Grass and Forage Plant Improvement in the Tropics and Sub-Tropics

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Key points

1. The majority of tropical and subtropical forage grass genera and/or species have not yet been collected, or need further collection to be representative of their natural distribution.
2. New biotechnological techniques will only result in the release of superior forage cultivars if supported by strong breeding programs.
3. More funding and investment in the formation of strong public research teams in forage conservation and improvement are needed to guarantee the sustainability of tropical and subtropical pasture-based livestock systems in the future.
4. The creation of a permanent international working group on tropical and subtropical forages is essential to assist the International Plant Genetic Resources Institute (IPGRI) in prioritising collection, conservation, evaluation and adoption in the tropical/subtropical world for the benefit of mankind.

Keywords: apomixis, genetic improvement, molecular breeding, selection

Introduction

Approximately half the world bovine meat production comes from tropical and subtropical countries (Table 1). These countries have had a 200% increase in beef and veal production in the last 40 years, compared with world production which exhibited an increase of 91% (FAOSTAT data, 2004). According to Pinstrup-Andersen & Pandya-Lorch (1997), world population is projected to rise to 8 billion by 2020, resulting in an increase of 110% in demands for food, cereals and meat, mostly in developing countries. These numbers pose a challenge and an opportunity for R&D, and innovation in production of quality animal products and encompasses animal nutrition, animal health, and particularly pasture and forage science.

Table 1 Comparison of beef and veal production in the tropics and subtropics to that of the world in the last 40 years (FAOSTAT data, 2004)

Beef and Veal Production (Mt)	Year		Percent increase
	1963	2003	
Tropics and subtropics[1]	10,352,277	30,873,627	200
World	30,855,743	58,922,239	91

[1]Estimate since some countries contain both subtropical and temperate areas.

Animal production in tropical and subtropical areas of the world is largely dependent on either native or planted pastures; therefore the demand for high quality, productive and adapted forages is high. Brazil, for instance, has the number one commercial beef cattle herd in the world (~189 million heads in 2003), and the evolution of improved pastures has occupied extensive areas of native grasslands, especially in the savannahs of central Brazil. Native pastures decreased from 103 to 78 million ha from 1970 to 1996, while cultivated pastures increased from 30 to 100 million ha in this period (IBGE Censo Agropecuário, 1995-1996). Poor management and overgrazing due to an increase in the demand for animal products has resulted in large expanses of degraded pastures. The long dry season in many tropical and subtropical countries has also contributed to pasture degradation, and due to a lack of available adapted forages the sustainability of pasture systems in these regions is limited.

Species and genetic resources

The main institutions responsible for the development of tropical pastures in the last 30 years were the International Centre for Tropical Agriculture (CIAT) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which played a fundamental role in collecting, maintaining, exchanging and evaluating forage germplasm, especially legumes (Miles, 2001; Valle, 2001).

Forage germplasm collection in tropical and subtropical regions were a priority in the 1970's and 1980's, and CIAT and CSIRO together with the International Livestock Research Institute (ILRI) and the International Plant Genetic Resources Institute (IPGRI) with the collaboration of many national Institutes gathered vital forage genetic resources (Schultze-Kraft *et al*., 1993; Hanson & Maass, 1999). It is estimated that the three main international forage genebanks in the world hold about 30,000 accessions of the main forage species (Hanson & Maass, 1999), with 2.5 to 8.5 times more legumes than grasses. The bank with the most legume accessions is CIAT (15,981) and with the most grasses is CSIRO (2,666). The most representative genera in these banks are *Brachiaria* and *Panicum* in the grasses, and *Stylosanthes*, *Desmodium* and *Centrosema* in the legumes.

In recent times, a shortage of funding and staff have jeopardised the conservation of this germplasm for future generations, which is causing serious concern among pasture scientists worldwide (Valle, 2001). In addition, the diversity may considerably less than the numbers suggest since estimates of 30% duplication of accessions in these genebanks has been reported (Hanson & Maass, 1999). Furthermore, tropical grass seeds are known to lose their viability - even under ideal storage. Budget restrictions in the late 1990's, cut back CIAT's and CSIRO's staff and funds for adequately maintaining and renewing these collections, thus 15,000 accessions of the least important genera in the Australian Tropical Forage Genetic Resource Centre (ATFGRC) were sent to CIAT and ILRI for storage.

Apomictic forage grasses display wide variation in nature, however, little of this diversity has been sampled (Savidan, 2000). The first species intensively collected was *Panicum maximum* in East Africa by the Institute de Recherche pour le Developpment[3] (Combes & Pernès, 1970), and later by Hojito & Horibata (1982). Thus the diversity of this species is well represented in *ex situ* collections (Savidan *et al*., 1989). *Brachiaria* was also extensively collected by CIAT in East Africa (Keller-Grein *et al*., 1996), but important species (*B. mutica* for example) and sexual pools of *B. brizantha* and *B. humidicola* are still lacking, thus the existing collection cannot be considered representative of the natural distribution. The genus *Paspalum* spp. of South American origin, has also been extensively collected by the Brazilian Agricultural Research Corporation (Embrapa) but the complete collection of ca. 1600 accessions has not been fully characterized to determine if this genebank is representative or not.

[3]Former ORSTOM (Office de la Recherche Scientifique et Technique d'Outre-Mer).

Genera such as *Pennisetum*, *Hyparrhenia*, *Melinis*, *Setaria*, *Andropogon*, *Hemarthria*, *Chloris*, and *Cenchrus* should be included in forthcoming collecting efforts to ensure enough diversity for future improvement efforts, and sexual forms within apomictic *Hyparrhenia* and *Melinis* must be sought (Jank *et al*., in press). It is essential that germplasm development from collection, plot evaluation, regional trials and animal performance trials in pastures through to its use by farmers be considered of high priority for the adoption of forage-based technologies (Peters & Lascano, 2003).

The creation of an IPGRI-sponsored, permanent international working group on forages, as recommended since 1979, and emphasised by Schultze-Kraft *et al*., (1993) and Valle (2001), is strongly urged by the scientific community. This group could be a forum for discussions and communication among forage germplasm specialists, and assist IPGRI in taking action to guarantee forage collection, conservation, evaluation and adoption in the tropical/subtropical world for the benefit of mankind.

Breeding targets and priorities

Breeding is the key for the future development of superior forages, as selection programmes based on large germplasm progress and superior accessions are released. The tropical world has still to profit from the genetic manipulation of tropical forages through breeding. Most cultivars of tropical grasses available commercially are wild ecotypes selected from natural diversity (Hacker & Jank, 1998). Others on the verge of release may still add to several production systems before hybrids come along. However, breeding is only justified after the germplasm of a genus or species has been explored and specific problems identified (Cameron, 1983). According to Miles (2001), it is premature to begin hybridisation programs of a species prior to its wide use in production systems, or before large germplasm resources are available and have been evaluated, or without knowledge of the biology and genetics of the species.

Breeding of forages has similar targets to those of crops, e.g. increased productivity and quality, resistance to pests and diseases, efficient use of fertilisers, and adaptation to edaphic and climatic stresses. However, there are additional requisites since forages have no intrinsic value unless converted into animal products (meat, milk, hide, calves), which implies indirect evaluation to verify worth. Through the years, selection has involved the choice of the most vigorous plants, and field-oriented selection has contributed greatly to genetic advance (Busey, 1989).

The main objective of forage breeding programs thus far has been improvement of forage yield and quality (Hacker, 1986; Oram, 1986; Vogel *et al*., 1989; Burton *et al*., 1993; Hacker & Jank, 1998; Jank *et al*., 2001). Whereas direct selection has resulted in the selection and release of cultivars 80 to 130% more productive in terms of leaf yield than the commercial standard, bred cultivars have also led to increases of 26 and 47% in digestibility and productivity respectively (Burton, 1989; Burton *et al*., 1993; Jank *et al*., in press). Improvement for yield and quality had direct benefits to the farmers by improving animal performance. Thus the adoption of released cultivars have resulted in increases in 6% milk production, and 25 to 28% liveweight gain per area (Lowe *et al*., 1991; Euclides *et al*., 1993).

Leaf yield, leaf percentage, seed production and aggressive regrowth are the objectives of *P. maximum* breeding in Brazil (Jank *et al*., 2001, 2004; Muir & Jank, 2004; Resende *et al*., 2004). The *Brachiaria* breeding programs at CIAT and Embrapa, intend to select hybrids which combine the qualities of three species: the high forage quality and determined flowering cycle of *B. ruziziensis*, the yield and resistance to spittlebug of *B. brizantha* and the vigour and adaptation to acid, infertile soils of *B. decumbens* (Miles & Valle, 1996; Miles, 1999; Valle *et al.*, 2000; Peters & Lascano, 2003). At CIAT the *Brachiaria* breeding program also involves *Rhizoctonia* resistance, tolerance to drought, and increased seed production (Peters & Lascano, 2003).

Ease of management through grazing and control of excessive stemmy herbage during periods of active growth were the objectives of the CIAT *A. gayanus* improvement project. Although synthetic lines were developed, they were not commercialised and the programme was discontinued. In Brazil, *A. gayanus* cv. Baeti was bred for quick establishment and stand uniformity (Batista & Godoy, 1995). After the release of this cultivar the programme was also discontinued.

Seed production is also a factor considered in breeding programs (Hacker, 1991a,b; Hacker *et al*., 1993; Diz & Schank, 1993). Poor seed production results in high seed prices and consequently little adoption. This was the case with many good legume cultivars released, e.g. the *Brachiaria* hybrid cv. Mulato (Miles, 1999). Despite the use of vegetative propagation in certain systems and in some countries, seed producing cultivars are easier to establish, faster to be adopted and much more widespread. In this sense, one of the objectives of the *P. purpureum* breeding programmes in Florida and Brazil is selection of seed producing cultivars, which are obtained by crossing *P. purpureum* and *P. glaucum* (L.) R. Br. The progenies from this cross are triploid and sterile, but once doubled by colchicine become hexaploid and fertile. The improvement of this species also involves the incorporation of apomixis and maintenance of perenniality. The species reproduces sexually and can be crossed with *P. squamulatum* Fresen which reproduces apomictically (Pereira *et al*., 2001). The hybrid vigour is fixed in apomictics and the hybrid may be released as a cultivar for pastures or for cut and carry.

Other traits bred and selected for are leafiness, establishment, stand uniformity in sexual reproducing plants, late flowering, spring productivity and winter survival, early growth and regrowth, adaptation, mineral composition and disease resistance (Table 2).

Contrary to the scenario with cool-season forages, there has been very little or no consideration given to the impact on the environment as a consequence of livestock production, either in terms of water requirements or diffuse pollution. For this reason, environmental impact is not a priority in the breeding programs of most tropical and subtropical forage grasses and legumes. However, the principle of 'Best Production Practices', which comprises environmental and social concerns, are a market demand and are being incorporated into beef supply chains in Brazil and other major beef/milk producing and exporter countries in the tropics.

Table 2 Main tropical and subtropical genera and summarised objectives in the breeding of tropical forage species[1]

Species	Breeding target	References
Andropogon gayanus	Establishment, uniformity,late flowering, vigour	Batista & Godoy, 1995; Grof (*pers. comm.*).
Brachiaria spp.	Spittlebug resistance, nutritive value, adaptation to acid soils, seed yield, Rhizoctonia resistance	Miles & Valle, (1996); Valle *et al*., (2000); Miles, (1999); Peters & Lascano, (2003)
Cenchrus ciliaris	Digestibility, overall performance, spring productivity, winter survival	Pengelly *et al*., (1992); Hacker *et al*., (1995); Hussey & Bashaw, (1996)
Chloris gayana	Early growth and regrowth, mineral composition	Nakagawa *et al*., (1993); Jones *et al*., (1995)
Cynodon spp.	Yield and digestibility	Burton & Monson, (1988); Burton *et al*., (1993)
Digitaria spp.	Digestibility, seed yield and quality, leafiness, spring yield	Hacker, (1986); Terblanche *et al*., (1996); Hacker *et al*., (1993; 1995)
Hemarthria altissima	Yield, winterhardiness	Oakes, (1979); Quesenberry *et al*., (1978)
Panicum maximum	Leaf yield, adaptation, leafiness, regrowth, seed production	Machado *et al*., (1988); Sato *et al*., (1993); Jank *et al*., (2001, 2004); Muir & Jank, (2004); Resende *et al*., (2004)
Paspalum spp.	Yield, nutritive value, ergot resistance	Burton, (1989); Schrauf *et al*., (2003); Batista & Godoy, (2000); Venuto *et al*., (2003)
Pennisetum spp.	Seed production, growth habit, spittlebug resistance, resistance to 'kikuyu yellows' (virus?), digestibility	Pereira *et al*., (2001); Wouv *et al*., (1999); Diz & Schank, (1993); Luckett *et al*., (1996)
Setaria sphacelata	Yield, seed production, winter greenness	Hacker, (1991a; 1991b); Oram, (1986; 1990); Jank *et al*., (2002)

[1]Some programs are the result of direct germplasm selection, and some have been discontinued.

Tropical forage breeding has been reviewed by many authors (Cameron, 1983; Miles, 2001; Pereira *et al*., 2001; Valle, 2001, Miles *et al*., 2004) who identify the most important factors limiting progress are as follows:

- Access to germplasm representing natural variation, especially of exotic grasses.
- Large number of candidate species/genera of legumes with little to no information about the biology, genetic variation and agronomy.
- Important species with a complex reproductive structure (polyploidy, apomixis), non-domesticated (no seed retention, anti-quality compounds etc.) and dependence on existing breeding methods not necessarily efficient for the particular program.
- Complex criteria of merit and deficient screening techniques. Unlike cereals, forages have no specific point in time when yield can be fully evaluated, due to conversion into animal

products over their growth curve. Merit depends on co-ordinated effort of teamwork, over a wide range of ecosystems to ascertain G x E interaction, and involves time-consuming and costly evaluations of pastures under grazing.

- Little knowledge of genetic control of agronomic traits to be improved.
- Lack of forage breeding courses or tropical forage breeders, thus no academic expertise being developed; few genera/species being tackled.
- Funding and personnel limitations. Complete reliance on public research institutes for the activity with little input from the private sector, the direct beneficiary of the activity.

Research needs and prospects for using new technologies

Tropical and subtropical forage grasses still need to undergo domestication, the process which requires continuous cycles of cultivation and selection to fit human needs. Common breeding and screening techniques to fit the fundamentals of forages also need to be developed to shorten the process of cultivar release.

Conventional breeding has contributed substantially to the genetic improvement of tropical and subtropical forages. With the development of DNA-based molecular markers (RFLP, RAPD, AFLP, SSR) and other biotechnological tools, new and numerous possibilities of utilisation are being pursued.

The main application of molecular markers in tropical forages thus far, has been in the evaluation of genetic divergence among accessions in germplasm banks and commercial cultivars, as in *Cynodon* spp. (Assefa *et al*., 1999; Karaca *et al*., 2002), *Paspalum notatum* (Daurelio *et al*., 2004), *C. gayana* (Perez *et al*., 1999; Ubi *et al*., 2003), *Pennisetum purpureum* (Smith *et al*., 1993). These studies are useful to direct germplasm collection and utilisation.

Another very important application of molecular markers is fingerprinting of genetic materials for the legal rights associated with the development and release of protected cultivars.

Genomic maps are being produced, and markers linked to economic important traits are being sought (Jessup *et al*., 2000 in *Cenchrus*), as the apomixis locus in many apomictic species permit early identification of reproductive modes of hybrid populations (Pessino *et al*., 1998; Casa *et al*., 2002; Goel *et al*., 2003). The greatest potential of these techniques are: a) the early identification of hybrids to reduce the time involved in the process of selection and release of cultivars; b) the determination of heredity mode in polyploid species (such as disomic or tetrasomic); c) the identification of apomictic or sexual hybrids in apomictic species of grasses; d) aid in the selection of resistance to pests and diseases; and e) aid in the selection of characters of difficult measurement (quantitative traits), such as factors associated to nutritional quality and digestibility, and adaptation to soil characteristics.

The use of quantitaive trait loci (QTL's) have not as yet proved useful, since low heritability of production related characters (associated with a high number of loci) in forages, may limit progress in subsequent generations (Bernardo, 2001).

The use of transgenesis in tropical forages should impact on many production systems, as the mechanisms of floral initiation, the biological clock, apical dominance, root development and other mechanisms being studied in various grain-crop grasses become better understood. Grasses in general, present a high degree of synteny in their genomes, thus specific information generated for *Zea mays* (maize), *Oryza* spp. (rice) or *Triticum* spp. (wheat) has potential application to forage grasses (Morgan *et al*., 2002). Transformation procedures have been developed for some forage grasses but need to be improved. The difficulty in transgenesis lies in identifying candidate genes to introgress, since most important traits are quantitative (Morgan *et al*., 2002).

The main impact of transgenesis will be in characters associated with nutritional quality (Hancock & Ulyatt, 2001; Spangenberg *et al*., 2001). The development of transgenic cultivars resistant to herbicides should impact on commercial seed production, reducing the cost of production and increasing purity and quality of the seeds. Also, cultivars resistant to pests and diseases are necessary in tropical conditions, and the incorporation of resistance genes is a shorter and more efficient method.

The genomic approach in studies with tropical forages depends on building infrastructure for the sequencing of the genome, and in obtaining the expressed sequence tags (EST) and segregating populations for the characters under study. This infrastructure is being built for grasses of the *Brachiaria* genus at Embrapa and CIAT. The main focus for these studies is to elucidate the complex molecular base of environmental stress tolerance, mainly aluminium tolerance. Another objectives of these studies is to develop QTL's that co-segregate with aluminium tolerance in the populations, to enhance the efficiency of breeding programs in the future. The data of EST's will be used for association studies with the available QTL's from genomes of different grasses, e.g. *Oryza* spp. (Ishitani *et al*., 2004), *Triticum* spp. and *Zea mays*.

However, it is important to say that these techniques will not result in the release of superior forages unless there are strong breeding programs to support them.

User participation in breeding

Livestock is an important component in many smallholder farming systems throughout the tropics (Pengelly *et al*., 2003). The demand for livestock products has increased in the past 10 years, and will increase even further in the future, which will have major impacts on household, farm and regional economics. New adapted forages to address these increasing demands are necessary, and the participation of farmers is essential in the evaluation and adoption of new cultivars (Stür *et al*., 2002). Smallholder farmers have different needs for forages, which need to be adapted to their utilisation systems as well as to labour availability. Selection in these systems should therefore include on-farm evaluation and participatory research, which is more effective and less time-consuming. The adoption of forage thus selected is also more effective and quicker to expand.

The main limitation to livestock productivity in many smallholder systems is the lack of adequate quality pastures, especially over the 5-7 months dry season (Gobius *et al*., 2001). Other limitations include the low efficiency of many systems, difficulty in accessing and adopting technology and poor farm management techniques. The reasons for the poor adoption are lack of tradition in using forages, the long-term or indirect benefits of using forages as compared to crops, and unavailability of planting material because of lack of demand for seeds (Kumwenda & Ngwira, 2003; Peters & Lascano, 2003; Roothaert *et al*., 2003). In some countries, these problems are being bypassed by Government incentives such as in NE Thailand (Gobius *et al*., 2001), where promotion of pasture use and farmer-awareness have demonstrated that livestock play a critical role in the sustainability and intensification of agricultural productivity (Kumwenda & Ngwira, 2003).

In SE Asia, forage research only began in 1992, with the selection of environmentally adapted germplasm through projects from CIAT, CSIRO, AusAid and the Asian Development Bank (Roothaerdt *et al*., 2003). Many projects are being developed in Africa and Southeast Asia to improve family welfare by implementing small-scale mixed farming systems with promising results (Ayele, 2003; Gobius *et al*., 2001; Roethardt *et al*., 2003; Stür *et al*., 2002).

Funding issues and training in plant breeding

It has been estimated, that the value of a breeding program is at least U$ 100,000 per year. Considering that the development of a forage cultivar takes around 10 years, because cultivars are perennial and so need to be evaluated over extended periods and under grazing, a new grass cultivar may represent an investment of over one million dollars (Vogel, 1989).

Until 2001, no private companies had been involved in tropical forage plant breeding (Miles, 2001), and most tropical forage releases have been funded exclusively by Government investments, with nil participation of private companies. Even in turf grasses, public plant breeding resulted in the release of the most widely used warm-season turf grasses (Busey, 1989) although private efforts have been notable in development and marketing.

In many cases, breeders have to seek external financing for continuing their research programmes. Thus many small projects, which integrate large breeding programs are being financed by both public and private organisations. Other sources of funding include seed companies, which invest in public plant breeding in return for the exclusiveness in commercialising the released cultivars. This is the case of UNIPASTO (Association for Promotion of Breeding Research in Tropical Forages) in Brazil, by which 40 seed companies finance breeding programmes developed by Embrapa, and the Seed Company Papalotla in Mexico, which finances the *Brachiaria* breeding program at CIAT.

The main revenue to breeding projects world-wide is from the royalties paid to the breeders from plant breeding rights and from seed sales (Dale, 2004). In many institutions, e.g. the University of Florida, royalties revert to the breeding programs, which permit their continuation. As more plant variety protection legislation is adopted in more countries, there will be a tendency for multinational seed companies to enter the tropical seed market (Miles, 2001). However difficulties in obtaining financing, and the limited numbers of researchers world-wide working with tropical forage plant breeding, have not permitted the expansion of many plant breeding programs, and at times, have resulted in the discontinuation of many programs. This has occurred especially so in Australia, with the retirement of active plant breeders.

Most Universities around the world have maize and other crop breeders in genetics and plant improvement departments, but only a very few, particularly in the USA actually have forage breeders. This results in biases towards crops of alogamous or autogamous reproduction, and forages or apomixis become the theme of only a few lectures. Much is yet to be done. Studies concerning apomixis and the apomictic grasses are too numerous to be omitted from genetic courses, especially since at present times many attempts are being made to transfer apomixis to important cultivated crops such as *Oryza sativa*, *Z. mays* and *Triticum* spp.

Concluding remarks

In the past, much was invested in tropical forage germplasm collection, conservation, distribution and evaluation. Many cultivars have been released, and a smaller proportion adopted in the various countries. A number of important advances in pasture/livestock research made in the last decade are expected to have a major impact on the productivity, persistence and sustainability of pasture-based livestock systems in tropical and subtropical areas.

However, the downsizing of international pasture research programmes, have left germplasm conservation under extreme pressure. Therefore, it is of major importance that more progressive and large national programmes covering a range of agroecosystems (e.g. Embrapa in Brazil), assume the leading role in this area of research. Furthermore, action must be undertaken to create an international working group on forages to guarantee investments in forage research and adoption worldwide. The continuation of sustainable pasture based livestock systems in the tropics and subtropics depends on research to achieve further progress.

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