CONSERVATION OF TROPICAL FORAGE GENETIC RESOURCES

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ABSTRACT

Tropical forage genetic resources are essential material for the development of adapted pasture crops. Collection, conservation and characterization of these resources are needed to preserve them for future generations. Collections were made after 1970 by CIAT, CSIRO and ILRI. The current status of these collections and present conservation methods are reviewed. Genetic diversity has been assessed using morphological characterization in collections of *Stylosanthes, Zornia, Macroptilium, Centrosema, Desmodium, Arachis, Chamaecrista, Brachiaria, Panicum, Pennisetum*, and *Sesbania*. Molecular characterization of *Stylosanthes, Brachiaria* and *Pennisetum* was a useful technique to assess genotypic variation. Areas for future research include identifying geographic and biological gaps in the collections by combining the use of new tools such as geographic information systems and molecular biology. More research on seed storage is needed to determine the optimum storage conditions for long-term conservation. Targeted characterization towards the end use as livestock feed, cover crop or green manure will ensure full utilization of this valuable resource.

KEYWORDS

Tropical forages, genetic resources, conservation, collection, characterization, grasses, legumes, fodder trees

INTRODUCTION

Tropical forages are widely distributed in natural rangelands, along roadsides and crop fields, on fallow ground and in disturbed areas throughout the tropics. Forages are important not only for livestock feed but to improve soil fertility through nitrogen fixation and mulch, to improve soil texture and water holding capacity, and for soil stabilization and prevention of erosion. It is interesting to note that despite the wide acceptance of the value of forages as part of sustainable crop-livestock farming systems in the tropics, sown pastures are limited to few high potential areas such as Australia and the savannas of South America, and rely on a very limited genetic base of few species. There are over a thousand forage species found in natural pastures in the tropics, but less than 60 are used commercially for sown pastures for livestock production. Fodder trees are even more limited in their use with only leucaena (*Leucaena leucocephala* (Lamk.) de Wit) being used on a wide scale. Recently, the problems caused by the leucaena psyllid have prompted an expansion in the production of other species such as gliricidia (*Gliricidia sepium* (Jacq.) Kunth ex Walp.), calliandra (*Calliandra calothyrsus* Meissn.) and sesbania (*Sesbania sesban* (L.) Merrill). There is a wide pool of forage germplasm available for future selection of adapted genotypes for further development as livestock feed.

Forages show a wide range of diversity and adaptation to environment and eco-region. The major centre of diversity for both herbaceous and fodder tree legumes is tropical America (Williams, 1983). Several major forage species which are now widely used throughout the tropics have originated from this region. These include Caribbean stylo (*Stylosanthes hamata* (L.) Taubert), Brazilian stylo (*S. guianensis* (Aubl.) Sw.), Townsville stylo (*S. humilis* Kunth), shrubby stylo, (*S. scabra* Vogel), centro (*Centrosema pubescens* Benth.), *Desmodium* species (*Desmodium intortum* (Miller) Urb. and *D. uncinatum* (Jacq.) DC.) and pinto peanut (*Arachis pintoi* Krapovickas & Greg.) among the herbaceous species. The most commonly used

fodder trees originating in this area are leucaena, gliricidia and calliandra. Sub-Saharan Africa is the centre of origin and diversity of most of the warm season perennial forage grasses, which originated in the extensive rangelands in the sub-humid and humid lowlands of this region. These include gamba grass (*Andropogon gayanus* Kunth), signal grass (*Brachiaria decumbens* Stapf) and related species of *Brachiaria*, buffel grass (*Cenchrus ciliaris* L.), Rhodes grass (*Chloris gayana* Kunth.), couch grass (*Cynodon dactylon* (L.) Pers), pangola grass (*Digitaria eriantha* Steud.), Guinea grass (*Panicum maximum* L.), kleingrass (*Panicum coloratum* L.), Kikuyu grass (*Pennisetum clandestinum* Chiov.), Napier grass (*P. purpureum* Schumach.) and setaria (*Setaria sphacelata* (Schumach.) Moss). These species have now been widely spread throughout the tropics and have gained importance in areas other than their centres of origin. An example is *Brachiaria*, which is sown on over 50 million hectares in tropical America (Miles et al., 1996).

FORAGE GERMPLASM COLLECTION

Considerable emphasis was placed on the collection of germplasm to assemble diverse collections of tropical forage species for use in the development of tropical forages for sown pastures during the 1970's and 1980's by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Centro Internacional de Agricultura Tropical (CIAT), International Livestock Centre for Africa (now the International Livestock Research Institute (ILRI)) and International Plant Genetic Resources Institute (IPGRI) in collaboration with national institutions. This focus on collection added several thousand new accessions to the existing collections held in the genebanks of CSIRO, CIAT and ILRI (Schultze-Kraft, 1985; Hanson and Lazier, 1989; Reid, 1993). These three international genebanks together hold an estimated 30000 distinct accessions of the major forage species (Table 1). Germplasm was also left in the country of collection but much of this has been lost as a result of inadequate storage conditions and funds to maintain the material, or through civil strife.

After an early phase of exploration, collections were focussed on genera and species already identified as important cultivated forages to broaden the germplasm base for selection of new cultivars. Species specific expeditions include the Centro Nacional de Pesquisa de Recursos Geneticos e Biotecnologia (CENARGEN), CIAT and CSIRO collection missions for *Stylosanthes* in Brazil in 1977 and 1980, the CENARGEN and CIAT collection missions for wild *Arachis* species in South America in the 1990's (Valls and Pizarro, 1994), and the ILRI, IPGRI and Tanzanian Agricultural Research Organization (TARO) collection mission for *Sesbania* in Tanzania in 1987. Forage germplasm was also collected on a geographic basis from areas rich in biodiversity, which had not been properly sampled. Examples of this type of collection are the CIAT missions in South East Asia (Schultze-Kraft et al., 1989; 1993) and the IPGRI missions in sub-Saharan Africa from 1984 to 1991. Such broad collection missions are very cost effective, especially where institutions such as IPGRI had on site collectors, who were able to sample over wide areas. Despite this emphasis on collection, there are still many areas which have not been sampled because they are inaccessible due to terrain, war or cost of collection. Much of the early collection was done along the roadsides, therefore there is still a need to go into the more remote areas and also to do more complete sampling in areas of high diversity.

The geographic coverage of the material in the collections is also very broad with samples from tropical areas of Central and South America, South East Asia and sub-Saharan Africa. Geographic information systems (GIS) offer the opportunity to identify areas for further collection but which have largely been neglected. As the eco-physiological adaptation characteristics of forages are to a large extent reflected in the environmental conditions under which they were collected, GIS studies provide insight into potential regions both for collection as well as for adaptation of species. On the basis of climate data alone, Jones et al. (1996) predicted a high probability of occurrence of *Stylosanthes guianensis* in southern Peru and northern Bolivia, where it has not yet been collected. Lascano et al. (1995a) used information on adaptive characteristics of *Leucaena* and GIS information on soils and climates to prepare a map of potential areas for use of *Leucaena* in tropical America.

Although many areas have been well covered, most of the collections have focussed on the sub-humid and humid lowlands or tropical highlands. Collections in the tropical highlands have largely been limited to sub-Saharan Africa and little collection has been done in the tropical highland areas of Asia or tropical America. While the range of adaptation of some tropical legumes and grasses reaches well into the mid-altitude highlands, there is a shortage of available germplasm for use in the true tropical highlands, where there is an urgent need for herbaceous materials to use for soil stabilization and reclaiming degraded areas, and for fodder tree species for use both for livestock production and as fuelwood in fragile highland ecosystems. Other gaps in the collections are fast growing fodder tree species with high palatability for livestock which can be used to replace leucaena, and drought-resistant species which can produce quality feed for dry season feeding. A newly developed shrub legume with high drought tolerance and acceptable forage quality is *Cratylia argentea* (Desv.) O. Kuntze (Pizarro and Coradin, 1996).

CONSERVATION OF GERMPLASM
Forage germplasm is usually stored in seed genebanks. Seeds are maintained in security base collections for long-term conservation and in medium-term conditions for distribution to users. The common forage legumes have orthodox seeds, which can be dried and stored at low temperatures for long periods without loss of viability. Many of these tropical legumes have hard seeds, which require scarification to allow imbibition and germination. Souza and Marcos-Filho (1993) found a correlation between hard-seededness and physiological quality in *Calopogonium mucunoides* Desv. which may be responsible for increased longevity. There is little published information on the storage of forage legume seeds. However, *Calopogonium mucunoides, Centrosema pubescens, Desmodium heterocarpon* (L.) DC. subsp. *ovalifolium* (Prain) Ohashi, *Pueraria phaseoloides* (Roxb.) Benth., and *Stylosanthes guianensis* seeds showed little loss of germination after storage for 16 years at -1°C (Bass, 1984).

The major constraint to the maintenance of large collections of forages in seed genebanks is in the production of seeds of distinct genotypes. Many forage species are out-crossing and require isolation for seed production. Even some species of legume, which were previously thought to be selfing may have considerable outcrossing. In some *Centrosema* species, for example, outcrossing rates of more than 50% have been found (Maass and Torres, 1992; Penteado et al., 1996). Sufficient plants should be used to represent the variation in the population and to produce the quantity of seeds needed. The variation in genebank samples depends on the number of plants initially sampled and the breeding system. Maintenance of large collections is expensive and land requirements are high, especially

for fodder tree species. In addition, many of the major forages are perennial and do not produce seeds during the first year. Therefore seed production may be slow and extensive, leading to delays in regeneration and unavailability of accessions for distribution until seeds can be produced.

Forage grasses are more difficult to conserve. Several important forage species have seeds which are relatively short-lived, including Guinea grass (Harty et al., 1983) and signal grass (Whiteman and Mendra, 1982). This has led to the conservation of major collections of these species in field genebanks (Maass and Ortiz Escobar, 1996). Other species of grasses are shy seeders, rarely producing seeds or producing few viable seeds. These species, which include pangola grass and Napier grass are also usually stored in field genebanks. Alternative methods for the management of these species, which are difficult to handle as seeds, have been examined. Methods have been developed for *in vitro* collection, slow growth storage and dissemination of couch grass (Ruredzo 1991; Hanson and Ruredzo, 1992), and for the storage and dissemination of pangola grass and Napier grass.

In situ conservation offers an additional means of conserving the diversity of many forage species. Although no major reserves have been established with the primary aim of conserving forage species, many forages are conserved *in situ* in national parks in sub-Saharan Africa as part of ecosystem and wildlife conservation efforts in the region. Research has concentrated on the assessment of vegetation composition and diversity at the species and genus level. However, if *in situ* conservation is to be used in the future, more information is required on the intraspecific diversity, distribution of diversity within species, and the effects of controlled grazing by wildlife and domestic ruminants on the changes in diversity within and between species.

DUPLICATION AND SECURITY
The safe storage of forage germplasm in *ex situ* collections for future use in the development of forages for improving livestock production is a major concern to present and future generations. The Convention on Biological Diversity sets out the framework and policies on ownership of, and access to, biodiversity and the equitable sharing of benefits arising from the use of that biodiversity. Currently, germplasm held in national genebanks is under national legislation and exchanged under bilateral agreements, whilst forage germplasm held in international centres' genebanks is under a multilateral system, which works within the framework of international agreements (IPGRI, 1996).

The material in the Consultative Group of International Agricultural Research (CGIAR) genebanks was collected before the Convention on Biological Diversity came into force. This germplasm is held in trust for future generations and as such is freely available on request. In 1994, the CGIAR Centres agreed to place their collections under the auspices of the Food and Agriculture Organization of the United Nations (FAO) as part of the global network of *ex situ* germplasm collections. The CGIAR Centres have agreed not to claim legal ownership, nor to seek any intellectual property rights over that germplasm, nor related information. In order to ensure the continued free availability of germplasm, the Centres have also agreed to pass on the same obligations to all future recipients of this germplasm. The Centres have agreed to store the germplasm according to international standards (FAO/IPGRI, 1994), to make it freely available and to ensure its duplication for long-term security under this agreement with FAO.

There is substantial duplication of accessions between the major

genebanks with up to 30% of the material being duplicated in one or more location. Much of the forage germplasm held in national programmes is also actively duplicated in the genebanks of CIAT and ILRI. In addition these two Centres have a policy to duplicate the material for safety in other secure long-term storage conditions. However, germplasm held in "active" duplication may be subjected to genetic changes through selection or outbreeding during regeneration. Penteado et al. (1996) found that the same accessions of *Centrosema* species sampled from CENARGEN and CIAT showed large differences in genetic composition.

GENETIC DIVERSITY IN AVAILABLE COLLECTIONS

The amount of diversity in storage in these collections is only now being assessed as accessions are assembled from several genebanks and systematically studied for a range of characters. Characterization can be done on morphological characters to identify groups of morphologically similar individuals. Molecular methods can also be used to determine distinct genotypes, elucidate species relationships or identify genetic duplicates in collections. Users can select either all accessions of a cluster of a specific type of direct interest or take one or two accessions from each cluster to screen a wider range of morphological variation.

The first characterization of tropical forages was done by Edye et al. (1974), who classified 287 accessions from 17 species in the stylo collection assembled by CSIRO for 35 morphological and 11 agronomic characters. Fifty-three distinct groups of accessions were identified, all except three groups being monospecific, indicating the distinctiveness of species within this genus. A collection of *Stylosanthes fruticosa* (Retz.) Alston was characterized using 18 morphological and agronomic characters. The 93 accessions were clustered into ten groups on form, leaf shape and spike length (Hakiza et al., 1988a). A similar study, where 22 characters were measured on a collection of 161 accessions of four species of *Zornia*, showed that distinct species were identified in the clustering, although 11 clusters were identified within the four species (Hakiza et al., 1988b). More recently, Pengelly and Eagles (1995) classified 53 accessions of *Macroptilium gracile* (Poeppiga ex Benth.) Urb. for 23 attributes into ten well defined groups of accessions based on amphicarpy, cotyledon node height, seedling leaf and pod shape, internode length and time to flowering. In another study, 108 accessions of the fodder tree *Sesbania sesban* were scored for 18 morphological attributes. This resulted in seven clusters based on flower and leaf characters and separated botanical varieties (Heering et al., 1996a).

Morphological characterization of collections of *Stylosanthes scabra* (Maass, 1989; Maass and Schultze-Kraft, 1993), *S. viscosa* Sw. (Keller-Grein and Schultze-Kraft, 1992), *Centrosema brasilianum* Benth. (Schultze-Kraft and Belalcazar, 1988), and *Desmodium heterocarpon* subsp. *ovalifolium* (Schultze-Kraft and Benavides, 1988) revealed large variation in the respective collections. The characterization of a large germplasm collection of *S. scabra* by Maass (1989) helped identify four major morpho-agronomic plant types, one of them now being suggested to be a new species. This species may well be the diploid progenitor of the allotetraploid *S. scabra* and has been tentatively called *Stylosanthes* sp. aff. *scabra* (Liu and Musial, 1996). This species is well adapted to heavy clays in subtropical climates (Date et al., 1996). Morphological characterization has also been done for the legumes *Arachis pintoi* (Maass et al., 1993) and *Chamaecrista* (Torres et al., 1995), and the grass *Brachiaria* (Valle et al., 1993).

Some of the major forage grasses have also been characterized using these methods. Pengelly et al. (1992) classified a collection of 322 accessions of buffel grass and related species on 11 agronomic attributes. This resulted in six groups which differed in rhizome development, plant maturity and yield but it was not possible to clearly identify species due to the wide variability seen in buffel grass. Of the many morphological attributes which can be used to characterize grasses, some provide better separation between accessions. A study in Napier grass indicated that culm and node diameter, leaf size, arista and spikelet length, diameter of rachis, leaf pubescence, ligule size, colour and pubescence, and roughness and indentation of leaf margins were useful characters for this species (Tcacenco and Lance, 1992). However, a comparative study of morphological characterization carried out in a large collection of *Brachiaria* species (Valle et al., 1993), indicated the limitations of this approach as only highly heritable characteristics have value for classification of a collection.

Heritable traits which are important contributors to the feeding value of the leaf material have also been characterized. These include anti-quality components, such as polyphenols, which occur particularly in shrubs and trees (Lascano et al., 1995b). Preliminary research on the use of polyphenolic profiles determined by use of high performance liquid chromatography (HPLC) on a limited number of accessions indicated that there were some distinct profiles which could classify accessions into groups but that they were not sufficiently unique to reliably identify accessions (Plumb et al., 1996). A larger collection of 103 accessions of *Sesbania sesban* was clustered into 10 groups based on the HPLC profiles (Heering et al., 1996b).

More recently molecular techniques have been applied to the characterization or "fingerprinting" of forage germplasm and elucidation of species relationships. Isozymes and native seed proteins studied by polyacrylamide gel electrophoresis in species of *Arachis* (Maass et al., 1993; Maass and Ocampo, 1995), *Stylosanthes* (B.L. Maass, S.I. Marulanda, and C.H. Ocampo, unpublished), and *Brachiaria* (Keller-Grein et al., 1996) were used to fingerprint almost 2000 accessions. In these studies large variation was encountered among accessions, and only few possible duplicates were determined. In addition, regions of diversity were identified, for example for both *Stylosanthes capitata* Vog. and *S. guianensis* in northern and northeastern Brazil (B.L. Maass, S.I. Marulanda, and C.H. Ocampo, unpublished data).

Randomly amplified polymorphic DNA (RAPD), a polymerase chain reaction (PCR) based technology has been used to study the variation within and between populations. This technique was applied to subpopulations of gliricidia to assess the variation present (Dawson et al., 1995) and to determine the amount of geneflow between populations. Cluster analysis showed that populations were more similar when geographically closer together, which suggests that seeds are dispersed and geneflow occurs over a relatively small area. A similar technique was applied to stylo species to elucidate species relationships (Kazan et al., 1993a; Gillies and Abbott, 1996). This technique was able to group species in a similar manner to using morphological and agronomic characterization. It also allowed species to be identified with a high degree of accuracy and to group closely related species as well as identify probable ancestors of allotetraploid species (Gillies and Abbott, 1996). However, low levels of polymorphism were found among accessions of the same species in a study of 20 accessions from *S. guianensis, S. hamata, S. humilis* and *S. scabra* (Kazan et al., 1993a) and in a study of 45 accessions of *S. guianensis* (Kazan et al., 1993b).

These techniques have also been applied to grasses. Genetic

relationships among *Brachiaria* species were compared, using RAPD primers (Tohme et al., 1996). The genotypes evaluated included 58 accessions of six species, which are of major interest to forage programs in tropical America and represent a diverse range of germplasm, both for ploidy level and for agronomic characteristics. The grouping pattern obtained is consistent with assigning *B. decumbens*, *B. brizantha* (A. Rich.) Stapf, and *B. ruziziensis* Germain & Evrard to one agamic complex, and *B. jubata* (Fig. & De Not.) Stapf, *B. humidicola* (Rendle) Schweick., and true *B. dictyoneura* (Fig. & De Not.) Stapf to another taxonomic group. A study using RAPDs to assess the variation in the collection of Napier grass indicated that individual genotypes could be accurately identified, even in material collected from different sources because Napier grass is propagated vegetatively (A. Lowe and J. Hanson, unpublished data).

These studies have indicated that a large amount of variation does occur within the material already collected and stored in the world's major forage genebanks. Molecular techniques provide many opportunities for future research. There is also the potential to identify genetic hotspots, where variation within populations is greatest for further collection or to identify those species with a very small genetic base, where further collection is required. Specific genes may be identified which control adaptive traits or tolerance to diseases. There is then the potential to transfer these genes to other related species and even to crop species.

FUTURE NEEDS
Despite considerable emphasis on collection, conservation and characterization of forage germplasm, there remain many opportunities and challenges for the future. There is still more useful germplasm which needs to be collected once geographic and biological gaps have been identified in current germplasm collections. Geographic coverage can be assessed and GIS used to identify other potential areas for collection and key areas of variation for further collection. Germplasm from the cool tropics has not been widely sampled and there is a need for further collections in the tropical highlands and subtropical regions, especially for woody species.

More research is required on seed storage to determine the most appropriate conditions for the wide range of forage species stored in genebanks. Studies on the effects of threshing, drying and storage temperature on longevity during storage are needed. Only very few species have been studied and yet all species are treated in a similar way. The effects of storage fungi on seed viability also requires study, together with emphasis on the elimination of seed-borne diseases to provide disease-free germplasm for distribution. Seed production remains a major bottleneck in making germplasm available to users. Research is required to determine the pollination mechanisms, breeding systems and isolation distances for lesser known forage species as well as the most appropriate management methods for seed production.

There still remains much to be done on the systematic characterization of the large *ex situ* collections of forages, which will provide information on the variation present and the amount of duplication. Targeted characterization to the end use as livestock feed, cover crops and green manure will provide important information to select superior genotypes for these uses. Molecular techniques will be particularly important for determination of species relationships and identification of duplicates.

REFERENCES
Bass, L.N. 1984. Storage of seeds of tropical legumes. Seed Sci. Technol. **12:** 395-402.

Date, R.A., L.A. Edye and C.J. Liu. 1996. *Stylosanthes* sp. aff. *scabra*: A potential new forage plant for northern Australia. Trop. Grassl. **30** (in press).

Dawson, I.K., A.J. Simons, R. Waugh and W. Powell. 1995. Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. Heredity **74:** 10-18.

Edye, L.A., R.L. Burt, C.H.L. Nicholson, R.J. Williams and W.T. Williams. 1974. Classification of the *Stylosanthes* collection. 1928-69. CSIRO Aust. Div. Trop. Agron. Tech. Pap. No. 15, 1-28.

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

Gillies, A.C.M. and R.J. Abbott. 1996. Phylogenetic relationships in the genus *Stylosanthes* (Leguminosae) based upon chloroplast DNA variation. Pl. Syst. Evol. **200:** 193-211.

Hakiza, J.J., J.R. Lazier and A.R. Sayers. 1988a. Characterization and evaluation of forage legumes in Ethiopia: Preliminary examination of variation between accessions of *Stylosanthes fruticosa* (Retz.) Alston. Pages 174-191 *in* B.H. Dzowela, ed. African Forage Plant Genetic Resources, Evaluation of Forage Germplasm and Extensive Livestock Production Systems. ILCA, Addis Ababa, Ethiopia.

Hakiza, J.J., J.R. Lazier and A.R. Sayers. 1988b. Characterization and preliminary evaluation of accessions of *Zornia* species from the ILCA collection. Pages 149-173 *in* B.H. Dzowela, ed. African Forage Plant Genetic Resources, Evaluation of Forage Germplasm and Extensive Livestock Production Systems. ILCA, Addis Ababa, Ethiopia.

Hanson, J. and R.J. Lazier. 1989. Forage germplasm at the International Livestock Centre for Africa (ILCA): an essential resource for evaluation and selection. Pages 265-266 *in* Proc. XVI Int. Grass. Congr., Nice, France.

Hanson, J. and T.J. Ruredzo. 1992. *In vitro* culture techniques for forage genetic resources. Pages 149-151 *in* J.P. Moss, ed. Biotechnology and crop improvement in Asia. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Harty, R.L., J.M. Hopkinson, B.H. English and J. Alder. 1983. Germination, dormancy and longevity in stored seeds of *Panicum maximum*. Seed Sci. Technol. **11:** 341-351.

Heering, H., S. Nokoe and Jemal Mohammed. 1996a. The classification of a *Sesbania sesban* collection. 1. Morphological attributes and their taxonomic significance. Trop. Grassl. **30**: 206-214.

Heering, H., J.D. Reed and J. Hanson. 1996b. Differences in *Sesbania sesban* accessions in relation to their phenolic concentration and HPLC fingerprints. J. Sci. Food Agric. **71:** 92-98.

IPGRI. 1996. Access to plant genetic resources and the equitable sharing of benefits: a contribution to the debate on systems for the exchange of germplasm. Issues in Genetic Resources No 4, International Plant Genetic Resources Institute, Rome, Italy.

Jones, P.G., R. Rebgetz, B.L. Maass and P.C. Kerridge. 1996. Genetic diversity in *Stylosanthes* species: a GIS mapping approach. Paper presented at the First International Symposium on Tropical Savannas, Brasília, DF, Brazil, 24-29 March 1996.

Kazan, K., J.M. Manners and D.F. Cameron. 1993a. Genetic variation in agronomically important species of *Stylosanthes* determined using amplified polymorphic DNA markers. Theor. Appl. Genet. **85:** 882-888.

Kazan, K., J.M. Manners and D.F. Cameron. 1993b. Genetic relationships and variation in the *Stylosanthes guianensis* (Aubl.) Sw. species complex assessed by random amplified polymorphic DNA. Genome **36:** 43-49.

Keller-Grein, G., B.L. Maass and J. Hanson. 1996. Natural variation in *Brachiaria* and existing germplasm collections. Pages 16-42 *in* J.W. Miles, B.L. Maass and C.B. do Valle, eds. *Brachiaria*: Biology, agronomy, and improvement. CIAT and Empresa Brasileira de Pesquisa Agropecuária/Centro Nacional de Pesquisa de Gado de Corte (EMBRAPA/CNPGC), Cali, Colombia.

Keller-Grein, G. and R. Schultze-Kraft, 1992. Preliminary agronomic evaluation of a *Stylosanthes viscosa* Sw. collection. Genet. Resour. Commun. **15:** 1-35.

Lascano, C.E., B.L. Maass, P.J. Argel and E. Víquez. 1995a. Leucaena in Central and South America. Pages 152-158 *in* H.M. Shelton, C.M. Piggin and J.L. Brewbaker, eds. *Leucaena* - opportunities and limitations. Proceedings of a workshop at Bogor, Indonesia, 24-29 January 1994. ACIAR proceedings no. 57. Canberra, Australia.

Lascano, C.E., B.L. Maass and G. Keller-Grein. 1995b. Forage quality of shrub legumes evaluated in acid soils. Pages 228-236 *in* D.O. Evans and L.T. Szott, eds. Nitrogen fixing trees for acid soils Nitrogen fixing tree research reports (Special issue). Winrock International and NFTA, Morrilton, Arkansas, USA.

Liu, C.J. and Musial, J.M. 1996. *Stylosanthes* sp. aff. *scabra*: a diploid progenitor of *Stylosanthes scabra*. Genome (in press).

Maass, B.L. 1989. Die tropische Weideleguminose *Stylosanthes scabra* Vog. - Variabilität, Leistungsstand und Möglichkeiten züchterischer Verbesserung. Sonderheft 97. Landbauforschung Völkenrode, Braunschweig, Germany. 189 p.

Maass, B.L. and C.H. Ocampo, 1995. Isozyme polymorphism provides fingerprints for germplasm of *Arachis glabrata* Bentham. Genet. Resour. Crop Evol. **42:** 77-82.

Maass, B.L. and A. Ortiz Escobar. 1996. Managing tropical forages field genebanks at CIAT. Paper presented at the IPGRI/SGRP/CIAT/FAO Consultation meeting on Management of Field and In Vitro Genebanks at CIAT, 15-20 January 1996. Cali, Colombia.

Maass, B.L. and R. Schultze-Kraft, 1993. Characterisation and preliminary evaluation of a large germplasm collection of the tropical forage legume *Stylosanthes scabra* Vog. Pages 2151-2153 *in* Proc. XVII Int. Grass. Cong., New Zealand and Australia.

Maass, B.L. and A.M. Torres. 1992. Outcrossing in the tropical forage legume *Centrosema brasilianum* (L.) Benth. Pages 465-466 *in* Abstr. XIII EUCARPIA Congress, Angers, France.

Maass, B.L., A.M. Torres and C.H. Ocampo, 1993. Morphological and isozyme characterisation of *Arachis pintoi* Krap. *et* Greg. *nom. nud.* germplasm. Euphytica **70:** 43-52.

Miles, J.W., B.L. Maass and C.B. do Valle (eds.). 1996. *Brachiaria*: Biology, agronomy, and improvement. CIAT and Empresa Brasileira de Pesquisa Agropecuária/Centro Nacional de Pesquisa de Gado de Corte (EMBRAPA/CNPGC), Cali, Colombia. 288 p.

Pengelly, B.C. and D.A. Eagles. 1995. Geographical distribution and diversity in a collection of the tropical legume *Macroptilium gracile* (Poeppiga ex Bentham) Urban. Aust. J. Agric. Res. **46:** 569-80.

Pengelly, B.C., J.B. Hacker and D.A. Eagles. 1992. The classification of a collection of buffel grasses and related species. Trop. Grassl. **26:** 1-6.

Penteado, M.I. de O., L.E. Sáenz de Mira and M. Pérez de la Vega. 1996. Genetic resources of *Centrosema* spp.: genetic changes associated to the handling of an active collection. Genet. Resour. Crop Evol. **43:** 85-90.

Pizarro, E.A. and L. Coradin (eds.). 1996. Potencial del género *Cratylia* como leguminosa forrajera. Proceedings of a workshop 17-20 July 1995, Brasilia, D.F., Brazil. Working document. CIAT, Cali, Colombia. 118 p.

Plumb, V.E., C.D. Wood, C. Gay, J. Hanson and J.H. Heering. 1996. The use of HPLC-derived phenolic profiles as a means of classifying *Sesbania* accessions. J. Sci. Food Agric. **70:** 481-486.

Reid, R. 1993. Establishing and sharing collections of a valuable global resource. Pages 77-82 *in* M.J. Baker, ed. Grasslands for our world. SIR Publishing, Wellington, New Zealand.

Ruredzo, T.J. 1991. A minimum facility method for *in vitro* collection of *Digitaria eriantha* ssp. *pentzii* and *Cynodon dactylon*. Trop. Grassl. **25:** 56-63.

Schultze-Kraft, R. 1985. Development of an international collection of tropical forage germplasm for acid soils. Pages 109-111 *in* Proc. XV Int. Grass. Cong., Kyoto, Japan.

Schultze-Kraft, R. and J. Belalcázar. 1988. Germplasm collection and preliminary evaluation of the pasture legume *Centrosema brasilianum* (L.) Benth. Tropic. Agric. (Trinidad) **65(2):** 137-144.

Schultze-Kraft, R. and G. Benavides, 1988. Germplasm collection and preliminary evaluation of *Desmodium ovalifolium* Wall. Genet. Resour. Commun. **12:** 1-20.

Schultze-Kraft, R., Ha Dinh Tuan and Nguyen Phung Ha. 1993. Collection of native forage legume germplasm in Vietnam. Pages 233-234 *in* Proc. XVII Int. Grass. Cong., New Zealand and Australia.

Schultze-Kraft, R., S. Pattanavibul, A. Gani, C. He and C.C. Wong. 1989. Collection of native germplasm resources of tropical forage legumes in Southeast Asia. Pages 271-272 *in* Proc. XVI Int. Grass. Cong., Nice, France.

Souza, F.H.D. and J. Marcos-Filho. 1993. Physiological characteristics associated with water imbibition by *Calopogonium mucunoides* Desv. seeds. Seed Sci. Technol. **21:** 561-572.

Tcacenco, F.A. and G.N. Lance. 1992. Selection of morphological traits for characterization of elephant grass accessions. Trop. Grassl. **26:** 145-155.

Tohme, J., N. Palacios, S. Lenis and W. Roca. 1996. Applications of biotechnology to *Brachiaria*. Pages 196-204 *in* J.W. Miles, B.L. Maass and C.B. do Valle, eds. *Brachiaria*: Biology, agronomy, and improvement. CIAT and Empresa Brasileira de Pesquisa Agropecuária/Centro Nacional de Pesquisa de Gado de Corte (EMBRAPA/CNPGC), Cali, Colombia.

Torres, A.M., J. Belalcázar, M. Andrade and A. Ortiz. 1995. Variabilidad del germoplasma del género *Chamaecrista* (Breyne) Moench (Leguminosae: Caesalpinioideae) conservado en el CIAT. Pages 403-407 *in* Memorias del I Congreso Nacional sobre Biodiversidad, Cali, Colombia, 4-8 December 1994.

Valle, C.B. do, B.L. Maass, C.B. Almeida and J.C.G. Costa. 1993. Morphological characterisation of *Brachiaria* germplasm. Pages 208-209 *in* Proc. XVII Int. Grass. Cong., New Zealand and Australia.

Valls, J.F.M. and E.A. Pizarro. 1994. Collection of wild *Arachis* germplasm. Pages 19-27 *in* P.C. Kerridge and B. Hardy, eds. Biology and agronomy of forage *Arachis*. CIAT, Cali, Colombia.

Whiteman, P.C. and K. Mendra. 1982. Effects of storage and seed treatments on germination of *Brachiaria decumbens*. Seed Sci. Technol. **10:** 233-242.

Williams, R.J. 1983. Tropical legumes. Pages 17-37 *in* J.G. McIvor and R.A. Bray, eds. Genetic Resources of Forage Plants. CSIRO, Melbourne, Australia.

Table 1
Germplasm of major genera of tropical grass and forage legumes held in three international *ex situ* collections.

Genus	CIAT		ILRI		CSIRO[a]	
	species (no.)	accessions (no.)	species (no.)	accessions (no.)	species (no.)	accessions (no.)
Tropical legumes						
Aeschynomene	32	1 002	11	156	28	445
Alysicarpus	9	273	10	255	11	314
Arachis	25	111	5	12	20	107
Calopogonium	4	536	3	41	5	62
Centrosema	34	2 423	12	325	26	1 020
Chamaecrista	16	310	5	102	12	109
Crotalaria	24	289	34	231	60	229
Desmodium	52	2 949	28	182	90	1 325
Galactia	12	578	2	4	13	187
Indigofera	16	237	32	268	47	297
Lablab	1	25	1	181	1	139
Lotononis	2	3	3	59	12	80
Macroptilium	10	613	10	71	15	678
Macrotyloma	6	52	5	39	9	166
Neonotonia	1	69	1	262	1	251
Phaseolus	3	33	7	290	16	161
Pueraria	4	261	2	7	3	17
Rhynchosia	14	448	13	143	38	325
Stylosanthes	25	3 598	15	1 151	33	2 184
Teramnus	5	388	4	66	8	282
Vigna	33	750	20	427	46	568
Zornia	17	1 033	10	283	24	267
Tropical grasses						
Andropogon	4	99	5	51	20	71
Brachiaria	25	684	23	663	20	136
Cenchrus	2	55	6	127	10	514
Chloris	4	54	9	123	20	162
Cynodon	3	17	4	112	7	56
Digitaria	11	28	13	59	37	436
Eragrostis	7	55	16	71	10	86
Hyparrhenia	11	59	11	38	6	34
Panicum	10	606	16	211	42	606
Paspalum	14	122	9	59	45	303
Pennisetum	9	55	19	241	10	127
Setaria	5	45	8	63	25	135
Tropical fodder trees and shrubs						
Acacia	8	23	69	180	30	70
Albizzia	2	7	10	23	15	30
Cajanus	1	54	1	156	9	75
Calliandra	3	21	2	6	7	25
Chamaecytisus	1	1	2	211	2	2
Codariocalyx	2	37	1	27	2	37
Cratylia	2	12	1	1	2	4
Desmanthus	7	95	7	112	11	440
Erythrina	9	63	10	38	4	9
Flemingia	6	147	1	6	44	7
Gliricida	1	9	1	88	1	52
Leucaena	11	203	20	148	17	453
Prosopis	1	11	4	11	5	9
Sesbania	12	38	25	414	34	238

[a] Data supplied by B Hacker, CSIRO, 1996