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Conservation of forage germplasm with an emphasis on application of tissue culture techniques in a genebank

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Introduction

Inadequate nutrition in livestock, resulting from feed shortages and the poor quality of available feed is the major constraint to increased livestock production in sub-Saharan Africa. Forage crops play an important role as animal feed resources and germplasm is being tested to select adapted forages for further development and use by small-holder farmers in Africa. A large pool of germplasm, which can be used for evaluation and selection, is essential for development of improved forages for animal feeds.

Conservation of germplasm has traditionally relied to a large extent on storage of seeds in genebanks. However, some forage species may take a long time to reach maturity and produce seeds or, in the case of grasses, have inadequate production of seeds for collection, multiplication, dissemination and utilization. *In vitro* culture techniques have a potential use for those tropical forage legumes and grasses which cannot be conveniently handled by conventional methods as an alternative method to field genebanks for genetic resources management.

Seed storage

The aims of good seed storage are to control loss of vigour and viability of the seeds and prevent infestation from diseases and insects. The length of time that a seed can remain vigourous and viable depends on the genotype, species, physiological state of the seed at the start of storage and the storage conditions.

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The two major environmental factors affecting longevity during storage are seed moisture content and temperature. Both work independently in their effect on longevity, but for orthodox seeds which can be dried, normally the lower the moisture content of the seeds and lower the storage temperature, the longer the seeds will maintain vigour and viability. A general guide to the effects of seed moisture content and temperature during seed storage were proposed by Harrington (1970). Each 1 % decrease in seed moisture content below 14 % and down to about 4 % moisture content doubles the life of the seed. Similarly longevity is doubled for every decrease of 5° C in temperature at temperatures between 50° and 0°. Experiments show that these simple rules provide a useful guide to estimate longevity during seed storage. More precise ways of estimating longevity have also been developed by Roberts (1972) by the use of a nomograph and formula, which take into account the major factors affecting longevity during storage.

Seed drying is probably the most important of these two factors. The moisture content of seeds is always in equilibrium with the ambient relative humidity of the store and therefore fluctuates. Seeds may be dried in dehumidified rooms or by using hot air. However, care must be taken not to damage seeds by using too great a temperature during the drying process. Once dried, seeds need to be stored in sealed containers to prevent them reabsorbing moisture from the surrounding air.

Storage of germplasm in genebanks requires very stringent conditions of low seed moisture content and low temperature to maintain seed viability and prevent deterioration of seeds, which may lead to genetic changes. A sufficient number of seeds of each accession must be stored to adequately represent the genetic variation of the accession and also allow sufficent seeds for monitoring and distribution. IBPGR has recommended that for materials showing little morphological variation 3000 seeds per accession are acceptable, but 4000 seeds are preferable. For heterogeneous accessions, a minimum of 4000 seeds per accession should be stored and 12000 seeds per accession are preferable.

Germplasm which is being used for distribution or in plant breeding programmes may need to be stored for several years in active collections. For active collections IBPGR have recommended that seeds should be dried to 7 % moisture content or less, sealed in moisture proof containers and stored at temperatures of less than 15°C. Seeds stored under these conditions should maintain viability for a minimum of 15 to 20 years. For long-term security storage IBPGR has recommended that seeds should be dried to between 3 % to 7 % moisture content, sealed in airtight containers and maintained in freezers at - 18°C or less. Under these conditions it is expected that viability can be maintained for much longer periods.

Table 1. Forage germplasm in the ILCA genebank

	Browse	Grasses	Legumes
Number of genera	119	59	58
Number of species	334	185	377
Number of accessions	1 607	2 661	6 854

Table 2. Major genera in the ILCA genebank

genus	no of accessions	genus	no of accessions
1. Legumes			
Alysicarpus	172	*Medicago*	224
Cassia	18	*Melilotus*	43
Calopogonium	39	*Neonotonia*	259
Canavalia	13	*Onobrychis*	28
Centrosema	326	*Ornithopus*	8
Chamaecrista	66	*Pisum*	126
Clitoria	25	*Phaseolus*	283
Crotalaria	182	*Pueraria*	7
Desmodium	166	*Rhynchosia*	137
Indigofera	153	*Stylosanthes*	1 127
Lathyrus	164	*Tephrosia*	116
Lablab	184	*Teramnus*	65
Lotononis	7	*Trifolium*	1 598
Lotus	28	*Vicia*	253
Lupinus	53	*Vigna*	417
Macroptilium	75	*Zornia*	255
2. Grasses			
Aristida	8	*Hyparrhenia*	38
Andropogon	44	*Lolium*	74
Agropyron	24	*Melinis*	15
Brachiaria	650	*Panicum*	139
Bromus	19	*Paspalum*	63
Cenchrus	87	*Pennisetum*	128
Chloris	103	*Phalaris*	22
Cynodon	28	*Phleum*	10
Dactylis	24	*Poa*	5
Digitaria	44	*Setaria*	67
Eragrostis	34	*Sorghum*	57
Echinochloa	22	*Urochloa*	31
Festuca	38		

Table 2 (continued). Major genera in the ILCA genebank

genus	no of accessions	genus	no of accessions
3. Browse			
Acacia	157	*Erythrina*	57
Aeschynomene	130	*Faidherbia*	12
Albizia	26	*Flemingia*	6
Atriplex	46	*Gliricidia*	89
Cajanus	157	*Leucaena*	174
Calliandra	6	*Prosopis*	13
Cordariocalyx	27	*Pseudarthria*	18
Chamaecytisus	38	*Sesbania*	291
Desmanthus	113	*Teline*	13

Forage seed storage at ILCA

The ILCA Forage Genetic Resources Section works with the acquisition, maintenance, characterisation and distribution of forage germplasm as part of the theme on resource assessment and services in the Animal Feed Resources Trust. The genebank holds more than 11,000 accessions from 896 species of 236 genera (Table 1) with a wide representation of the major genera identified for potential forage use (Table 2). About 40 % of this germplasm was collected by ILCA in co-operation with other institutes in Ethiopia, Kenya, Tanzania, Niger, Rwanda, Burundi, Zaire, Mali and Nigeria. The remaining germplasm was donated to ILCA by other major forage research institutes, particularly the Centro Internacional Agricultura Tropical (CIAT) in Colombia and the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia.

The ILCA genebank holds both an active collection for research and distribution of seeds and a base collection for long-term security storage. ILCA adheres to international standards for the conservation of seeds in genebanks (IBPGR, 1985) as part of Consultative Group on International Agricultural Research (CGIAR) policy on plant genetic resources (CGIAR, 1989).

The seeds are first dried to 5 % moisture content by spreading them in thin layers in porous containers in a dehumidified drying room of 45 m^3 at 20 % relative humidity and 15°C. Under these conditions it takes about two weeks for the seeds to reach the desired moisture content. Seed moisture content is determined by a modified International Seed Testing Association (ISTA) oven dying method (ISTA, 1985). ISTA recommendations are followed for determining the temperature of the test and for deciding if grinding of seeds is required, but the amount of seed material used is reduced to 0.5 g and the degree of accuracy on weighing increased by using an

analytical balance. This method is preferred to minimise the numbers of seeds destroyed by the test and is especially important in some small seeded species, such as *Trifolium*, where thousand seed weight is as low as 1 g. For those species not included in the ISTA rules, the low temperature method is used for oily seeds or whenever there is doubt.

After drying, the seeds are sealed in laminated aluminium foil bags to prevent re-absorption of moisture from the air. Quantities of up to 1 kg can be sealed in each bag, which is labelled with the accession number, lot number, genus and species names and the weight. The bags are sealed using a corrugated seal, foot-operated heat sealer. The bags of seeds are arranged in accession number order in cardboard tidy bins for storage in the cold room.

The active collection is stored in a large walk-in cold room of 150 m^3 at 8°C on movable shelving to economise on space. The cold-room is dehumidified to 30 % relative humidity so that seeds which are stored in paper or cloth bags awaiting drying and large bulk quantities of seeds can be stored without problem. The base collection is stored in freezers at -20°C.

Conservation in field genebanks

Several forage species rarely produce viable or full seeds or are sterile hybrids. These species are usually conserved as plants in field genebanks. This is the case with some important genera of forage grasses, including *Cynodon*, *Digitaria* and *Brachiaria*. Plants which are stored in field genebanks require considerable space, careful management, are expensive to maintain and are at risk from pests and diseases.

The field genebank is located at Zwai in the Ethiopian Rift Valley at the Forage Genetic Resources Unit seed multiplication site. The site is situated at 1 650 m altitude on slightly basic soils of pH 8.4. Irrigation is available from river water, so that the materials are fairly secure. The field genebank collection currently comprises more than 300 accessions of *Brachiaria* species which were collected between 1984 and 1985 in Africa on joint CIAT-ILCA collection missions. Seed production in these materials has been poor, although it is hoped that they will eventually produce seed for longer term storage.

Use of *in vitro* culture for germplasm management

Over the last decade *in vitro* plant culture has proved invaluable to crop plant improvement, providing solutions and alternative approaches to overcome constraints in management of genetic resources. *In vitro* techniques have been used for germplasm collection (IBPGR, 1984; Withers, 1987), disease elimination (Kartha, 1981,

1986), micropropagation (Hussey, 1983), germplasm dissemination and conservation (Withers, 1980). *In vitro* techniques have also been used for the creation of variability through somaclonal variation (Larkin & Scowcroft, 1981; Scowcroft, 1984), *in vitro* pollination, fertilisation, embryo culture, somatic hybridisation (Melchers, 1982; Melchers *et al.*; 1978) and genetic transformation (Wullems *et al.*, 1982). Haploid plants which are invaluable for plant breeding can be produced from *in vitro* anther and pollen culture (Sunderland & Cocking, 1978; Sunderland, 1979). Forage species have received much less attention but investigations in *in vitro* methods for management of forestry species (Hartney & Kabay, 1985; Bonga and Durzan, 1982) and on forage grasses at the Welsh Plant Breeding Station (Dale, 1978; 1980) and at ILCA (Ruredzo & Hanson, 1990) have shown that these techniques are equally useful in management of forage genetic resources and plant improvement.

Many of the early concerns about the stability and genetic integrity of plant *in vitro* cultures have now been resolved. Plants which are recovered from the non-adventitious growth of shoot tips, axillary buds, meristems, embryos, and gametes have been found to be genetically stable (Scowcroft, 1984). Plants which are directly regenerated from non-axillary regions of the plant are also stable, although their genotype will depend on the mother tissue (D'Amato, 1975). Indirect regeneration through a callus stage is believed to release somaclonal variation giving rise to genetic variants from the same tissue, which can include enhanced desirable characteristics (Larkin & Scowcroft, 1981). Plants which are recovered from these therefore need to be assessed for genetic stability and enhanced desirable characters.

In vitro culture research at ILCA

Work on *in vitro* culture technology has been in progress at ILCA since 1986, when the International Board for Plant Genetic Resources (IBPGR) and ILCA agreed to collaborate in a two year project to develop minimal facility methods for *in vitro* collection and *in vitro* slow growth methods for the conservation of *Cynodon aethiopicus*, *C. dactylon* and *Digitaria decumbens*. A suitable basal medium for grass species was identified for the growth of these species *in vitro* and suitable exogenous growth substances (auxins, cytokinins and gibberrellins) and carbon source for normal rates of growth *in vitro* were determined. Cultures of the forage grasses *Cynodon* and *Digitaria* were successfully initiated, multiplied and rooted, and slow growth conservation techniques using low temperature were developed for the species (Ruredzo & Hanson, 1990). It is now possible to maintain cultures at 15°C for up to 18 months without subculturing and to recover them into normal growth conditions. A minimal facility method for the transfer of these species to soil was developed and a minimal facility method for the collection of *C. dactylon* using locally available sterilising agents, antibiotics and fungicides as supplements to the media to control contamination was also developed and tested.

Table 3. Distribution of forage germplasm

Year	No of samples distributed	No of African recipients	No of other recipients
1984	98	25	17
1985	1 889	100	20
1986	5 250	169	32
1987	5 423	191	42
1988	6 155	185	61
1989	6 824	213	7
1990	3 633	142	29

In 1989 the work was expanded to multipurpose tree legumes in a project supported by the International Development Research Centre (IDRC) to develop *in vitro* culture methodology for rapid multiplication. *Sesbania sesban*, *Leucaena leucocephala* and *Erythrina brucei* were successfully initiated *in vitro* from nodal cuttings. Adventitious shoot regeneration was induced from somatic material without pre-existing meristems from embryo-derived cotyledons, hypocotyls, and embryo axes of *Sesbania sesban* (Ruredzo & Hanson, 1990) and from embryo-derived explants in *Faidherbia albida* and *Acacia tortilis*. Non-adventitious regeneration was successfully achieved from shoot tips in *Erythrina brucei*, *Faidherbia albida* and *Acacia tortilis*.

Distribution of germplasm

All seeds in the ILCA genebank are freely available in small quantities to *bone fide* forage research workers. This is done both directly and through networks, such as the African Feed Resources Network. Each year more than 5 000 seed samples are distributed in response to requests (Table 3), mostly in sub-Saharan Africa.

Duplication

ILCA has agreed to participate in the IBPGR global network of crop germplasm collections and has accepted the responsibility to maintain global collections of *Cenchrus*, *Digitaria* and *Lotononis* and African collections of *Neonotonia* and *Trifolium*. An approach has been made from CIAT to duplicate forage germplasm between the two institutes. Security duplication of accessions of *Neonotonia* species at the alternate base collection in the Royal Botanic Gardens, Kew in the UK commenced in 1987 and is on-going.

Seed multiplication and characterisation

Over the last four years, priority in the genetic resources work has changed from the collection and acquisition of forage germplasm toward the adequate characterisation and evaluation of the existing collection. Characterisation of germplasm is done whilst plants are in the field for seed increase. More than 2 500 accessions are handled in this way every year. Much of the collection is still represented by very small seed samples. Emphasis has been given to seed production activities to better characterise the material and make it available for utilization.

The gemplasm is multiplied at three sites in Ethiopia. Temperate materials are grown at the ILCA Headquaters site at 2 350 m altitude during the long rainy season from June to September. Tropical and sub-tropical materials are multiplied at two locations in the Ethiopian Rift Valley: at Zwai with a soil pH of 8 and an altitude of 1 650 m under irrigation; and at Soddo with a soil pH of 4.5 and an altitude of 1 850 m under rainfed conditions. Seed multiplication activities are carried out in close collaboration with the Herbage Seed Unit.

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