Genetic Resources

Beat Boller[1] and Stephanie L. Greene[2]

[1] Agroscope Reckenholz-Tänikon, Research Station ART, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland, beat.boller@art.admin.ch
[2] USDA, ARS National Temperate Forage Legume Genetic Resources Unit, 24106 North Bunn Road, Prosser, WA 99352, USA, stephanie.greene@ars.usda.gov

1 Introduction

Plant genetic resources (PGR) for food and agriculture consist of the diversity of genetic material contained in traditional varieties and modern cultivars grown by farmers as well as crop wild relatives and other wild plant species that can be used for food, feed for domestic animals, fiber, clothing, shelter, wood, timber, energy, etc. (FAO 1997). Fodder crop genetic resources broaden the FAO definition of PGR, which is based upon field crops. In maize and many other crop species, the wild form of the cultivated species no longer exists, since breeding for domesticity has resulted in plant species being unable to reproduce without the helpful hand of humans. Forages are less domesticated (Harlan 1983). Unlike many field crops, wild forms of common forage species still exist, as well as feral (naturalized) forms (populations that originated from forage crops, but that escaped to persist in the natural environment). Such wild populations are usually called "semi-natural" because they have developed in an agricultural situation, but without conscious selection. They would not fall in any of the categories of the definition of PGR as cited above but can be regarded similar to crop wild relatives. The closeness of wild and cultivated forms of fodder crop species makes a wealth of natural genetic variation readily accessible for use in breeding.

Evidently, PGR are indispensable for any breeding effort. At first and very obviously, PGR with desirable traits must be chosen to initiate the breeding process. The choice of this initial material is crucial for the programme because breeding is a long-lasting process, and many years of selection and recombination are needed before success can be assessed and finally, a new variety can be created. How much the origin of the starting material can influence the properties of a breeding programme has been extensively studied in an interesting example from New Zealand (Bahmani et al. 2001). Recent varieties derived since 1975 from the "Mangere" ecotype of perennial ryegrass differed fundamentally in yield potential, growth habit and behaviour under grazing from older varieties derived from the

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geographically distinct "Hawke's Bay" ecotype, which had previously dominated the New Zealand ryegrass seed market between 1936 and 1964. While the more recent varieties derived from the "Mangere" ecotype were higher yielding under cutting, partly due to a more erect growth habit and a greater proportion of fertile tillers, they were less adapted to grazing than the older varieties derived from the "Hawke's Bay" ecotype, leading to problems of persistence. It is interesting to note that this fundamental difference was only discovered after release of the more modern varieties. This observation points to the fact that the best possible knowledge of the properties of potential starting materials should be obtained before making the choice.

Second and less obviously, PGR are needed to add new variability to an existing breeding programme. A basic tenet of plant breeding is that gain from selection increases with an increase in additive genetic variance for a given character (Fehr 1987). Selection inevitably decreases additive genetic variance in breeding material. For example, when we select for disease resistance, we aim at eliminating susceptibility genes in order to obtain a population which is homozygous for major resistance genes. This resulting population has a high level of resistance and low variability in susceptibility to the disease. However, since we select only a limited number of individuals in each cycle, genetic diversity for all other traits is also affected. Rare alleles are rapidly lost and opportunities for selection decrease because the most frequent alleles become fixed in the population. Furthermore, in out crossing self-incompatible taxa, inbreeding depression may occur because there is a higher chance of homozygosity for deleterious recessive genes.

An infusion of exotic germplasm at this point will increase additive genetic variance. However, there will be a reduction in mean performance, as the population moves away from the selective peak reached through previous breeding efforts. The less adapted the PGR, the greater the drop in performance. With continuous selection within the broadened breeding population, performance will improve again. However, if introduced beneficial alleles are the same as those already present in the breeding population, further selection will return the population only back to the same selective peak, and no net gain in performance will be realized. Only if the introduced alleles are unique, can selection increase the level of performance to a higher selective peak. Therefore, PGR most likely to improve upon quantitative traits will be those accessions that possess favourable alleles not present in the breeding gene pool. Humphreys (2003) reviewed criteria and objectives of the use of new genetic material in a long-term breeding programme with a special focus on sustainability. Appropriate strategies for the use of PGR in the breeding programme will be discussed in Section 5 of this Chapter.

2 Types of Genetic Resources and Conservation Modes

2.1 Categories of PGR

Four basic categories are of potential importance for fodder crop and amenity grasses breeding programmes:

1. Wild relatives: Most fodder crops and amenity grasses belong to large genera with several more closely related species of potential interest in breeding. However, due to their relatively young history as crops, available genetic variability within the cultivated species of fodder crops and amenity grasses is generally still quite large. Nevertheless, the use of wild relatives in breeding programmes is of importance in allopolyploid species with complex systematic like alfalfa or white clover, and wild relatives have been used successfully to introgress specific characters into the cultivated species, such as the profuse flowering trait from *Trifolium nigrescens* into white clover (Marshall et al. 2008).
2. Wild and semi-natural forms of cultivated species: These two sub-categories are difficult to distinguish for most species because there is no clear borderline between wild and semi-natural forms. This is because permanent grassland in most relevant cases exists only as a consequence of human agricultural activity in zones where forests would be the natural vegetation. Adapted native grasses originating from non-agricultural habitats settle in permanent grassland together with naturalized populations of the same species which may have spread from an initial seeding. Therefore, such populations form a continuum from wild populations in non-agricultural habitats to populations of natural and semi-natural grassland. Rather than trying to assign them to an either wild or semi-natural origin, it is more appropriate to address such populations as *ecotypes*. Using the term "ecotype" implies populations which have adapted to a known environment after many years of natural selection, usually involving natural re-seeding but without deliberate human interference such as selection, seed harvest, or human-mediated seeding. For ecotypes, natural selection is the main driving force of genetic differentiation. Ecotype populations usually do not arise from an initial sowing of the species, neither sown as such nor as part of a seed mixture, but from spontaneous seedlings emerging gradually over the years through natural spreading. That is, human interference in the development of an ecotype is limited to actions of usual management practices, such as frequency and type of utilization, or intensity of fertilization. If sufficient cycles of recombination with local genetic material and natural selection have occurred, an initial sowing of a fodder crop variety may also give rise to an ecotype population. In ecotype studies, it is often postulated that a certain number of years must have elapsed since the last deliberate re-seeding before a population can be called an ecotype. The time span postulated ranks from 10 to 25 years. Examples of ecotype studies with relevance to fodder crops and amenity grasses breeding are discussed in Section 3.1.
3. Landraces: Populations which have adapted to a specific region or location, such as a farm (farm varieties, "Hofsorten") by repeated seed harvest and human-mediated re-seeding in the same region or location. The term "landrace" implies that human interference plays an important role in the development of the population. In the case of landraces, human actions are usually carried out deliberately to improve local adaptation, e.g. by re-seeding the surfaces with locally produced seed, and by carrying out seed harvest after several years of utilization as forage to improve persistency. Prominent examples of highly valuable, traditional landraces are alfalfa in Italy (Torricelli et al. 2003), timothy in Norway (Schjelderup

et al. 1994), and red clover in Switzerland (Boller et al. 2003; Hermann et al. 2003; Kölliker et al. 2003).

4. Varieties: Any cultivated variety (cultivar), whether freely available on the market, protected by plant breeder's rights, or having become obsolete and stored in gene banks, can be used in breeding without any restriction. The right to freely use even protected varieties as PGR in breeding is called "breeder's exemption" and is an important provision of the international convention for the protection of new varieties of plants (UPOV 1991). For use in breeding, varieties have the advantage of being precisely described through the registration procedure for distinctness, uniformity and stability (DUS), and usually have been evaluated extensively in official tests for their value for cultivation and use (VCU). Furthermore, commercially successful varieties have proven their ability to give satisfactory seed yields. These properties render cultivars very popular as PGR in fodder crop breeding. Once a variety has ceased being produced for the market, and thus has become "obsolete", an appropriate seed sample is added to the gene bank collection of the respective country. The gene bank will then assume from the breeder the responsibility for long-term maintenance of the variety's integrity, and for securing availability of seed for use in breeding or research. During the commercial lifetime of a variety, seed samples can easily be obtained from the breeders who exchange their varieties free of charge as a voluntary service to their peers.

2.2 Modes of PGR Conservation

Two modes of conservation are of importance for fodder crop and amenity grasses PGR: While all types of PGR are maintained *ex situ* as seed samples in gene banks, wild relatives, ecotypes and landraces can also be maintained *in situ* (referred to as "on farm" in the case of landraces). The two approaches differ fundamentally in their objectives regarding the genetic make-up of PGR. In *ex situ* conservation, maintaining the genetic integrity of the original seed sample is a major concern and all measures of collection, storage, regeneration, and distribution aim at keeping presence and frequency of alleles within the population as constant as possible. Conversely, the objective of *in situ* conservation is to maintain the environment which has allowed the development of the distinctive properties of the PGR. In the case of *in situ* conservation, genetic evolution is deliberately made possible in order to allow a further development of PGR to even better match the requirements of their specific environment. The common objective of the two strategies is to conserve a maximum of different alleles and the largest possible amount of genotypic diversity with as few individuals as possible (Hayward and Sackville Hamilton 1997).

3 Genetic Resources Maintained *In Situ*

3.1 Breeding Importance of *In Situ* Germplasm

Historically, genetic resources growing *in situ* have been by far the most important sources of germplasm used in breeding of fodder and amenity grasses and most

perennial legumes, with the exception of alfalfa and red clover which have a longer tradition of being cultivated as sown crops. In the search for well adapted and persistent genetic materials, breeders have systematically explored permanent grassland in their target regions to collect ecotypes. They followed the recommendation of Hertzsch (1959) that "suitable starting plants will be found on old permanent grassland with an association of species which is typical for the respective situation". His discussion about the starting material for grass breeding clearly pointed to the great potential value of diverse natural permanent meadows and pastures as reservoirs of well-adapted populations of grassland species. Undoubtedly, the use of adapted genetic material collected in permanent grassland has been of great benefit to early fodder crop breeding. It has dramatically improved persistency of fodder grasses compared to the often exotic provenances of grass seed that had been used previously. The bulletin of perennial forage plants listed in the French national catalogue in 1984 (I.N.R.A. 1984) lists ecotypes as the material of origin for the large majority of varieties for which an unequivocal origin was declared (Table 1).

Table 1 Declared origin of varieties in the official French catalogue of fodder plant varieties accepted 1957–1984 (I.N.R.A. 1984)

	Number of varieties originating from:			
Species	Ecotypes	Varieties or landraces	Ecotypes and varieties	Breeding material of diffuse origin
Dactylis glomerata	12	0	0	1
Festuca arundinacea	11	0	0	6
Festuca pratensis	4	1	1	1
Phleum pratense	5	0	3	1
Lolium perenne	10	1	8	9
Lolium multiflorum ssp. *italicum*	5	1	3	10
Total	47	3	15	28

Nowadays, breeders rely much more on crosses between varieties as starting materials for their breeding, rather than introducing newly collected material. However, since most of the older varieties have been derived from an original collection in grassland, we can assume that the majority of varieties currently in use trace back at least partly to breeding material originally created from collections of PGR *in situ*. This is reflected by the small genetic distance between cultivars and ecotypes found in molecular studies, for example in perennial ryegrass (Bolaric et al. 2005; McGrath et al. 2007), meadow fescue and Italian ryegrass (Peter-Schmid et al. 2008b).

In modern forage crop and amenity grasses breeding, collected material of ecotypes still keeps its significance as a source of hitherto unused genetic variation for particular traits of interest. For example, Beuselinck (2004) used material of wild accessions from Morocco to introduce the rhizomatous growth character into *Lotus corniculatus*. Swiss ecotypes of *Lolium multiflorum* were used successfully to create a variety with improved early spring growth and resistance to snow mould diseases, and a variety which combines a strong tendency to form inflorescences in the seeding year with excellent persistence (Boller et al. 2005a).

3.2 Grassland-Dominated Regions as Centres of Diversity

Grassland-dominated regions provide the most diverse opportunities for collecting PGR of fodder crops and amenity grasses *in situ*. For most temperate species of interest, non-irrigated permanent grasslands are concentrated in zones with at least 800 mm of annual rainfall and about 9°C annual mean temperature. This is particularly true for more intensively utilized grassland. Areas suitable for intensive agriculture in zones with 600–800 mm of annual rainfall would still provide good conditions for permanent grassland, however, such land was turned into arable land wherever possible. If livestock is reared in these zones, temporary grassland and arable forage crops provide the main part of feed rather than natural grassland.

Grassland-dominated regions occur in temperate zones around the world. For example, northern Africa, temperate Asia, as well as the Atlantic islands of Macaronesia belong to the area of natural distribution of important grassland species. In Europe, temperate grassland-dominated regions are found in the Pyrénées, on the British Isles and the Balkan, but are particularly diverse in the zone between the northern foothill of the Alps and the coastal zones of the Atlantic and Baltic sea (Figure 1). In the following discussion, we will focus on Europe from the Alps northwards as one of the major centres of diversity of fodder crops and amenity grasses. Most temperate grassland species are truly indigenous to that region, while some most likely have developed naturalized populations from historic introduction as a fodder crop. Scholz (1975) suggested the latter status for *Arrhenatherum elatius, Alopecurus pratensis* and *Phleum pratense* for the whole of Europe, and also for *Lolium perenne, L. multiflorum* and *Trisetum flavescens* for many regions where these species are restricted to man-made habitats.

Apart from climatic and edaphic factors, grassland management contributes substantially to the diversity of grassland plant communities. Intensive management, specifically a high grazing pressure, tends to decrease species diversity. In recent years, targeted programmes aiming at a more relaxed grassland management as part of agri-environment measures have been established to increase biodiversity. However, the response of the plant communities to reduced management intensity is slow (Marriott et al. 2004). Nösberger et al. (1998) concluded that management for habitat heterogeneity at all scales will conserve most of the biotic diversity at a site. Such a system considers diversity not only on a small scale of cutting or fertilization regimes of a particular field, but includes the larger scale of landscape

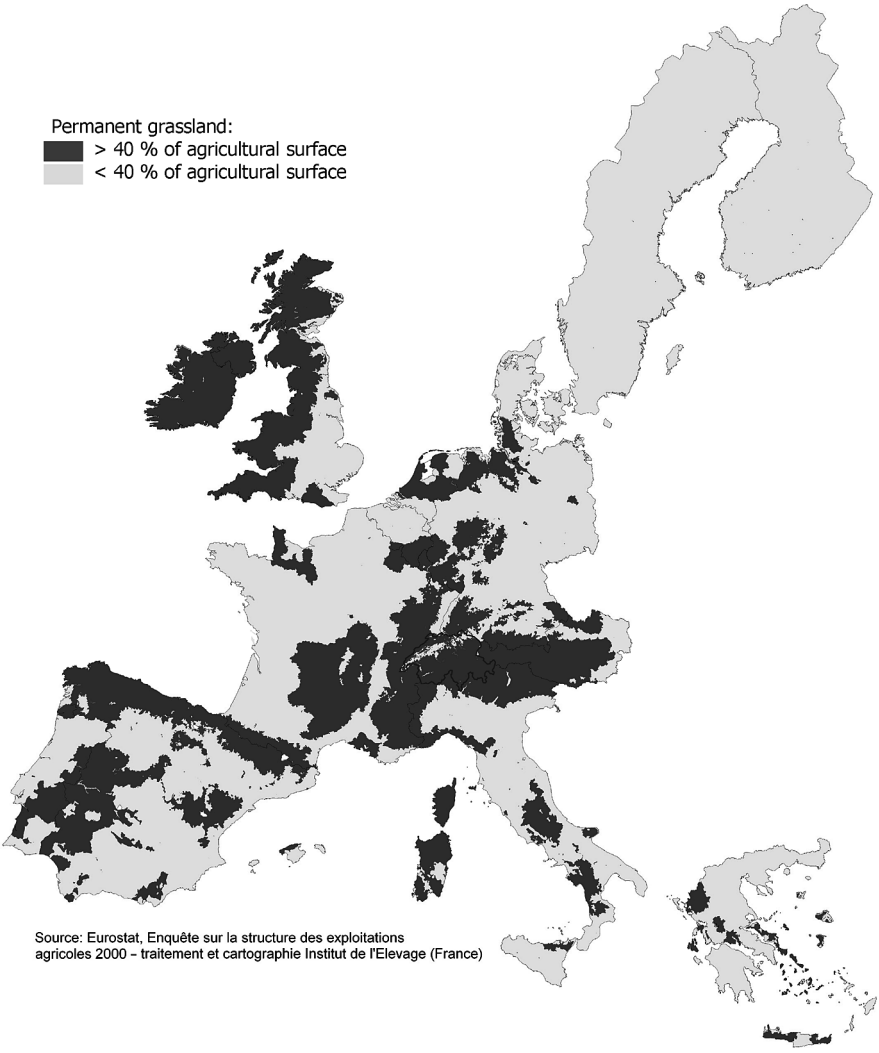

Fig. 1 Regions of Europe with important proportions of permanent grassland per agricultural surface. Adopted from Pflimlin et al. (2005); Swiss data supplemented by E. Szerencsits

structure. It will allow for intensive management of the most favourable pastures to produce high-quality ruminant feed, along with maintenance of infrequently cut hay meadows providing opportunities for environmental services (Nösberger and Rodriguez 1998). Clearly, maintaining diverse grassland on the landscape scale in such a way will also assist *in situ* conservation of PGR of fodder crops and amenity grasses.

3.3 Criteria and Strategies for Collecting PGR *In Situ*

Depending on breeding objectives, two basic criteria need to be considered in making a strategy for collecting fodder crops PGR *in situ*:

1. When the primary objective is to enlarge genetic diversity of the breeding programme, the relative degree of genetic variation within and among sampling sites will affect decisions about the number of sites to be visited and the number of individuals to sample per site.
2. When the objective is to find new genes affecting particular characters, the choice of collecting sites will be influenced by the presence of environmental or management factors that are selective forces for traits of interest.

Genetic variation within and among ecotype populations has been studied using molecular markers for a number of grassland species (Table 2). Although different marker systems have been used to assess diversity and this may affect the estimates, a general picture emerges from these studies showing that the variation within populations accounts for at least 60 and up to 98% of the total genetic variation. This suggests that sampling a large number of sites with just a few individuals is less effective for capturing genetic diversity than sampling fewer sites with a higher number of individuals.

Table 2 Contribution of among and within populations variance in marker-based studies of genetic diversity of grassland ecotypes, based on analysis of molecular variance (AMOVA)

Source	Species	Marker system	No. of ecotype pops.	No. of indiv. per pop.	AMOVA: % of variance	
					Among pops.	Within pops.
Bolaric et al. (2005)	*Lolium perenne*	RAPD	22	20	29	71
Rudmann-Maurer et al. (2007)	*Poa alpina*	SSR	54	8	25	75
Reisch et al. (2003)	*Sesleria albicans*	RAPD	25	4	38	62
Peter-Schmid et al. (2008a)	*Lolium multiflorum*	SSR	12	23	2	98
	Festuca pratensis	SSR	12	23	4	96
McGrath et al. (2007)	*Lolium perenne*	cpSSR	61	16	37	63
Fjellheim et al. (2005)	*Festuca pratensis*	AFLP	15	20	31	69

Molecular studies published so far do not point to a specific strategy in the search for sites with a high genetic variability. In a long-term experiment at two locations, it was shown that genetic variation within ecotype populations of *Festuca pratensis* was negatively affected by a higher intensity of agricultural management, namely, by an increase of defoliation and fertilization frequency (Kölliker et al. 1998). However, this effect was not observed in a larger collection of ecotype populations of the same species (Peter-Schmid et al. 2008b). Rudmann-Maurer et al. (2007) did not find an effect of fertilization intensity on genetic diversity of *Poa alpina* ecotype populations, nor did they find a reduction of genetic diversity with decreasing abundance of the species in the sward.

However, molecular marker systems which are suitable to describe genetic diversity, such as RAPD, AFLP or SSR, deal with anonymous loci and are often located in non-coding regions of the genome. Although morpho-physiological characters are more cumbersome to assess and need replications in space and time to yield accurate results, they have been helpful in pointing to a specific strategy of sampling many sites to capture extremes in trait values. When the variability of morpho-physiological characters of a comparable set of ecotypes was assessed in relation to molecular marker variability, usually, a larger among populations variability was observed for the morphological characters than would have been expected from the variability of molecular markers. For example, Fjellheim et al. (2007) with Norwegian and Peter-Schmid et al. (2008b) with Swiss material found ecotypes of *F. pratensis* to exhibit larger ranges of mean values for 16 out of 19 and 13 out of 15 morphological characters investigated, respectively, than for cultivars, whereas the opposite was true for molecular marker diversity (Fjelllheim et al. 2005, Peter-Schmid et al. 2008a). This implies that sampling ecotype populations from a larger range of sites will increase the chance of including the extremes for the traits of interest.

The influence of environmental factors as selective forces at the genotypic level is at the base of the concept of ecotypic differentiation. It generally holds true that ecotypes from contrasting climates are better adapted to climatic conditions which are more similar to those at their origin. Winter hardiness and resistance to heat or drought are typical examples. Resistance to biotic stress can be expected in a climate which is favourable for the pathogen. A well-documented example is crown rust in ryegrasses and fescues which affects populations from high altitude much more strongly than populations from low altitude, where the pathogen *Puccinia coronata* finds better conditions for survival and spreading (e.g., Peter-Schmid et al. 2008b, Balfourier and Charmet 1991).

However, management factors can dramatically override the effects of environment and lead to strong differentiation on a small spatial scale. Tyler (1988) demonstrated this with an example of *L. perenne* sampled either within a hay meadow or on a path leading through that meadow. The population from the path flowered 30 days later, produced over five times less dry matter in spring, but suffered five times less from a freezing test than that from within the meadow. These differences were as large as the range observed in a more general way between southern and northern ecotypes from the whole of Europe. From this and other

similar examples, it can be concluded that collections should be made in zones with a climate similar to that of the target region of the breeding programme, but management history of the sites should be taken strongly into account.

An obvious way to infer management history of a potential sampling site is to assess floristic composition of the vegetation present. Plant species composition at a given moment is a good indicator of management factors which have been effective at the site over previous years. It is reasonable to assume that management factors affecting plant species composition will affect genetic differentiation of ecotypes in a similar way, and therefore, similar ecotypes of a species can be expected in grasslands of similar floristic composition or vegetation classification. However, this plausible correlation has not been studied to a great extent. A recent study with *L. multiflorum* (Boller et al. 2009) suggested that ecotypes from sites the vegetation of which was classified as *Lolietum multiflori* were more productive and showed better resistance against bacterial wilt and snow mould than ecotypes from *Arrhenatherion* sites. This suggests that the chances of finding well-adapted germplasm of a species can be increased by choosing collecting sites with a floristic composition pointing to agricultural management similar to that of the target use. Additionally, visiting places with contrasting floristic composition appears to be a good way to increase genetic diversity within a collection of a species.

3.4 Protection of PGR *In Situ*

During the past few decades, the protection of fodder crops and amenity grasses PGR maintained *in situ* has received increasing interest. This may be exemplified by two, temporally spaced reviews of PGR activities of the same institution, namely the former Welsh Plant Breeding Station in Aberystwyth (UK), nowadays the Institute of Biological, Environmental and Rural Sciences. While Tyler (1988) described naturally occurring ecotypes of forage grasses as "a seemingly limitless gene pool of variation on which the breeder can draw", Humphreys (2003) stated that "although it is diminishing year by year through genetic erosion, a wide range of valuable genetic resources are still available in the natural or semi-natural grasslands of Europe". He listed eight types of habitat risks which would justify collections to conserve adapted gene complexes, among which "ploughing and re-seeding" and "management change" were the two most important. The potential need of protection of forage grass populations as PGR is therefore seen in the same context as the protection of habitats to maintain biodiversity at the plant and animal species, as well as the ecosystem level, where general intensification of agricultural production is regarded the major threat to the conservation value of grassland (Marriott et al. 2004).

Nösberger (1994) presented an approach of promoting an individual farm-based grassland system with a varied, site-specific management supporting long-term, floristic stability of grassland as a promising way of encouraging farmers to contribute to the maintenance of biodiversity. On a landscape scale, such a system results in a diversity of habitats (see Figure 2) which creates opportunities for *in*

Fig. 2 Permanent grassland of varying intensity of management provides opportunities for *in situ* conservation in grassland-dominated regions such as in Northeastern Switzerland (Photo G. Brändle)

situ conservation of diverse populations of grassland species. Options to achieve both agricultural and nature conservation objectives in grassland systems were discussed in a similar way by Wilkins and Harvey (1994). The need for financial compensation to farmers for the economic constraints of such systems has been recognized and is implemented in modern agricultural policies, e.g. in the Common Agricultural Policy (CAP) of the European Union. Whether or not such an overall strategy is sufficient to adequately protect *in situ* conserved PGR remains open to question.

Programmes for promoting biodiversity with a focus on nature conservation concentrate on extensively managed, species-rich grassland. However, more intensively managed grassland may hold ecotypes which are of greater interest for future fodder crop breeding. Peter-Schmid et al. (2008a) showed that Swiss ecotype populations of *F. pratensis* from extensively managed habitats with a high nature conservation value contained significantly less rare alleles than populations from habitats managed more intensively. Management intensity also had a significant influence on morphological characters (Peter-Schmid et al. 2008b). Different conclusions were drawn by Van Treuren et al. (2005) for ecotypes of *L. perenne* and *Trifolium repens* from old Dutch grasslands. They concluded that no specific conservation measures were needed for ecotypes from pastures in agricultural use because they did not differ basically from ecotypes from nature conservation areas. This may reflect the absence of habitat fragmentation in Dutch grasslands, preventing strong ecotypic differentiation. In regions with a more variable agricultural landscape, the protection of fodder crop PGR in grassland of agricultural use with different levels of intensity appears to deserve adequate attention.

4 Genetic Resources Maintained *Ex Situ*

Ex situ germplasm collections serve a dual purpose. They are an important tool for conserving genetic diversity and they provide genetic resources for a broad range of users (Greene and Morris 2001). *Ex situ* conservation activities can be divided into the following general categories: acquisition, maintenance, evaluation and distribution.

4.1 Forage Germplasm Acquisition

Germplasm is collected to either fill gaps in existing collections or to protect forages at risk of disappearing. The objective in germplasm collection is to sample the most amount of diversity with a manageable amount of accessions. The strategy proposed by Marshall and Brown (1983) for forage species includes collecting seed from 50 to 100 individuals at each site, and sampling as many sites as possible to capture the range of environmental diversity. Sackville Hamilton and Chorlton (1995) outline strategies for collecting vegetative samples of forage grasses and legumes. Vegetative sampling requires additional effort to produce seed but may reduce effects of sampling time on preferential sampling of either early or late flowering genotypes. A possible compromise is to collect tillers with inflorescences of varied ripeness, immerse them in tap water and allow them to set seed in appropriate isolation. Collection sites and collecting details need to be thoroughly documented. Information such as geographic coordinates, ecological site description and improvement status (i.e. wild, landrace, cultivar) help plant breeders selecting germplasm since adaptation can be inferred from this data (Steiner and Greene 1996). Recent acquisition objectives for forages have focused on collecting wild relatives and landrace germplasm throughout Central Asia, in countries once part of the former Soviet Union (Street 2002, Greene et al. 2005). Efforts have also focused on collecting landraces and wild species from European countries (e.g. Chorlton et al. 2000, Annicchiarico 2006, Pederson et al. 1999). Francis (1999) provides a list of forage species that should be collected in the Mediterranean to broaden adaptation to specific edaphic conditions. Future acquisition efforts undoubtedly need to focus on forages that will expand current ecological niches to meet the challenges of global climate change.

4.2 Storing and Regenerating Forage Genetic Resources

Ex situ conservation of forages usually involves the storage of seed in gene banks. Standard temperatures for active collections are 0–4° C and for base collections −18°C, both at 3–7% seed moisture. Longevity in seed storage is dependant on seed increase conditions and initial germination going into storage. If seed is increased under optimal conditions and initial germination is >95%, longevity in storage for many forage species is forecasted to be 100 years or longer when stored at −18°C

(Sackville-Hamilton et al. 1998). Cryopreservation (−196°C in liquid nitrogen) of seed, meristem and callus is possible but is usually used for vegetatively propagated grasses (Cachită and Crăciun 1995, Reed et al. 2006). Germplasm storage conditions should be optimized to maximize longevity, since seed regeneration is the costliest activity of maintaining germplasm. Inevitably, newly collected seeds need to be increased for distribution, and have to be regenerated when seed quantity or viability drops below threshold limits. Careful consideration needs to be given to regeneration to minimize any genetic change during the process. This is especially true for forages, since they are largely out crossers and genetically heterogeneous. Genetic change can occur through genetic drift, selection, and contamination with alien genes (Sackville-Hamilton 1998). The effects of selection and contamination have the most impact (Sackville-Hamilton 1998, Van Treuren et al. 2006). Preferred and accepted standards for the different steps of regeneration were compiled by Sackville-Hamilton et al. (1998), and these standards are under continuous revision by the ECPGR working group on forages (Boller et al. 2005b, see also http://www.ecpgr.cgiar.org/Workgroups/forages/forages.htm). Choosing regeneration environments close to the original sampling environment minimizes selection pressure. However, costs of regeneration and accession sensitivity to environment also need to be considered (Hinton-Jones et al. 2007). Contamination by alien genes can be avoided in wind pollinated grasses by spatial isolation, and this can be improved by a tall barrier crop such as rye. Based on results of a recent international study (Marum et al. 2007), an isolation distance of 30 m with an efficient barrier crop is sufficient to limit alien contamination to 1% of pollination events. Contamination can be avoided in outcrossing legumes, which are mainly insect pollinated using isolation cages (Figure 3).

Differential seed production among maternal plants, as well as differential pollen production among paternal plants can decrease effective population size (N_e), causing population change through drift and selection (Johnson et al. 2002, Van

Fig. 3 Series of pollination cages for isolated seed regeneration of alfalfa accessions at Prosser (USA) (Photo S. Greene)

Treuren et al. 2006). This can be mitigated by harvesting equal amounts of seed or inflorescences from maternal plants (Johnson et al. 2004) or equalizing pollen contribution (Van Treuren et al. 2006). The additional labour costs of implementing these practices are substantial. The ECPGR forage working group recommends either keeping seed harvested from individual plants separate for samples going into the base or duplicate (i.e. regeneration) collection and making a bulk sample for distribution, or using at least 100 plants for regeneration when harvesting seed as bulk. Typically, harvesting 100 plants as a bulk limits overwhelming contribution to seed yield of some very big plants in the same way as making a balanced bulk of 30 plants harvested separately (Boller et al. 2009). Number of plants harvested, specific protocol used (for growing and harvesting accessions), and occurrence of any unusual environmental conditions should always be documented so curators and users can gauge for themselves the quality of the regeneration process.

4.3 Germplasm Evaluation

For PGR to be useful to plant breeders, accessions in *ex situ* collections need to be characterized and evaluated, and this information needs to be readily available to users of the collection. Generally, characterization focuses on traits that are simply inherited while evaluation focuses on traits that have quantitative inheritance. International Descriptor Lists have been published for forage grasses, forage legumes, annual medic sps., *Panicum miliaceum, P. sumatrense*, *Setaria italica*, *S. pumila* and *T. repens* (http://www.bioversityinternational.org/scientific_information/themes/germplasm_documentation/crop_descriptors/#c462). This provides a starting point for characterizing accessions in a standard format. Depending on resources, forage collections frequently have more data available. For example, over 1000 accessions of the USDA *Medicago sativa* collection have been evaluated for 13 diseases, 7 insects, 27 agronomic traits, 7 feed quality traits and 5 abiotic stress-tolerant traits (Bauchan and Greene 2002). This data can be queried and/or downloaded from GRIN (www.ars-grin.gov/npgs). In Australia, 20,997 annual medic accessions have been evaluated for 27 agronomic traits (Skinner et al. 1999). Qualitative traits can generally be collected during regeneration, but may not be of interest to plant breeders who are seeking disease, insect or abiotic stress resistance. Unfortunately the cost of germplasm evaluation is high, and frequently outside the scope of curators, who must focus on maintenance (Chapman 1989). This is particularly true for forages which generally exhibit large genotype by environment interactions due to their out crossing nature (Breese 1969). Another difficulty is that many forages are grown in a sward which adds additional cost in evaluating traits such as interplant competition, persistence and sward growth pattern, grazing tolerance and yield. Cost-effective protocols have been proposed including unreplicated designs, use of spaced plants and “micro-plots” (Annicchiarico 2004, Tyler et al. 1987, Rhodes 1987).

4.4 Major *Ex Situ* Collections

Ex situ genetic resource collections are unique in that they conserve important genetic diversity, but importantly, make it readily available to plant breeders and researchers. Table 3 lists PGR collections around the world with major holdings of temperate forage legumes and grasses with >1000 accessions. Contact information for requesting germplasm is also listed; in the case of decentralized collections like ECPGR, the easiest way to obtain seed is to contact the respective genebank curator. In the future, plant breeders will be able to do "one-stop-shopping", once a central internet portal is developed that links the world's genebanks (Global Crop Diversity Trust, 2009). Information on individual accessions is vital for helping plant breeders select germplasm with desirable characteristics. Most germplasm collections have passport and evaluation data, held either in local databases or online databases. The Germplasm Resources Information System (GRIN) database covers the United States collections (www.ars-grin.gov/cgi-bin/npgs/html/croplist.pl). Users can query online using a wide range of morphological, agronomic, disease- and insect-resistance descriptors to select accessions. The European Cooperative Programme for Plant Genetic Resource (ECPGR) has databases for the following forage grasses: *Agropyron*, *Agrostis*, *Arrhenatherum*, *Bromus*, *Dactylis*, *Festuca*, forage grasses (minor), *Lolium*, *Phalaris*, *Phleum*, *Poa*, *Trisetum* and forage legumes: forage legumes (minor), *Lathyrus*, *Lupinus*, *Medicago* (annual), *Medicago* (perennial) *Trifolium alexandrinum*, *T. resupinatum*, *T. pratense*, *T. repens*, *T. subterraneum*, *Vicia* and *Vigna* (www.ecpgr.cgiar.org/, click on Germplasm Databases). A database of genebanks can be found at www.bioversityinternational.org/scientific_information/information_sources/, and click on Germplasm Databases.

5 Strategies for Using PGR in Breeding

Historically, genetic resources have been instrumental in developing modern high-yielding forage varieties around the world. In New Zealand, for example, it has been estimated that germplasm introduced over the last 30–40 years contributes annually $1 billion to exports in grassland agriculture (Lancashire 2006). Although the value of PGR is well recognized, most plant breeders are hesitant to incorporate unadapted PGR into their programmes. Humphreys (2003) suggested the reasons are due to "the widening gap between improved/unimproved material, poor characterization of PGR, transfer of adverse traits, genetic disruption of background genotypes, genetic complexity of some traits and a slow rate of introgression". However, the benefits of broadening the genetic base of our cultivated fodder and amenity grasses, especially as we enter an era of uncertain environmental change, challenge us to build on traditional and new strategies to introgress PGR into breeding programmes. These strategies range from judicious selection of PGR, use of pre-breeding or population improvement schemes, to use of the latest molecular techniques to carry out "precision" breeding.

Table 3 *Ex situ* collections having major holdings of forage legumes and grasses

Country/international organization	Number of accessions	Description	Contact
Australia			
Australian Medicago Genetic Resource Centre, Adelaide, South Australia	45,640	Temperate pasture legumes of Mediterranean origin and Australian native forage species. Major genera: *Medicago* (mainly annual species), *Trifolium*, *Lotus*, *Astragalus*, *Hedysarum*, *Trigonella*, *Atriplex*, *Onobrychis*, *Melilotus*; >1500 amenity grasses	www.sardi.sa.gov.au/pastures/genetic_resources
Genetic Resource Centre for Temperate Pasture Legumes, South Perth, Western Australia	9,184	Pasture legumes collected from temperate regions around the world. Major genera: *Trifolium* (mainly annual species), *Ornithopus* and *Biserrula*	www.agric.wa.gov.au/content/PAST/PL/GENETIC_RESOURCE_INDEX.HTM
CGIAR			
International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria	20,031	Forage and range species collected mainly between 45° and 30° latitude in Europe, Africa, Middle East and central Asia. Major genera: *Medicago* (annual species), *Trifolium, Astragalus, Onobrychis, Trigonella, Scorpiurus*	singer.cgiar.org/index.jsp

Table 3 (continued)

Country/international organization	Number of accessions	Description	Contact
Europe			
European Cooperative Programme for Plant Genetic Resources (ECPGR)	56,444	Forage legume and grass germplasm housed in over 35 institutes throughout Europe. Institute listings can be accessed at http://eurisco.ecpgr.org/. Major genera: *Trifolium*, *Festuca*, *Lolium*, *Dactylis*, *Medicago*. Strong representation of landraces and varieties developed in individual European countries, as well as local ecotypes.	www.ecpgr.cgiar.org/
United States			
USDA National Plant Germplasm System	33,142	Forage legume and grasses collected from temperate areas around the world. Range and wild species endemic to the United States. Major genera: *Medicago*, *Trifolium*, *Festuca*, *Elymus*, *Dactylis*, *Lolium*, *Eragrostis*, *Poa*, *Bromus*	www.ars-grin.gov/npgs/

5.1 Choice of PGR

The plant breeders' ideal PGR and the ideal PGR to benefit a breeding programme in the long term are generally opposed. Plant breeders are drawn to PGR for use in fodder crop or amenity grasses breeding programmes, that are genetically diverse, have promising characteristics (in view of the breeding objectives) and add genetic variability to the currently active breeding material. Furthermore, they should be reasonably adapted to the target environment. However, the greatest benefit, in terms of improving populations beyond current performance standards, and adding genetic variability would be from PGR that are most divergent from the germplasm used in the active breeding programme. However, these are in general, poorly adapted. Moreover, their characteristics are mostly unknown, at least in their response to the target environment. At the other end of the scale, new varieties from an outside breeding programme which have proven their value in recent official variety trials would be ideal candidates in the search for well-documented promising characteristics. However, their potential to add genetic diversity to the pre-existing current breeding material is doubtful. On the one hand, new varieties from a successful breeding programme are likely to be closely related to older varieties of the same programme which had already been used previously. On the other hand, older varieties of one's own breeding programme may have been used in the development of outside breeding programmes. Furthermore, exchange of breeding materials due to changing alliances among breeding companies has probably decreased genetic diversity between the breeding pools used by different breeders.

Selection from local and introduced ecotypes (especially if germplasm originates from a different gene pool than the breeding pool) can be expected to provide a good compromise between the requirement of adding genetic diversity and that of offering promising characteristics along with good adaptation to the target environment. If environmental data of their origin are known, their adaptation can be matched with the target environment of the breeding programme. Increasingly, germplasm collections are improving on the quality of passport data, especially more precise information on latitude and longitude. Text descriptions of older collection site locations are being converted into geographic map coordinates and new collection sites are being documented with GPS. This allows plant breeders to more easily select germplasm adapted to their target environment. Once GIS-based applications are coupled to genebank collection databases, selection of adapted germplasm will be further facilitated (Greene et al. 2007). Interesting characteristics are usually less well known than with varieties but may be derived from knowledge of environmental and management factors of the site the ecotype originate from.

In situations where adapted ecotypes do not provide the needed variation, plant breeders must turn to unadapted material, and in some cases (especially in grasses), utilize germplasm of different species and even genera. Plant breeders rarely have the resources to evaluate entire collections for traits of interest. The choice of PGR can be simplified by carrying out an initial screening of a core collection, if available. The core collection concept was proposed by Frankel (1984) who suggested

that a subset of accessions from a germplasm collection could be identified that represented the majority of genetic variation in a collection. A core collection that contained 5–10% of the accessions should retain over 75% of the variation of the entire collection (Brown 1989). There are numerous ways to develop core collections but the general strategy is to hierarchically stratify the collection into classes of accessions that share common characters. Characters can include ecologic or geographic origin, phenotypic characters, molecular marker data or a combination. Once the collection is classified, accessions are sampled from each class, using a number of different sampling strategies (Van Hintum 1999). Theoretically, after evaluating a core collection and identifying useful accessions, plant breeders can go back to the whole collection and screen those accessions in the same class(es) as those identified in the core subset. Core collections have been developed for most of the major forage germplasm collections. The USDA germplasm collections have core collections of red clover (Kouame and Quesenberry 1993), white clover, birdsfoot trefoil (Steiner et al. 2001), annual medic (Diwan et al. 1994), alfalfa (Basigalup et al. 1995) and *Poa pratensis* (Johnson et al. 1999). A core collection has been developed for the Australian Annual Medic Germplasm collection (Skinner et al. 1999) and *Medicago truncatula* collection (Ellwood 2006). A core collection for *M. truncatula* has also been developed for the French collection (Ronfort et al. 2006). A core collection is also being developed for the European collection of *L. perenne* (Maggioni et al. 1998).

5.2 Pre-breeding Strategies

Although careful choice of PGR can help overcome some of the challenges of using PGR, to truly capitalize on the presence of unique alleles outside of the breeding gene pool, plant breeders need to adopt strategies that will allow the introgression of unadapted germplasm into the breeding programme as efficiently as possible. Traditionally, the approach has been hybridization and backcrossing to the elite germplasm. Test cross evaluation allows breeders to further evaluate unadapted germplasm, especially for quantitiative characters such as yield, as well as start of the pre-breeding process. Crosses are made between unadapted germplasm and elite genotypes and the progeny evaluated for the trait of interest (for example, Bhandari et al. 2007, Maureira et al. 2004). This can uncover potential traits that might be masked in unadapted germplasm. Also, estimates of GCA and SCA can provide insights into additive and non additive gene action and suggest which unadapted germplasm might be most beneficial to incorporate into a breeding programme. Test cross progeny can be bulked to form composite populations or gene pools. Williams et al. (2007) developed several white clover varieties in New Zealand using this technique. The formation of regional gene pools was proposed to broaden the genetic base of alfalfa in the United States (Barnes et al. 1977). Regional genepools of perennial ryegrass were established in France (Charmet and Balfourier 1995). A further pre-breeding approach was undertaken in Germany where 800 Polish genebank

accessions were evaluated and genepools created according to the same time of flowering (Paul 1989).

Interspecific, and even intergeneric hybridization, has been successfully employed, and varieties released in several grasses, most notably in *Lolium* and *Festuca* species (Humphreys 2003, Humphreys et al. 2006). Interspecific hybridization has also been used to transfer traits such as rhizome production, seed production and persistence in *Trifolium* (Abberton 2007), and disease resistance and pod coiling in *Medicago* (Armour et al. 2008). Although no varieties have been yet commercialized, this can be expected in the near future (Williams and Hussain 2008).

Advances in genomics should make introgression of unadapted germplasm much more efficient. Germplasm collections may be "mined" for desirable genes to introgress into elite lines (Tanksley and McCouch 1997). The basic strategy involves the development of genetic linkage maps. The location of targeted genes and quantitative trait loci (QTL) can then be identified. Molecular markers that are closely associated with the gene of interest will then allow for marker-assisted selection (MAS). In forages, rapid progress is being made to develop these genomic tools for use in breeding programmes (see Chapter 4). However, before these genomic tools will truly allow plant breeders to more effectively and efficiently utilize PGR to improve production and broaden the genetic base of cultivated fodder and amenity grasses, functional markers must replace the anonymous markers which are still dominating research into localizing QTLs (Kölliker et al. 2009).

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