

GENETIC RESOURCES FOR TEMPERATE AREAS - ACHIEVEMENTS AND PERSPECTIVES

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Abstract

The processes of natural evolution and domestication of temperate forage genetic resources resulted in a build-up of genetic diversity, being remarkable in natural populations of grasses and legumes from Central Europe and the Mediterranean basin. Worldwide utilization of few species caused further increment in intraspecific genetic differentiation, meanwhile landraces became adapted to local agricultural production systems. Highlights are given to the risk of genetic erosion of indigenous grasslands as a consequence of the ecosystem modifications imposed by agriculture, as well as the drastic reduction of intraspecific genetic diversity caused by the process of plant breeding and environmental leveling in modern times. The balance of the *ex situ* germplasm collections and the genetic status of the main collections are reviewed. The largest collections of the most relevant legumes and grasses are held by Oceania (>55,000 accessions) and Central Europe (>31,000 accessions), respectively. In contrast, few attempts to collect and characterize indigenous species well adapted to marginal areas have been performed outside the Mediterranean. There are many potentially useful accessions and natural populations suitable for forage production already stored in gene banks, but financial constraints usually limit germplasm evaluation and characterization. The development of core subsets will optimize efficiency in management and use of collections, encouraging germplasm enhancement and utilization. The stratification strategy to sample useful germplasm for plant breeding and the relevance of geographical factors to classify collections are highlighted.

Keywords: Genetic resources, temperate forages, core collection, grasses, legumes, *ex situ* collections

Introduction

Grasslands have been regarded as a natural resource to be exploited with minimum investment, and consequently the outputs obtained have generally been low and unpredictable. However, they cover over 3200 million hectares, about one fifth of the global land surface, and support much of world production of meat, milk and wool (Kumar, 1952; FAO, 1993; Pearson and Ison, 1997). Besides the fact that major producers depend on the abundance of extensive natural grasslands, a large number of countries still report lack of information about indigenous genetic resources, the need for assessing the existing diversity, and the critical relevance of filling certain identified gaps in existing collections (FAO, 1996). The absence of good inventories of existing collections is an obstacle for planning and allocating priorities to collection and conservation activities. Despite these difficulties, there has been an increased interest of forage

plant breeders in the adequate development and utilization of genetic resources in recent times (Charmet *et al.*, 1997).

The rate and extent of degradation of natural resources such as soils and grasslands have concerned scientist throughout the XX century. Much of the soil degradation is taking place on agricultural lands, whereas species richness in temperate grasslands can rapidly be impoverished by overgrazing, leading to degrading processes difficult to revert (Hadley, 1993; Cocks, 1995; Pinstup-Andersen and Pandya-Lorch, 1997). Although many other factors pose systematic pressures over species survival, overgrazing in Australia has presumably caused the extinction of 34 plant species, and continue to threat more than 100 endangered species (Frankel *et al.*, 1995).

The development of genetic resources has had a remarkably successful history for the major temperate grain crops, clearly identifying the priorities for *ex situ* conservation and the concomitant increment of a wide range of germplasm made available for breeders (Brush, 1999). Despite the worldwide economical relevance of pastures, few programs have promoted strong action to meet effective and efficient genetic resources management for forage species. The general trend to hold large *ex situ* collections within few genus whereas a large number of wild forage species are represented by a small number of samples will certainly continue, as well as the strong bias towards species of Mediterranean and European origin (FAO, 1996). The promotion of national gene banks over the last 20 years has not reverted this tendency, but it has had the benefit of widening the range of species as well as regions where collecting activities have been developed (IBPGR, 1991; FAO, 1996). The environmental leveling and the concomitant global approach of plant breeding, the size and adequacy of *ex situ* collection and their extended use through the implementation of core subsets, the priorities of collecting activities (species as well as genetic status), will certainly be issues of critical importance for the development of a sustainable base of genetic resources on temperate forage species.

Genetic diversity for temperate grasslands

The great diversity of grass and legume species that form the grassland canopy in Europe and the Mediterranean area perform an invaluable role in grassland farming in many regions of the world. The most relevant grass species (e.g. perennial ryegrass - *Lolium perenne*, Italian ryegrass - *L.multiflorum*, tall fescue - *Festuca*, cocksfoot - *Dactylis glomerata*, timothy - *Phleum pratense*) were molded by climatic change and plant migration in Central and Northern Europe, where large areas of natural forests were replaced by a new pattern of grasslands after crop cultivation (Spedding and Diekmahn, 1972; Pearson and Ison, 1997). On the opposite, herbage legumes (e.g. alfalfa - *Medicago sativa*, white clover - *Trifolium repens*, red clover - *T.pratense*, subclover - *T.subterraneum*) were derived primarily from virgin grasslands in the Eastern Mediterranean and adjoining countries, spreading gradually into other temperate parts of the world by their deliberate and organized introduction. White clover, alfalfa and red clover have an almost cosmopolitan distribution, whereas other legumes such as annual clovers (*Trifolium* spp.) and medics (*Medicago* spp.), trefoils (*Lotus* spp.), are less intensively used in grassland farming (Taylor, 1985; Michaut *et al.*, 1988; Hawkes, 1995; Williams, 1996; Frame *et al.*, 1998).

The remarkable adaptability of most domesticated forage species determined the great expansion of pastoral activities to diverse climatic conditions. Large temperate regions of America, Africa and Oceania were covered either with natural forest or indigenous grass canopies before European species were introduced. Following a similar transformation of the ecosystem of Northern Europe, perennial ryegrass and white clover pastures replaced natural forest in humid areas of New Zealand and Southern Chile. On the contrary, large areas of alfalfa

were cultivated on arable lands of US and Argentina previously covered with natural grasslands, while annual clovers and medics were successfully grown in Australia's dry lands (Wratt and Smith, 1983; FAO, 1996; Frame *et al.*, 1998).

The small proportion of cultivated pastures in relation to the area of arable lands available echoes the difficulties to accomplish persistence with the introduced legume species (FAO, 1993; 1996). The fluctuations in the cultivated area of the main legumes, that clearly reflect the complexity of the biotic and abiotic factors that affect persistence, has been highlighted in diverse reviews (Williams, 1987; Hanson *et al.*, 1988; Marten *et al.*, 1989; Williams, 1996; Frame *et al.*, 1998). For instance, the area of alfalfa in Argentina greatly fluctuated after reached a peak of over eight million hectares in the 1920s, being reduced to less than four millions hectares in the 1970s (Boerger, 1937; Hijano and Basigalup, 1995). The primary determinant for this decline has been the susceptibility of local cultivars to four species of aphid previously unknown in the region, although other factors such as soil degradation, root and crown diseases, also reduced stands and therefore forage productivity (Aragón and Imwinkelfied, 1995). Not surprisingly, natural grasslands still sustain animal production in extensive farming systems such as the prairies of North America or the pampas of South America (Pearson and Ison, 1997). By their extreme dominance, the grasses constitute the fundamental elements in the pampas, whereas forbs, bushes and legumes come next (Parodi, 1930; Rosengurtt, 1943; Burkart, 1969; Burkart *et al.*, 1987; Izaguirre, 1995). In spite of this diversity, collecting and breeding of indigenous species did not receive as much attention in the past as breeding of introduced species (Cragnaz, 1990; García *et al.*, 1991; Bayce *et al.*, 1998; Berretta, 1998). Concomitantly, the contribution of indigenous grasses to world agriculture has been spare: *Bromus unioloides*, *Paspalum dilatatum*, a winter and subtropical grass, respectively (Parodi, 1930; Spangenberg *et al.*, 1941; Burkart *et al.*, 1987).

Domestication of wild species

Overall, the number of food crops tends to decrease, remaining those that are most efficient, whereas the trend in forage research within the last three decades has been the opposite (Taylor, 1985; Stewart, 1992; Diwan *et al.*, 1994; Cocks, 1995). The main reason for the development of new species has been the demand for sustainable farming systems that could not be accomplished with the presently cultivated species. Essential characteristics for sustainability, such as forage productivity under stressful environments or persistence of perennial legumes, have challenge breeding programs for decades (Busbice *et al.*, 1972; Martens *et al.*, 1989; Eagles *et al.*, 1997). There is no doubt that the equilibrium of indigenous species with biotic and environmental stresses could greatly contribute to forage production; likewise, the understanding of the underlying mechanisms that contribute to this balance could greatly contribute to determine the breeding strategy for introduced species. However, the need to identify the conditions that are limiting production in each species individually greatly delay research progress and raise more difficulties in the selection of the best genetic resources strategy for stressful environments.

Peasant farming throughout centuries domesticated most plant species presently utilized for forage production, while domestication in modern times is generally developed through long research studies. Even though research procedures are based upon the same principles utilized for other crops, breeders have to overcome new challenges. Firstly, the development for many indigenous species requires assembling germplasm that may be utilized as a source nursery. Problems arise from the breeding potential of the intraspecific variability, the diverse mode of

reproduction of the different species, irregularities in seed setting, the perennial nature of most forage species, the maintenance of seed viability (Taylor, 1985; Vogel and Pedersen, 1993). These challenges pressure on breeding program, which have difficulties to reach farms with advanced cultivars of new species, and place their collections at a high risk of loss. In contrast with food crops, forage plant breeders still have the advantage to collect volunteer species with specific adaptation to stressful environments (e.g. soil types, low fertility, summer drought), which could sometimes be taken into cultivation with almost no breeding. For example, the multiplication of an annual trefoil probably introduced unconsciously in Uruguayan natural grasslands, led to the first cultivar of *Lotus subbiflorus* 'El Rincón'. The main reasons for its success in extensive farming systems are the tolerance to acidic soils as well as the adaptation to intensive and continuous grazing (Risso and Carambula, 1998). There is a large reservoir of species to be explored, particularly as sustainable alternatives for marginal areas where the main forage species could not persist.

Regardless of the great diversity of indigenous grass and legumes species with forage potential and the interest of breeders, the successful development of new cultivated species has been sparse outside the Mediterranean area. Although studies on indigenous grasses were already under progress in the 1930s, *Bromus unioloides* has been the most promising winter grass domesticated in South America, with 20 cultivars in OECD Cultivar List 1998 (Boerger, 1943; Cragnaz, 1990). The richness of this genus is reflected in the cultivars from recently domesticated species, comprising *B.stamineus* 'Grasslands Gala' and *B.auleticus* 'INIA Tabobá' and 'Potrillo' (Stewart, 1992; Bemhaja and Olmos, 1996). The former has been domesticated in New Zealand and the latter in Uruguay, although both species have their origin in South America. The sparse outcome possibly reflects the limited genetic diversity of minor species presently stored in *ex situ* collections, as well as the focus of most germplasm research, generally centered on pre-breeding activities, e.g. *Trifolium* species in North America (Taylor, 1995; Quesemberry and Taylor, 1997; Quesemberry *et al.*, 2000), grasses in North and South America (Dewey, 1989; Phan and Smith, 1997; Bayce *et al.*, 1998).

Too much emphasis has been given to the major temperate food crops in framing genetic resources and there are many locally adapted forage species where collections are nonexistent, small, or poorly representative of the species (IBPGR, 1995; Alonso *et al.*, 1998; Quesemberry *et al.*, 2000). Furthermore, most germplasm

Europe and the Mediterranean, centers of diversity of the main cultivated species (e.g. Taylor, 1985; Charmet *et al.*, 1993; Casler, 1995; Piano *et al.*, 1996; Williams, 1996; Ruiz *et al.*, 1999). In addition, most countries that developed genetic resources programs have at the present time a large proportion of domesticated species in their collections (FAO, 1996). At the regional level, the effort has been more sporadic and generally less successful, but with a number of notable exceptions including the UNDP/IBPGR European Co-operative Program for the Conservation and Exchange of Crop Genetic Resources (IBPGR, 1989). In view of the relevance of the genus *Bromus* for South America, PROCISUR (a co-operative program between Argentina, Bolivia, Brazil, Paraguay and Uruguay) sponsored a joint collecting program that includes conservation and characterization activities (Cragnaz, 1990; Oliva *et al.*, 1993; Oliveira and Moraes, 1993; Puignau and Da Cunha, 1996; Massa *et al.*, 1997; Bayse *et al.*, 1998). Seed of low viability and slow establishment are among the major constraints related to this genus domestication.

Crops are grown increasingly at the limit of their adaptation, where the ability to survive periods of environmental constraint is an essential characteristic for success, e.g. perennial species adapted to environments with moisture deficit. Although intraspecific genetic variation for drought tolerance exists in perennial grasses as well as legumes (e.g. perennial ryegrass -

Thomas and Evans, 1989; Wedderburn *et al.*, 1990; white clover - Woodfield *et al.*, 1996), pastures in sustainable farming systems will possibly require the search for species with superior drought resistance. Drought tolerance is generally associated with plant characteristics such as early maturity, deep root systems, reduced leaf area during periods of high temperature and dry soil, although the ability to tolerate other stresses also contributes to plant survival (e.g. soil hardness, unavailability of mineral nutrients). In addition, naturally drought resistant species may develop specific mechanisms of survival. For instance, the development of storage roots in *Trifolium somalense*, indigenous to Ethiopia, and *T. polymorphum*, indigenous to Southern Brazil, Uruguay, Argentina, confer great resistance to summer moisture deficits (Mengistu, 1988; Coll and Zarza, 1992; Izaguirre, 1995).

Threats to genetic diversity

Genetic diversity of cultivated species has undergone dynamic changes at specific and intraspecific levels. The initial trend in agriculture has been a decrease in the number of species used, accompanied by an increase in intraspecific diversity due mainly to the utilization in scientific breeding of locally adapted landraces (Brush, 1999). However, in recent times intensive agriculture brought about a severe reduction in environmental diversity, whereas scientific plant breeding developed in an almost world-wide scale has had the effect of restricting intraspecific diversity everywhere, being remarkable in the main cereal crops (Frankel *et al.*, 1995).

Likewise, cultivars of many forage species have been developed from a limited genetic base. Pedigrees of many red clover US cultivars are closely related, revealing common population origin (Sjodin, 1996); not surprisingly, Berg and Leath (1996) observed only small differences in cultivars susceptibility to *Stemphylium* leaf spot. Further examples could be observed in the genetic structure of crimson clover (*Trifolium incarnatum*) and UK white clover cultivars. Most modern US and European cultivars of crimson clover have relatively few distinct genetic sources, despite the potentially large variability available in the Mediterranean countries as well as *ex situ* collections (Frison and Serwinsky, 1995; Steiner *et al.*, 1998). Another example of narrow genetic base was given by the susceptibility of all white clover cultivars bred in the UK to clover rot (*Sclerotinia trifoliorum*) whereas all resistant cultivars were from Continental origin (NIAB, 1974). Furthermore, the inclusion of distinct sources of germplasm as well as the hybridization with related species (*Medicago falcata*, *M. varia*) had initially widened genetic diversity in alfalfa US cultivars, but their worldwide distribution and the concomitant replacement of landraces/local cultivars had finally reduced intraspecific diversity (Barnes *et al.*, 1977; Basigalup and Hijano, 1995; Piano *et al.*, 1996).

Most of the research on genetic erosion has been carried out in the major cereal crops, particularly in the Mediterranean areas where Vavilov performed the first collecting expeditions, being his records the foundation stone for several studies on genetic erosion (Frankel and Brown, 1984; Damania and Valkoun, 1991; Hawkes, 1995; Brush, 1999). Overall, considerable amount of variability still existed in the wild species of cereals and food legumes, but landraces had largely been lost due to replacement by advanced cultivars. Similarly, the issue of genetic erosion could have been overstated concerning most of the wild forage species, although information on the actual extinction of forage species is certainly incomplete (Cocks, 1995). Wild species, which exhibit ecological adaptation and highly localized pattern of distribution (specialist species), are most at risk for genetic erosion than those with a widespread distribution and high level of intraspecific diversity (generalist species). The *Trifolium* species indigenous to the North America could be a good example of specialist species, since several species considered

potentially threatened or endangered were identified in very specific microhabitats (Quesemberry and Taylor, 1997). Even though the generalist species usually remain abundant, the understanding of the balance between environmental changes and species survival will be essential for planning future germplasm collections based on their genetic erosion risk.

Genetic erosion, usually defined as the result of habitat destruction, reduces the number of species present in addition to the variability within species. In this context, the Mediterranean basin appears to be a prime candidate for genetic erosion, due to its role as a source of diversity for most cultivated species (Cocks, 1995; Brush, 1999). However, the reduction in genetic diversity is emerging in most temperate areas of the world, where the better grasslands are being plowed for cereals and overgrazing is destroying poorer grasslands (FAO, 1996). In addition, genetic uniformity of modern varieties and their widespread distribution throughout diverse ecosystems will probably become the most relevant feature of genetic erosion.

The value of landraces for forage breeding

Conservation of cultivated forms is a vitally important source of genetic variation for pasture improvement. As germplasm was introduced to new regions, natural selection as well as introgression from local populations led into the development of numerous locally adapted ecotypes and farmers landraces with the consequent raises in intraspecific variability. The approach for landraces conservation has been highly successful in grain crops, where efforts on *ex situ* conservation of the genetic variability has been concentrated on a reduced number of species and populations of grain crops (Frankel *et al.*, 1995). The essential role that landraces has played as sources of variation for plant breeding, and the fact that they were often under imminent threat of extinction, were the main reasons for this effort. In contrast, there has been few attempts to preserve landraces in forage species, be *ex situ* collections of perennial species in European countries (e.g. Poland, France; FAO, 1996; Charmet *et al.*, 1997).

How representative currently *ex situ* collections are of total diversity existing *in situ* is a question impossible to answer precisely. Nevertheless, the frequent inadequacy of landraces conservation in forage species contrasts with the high number of breeding programs that regularly incorporate them in order to enhance local adaptation. For example, over 90 percent of Russian red clover cultivars have been selected from local populations whereas breeding of *Lotus corniculatus* in Uruguay has relied on locally adapted populations (Williams, 1996; Rebuffo and Altier, 1997). Further examples of the utilization of landraces in forage breeding have been extensively reviewed (e.g. white clover - Williams, 1987; alfalfa - Michaud *et al.*, 1988; red clover - Williams, 1996). In recent years, however, it has become increasingly obvious that the time for collecting genetically diverse landraces is passing quickly for most of the major forage species. Indeed reports of the replacement of landraces by advanced cultivars are quite frequent in the literature, particularly in alfalfa (Ulanovsky *et al.*, 1990; Smith *et al.*, 1995; Rossanigo *et al.*, 1995; Piano *et al.*, 1996).

Farmers' knowledge can further enhanced the genetic value of a germplasm collection, providing useful passport information for breeders. Diverse strategies used in landraces germplasm collection rely on the information provided by local people, e.g. the type of the plant, seed origin, stand persistency. Indeed old stands under grazing are commonly used for germplasm collection in forage species (e.g. Itria and Tiranti, 1978; Wedderburn *et al.*, 1989; Widdup and Ryan, 1991; Basigalup and Hijano, 1995), in addition to the collection of farmers' seed lots (e.g. Ulanovsky *et al.*, 1990; Smith *et al.*, 1995). Their location as well as accurate

passport information (e.g. stand age, grazing management, seed origin) is certainly based on farmers' knowledge. Therefore, the trend to a rapid reduction in the number of farms poses further pressure over conservation of landraces.

We must recognize that today landraces are no longer the only breeding resources, giving way to breeders' populations and to crop-related wild species, especially in breeding for resistance to diseases and pests (Williams, 1987; Frankel *et al.*, 1995; Taylor and Quesemberry, 1996). However, analytical breeding has been largely unsuccessful in the case of stressful environments, characterized by high variability in climatic stresses, whereas natural selection has been able to combine escape and avoidance traits into the combination of genotypes that form landraces. Even though the local populations could be less productive than advanced cultivars in favorable environments, under adverse conditions (e.g. low fertility soils, water stress, intensive grazing) landraces can still outyield them (Ceccarelli *et al.*, 1991; Piano *et al.*, 1996). In addition to this specific genetic adaptation, landraces are genetically diverse and dynamic, combining escape and avoidance traits through long-term climatic and biological changes (Frankel *et al.*, 1995). Modernization of agriculture, exclusion of marginal areas from cultivation and the wide access of new cultivars have placed at serious risk the maintenance of coevolutionary selection pressures, which characterize the development of landraces populations. Not surprisingly, conservation *in situ* (e.g. reserves, on farm conservation) along with *ex situ* collections have been repeatedly proposed in diverse scientific as well as political forum, particularly in the most fragile ecosystems (FAO, 1996; Brush, 1999).

***Ex situ* conservation**

Temperate grasslands and pastures are among the world's major areas of production, yet in comparison with the cereal food crops, little effort has been made to conserve the great diversity of wild and cultivated forms of forage species (Table 1). Even though the International Plant Genetic Resources Institute (IPGRI) sponsored many collecting expeditions, and promoted the development of a large number of regional and country gene banks, forage species still represent less than 10% of all *ex situ* accessions whereas over 40% are cereals (FAO, 1996).

There is a notable unbalance in the size of the *ex situ* collections from different regions and countries, even though information on accession numbers is incomplete and certainly excludes small collections as well as many active gene banks handled by breeders (Table 2). Over 40 percent of all *ex situ* accessions of the genus *Trifolium*, *Medicago* and *Lolium* are assembled in Australia and New Zealand, while other perennial grasses collections are concentrated in Central Europe. ICARDA and VIR are also relevant Institutes related to the conservation *ex situ* of temperate pastures. This concentration of germplasm collections contrasts with South America countries, where the forage gene banks have rather small forage collections. The most significant collections, held in Argentina and Chile, are by and large linked to active breeding programs working with introduced species (Demagnet and Contreras, 1988; Cubillos and Suzuki, 1991; Craganz, 1990; Alonso and Clausen, 1998).

Crop species range from those only in the initial stage of intensive plant improvement, as many of the species used in pasture and range lands, to species like cereals which have been transformed by introduction and intensive breeding on a near-global scale (Frankel and Brown, 1984). The extent of this modification is reflected in the biological status of *ex situ* collections, although this information (whether wild, old variety, landraces, selection, hybrid, introduction) is not often adequately documented. The European Co-operative Program for the Conservation and Exchange of Crop Genetic Resources has documented the most comprehensive database

information for forage species, even though passport information of large collections (e.g. VIR) is insufficient. The breakdown of European *ex situ* collections by type of accessions given in Table 3 undoubtedly indicates the sparse development for most of the forage genus. Wild accessions represent a high proportion in legumes as well as grasses, a divergent feature with the main cereals collections where wild relatives are usually less than 4 percent (FAO, 1996). Advanced cultivars and breeding lines represent an extremely low proportion of the total legume accessions, being red clover and alfalfa the exceptions, with 12 and 15 percent of all recorded accessions as product of modern breeding. With regards to landraces and ecotypes, they are poorly and unevenly represented. Despite their generalized used as source populations for modern breeding, remarkable efforts have been only taken for perennial grasses (more than 40 percent of *Dactylis*, *Festuca* and *Phleum*) and white clover, whereas they represent a rather small proportion for other legumes. The main collections analyzed previously have an extremely high number of species suitable for forage production that are represented by few accessions, whereas other species are only stored in few national gene bank. Revert the balance of present collections will require international co-ordination for issues such as identification of collection gaps and assignation of priorities to endangered or threaten species or environments.

There is likely a high degree of duplication between the *ex situ* collections of European countries, New Zealand and US. One obvious means of improving the efficiency of gene banks would be to identify, as far as possible, redundant duplicate accessions within and between collections. While the identification of duplicates may be conceptually easy in the presence of adequate passport information, this is not the case in forages and the task can become discouraging tedious. In addition, even if all duplicates within collection were eliminated, the gains in efficiency from reduction in entry number are likely to be modest for many collections.

Core collections

The characterization and evaluation of germplasm collections are a continuing and essential process in many countries. However, the large size of germplasm collections and financial constraints, has been the major limitations reducing the effectiveness of germplasm evaluation and characterization, discouraging germplasm enhancement and utilization. To minimize these limitations Frankel (1984) and Frankel and Brown (1984), developed the core collection concept, and later Brown (1989a,b) developed its theory. A core collection consists of a set of accessions selected to represent the genetic diversity of a crop species and its relatives with minimum redundancy. The rationale of the proposal is to set up a hierarchical structure in the collection where a representative small subset (core) provides efficient access to the collection's diversity, while the remaining fraction is not eliminated but retained as a reserve collection (Brown, 1989a). This strategy minimizes the cost of genetic conservation, while insuring the preservation of maximum genetic variation. It also allows for rapid evaluation of germplasm, and better access to the base collection, reducing the overall cost of the process.

A considerable number of core collections have been proposed for different germplasm collections (Charmet *et al.*, 1993; Hamon *et al.*, 1995; Ortiz *et al.*, 1998; Abadie *et al.*, 1999; Malosetti *et al.*, 2000). Brown and Spillane (1999), based on a survey conducted by IBPGR, report core collections developed for 11 forage species: alfalfa, annual medics, berseen clover (*Trifolium alexandrinum*), Kentucky bluegrass (*Poa pratensis*), red clover, ryegrass, shaftal clover (*Trifolium resupinatum*), subclover, sweetclover (*Melilotus*), trefoil and white clover. Most of these collections have been developed by the USDA system. Two recent examples are the core collections developed for *Poa pratensis* (Johnson *et al.*, 1999), and annual medics

(Skinner *et al.*, 1999). Moreover, there have been reports on the use of previously established core collections for searching valuable traits in forage breeding programs (Bouton, 1996; Jung *et al.*, 1997; O'Neill and Bauchan, 2000). However, considering the high number of large collections of forage crops reported by FAO (1996), there is still plenty of room for the use of this methodology.

The development of a Core Collection is basically a sampling exercise that tries to assure the conservation of the alleles present in the base collection. To consider that, it is important to address two issues: a) whether all the alleles in the base collection are equally important to be included in the core, and b) whether a reasonable proportion of target alleles can be included in the core. Regarding the first issue, Allard (1992) found that allele frequencies of loci affecting morphological, disease resistance, allozyme and RFLPs variants were highly correlated with adaptability and productivity. The following three alleles classes are mentioned: i) alleles that are present in a high proportion of accessions which make a significant contribution to wide adaptation, ii) alleles that are present in intermediate to high frequencies but not widely distributed, which specially contribute to adaptedness to specific environments, and iii) alleles that are absent or at low frequency in nearly all accessions and so are supposed to contribute little or nothing to adaptation (Allard, 1992; Brown, 1989a). Therefore, class i and ii alleles are of high priority in sampling useful germplasm for plant breeding, because they control traits of agronomic importance contributing to productivity and resistance or tolerance to biotic and abiotic stresses. Class iii alleles constitute a special case, because even when they generally offer little value for breeding, they should not be ignored, because they can in certain cases contribute with quality traits.

With regard to the issue of including a reasonable proportion of target alleles in the core, any sampling process will almost certainly include class i alleles, and will have low chances of including rare alleles. So, special attention should be put in the conservation of class ii alleles, through an adequate stratification of the collection. If a specific rare variant exists in a collection, finding it depends mainly on sample size and luck, being largely unaffected by strategy (Brown, 1995). Probably a systematic search of the collection is still the best alternative, with the disadvantage of being expensive.

Stratified random sampling is the procedure recommended by most authors to develop a core collection (Brown, 1989a; Spagnoletti Zeuli and Qualset, 1993; Crossa *et al.*, 1994; Brown 1995; Crossa *et al.*, 1995; Harch *et al.*, 1995; Franco *et al.*, 1998; Johnson *et al.*, 1999). The two main axis to be considered in the stratified random sampling procedure are geographic distribution and genotypic composition (Brown, 1989a; Crossa *et al.*, 1994; Hintum, 1995; Abadie *et al.*, 1998).

In the process of developing core collections, the importance of geographical factors for properly classifying and characterizing large germplasm collections becomes evident. Quite a few core collections were developed using ecogeographical origin as a major component for germplasm classification and stratification (Charmet *et al.*, 1993; Spagnoletti Zeuli and Qualset, 1993; Cordeiro *et al.*, 1995; Tohme *et al.*, 1995; Abadie *et al.*, 1998). Wild genetic resources and landraces may show site-specific resistance to biotic and abiotic stress. By knowing the prevailing environmental conditions at the collection site, inferences can be made with regard to degrees of adaptation. Many studies support the hypothesis that resistance to abiotic stresses may be found in accessions previously exposed to the specific environmental stress (Hawtin *et al.*, 1996; Sayed, 1985; Beebe *et al.*, 1997). In addition, isolation by distance can contribute to genetic differentiation, especially for open-pollinated species. That is, populations that are located closer are more likely to share common genetic backgrounds, while distant populations,

even when similar phenotypically, are likely to have different genetic backgrounds that have been selected independently leading to similar adaptations.

Recently, Balfourier *et al.* (1998) compared several sampling strategies including geostatistical analysis for developing core collections of natural populations of perennial ryegrass, a naturally open pollinated species, and annual medics, a naturally self-pollinated species. The comparisons for the two species showed that the strategies that took into account the spatial structure of diversity gave core collections with best retention statistics for morphological and agronomical traits.

One of the major limitations for the use of geographical factors for germplasm classification is that passport data usually lack environmental factors of the collection site for the individual accessions. In situations like this, the use of geographical information can be not so informative, leading to poor classifications. Johnson *et al.* (1999), developing a core collection for Kentucky bluegrass, observed that the strategies using geographical classification were no different from a random sample, while those using clustering on morphological and agronomical data produced improved retention statistics. They attributed these results to the lack of precise geographical and ecological information in their data set. This can be ameliorated with the use of Geographic Information Systems (GIS), as these systems operate with digitized maps storing environmental information of large areas, that includes vegetation, pedological surveys, climatical and geomorphological data. The simultaneous plotting of the geographical location of the germplasm and digitized maps in GIS enables a fair inference about the environmental characteristics of the collection site (Jones *et al.*, 1997; Guarino *et al.*, 1999). An additional advantage of the system is the possibility of continuously updating its digital database.

There are a number of practical reasons for core collections, but all of them imply saving resources due to the economy of size, allowing to assign saved resources to other germplasm related activities (Morales *et al.*, 1995; Brown, 1995). For curators, the core allows to set up priorities for activities as germination test, regeneration, and stock management (e.g. how to increase the collection, or in the identification of duplicates). For breeders, the core represents a logical first step for the screening of desirable alleles in the collection. Moreover, the time and financial resources needed to evaluate a new trait in the collection are reduced, allowing to increase the number of characters evaluated and widening the set of techniques used (e.g. molecular markers).

Future trends

The implicit purpose in preserving the genetic diversity is to develop technology that will enable farmers to produce in a manner that is sustainable and economically viable. Since it is far easier to accomplish this aim through the establishment of *ex situ* gene banks, the size of grasses and legumes collections will continue to increase. The generalized utilization of tools such as core collections, digitized maps for passport information or biotechnology techniques for evaluation of genetic diversity, will ensure a more efficient utilization of the resources available for collection and characterization, being particularly relevant at the time of defining collection gaps.

Even on the modest scale, the preservation of genetic diversity involves substantial costs in scientific effort, organization and resources. The problems of managing increasing species diversity are just emerging, particularly for the *ex situ* collections of wild species. The great concern about species extinction caused by deforestation, agriculture, grazing, brought a concomitant increment in the size as well as species diversity of *ex situ* collections. At the mean

time, regeneration for a large number of old accessions is urgently required. In the near future the arising questions on conservation strategies will certainly include issues such as: the confrontation of resampling conserved sources *in situ* instead of regenerating accessions; the efficacy of regeneration procedures to preserve genetic diversity and stability of the accessions; the identification of interspecific and intraspecific gaps of value for future breeding.

In situ conservation is widely regarded as the method of choice for the preservation of wild species. The preservation of representative populations throughout their natural geographic range should involve the establishment of special genetic reserves, which are mainly located in countries with limited resources for genetic research. The genetic composition and structure of populations in reserves could be of primary research interest to disclosure co-evolutionary selection pressures in stressful environments, yet the development of *in situ* conservation programs with tangible results will require the joint effort of farmers, countries and international organizations. Progress in the establishment of soundly based *in situ* conservation programs for grasslands could be difficult and time consuming, but it is certainly a priority need for centers of diversity.

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Table 1 - Total accessions number for the major cereals and temperate forage crops grouped by categories. (Source: FAO, 1996).

Cereals	Accessions No.	Forage Legumes	Accessions No.	Forage Grasses	Accessions No.
<i>Triticum</i>	788,654	<i>Trifolium</i>	78,405	<i>Lolium</i>	38,149
<i>Hordeum</i>	486,724	<i>Medicago</i>	52,764	<i>Festuca</i>	23,725
<i>Oryza</i>	420,341	<i>Vicia</i>	26,244	<i>Phleum</i>	9,127
<i>Zea</i>	261,584	<i>Lathyrus</i>	13,253	<i>Poa</i>	7,922
<i>Avena</i>	223,287	<i>Lotus</i>	3,636	<i>Dactylis</i>	6,594
<i>Sorghum</i>	168,550			<i>Elymus</i>	2,665
<i>Triticale</i>	40,131			<i>Bromus</i>	2,097

Table 2 - Accessions number of the main collections of germplasm for the major forage legume and grasses. (Source: FAO, 1996).

Genus	Oceania	Central Europe	ICARDA	France Spain	Japan	VIR	US Canada	Others
Legumes								
<i>Trifolium</i>	32,000	3,652	3,401	3,879		5,131	5,173	24,099
<i>Medicago</i>	22,000	1,292	8,456	1,140				17,949
<i>Vicia</i>		1,541	5,353	2,264		3,138		12,114
<i>Lathyrus</i>			1,682	3,627				7,944
<i>Lotus</i>	1,800							1,836
Grasses								
<i>Lolium</i>	18,000	3,814			1,055			15,335
<i>Festuca</i>	1,100	4,484			3,896		1,195	13,050
<i>Dactylis</i>		11,450			5,739	1,072	1,044	6,594
<i>Phleum</i>		5,004						
<i>Poa</i>		2,329						5,593
<i>Bromus</i>	2,220	5,045						2,067
<i>Elymus</i>							2,239	467

Table 3 - European germplasm collection for the major cultivated forage crops. Number of institutes involved, species within the genus and accessions, and the accession cataloguing expressed as a percentage of the accession number. (Data synthesized from Bettencourt and Perret, 1986; IBPGR, 1991; Frison and Serwinski, 1995).

Crops	Institute No.	Species No.	Accessions No.	Accessions cataloguing				
				Cultivars/ breeding lines	Land-races	Eco-types	Wild	Unclassified
Grasses								
<i>Lolium perenne</i>	29	1	14,209	18	27	1	28	27
<i>Lolium multiflorum</i>	23	1	2,659	28	23	1	18	30
<i>Dactylis</i>	38	7	16,015	11	46	0	23	19
<i>Festuca</i>	42	56	12,763	12	43	0	20	25
<i>Phleum</i>	27	11	7,938	15	40	0	20	26
<i>Bromus</i>	26	44	790	7	13	12	15	65
Legumes								
<i>Trifolium pratense</i>	37	1	7,462	12	11	0	9	69
<i>T.subterraneum</i>	16	1	6,107	5	14	0	65	15
<i>Trifolium repens</i>	32	1	3,383	6	40	0	10	45
<i>Medicago</i> perennials	36	14	7,441	15	17	0	6	63
<i>Medicago</i> annuals	26	59	5,466	6	16	0	59	19