Yes		Yes – when started			Yes	Regen. not yet initiated Funds, manpower

[1]CR, Country Report; WIEWS, World Information and Early Warning System; na, not available.
[2]LT, long-term; MT, medium-term; ST, short-term; NR, no report.
[3]Information obtained from.
[4]Does not include accessions stored in Nordic Gene Bank.
[5]Accessions in National Genebank only. Does not include accessions stored by other institutes.
[6]Accessions in base collection only.
[7]Includes base and active collections.

Appendix 3. Americas and Caribbean – regeneration information

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
North America										
Canada	100 000[4]	212 061	LT, MT, ST	Yes		Yes	Yes	85		25 000 samples tested to date
USA	550 000	519 726	LT, MT, ST	Yes	19% of active collection[5]	Yes		85	Yes	Backlog of samples for regen. due to lack of suitable conditions at some sites. Need for arid zone, long-growth-season regen. site Main limitation lack of manpower & facilities for cross-pollinating spp.
Latin America and Caribbean										
Argentina	30 000	26 318	LT, MT, ST	Yes		Yes			Yes	
Barbados	2868[6]	na	ST	Yes		Yes				Management, funding
Bolivia	7212[7]	12 429	ST	Yes	70	Yes		Varies with species		
Brazil	194 000	151 365	LT, MT, ST	Yes	64	Yes				Funds, infrastructure & training
Chile	36 000	3790	LT, MT, ST	Yes		Yes			Yes	Funds, manpower
Columbia	85 000[8]	34 185	ST	Yes	80	Yes		60–70	Yes	Contamination may occur due to lack of suitable facilities
Costa Rica	na	4829	MT, ST	Yes						Funds
Cuba	18 668	12 408	LT, MT, ST	Yes	70	Yes				Problems with electricity supply for cold stores Aim to improve base collection to reduce frequency of regen.
Dominican Rp	na	2024	ST	Yes						No LT store, lack of facilities
Ecuador	12 769[9]	35 780	MT, ST	Yes	30					No information
Guatemala	na	2724	ST	Yes	60	Yes – for main crops			Yes	Some accns duplic. in IARCs No LT store. Some accns lost due to lack of funding and manpower

Appendix 3. continued

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Honduras	4457	4257	ST	Yes		Yes				No LT store Funding, manpower, facilities
Jamaica	NR	795								
Mexico	55 337[10]	103 305	LT, MT, ST	Yes	48	Yes				Funding, manpower
Nicaragua	na	2207	MT, ST	Yes	15	Yes		70	Yes	No LT store Funding, facilities, especially for cross-pollinating spp.
Peru	51 758[8]	19 423	MT, ST	Yes		Yes		50–60		Manpower, facilities
Puerto Rico	NR	4000								
Trinidad & Tobago	na	2315	ST	Yes						Funds No LT store
Uruguay	na	1256	LT, MT, ST		100					Manpower, facilities Problems with regen. cross-pollinating spp.
Venezuela	14 647	15 356	LT, MT, ST	Yes						Lack of space for regen. cross-pollinating spp.

na, information not available.
NR, no Country Report submitted.
[1]CR, Country Report; WIEWS, World Information Early Warning System.
[2]LT, long-term; MT, medium-term; ST, short-term.
[3]Information from.
[4]Includes germplasm in national genebanks only, not in private, NGO etc. genebanks.
[5]60 555 accessions are in base collection only; these need to be multiplied for duplication purposes.
[6]Does not include seed accessions of West Indies Central Sugar Cane Breeding Station.
[7]Does not include working collections of research institutes.
[8]Includes working collections.
[9]Does not include working collections.
[10]Does not include CIMMYT collection.

Appendix 4. Asia – regeneration information

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Bangladesh	22 565	10 673	LT, MT, ST	Yes	90	Yes		85	No	Manpower, facilities, funds, information
Bhutan	NR	40								
Cambodia	na	2155	LT	No						Only rice germplasm stored Duplicated at IRRI
China	350 000[4]	300 000	LT, MT, ST	Yes	Not yet required	Yes				Viability tested for last 8 years. Not yet needed to regen. acc.
D.R. Korea	100 000	na	LT, ST	Yes		Yes				Limited facilities for LT storage Most germplasm under ST storage Regen. required. frequently
India	144 109[5]	244 337	LT, MT, ST		63					No information
Indonesia	na	26 828								No information
Japan	202 581[6]	103 960	LT, MT, ST	Yes	4					No information given
Korea Rep.	120 000	115 837	LT, ST	Yes	50	Yes		80	Yes	Land, facilities, manpower Problems with cross-pollinating crops.
Malaysia	38 255	31 225	LT, MT, ST	Yes				80 – rice		No information
Mongolia	24 000	na	ST	Yes				85		No LT storage, regen. Required. Often accns lost
Myanmar	8000	na	MT, ST	Yes				85		No information
Nepal	8383	8042	ST	Yes	30					Viability monitoring since 1994 No isolation facility for cross-pollinating crops Some accns duplicated in IARCs
Pakistan	18 000	na	LT, MT, ST	Yes	48	Yes		85	Yes	All accns duplicated in IARCs
Philippines	45 898	59 399	LT, MT, ST	Yes				85	Yes	No information

Appendix 4. continued

Country	No. of Accessions[1] CR	WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Sri Lanka	11 205	11 781	MT, ST	Initial mult. only	30	Yes		85	Yes	LT store not up to intl standard Information required on reproduction of wild spp. Rice germplasm duplicated at IRRI
Taiwan	NR	14 368								
Thailand	14 351[7]	32 404	LT, MT, ST	Yes	4	Yes			Yes	Land, facilities, manpower, funds. Prefer not to keep material which cannot regenerate Not confident with cross-pollinating spp.
Turkmenistan	4832		ST	Yes		Yes				No LT storage facilities Manpower, funds
Uzbekistan	50 000		ST	Yes		Yes				No LT store Funds, facilities Problems with regenerating cross-pollinating crops
Viet Nam	13 200	21 493	MT, ST	Yes	30			85		Poor storage facilities Aim to regenerate 20% of collection every year

[1]CR, Country Report; WIEWS, World Information and Early Warning System; na, not available.
[2]LT, long-term; MT, medium-term; ST, short-term; NR, no report.
[3]Information obtained from.
[4]Includes 50 000 accessions introduced from outside China.
[5]Does not include working collections.
[6]Includes active and base collections.
[7]Information for National Rice Seed Store and National Genebank only.

Appendix 5. Africa – regeneration information

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Angola	328	na	LT	No	100					Funding, manpower
Benin	na	2453	ST	Yes						No LT storage Regen. required every 3–4 years Funding
Botswana	1400	3390	LT, ST	Yes						Limited LT storage Some duplicated at IARCs
Burkina Faso	450	850	MT, ST	Yes		Yes				Facilities, particularly for cross-pollinating spp. Manpower, funds
Cameroon	na	2329	LT, ST	Yes		Yes				Some duplicated at IARCs
Congo	1755	634	ST							No LT storage Only active collections regularly regenerated
Côte d'Ivoire	22 498	16 387	ST							No LT storage
Eritrea	1087	na	MT, ST	Not yet started						
Ethiopia	54 000	47 606	LT, ST	Yes	8	Yes		85	Yes	Funding, land, manpower
Ghana	na	2987	LT, ST	Yes		Yes		85		Funds, isolation facilities
Guinea	na	899	MT, ST	Yes		Yes			Yes	Facilities Some duplicated at IARCs
Kenya	na	50 037	ST, MT, LT	Yes	60	Yes		85		Funding
Madagascar	15 000[4]	4046	ST	Yes		Yes				LT store unreliable Funding Some duplicated in IARCs
Malawi	1300[5]	11 421	LT	Yes	80			84	No	Funding Land for isolation, particularly for sunflower Technical information
Mauritius	228	195	ST, MT, LT	No	25					Manpower
Morocco	20 470	na	ST, MT, LT	Yes		Yes			Yes	
Mozambique	675	1872	ST	Yes		Yes		70	Yes	Funds, facilities
Namibia	1100	1515	LT, ST	No				85 (cvs) 65 (wild spp.)		Accns duplicated in regional genebank New collection, accns do not need regenerating yet Manpower

Appendix 5. continued

Country	No. of Accessions[1] CR	No. of Accessions[1] WIEWS	Type of storage[2]	Regeneration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germination % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Nigeria	NR	12 324								
Senegal	12 000[4]	1011	ST, MT	Yes					No	No LT store Regen. required every 3–4 y Funds
Seychelles	100	369	Field	Yes		No				Funding Technical information
Sierra Leone	623	1848	ST	Yes						No LT storage Facilities Lack of government support
South Africa	48 918[4]	19 794	LT, ST, MT	Yes		Yes				Some LT stores unreliable Accns lost due to lack of regen. Land, funds, manpower
Sudan	3500	5178	LT, ST	No						Cereals duplicated at ICRISAT Funding, manpower
Tanzania	na	2510	LT	No						Seed store not yet fully equipped
Togo	4000	333	ST	Yes						Manpower, facilities
Tunisia	1768	na	ST	Yes		Yes				PGR only used in crop improvement programme
Uganda	na	11 483		Yes	80			90% of prev. test		Facilities, manpower, funds
Zaire	na	18 830	MT, ST	Yes						No LT store Accns regen. every 2–3 y
Zambia	4619	5901	MT, ST	Yes	84			85		No LT store Lack of facilities for out-crossing crops; no irrigation
Zimbabwe	45 698[4]	2886	ST, MT	Yes		Yes		70 – tobacco		Germplasm all in working collections Facilities, manpower

[1]CR, Country Report; WIEWS, World Information and Early Warning System.
[2]LT, long-term; MT, medium-term; ST, short-term.
[3]Information obtained from WIEWS.
[4]Includes working collections.
[5]National genebank only.
na, information not available.
NR, no report.

Appendix 6. Near East – regeneration information

Country	No. of Accessions[1] CR	WIEWS	Type of storage[2]	Regen-eration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germina-tion % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
Afghanistan	NR	2965								
Egypt	8000	8914	LT, MT, ST	Yes	100	Yes				LT store unreliable Active collections only regen.
Iran	40 000	39 050	LT, ST	Yes		Yes			Yes	Funds, manpower, facilities Difficulties in preventing contamination of outcrossing spp.
Iraq	6400	1143	LT, ST	Yes	80	Yes				Some accns duplicated at IARCs Problem regen. outcrossing spp. Funds, equipment
Jordan	3588	na								All accns stored at ICARDA for regen.
Kazakhstan	33 000	na	ST	Yes		Yes			Yes	No LT store. Accns regen. every 1–5 years
Libyan Arab Jamahiriya	NR	2313			5					
Oman	NR	238								
Syrian Arab Republic	8750	4621	MT	Yes						No information
Yemen, Republic of	2500	4229	ST							No LT store Accns have to regen. frequently

[1]CR, Country Report; WIEWS, World Information and Early Warning System.
[2]LT, long-term; MT, medium-term; ST, short-term.
[3]Information obtained from.

Appendix 7. South-west Pacific – regeneration information

Country	No. of Accessions[1]		Type of storage[2]	Regen-eration ongoing?	% to be regenerated[3]	Specialist scientist involved?	Private sector involved?	Germina-tion % of seed for regen.	Fresh & old seed kept separate?	Major constraints/comments
	CR	WIEWS								
Australia	NR	94 768								
Fiji	NR	943								
New Zealand	NR	28 914			50					
Papua New Guinea	3873	5656	ST	Yes						No LT store Regular regen. required. for all accns Accns being lost Funding
Solomon Islands	500	1130	ST Field	Yes						No LT storage Field collection continually being regen. but accns being lost
Vanuatu	NR	664								

[1]CR, Country Report; WIEWS, World Information and Early Warning System.
[2]LT, long-term; MT, medium-term; ST, short-term.
[3]Information obtained from.
NR, no report.

Analysis of information on seed germplasm regeneration practices

Nestor C. Altoveros and V. Ramanatha Rao

Introduction

The majority of the germplasm collections in genebanks all over the world are conserved in the form of seed. The maintenance of sufficient seed quantities through regeneration poses the problem of preserving of the genetic integrity and genetic diversity in the collections. All the steps involved in the regeneration of seed material, as well as biotic and abiotic factors, can lead to changes in the genetic constitution and loss of variability of the accessions conserved. Genetic drift and shift can occur as a result of methods used for sampling the seeds to be sown, due to biotic and abiotic stresses to which the seeds and growing plants are subjected; inappropriate isolation and pollination techniques employed; harvesting, processing and restocking methods used; human error; and as a consequence of differences in competitiveness and fecundity of the plants. There is presently much conjecture and uncertainty over regeneration procedures. Significant results have been obtained for some major crops and rationalization of procedures has been recommended. The theoretical basis has been formulated for certain aspects of regeneration, including for example regeneration subsample sizes and frequencies of alleles over regeneration cycles.

The degree with which this information, which is scattered throughout the literature, has been assimilated by curators of germplasm collections, and how it is applied to the practical aspects of regeneration, is at present a matter of speculation. Knowledge on the current regeneration practices employed by genebank managers as they agree or differ with the available recommended practices is therefore of critical importance to provide an idea of what is happening to the vast germplasm collections accumulated over the years. It will also provide a basis for determining where information is lacking, and where intervention will arrest the 'decay of variability' in the genebanks.

Regeneration procedures and management survey

With these considerations in mind, the International Plant Genetic Resources Institute (IPGRI) has conducted a survey on germplasm seed regeneration practices. A questionnaire was sent out at the end of 1989 to genebank curators in 320 institutes listed in the Conservation Database of IPGRI. The items in the questionnaire can be divided into the following categories:

- information on the curator and the genebank;
- species, type of collection and number of accessions;
- seed storage management practices;
- regeneration practices, including cultural management;
- information on the reproductive biology of the species in the collection.

Further communications were sent out until March 1990 to the curators for clarifications and for details not provided in the original replies. The responses were incorporated in a database on regeneration, and analysed to determine seed storage conditions and practices as they affect the need for regeneration. In this paper an attempt is made to determine the actual regeneration practices in use for different types of species, collections and samples covered by the survey. Common and different regeneration practices for different types of species, collections and samples were identified and described. The genetic principles underlying regeneration practices were defined and discussed, the theoretical aspects were compared with the actual practices, and the differences between the two were interpreted and explained. Although the survey included field genebanks and *in vitro* conservation facilities, only seed genebanks are considered in this paper. For clarity, mention may be made of the number, percentages, etc., as related to the field genebank and *in vitro,* but they are not part of the analysis.

Discussion of the survey results

Number of genebanks and species/accessions

Responses were received from 125 curators in 78 genebanks from 42 countries, consisting of 72 genebanks from 36 national programmes and six from international agricultural research centres (IARCs). The number of responding genebanks per country ranged from one (28 countries), two (five countries), three (four countries), four (two countries), five (one country), six (one country) and nine (one country) (Table 1). Curators per genebank ranged from one to 14. There were 74 curators managing single species or single crop collections, while 45 were managing collections of two to over 1000 species. Six curators gave no information on the number and identity of species they were managing.

The total number of accessions maintained by the curators ranged from 30 to 79 527. There were 77 curators (66%) managing up to 5000 accessions. The number of accessions in the base and active collections ranged from 60 to 79 557, and 30 to 79 557, respectively. There were 57 and 105 curators managing base and active collections, respectively (Table 2).

Type of collections and storage environment

Collections were classified as base, active, and other. Listed under other collections were safety collections, strategic test collections, genetic stocks, working collections, field collections, vegetative collections, and collections for research. Twelve curators managed base collections only, 60 managed active collections only, and 49 managed both base and active collections. Therefore 61 curators managed base collections and 109 managed active collections. Five respondents did not specify the type of collection they managed. Of the 61 curators managing base collections, 35 stored their materials at –24°C to –10°C, 10 at 0 to 7°C, six at ambient conditions, and three used other methods (field or *in vitro* collection). Seven curators did not specify the storage temperature (Table 3). Only 19 curators provided information on humidity during storage. Three curators stored seeds at 27–45% RH and –20 to –10°C, eight at 20–50% RH and 0–4°C, one at ambient RH and –20°C, one at 50% RH and ambient temperature, and six at ambient RH and temperature (Table 4). Only six curators indicated the seed moisture content during storage, both for base and active collections. Two stored seeds at 5% MC, and one each at 4–6%, 4–8%, 5–7% and 5–8%. The relationship

Table 1. Countries represented, numbers of genebanks and of curators per country

Country	No. of genebanks	No. of curators	Country	No. of genebanks	No. of curators
Argentina	1	3	India	3	7
Australia	1	1	Israel	1	1
Belgium	1	1	Japan	1	1
Bulgaria	1	1	Morocco	1	1
China	3	3	Mexico	1	1
Colombia	1	1	Netherlands	2	6
Costa Rica	1	1	Norway	1	1
Czech Republic	3	5	New Zealand	1	1
Cyprus	1	1	Pakistan	2	2
Germany	4	5	Peru	1	1
Denmark	1	1	Philippines	1	1
Ecuador	1	2	Poland	9	11
Egypt	2	2	Russia	1	1
Spain	1	1	Sweden	2	2
Ethiopia	2	2	Syria	1	1
France	6	12	Thailand	1	1
Great Britain	4	5	Turkey	3	3
Ghana	1	1	Taiwan	1	1
Greece	1	6	USA	5	14
Hungary	1	8	Yemen	1	1
Indonesia	1	1	Yugoslavia	1	2
			Total	**78**	**125**

Table 2. Curators classified according to number of accessions managed

	Type of collection					
	Base		Active		Whole collection	
Number of accessions	No. curators	Percent	No. curators	Percent	No. curators	Percent
Up to 1000	13	22	36	23	38	32
1001–5000	18	32	33	32	39	33
5001–10 000	11	19	15	14	18	15
10 001–20 000	11	19	13	12	14	12
20 001–30 000	2	4	2	2	2	2
30 000–40 000	1	2	4	4	4	3
Over 40 000	1	2	2	2	2	2
Total	**57**		**105**		**117**	

Table 3. Storage temperature used for base and active collections

	No. curators	
Temperature range (°C)	Base collection	Active collection
–24 to –10	35	11
–5 to 0	–	2
0 to 5	9	64
6 to 10	1	8
11 to 15	–	2
Ambient	6	9
Not specified	7	8
Field collection	3	5
Total	**61**	**109**

Table 4. Relative humidity and temperature in storage rooms for base and active collections

Temperature range (°C)	RH (%)	No. curators
–24 to –10	27–45	3
–20	Ambient	1
0–5	20–50	8
Ambient	50	1
Ambient	Ambient	6
Total		19

Table 5. Minimum number of seeds stored for base and active collections of species described as being cross-pollinated

	Base collection		Active collection	
Seed number	No. species	No. curators	No. species	No. curators
<8000	27	24	60	34
8000–12 000	4	3	19	7
>12 000	27	4	65	12
Total	**58**	**31**	**144**	**53**

Table 6. Maximum number of seeds stored for base and active collections of species described as being cross-pollinated

	Base collection		Active collection	
Seed number	No. species	No. curators	No. species	No. curators
<8000	19	16	37	24
8000–12 000	15	12	28	15
>12 000	27	4	78	16
Total	**61**	**32**		

between relative humidity and seed moisture content with packaging was not very clear. This was because even the genebanks which practised fairly strict control on RH were using hermetically sealed cans or alfoil bags. Additionally, some genebanks that were using paper or cloth bags had no control of RH.

Of the 104 curators managing active collections, 13 stored their materials below 0°C, 64 at 0–5°C, eight at 6–10°C, two at 11–15°C and nine at ambient temperature. Eight curators did not specify the storage temperature (Table 3).

Only 19 curators provided information on the humidity of their storage rooms (base and active), which is presented in Table 4 together with the storage temperatures. Only six curators indicated the seed moisture content during storage, which ranged from 4 to 8%. Two curators indicated moisture contents of up to 8% for seeds in base collections.

Quantity of seed

The majority of the curators (77% for base and 64% for active collections) kept the actual minimum seed numbers lower than the recommended 8000 seeds per accession for allogamous species (Tables 5 and 6). The number of curators keeping fewer than 8000 seeds decreased to 50% for base and 44% for active collections when the maximum number of seeds stored was considered. The majority of the curators (68% for base and 62.5% for active collections) kept an equal or greater number than the recommended 3000 to 4000 seeds per accession for autogamous species (Tables 7 and 8). The number of curators who kept the minimum number of seeds lower than the recommended quantity is still high (12 out of 37 for base, and 21 out of 56 for active collections). The same trend is observed for the maximum number of seeds kept (nine out of 37 for base, and 17 out of 48 for active collections).

Tables 9 and 10 present the minimum and maximum number of seeds stored by curators in base and active collections for species or groups of species which they described as being predominantly cross-pollinated. On the basis of the minimum number of seeds stored, seven of the 17 curators holding base collections treated the often cross-pollinated materials as self-pollinating, two treated them as cross-pollinating, and eight treated them as between the two. On the same basis, 10 of the 27 curators holding active collections treated the materials as self-pollinating, 11 treated them as cross-pollinating, and six treated them as between the two. On the basis of maximum number of seeds stored, five of the 18 curators who managed base collections treated the materials as self-pollinating, eight treated them as cross-pollinating, and five treated them as between the two. In the case of active collections, six of the 26 curators treated the materials as self-pollinating, 14 as cross-pollinating, and 10 as between the two.

Table 7. Minimum number of seeds stored for base and active collections of species described as being self-pollinated

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	23	12	48	21
3000–4000	26	13	39	15
>4000	24	12	50	20
Total	**73**	**37**	**137**	**56**

Table 8. Maximum number of seeds stored for base and active collections of species described as being self-pollinated

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	20	9	26	17
3000–4000	9	7	26	9
>4000	46	21	75	22
Total	**75**	**37**	**127**	**58**

Table 9. Minimum number of seeds stored for base and active collections of species described as being predominantly cross-pollinated

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	7	4	9	6
3000–4000	4	3	7	4
8000–12 000	4	2	7	4
>12 000	–	–	14	7

Table 10. Maximum number of seeds stored for base and active collections of species described as being often cross-pollinated

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	5	3	7	4
3000–4000	2	2	2	2
8000–12 000	7	6	10	7
>12 000	6	2	15	7

Table 11. Minimum number of seeds stored for base and active collections of species described as self- or predominantly cross-pollinated, or both

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	3	3	10	6
3000–4000	–	–	–	–
>4000	2	2	2	2
Total	**5**	**5**	**12**	**8**

Table 12. Maximum number of seeds stored for base and active collections of species described as self- or predominantly cross-pollinated, or both

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	1	1	7	3
3000–4000	1	1	2	2
>4000	2	2	2	2
Total	4	4	11	7

Table 13. Minimum number of seeds stored for base and active collections of species described as cross-, self-, or predominantly cross-pollinated

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	1	1	9	5
3000–4000	2	2	75	2
8000–12 000	–	–	1	1
>12 000	1	1	1	1

Table 14. Maximum number of seeds stored for base and active collections of species described as cross-, self-, or predominantly cross-pollinated

	Base collection		Active collection	
Seed number	**No. species**	**No. curators**	**No. species**	**No. curators**
<3000	2	2	7	4
3000–4000	3	3	3	3
8000–12 000	1	1	1	1
>12 000	1	1	4	3

The wide and even spread in the minimum and maximum number of seeds stored for both the base and active collections may be a reflection of the uncertainty on how predominantly cross-pollinated species are to be treated, i.e. like self-pollinators, like cross-pollinators, or between the two. The heterogeneity of the population structure in predominantly cross-pollinated plants may be expected to be more than that of self-pollinators. It might therefore be safer to store more seeds than recommended for self-pollinators, to ensure that a representation of all genotypic combinations in the population is captured. On the basis of number of seeds stored, half of the curators of base collections treated the materials as self-pollinating, and the other half as between self- and cross-pollinating. On the other hand, the majority of the curators of active collections (75 and 71% for minimum and maximum number of seeds stored, respectively) treated the materials as self-pollinating (Tables 11 and 12). The minimum and maximum number of seeds stored by curators in base and active collections for species or groups of species described as being either cross-pollinated, self-pollinated or predominantly cross-pollinated are presented in Tables 13 and 14. The curators were almost evenly divided as to whether they should treat the species as self- or cross-pollinated, perhaps a reflection of their uncertainty regarding the pollination behaviour of the species.

Frequency of regeneration

The frequency of regeneration of accessions will depend on storage conditions as they affect seed quality, the rate by which seed stocks are depleted, and the amount of seeds maintained. To determine if the need to regenerate is dependent on the amount of seeds stored by the curators, the frequency of regenerations carried out in the past was analysed (Tables 15 and 16). The data indicate that regeneration had been carried in a great majority of the collections held by all curators. If the frequency of regeneration is considered, it can be noted that one to three regenerations are the most common. This becomes understandable if we consider the common experience of most genebanks of having limited initial numbers of seeds of an accession, in which case one or two regenerations will need to be carried out to bring the seed numbers to acceptable levels. However, there are still an appreciable number of collections where the number of seeds stored is less than 3000 and which have been regenerated from four to 11 times, indicating that the relatively small number of seeds kept may in fact necessitate more frequent regeneration.

Reasons for regeneration

Table 17 presents the reasons given by curators for undertaking regeneration of their collection – viability and quantity of seed were the main reasons cited. Other reasons cited were distribution, research, use of the material, limited initial seed quantities, length of time the seeds have been under storage, pest infestation, availability of facilities, time and cost of regeneration. All these 'other reasons' are different expressions of drop in viability or seed quantity. As discussed above, seed viability as it impacts on the need to regenerate germplasm is a function of conditions of storage, i.e. temperature and seed moisture content or relative humidity. The need to regenerate due to depleted seed numbers will depend on how much seed was stored in the first place (see above), and the rate by which seeds are withdrawn for distribution and use. In theory, the latter should not be a problem in base collections (if we consider the purpose of these collections, i.e. long-term conservation, and that these collections should not be touched for distribution and utilization). However, if we look at the frequency with which base collections are regenerated and the reasons given for the need to regenerate, it can be seen that a significant proportion of base collections were in fact treated as active collections and seeds were distributed and utilized, which will logically result in the depletion of seed stocks and the need to regenerate more often.

Sample size for regeneration

The minimum and maximum number of plants grown during regeneration of species or species groups perceived by curators as cross-pollinating is given in Table 18. Based on the

Table 15. Minimum number of seeds stored, number of collections, and frequencies of regeneration carried out by germplasm curators

Minimum no. seeds stored	Number of germplasm collections (frequency of regeneration)					
	Minimum			Maximum		
	0	1–3	4–11	0	1–3	4–11
<500	15	17	7	15	16	13
500<1000	1	–	2	1	–	2
999<2000	2	6	3	2	5	3
999<3000	2	16	6	2	9	13
≥3000	23	125	8	17	112	22
Total	**43**	**164**	**23**	**37**	**142**	**53**

Table 16. Maximum number of seeds stored, number of collections, and minimum and maximum frequencies of regeneration carried out by germplasm curators

Maximum no. seeds stored	Number of germplasm collections (frequency of regeneration)					
	Minimum			Maximum		
	0	1–3	4–11	0	1–3	4–11
<500	12	12	3	12	8	9
500<1000	0	0	2	0	0	2
999<2000	2	7	5	2	6	5
1999<3000	0	11	6	0	11	6
≥3000	32	134	8	22	124	22
Total	**46**	**164**	**24**	**36**	**149**	**44**

Table 17. Criteria cited by curators for regenerating germplasm in base and active collections

Criterion	Base collection		Active collection	
	No. collections	No. curators	No. collections	No. curators
Viability	243	44	464	73
Quantity	106	39	288	59
Research	110	2	120	4
Use	9	5	22	12
Distribution	11	2	21	4
Other	17	9	24	12

Table 18. Minimum and maximum number of plants grown during regeneration of species or group of species perceived to be cross-pollinating

Plants	Minimum		Maximum	
	No. collections	No. curators	No. collections	No. curators
<25	36	18	30	13
25–50	59	24	50	21
>50	67	22	82	32
Total	**162**	**64**	**162**	**66**

Table 19. Minimum and maximum number of plants grown during regeneration of species or group of species perceived to be self-pollinating

No. plants grown	Minimum		Maximum	
	No. collections	No. curators	No. collections	No. curators
<20	30	11	12	7
<40	65	23	27	15
20≤60	65	26	58	21
>60	65	34	90	40
Total	**160**	**71**	**160**	**68**

minimum number of plants grown, 28% of 64 curators grew fewer than 25 plants during regeneration. Based on the maximum number of plants grown, 20% of 66 curators grew fewer than 25 plants.

Table 19 presents the minimum and maximum number of plants grown during regeneration of species or group of species perceived by curators as self-pollinating. Based on the minimum number of plants grown, 16% of 71 curators grew fewer than 20 plants during regeneration, and 52% grew fewer than 60 plants. Based on the maximum number of plants grown, 10% of 68 curators grew fewer than 20 plants, and 41% grew fewer than 60 plants.

The minimum and maximum number of plants grown during regeneration of species or species groups perceived by curators as predominantly cross-pollinating, grouped on the basis of the recommended number of plants that should be grown when regenerating cross- and self-pollinating species, also varied significantly (Table 20). When the grouping was based on the recommendation for self-pollinating species, 5% of the curators grew fewer than the recommended minimum number (20 plants) needed for the maintenance of heterogeneous accessions; 24–35% grew fewer than the minimum (40 plants) required when the pedigree method is used; and 48–65% grew fewer than the minimum (60 plants) required when the bulk method is used. When based on the recommendation for cross-pollinating species, 10% of the curators grew fewer than the recommended number (25 plants).

Table 21 presents the minimum and maximum number of plants grown during regeneration of species or species groups, the breeding systems of which were perceived by curators as mixtures of cross-, self-, and predominantly cross-pollinating, and grouped according to the recommended number of plants that should be grown when regenerating cross- and self-pollinating species. When the grouping was based on the recommendation for cross-pollinating species, considering the minimum and maximum number of plants grown, 38 and 36% of the curators, respectively, grew fewer than the recommended number. When it was based on the recommendation for self-pollinating species, the figures were 24 and 18%. From one-fifth to a quarter of curators maintaining germplasm collections of cross-pollinating species, or groups of species, failed to maintain a regeneration sample size large enough to minimize the loss of alleles occurring at frequencies of 5% or lower. This occurred despite the fact that recommendations for regeneration sample size can be found in the literature.

From 10 to 16% of the curators maintaining self-pollinated material grew fewer than the minimum number of plants required to ensure maintenance of the original genotypic constitution of heterogeneous accessions, irrespective of the method of multiplication used. Assuming that all curators used the pedigree method, 22–32% of the curators grew fewer than the minimum number of plants required. If all the curators used the bulk method, 41–52% of the curators grew fewer than the minimum number of plants required.

When the results of the survey for cross-pollinated and self-pollinated species or groups of species are compared, it can be seen that a greater proportion of curators grew fewer than the minimum recommended number of plants when regenerating the latter. One possible reason is the perception that it requires fewer plants to maintain the genetic integrity in self-pollinating accessions, perhaps equating autogamy with homogeneity. What is perhaps not taken into account is the fact that a population of self-pollinators may consist of a great number of homozygous individuals which differ genotypically and genetically from each other (i.e. heterogeneous), and alleles will have been fixed in homozygous condition in the individuals. Therefore it will require a larger sample of individuals to ensure that all, or almost all, of the fixed alleles will be represented when the population is regenerated.

Breeding systems and pollination control

Table 22 presents the breeding systems as they are perceived by curators, as they are reported in the literature, and the number of corresponding collections. There were 121 cases where the breeding system was perceived by the curators as cross-pollinated and where information on the breeding system is available in the literature. In a great majority of cases

(114), the two agree. There was a disparity in one species which was reported in the literature as self-pollinated but was treated as cross-pollinated; three cases which were reported as predominantly cross-pollinated; and three which were reported as either cross- or self-pollinated depending on the genotype. There were 76 cases where the breeding system was perceived by the curators as self-pollinated and where information is available in the literature. In 22 cases there was a disparity between the two. There were 30 cases for

Table 20. Minimum and maximum number of plants grown during regeneration of species or group of species perceived to be predominantly cross-pollinating

	Minimum		Maximum	
No. plants grown	No. collections	No. curators	No. collections	No. curators
<20	4	1	4	1
<40	8	7	14	5
40–60	26	12	20	9
>60	8	7	14	11
Total	**38**	**20**	**38**	**21**
<25	8	2	8	2
25–50	22	11	16	8
>50	8	7	14	11
Total	**38**	**20**	**38**	**21**

Table 21. Minimum and maximum number of plants grown during regeneration of species or group of species perceived to be either cross-, self-, predominantly cross-pollinating, or mixtures of the three

	Minimum		Maximum	
No. plants grown	No. collections	No. curators	No. collections	No. curators
<20	8	5	7	4
20–60	89	10	88	10
>60	6	6	8	8
Total	**103**	**21**	**103**	**22**
<25	88	8	14	8
25–50	9	7	79	5
>50	6	6	10	9
Total	**103**	**21**	**103**	**22**

Table 22. Breeding system as perceived and as reported

Breeding system		
As perceived by curators	As reported in the literature	No. collections
Cross-pollinating	Cross-pollinating	114
Cross-pollinating	Self-pollinating	1
Cross-pollinating	Predominantly cross-pollinating	3
Cross-pollinating	Cross/self-pollinating	3
Self-pollinating	Self-pollinating	54
Self-pollinating	Cross-pollinating	9
Self-pollinating	Predominantly cross-pollinating	8
Predominantly cross-pollinating	Predominantly cross-pollinating	13
Predominantly cross-pollinating	Cross-pollinating	14
Predominantly cross-pollinating	Self-pollinating	3

Table 23. Requirement for isolation, as indicated, during regeneration of germplasm collections with different breeding systems

	No. collections	
Breeding system	Isolation required	Isolation not required
Cross-pollinated	148	14
Self-pollinated	37	129
Predominantly cross-pollinated	28	13
Mixture of above	15	76

Table 24. Pollinating agents in 36 species as indicated by curators and as reported in the literature

Species	Pollinating agent[1] according to: Curator	Literature	References
Allium cepa	–	B	Wojtowski *et al.* (1979)
Bauhinia esculenta	BO	BO	Hokche and Ramirez (1990)
Beta vulgaris	W	IW	Free *et al.* (1975)
Brassica oleracea	F	B	Hussein and Abdel Aal (1982)
Cassia sp.	–	B	Buchmann (1974)
Chamaechrista sp.	–-	B	Pinheiro *et al.* (1988)
Cichorium endivia	BI	B	Marletto *et al.* (1988)
Cichorium intybus	W	B	Marletto *et al.* (1988)
Coronilla varia	–	B	Ptacek and Hofbauer (1973)
Cracca sp.	–	B	Ricciardelli d'Albore (1983a, b)
Crotalaria spp.	BO	BI	Abrol and Kapil (1988); Grewal and Singh (1979); Vidal *et al.* (1988)
Cucumis melo	B	B	Grewal and Sidhu (1978)
Cuphea viscosissima	B	B	Parker and Tepedino (1990)
Cytisus spp.	BO	B	Christoffersen and Brander (1990)
Dactylis glomerata	–	W	Naghedi-Ahmadi (1977)
Daucus carota	I	BF	Wilson *et al.* (1991); Ottoson (1984)
		BI	Wojtowski *et al.* (1979)
Erythrina sp.	–	Bird	Guillarmod *et al.* (1979); Wesley (1987)
Gossypium arboreum	BI	I	Tanda (1983); Tanda and Goyal (1979)
Helianthus annuus	BI	B	Hussein and Abdel Aal (1982); Simpson and Neff (1987); Stamm and Schuster (1989)
Indigofera spp.	BO	B	Atmowidjojo and Adisoemarto (1986)
Lagenaria siceraria	B	I	Shrivastava (1991)
Luffa acutangula	B	I	Shrivastava (1991)
Lupinus sp.	W	B	Dimitrov (1990); Williams (1987)
Melilotus albus	B	B	Kropacova and Miklik (1970)
Melilotus officinalis	B	BI	Ganiev (1984); Ricciardelli d'Albore (1983a, b)
Momordica charantia	B	BI	Grewal and Sidhu (1978); Shrivastava (1991)
Parkinsonia sp.	–	B	Jones and Buchmann (1974)
Paspalum scrobiculatum	W	BW	Adams *et al.* (1981)
Petroselinium crispum	BIW	I	Anasiewicz (1982); Burgett (1980); El Berry *et al.* (1974); Ricciardelli d'Albore (1986)
Phaseolus coccineus	BI	B	Quagliotti and Marletto (1987)
Prosopis sp.	–	B	Genise *et al.* (1990); Habit *et al.* (1981); Simpson and Neff (1987)
Raphanus sativus	I	BI	Hussein and Abdel Aal (1982)
Stylosanthes sp.	–	B	Pereira-Noronha *et al.* (1982)
Trifolium sp.	–	B	Ricciardelli d'Albore (1983a, b) (*pratense*)
Trifolium alexandrium	–	B	Hussein and Abdel Aal (1982)
Vicia faba	B	B	Hussein and Abdel Aal (1982)

[1]B=bee; I=insect; F=fly; W=wind; O=other.

those perceived as predominantly cross-pollinated, and there was a disparity in 17 cases. All in all, there were 46 instances out of 227, representing 46 collections, where there were differences in the breeding system of species, or groups of species, as the curators perceived it and as reported in the literature. The pollination behaviour of plant species where information was readily available in the literature is presented in Appendix 1. This clearly demonstrates that (i) initially there is a need to establish the breeding system of a species at the site of regeneration; (ii) accessions of the same species can differ as to the extent of a particular system; and (iii) adequate information for many species is either not available or is scanty.

Curators differed on whether or not isolation is required for collections which they described as self-pollinating, cross-pollinating, predominantly cross-pollinating, or mixtures of the three (Table 23). The curators indicated that no isolation is required in 14 collections of cross-pollinated species (10% of the total), in 13 collections (32%) of predominantly cross-pollinated species, and in 76 collections (84%) of species where the breeding system is a

mixture of the three. On the other hand, isolation is deemed necessary in 37 collections (22%) of self-pollinated species.

In the first two cases, the non-provision of isolation when it is in fact necessary could mean the danger of contamination by foreign pollen, thereby affecting the genetic integrity of the accessions after regeneration. In the third case, the assumption of self-pollination by a majority of the curators would mean that safeguards against contamination by foreign pollen are not provided. If it so happens that the plants are actually cross-pollinating, the absence of isolation during regeneration will result in the contamination of the accession by foreign genotypes. In the fourth case, the provision of isolation when it is not necessary will not affect the genetic structure of the self-pollinating accessions. It would, however, make the regeneration process costlier and more time-demanding.

A search of the literature provided information on the pollinating agents in 36 cross-pollinated species which were included in the collections of the curators in the survey. There was agreement between the curators and the literature on the pollinating agents of 27 species. In seven species, information on the pollinating agents was available in the literature but was not known by the curators. In three species, the pollinating agents cited by the curators disagreed with the literature report (Table 24). Once again it is not certain who is correct, and this emphasizes the need to ascertain and use the appropriate pollen vector.

Conclusions

This analysis raises various questions regarding the procedures and management practices of germplasm accessions in genebanks. Some of these are related to accession history, breeding systems, floral biology and pollination control. A number of questions are related to management issues such as the regeneration load and number of accessions conserved in a genebank, and the availability of resources (funds and human resources). Many problems arise from the frequently small size of the initial samples and the quality of seeds conserved. The latter is determined by a number of pre- and post-harvest handling techniques. It is recognised that the survey was conducted some time ago, but the information that has been gathered through visits over the past 5 years has helped to confirm the existence of (serious) problems in many *ex situ* collections. Consequently, germplasm seed regeneration deserves much more attention than it has been receiving from the PGR community. This is essential if we wish to safeguard the significant investment that has been made in collecting and conserving millions of accessions worldwide.

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Appendix 1

Pollination behaviour of some plant species

Here an attempt is made to list the agricultural species in terms of their pollination behaviour. However, it must be noted that, in most cases, the information is somewhat anecdotal, based on general observations by several workers. It is also known that the pollination behaviour of a number of species tends to vary depending on the environment in which they are grown. Hence, when there is a doubt, it will be advisable to do some preliminary testing of the pollination behaviour of the species in question at the regeneration site.

IPGRI will greatly appreciate receiving information on breeding systems / pollination behaviour, outcrossing rates, etc., on any flowering plants. This information can be sent to V. Ramanatha Rao, IPGRI (E-mail: v.rao@cgiar.org).

Self-pollinated crop plants

Annual fescue – *Festuca* spp.
Apricot – *Prunus armenica*
Barley – *Hordeum vulgare*
Berseem or Egyptian clover – *Trifolium alexandrium*
Black gram / Urd – *Vigna mungo*
Buckwheat, Bitter – *Fagopyrum tataricum*
Centro – *Centrosema* spp. (some level of outcrossing occurs)
Chickpea – *Cicer arietinum*
Chilli pepper – *Capsicum annuum, C. frutescens*
Citrus – *Citrus* spp.
Coffee, arabica – *Coffea arabica* (50% pollination before flower opens)
Cotton – *Gossypium hirsutum* and *G. barbadense*. Frequently >10% outcrossing can occur
Cowpea – *Vigna unguiculata*
Crotalaria - *Crotalaria juncea*
Eggplant – *Solanum melongena*. Some genotypic variation for outcrossing, in the range 6–7%
Endive, *Chicorium endivia*
Flax or linseed, *Linum usitatissimum*: some cross-pollination can occur
Foxtail millet – *Setaria italica*
French / common bean – *Phaseolus vulgaris*
Grasspea, chickling vetch – *Lathyrus sativus*: significant level of outcrossing occurs
Groundnut – *Arachis hypogaea*: low outcrossing (1.5%) may occur
Kodo millet, *Paspalum scrobiculatum*: mostly cleistogamous
Lentil – *Lens culinaris* (predominantly selfing species)
Lettuce - *Lactuca sativa*
Leucaena – *Leucaena leucocephala* (most other *Leuceana* species outcrossing)
Lima bean – *Phaseolus lunatus*
Lupin – *Lupinus angustifolius* and in *L. mutabilis* some outcrossing occurs; in *L. albus* up to 9%
Mungbean / Greengram – *Vigna radiata*
Narbo bean – *Vicia narbonensis*: predominantly selfing
Nectarine
Oats – *Avena sativa*, related species variable
Okra – *Abelmoschus* spp.
Parsnip – *Pastinaca sativa*: cultivated ones self-pollinated
Peas – *Pisum sativum*
Peach – *Prunus persica*
Rice – *Oryza sativa* some wild rices outcrossing up to 50%
Sesame – *Sesamum indicum*

Slender wheat-grass, *Elymus trachycaulum*
Soybean – *Glycine max*
Strawberry clover – *Trifolium fragiferum* (self-incompatible forms occur)
Stylos – *Stylosathus* spp.: outcrossing can range between 2 and 22%
Subterranean clover – *Trifolium subterraneum*
Sweet clover – *Melilotus alba* (tripping needed)
Tobacco – *Nicotiana tabacum*. However, some *Nicotiana* species are self-incompatible, outcrossing moderate to high
Tomato – *Lycopersicon esculentum*. Cultivated tomato is known to outcross in its centre of origin and at a few other locations. Some self-incompatible species with moderate to high outcrossing occur
Velvet bean
Vetch (common, *Vicia sativa*, hairy, and pannonico)
Wheat – *Triticum* spp.
Winged bean – *Psophocarpus tetragonolobus*: outcrossing ranging between 0.3 and 7.6% recorded due to environmental conditions
Yellow sweet clover – *Melilotus indica*

Cross-pollinated crop plants

Adlay / Job's tears – *Coix lachryma-jobi*
Alfalfa / Lucerne – *Medicago sativa*: self-incompatible in some degree or self-incompatible strains occur, upon self-fertilization seed pods are partly coiled or straight
Almond – *Prunus dulcis*: strongly self-incompatible
Alsike clover: strongly self-incompatible
Amaranths (grain) – *Amaranthus* spp: monoecious or monoecious strains occur
American grapes – *Muscandia rotundifolia*: dioecious, monoecious strains occur
Andropogon – *Andropogon gayanus*
Apple – *Malus* spp.: self-incompatible in some degree or self-incompatible strains occur
Asparagus – *Asparagus officinalis*: dioecious
Avocado – *Persea americana*: self-incompatible in some degree or self-incompatible strains occur
Banana – *Musa* spp.: monoecious or monoecious strains occur, parthenocarpic
Birdsfoot trefoil – *Lotus corniculatus*: self-incompatible in some degree or self-incompatible strains occur
Blackberry: monoecious or monoecious strains occur
Black mustard – *Brassica nigra*: strongly self-incompatible
Bluberry – *Vaccinium* spp.
Bottle gourd – *Lagenaria siceraria*: monoecious
Broccoli, *Brassica oleracea* var. *italica*: strongly self-incompatible
Brown mustard – *Brassica juncea*: strongly self-incompatible
Brussels sprouts – *Brassica oleracea* var. *gemmifera*: strongly self-incompatible
Buckwheat, common – *Fagopyrum esculentum*
Buffalo grass – *Paspalum conjugatum*
Cabbage – *Brassica oleracea* var. *capitata*: strongly self-incompatible
Cacao – *Theobroma cacao*: self-incompatible
Carrot – *Daucus carota*: some level of geitonogamy occurs
Cashew nut - *Anacardium occidentale*
Castorbean – *Ricinus communis*: monoecious or monoecious strains occur
Cauliflower – *Brassica oleracea* var. *botrytis*: strongly self-incompatible
Celery – *Apium graveolens*
Chard – *Beta vulgaris* ssp. *cicla*
Cherry – *Prunus* spp.: strongly self-incompatible
Chestnut: monoecious or monoecious strains occur
Chicory – *Chicorium intybus*: strongly self-incompatible

Chinese cabbage – *Brassica campestris*: strongly self-incompatible
Clove – *Syzygium aromaticum*: self-incompatible in some degree or self-incompatible strains occur
Coconut – *Cocos nucifera* (dwarf forms self-pollinated)
Coffee, robusta – *Coffea robusta*: self-sterile
Collard: strongly self-incompatible
Crimson clover – *Trifolium incarnatum*: cross-pollinated, but self-fertile upon tripping
Cucumber – *Cucumis sativus*: monoecious or monoecious strains occur
Date palm – *Phoenix dactylifera:* dioecious
Ethiopian mustard – *Brassica carinata*: strongly self-incompatible
Faba bean – *Vicia faba*: outcrossing ranges 4–80%
Fig: effectively dioecious, parthenocarpic
Grapes – *Vitis vinifera*: monoecious strains occur
Hemp – *Cannabis sativa*: diocecious
Hops - *Humulus lupulus* : onoecious or monoecious strains occur
Jerusalem artichoke or topinambour - *Helinathus tuberosus* : self-incompatible in some degree or self-incompatible strains occur.
Kale – *Brassica campestris* var. *acephala*: strongly self-incompatible
Kiwi - *Actinidia deliciosa* : dioeceous
Kohlrabi – *Brassica oleracea* var. *gongylodes*: strongly self-incompatible
Kura clover – *Trifoloium ambiguum*: self-incompatible in some degree or self-incompatible strains occur
Lolium, Rye grass – *Lolium* spp., *L. perenne*: outbreeder, annual; *L. temulentum*: inbreeder
Maize – *Zea mays*: monoecious
Mango – *Mangifera indica*: self-incompatible in some degree or self-incompatible strains occur
Meadow fescue – *Festuca pratensis*: self-incompatible in some degree or self-incompatible strains occur
Musk melon, cantaloupe, rock melon – *Cucumis melo*: monoecious
Oil palm – *Elaeis guineenis*: monoecious
Olive – *Olea europae*: self-incompatible in some degree or self-incompatible strains occur
Onion – *Allium cepa* and most other *Allium* species
Orchardgrass, Cocksfoot – *Dactylis glomerata*: self-incompatible in some degree or self-incompatible strains occur
Papaya – *Carica papya*: dioecious
Parsley – *Petroselinum crispum*
Pear – *Pyrus* spp.: self-incompatible in some degree or self-incompatible strains occur
Pearl millet – *Pennisetum glaucum*
Pecan: monoecious or monoecious strains occur
Pigeonpea – *Cajanus cajan*
Pistachio – *Pistacia vira*: dioecious
Plum – *Prunus* spp.: self-incompatible in some degree or self-incompatible strains occur
Potato – *Solanum tuberosum*. (Cultivated tuberosum and Andigena groups): self-compatible outbreeders; wild and cultivated diploids: self-incompatible outbreeders; wild allopolyploids: self-compatible inbreeders
Pumpkin or winter squash – *Cucurbita maxima*, *C. moschata* and *C. mixta*: monoecious
Radish – *Raphanus sativus*: strongly self-incompatible
Rai or wild turnip – *Brassica tournefortii*: strongly self-incompatible
Red clover – *Trifolium pratense*: largely self-sterile, strongly self-incompatible
Rhubarb: monoecious or monoecious strains occur
Rubber – *Hevea brasiliensis*: sterile to self-sterile
Rutabaga: strongly self-incompatible
Rye – *Secale cereale*: strongly self-incompatible
Safflower – *Carthamus tinctorius* (some related species self-incompatible)
Sarson, yellow-seeded – *Brassica campestris* ssp. *trolocularis*: strongly self-incompatible

Scarlet runner: bean – *Phaseolus coccineus*: self-incompatible in some degree or self-incompatible strains occur
Smooth bromegrass – *Bromus inermis*: self-incompatible in some degree or self-incompatible strains occur
Slender trefoil – *Lotus tenuis:* self-incompatible in some degree or self-incompatible strains occur
Sorghum – *Sorghum bicolor*: some selfing occurs
Spinach, *Spinacia oleracea*: doecious
Squashes – *Cucurbita* spp.: monoecious
Strawberry clover – *Trifolium fragiferum*: self-incompatible in some degree or self-incompatible strains occur.
Strawberry – *Fragaria ananassa*: monoecious or monoecious strains occur
Sugarbeet – *Beta vulgaris*: self-incompatible in some degree or self-incompatible strains occur
Sunflower – *Helianthus annuus*: self-incompatible in some degree or self-incompatible strains occur
Sweet potato – *Ipomoea batatas*: self-incompatible in some degree or self-incompatible strains occur
Tall fescue – *Festuca arundinacea*: self-incompatible in some degree or self-incompatible strains occur
Tea – *Camellia sinensis*: self-sterile
Timothy grass – *Poa pratense*: self-incompatible in some degree or self-incompatible strains occur
Toria or Indian rape – *Brassica campestris* ssp. *dochotoma*: strongly self-incompatible
Turnip - *Brassica campestris* : strongly self-incompatible
Turnip rape - *Brassica campestris* ssp. *oleifera*: strongly self-incompatible
Walnut: monoecious or monoecious forms occur
Watermelon – *Citrullus lanatus*: monoecious
Wax gourd – *Benincasa hispida*: monoecious
White clover – *Trifolium repens*: strongly self-incompatible
White mustard - *Sinapis alba* : self-incompatible in some degree or self-incompatible strains occur
Yams – *Dioscorea* spp: dioecious; hermaphrodite flowers occur

Most B*rassica* species are self-incompatible, outcrossing up to 100%

Variable species

Quinoa – *Chenopodium* spp: gynomonoecy, hermaphrodite flowers occur (up to 99%, virtual cleistogamy to complete self-incompatibility
Kapok – *Ceiba pentandra*: pollination by bats and bees, autogamy, geitonogamy and allogamy occur
Cenchrus ciliaris: apomixis
Raspberry – *Rubus* spp.: self-incompatible or self-compatible forms occur, sexual or subsexual, dioeceous forms occur

Conservation, evaluation and use of maize genetic resources

Wilfredo Salhuana

Introduction

The term 'genetic diversity' is in common parlance. However, for genetic diversity to be useful in plant breeding in order to serve farmers and consumers, it must encompass genetic variability that is not present in the materials breeders are currently working with. It is necessary to have new sources of germplasm for present and future uses since environmental conditions, disease pressure, technologies, and demands from the farmer and consumer are constantly changing. It is strongly advisable that the new sources of germplasm have yield potential or some other useful trait or traits so that breeders can be encouraged to use sources of new genetic diversity in their programmes.

If we only continue to add accessions into genebanks and maintain their viability, without having a minimum level of information on the material, then the collections will, for the majority, continue as unused stocks of seed. An immediate problem that must be dealt with is the fact that maintenance of viability for many of the collections is often barely adequate. It is necessary to have sufficient quantity of viable seed to work with and to evaluate germplasm collections. Firstly, we must evaluate the existing populations to select a few from the many thousands available and then publish the results of this evaluation. Secondly, begin a programme of germplasm enhancement selecting for yield and other desirable characteristics that are demanded by farmers and markets.

These are neither easy nor rapidly completed tasks. Great amounts of time, effort and patience are required. Decades of regeneration, evaluation, and pre-breeding are needed to work with just a handful of the many populations that are stored in genebanks or that are used in agriculture as landraces or local varieties. However, these efforts are crucial so that germplasm resources can be more effectively and widely available and used by breeders, and as a consequence be made available to farmers.

All the resources available must be used in order to improve upon the current practices. The most efficient way to achieve this goal is by a well planned programme that should include all institutions interested in participating in regeneration, evaluation, publishing the results, and enhancement.

Regeneration

This task is very difficult to implement for one institution, since it is necessary to have sufficient environmental conditions, land, personnel, storage facilities, financial resources, etc. For these reasons it is convenient to join resources in order to use them in the most efficient way. To do this requires the implementation of a well coordinated plan based on partnerships between national and international programmes which would enhance the capacity of these programmes to conserve and regenerate vulnerable *ex situ* collections. Any plan would need to consider a budget that would encompass all of the costs for the years the plan would be in use. This budget would have to show the sharing of all costs such as personnel and the facilities needed to carry out the work. It is important to establish a methodology that permits each accession to maintain the frequency of the alleles without modifications.

Choosing an environment for increasing seed potential which corresponds to the site at which the collection was obtained is crucial in regeneration. This practice will demonstrate the possibility of natural and artificial selection and increase the amount of seed. Due to the difference in environmental conditions in which maize is grown (especially altitude and latitude) and the lack of adaptability of accessions when they are planted in locations that are not similar to the original conditions, it is necessary to undertake joint action in order to ensure the provision of correct climatic conditions for regeneration. It would be convenient to specify a few countries that have all the environmental conditions required for the

Table 1. Collections and number of countries (in parentheses) for each homologous area

	Homologous area[1]					
Country	**1**	**2**	**3**	**4**	**5**	**Total**
Bolivia	195 (8)		145 (4)	73 (2)		413 (14)
Brazil	341 (6)				64 (5)	405 (11)
Colombia	112 (9)	76 (2)			17 (1)	251 (8)
Chile	19 (1)		46 (1)		63 (4)	82 (5)
Guatemala	131 (5)	156 (3)				484 (9)
Mexico	242 (3)	22 (1)			15 (1)	369 (10)
Paraguay	254 (4)		59 (3)	22 (1)	24 (2)	254 (4)
Peru	121 (4)	82 (2)				265 (9)
Uruguay	268 (7)					322 (11)
USA	113 (7)				54 (4)	124 (8)
Venezuela	12 (1)				11 (1)	12 (1)
Total	1990 (50)	336 (8)	312 (11)	95 (3)	248 (18)	2981 (90)

[1]Homologous areas: 1, <1200 m and below 26°N or S; 2, 1200–1900 m and below 26°N or S; 3, 1900–2600 m and below 26°N or S; 4, >2600 m and below 26°N or S; 5, above 26°N or S.

regeneration of the accessions. Passport data will help in selecting the best locations for regeneration. In order to better select a location it is advisable to do a preliminary adaptation test in several locations that have been chosen for regeneration, with a few collections representative of the races. In the Latin American Maize Project (LAMP), an adaptability test was conducted in several countries with collections that represent races of the different countries. Table 1 presents a number of collections and the number of countries for each homologous area in the appropriate locations of each country.

The number of days to shed and silk was taken as a measure of adaptability of the collections and of course as a consequence of the race. The number of days was transformed to heat units. This not only helped to establish the location for regeneration, but also to find the location where the yield trials should be grown for evaluation.

It is necessary to know the status of the genebanks in order to know the existing number of accessions and the quantity and quality of the seed for each accession. This will help to determine the list of accessions that need regeneration. Thereafter, the list of accessions will be examined and duplicates eliminated, based on the available information and the experience of the researchers handling the material. Once the accessions and the locations are chosen, the seeds will be planted in well prepared fields, with good irrigation, well fertilized, avoiding extreme heat and drought conditions and where good disease and insect control has been established.

Once the location for the regeneration is selected, then a sample must be chosen that is of sufficient size. The current consensus is that effective population sizes for cross-pollinated species should be at least 200 per cycle, which means that field samples of 400–500 plants per cycle are required for each regeneration. Use of the adequate location and appropriate techniques avoids contamination and reduces selection. The recommended procedure for pollinating is chain-crossing using each plant as a male and female. The efficiency of the pollination could be affected by the poor adaptability of the accessions in the different locations chosen for regeneration. This could be caused by asynchrony between silk and shed, resulting in not using all the plants for pollination. The behaviour of some of the races, such as Piricinico, Choco, and Montana, can vary from location to location in the regeneration process. In some, seed increase is difficult due to problems of adaptation. These races are very difficult to pollinate because of the difficulty in synchronization and result in poor quality seed. Another important factor is that the temperature at the time of pollination should not be >36°C or 97°F.

Processing of seed is done according to well established procedures. After the ears are harvested, they are classified and evaluated. A picture is taken of the most representative

ears. The number of ears is counted before shelling the bulk, then equal quantities of seeds from each pollinated ear are taken to form a balanced sample. The ears that have few kernels must not be included in the count of ears because this can be a product of contamination. The size of the balanced sample is chosen depending on the needs of the bank. The sample has to be very clean, and is dried to about 10% seed moisture content. It will be advisable to treat the seed with photoxin or apply any insecticide to avoid future damage to the kernels. The sample needs to have appropriate labels outside and inside the bag. If possible, aluminium bags are used to store the material in the cold rooms. It is recommended to send samples of the accessions to long-term storage facilities as backup samples.

It is possible to make a preliminary evaluation of certain characteristics, especially of the flower time, during the process of regeneration. A standardized format to collect data in the field and during the process of shelling needs to be established. It will be useful to develop a rational programme that can be made available for all the collaborators to input and search for data. This will permit everybody to follow the same system and enable them to exchange information easily via diskette or through electronic mail. It is convenient to exchange information whenever there is an update, so an information network will be created which may also facilitate germplasm exchange. It is advisable to review every year, with all the collaborators, the advancement in the regeneration process, examine the problems and discuss how to solve them to improve the system.

The concept of core collections and of active and long-term collections needs to be considered. When we use core collections, it does not mean that we are going to stop using the individual collections. We continue to use active and long-term collections, but having the core collection may help in the regeneration of seeds.

The minimum required facilities for the regeneration process are: dryer, seed counter, shelling and cleaning equipment, cold storage room, aluminium foil bags, pollination and shoot bags.

The existing phytosanitary regulations, which vary from country to country, make exchange of germplasm difficult. These regulations need to be followed and respected, but it is necessary to discuss with representatives of each government to make the necessary arrangements so that the germplasm is exchanged quickly but safely. The idea is that the location in which the regeneration activity takes place will act like a quarantine site that can be visited any time by the inspectors, and if any symptoms of disease appear the plants are eliminated.

Cooperative activities in maize regeneration

Various examples can be cited of joint initiatives.

Regeneration of accessions by Pioneer for CIMMYT

CIMMYT has the responsibility of conserving the world's maize genetic material, and holds around 10 500 accessions in its maize genebank. Pioneer considers that it would be beneficial to help in the important but difficult task of regenerating the accessions at CIMMYT. With the goal of regenerating all the material that was in danger of being lost, a list was compiled of the accessions that exist only in this bank which have poor germination or very little seed. After compiling this list, seeds of the first 300 accessions were sent to Homestead, Florida for regeneration. In subsequent years, CIMMYT has sent more accessions for regeneration (Table 2).

Since CIMMYT at that time did not have an adequate cold room facility to preserve the material for long periods of time, it was decided to send 500 g samples of seed to the Plant Introduction Station, Beltsville. Plant Introduction (PI) numbers were assigned and samples were sent to the National Seed Storage Laboratory at Fort Collins, Colorado. Pioneer stored a sample of 250 g of each accession. The bulk of the seed (which was usually around 1–7 kg) was returned to CIMMYT for preservation and distribution, particularly to national programmes throughout the world. In addition, various agronomic characteristics were

Table 2. Accessions sent from CIMMYT for regeneration

Year	No. accessions
1981	107
1982	300
1983	350
1984	300
1985	328
1986	300
1987	300
Total	**1985**

recorded in the course of the regeneration. These were published and distributed in two catalogues by Pioneer.

Latin America–North Carolina State University project for regeneration

The Latin America–North Carolina State University project, financed by USDA–ARS, regenerated national and international accessions held by national programmes in Colombia, Mexico and Peru at a rate of 1000 accessions per year.

Regenerating endangered Latin American Maize Germplasm project

One of the accomplishments of the LAMP project was to determine the status of genebanks in Latin America: the number of accessions, the quantity and quality of the seed for each accession, and a list of accessions that needed to be regenerated. As a result of this information, another project was developed by USAID/USDA/CIMMYT, called Regenerating Endangered Latin American Maize Germplasm. Thirteen countries are participating in this regeneration project, and nearly 7432 endangered landrace accessions are being regenerated.

Latin American Maize Project (LAMP)

The LAMP project has demonstrated very clearly the precarious status of Latin America's maize germplasm resources. Only a portion of the total accessions held in Latin American maize banks is viable and has sufficient seed for testing.

In 1987 Pioneer, recognising that the preservation, documentation, distribution and evaluation of accessions in different genebanks must be done through coordinated efforts among the different national and international organizations involved, provided $1.5 million to the USDA–ARS to carry out a 5-year maize evaluation project. This effort was named the Latin American Maize Project (LAMP), and was the first coordinated international project to deal with the evaluation of genetic resources of a major world crop species. LAMP is based on the cooperative effort of 12 countries: Argentina, Bolivia, Brazil, Colombia, Chile, Guatemala, Mexico, Paraguay, Peru, USA, Uruguay and Venezuela. The main objective of LAMP is to evaluate the agronomic characteristics of over 14 000 accessions found in Latin American and US genebanks so they might then be used in breeding programmes.

Under LAMP, the varying responses of accessions to different environmental conditions (primarily altitude and latitude) were recognised and five homologous areas (HAs) were defined as:

- HA1, below 1200 m and below 26°N or S
- HA2, 1200–1900 m and below 26°N or S
- HA3, 1900–2600 m and below 26°N or S
- HA4, above 2600 m and below 26°N or S
- HA5, above 26°N or S

LAMP established a five-stage evaluation sequence. The first two stages were for reducing the number of accessions to a practical, feasible number in order to cross them with testers to determine combining ability. In the third stage the selected material was exchanged between countries in order to cross with the chosen tester. In stage 4, test-crosses, check hybrids and varieties were planted in replicated trials in each of the HAs and data were recorded for 17 traits, including yield. From the stage 4 test-cross trials, 100 tropical accessions were selected in HA1 and 78 temperate accessions in HA5.

As a consequence of LAMP there now exists a more precise determination of the status of maize stored in germplasm banks in Latin America with respect to:

- the number of accessions in each genebank;
- the quantity and quality of seed for each accession;
- the identity of accessions that need regeneration;
- the adaptability of the accessions and races to permit a more thorough and effective exchange of germplasm between regions;
- performance, agronomic, disease, and insect resistance information on selected accessions.

The data collected during the first two stages have been published in two catalogues and distributed on CD-ROM. Also, *Stage 4 Results From Homologous Areas 1 and 5* were published as a printed catalogue and were digitized on a CD-ROM along with maize genebank inventories from CIMMYT, USDA–ARS (Germplasm Resources Information Network) and Agriculture and Agri Foods Canada, and other crops from USDA–ARS.

Evaluation

Very little effort has been made towards one of the most important genetic resource activities, i.e. evaluation. The lack of knowledge on certain agronomic characteristics has made some accessions unusable. LAMP has made it possible to gain some knowledge of certain agronomic characteristics, especially yield.

Germplasm enhancement

US germplasm enhancement project

A cooperative project between 21 private companies and 19 universities was started in 1994 to adapt and enhance the selected accessions from LAMP. Most of the germplasm was unadapted and required enhancement over a long period through conversion and selective adaptation by corn breeders at numerous environments throughout the major corn-growing regions of the USA. The total process was too large and long-term for public or private institutions to accomplish individually, so necessitated a joint effort by several collaborators. Throughout years of investigations, seed companies have developed inbred lines and hybrids that have demonstrated an increase in maize productivity. Possibly the most productive maize germplasm in the world is now found in these lines and hybrids. For the LAMP material to be more useful, it was important for the accessions to be crossed with commercial-level proprietary inbred lines. The companies have since made crosses of the LAMP material with their proprietary inbred lines. Their collaboration was enhanced by providing in-kind support to allow the necessary replication, nurseries, winter nurseries and environments for selective adaptation.

Having been crossed with proprietary inbred lines, this summer the accessions will be crossed to a second inbred line of another company in order to develop 75% temperate material for higher yield potential, improved agronomic characteristics, and the added adaptability needed for further breeding. This is a unique case of collaboration in which 19 public entities and 21 private seed companies are participating with the objective of increasing the productivity and genetic diversity of maize grown in the USA. Contributions to the project by private seed companies are very significant, and include the making of crosses of LAMP germplasm with elite proprietary inbreds, exchanging complex crosses that

include proprietary germplasm, and evaluating the hybrids involving the newly derived lines.

Molecular techniques for plant genetic resources

Pioneer, in collaboration with members of private industry and the public sector, is developing a set of primers that will allow amplification of microsatellite or simple sequence repeat (SSR) molecular markers for maize The goal is to have 60–100 SSR loci which can provide the next generation of characterizing germplasm and which will be much more cost-effective to use than RFLPs. We have already placed 50 SSRs in the public domain and researchers at CIMMYT and the Plant Introduction Station at Ames, Iowa are beginning to use them.

Managerial tools for seed regeneration

Mark P. Widrlechner

Introduction

Any discussion of current practices and past experiences in seed regeneration for plant germplasm conservation should begin with a reflection on institutional contexts. While we may share many goals in the preservation of plant biodiversity, the practices and experiences brought to this conference are shaped by our diverse institutional missions, cultures and goals. Any recommendation that I might make here today and any consensus reached in our meeting should be viewed as friendly advice to be adapted to each institution's own mission and overall goals. For example, programmes with missions highly focused in support of specific crop improvement projects may rightly view my comments regarding relations with a broader user community as only marginally relevant.

Users and demand

As we examine factors to be considered when planning regeneration schemes and the resources that might be mobilized to overcome constraints on the successful regeneration of germplasm, let us first consider the potential and actual roles played by the germplasm user community. Users play critical roles as advisers to, and advocates for, *ex situ* germplasm conservation and as the drivers of demand for our collections.

For many crops, there is a large body of expertise on plant culture and protection, genetics, systematics, breeding biology, seed production, and utilization. This expertise is multidisciplinary and is diffused among many researchers, who individually may or may not be aware of pertinent germplasm collections. Some researchers with long-standing knowledge of collections and curators regularly request germplasm from our institutions; many others have less contact with, or understanding of, our institutions; whereas still others are totally ignorant of our collections or how well documented and evaluated germplasm can contribute to their research.

When curators plan regeneration programmes and confront the physical, financial, and political constraints that may impede such plans, they should be able to bring to bear the combined expertise and influence of researchers and other users. By developing a network of 40 commodity-oriented Crop Germplasm Committees, the US National Plant Germplasm System (NPGS) has organized a valuable mechanism for convening teams of experts to advise curators on a broad range of managerial issues (Anonymous 1992), including aspects of seed regeneration. Well-crafted surveys of potential and actual germplasm users (McFerson *et al.* 1996) can also provide advice for curators when such expert committees are not easily assembled. And, finally, curators may benefit by publicizing their work to those likely to be ignorant of germplasm collections and their significance.

Building strong and mutually beneficial relationships with the broadest possible range of germplasm users will help ensure the long-term success of *ex situ* conservation. Should resources or national priorities shift away from one discipline towards another, it would be wise for germplasm managers to remain flexible in meeting the needs of all pertinent users. To do so, managers of national and international germplasm programmes should be very interested in a disciplinary analysis of users and trends over time.

Such a disciplinary analysis fits in well with a more comprehensive analysis of demand. Demand is a key criterion for setting regeneration priorities and deserves close scrutiny. A germplasm collection’s value is entwined with its present and future uses. To maximize value, regeneration must be adequate for both long-term conservation and to meet users’ requests.

Managerial decisions regarding regeneration can occur *ad hoc* in response to unmet requests or, preferably, through more systematic long-term demand analyses (Bretting and Widrlechner 1994, 1995). An effective demand analysis should consider patterns of demand

by taxon, accession and end use, ideally by examining a period, perhaps 5 or more years, long enough to temper short-term fluctuations. Unmet requests should also be documented and quantified. From these analyses, the quantity of seed needed to meet past demand can be calculated, and this quantity can serve as one predictor of future demand.

Other factors to be considered for projecting future demand include:

- an awareness of new threats to crop production, such as recently discovered virulent pathogens or insect pests;
- a realization that, as our collections are better characterized and more thoroughly evaluated and as curators learn more about them, requests should become more highly focused;
- an evaluation of the role that core collections or other special subsets may have in directing and managing demand;
- informed forecasts of upcoming changes in germplasm use, such as developments in new crops, large-scale germplasm evaluation programmes, impending retirements of plant breeders, curators, or other significant users, and shifts in national disciplinary priorities.

Setting priorities for germplasm regeneration

Although projections of future demand should guide plans for germplasm regeneration, there are inherent risks in trying to plan for an uncertain future (Bretting and Widrlechner 1994). Other factors must also be weighed. For example, those accessions that help maximize available genetic diversity may receive high priority. For collections containing core subsets carefully chosen to maximize genetic diversity [see Schoen and Brown (1993) for a discussion of strategies and Erskine and Muehlbauer (1991) and Tohme *et al.* (1995) for two examples], priority can be given to core accessions. Or, if those accessions have already been regenerated, others with novel genotypes or adaptations may be placed first in the regeneration queue. In collections organized by genus or family, diversity might be maximized by regenerating those species or genera most divergent from taxa presently available for distribution.

Another approach, somewhat different from maximizing genetic diversity within a collection, is to maximize the degree to which collections at various institutions are unique. Genebank holdings for many crops are extensively duplicated among institutions (Williams 1989). If duplicated accessions are readily available from other sources, perhaps they should receive lower priority for regeneration. Between the issues of outright duplication and genetic uniqueness lies a middle ground of institutional overlap in the historical, cultural and geographical aspects of germplasm and its associated information. We should recognize that germplasm is more than just genes or gene products. Cultivated germplasm has a human cultural context and, especially with traditional societies, so may many wild species. Finally, should germplasm accessions with more complete or accurate passport, characterization and/or evaluation data be given priority for regeneration over those samples with lower quality documentation? All other factors being equal, I would answer 'yes'.

One of the most common challenges faced by curators was noted by Deputy Director Iwanaga in his invitation letter to us: "Two key factors that determine the frequency of regeneration are the viability of the accession and quantity of seed held. Which factor predominates when deciding to regenerate the accessions in your genebank?". A small, unscientific poll of curators at five NPGS sites produced three replies that viability and quantity are equally important in a decision to regenerate. In contrast, another response suggested that viability would be the driving factor when low, but that otherwise quantity would be the key factor. From a very different perspective, a curator of genetic stocks indicated that more compelling than either quantity or viability was that regeneration should occur so that the curator "can observe the mutant traits, otherwise there would be no institutional memory as to how a particular trait behaves".

I believe that there is no single best answer to Dr Iwanaga's question. Rather, the optimal solution will vary according to the characteristics of the particular accessions managed. Breese (1989) reviewed many of the factors influencing the development of optimal solutions. For example, for crops with highly heterogeneous accessions (often the case with allogamous species), quantity becomes more important, both because of statistical sampling concerns and the need to conserve sufficient numbers of cross-compatible individuals. For crops in which seed deterioration is relatively rapid, unpredictable or difficult to monitor, viability is more important. When the two factors are considered equally important, it may be useful for management purposes to express seed quantities on a live-seed basis, but I know of no NPGS site that has adopted this approach.

One recurring problem for setting regeneration priorities for original samples by quantity and viability is that original samples are often so small that seeds cannot be sacrificed for viability tests. If viability tests are conducted and the resulting germination-test seedlings serve as plants for regeneration, then there is probably no prioritization. For such cases, non-destructive testing of small seedlots is a crucial topic for future research.

To end this overview of ways to set regeneration priorities, we must consider the challenges created by dynamic constraints and technologies. Curators must weigh the probability of successful regeneration under current protocols against the probable outcomes resulting from new regeneration technologies or by future access to controlled environments or other more optimal growing sites (either *ex situ* or through coordinated *in situ* conservation efforts). No curator should attempt regeneration when the probability of outright failure or drastic selective change is high, if better protocols can be followed in the near future and the seeds are viable and well stored. The success rates of current protocols should be monitored frequently and new protocols compared by their relative success rates standardized by input costs.

Refining regeneration protocols

I will now examine the development of new regeneration protocols, citing examples gleaned from my experiences as Horticulturist at the North Central Regional Plant Introduction Station (NCRPIS). These examples fit into three general areas of applied research: insect pollinators, high-density pot culture, and mating-scheme evaluation, and a fourth area just now emerging: geographic information systems (GIS). Our experiences in developing and refining protocols can be applied to many crops and generally rely on widely available technologies.

Our site focuses on seed regeneration of allogamous crops and their wild relatives, and consequently most of our accessions are highly heterogeneous and heterozygous. Conserving the genetic diversity within such accessions presents challenges more complex than for homogeneous germplasm.

During the late 1970s, the NCRPIS developed a regeneration system primarily for vegetable crops, employing screened cages with specially designed small hives of honey bees (Ellis *et al.* 1981). We later constructed larger cages to accommodate wild *Helianthus.* In addition to reducing net cost per regenerated seed relative to those produced by hand pollination, the cages protect *Cucumis* from beetle-transmitted bacterial wilt. We have tested the system's ability to restrict gene flow (Wilson 1989); compared seeds produced by various races of honey bees (Wilson and Collison 1988); and documented improvements in regeneration quantity and quality (Wilson *et al.* 1991; Widrlechner *et al.* 1992).

From modest beginnings, the insect-cage regeneration programme has expanded to its present size of about 800 cages per year. During this expansion, we developed expertise in beekeeping, with particular emphasis on increasing our self-sufficiency in maintaining honey bee colonies.

In recent years, we have located so many hives on our research farm that local nectar and pollen resources cannot maintain the hives, necessitating labour-intensive artificial feeding and off-site bee yards. This has given an impetus to a small research project on plants native to our region that produce large quantities of nectar (Ayers and Widrlechner 1994). Beyond

the inadequacy of local bee forage, other very important limitations to honey bee survival in field cages should be noted. Honey bees are social insects, with more than 5000 worker bees needed for ongoing colony maintenance. This number is much greater than that needed to effect pollination among the 100 or fewer plants in a cage. In addition, there are many plants with floral morphologies more suited for pollination by insects other than honey bees.

For all these reasons, we are testing other insects, such as flies, bumble bees and solitary bees, as pollinators in cages. In some cases these may be used in combination with honey bees (Wilson *et al.* 1991); in others, they may be more efficient substitutes for honey bees (Wilson and Roath 1992; C. Abel, personal communication).

The NCRPIS location at 42°N latitude, in a region with a continental climate, greatly reduces success rates for field regeneration of plants requiring a photoperiod shorter than 12.5 h to induce flowering. Accordingly, we cooperate with a low latitude site in Puerto Rico (18°N) to regenerate short-day maize. For short-day amaranths, we have instead developed a protocol for cultivating large populations at high density in containers under plastic tents in a greenhouse during the short days of winter (Brenner 1993; Williams and Brenner 1995). The advantages of pot culture in germplasm regeneration and evaluation are often overlooked (Spoor and Simmonds 1993). We are now testing this protocol's applicability to small, rapidly flowering plants with autogamous or mixed mating systems. This sort of greenhouse regeneration programme can facilitate more complete seasonal use of structures primarily designed for other purposes, such as starting seedlings for field plots or conducting experiments under longer photoperiods.

Many of the maize accessions that can be regenerated under our field conditions are heterogeneous landraces that require large populations and well-designed mating schemes for hand pollination. Various mating schemes have been proposed and their genetic consequences theoretically tested (Crossa *et al.* 1994). A doctoral candidate at Iowa State University is now deploying isozymes to track changes in gene frequency and population structure in maize accessions after they have been subjected to various mating schemes. When combined with practical information on time and labour investments, we should be able to apply his results to determine the most cost-effective protocols for conserving diversity in maize landraces.

GIS are rapidly gaining prominence as tools to manipulate complex, site-specific data sets. Wild plants, weeds and landraces all have evolved in response to ecogeographic variables, and such accessions can be linked to pertinent environmental data through GIS. Evaluation data from modern varieties are also collected under well characterized environments at defined locations. Some applications of GIS for refining plant exploration and increasing the potential value of collections have recently been outlined by Guarino (1995). Knowledge about the climatic and edaphic determinants of plant performance can also refine targets for future exploration (Widrlechner 1994) and help match germplasm more appropriately to geographically diverse users (Pollak and Corbett 1993). Perhaps curators will soon use GIS to develop models for coordinating field regenerations among multiple locations in national or international networks. Earlier this year, the NPGS formed an *ad hoc* committee to examine how GIS could assist germplasm managers and to design prototype applications. Applications of GIS to regeneration management will probably be unimportant until a higher proportion of verified accession locality data are incorporated into our national database, the Germplasm Resources Information Network (GRIN).

Refining post-harvest seed management protocols

The timing of harvest, the interval between harvest and storage, and the methods for cleaning and preparing seeds for storage can all influence seed quality and longevity. Protocols for seed drying and vigour testing are widely studied within the discipline of seed science. But it is my impression that these studies generally examine the seeds of modern commercial varieties of the world's major crops. The seeds with orthodox storage characteristics that present the greatest managerial difficulties are often those that have

received the least attention in seed science research. Heterogeneous landraces and semi-domesticated taxa pose special impediments for seed science research and for developing post-harvest protocols that produce high-quality samples without decreasing genetic variation. Landraces may vary widely within populations for seed size, shape, density and dormancy characteristics. Seeds of wild taxa may be even more problematic. At the NCRPIS, we curate genera, such as *Chamaebatiaria*, *Holodiscus* and *Spiraea* (Rosaceae), *Jamesia* (Saxifragaceae), and *Tridens* (Poaceae), in which the visual recognition of individual seeds can be very difficult even under 10× magnification, and other genera in the Lamiaceae and Caryophyllaceae with seeds so small that they pass through our finest seed-cleaning screens.

These limitations to basic research and more applied post-harvest protocols also apply to published seed-testing standards, which are often based on experiments with commercial seedlots. The *Handbook of Seed Technology for Genebanks* (Ellis *et al.* 1985), a rich assemblage of data and general advice, presents strategies for both post-harvest handling and viability testing. But for many taxa, Ellis *et al.* (1985) rely heavily on national and international standards and present perhaps too little information or advice on ways to cope with variability within and among accessions.

Crop-specific curators and some critical managerial issues they face

Many of the accomplishments of the NCRPIS result from actions begun about 15 years ago by Dr Raymond Clark, at that time the Station's Research Leader/Coordinator, with the support of the NC-7 Regional Technical Advisory Committee, to develop a team of crop-specific curators. Today our team includes six full-time curators and myself, with part-time curatorial responsibility for certain ornamental genera, collectively comprising 52 years of curatorial experience. We organize curatorial responsibilities by genus grouped into crop categories, such as vegetables, pseudocereals and forage legumes. This is consistent with a national system that divides responsibility taxonomically among sites and receives advice from a network of 40 Crop Germplasm Committees organized by a combination of end-use and taxonomic groupings. In this way, the subtleties of diversity within particular crops and their user communities can be learned and harnessed to produce better seeds and to meet users' needs. Without a crop-specific focus, it is difficult to imagine how this plethora of information could be organized or how managers could develop a high degree of specialized expertise, especially related to the intricacies of regeneration.

I believe that regenerations, post-harvest processing, and initial viability testing should be entrusted to crop-specific curators who, with experience, are best qualified to recognise differences among accessions and to work with other experts to develop or refine suitable protocols. Crop-specific curators removed from day-to-day regeneration management would tend to have less understanding of practical constraints. And conversely, regeneration experts without a crop focus would be unlikely to relate their experiences to patterns of genetic variation or adaptation within taxa or to communicate as effectively with the user community. Ideally, networks of crop-specific curators should form to foster rapid and frequent exchange of curatorial observations and strategies. Perhaps they could be organized like the working groups of the European Cooperative Programme for Crop Genetic Resources Networks, or more informal groups linked by the Internet.

Finally, I will conclude by briefly mentioning some critical research areas directly related to the above remarks, along with a few other issues raised by my NPGS colleagues. Germplasm demand and germplasm regeneration should be linked. We know that patterns of demand among collections vary widely and we expect them to be dynamic. But very few analytical tools for assessing demand or projecting future demand have been widely disseminated or empirically tested. Can IPGRI help develop such analytical tools and/or convene working groups of curators and others best able to forecast future trends in plant science research and crop improvement?

Because protocols to balance factors such as seed quantity and quality, or the number of accessions regenerated and population size, are greatly influenced by patterns of genetic diversity, breeding systems, seed longevity, and regeneration conditions, any such protocols

must be crop-specific. It is not likely that much progress can be made on these topics by following general prescriptions, but perhaps, just as IBPGR sponsored the development of descriptor lists, IPGRI might consider similar crop-specific examinations of regeneration issues. As mentioned earlier, related to the development of crop-specific protocols is the need for non-destructive viability testing of small samples.

On so many levels, from breeding biology to seed physiology, lack of information about the inherent characteristics of wild and weedy taxa is reducing the efficacy of regeneration programmes. The potential value of secondary and tertiary gene pools for crop improvement is increasing through developments in genetic transformation, somatic hybridization and other biotechnologies. Thus wild and weedy crop relatives deserve increased attention for basic and applied research into optimal seed propagation.

Biotechnological advances have made many classes of molecular genetic markers increasingly available. Genetic markers are proven tools for documenting trueness to type and other population changes during the course of regeneration (Bretting and Widrlechner 1995). As new classes of markers are characterized and as the relative costs of deploying various markers change, who will translate these developments to the best advantage of curators? Before leaving the subject of biotechnology, I also wonder what its role may be in rescuing samples with low variability and very limited seed amounts, either through regenerating intact plants or by capturing genetic information without direct regeneration.

All of these lines of research will have greater influence if we can work together to foster the discipline of germplasm conservation by educating an expanding corps of crop-specific curators. Ultimately, the investment in curators should produce the highest returns, for it is through their practical experience and scientific judgement that research results can best be applied.

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Sample size and effective population size in seed regeneration of monoecious species

J. Crossa

Introduction

To preserve the genetic variability of genebank collections during seed regeneration, it is important that the sampling of accessions is being done efficiently, and that the population or sample to be used for the regeneration process be of sufficient size to maintain as much genetic diversity as is practicable. Large samples are expensive and difficult to manage, but if the samples are too small, valuable alleles may be lost through random changes in allele frequency (random genetic drift). In regenerating seed, it is important for genebank managers to know the size of a sample of a genebank accession needed to obtain one or more rare alleles with a certain probability, and how this sample will affect the genetic integrity of the accession in terms of changes in allele frequency and inbreeding depression. Maintaining allelic diversity during regeneration depends, among other things, on three main factors (Crossa 1989; Crossa *et al*. 1993):

- sampling procedures
- random genetic drift due to sampling
- seed viability.

This paper aims to:

- examine the practical sample size in the regeneration of seed stocks;
- discuss the effect of random genetic drift and bottlenecks in seed regeneration; and
- describe some practical options to address these issues.

Practical sample size

Using probability theory, models can be derived that address issues which arise in determining the sample size of an accession to be regenerated. Results have shown that the appropriate size depends more on the frequency of the rare allele or alleles than on their number. Making the reasonable assumption that k–1 alleles occur at an identical low frequency of p_0 and that the k^{th} allele occurs at a frequency 1-[(k–1)p_0], Crossa *et al*. (1993) showed that for loci with two, three or four alleles, each with p_0= 0.05, 89–110 individuals are required if at least one allele at each of 10 loci is to be retained with a 90% probability. If 100 loci are involved, 134–155 individuals are required. For two, three or four alleles, 10 loci and p_0=0.003, 150–180 individuals are required; for 100 loci, 225–255 individuals are needed. Sample sizes of 160–210 individuals are required to retain alleles at frequencies of 0.05 in each of 150 loci, with a 90–95% probability.

Assuming two alleles at each of the 20 000 loci and one of them at a 0.05 frequency, 186 individuals will preserve this allele at each loci with a 95% probability. This is similar to the numbers given by Lawrence *et al*. (1995). An extensive computer program was developed by Hernandez and Crossa (1993) for computing the optimum sample size under given numbers of alleles per locus, numbers of loci, probability levels, and allele frequencies.

Random genetic drift and the variance effective population size

Unpredictable changes in allele frequency caused by sampling error in small populations (random genetic drift) leads to a continuous fixation and loss of alleles and reduces the proportion of heterozygous individuals in the populations. These random changes in allele frequency occurring in finite populations subject to sampling error are quantified and predicted using the parameter called ‘effective population size’.

In a large mating population of N individuals, the reduced number of parents whose offspring will constitute the next generation is referred to as the effective population size, *Ne*. Historically, there have been two approaches to quantify *Ne*. The first is related to the inbreeding occurring in a breeding population and is called ‘inbreeding effective population

size' [$Ne_{(i)}$]. The second is concerned with the sampling variance of allelic frequency in a breeding population and is called 'variance effective population size' [$Ne_{(v)}$]. Several factors affect the effective size of a population: number of gametes contributed per individual in the parental population; number of offspring per generation; and number of individuals per generation. The effective population size, taken as a measure of the genetic representativeness of a seed sample, can be adapted to specific aspects of genetic resources preservation such as seed regeneration (Crossa and Vencovsky 1994).

Probability models and computational formulae for the variance in the number of contributed gametes and the variance effective population size in monoecious species have been developed by Crossa and Vencovsky (1994). Four basic alternative sampling procedures of female and male gametes were distinguished by Crossa and Vencovsky for a germination rate of u and a large parental population size (N):

Case 1

Pollination is random and an unequal number of seeds are taken from each pollinated ear. For this case

$$Ne_{(v)} = Nu$$

Case 2

Pollination is random but equal numbers of seeds are taken from each ear, such that

$$Ne_{(v)} = N[4u/(4-u)]$$

Case 3

Pollination is controlled (chain crosses, plant-to-plant crosses) but unequal numbers of seeds are taken from each ear. For this case

$$Ne_{(v)} = N[4u/(4-u)]$$

Case 4

Pollination is controlled and equal numbers of seeds are taken from each pollinated ear. Here

$$Ne_{(v)} = N[2u/(2-u)]$$

In germplasm seed regeneration, where population size is constant due to gametic control, its positive effect is more evident when there is no great loss in germination rate (u = 1). Field pollination procedures, such as plant-to-plant crosses (with or without reciprocals) and chain crossing, and taking equal numbers of seeds from each ear (control of the female gametes), provide

$$Ne_{(v)} = N[2u/(2-u)] = 2N \text{ for } u = 1$$

The bottleneck effect when regenerating small accessions

The bottleneck effect occurs when a small number of individuals are used to produce the next generation. This results in allelic frequencies that differ from those in the original population. Rare alleles occurring at low frequencies have a high probability of being lost as a result of such a bottleneck, although the average heterozygosity is influenced more by the rate of population growth after the bottleneck occurs than by the size of the bottleneck.

Theoretical studies on the effect of the bottlenecks on average heterozygosity and on the loss of alleles have shown that the amount of reduction in heterozygosity per locus depends on (i) the size of the bottleneck, and (ii) the rate of population growth after the founder effect. On the other hand, the loss in the average number of alleles per locus is highly affected by bottleneck size, but not so much by the rate of population growth (Nei *et al*. 1975; Crossa *et al*. 1992).

When the genetic variance of a quantitative trait is controlled by additive gene effect, the bottleneck effect should decrease the variation in proportion $1/Ne$. However, if genetic variability is partly due to non-additive gene effects (dominance and epistasis), the effect of N will not be a simple relationship (Barret and Husband, 1990) in the sense that additive genetic variance might temporarily increase after the bottleneck.

It is common for maize genebanks to have to regenerate accessions of less than 10 ears each. The effective population size is highly dependent on the sex that is less numerous, in this case the females, and the effective population size is four times the number of females, that is, $Ne = 4(10) = 40$. This may represent a severe bottleneck, possibly causing some rare alleles with a frequency of 0.05 or less to be lost. One can expect that, for a certain level of polymorphism, the mean number of alleles in the sample of 10 ears will be about half that of the original population, and the loss of alleles in subsequent cycles of regeneration is reduced if the population grows rapidly. Therefore, it is important in subsequent cycles of regeneration to increase the population size as much as possible (to at least 100 ears), to prevent a large additional reduction in heterozygosity and a further increase in the rate at which alleles are lost.

Practical methods for regenerating maize accessions

An optimal and practical procedure for germplasm regeneration should control the number of pollen plants through controlled hand pollination (plant-to-plant crosses, chain-crosses, etc.), and the number of seed plants by taking equal numbers of seeds from each pollinated ear. Because of limited resources, ideal procedures for seed regeneration may be highly impractical and very costly, requiring extensive land, labour and management resources.

A practical procedure for regenerating maize accessions may be the one described by Crossa *et al*. (1993). Suppose we have 200 maize ears, and two kernels are taken at random from each ear and put into a packet. Repeat this until four or five packets are complete. Plant two packets (of 400 kernels each) in two different field blocks; make 200 plant-to-plant crosses using each plant as a female or male, but not as both. Repeat the operation for the second packet. Ears are harvested from one block and, if necessary, a total of 200 ears are obtained by harvesting additional ears from the other block. For this case $N = 400$ and $Ne = 4N = 1600$ individuals, because the accession is treated as a dioecious species, each plant being used only as male or female, but not both. Other practical alternatives can be created by selecting more than two seeds per ear and considering seed loss due to poor germination (Crossa *et al*. 1993).

Conclusions

For germplasm seed regeneration, a sample size of 130–200 individuals will give a high probability of retaining rare alleles at low frequencies in most of the loci. During regeneration, genetic drift becomes an important factor determining allele loss. While regenerating accessions of small size, a bottleneck effect is inevitable. Therefore, during subsequent regenerations of such accessions, it is important to increase the population as much as possible in order to prevent further loss of alleles. Adequate field procedures for controlling the number of pollen and seed plants, that will increase effective population size (*Ne*), are recommended in this paper.

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Seed quality considerations in germplasm regeneration

N. Kameswara Rao and D.V.S.S.R. Sastry

Introduction

Regeneration of germplasm is one of the most crucial processes in genebank management. It is costly in terms of resources and time, and it involves the risk of genetic drift due to sampling errors and genetic shift due to selection, which are compounded over each regeneration. Many landraces are heterogeneous mixtures of genotypes, and the problems of genetic drift and shift are even greater. Breese (1989) has summarized the various factors that could cause genetic shifts during the course of germplasm regeneration. Genetic shifts can occur in heterogeneous germplasm accessions during germination due to differences in longevity or the degree of dormancy of constituent genotypes; at the seedling stage and during crop growth due to genotypic differences in interaction with soil and climatic factors, susceptibility to diseases and pests, and competition; and at flowering and maturation phases due to differential flowering and maturity. Efficient management of seed germplasm collections therefore entails minimizing the frequency of regeneration. This can be achieved by maximizing seed longevity. It is known that potential longevity of seeds depends on initial quality. Storage conditions determine the extent to which potential longevity can be maximized, therefore the full benefits of any good storage system are not likely to be realized unless the seeds that go into the store have high initial vigour. Several pre- and post-harvest factors, such as crop management, seed production environment, maturity, harvest and drying practices, influence initial vigour and therefore the subsequent longevity of seed lots regenerated from germplasm grow-outs.

Crop management

Optimal cultural conditions, including soil fertility, moisture supply and plant density during crop growth, can affect the initial quality of seeds. Unfortunately most of the studies on crop management practices were focused on yield and nutritional quality and only limited information is available about their effect on seed longevity.

Soil fertility

Nitrogen, phosphorous and potassium in soil play an important role in the development of the plant and in determining seed quality. Deficiencies of the minor elements calcium, boron and manganese are known to produce characteristic damage to seeds, impairing longevity. For example, soyabeans produced in soils with potassium deficiency had lower quality (Crittenden and Svec 1974), while pea seeds harvested from a boron-deficient area produced abnormal seedlings (Leggatt 1948). In groundnut, discoloration of the cotyledons is associated with boron deficiency, while discoloration of the plumule is associated with calcium deficiency (Cox and Reid 1964). The application of gypsum to peanuts at early bloom stage, which is a standard practice, reduces the incidence of pops and unsound kernels. It is therefore important to identify the optimal nutritional requirements of the seed crop and to fertilize the soil accordingly. Apart from fertility, soil pH has significant effect on seed production as observed in the wild species of groundnut (Dr A.K. Singh, personal communication).

Moisture stress

Acute deficiency in moisture supply during seed development and maturation interrupts seed development and usually results in inferior seeds. In pearl millet, seeds harvested from plots subjected to drought stress at the time of flowering had reduced storage longevity compared to controls (Kameswara Rao, unpublished data). In soyabean, moisture stress during the pod-filling stage reduces the size of seeds as well as their germination (Shaw and Laing 1966). In groundnut, drought during pod development leads to severe loss of viability

during storage (Cox *et al*. 1976; Vanangamudi *et al.* 1987). In addition to drought stress, soil temperature also influences maturation rate, thus indirectly affecting seed quality (Sanders *et al*. 1985; Nautiyal *et al*. 1991). Therefore, for any crop, the water management strategy should consider the rainfall probability and seasonal distribution from long-term records; careful selection of planting date to avoid stress at flowering or pod-filling stage; and supplying additional water by irrigation, if necessary.

Plant density

Growing plants at lower density minimizes competition that could lead to selective elimination of genotypes and maximizes the seed output per plant, which is particularly important when small numbers of plants are grown. Plant density also influences the relative humidity of the microclimate within the crop canopy and thus the disease and pest incidence, affecting seed quality. In pigeonpea, seed vigour after accelerated ageing was highest with six plants per metre of row and 0.4 m between rows (Pedroso *et al.* 1988). Chavez and Mendoza (1985) found that seeds from groundnut intercropped with sugarcane had low germination and seedling vigour. The microclimate characterized by high relative humidity contributed to the deterioration in seed quality.

Seed production environment

Climatic conditions

Climatic conditions during seed formation and maturation affect the initial quality and potential longevity of seeds. Geographical areas with low precipitation and low relative humidity during seed ripening and maturation are favourable for seed production (Delouche 1980). The incidence of pests and diseases is usually low and seed quality is good when produced under these conditions. On the other hand, frequent rainfall combined with high temperatures common to tropical and sub-tropical regions are detrimental to the production of quality seeds. Extremes of temperature occurring during maturation, such as hot, dry weather (Tekrony *et al.* 1980) or frost (Moorse *et al.* 1950), can have an adverse effect on seed quality. Early, sustained freezing during seed development was reported to cause serious damage in corn (Delouche 1980). A temperature between 18 and 19°C for 4–5 weeks was found to be most favourable for wheat seed quality (Agrawal 1986).

Germplasm collections contain accessions originating from a wide range of environments, and often the seed production environment at the site of regeneration may not be optimal for all accessions. In rice, for example, the potential longevity of japonica cultivars which originated in temperate regions was found to be less when produced in warmer seed production regimes compared to cooler regimes (Ellis *et al.* 1993; Kameswara Rao and Jackson 1996b). Therefore, it would be ideal to regenerate germplasm in near-optimum locations, to meet the requirements of specific cultivars. In countries such as the USA, China and India, with a sufficiently diverse climate, establishment of seed production in near-optimum areas may not be a problem.

Altering planting dates to allow the critical stages of seed maturation to coincide with the favourable segments of the field environment may prove feasible for improving seed quality to some extent under such circumstances. For example, in the japonicas, seeds of highest quality and longevity were obtained when planted in October in Los Baños, Philippines, as seed ripening coincided with the coolest time of the year (Kameswara Rao and Jackson, unpublished data). In Columbia, USA, soyabeans from late plantings mature after the hot, dry weather, and consequently, have high quality compared to those from early plantings which mature during hot weather (Green *et al*. 1965). Hot weather during seed maturation was reported to result in seed coat wrinkling, green seeds and reduced germination in soyabean (Moorse *et al*. 1950; Costa 1979). In regions such as West Africa, with bimodal rainfall, seed quality was better in soyabean when planted in the minor season (September–November) compared to those planted in the major season (April–August) (Mercer-Quarshie and Nsowah 1975; Nangju 1977). In the major season, seed ripens during rains, thus the quality suffers greatly due to weathering. The photoperiodic conditions during growth may

also affect seed morphology, for example seed coat thickness, and consequently the physiological processes occurring during the early stages of germination (Gutterman 1973; Puri and Hardman 1976). Light intensity also affects pollination as well as ripening and drying of seeds. Similarly, strong winds are unfavourable for seed production as they cause lodging of plants and shattering of seeds.

Microenvironment

Microclimate, influenced by plant density and the pollination control mechanisms such as caging and bagging, can have significant effects on seed quality. In pearl millet, seeds regenerated by cluster bagging deteriorate faster than open-pollinated or selfed seedlots (Kameswara Rao, unpublished data). In cluster bagging, emerging spikes from three to four plants are enclosed in a paper bag for about 4–6 weeks, and the microclimate within the bags characterized by high humidity could lead to loss in quality of the seeds. High rates of nitrogen fertilizer, irrigation, narrow spacing and other practices which contribute to a dense canopy and high humidity within the canopy increase the degree of deterioration, as shown for cotton (Caldwell 1972). In soyabean, seeds on plants that were shaded to remove 50% of the incident sunlight deteriorated at a much slower rate than seeds from unshaded plots, because of the more stable microenvironment around the shaded plants (Mondragon and Potts 1974).

Diseases and insects associated with particular environments become part of the total climate pattern and may cause severe damage to developing seeds, especially in tropical and subtropical climates with high rainfall and temperature. Microorganisms, especially field fungi, invade the seeds during or after ripening and during harvesting operations (Christensen 1972) and cause weakening and death of ovules and embryos, seed discoloration and shrivelling of seeds. Seedborne pathogenic bacteria and viruses have not been studied for their effects on seed quality despite their significant impact on yield. Insects, besides providing openings for subsequent invasion of pathogens and moisture, also transmit diseases directly.

Seed maturity

Immature seeds are known to be inferior to mature seeds in vigour and viability. On the other hand, harvest delays beyond optimum maturity contribute to weathering and loss in quality. Therefore, timely harvest is extremely important in order to obtain seeds of high quality with maximum potential longevity. It has been generally recognized that the maximum quality is realized at physiological maturity, defined as the stage at which seeds attain maximum dry weight and full germination capacity (Shaw and Loomis 1950; Harrington 1972). Although it appears simple for individual seeds, many plants have an indeterminate flowering pattern, and seeds with varying degrees of maturity and therefore of different storage potential occur on the same plant. In such cases, the problem of harvesting seeds with uniform ripeness can be effectively met by tagging and harvesting heads individually. Although seeds attain maximum vigour and viability at physiological maturity, the seed moisture content will remain usually high (32–35% in cereals and 50–55% in legumes) and make harvesting and threshing difficult without mechanical injuries. Also, seeds are likely to suffer desiccation injury during drying for subsequent storage. Therefore, seeds are generally allowed to dry until they reach harvest maturity, i.e. the moisture content at which they can be effectively harvested and threshed mechanically with the least amount of damage. There is growing evidence which suggests that developing seeds attain maximum potential longevity after the end of the grain-filling period, now defined as mass maturity (Ellis and Pieta Filho 1992). Preliminary studies on pearl millet and sorghum, and evidence from a wide range of other crops including rice, wheat, barley and soyabean, indicate that seeds attain maximum potential longevity some 1–2 weeks after physiological maturity or the end of the grain-filling period (Kameswara Rao and Jackson 1996a). The stage during seed development at which potential longevity is maximum is now defined as storage maturity.

Harvesting and drying practices

Drying

In semi-arid and arid climates, where the ambient relative humidity of the atmosphere is low, maturation drying reduces the seed moisture content to reasonably low levels (12–15%). Over-drying, however, can cause problems such as cracks and rupture of testa during threshing and handling, as seen in many legumes, particularly chickpea and soyabean. Therefore seeds must be harvested and threshed before their moisture content becomes too low. Critical damage was reported to be the least when seeds were harvested at 16–18% moisture in many crops, including corn, soyabean, small grains and groundnut. In tropical and subtropical environments where ambient relative humidity is high, some post-harvest drying becomes necessary. Sun-drying and/or systems based on forced ventilation with heated air are generally used to reduce moisture content. However, there is likely to be some detrimental effect on overall seed quality with these methods. For long-term conservation, as required for germplasm accessions, it is recommended that seeds be dried at low temperature (15°C) and relative humidity (15%) to avoid any adverse affects of drying on the initial quality and subsequent longevity (Chromarty *et al*. 1982). However, unless carefully regulated, drying to such low moisture contents can sometimes cause problems such as cleavage damage, as observed in soyabean (Chromarty *et al*. 1982) and in chickpeas with round seeds (Kameswara Rao *et al*. 1990).

Mechanical damage

Harvesting method, particularly with respect to mechanical damage to seed, is an important determinant of seed quality. Mechanical injuries predispose the seeds to microbial attack which could accelerate their deterioration when stored under poor conditions. For many crops, the quality of seeds obtained by hand threshing was reported to be superior as compared to mechanical threshing. Some factors responsible for mechanical injuries to seeds during threshing are: seed size, resilience, friction, comprehensive strength, and rupture strength of seed in relation to seed moisture content, internal friction and specific weight. Perl and Luria (1978) observed that even slight injuries can promote infection, abnormal seedlings, and loss of viability and field emergence capacity. Usually seeds with hairline cracks and other such microscopic damage escape separation based on size and shape criteria during seed processing and affect the overall quality of seed lots.

Seeds vary widely with reference to both the extent and intensity of damage and for different reasons. Seeds that are spherical in shape are better protected against mechanical injuries than seeds that are elongated or irregularly shaped (Moore 1972). Large-seeded legumes are particularly susceptible to injuries that reduce viability. In sorghum and maize, the lower portion of the germ extends beyond the general outline of the endosperm, and as a result the radicles are often damaged. The natural protrusion of the tip of the radicle in groundnut and chickpea seeds promotes root injuries which lead to accelerated deterioration and loss of viability. The moisture content of individual seeds at the time of harvest also causes wide differences in the extent and seriousness of injuries: for example, mechanical impacts can be destructive to cell membranes under drying stress. Bunch (1960) reported that corn shelled at 14% kernel moisture encountered less damage than shelling at 10% moisture content. At 4–5% kernel moisture content, the damage was nearly 100%. Clearly, the cylinder speed and clearance between the beater bars and cylinder drum need to be regulated depending on the moisture level and seed size of the accession.

Conclusions

Seeds are a product of the seed production environment as well as the genetic constitution of the parent plants. The complex of environmental conditions, including soil and climate, frequently override the expression of genetic characters, causing the seed to exhibit additional traits which reduce seed quality. Therefore, to improve seed quality, germplasm regeneration programmes should stress improved management and production practices. Seeds of high quality can be obtained by planting in suitable areas at appropriate times

under optimal conditions, following proper harvesting and drying techniques and careful handling and processing, and ensuring minimum deterioration before reaching appropriate storage conditions. The time from harvest to storage is critical: seeds should be processed as quickly as possible, and until such time they should be stored under conditions which minimize pre-storage deterioration. The longer the interval, the greater the risk of infestation. It is well known that good storage conditions can only delay seed deterioration, but cannot stop the process altogether. Therefore, in order to experience the full benefits of any storage system, the quality of the seeds entering the store should be of the highest. Although optimum seed production practices for high quality seeds were listed for a number of important food crops, these broad general outlines are more apt for commercial seed lots where, unlike germplasm collections, the variation in morphoagronomic characters for a given species is limited, and at the same time it is difficult to evolve recommendations for individual accessions. Nevertheless, accessions can be grouped based on geographic origin, maturity, seed size / mass, etc., and their response to various pre- and post-harvest factors that influence seed quality can be studied in order to develop general guidelines for the production of good quality seeds with maximum potential longevity, in order to minimize the frequency of germplasm regenerations.

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Introduction to the Consultation

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The title of the meeting, Consultation Meeting on the Regeneration of Germplasm of Seed Crops and their Wild Relatives, is self explanatory. However, below I will provide the background to information, explain the objectives and point out possible outputs.

Background

There is a general agreement that the maintenance of the genetic diversity and integrity of the material in *ex situ* collections is a very important aspect of conservation of plant genetic resources. At the same time, it is also recognised that regeneration of accessions, although a vital aspect of genebank-related activities, has not received the attention it deserves. The basic theoretical framework for seed regeneration is based on the results of plant breeding experiments dealing with mixtures and composites and with fairly large populations which are not, strictly speaking, equivalent to much smaller 'populations' that most of the genebank managers deal with. Information which is basic to the development of appropriate procedures for seed germplasm regeneration, such as reproductive biology, breeding systems, and the structure and distribution of the genetic diversity of the material to be conserved, is lacking for many crop genepools.

Additionally, the causes of genetic drift, genetic shift and mutation during the regeneration process need to be identified, their effects on the integrity of the sample quantified, and methods developed to mitigate their effects. Also, the effect of the presence of seedborne pathogens on the maintenance of genetic integrity of *ex situ* collections needs further investigation. In the case of cross-pollinated species, questions remain on what may be the most effective isolation techniques, pollination control methods and mating techniques.

The effects of genetic shift (due to selection pressures) and drift (random loss of alleles due to sampling) mount with each regeneration cycle, and therefore the frequency of regeneration has to be kept to a minimum. Procedures are needed to manage and carry out regeneration which optimize the maintenance of the genetic integrity of the accessions conserved. Such procedures need to be practical and economic, in particular to aid the national genetic resources programmes. However, the methods and management of regeneration will depend on

- the breeding system and genetic structure of the population, and
- the environment and circumstances of the place of regeneration.

Rather than attempting to produce crop- or species-specific guidelines, it may be important to develop a general decision guide to seed germplasm regeneration that will provide genebank staff with different options that are scientifically sound and cost-effective for the species with which they deal and in their specific situations.

These options should also provide information as to the genetic fate of the material. Much knowledge and experience is available among the genebank and plant breeding communities, but it needs to be gathered, synthesized and shared. Hence one of the underlying objectives of this Consultation meeting is sharing of experiences among the participants, and continued interaction between them after the Consultation meeting may provide a sort of low-level network through correspondence and shared activities that will greatly assist germplasm regeneration work in the genebanks around the world.

Objectives of the meeting

1. Review the theoretical basis and current practices of seed germplasm regeneration, and identify critical problems and possible solutions.

An attempt will be made by the experts present at the Consultation to think through some of the theoretical aspects which form the basis for germplasm seed regeneration. These are largely based on quantitative genetical theories of breeding populations as well as on the neutral allele theory. This is expected to help to identify critical problems, constraints and possible solutions, and ideas for future work to unravel some problems.

2. Identify the critical criteria in genebank manager/curator decisions on implementing regeneration procedures that are both scientifically sound and cost-effective for the respective species and under special circumstances, and develop a general guide to decision-making in seed germplasm regeneration as an aid to genebank staff.

   The other major objective – probably the more interesting one – is one that is more applied, to identify the critical areas that pose a problem in the practice of germplasm seed regeneration. We need cost-effective but scientifically sound procedures to regenerate germplasm. There appears to be a tendency to follow the easiest method, without giving much thought to the genetic consequences of the actions taken in a genebank. At the end of the day, we are expected to maintain the genetic diversity in the material collected and conserved. We need to think more imaginatively and make genebank work more innovative (to eliminate the word routine from genebank-related activities). It is expected that, at the end of the meeting, we should be able to put together a decision guide, a guide that basically provides information on what happens to an accession when a particular method is followed to regenerate it.

3. Identify aspects that need further information and/or the improvement of regeneration procedures, and identify the opportunities to gather the necessary information and/or carry out the research.

   There are clearly a number of areas bothering us about germplasm regeneration. These areas need further investigation, so that what the genebank curators do while regenerating germplasm seed becomes scientifically sound. We also need to investigate how we can best regenerate an accession and at the same time keep the cost of regeneration low enough to be affordable by most national programmes and genebanks. We need to remember that while costs are important, our efforts are also aimed at safeguarding the investment that has already been made – an investment of time and money that has gone into collecting and assembling a vast number of germplasm accessions.

4. Consider the current regeneration needs of existing *ex situ* seed collections and recommend appropriate measures, including the use of collaborative mechanisms and opportunities for regeneration.

   There have been several estimates of the number of accessions that need urgent regeneration (not multiplication) in genebanks around the world. One of the estimates arrives at a number around 1 million, and the funds required will depend on the cost per accession in any given area and the species under consideration. Where will the funding come from for such an enormous task? Even if funds are available, are the available methods good enough to make sure that the funds are spent wisely?

The only way that we can meet these demands is through collaboration and cooperation. We need to identify the opportunities that combine use and regeneration effectively and work closely so that the germplasm is effectively conserved and efficiently used.

Outputs

Firstly, an exchange of information and ideas on germplasm regeneration. This is expected to lead to either formal or informal linkages among the participants, with or without the involvement of IPGRI.

Secondly, a framework for seed germplasm regeneration guidelines will be developed. Such a decision guide should bring together scientific/genetic principles and practical applications,

and be easily followed by the technicians who operate on a day-to-day basis in a genebank. It should provide information on the options available and their genetic and practical implications. The decision guide should be able to provide guidance on the decisions and experiments that genebank managers must make or conduct to regenerate germplasm in a scientifically sound and cost-effective manner. Knowledge of what happens to the material conserved if one follows certain procedure(s) is the most important aspect of such a decision guide. This will allow genebank managers to make appropriate choices based on the skills and resources available to them, and be aware of the consequences (mainly genetic) of their actions. An element of risk is always there, but at least the proposed decision guide will indicate the level of risk and will assist genebank managers to make well informed decisions.

The third expected output is to develop some sort of action plan. Since genebank managers, geneticists and plant breeders are present at this Consultation meeting, we can expect a research agenda to address key issues and gaps in knowledge on regeneration procedures that are critical to issues discussed. Such procedures will assist most of us to better conserve the germplasm. While developing the agenda, it may be possible to agree on some of the activities to be carried out on a collaborative basis.

Lastly, we hope that some suggestions for enhanced strategies and collaborative actions to address the seed regeneration needs of current *ex situ* collections of crop plant genetic resources will be forthcoming. This assumes importance in the context of the proposed IV International Technical Conference on Plant Genetic Resources in Food and Agriculture that FAO has planned for June 1996. The International Conference and Programme on Plant Genetic Resources (ICPPGR) has been in touch with a number of experts and national programmes to ascertain regeneration needs as well as costs involved. Within the activities related to ICPPGR, there may be a scope for developing programmes for assistance to carry out regeneration of material that is most urgent.

Framework for the management and regeneration of seed germplasm collections

Reports of the Working Groups

With the overall aim of maintaining the genetic integrity of accessions during *ex situ* conservation, as close to original as possible and in the most-cost effective manner, the Consultation Meeting decided to form three Working Groups with the following topics:

1. Minimize the regeneration requirement of the collection
2. Minimize the regeneration frequency of accessions in the collection
3. Conduct cost-effective regeneration of the accessions

Each of the Working Groups reported the outcome of their deliberations to the plenary, and summaries are presented below.

Working Group 1. Minimize the regeneration requirement of the collection

Objective

Control the size of the collection. Aspects such as the inclusion of accessions into the collection and minimization of redundancy should be considered.

Requirements

- A well defined, established policy on the acquisition of accessions (collecting/introduction).
- A well defined, established policy on the continuing conservation of accessions within the collection, including limiting the redundancy.

These operational policies will depend on national conservation priorities and policies, including national policy and strategy for inter-sectorial and inter-institutional collaboration within the country on the conservation of genetic resources (GR) and for international collaboration in GR.

1.1 Considerations in establishing national conservation priorities and policies.

- Conservation of germplasm that is unique to the country is required under international agreements such as the Convention. In addition, priority should be given to germplasm that has importance for meeting national development needs.
- Coordination among government, non-government and private institutions within-country to share efforts and expertise on germplasm conservation.
- Conservation strategies that integrate *in situ* and the different available *ex situ* methods in a complementary and cost-effective manner.
- Coordination at regional and global levels to ensure rational, cost-effective conservation, and access to and the exchange of germplasm. Organization of safety duplication of collections; sharing of facilities for long-term conservation.
- Species or species-group focus on the conservation of genetic resources to ensure the involvement of species experts. Training activities should be on a crop-specific basis.

1.2 Considerations in defining and establishing operational policies on the acquisition of accessions into the collection.

- Acquisition rate should be matched with the conservation capacity (physical facilities, human and financial resources and seasonal work capacities) of the genebank, including the capacity to carry out regeneration.

- Obligatory versus optional initial regeneration: when initial seed quality is high, priority should be given to storing the accession directly in a base collection (as long as sample quantity meets base collection requirements) in order to maintain the original sample (genetic integrity) for as long as possible. Some genebanks have a policy of obligatory initial regeneration in order to control initial seed quality and to grow out the accession for verification of species and accession against passport data, and undertake their own characterization.

  Constraint: the difficulty in ascertaining initial seed quality. Seed ageing may not be apparent from initial germination tests.

1.3 Considerations specific to operational policies on:

1.3.1 Collecting

- Attention to germplasm that is unique to the country, which is under threat or has special features of interest to national plant improvement / agricultural development plans.
- Utilize procedures that maximize initial seed quality and optimize initial seed quantity in order to meet requirements for division and storage of the sample without need for an initial regeneration.

  Constraints: lack of knowledge on the factors effecting initial seed quality and on procedures to maximize quality at time of collecting and during handling prior to storage; and lack of information about the breeding systems and patterns of genetic diversity in species that guide decisions on sample size to collect and store.
- International collecting: undertake an initial regeneration in collaboration with the country of origin, preferably within the country of origin, in order to avoid splitting original samples that will then need independent regeneration and thus incurring greater expense and greater risk to accession genetic integrity at two locations, one of which may not be optimal for growth of the species. Collaboration on initial regeneration also provides an opportunity for training and cooperative characterization of the germplasm.

1.3.2 Introduction

- Special attention should be given to germplasm important for national needs or for meeting international obligations.
- Utilize existing, or establish, mechanisms allowing access to accessions stored elsewhere as needed, rather than maintaining a duplicate. Participate in networks and foster multilateral and bilateral agreements for access to germplasm.
  Constraints: depends on national policies and global access arrangements.
- Compare passport records (including information on accession history and, where available, characterization and evaluation data) of new to-be-added accessions with existing acquisitions, to avoid introducing duplicate accessions.
  Constraint: the difficulties of identifying true duplicates.
- Record complete passport data: obtain all available identifiers (collector's number, donor accession number / s) and information on the history of the accession.

  Constraint: information that enables curators to make cost-effective decisions on whether to keep the introduction, conserve it as base and / or active collection, and what sample sizes to store and use for regeneration, usually does not accompany introductions and currently is rarely part of collection / genebank management documentation systems.

1.4 Considerations in defining and establishing operational policies on conserving accessions in the collection

1.4.1 Rationalization of collections – avoiding/eliminating redundant accessions. Examination of available information on the accession (passport, characterization and evaluation data; accession history) and further morphological and molecular characterization to identify duplicates. Elimination, bulking or grouping of duplicates. Opportunities to rationalize among collections within the framework of networks and cooperative programmes.

Constraints: lack of information on the history of the accession; difficulty of determining duplicates.

1.4.2 Conservation as base, active, working or duplicate collection. The base collection is defined as a set of accessions, each of which should be distinct and, in terms of genetic integrity, as close as possible to the sample provided originally, which is preserved for the long-term future and from which no seeds are taken for distribution (FAO–IPGRI 1994). The active collection comprises accessions which are (immediately) available for multiplication and distribution for use (FAO–IPGRI 1994). Safety or duplicate collections comprise accessions deposited at a location different from that of the base or active collection, for safety reasons. In the case of base and safety duplicate collections which are held under a long-term conservation commitment, the inclusion of accessions should be in accordance with national or institutional policies on acquisition and conservation. This may also be the case for active collections but, in general, working collections will include material that is not under genebank conservation policies and procedures.

Working Group 2. Minimize the regeneration frequency of accessions in the collection

Objective

Maximize initial quality and optimize initial quantity of the accession, and optimize the maintenance of viability and seed quantity in storage.

Requirements

- A management approach and documentation system that allows for decision-making on the maintenance of accessions at the individual accession level and flexibility in the choice of options (within the constraints of existing facilities and resources).
- Linking base and active collections and matching storage conditions to required accession storage life, to reduce regeneration and costs.

2.1 Considerations on accessing a sample into the genebank.

2.1.1 Acquisition and conservation policies should take into account the species, its nature, origin and uniqueness, and the type of collections implemented (base, active, duplicate, working).

Options:

- Sample meets the requirements of genebank acquisition and conservation policies – access into genebank.
- Sample does not meet the requirements – assign a temporary number; place in working collection.

2.1.2 Quantity and quality of the sample and of the information accompanying it: number of seeds in the sample, initial viability and health status, quantity and quality of passport data, including information on the history of the accession.

Requirement: initial viability test, seed health check, information on sample history; examination of accompanying data.

Constraints: difficulties in assessing quality and obtaining detailed sample history.

Options:

- Genebank procedures include obligatory initial regeneration – assign temporary number, store temporarily, prioritize and register for regeneration.

- Quality and quantity of seed and data meet requirements for storage – access and process for storage.
- Sample does not meet requirements for storage – assign temporary number, and:
  if data requirements are not met – hold temporarily and request information from donor;
  if quality (viability, seed health) is critical – regenerate immediately;
  if quality is poor – assign first priority for regeneration;
  if quantity of seed is critically low – regenerate immediately, using germinated seed from viability test if necessary;
  if seed quantity is limited – assign second priority for regeneration.

2.2 Considerations in maintaining and managing accessions in base and active collections and duplicating accessions.

2.2.1 Genebank/institutional priorities and procedures in establishing base, active and duplicate collections.
Options:
- First priority is generally given to placing the accessions in the base collection, where they will be stored and managed under optimum conditions to ensure that the genetic integrity remains as close to original as possible (refer to definition of a base collection).
- Priority for safety duplication will depend on the uniqueness of the accession. High priority should be accorded to newly collected samples and material that is not likely to be found in other genebanks.

2.2.2 Storage conditions for base and active collections.

Base collection: storage conditions should be optimum (the recommended conditions for long-term conservation whenever possible are 3–7% seed moisture content, depending on the species, and stored at –18°C or below (FAO–IPGRI 1994) in order to maximize accession longevity and thereby minimize the frequency of regeneration due to loss of viability in storage.

Active collection: storage conditions should provide the sample life-span in storage as dictated by the demand on the accession(s), so that regeneration of the sample is determined by need to increase seed stock, not viability. Recommended are conditions which allow seed viability to remain above at least 65% for 10–20 years (FAO–IPGRI 1994).
Options:
For accessions in high demand: medium/short-term storage conditions. High regeneration (multiplication) frequency will be inevitable if the need to generate additional seed stock for distribution is to be met. Thus, storage conditions need only ensure a sample storage life equivalent to the regeneration interval. Storage under unnecessarily stringent conditions is not economic. In the case of species with intrinsically good longevity (and at locations favourable to seed longevity), storage of accessions in very high demand may be most cost-effectively maintained in large quantities maintainable in large quantities at ambient conditions.
Constraints:
- Type of facilities available, and limitations in matching storage conditions to storage life requirements of individual accessions when storing a large number of accessions in the same store, that may have different intrinsic storage lives and significantly different regeneration intervals due to demand requirements.
- Storage space available, and limitations to storing quantities large enough to meet demand over a longer period as a strategy to minimize the frequency of regeneration.
- The land, funds and trained personnel to multiply large seed quantities.

For accessions in low demand: long-term storage conditions. Storage under long-term conditions optimizes storage life and thereby reduces the cycles of regeneration needed to maintain sample viability whilst seed stocks remain sufficient to meet demand.
Options:

- Keep base collection sample and active collection sample separately in long-term store in case of different regeneration lots.
- Keep just one sample if an original sample or from the same regeneration lot. Manage as a base collection, but hold stock above the minimum number for base collection.

2.2.3 Sample sizes for base, active and duplicate collections.

Base collection: the minimum seed quantity must be sufficient to meet needs for viability monitoring and regenerating the accession to re-establish the active collection from the base, when necessary, and regenerate the base when viability drops below the threshold set. Determining the appropriate quantity for an accession will be based on:

- amount of seed needed for each test
- frequency of the tests
- amount of seeds needed to ensure the accomplishment of a sample that duly represents the accession (basic unit)
- the frequency that the active collection is likely to have to be re-established from the base collection during the life-span of the accession (i.e. before it has to be regenerated due to loss of viability).

FAO–IPGRI (1994) recommend 1000 viable seeds for base collection as an absolute minimum.
Safety of duplicate collection: the minimum sample size must be sufficient to ensure the accomplishment of the regeneration of a representative sample of the original accession.
Active collection: the sample quantity must provide enough seed for:

- viability monitoring: which will depend on the frequency of tests and number of seeds required for each test.
- supplying samples for distribution for use, characterization, evaluation and research: which will depend on the level of demand on the accession and the amount of seed in each distribution.

2.2.4 Monitoring sample quantity and viability, and thresholds for regeneration.

Stock control: through an adequate management documentation system that records individual seed movements.
Viability: germination test.
Type of test: standard (ISTA regulation; genebank standards, etc.); sequential or others; viability monitoring (FAO–IPGRI 1994). Please note that the following aspects might influence the frequency of testing, the sample size, etc.:

experience
species
seed quality
storage conditions
sample heterogeneity.

Frequency: very frequent – increase in workload; infrequent – risk of losing accessions.
Strategy of testing: stratified – risk of loss; less cost; less seed; less regeneration; stratify according to lot.
Threshold:

species
heterogeneity
initial viability

low – risk of loss
high – increased frequency of regeneration.

Working Group 3. Conduct cost-effective regeneration of the accessions

The outline below has been used as the basis for the preparation of the decision guide on regeneration of accessions in seed collections (Sackville Hamilton and Chorlton 1997).

3.1 Main points

3.1.1 *A priori* information
- What is known about the species
- Accession history prior to regeneration

3.1.2 Considerations after sample is available for examination and regeneration

3.1.2.1 Context to choice of procedures
- Regeneration environment
- Population genetics / breeding system
- Minimum seed quantity required for successful regeneration
- Seed health (exchange / quarantine context)

3.1.2.2 Procedures for:
- Evaluation of available regeneration environments
- Assessing quality of original sample (or subsequent samples
- to be used regeneration)
- Crop management
- Post-harvest management
- Seed health

3.1.3 Analysis of resources and cost-effective application
- Current resource assessment
- Need for long-term outlook
- Within-collection cost-effectiveness

3.2 Detailed outline

3.2.1 *A priori* information

3.2.1.1 What is known about the species regarding:

- Adaptation
  General
  For seed production
  Climatic, edaphic and photoperiod conditions where “native”
  “Ideal”, climatic, edaphic and photoperiod conditions. Phenology in relation to climatic, edaphic and photoperiod conditions
- Seed physiology
  Storage
  Dormancy and germination
- Growth morphology
- Significant biotic stresses

- Genetic structure
  Breeding system
  Fecundity
  Population size
- Farmer management (for cultivated taxa)
- Seed health
- Risk assessment
  Weediness
  Other gene flow problems

3.2.1.2 Accession history prior to regeneration

- Collection techniques
- Past bottlenecks
- Degree of homogeneity and how diversity has been managed
- Seed health / quarantine status
- Seed viability and actual number of live seeds

3.2.2 Considerations after sample is available for examination and regeneration

3.2.2.1 Context to choice of procedures

- Regeneration environment
  Access to desirable sites
  Access to controlled environments (linkages to collaboration is appropriate environments are not available, also see 3.2.3.1)

- Population genetics / breeding system
  Applied and theoretical population genetics
  (i) Population size
  - statistical sampling
  - base unit
  "**An accession-specific population size, reflecting the effective population size given a certain mating system, needed to preserve diversity under certain assumptions**"
  Assumptions:
  Certain minimum seed quantity required (See 3.2.2.1)
  Frequency of rarest alleles to be conserved
  Number of loci (linkages to conservation policies)
  Genetic structure of population
  Probability of accomplishment
  (ii) Change caused by "selective" conditions – linkages to Evaluation of regeneration environments and subdividing samples (3.2.2.2)
  Actual assessments of population characteristics
  (i) Tools (genetic markers and appropriate statistical interpretation)
  (ii) Patterns of genetic variation (and by inference)
  (iii) Breeding systems (and by inference)

- Minimum seed quantity required for successful regeneration – (linkages to accession history and sample size)

Production targets to:
Meet long-term preservation of genetic integrity - i.e. provide future samples for regeneration

Meet demand - linkages to distribution management and population genetics (providing user with "adequate" sample)
Meet need for viability monitoring
Safeguard against loss of viability (multiplier)
Safeguard against seed losses in crop management and post-harvest procedures (multiplier)

- Seed health (exchange / quarantine context) – linkage to acquisition policy
  Access to current international regulations - linkage to collaboration with plant health agencies
  Internal standards / self-regulation

3.2.2.2 Procedures for:

- Evaluation of regeneration environments
  Measures of climate, photoperiod, and edaphic conditions for given locations and growing seasons must be examined in the context of *a priori* information about the plants and the past experience.

- Assessing the quality of original sample (or subsequent samples used for regeneration)
  Quantity
  Quality
    - Viability
    - Seed health - linkage to seed health procedures (3.2.2.2)

  Identity verification (if possible by seed) - linkage to inactivation policy
  Assessments of heterogeneity (in relation to accession history)

- Crop Management
  General agronomy / horticulture
    - Soils (biotic & abiotic characteristics)
    - Fertility and water management
    - Field preparation
    - Weed, insect and disease control
    - Timing of planting and harvest
    - Stand and plant density (thinning, plant habit management)
    - Seed pre-treatments / procedures for improvement of the seed germination rate
    - Seedling cultivation (for transplanted crops)
    - Microclimate manipulation (windbreaks, shading, etc.)

  Pollination management
    - Isolation (distance, temporal, cages and other physical barriers)
    - Insect pollinator management
    - Hand pollination procedures – linkages to population genetics

  Verification of accession identity based on whole plant - (linkage to inactivation policy)
  Impacts of crop management decisions on:
    - Seed quality (viability, vigour and "storability"; genetic integrity –representation)
    - Seed health
    - Seed quantity

  Cautions regarding:
    - Roguing to preserve genetic integrity (linkage to verification of accession identity)
    - "Splitting" as a tool to preserve rare variants and to simplify evaluation

- Post-harvest management
  Chronology (more-or-less)

- Transport of harvested materials
- Initial drying
- Threshing / seed extraction
- Cleaning
- Drying and moisture testing
- Initial viability testing (assessment of dormancy)
- Final moisture equilibration
- Special post-harvest treatment, such as after-ripening
- Quantification and information management - linkages to verification of accession identity and inactivation policy.
- Cautions about seed protection treatments linkage to seed health (fumigants / pesticides)

Assessment of storage conditions at each step above
Impacts of post-harvest management decisions on quality and quantity

- Seed health
  Procedures to control during regeneration
  - Pathogens affecting initial quantity and quality
  - Pathogens affecting storage quality
  - Seed transmitted pathogens and other pests

  Procedures for elimination of extant seed-transmitted pathogens and other seed-transmitted pests.
  Impacts of seed health management decisions on quality and quantity

3.2.3 Analysis of resources and cost-effective application

3.2.3.1 Current resource assessment - linkage to collaboration

- Facilities
  Seed storage
  Land and controlled environments
  Other general infrastructure
- Funding / financial resources
- Human resources
  Skills, knowledge, experience
  Quantity
- Information management
- Institutional management policies

3.2.3.2 Need for a long-term outlook

- Security of current status over time
- New opportunities

3.2.3.3 Within a particular collection, quantify the resources needed to ensure a certain level of regeneration success for particular techniques. The measurement of 'success' must be linked to conservation policies.

3.3. Research topics

3.3.1 Many species (especially wild relatives) lack basic information on cultivation; mating systems and patterns of genetic diversity; flower biology and seed production and seed physiology, including storage characteristics, dormancy and (see point 3.2.1.1) germination.

3.3.2 Geographic Information Systems (GIS) in relation to the choice of regeneration, sites etc. (see point 3.2.2.1 and others).

3.3.3 Environments (see 3.2.2.2)

3.3.4 How to optimally employ various markers for genetic assessment (see 3.2.2.1)

3.3.5 Degree of genetic control of dormancy / viability (3.2.1.1)

3.3.6 Tools for demand analysis and forecasting (3.2.2.2). Factors affecting initial seed quality and produces to maximize quality in the field and during handling prior to storage.

3.3.7 Techniques to minimize selection for various regeneration procedures (3.2.2.2)

3.3.8 Non-destructive viability testing (3.2.2.2)

3.3.9 Cost effectiveness and success of isolation / pollination control and 3.2.3.1 techniques (3.2.2.2).

3.3.10 Research on seed drying (3.2.2.2).

3.3.11 Rescue of deteriorated samples (3.2.2.2).

3.3.12 Actual seed deterioration curves under medium and long-term (3.2.2.3).

3.3.13 Methods to "clean up" seedborne pathogens that preserve genetic integrity (3.2.2.2).

3.3.14 Role of pathogens in seed longevity (3.2.2.2).

3.3.15 Modelling to optimize cost-effective application of accession- specific management (3.2.3).

3.3.16 Base unit. Information on location and sample size at the time of collecting, during quarantine and during subsequent regenerations, the number of regenerations and how the accession has been managed in collection (splitting or bulking of accessions) is important for curator decisions on sample size and sites for further regeneration and sample sizes to store (3.2.2.1).

3.3.17 Cost-effectiveness of various models of viability monitoring (3.2.3).

3.3.18 Cost-effectiveness of schemes to organize active & base collections (3.2.3).

Role of expert systems in integrating all factors for resource utilization and effectiveness (3.2.3). Strategies and methods to rationalize collections.

References

FAO–IPGRI, 1994. Genebank Standards. Food and Agriculture Organization of the United Nations, Rome / International Plant Genetic Resources Institute, Rome.

Sackville Hamilton, N.R. and K.H. Chorlton. 1997. Regeneration of accessions in seed collections: a decision guide. Handbook for Genebanks No. 5. International Plant Genetic Resources Institute, Rome.

Programme

4 December

08h30–09h00 Registration

Inaugural Session: Chair, A.C. Guedes

09h00–09h30 Welcome addresses:
ICRISAT, J.W. Stenhouse
IPGRI, J.M.M. Engels
FAO, M.N. Anishetty
SGRP, Jane Toll

09h30–09h50 Introduction to the Consultation: V. Ramanatha Rao

Objectives:
1. Identifying criteria and options in curator/genebank manager decisions on managing and carrying out regeneration, and formulating a curator decision framework.
2. Identifying topics requiring further information and/or research and opportunities to gather the information and/or carry out research
3. Proposing strategies and mechanisms for addressing the regeneration needs of existing collections.

09h50–10h00 Group photo

10h00–10h30 *Tea/coffee break*

Session I: Country Reports

Chair, M. Widrlechner; Rapporteur, E. Weltzein

10h30–12h00 India, B.B. Singh
China, Fan Chuanzhu
Ecuador, R. Castillo
Philippines, N. Altoveros

12h00–12h30 Discussion and synthesis of points raised

12h30–13h30 *Lunch*

Session I: Country Reports (Continued)

Chair, N. Altoveros; Rapporteur, P. Remanandan

13h30–14h30 Australia, P. Lawrence
Germany, K. Specht
USA, M. Widrlechner

14h30–15h00 Discussion and synthesis of points raised

15h00–15h30 *Tea/coffee break*

Session I: Country Reports (Continued)

Chair, R. Castillo; Rapporteur, K.E. Prasada Rao

15h30–16h30 Brazil, A.C. Guedes
Ethiopia, H. Kebede
Turkey, A. Tan

16h30–17h00 Discussion and synthesis of points raised

5 December

Session I: Country Reports (Continued)

Chair, A. Tan; Rapporteur, A.K. Singh

08h30–09h10 Bulgaria, S. Stoyanova
Kenya, J. Chewya

09h10–09h30 Discussion and synthesis of points raised

09h30–10h00 *Tea/coffee break*

Session II: Reports from IARCs
Chair, K. Specht; Rapporteur, N. Kameswara Rao
10h00–12h30 CIMMYT, J. Crossa
ICARDA, B. Humeid
ICRISAT, J.W. Stenhouse
IITA, J. Hughes
IRRI, R. Reano
AVRDC, L.M. Engle
12h30–13h30 *Lunch*
13h30–14h00 Discussion and synthesis of points raised
Session III: Presentations on regeneration experience and studies
Chair, P. Lawrence; Rapporteur, B.B. Singh
14h00–15h00 The global regeneration need: evidence collated from Country Reports to the IVth Technical Conference (FAO), M.N. Anishetty
The experience of the Latin American Maize Project in addressing a regeneration need, collaboratively, W. Salhuana
The analysis of information of regeneration practices obtained by questionnaires from 200 institutes, N. Altoveros
15h00–15h30 *Tea/coffee break*
Session IV: General discussion
Chair, J.W. Stenhouse; Rapporteur, P.N. Mathur
15h30–17h30 General discussion on all presentations including:
- synthesis of topics raised in presentations
- identification of issues for discussion in Working Groups

6 December
Session V: Introductory presentations to the Working Group sessions
Chair, V. Ramanatha Rao; Rapporteur, H.F.W. Rattunde
08h30–10h00 Overview of the genetic principles in regeneration, J. Crossa
Overview of the seed viability principles, N.K. Rao
Approaches to developing guidelines to regeneration, J. Toll/Tay Ying Sung
10h00–10h30 *Tea/coffee break*
10h30–12h30 Working Group sessions

It is proposed to form two Working Groups:
Group A - Managing regeneration
Group B - Regeneration procedures
The groups are expected to:
- ***identify the key criteria and possible options in curator decisions on managing***
- ***regeneration (Group A) and on procedures to carry-out regeneration cost-effectively (Group B);***
- ***identify aspects that need further information and/or research; and***
- ***examine measures to address current regeneration needs.***

12h30–13h30 *Lunch*
13h30–17h30 Working Group sessions continued

7 December
08h30–10h00 Introduction to ICRISAT Genetic Resources Division (tour of facilities and fields)
10h00–10h30 *Tea/coffee break*
Session VI: Reports of the Working Groups
Chair, J.M.M. Engels; Rapporteur, L.M. Engle

10h30–11h00 Presentation of Working Group findings
11h00–12h30 Discussion of Working Group findings
12h30–13h30 *Lunch*
13h30–15h00 Discussion of Working Group findings (continued)
Formulation of meeting outputs / conclusions according to objectives:

- development of a curator decision framework for managing and carrying-out regeneration
- preparation of a list of priority research and information needs and the proposals / agreements required to undertake these
- propositions for strategies / mechanisms to address regeneration needs in existing collections

15h00–15h30 *Tea/coffee break*

Closing Session

Chair, Jane Toll
15h30 Closing remarks, D.E. Byth

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