PN-AAU-759

IBPGR ADVISORY COMMITTEE ON SEED STORAGE

Report of the Third Meeting

International Board for Plant Genetic Resources

AGPG/IBPGR/84/74
April 1985

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

IBPGR ADVISORY COMMITTEE ON SEED STORAGE

Third Meeting
4-6 April 1984

REPORT

IBPGR Secretariat
Rome, 1985

The International Board for Plant Genetic Resources (IBPGR) is an automous international scientific organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR). The IBPGR was established by the CGIAR in 1974 and its Executive Secretariat is provided by the Food and Agricultural Organization of the United Nations. The basic function of the IBPGR is to promote and coordinate an international network of of genetic resources centres to further the collection, conservation, documentation, evaluation and use of plant germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world. The Consultative Group mobilizes financial support from its members to meet the budgetary requirements of the Board.

IBPGR Executive Secretariat
Crop Genetic Resources Centre
Plant Production and Protection Division
Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla, 00100 Rome, Italy

© International Board for Plant Genetic Resources, 1985

CONTENTS

		Page
CONTENTS		iii
INTRODUCTION		1
REPORT		1
APPENDIX I	IBPGR Advisory Committee on Seed Storage	9
APPENDIX II	Agenda	11
APPENDIX III	Maintaining Viability During Collecting Expeditions	13

INTRODUCTION

1. The third meeting of the IBPGR Advisory Committee on Seed Storage was held 4-6 April 1984 at the University of Reading, UK, by kind invitation of the Chairman of the Committee, Professor E.H. Roberts. The following members were present, in addition to the Chairman: Prof. J.D. Bewley, Prof. H.F. Chin, Dr. A.G. Gordon, Mrs. E. Plli-Sevilla, Mr. R.D. Smith, Dr. P.C. Stanwood, and Dr. J.T. Williams. Mr. A.S. Cromarty and Dr. R.H. Ellis attended at the invitation of the Committee. Prof. J.P. Cooper attended on behalf of the IBPGR. Dr. K.L. Tao, of the IBPGR Secretariat, acted as Technical Secretary. Addresses of the Committee members are shown in Appendix I. The agenda, as adopted, is shown in Appendix II.

2. The Committee had been convened to establish standards which would be appropriate for genebanks operating within the international network. It was thought important to identify minimal standards necessary to ensure the safety of valuable genetic resources and also the safety of personnel working in genebanks. For both purposes there need to be standards not only for the equipment but also for the procedures adopted. The Committee recognized that it would not always be possible for genebanks to meet immediately all the standards outlined in the following paragraphs, but the specification of standards would be an encouragement to the development of facilities and procedures.

One of the problems of establishing minimal standards is that they might come to be considered ideal, and therefore their existence might block initiatives to further improvement. Because of this the Committee decided that, for some criteria, two standards would be specified:

(i) 'Acceptable': in many cases minimal but adequate for storage; and

(ii) 'Preferred': a higher standard to be aimed for.

REPORT

AVAILABILITY OF MATERIAL

3. The principle of the IBPGR that material should be freely available, together with related information, must be adhered to for all active collections. It should apply to base collections when the material is not known to exist from other sources (active collections) or is not easily available from some other non-base collection, and free availability should be restricted to plant improvement programmes. Users, however, should be discouraged from applying for samples to base collections.

4. While it is clear that most of the material held in genebanks will be part of the 'core' collection which is considered to be generally available as outlined in paragraph 3, some genebanks may also hold certain material on behalf of other institutions which would not be generally available. Such 'non-core' material might, for example, include narcotics, breeders' advanced lines, samples held for plant variety rights registration, etc. Genebank managers maintaining base collections should therefore identify the accessions which form the core collection which are available for international registration.

STORAGE STANDARDS FOR BASE COLLECTIONS

5. Safety of installation:

(i) Power supply (with reference to the essential parts of the store and equipment, and not offices)

Acceptable: A genebank should have a stable and continuous electricity

supply and should be equipped with an adequate back-up generator with a fuel supply for several weeks of operation. In countries where electric voltage fluctuates markedly, a stabilizer is required to stabilize the power supply.

Preferred: Two back-up generators.

(ii) Fire precautions

Acceptable: Adequate fire precautions as suggested in The Design of Seed Storage Facilities for Genetic Conservation[1/] (referred to subsequently in this report as DSSF) are needed. They should be tested from time to time. Particular attention should be paid to: maintaining appropriate fire fighting equipment (and trained personnel); lightening conductor/rod; alarm system; high temperature cut-out for the cooling system; and compressor, which should be mounted behind a wall. Alternative arrangements for storage should be available for implementation after a major catastrophe e.g. the use of local commercial stores.

(iii) Security

There should be adequate security arrangements for the protection of the facility.

(iv) Refrigeration standards and equipment

Acceptable: Refrigeration standards and equipment must conform to the "DSSF" specifications. Attention should be paid to the local availability of refrigerant.

Preferred: There should be back-up refrigeration equipment.

(v) Construction and insulation standards

The construction standard and testing of the facility and the cold room should follow the guidance provided by "DSSF".

6. Safety of personnel: Protective clothing should be used; recognized procedures and instructions should be given to staff; adequate precautions should be provided (alarms, ability to open the cold store from inside, etc.)

7. Storage containers: Containers should be hermetically sealed and be water/vapour proof. Cans, bottles and aluminium foil are all acceptable (see "DSSF") but should be quality-tested to ensure they are in fact moisture-proof.

8. Storage temperature:

(i) Limits

Acceptable: Sub-zero (<0°C).

Preferred: -18°C or less.

(ii) Monitoring

Acceptable: Temperature should be checked regularly against a certified thermometer.

Preferred: Temperature should be checked regularly against a certified

---

1/ Cromarty, A.S., R.H. Ellis and E.H. Roberts. 1982. IBPGR, Rome.

thermometer and an alarm system installed which is triggered if the temperature rises above a specified level.

9. Seed moisture content: A single acceptable standard is appropriate for most species: seed moisture content typically should be 3-7%, attained using recognized procedures, except where there is strong evidence that, for a particular species, this is inappropriate (e.g. soyabean should be 7-8%). In many cases where it was originally claimed that low moisture content might be detrimental to seed longevity, subsequent investigations have shown such worries to be unfounded if attention is given to points 7, 8, 9, and 10 in paragraph 12.

10. Methods and equipment for moisture content determination: Seed moisture should be determined according to methods advocated in ISTA[1/] rules except that small sample sizes are used. The small sample size should be matched with an increase in balance sensitivity. If other methods are used, they should be calibrated against the ISTA standard.

11. Accession sizes: The figures provided below assume that the accessions are above the regeneration standard estimated on a full seed basis. While it is recognized that for some accessions it may be difficult to achieve the acceptable standard without instituting a seed-multiplication cycle, the amounts suggested are nevertheless considered necessary for the normal operations of a genebank and to allow some degree of insurance.[2/]

(i) Genetically homogeneous accessions

Acceptable: Normally 3000 seeds.

Preferred: Normally 4000 seeds.

1/ International Seed Testing Association

2/ In normal operations some seed may be needed for moisture content determination and 200 to 400 seeds are required for the initial viability test. Further samples, averaging at least 200 seeds per sample, will be required for each subsequent monitoring test during storage. To allow for accidents, at least 400 seeds should be retained for regeneration (and more for genetically heterogeneous accessions, see below) when the accession approaches depletion. Further samples will be required to replenish losses from active collections or supply needs where no active collection exists.

More seed will be required for genetically heterogeneous accessions. The required number of extra seed depends on the assumptions made and the policies arising from these assumptions. For example, if a gene is present within an accession at a frequency of 0.05, a sample of 59 seeds would be sufficient to ensure a 95% probability of the gene being present within at least one seed in the sample. If, however, there were 20 different genes with similar low frequencies within the accession, the probability would only be 0.358 that this size sample would contain all 20. To ensure a 95% probability of any one of these genes being present in at least one seed would require a sample size of 117. However, when seeds are sown, not all survive to maturity: 75% field survival would be a high figure for seeds of 85% viability. Thus to achieve the aims assumed in the last example, 156 seeds would be needed. Obviously if the gene frequencies were less than 0.05, or if one demanded a higher probability of their presence than 95%, then the samples size required would be much greater. Some flexibility is needed as it is difficult to anticipate future demands and policies, but it would seem that samples of heterogeneous accessions of between 100 and 160 seeds might be considered a suitable compromise for distribution, multiplication or regeneration. Hence larger accession sizes are suggested for genetically heterogeneous accessions, even though 100 seeds for distribution might be considered satisfactory.

(ii) Genetically heterogeneous accessions

Acceptable: 4000 seeds but with the intention to increase stocks to the preferred number.

Preferred: Normally 12,000 seeds.

12. Regeneration standards: It is noted that a number of wild species, forestry species and others have putative low viability. Setting an 85% regeneration standard may appear highly optimistic for those species which have gained a reputation for setting seed of low viability. Nonetheless, the collective experience of the committee encourages the view that in the great majority of cases close examination of the following 10 points will provide a rational explanation for many of these reputations:

1 The maturity status of the seed at harvest;
2 The lateness of the harvest in relation to seed maturity;
3 The immediate post-harvest storage conditions of the seed;
4 The possibility of a high proportion of morphologically intact but ungerminable seed, due either to embryo abortion or bruchid infestation;
5 The seed extraction and cleaning processes adopted;
6 The use of insecticidal or fungicidal seed treatments;
7 The seed drying procedures employed;
8 The possibility of inadvertent use of truncated germination tests;
9 The possibility of unwitting use of inappropriate dormancy breaking treatments;
10 The procedures used in germination tests which produce rehydration injury.

In view of the value and importance of the material held in genebanks, the committee has adopted an 85% regeneration level for all species to encourage the adoption of those simple modifications to seed handling procedures which will be needed to overcome these difficulties. The effort of the genebanks in producing high quality seed will be rewarded by substantial increases in storage life.

In the unexpected event that investigative efforts can be shown to have failed in overcoming these problems of low initial viability, lower regeneration standards will, of necessity, be acceptable.

13. Methods of regeneration: Curators should, as part of Management, consult with crop experts on techniques for regeneration and should use them in their own regeneration programmes or include instructions when seed is sent to other institutions for growing out.

14. Viability monitoring:

(i) Responsibility

In the past there has been the concept of a 'black box' type duplication of collections, with the samples in the 'black box' not monitored. This should be vigorously discouraged. The curator of each base collection has the responsibility to monitor viability. Due to variation in storage conditions, duplicated samples stored in different genebanks may deteriorate at different rates. Therefore each genebank should be responsible for conducting viability tests (including initial and monitoring tests for each accession).

(ii) Germination test principles

Normally germination tests are carried out when samples are received and monitored at intervals subsequently. The germination test result should be reported as percentage of true seeds which are capable of producing a seedling. Genebanks should take steps to remove empty seeds and to remove dormancy so that true estimates are obtained. Advice on these matters

will be found in a publication under preparation.1/

(iii) Initial germination test method: (assuming a reasonable technique is available for the species being tested)

Acceptable: An initial germination test is normally conducted by germinating 200 seeds (minimum of 2 replications). The result of this test can be accepted if germination is above 90% (180 seed germinated). Otherwise, an additional 200 seeds (minimum of 2 replications) will be tested; the average germination of the two tests is reported as the overall test result. Alternative methods will be considered on merit.

(iv) Viability monitoring period

Genebanks should regularly conduct monitoring tests. Under preferred storage conditions, the first monitoring test is conducted after 10 years for seeds with high initial germination percentage. Species known to have poor storage life or accessions of poor initial quality should be tested after 5 years. The interval between later tests should be based on experience, but in many cases may well be greater than 10 years.

(v) Viability monitoring test methods

Acceptable: Same as the initial viability test (batch testing).

Preferred: Use of the sequential test method as published by IBPGR.2/

In fixed batch testing there is the risk of curators regenerating when it is not necessary (i.e. when the true viability of the accession is above the regeneration standard) and also the risk that viability sometimes will have fallen below the regeneration standard. The aim is to save germplasm and not to regenerate until necessary; hence the preferred method in which the number of seeds employed is minimized while maintaining adequate accuracy, should be used whenever possible.

(vi) Equipment for germination tests, detection of empty seeds and removing dormancy

Genebanks should have adequate equipment in the seed laboratory to conduct adequate tests on the species for which they are responsible. Essential equipment includes germinators and/or germination rooms, incubators, balances and grinders. Further additional equipment is necessary (e.g. X-ray) if the genebank has responsibility for species which produce empty seeds.

15. Handling before storage:

(i) Handling procedures before storage

Acceptable: Prior to seed drying and storage, accessions should be kept in a pre-processing room with controlled temperature and humidity (see "DSSF"). Although low temperature and low relative humidity decrease the rate of seed deterioration and damage from insects, the rate of deterioration nevertheless proceeds much faster than under long-term

---

1/ Ellis, R.H., Hong, T.D. and Roberts, E.H. 1985. Manual of Seed Technology for Genebanks IBPGR, Rome.

2/ R.H. Ellis, E.H. Roberts and J. Whitehead. 1980. A new, more economic and accurate approach to monitoring the viability of accessions during storage in seed banks. Pl. Genet. Resources Newsl. 41:3-18.

storage conditions; therefore the period of temporary storage should be minimized.

(ii) Drying procedures and equipment (see "DSSF")

Acceptable: In certain circumstances for particular crops in certain geographical locations forced ventilation warm air drying would be acceptable. However, the technique is not recommended for new installations.

Preferred: Seed dried at 15°C and 10-15% RH in a drying room or a drying cabinet.

(iii) Seed cleaning

Seed should be cleaned before storage, using techniques which ensure minimal seed damage. Any waste materials should be properly disposed of.

16. Information about samples

(i) Types of data

Four types of data are necessary for each accession:

a. Passport: Passport data remain unchanged for each accession, and should include identification number, scientific name, pedigree/cultivar name, geographic location and natural habitat of collection, etc.

b. Characterization: (including preliminary evaluation data) Characteristics which are normally highly heritable.

c. Further evaluation: Screening of characteristics useful to breeders.

d. Management: These should include: current quantity of seeds (weight and number); date of receipt; date stored; location of accession; local control number; initial testing results, dates and results of all monitoring tests, and proposed date of next monitoring test; number of regenerations; location of duplicates; why an accession has been lost or is no longer available; data on release of sample (quantity and location); and (sites) of active collections related to the base collection.

Acceptable: A genebank should hold passport data and management data.

Preferred: In addition to passport and management data, genebanks should also hold the characterization and evaluation data or be able to identify the location of such data.

17. Seed testing laboratory: In addition to storage and drying facilities a genebank will need to include adequate space of suitable design for seed testing laboratories, office records, maintenance workshop, etc.

18. Genebank personnel: The genebank should indicate the number of species/accessions present and the anticipated annual growth pattern over the next 10 years. Based on this information, an assessment should be made of the anticipated work load, the number of technical and other staff needed, and the intended management structure.

Guidance is available in ISTA publications. Although genebanks differ in requirements, the suggestions in Table 1 are offered as guidelines.

19. Arrangements for duplicating samples: A genebank must make arrangements to store duplicate samples in at least one other base genebank. It is preferred that this other base collection is in another country.

20. Standards for storage in liquid nitrogen: Should a particular genebank have plans for using LN_2 for storage, details of the procedures should be provided.

STORAGE STANDARDS FOR ACTIVE COLLECTIONS

21. The aim should be to store an active collection of good quality seed and maintain viability at more than 85% for 10-20 years. Accessions may be held either in unsealed or sealed storage containers.

22. Unsealed storage: Seeds should be stored in conditions of not more than 10°C and 20% RH. Because the full capacity of a standard air conditioner in tropical areas is 50% RH and 10°C, special equipment would be required in that zone. However such storage is not to be encouraged. In tropical areas, sealed storage is recommended.

23. Sealed storage: Seeds of most species should be dried to 7% seed moisture or less and should be stored at less than 15°C in a sealed container (if scientifically recognized as a poor storer, the temperature should not exceed 5°C and seeds should be dried to 4%). The temperature may fluctuate, but must not rise above 15°C.

24. Monitoring tests and intervals: Monitoring tests for viability should be conducted every 5 years or more often.

25. Standards for exchange and distribution:

(i) Seeds should not be sent out in open packets but in suitable (preferably moisture proof) containers.
(ii) Adequate information, such as passport data and (if required) evaluation data should accompany the sample.
(iii) Special details of germination methods should be provided.

Table 1. Number of workers in a genebank handling 10,000 to 200,000 accessions1/

	10,000	100,000	200,000
1. Director/curator2/	1	1	1
2. Assistant director	0	1	1
3. Clerical staff	1	2	3
4. Information handling	1	2	3
5. Technical staff			
Maintenance	1/2	5	10
Growth3/	1/2	5	10
6. Scientific4/	1	2	3
6.1 Botanist/taxonomist	-	1	1

1/ The number of workers is for an average genebank. If the plant materials held are difficult to handle, the number of workers should be increased.
2/ A plant physiologist with management ability is preferred.
3/ If the growth rate of number of accessions is >10% per year these figures would need modification.
4/ An appropriate order of filling posts would be: 1) seed physiologist, 2) seed pathologist, 3) botanist.

REGISTRATION OF GENEBANKS

26. As a means towards ensuring the safety and general accessibility of plant genetic resources, the Committee recommends that IBPGR should develop a register of those genebanks which have achieved, or intend to achieve, most of the acceptable standards outlined in this report. It recognizes, however, that there may be practical constraints to the immediate implementation of all the standards. Thus the Secretariat, in consultation with individual genebanks and the Advisory Committee on Seed Storage, could advise the Board on the extent to which it is possible for any genebank to meet acceptable standards, and therefore whether, taking all factors into account, a genebank could be considered to be operating or intending to operate within the spirit of these standards.

OTHER BUSINESS

27. Standards for liquid nitrogen storage will be developed at a future meeting. Research standards, however, have been annexed to the report of the Second Meeting of this Committee. In this respect the committee recognizes the years of research on cryopreservation at the National Seed Storage Laboratory, Fort Collins, Colorado, USA. The Committee recommends that this activity be given encouragement and that it be recognized as a major international research centre for further development of this work.

28. Appendix II in the Design of Seed Storage Facilities for Genetic Conservation was to be updated and details published in the IBPGR/FAO Plant Genetic Resources Newsletter.1/

29. Both the Advisory Committee on Seed Storage and a Consultation on Practical Constraints Affecting the Collection and Exchange of Wild Species and Primitive Cultivars2/ requested a specialist paper on the careful handling of seeds during collection missions in order to maintain maximum viability. This report by Mr. R.D. Smith of the Committee was endorsed and is attached as Appendix III.

1/ Ellis, R.H. 1984. Revised table of seed storage characteristics. Pl. Genet. Resources Newsl., 58:16-33.

2/ IBPGR Secretariat Consultation, held 14-15 March 1983 (AGPG:IBPGR 83/49)

APPENDIX I

IBPGR ADVISORY COMMITTEE ON SEED STORAGE

Chairman

Professor E.H. Roberts
Department of Agriculture and Horticulture
University of Reading
Earley Gate
Reading RG6 2AT
UK

Members

Prof. J.D. Bewley
Biology Department
University of Calgary
2500 University Drive N.W.
Calgary, Alberta T2N 1N4
Canada

Prof. H.F. Chin
Department of Agronomy
Faculty of Agriculture
University Pertanian Malaysia
Serdang, Selangor
Malaysia

Dr. A.G. Gordon
Economic Forestry Group
(Nurseries) Ltd.
Maelor Nursery
Connery Lane
Bronington
Whitchurch, Salop. SY13 3EZ
UK

Mrs. E. Pili-Sevilla
Seed Quality Control Services
Ministry of Agriculture
Bureau of Plant Industry
Manila
Philippines

Mr. R.D. Smith
Royal Botanic Gardens, Kew
Wakehurst Place
Haywards Heath
Sussex RH17 6TN
UK

Dr. P.C. Stanwood
National Seed Storage
Laboratory
Colorado State University
Fort Collins, Colorado 80523
USA

Dr. J.T. Williams
International Board for Plant
Genetic Resources
Food and Agriculture Organization
Via delle Terme di Caracalla
00100 Rome
Italy

APPENDIX II

AGENDA

I. Availability of Material (must be assured, to whom?)

II. Storage Standards for Base Collections: acceptable, preferred.

1. Safety of installation for seeds

   1.1 Power supply, including back-up arrangements
   1.2 Fire precautions
   1.3 Security, flood, earthquakes, civil unrest, etc.
   1.4 Refrigeration standards and equipment
   1.5 Construction and insulation standards

2. Safety of personnel

   2.1 Protective clothing
   2.2 Procedures for work in cold store
   2.3 Precautions (alarms, exits, emergency lighting)

3. Storage containers

   3.1 Types
   3.2 Quality tests

4. Storage temperature

   4.1 Limits
   4.2 Monitoring arrangements (alarms)

5. Seed moisture content

   5.1 Limits
   5.2 Methods and equipment for moisture content determination

6. Accession sizes

   6.1 Genetically homogeneous accessions
   6.2 Genetically heterogeneous accessions

7. Regeneration

   7.1 Standards (viability percentage at which regeneration is instituted)
   7.2 Methods (How many seeds? Isolation. Biparental mating, etc.)

8. Viability monitoring

   8.1 Responsibility (who has it if material is held at two or more centres?)
   8.2 Germination test principles (definition of viability, empty seeds, dormancy, etc.)
   8.3 Method for initial germination test (number of seeds, etc.)
   8.4 Viability monitoring period
   8.5 Methods for viability monitoring test (sequential, fixed size)
   8.6 Equipment for germination tests, detection of empty seeds, removing dormancy)

9. Handling before storage

   9.1 Handling procedures before storage
   9.2 Drying procedures and equipment
   9.3 Seed cleaning, purity and equipment

10. Information about sample

11. Seed testing laboratory

   11.1 Standards arising from 5, 8 and 9
   11.2 Genebank personnel (seed physiologist, estimate of work load, technical assistance)

12. Arrangements for duplicating samples (in another IBPGR designated centre/other)

13. Standards for liquid nitrogen storage

III. Standards for Active Collections

1. Unsealed storage (temperature, RH)
2. Sealed storage (temperature, moisture content)
3. Monitoring tests and intervals
4. Standards for exchange and distribution

IV. Registration of Genebanks

V. Other business

APPENDIX III

MAINTAINING VIABILITY DURING COLLECTING EXPEDITIONS

by

R.D. Smith

This paper accepts that seed collecting, like politics, is the art of the possible. Thus a framework of information is offered which collectors can balance against the practical constraints which will become dominant in the field. Hopefully, this advice will allow viability losses in the field to be minimised and the effectiveness of the mission to be maximised. However, much of the following advice is unproven and collectors' comments upon it will be most welcome.

The problems facing collectors in their attempts to maintain seed viability in the field will be determined by:

1. The maturity of the seed at harvest: relatively immature seeds having much higher moisture contents than mature seeds.

2. The climate of the region in which the collections are being made.

3. The logistics of the expedition.

Before each of these problems can be considered and advice offered on how they may be overcome, it will be useful to briefly reaffirm that the factors controlling the maintenance of seed viability are temperature, moisture content, gaseous environment and time.

Temperature

For the vast majority of species, it is true that at all moisture contents the higher the storage temperature above 0°C the shorter will be the lifespan of the seeds. However, for a very few species such as the Dipterocarps of Southeast Asia, sudden seed death will occur below temperatures close to 14°C. Fortunately, there is no record of such behaviour of the seed of the major crop plants.

All seeds can experience sudden death at temperatures below 0°C due to chilling injury if their moisture content is sufficiently high. Lower temperatures are needed to bring about the same chilling injury if the moisture content is reduced. For example, in barley, chilling injury will occur at 0°C if the seed has a moisture content of 25%: at -10°C if the seed has a moisture content of 20% and at -20°C if the seed is at 16% moisture content. Thus, attempting to preserve the viability of any seed of unknown moisture content by cooling is potentially dangerous and should not be undertaken unless the seed is known to be sufficiently dry.

Moisture Content and Gaseous Environment

The effects of these factors can be presented schematically in Figure 1.

At moisture contents above the critical level (position C around 18% m.c. +) the gaseous environment exerts a major effect with the availability of oxygen determining whether or not sufficient respiration is taking place to power the repair of any accumulated biological damage.

Storing high moisture content seed in relatively gas tight containers will result in the rate of loss of seed viability moving from position A to position B in the figure as the seeds respire and consume the available oxygen, effectively replacing the aerobic environment with an anaerobic one. However, if the build-up of anaerobic conditions is avoided by holding seed in containers which allow the free diffusion of oxygen to the

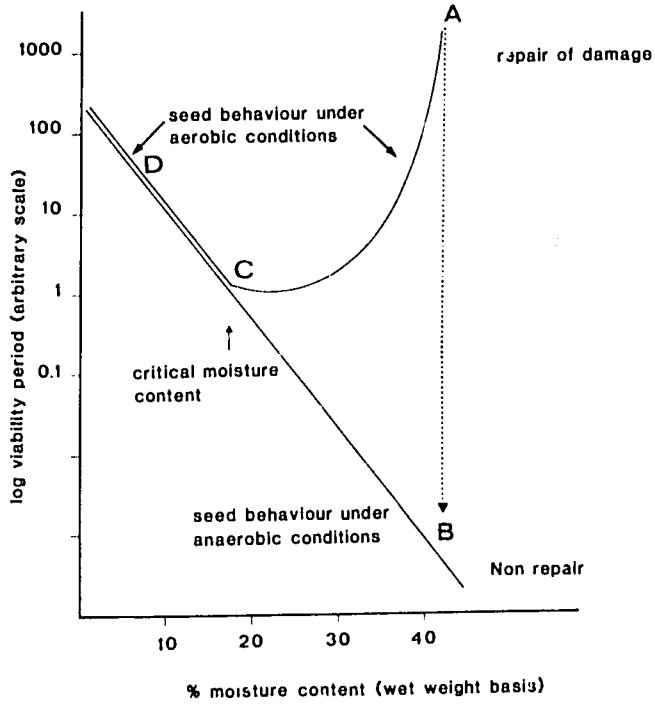

Figure 1.

seed (preventing the build-up of anaerobic conditions) water will be lost from the seed until its moisture content is in equilibrium with the surrounding air. Figure 1 shows that allowing the moisture content to fall from A to C will speed up the loss of viability; only further drying from C to D will again increase seed longevity.

Thus the problem for the seed collector is either to maintain seed at moisture content A or to dry to moisture content D as speedily as possible but without using heat which will in turn speed up loss of viability.

The Influence of the Maturity of the Seed

In barley, the harvesting of green, relatively immature grains does not affect their subsequent storability when compared with that of mature grains. There is no evidence to suggest that this is not also the case in other species. Thus, collecting relatively unripe seed is useful. However, Figure 2 suggests that there may be practical problems in the subsequent handling of the seed.

Taking the dry weight of the seed as a measure of maturity, then at 45 days the seed is very nearly fully developed yet it still has a moisture content of 30% and can be assumed to be moist enough to repair. Thus, provision will have to be made to ensure that the seed is maintained in an aerobic state. At 50 days, the seed has dried to around the critical moisture content where the rate of loss of viability is maximal irrespective of gaseous environment. Thus, the seeds will need to be dried further at some speed to maintain viability. At 60 days, the moisture content has fallen further and thus the need for urgent drying has fallen to what may be an acceptable level.

A similar pattern of seed development is considered typical for the cultivated crops, such as grains and pulses which have been selected so that their seeds do not disperse themselves. Amongst the wild relatives of these crops, however, self-dispersal still occurs. Direct measurement of seed moisture content at the time of shattering

(Table 2) shows that when the seeds are still to be found on the plant, the seed moisture contents are close to or in excess of the critical moisture contents, making them vulnerable to damage caused by the adoption of inappropriate collecting techniques. When seed dispersed inside fruits or cones are considered, the seed moisture contents are such that the seeds will always be in the repair mode and therefore in need of oxygen. As was said earlier the problem for the collector is either:

(a) to maintain the seeds aerated at high moisture content so that they repair any damage; or

(b) quickly dry the seed to a sufficiently low moisture content to reduce the rate of loss of viability to tolerable levels.

Option (a) can only be applied practically to seeds dispersed inside fruits and cones where the seeds exhibit dormancy (e.g. Malus, Pyrus and members of the Solanaceae). Seeds of cultivated pumpkin on the other hand, can and do germinate readily within the fruit.

The logistic problem of collecting and holding mould-free and aerated, the fruits of anything larger than a Solanaceous berry are extremely problematical. Whilst there will be some further development of this possibility later, the greater part of this paper is devoted to developing appropriate portable field techniques for seed drying.

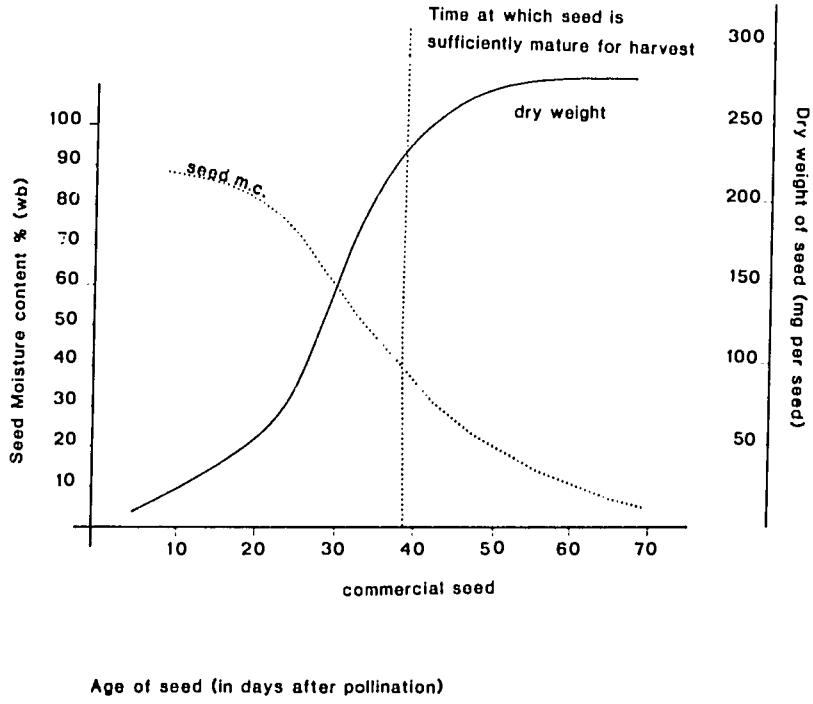

Figure 2.

Table 2. Direct measurement of seed moisture content at time of shattering

Genus	Location of collection	Condition	Moisture content (%)
Dactylis glomerata	UK	Just mature.	28.3
		2 weeks later	15.1
Melica uniflora	UK	Shattering	38.4
Bromus sterilis	UK	Shattering	54.2
Bothrioclina fusca	Kenya	Air dried in showery weather	30.9
		Sun dried 3 days	5.9
Echinopsis amplexicaulis	Kenya	At harvest	43.2
		1 day sun drying 4 hrs	8.0
		In cotton bag in vehicle	19.1
		Sealed over silica gel	12.0
Pithecellobium	Brazil	Pod brown but unopened	44
		Pod open, seed still attached via funicle	15
Lathyrus sphaericus	UK (glass house)	Pod ripe on point of explosive dispersal	16.1
Lathyrus sp.	UK (glass house)	Pod ripe on point of explosive dispersal	14.3
Nicandraphysaloides	UK	Green pod/white seeds	47
		Light brown pod/green seeds	32.8
		Pod brown and dry seeds brown	29.0
Coffee	Kenya	Commercial harvest	40
Apple	UK	At harvest	45
Cherry	UK	Sold as fruit	

Table 2. Direct measurement of seed moisture content at time of shattering (cont'd.)

Genus	Location of collection	Condition	Moisture content (%)
Cherry (continued)		Stone	40
		Embryo alone	60
Tomato		Commercial seed extraction	50
Agathis macrophylla	Solomon Islands	On cone shattering	67
Pumpkin	UK	Ripe fruit at maturity	80

THE EFFECTS OF THE AMBIENT CLIMATE DURING COLLECTING

Mature seeds are hygroscopic: that is, they will either take up or give out moisture to the surrounding air until the relative humidity of the air and the seed moisture content are in equilibrium. Thus meteorological data of mean monthly temperatures and relative humidities can be combined with equilibrium moisture content curves to provide temperature and moisture content values for substitution in the appropriate viability equations. Thus, the relative adversity of the ambient collecting conditions can be identified. Barley and onion seed values are used in the examples presented in Table 3 as the most inherently long-lived and inherently short-lived of seeds so far identified. Thus, barley will represent the best case and onion the worst.

Such a procedure is necessarily a gross approximation based on the assumption that the seeds are constantly in equilibrium with the surrounding air. In the light of the previous section, this need not necessarily be so at harvest. However, the patterns of loss of viability calculated in this way closely mirror the observed losses under open storage conditions. The results are useful for comparison, at least, where the errors should cancel out. Two comparisons can be usefully made.

The first is between the calculated field losses and those which could be expected in the controlled drying facilities of a recipient genebank. IBPGR currently recommends such facilities to run at 15°C and 15% RH under which conditions onion and barley could be expected to lose 0.005 σ units and 0.0005 σ units per month respectively. Comparison of these figures with those calculated for field conditions suggest that even the best field conditions observed at harvest time (Thessaloniki) are 20-60 times worse. Thus, as much damage will be done in a day or less under field conditions as is done in a month under dry room conditions. Thus, the need for maintaining seed viability in the field can be seen.

Such figures also have value in "calibrating" the adversity to seed preservation of areas and climates of which the collector has little or no previous experience. If the expected field losses for the month of harvest, the most likely collecting time, are expressed as a ratio of one another, a collector who has experience in making successful collections in one environment can calibrate the others against it. For example, if the collector had experience in collecting around Cambridge then when collecting around Thessaloniki twice as much time could be taken as in Cambridge without any more adverse effects. However, in Kathmandu, the same objectives would have to be achieved in half the time.

By addition of the losses for the intervening months, these figures can also be

Table 3. Effects of the ambient climate during collecting

Month	temp. °C	RH %	Onion m.c.	Onion σ loss/month	Barley m.c.	Barley σ loss/month
Cambridge, UK						
J	3.5	90.5	19+	"repair"	18.8	.16
F	3.9	88.5	18.1	O_2 needed	17.6	.10
M	6.1	85.0	16.0	.09	16.3	.08
A	8.7	74.0	12.2	.04	13.7	.04
M	11.9	75.0	12.5	.08	13.9	.06
J	15.1	75.0	12.5	.09	13.9	.08
J	17.0	76.5	12.9	.15	14.2	.13
A	16.7	79.0	13.6	.17	14.6	.15
S	14.4	82.5	14.7	.16	15.6	.16
O	10.4	86.0	16.7	.16	16.6	.14
N	6.7	89.5	18.8	"repair"	18.3	.16
D	4.5	91.0	19+	O_2 needed	19.3	.19
Thessaloniki, Greece						
J	5.7	78.0	13.3	.04	14.2	.05
F	7.1	70.5	11.4	.03	13.3	.02
M	9.8	70.0	11.2	.02	13.1	.02
A	14.6	66.5	10.6	.05	12.6	.04
M	19.5	65.0	10.4	.08	12.4	.08
J	23.9	58.0	9.6	.13	11.4	.08
J	26.7	55.5	9.4	.17	10.8	.08
A	26.4	56.5	9.5	.17	11.3	.10
S	22.4	63.5	10.2	.14	12.3	.11
O	17.1	71.5	11.6	.10	13.4	.10
N	12.3	77.5	13.2	.09	14.1	.07
D	7.3	79.5	13.8	.05	14.6	.05
Kathmandu, Nepal						
J	10.1	79.5	13.8	.08	14.6	.07
F	11.8	79.0	13.5	.09	14.5	.07
M	16.2	63.0	10.0	.05	12.2	.04
A	20.2	61.0	9.9	.08	11.9	.07
M	24.9	66.5	10.7	.21	12.7	.19
J	24.6	75.5	12.5	.35	13.9	.26
J	24.6	84.0	15.5	.75	16.0	.75
A	24.4	85.5	16.4	.95	16.5	.85
S	23.8	84.5	15.8	.72	16.3	.70
O	20.2	84.5	15.8	.45	16.3	.46
N	15.4	84.0	15.5	.22	16.0	.20
D	11.2	81.0	14.3	.12	15.0	.10
Douala, Cameroon						
J	26.4	84.5	15.7	1.0	16.2	1.06
F	26.7	85.5	16.5	1.2	16.5	1.1
M	26.4	85.5	16.5	1.2	16.5	1.1
A	26.4	85.0	16.0	1.05	16.3	1.0
M	26.4	87.0	17.3	1.45	17.0	1.26
J	25.3	88.5	18.3		17.7	1.21
J	24.2	91.0	19+	repair	19.0	1.70
A	24.2	90.0	19.0	O_2 needed	18.8	1.60
S	24.7	89.5	18.7		18.5	1.56
O	24.6	89.0	18.5		18.0	1.21
N	25.8	87.5	17.7	1.45	17.3	1.18
D	26.1	86.5	16.8	1.24	16.7	1.05

used to estimate the likely accumulation of damage since harvest and thus decisions on whether market samples are worth taking, can be made rationally.

The question now arises: What are acceptable losses of viabililty in the field? Only when a greater body of collecting experience has been gained will a rational and practically achievable level be capable of being set. Any value developed now will necessarily only be a vague estimate.

In light of the field losses expected around Thessaloniki and Cambridge a level of 0.10 probits per month is suggested.

Inspection of the calculated field losses, coupled with a consideration of the seed size of the target species will quickly show whether or not active drying is necessary. Provided the seed size is as small or smaller than onion seed it will equilibrate to the moisture content value used in the calculation within one day even if it is at 30% moisture content on harvest.

Larger seeds equilibrate much more slowly. Seed with sizes between that of onion and barley will still need to be dried using silica gel even if the equilibrium moisture content would be acceptable, to eliminate the risk during the drying process of damage to immature seed harvested at high moisture contents. One kilogram of silica gel will be needed to dry each kilogram of seed harvested to lose moisture contents which will reduce viability losses to tolerable levels. This drying should occur within 24 hours.

Seeds larger than barley grains and harvested at 30% moisture content cannot be dried to the tolerable ambient level within an acceptable short time without using large amounts of silica gel (14 kg silica gel/1 kg seed). Thus successful drying during the collecting mission for seeds larger than barley grains appears impossible. The only practical solution appears to be to reduce the time spent under these adverse conditions by removing them to a base station (either temporary or permanent) with proper drying facilities. This idea is developed further in the consideration of logistics.

Where the field losses are calculated to be unacceptable, the same drying procedures will need to be used for all seed smaller than barley grains.

All the drying calculations have been made assuming that the seed is in a thin layer to allow the unimpeded diffusion of the water vapour. Thus, collectors keeping the seed in relatively impermeable packets such as those made from glazed filled paper or densely woven fabric will interfere with this process and slow it down. Thus, the damage accumulated will be greater than need be.

Allowing the seed to form a densely packed mass will also slow the process by impeding diffusion. Therefore seed collectors will need to ensure that these adverse practices are reduced to a minimum if they wish to ensure that the seed dries to the tolerable moisture content within 24 hours.

One possible way to overcome this would be to place alternate layers of packeted silica gel with packeted seed. Perhaps more conveniently, specialist back-packs need to be designed to enable this alternation to take place conveniently. When silica gel absorbs water it gives off a considerable amount of heat of absorption. Therefore during the drying period the seed and silica gel should be kept in such a way that this heat is easily dissipated, thus avoiding any significant increase in temperature.

Rapid drying is necessary if the amount of silica gel required is to be kept to a minimum by exploiting its capacity for regeneration.

Once 1 kg of seed has been dried to the tolerable level within the 24 hour period by 1 kg of silica gel, the dried seed can be removed from the silica gel and packed tightly into a heavy duty (500 gauge) polythene bag which can be kept sealed by doubling

over the top twice and holding with adhesive tape. Possible further additions can then be easily made. If a cobalt chloride RH test paper is included with the seed, the collector can be sure that a tolerable seed moisture content is maintained by monitoring the internal RH. A tolerable level will be maintained if the internal RH does not exceed 50%.

The exhausted kilogram of silica gel will become available for regeneration over the next 24 hours before the whole process is repeated. Thus, if collectors can predetermine their expected daily haul of seed which are of similar size or smaller than barley, then double that weight of silica gel will need to be taken to ensure adequate drying.

It has been suggested that dried cereals or pulses could be used as desiccants on collecting missions, allowing for local purchase followed by abandonment. Unfortunately, their desiccant power is approximately half that of silica gel, thus twice the weight will be required. The problems associated with the increased weight suggest that this approach will be less practical than carrying silica gel.

Problems of Fungal Activity During The Collecting Mission

The effects of fungicidal treatment on subsequent longevity have not been extensively investigated. Where good evidence is available, all treatments of the fungicides investigated were immediately damaging. Thus they should not be used unless this is a specific requirement in order to obtain a quarantine certificate. Provided that the moisture content of the seed is kept to the tolerable level, the available evidence suggests that neither fungal nor bacterial activity will occur in the dried seed and thus should present no problems.

The Problems of Insect Infestation

The conventional wisdom is that those species of insect which develop within seeds and emerge, are not capable of reinvading intact seed. Thus they should not present a threat to any collection. However, some claims have been made that this may not be so for some of the tropical species of insect.

Invasion of the collections by those species which are storage pests is unlikely if the seed is kept at the tolerable moisture levels recommended here.

As with fungicides the effect of insecticides on subsequent seed storage is not fully investigated. Therefore their use should be avoided. Should there be a need to destroy the insects this can be achieved by exposing the seeds to deep-freeze sub-zero temperatures, provided that the seeds have been dried to the tolerable level. The developing and mature insects (though not their eggs) should be killed by this process within one week.

Exploiting Encounters (if any) with Refrigerators or Deep-Freeze Facilities en Route

Once the seed has been dried to the tolerable level, no risk is attached to lowering the seed to deep-freeze temperatures. Thus, if such facilities were encountered, it would be possible to cool the bags of dried seed down to low temperature levels overnight. If these cooled seeds were subsequently placed in insulated containers, then they may well take sufficient time to rewarm to the ambient to justify the effort. Fortunately, the lightweight air freight containers made from expanded polystyrene have good insulation properties allowing them to be used to bring collecting materials into the country and as seed containers during and after the mission. Their insulation properties should be improved if they are lined with reflective mirror foil of the kind used in and easily obtainable as survival blankets.

It should be remembered that in the majority of tropical and sub-tropical countries, even air at 50% RH, as inside the dried seed bank, holds sufficient water that cooling it to just refrigerator temperatures will saturate the air and condensation must occur. This will be seen as a slight fogging on the inside of the bag. However, the absolute amount of water to condense will be so small that when it is taken up by

the seed it will only marginally increase the seed moisture content and this can be ignored provided the bag is properly sealed to prevent continuous diffusion into the bag (and hence the seed) of the air within the refrigerator.

LOGISTICS

The preceding considerations have kept time constant at one month and adjusted the moisture content to limit viability losses to tolerable levels. However, time is just as important a variable in determining expected losses of viability as temperature and moisture content. Thus, where the logistics of the mission are such that they will allow time for the seed to experience alteration of ambient conditions, then alternative strategies can be considered which negate the need for field drying with its attendant problems of regenerating the silica gel.

If this is the case, it becomes useful to estimate the number of days at ambient conditions which will reseult in a loss of viability of 0.10 probit units. From Table 3 it can be seen that for the harvest month of July in Kathmandu, this will be around four days for both barley and onion. Around Cambridge this will be approximately 20 days for both. Thus, in climates similar to that of Cambridge, the time between harvest and arrival at a controlled drying facility can be kept down to around two weeks (erring on the safe side to allow for immature harvesting of seeds at moisture contents above the expected value) then the damage will be the same as if the seed had been dried to the tolerable level and retained by the collectors for a month. Such an approach does not appear impractical.

In Kathmandu, this solution appears to be unsatisfactory in comparison to field drying for trips of one month. However, if the collecting mission is to be away from base for three months, then the allowable period at ambient conditions to accumulate similar damage becomes 12 days. Only an investigation of the proposed route, the frequency of internal airline services, the availability of both porters and reliable air courier services will determine whether such a system may be feaslble.

It is perhaps the combination of the two possible approaches which offers the best solution for the preservation of seed viability under field conditions of:

a) seeds greater in size than barley seeds where drying will be prohibitively slow and involve considerable weights of silica gel;

b) seeds as small or smaller than barley in remote climatically adverse locations;

c) seeds borne inside fruits and therefore probably above 30% moisture content when harvested.

The combined system would involve a portable base which remained in a single location for about a week. This base would be responsible for the seed extraction, seed drying and despatch. The seed collectors would then be free to make forays into even more remote locations free from the need for field drying. Instead, they would be responsible for the speedy return of the seed to the base in order to avoid the need for drying. Such a configuration at least then makes possible any attempts to develop thin layer sun drying, always assuming that the damage accumulated by sun drying will be less than that achieved when drying by silica gel.

Transport of intact fruits containing the necessary seeds, followed by cleaning at base, appears more convenient than field drying.

The need to restrict drying times to 24 hours does not become so acute. The possibility of finding locations where the operation of specialist equipment to further reduce losses of viability becomes more possible.

If none of the suggestions so far made appear to be practical, the only possible solution for seeds of a size greater than barley at moisture contents well in excess of

30% appears to be to reduce the weight of the actual material collected each day. This could be achieved only if accurate sampling of the majority of genetic diversity was assured, by making collections of one seed from each of some 50-100 parent plants. For safety's sake, this should perhaps be done in triplicate. The smaller seed collections could then be placed over 2 kg of silica gel and dried for one week. In such a way the total amount of silica gel which the collectors need to take with them would be kept within a "reasonable" level.