Efforts to sustain *ex situ* collections: technological aspects

Florent Engelmann and Jan Engels
International Plant Genetic Resources Institute (IPGRI), Rome, Italy

Introduction

Europe occupies a very important position as regards the *ex situ* conservation of plant genetic resources for food and agriculture (PGRFA), since 38% of the genebanks identified in the Report on the State of the World's Plant Genetic Resources for Food and Agriculture (FAO 1996) are located in European countries (Table 1). According to the same report, these genebanks hold 35% of the world accessions which are conserved *ex situ* in seed, field and *in vitro* collections, i.e. more than any other single region.

A large number of the main crops cultivated in Europe produce orthodox seeds, which can be stored in seed genebanks for extended time spans at low temperature, preferably at -18°C or lower, after partial desiccation to a moisture content of 3-7% (fresh weight basis) (FAO/IPGRI 1994). However, there are also important crops which present problems with regard to seed storage. Many species produce recalcitrant or intermediate seeds which are unable to withstand much desiccation and are often sensitive to chilling, and thus cannot be stored dry at low temperature (Roberts 1973; Ellis *et al.* 1990). Some crop species do not produce seeds or have sterile genotypes. In the case of many temperate fruit trees, seeds are highly heterozygous, and thus of little interest for the conservation of particular gene combinations (Engelmann 1997a). These crops are usually propagated vegetatively to maintain clonal genotypes, and are conserved as whole plants in field genebanks.

This paper provides a brief overview of the current problems and constraints with *ex situ* conservation of PGRFA and presents the main activities performed by IPGRI to improve the technologies of *ex situ* conservation.

Table 1. Genebanks and accessions in Europe, compared with the rest of the world and the CGIAR (adapted from FAO 1996: Table 3.1. Genebanks and accessions in *ex situ* collections, by region).

	Accessions		Genebanks	
	Number	%	Number	%
Europe	1 934 574	35	496	38
Total	5 554 505	100	1308	100
CGIAR total	593 191		12	

Current problems and constraints with ex situ *conservation of PGRFA*[34]

Ex situ conservation of PGRFA is faced with different categories of problems related to the nature and diversity of the genetic resources conserved, the existing storage infrastructures and their operations, the characterization and documentation of germplasm and the conservation methodologies employed.

Diversity of genetic resources conserved *ex situ*

Species coverage

Major crops, which frequently produce orthodox seeds, are predominant in *ex situ* collections. Minor crops and species with non-orthodox seeds and vegetatively propagated ones are under-represented. Cereals and food legumes are the two largest categories of crops stored *ex situ*,

[34] Most of the background information included in this section is adapted from the Report on the State of the World's Plant Genetic Resources for Food and Agriculture (FAO 1996).

with around 48 and 16% of global accessions conserved in genebanks, respectively. By contrast, forages, vegetables, root and tubers, fruits and forages account for less than 10, 8, 4 and 4%, respectively, and medicinal, spice, aromatic and ornamental species are hardly present in *ex situ* collections.

Type of accessions

Most of the *ex situ* collections have been established by breeders, or have incorporated breeders' working collections. As a result, a large amount of accessions consists of breeding lines and advanced cultivars, whereas landraces, wild or weedy plants and wild relatives of cultivated plants are generally under-represented in germplasm collections.

Representativity of *ex situ* collections

As no inventory of the total genetic diversity PGRFA exists, it is impossible to assess with accuracy the representativity of *ex situ* collections in comparison with the diversity existing *in situ*. Nevertheless, the coverage of cereals is considered relatively complete, while the coverage of most vegetables, root and tubers, fruits and forages is considered low and that of wild relatives very low.

Infrastructural and operational problems

Existing facilities

With regard to seed storage facilities, the situation in Europe is contrasted with long-term storage facilities available in most Western Europe countries, whereas many eastern European countries have only access to short- or medium-term storage facilities (Table 2). In many instances, the latter genebanks are faced with problems including unreliable electricity supply, lack of appropriate equipment or difficulties to maintain the existing equipment. This has an immediate detrimental effect on conservation costs since regeneration of the material stored under suboptimal conditions has to be performed more frequently.

Duplication of collections

Even though the information available is incomplete, it appears that most germplasm collections are not or only partly duplicated for safety. There is no information either about how many accessions maintained in *ex situ* collections are unique and how many are duplicates, but it is generally assumed that the degree of redundancy, both within and between collections, is relatively high.[35]

Regeneration

Even when seeds are stored under optimal conditions, their viability declines progressively. Therefore regular viability monitoring is needed and regular regeneration is required to maintain seed stocks with sufficiently high viability. Even though the situation in Europe is on average less critical than in other regions, regeneration is an important problem in many eastern and southern European countries owing to (by order of importance) inadequate storage facilities, funding and staffing constraints, problems with regenerating cross-pollinating species and facility constraints.

[35] For more details regarding duplication of collections in Europe refer e.g. to Knüppfer (1997) and Maggioni and Gass (1998).

NUM .155826
DA .990830
INV1 .MO 010055826
COTE1 .M 076AMEPLA BRA/1
NIVBIB .1
TYPBIB .B
INDBIB .K
LAN .ENG
ISBN .92-9043-406-6
NIV .S
AUS .Gass, T. (comp.); Frese, L. (comp.); Begemann, F. (comp.); Lipman, E.
.(comp.)
AMS ./1IPGRI/2Institut International des Ressources Phytogénétiques/4Rome/
.ITA (ed.)
TITORS .Implementation of the global plan of action in europe - Conservation
.and sustainable utilization of plant genetic resources for food and
.agriculture. Proceedings of the european symposium
NOMCG .European Symposium on Plant Genetic Resources for Food and Agricultur
LOCG .Braunschweig
PAYSCG .DEU

indexation du global

DACG .1998/06/30; 1998/07/03
BIBLIOGR.+
TABL .+
LED .Rome (ITA)
ED .IPGRI
DP .1999
PAGE .404 p.
NOTE .European Programme for Crop Genetic Resources Networks (ECP/GR)
CHAP .076AMEPLA
DES .RESSOURCES GENETIQUES; PLANTE; AGRICULTURE; ALIMENTATION;
.CONSERVATION DES RESSOURCES GENETIQUES; COLLECTION BOTANIQUE;
.AMELIORATION DES PLANTES; SEMENCE; LEGISLATION; PROGRAMME DE
.RECHERCHE; BIODIVERSITE; ORGANISME DE RECHERCHE
CDE .CONSERVATION IN SITU; CONSERVATION EX SITU; BANQUE DE GENES; RESEAU
.DE RECHERCHE
GEO .EUROPE
MODAC .D
NOMIND .ambert
QUISAISI.naudy
FINENREG.U990831
ARELIRE .Y

Table 2. *Ex situ* storage facilities and number of accessions conserved in Western and Eastern Europe genebanks. LT: long-term; MT: medium-term; ST: short-term (adapted from FAO (1996): Appendix 1. Status by Country of National legislation, programmes and activities for PGRFA)

Country	Conservation *ex situ*	Genebank accessions
Western Europe		
Austria	LT managed	7 891
Belgium	LT managed	9 750
France	LT managed	249 389
Germany	LT managed	200 000
Greece	LT storage or MT/LT	17 556
Ireland	LT storage or MT/LT	2 758
Italy	LT managed	80 000
Netherlands	LT managed	67 374
Portugal	LT managed	29 361
Spain	LT managed	78 174
Sweden	LT managed	89 206
Switzerland	LT managed	17 000
United Kingdom	LT managed	114 495
Eastern Europe		
Albania	ST/MT storage	20 000†
Armenia	ST/MT storage	2 000
Belarus	ST/MT storage	4 000
Bulgaria	LT storage or MT/LT	55 420
Croatia	ST/MT storage	15 336
Czech Republic	LT managed	51 571
Estonia	ST/MT storage	3 000‡
Hungary	LT managed	75 170
Latvia	ST/MT storage	9 730‡
Lithuania	ST/MT storage	12 821‡
Moldova	ST/MT storage	6 000
Poland	LT managed	91 802
Romania	LT managed	93 000
Russia	ST/MT storage	333 000
Slovakia	LT storage or MT/LT	14 547
Ukraine	LT storage or MT/LT	136 400
Yugoslavia	ST/MT storage	38 000§

† LT genebank was established in 1998.
‡ LT facilities established in 1996 (Lithuania), 1997 (Latvia) and 1999 (Estonia).
§ LT facilities under construction in 1998.

Characterization and documentation

In comparison with other regions, European countries are generally in a better situation as concerns both the existence of fully computerized documentation systems and the percentage of germplasm characterized, even though different situations are found between European countries (Table 3). An important improvement in the area of documentation is the development of integrated, compatible systems allowing easy exchange of information. This matter is being given due attention in most of the existing European crop genetic resources networks.[36]

Table 3. Examples of extent of characterization in European *ex situ* collections (adapted from FAO 1996: Fig. 3.4. The extent of characterization of *ex situ* collections: selected countries)

Country	% of collection characterized
Ukraine	50
Netherlands	100
Moldova	60
Czech Republic	60
Bulgaria	45

[36] See also Lipman *et al.* (1997) and sections on information systems of this volume.

Conservation methodologies

The traditional method for conserving the genetic resources of recalcitrant seed and vegetatively propagated species is as whole plants in the field. There are, however, several serious problems with field genebanks, including exposure to natural disasters, attacks by pests and pathogens, and high maintenance costs (Withers and Engels 1990). Moreover, germplasm distribution and exchange from field genebanks is difficult because of the vegetative nature of the material and the greater risks of disease transfer (Withers and Engelmann 1997). *In vitro* slow growth storage and cryopreservation in liquid nitrogen offer safe and efficient complementary options to field genebank conservation. Cryopreservation represents the only safe long-term conservation option available for these problem species. Slow growth storage and cryopreservation protocols are being developed for an increasing number of crops but their routine use for genetic resources conservation is still restricted to a limited number of cases (Engelmann 1997b).

In the case of orthodox seed-producing species, the development of low-input storage technologies such as ultra-dry seed storage would allow genebanks with limited facilities to conserve their seed collections more efficiently.

In conclusion, *ex situ* conservation needs to be strengthened and made more cost-effective by:

- developing and/or improving conservation technologies, in particular *in vitro* slow growth and cryopreservation techniques for non-orthodox seed species and vegetatively propagated crops
- developing more effective germplasm management procedures
- reducing unnecessary duplication of accessions within genebanks and in networks for a given crop species
- developing core collections to facilitate the use of germplasm collections
- developing better and more accessible information and documentation systems.

IPGRI activities for technological improvement of ex situ *conservation*

IPGRI activities focus on three main areas for the improvement of *ex situ* conservation: locating and assessment of genetic diversity, developing conservation technologies, and management and use of germplasm collections. In all these areas, activities include both the implementation of research projects and the production of technical and scientific publications.

Locating and assessing genetic diversity

Current research activities in this area which are of direct relevance to *ex situ* conservation concern the development or adaptation of effective methods of measuring and describing genetic diversity, including morpho-agronomic, biochemical and molecular methods, useful traits and ethnobotanical techniques, etc. (IPGRI 1998). Recent publications in this field include a Technical Bulletin and a report of a workshop on molecular tools for plant genetic resources conservation (Ayad *et al.* 1997; Karp *et al.* 1997).

In addition, IPGRI is currently conducting research in the following areas (IPGRI 1998): the development and application of specific location methods combining agro-ecological, ecogeographic surveying with GIS and landscape analysis and ethnobotanical approaches; studies on the major factors that affect the extent and distribution of genetic diversity (including sociocultural and gender aspects); locating useful adaptive traits through targeted collecting methods and prediction of where useful traits may be located; and the development of methods for monitoring genetic erosion, including sociocultural and gender aspects.

Conservation technologies

Orthodox seed research

Several research projects focus on orthodox seed storage research (Engels and Engelmann 1998; IPGRI 1998). A project is investigating several aspects related to the interaction between storage temperature and optimal seed moisture content for lettuce seed as a model. The use of the ultra-dry seed technology for storing seed at room temperature is being evaluated (Walters and Engels 1998). The effect of agricultural practices on seed quality for storage is being investigated, as well as the possibility of using direct or indirect sun light for drying seeds. A project will evaluate the possibility of using freeze-drying for storing orthodox seeds.

Recent publications on seed research include a seed storage compendium (Hong *et al.* 1996) and a Technical Bulletin on the determination of seed storage behaviour (Hong and Ellis 1996). A Newsletter which reports on the progress of a global project which screens tropical forest tree seeds for their storage behaviour is published twice a year (IPGRI/DFSC 1998). Additional Technical Bulletins are in preparation, focusing notably on seed moisture content testing and seed desiccation techniques.

In vitro conservation and cryopreservation research

Various research projects focus on the development and/or adaptation of *in vitro* conservation techniques for vegetatively propagated crops and non-orthodox seed species (Engelmann 1997c; IPGRI 1998). In the case of slow growth storage, research projects aim at improving and simplifying existing protocols and at applying them to a larger number of species. Cryopreservation research focuses on the establishment of protocols for additional species and on large-scale testing and routine application of existing protocols to several model crops in the genebank context. Recently, a status report on the development and application of *in vitro* techniques for the conservation and use of plant genetic resources has been produced (Ashmore 1997). A Technical Bulletin on low-technology cryopreservation techniques is in preparation.

Complementary conservation strategies

In the case of PGRFA, *ex situ* conservation has been the customary practice to date. This perspective has now broadened to take account of the role of *in situ* conservation, including on-farm management of genetic diversity, which allows the process of crop evolution and adaptation to continue. It is now recognized that any conservation strategy will employ a combination of complementary methods, from nature reserves to genebanks, which will allow an increase in its cost-efficiency and safety. The appropriate strategy and the mix of methods depends on factors such as the biological characteristics of the species, their present management, available infrastructure for conservation, number of accessions in a given collection and geographic sites of collections, the purpose of conservation, the accessibility of germplasm and legal and administrative policies. In addition, the aspect of the use of the genetic resources is a very important factor to be considered while deciding how best to conserve such resources and users should be fully consulted when designing a conservation strategy. Research has been initiated on a few selected strategic case studies, which will allow the development of appropriate decision-making tools for the application of complementary conservation strategies under different conditions and for a range of different species (Nissilä *et al.* 1999).

Management and use of germplasm collections

Germplasm management encompasses various management issues such as acquisition, conservation, regeneration, characterization and distribution of accessions. Regeneration in particular is a critical procedure as it carries risks, such as genetic shift and drift which may compromise the genetic integrity of the samples. Some of these risks are inherent to the sample selection procedures followed, including the consequences of the number of plants per accession grown out, especially in the case of heterogenous accessions. Other risks to genetic integrity might be caused by the effects of diseases, pests and abiotic stresses which regenerated samples may be exposed to. Therefore, collections must be managed with the aim of minimizing the frequency of regeneration. Development of appropriate strategies towards this end is recognized as a priority area for continued investigation. In addition, research is needed to evaluate the level and causes of genetic shift and drift during regeneration. Improvement of cultivation procedures are also necessary to minimize the risks of such genetic changes, including enhancement of pollination, reduction of plant competition, determination of optimal population sizes, etc. In recognition of these needs, IPGRI performs several research projects aiming at improving regeneration procedures (IPGRI 1998). IPGRI also recently published guidelines aimed to assist genebank curators by providing decision criteria and options in this area (Sackville Hamilton and Chorlton 1997).

In the case of *in vitro* collections, the management problems relate to slow growth storage in which the maintenance of the genetic stability of the cultures and reduction of the workload of subculturing to the minimum are main objectives. The procedures worked out for managing cassava under slow growth (IPGRI/CIAT 1994) need to be applied to other cassava collections and down-streamed for wider application to other species. With this objective, IPGRI is currently preparing guidelines which will assist genebank curators in the management of field and *in vitro* germplasm collections (Reed and Bateson 1998).

Documentation

Activities focus on the development of methodologies and applications to support and improve the documentation of plant genetic resources. Consecutive versions of a Genebank Management System Software to facilitate the documentation of plant genetic resources and to increase the use of data on these resources (Perry *et al.* 1993) have been made available since 1993. IPGRI also produces crop descriptor lists, which are now available for more than 80 different crops. The most recent volumes have been published for pistachio, tea, yam and grapevine (IPGRI 1997a, 1997b, 1997c, 1997d). A list of multicrop passport descriptors was also recently developed in collaboration with FAO (Hazekamp *et al.* 1997). This list aims to provide an initial set of passport descriptors that can be used for all crops.

Germplasm health

IPGRI's research projects in the area of germplasm health focus on the development of new methods of pathogen detection and therapy, and on the adaptation of existing methods to the circumstances in developing countries (IPGRI 1998). Another important activity is the production, in collaboration with FAO, of the Technical Guidelines for the Safe Movement of Germplasm. The purpose of these guidelines is to provide relevant information on procedures that will help to ensure phytosanitary safety when germplasm is moved internationally. Guidelines have been produced for around 20 different crops, and the latest volumes published include *Eucalyptus* spp. (Ciesla *et al.* 1996), *Musa* spp. (Diekmann and Putter 1996a), stone fruits (Diekmann and Putter 1996b) and *Allium* spp. (Diekmann 1997).

Core collections

Core collections provide an effective approach to improved access to the diversity of a crop genepool and in particular to that present in large genebank collections. They consist of limited sets of accessions derived from an existing collection chosen to represent the genetic spectrum of the whole collection (Hodgkin *et al.* 1995). The core should include as much as possible of the genetic diversity found in the whole collection. Several research projects are underway to improve methodological aspects of the establishment and use of core collections (IPGRI 1998). A global survey of the core collections developed worldwide is currently being performed. Finally, a Technical Bulletin on the establishment of core collections is under development.

Conclusion

Substantial research in all of the routine germplasm conservation areas will be required to improve the effectiveness and efficiency of *ex situ* conservation in Europe and in other regions through the development of adequate technologies. This will be greatly facilitated through coordinated international collaborative research efforts.

IPGRI is prepared to continue to actively participate in this endeavour by providing assistance to the plant genetic resource community, among others, by translating research results into practical products directly applicable in genebanks. IPGRI can also assist in facilitating linkages between advanced research organizations and National Programmes in developing countries.

References

Ashmore, S.E. 1997. Status report on the development and application of *in vitro* techniques for the conservation and use of plant genetic resources. International Plant Genetic Resources Institute, Rome, Italy.

Ayad, W.G., T. Hodgkin, A. Jaradat and V.R. Rao (eds.). 1997. Molecular Genetic Techniques for Plant Genetic Resources: Report of an IPGRI Workshop, 9-11 October 1995, Rome, Italy. International Plant Genetic Resources Institute, Rome.

Ciesla, W.M., M. Diekmann and C.A.J. Putter (eds.). 1996. FAO/IPGRI Technical Guidelines for the Safe Movement of *Eucalyptus* spp. germplasm. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome, Italy.

Diekmann, M. (ed.). 1997. FAO/IPGRI Technical Guidelines for the Safe Movement of *Allium* spp. germplasm. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome, Italy.

Diekmann, M. and C.A.J. Putter (eds.). 1996a. FAO/IPGRI Technical Guidelines for the Safe Movement of *Musa* spp. germplasm (2nd edn.). Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome, Italy.

Diekmann, M. and C.A.J. Putter (eds.). 1996b. FAO/IPGRI Technical Guidelines for the Safe Movement of stone fruit germplasm. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome, Italy.

Ellis R.E., T. Hong and E.H. Roberts. 1990. An intermediate category of seed storage behaviour? I. Coffee. J. Exp. Bot. 41:1167-74.

Engelmann, F. 1997a. Importance of desiccation for the cryopreservation of recalcitrant seed and vegetatively propagated species. Plant Genet. Resour. Newsl. 112:9-18.

Engelmann, F. 1997b. *In vitro* conservation methods. Pp. 119-161 *in* Biotechnology and Plant Genetic Resources: Conservation and Use (J.A. Callow, B.V. Ford-Lloyd and J.H. Newburry, eds.). CAB International, Wallingford, UK.

Engelmann, F. 1997c. Research activities of the International Plant Genetic Resources Institute in the area of *in vitro* conservation of plant genetic resources. Plant Tissue Cult. Biotechnol. 3:46-52.

Engels, J.M.M. and F. Engelmann. 1998. Priority areas in seed conservation research – an IPGRI perspective. Pp. 153-168 *in* Progress in Seed Research. Proceedings of the 2nd International Conference on Seed Science and Technology, Zhongshan University, Guangzhou, China, 12-16 May 1997 (A.G. Taylor and Xue-Lin Huang, eds.). Cornwell University, USA.

FAO. 1996. Report on the State of the World's Plant Genetic Resources for Food and Agriculture prepared for the International Technical Conference on Plant Genetic Resources, Leipzig, Germany, 173-23 June 1996. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO/IPGRI. 1994. Genebank Standards. Food and Agriculture Organization of the United Nations /International Plant Genetic Resources Institute, Rome, Italy.

Hazekamp, Th., J. Serwinški and A. Alercia. 1997. Multi-crop Passport Descriptors. Pp. 35-39 *in* Central Crop Databases: Tools for Plant Genetic Resources Management (E. Lipman, M.W.M. Jongen, Th.J.L. van Hintum, T. Gass and L. Maggioni, compilers). International Plant Genetic Resources Institute, Rome, Italy/CGN, Wageningen, The Netherlands.

Hodgkin, T., A.H.D. Brown, T.J.L. van Hintum and E.A.V. Morales (eds.) 1995. Core Collections of Plant Genetic Resources. John Wiley & Sons, Chichester.

Hong, T.D. and R.H. Ellis. 1996. A protocol to determine seed storage behaviour. IPGRI Technical Bulletin No. 1 (J.M.M. Engels and J. Toll, vol. eds.). International Plant Genetic Resources Institute, Rome, Italy.

Hong, T.D., S. Linington and R.H. Ellis. 1996. Seed Storage Behaviour: a Compendium. Handbooks for Genebanks No. 4. International Plant Genetic Resources Institute, Rome, Italy.

IPGRI. 1997a. Descriptors for Pistachio (*Pistacia vera* L.). International Plant Genetic Resources Institute, Rome, Italy.

IPGRI. 1997b. Descriptors for tea (*Camelia sinensis*). International Plant Genetic Resources Institute, Rome, Italy.

IPGRI. 1997c. Descriptors for yam (*Dioscorea* spp.). International Plant Genetic Resources Institute, Rome, Italy.

IPGRI. 1997. Descriptors for grapevine (*Vitis* spp.). International Plant Genetic Resources Institute, Rome, Italy.

IPGRI. 1998. Annual Report 1997. International Plant Genetic Resources Institute, Rome, Italy.

IPGRI/CIAT. 1994. Establishment and operation of a pilot *in vitro* active genebank. Report of a CIAT-IBPGR Collaborative Project using cassava (*Manihot esculenta* Crantz) as a model. IPGRI, Rome, Italy / CIAT, Cali, Colombia.

IPGRI/DFSC. 1998. "Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds". Newsletter 4 (April 1998).

Karp, A., S. Kresovich, K.V. Bhat, W.G. Ayad and T. Hodgkin (eds.). 1997. Molecular Tools in Plant Genetic Resources Conservation: a Guide to Technologies. IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome, Italy.

Knüppfer, H. 1997. Using Central Crop Databases: searching for duplicates and gaps. Pp. 59-68 *in* Central Crop Databases: Tools for Plant Genetic Resources Management.(E. Lipman, M.W.M. Jongen, Th.J.L. van Hintum, T. Gass and L. Maggioni, compilers). International Plant Genetic Resources Institute, Rome, Italy/CGN, Wageningen, The Netherlands.

Lipman, E., M.W.M. Jongen, Th.J.L. van Hintum, T. Gass and L. Maggioni, compilers. 1997. Central Crop Databases: Tools for Plant Genetic Resources Management. International Plant Genetic Resources Institute, Rome, Italy/CGN, Wageningen, The Netherlands.

Maggioni, L. and T. Gass. 1998. Safety-duplication of germplasm collections in Europe. Pp. 96-102 *in* Report of a Working Group on Forages. Sixth meeting, 6-8 March 1997, Beitostølen, Norway (L. Maggioni, P. Marum, R. Sackville Hamilton, I. Thomas, T. Gass and E. Lipman, compilers). International Plant Genetic Resources Institute, Rome, Italy.

Nissilä, E.A.J., V. Ramanatha Rao, F. Engelmann and K.W. Riley. 1999. *Ex situ* strategies for complementary conservation of Asian sweet potatoes. Plant Genet. Resour. Newsl. 117:1-11.

Perry, M.C., K.A. Painting and W.G. Ayad. 1993. Genebank Management System Software User's Guide. International Board for Plant Genetic Resources, Rome, Italy.

Reed, B.M. and J. Bateson. 1998. Guidelines for the management of field and *in vitro* germplasm collections. Handbook for Genebanks No. 6. International Plant Genetic Resources Institute, Rome, Italy.

Roberts, E.H. 1973. Predicting the viability of seeds. Seed Sci. Technol. 1:499-514.

Sackville Hamilton, N.R. and K.H. Chorlton. 1997. Regeneration of accessions in seed collections: a decision guide. IPGRI/FAO Handbooks for Genebanks 5. International Plant Genetic Resources Institute, Rome, Italy.

Walters, C. and J. Engels. 1998. The effects of storing seeds under extremely dry conditions. Seed Sci. Res. 8, Supplement 1:3-8.

Withers, L.A. and F. Engelmann. 1997. *In vitro* conservation of plant genetic resources. Pp. 57-88 *in* Agricultural Biotechnology (A. Altman, ed.). Marcel Dekker Inc., New York.

Withers, L.A. and J.M.M. Engels. 1990. The test tube genebank – a safe alternative to field conservation. IBPGR Newsletter for Asia and the Pacific 3:1-2.