

Utilization of plant genetic resources in breeding for sustainability

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Abstract

UK agriculture is undergoing significant change with reduced subsidies for food production, increasing consumer demands for food safety and traceability, and environmental concerns including climate and demographic change. The International Treaty on Plant Genetic Resources for Food and Agriculture adopted by the United Nations Food and Agriculture Organisation supports the use of genetic resources for research and breeding. Mining genetic resources for useful genetic variation is perceived as a major benefit of genebanks. However, utilization by breeders may be constrained by poor characterization of genetic resources, a widening gap between improved and unimproved material, and the disruption of well-adapted genotypes during introgression. Breeders working with grasses and forage legumes for sustainable agriculture are fortunate in the wealth of genetic variation available both within the primary species of interest and among related species. New DNA technologies allow more targeted approaches to the use of these genetic resources. Possibilities for gene transfer between related species using conventional techniques expand the available gene pools while potential use of genetic transformation extend these even further.

Keywords: forage grasses; forage legumes; introgression; plant breeding; sustainability

Objectives in breeding for sustainability

Significant changes are taking place in UK agriculture with regard to, for example, CAP and GATT reform; reduced subsidies for food production; increasing consumer demands for safer food and traceability; environmental concerns including problems of climate change; and shifts in world demography that may reduce supply but increase demand. Increasingly agricultural systems will need to conserve natural resources, cause less pollution, deliver a range of outputs (including those with recreational, aesthetic and renewable resource value), and underpin the conservation and sustainable use of biological diversity. This will, inevitably, lead to multifunctional land use. However, economic, environmental and social

benefits are still likely to depend on viable agriculture involving food production as a major source of on-farm income but with issues of quality, safety, animal welfare and shifting of added value up the food chain of increasing importance. In this context work is carried out at the Institute of Grassland and Environmental Research (IGER; <http://www.iger.bbsrc.ac.uk/igerweb/>) on improving forage and amenity grasses and forage legumes, and examples of this work are given in this paper.

As well as providing a primary feed resource for livestock, grassland helps to maintain soil fertility; it protects and conserves soil and water resources; provides a habitat for wildlife (plants and animals); and contributes to an attractive landscape. Horses also consume significant amounts of forage, but their nutritional requirements differ from ruminants (cattle and sheep) in important respects (Longland, 2001). Forage breeders face a wide range of challenges in establishing priorities concerning,

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for example: (i) interactions with environmental factors (reducing nitrogen loss and phosphate pollution; improving soil quality and protecting against erosion; and coping with climate change); (ii) interactions with changes in land management (extensive versus intensive use, organic farming systems and multifunctional land use); (iii) changes in value (product quality versus quantity, biomass and industrial processing, and alternative products such as pharmaceuticals). Greater understanding of ecological interactions and economic benefits are also needed to maintain biodiversity and protect the landscape. Decisions have to be made on what species are most fit for purpose, whether there is a role for genetic improvement within them, and whether economically viable variety and seed production can be achieved. Interactions with symbiotic rhizobia and mycorrhiza in soil will also be considered as possible breeding targets.

Grassland has recreational, functional or landscape value in addition to its use in agriculture. In the UK 'amenity' grassland is widely distributed over about 4% of the land surface. About half is semi-natural grassland and the remainder is sown and maintained on a regular basis. It comprises areas differing widely in management and use, such as nature reserves, parks, domestic lawns, road verges, airfields, dam-faces, reclaimed industrial waste-land as well as more intensively managed sports areas such as football pitches, golf courses and school playing fields. In regularly maintained amenity grassland, mowing accounts for about 60% of the maintenance costs and fertilizer use for about 15%. The remaining 25% is used for the control of weeds, pests and diseases and on renovation. Amenity grassland managers must try to minimize input costs without risking sward quality, value and longevity. Over the past 30 years considerable advances have been made in breeding slow-growing, hard-wearing grasses for intensively used areas and persistent fine-leaved grasses with good tolerance of pests and diseases, tolerance of climatic and edaphic stresses, and attractive colour and appearance throughout the year. This has helped to ease management problems and reduce costs. In more natural grassland greater understanding of ecological interactions is helping managers to maintain high levels of biodiversity while retaining important aspects of landscape appearance and protection.

Alternative uses for forage grasses and legumes include biomass for energy, fermentation, ethanol production, biogas and bioelectricity; fibre production (for acoustic and thermal insulation and fibre-reinforced material); protein concentrates for monogastrics; and phytochemical production including pharmaceuticals. Natural products play a major role in pharmaceuticals, agrochemicals, food supplements or additives, herbal medicines, and

are widely used in cosmetics. Almost one-third of the world's leading prescription drugs are based on compounds derived from plants but they are still a largely unexplored resource in the pharmaceutical, agrochemical and other industries. Obtaining pharmaceutical products from forage species creates challenges in applying appropriate screening techniques (including metabolomic approaches) and in identifying potentially valuable sources of genetic variation.

Progress has already been made in addressing some objectives associated with sustainability. Grasses have been bred that accumulate 10–15% more sugars in leaves and stems and provide additional energy to improve the efficiency of plant protein conversion in the rumen. Feeding high-sugar grasses to dairy cows improves milk production and milk protein content but also reduces the partitioning of excess N into excreted dung and urine (Miller *et al.*, 2001). New white clover varieties have been shown to improve soil structure by reducing soil bulk density; improving fertilizer recovery; improving nutrient flux to support grass growth; and enhancing biologically driven processes involving plant × microflora interactions (Witty and Mytton, 2001). Work on red clover selections indicates advantages with regard to protein stability in silage associated with production of polyphenol oxidase (PPO) (Winters and Minchin, 2002).

Availability of genetic resources

The International Treaty on Plant Genetic Resources for Food and Agriculture adopted by the United Nations Food and Agriculture Organisation supports the use of genetic resources for research and breeding. Mining genetic resources for useful variation is perceived as a major benefit of genebanks although there may be difficulties in utilization by breeders because of, for example, the disruption of well-adapted genotypes during introgression. IGER maintains a Genetic Resources Collection (<http://www.igergru.bbsrc.ac.uk/>) in order to conserve, study, document and supply the variation required by breeding and research programmes at IGER, at other national institutes and by the international scientific community. The IGER genebank consists of a documented collection of over 10,000 accessions of temperate forage grasses and legumes and comprises both agricultural (*Lolium*, *Festuca*, *Dactylis* and *Trifolium*) and amenity (fine-leaved *Festuca*, *Poa* and *Agrostis*) species. New accessions may be acquired either passively via international seed exchange or actively by plant collection (Table 1). Having identified specific geographical locations, specific collection sites are selected with regard to variation in habitat (river-sides, paths, woodland, cliffs), management (cattle-grazed, sheep-grazed, mown) and altitude (aspect

Table 1. IGER overseas forage collections 1962–1998

Year	Region	Objective
1962	Tunisia, Morocco	<i>Festuca</i> chromosome races
1962	SE France, C Italy	Extend range of <i>Lolium</i>
1963	N Portugal, N Spain	Extend species diversity
1967	N Italy	<i>Lolium</i> 'in-season' growth
1971	Belgium, France, Switzerland	Early spring growth
1974	N Italy, Belgium	High summer growth
1977	NW Spain	<i>Dactylis</i> chromosome races
1980	Romania	Exchange visit
1981	Norway	Increase latitudinal coverage
1981	Ireland	EEC conservation collection
1982	Switzerland	Early spring growth
1986	Austria, Germany	Extend range in E Alps
1990	Poland	Extend range in E Europe
1992	Czech Republic, Slovakia	Habitat diversity
1993	Bulgaria	Genetic erosion
1995	Portugal	Extend range of habitats
1998	NE Italy	Habitat diversity

and slope). Collections aim to sample typical habitats or managements within a region, a range of altitudes, slopes or intensities of management, and unusual or isolated sites within the region. Each collection site is fully documented in terms of precise location, site description, relief, general and specific habitat type, soil type and site management. UK grass populations are under constant threat from habitat loss (agricultural improvement, mineral extraction, urban expansion) and from management change (diversification, 'set-aside', water/flood meadows). Therefore collections have also been made to conserve adapted gene complexes (Table 2).

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. Most temperate grass species belong to two major polyploid complexes, *Dactylis* and *Lolium/Festuca*. Within species complexes, polyploids generally have a higher genetic diversity and are distributed over a wider range of habitats than related diploids, often predominating at the edges of geographical distributions. In *Dactylis*, it is evident that considerable adaptive radiation has occurred among diploid species in the varied topography and climate of the Mediterranean basin and central southern Europe (Borrill, 1976; Lumaret, 1988). During the evolution of the genus it appears that hybridization and polyploidization events involving genetically differentiated diploid species produced more versatile tetraploids that extended the range of *Dactylis* further to the north, west and east of Europe. Within *Festuca* there

Table 2. IGER UK forage collections 1959–1999

Year	Region	Habitat risk
1959	Monmouth moors	Industrialization
1962	N Wales	Ploughing and re-seeding
1976/77	C, E and SE England	Ploughing and re-seeding
1976/77	N and mid-Wales	Ploughing and re-seeding
1979	Mid-Wales and the Marches	Ploughing and re-seeding
1980	SW England and Thames valley	Flood control
1980	NE Wales	Management change
1980/90	Upper Thames valley	Management change
1981	Derbyshire Peak District	Removal of sheep
1982	SE England	Arable farming
1983	N Wales	Management change
1984/85/87	SW England	Management change
1985	Yorkshire Dales	Quarrying
1986	SW England	Management change
1986	N and mid-Wales	Management change
1998/99	Welsh islands	Isolated populations

are about 100 species, widely distributed in temperate regions. The distribution of diploid species is more restricted than related polyploids. Hexaploid *F. rubra* extends well beyond the northern limit of diploid *F. pratensis* in Scandinavia. In the south of Europe, hexaploid tall fescue extends beyond the range of diploid ryegrasses and fescues through the Mediterranean Basin to North Africa where higher polyploid forms are also found (Tyler, 1988). At a more local level, the tetraploid form of *F. pratensis* var. *apennina* is found at altitudes above 1800 m in the Swiss and Italian Alps where the diploid form is absent (Tyler, 1988). The most useful agricultural species are *F. arundinacea* (hexaploid, $2n = 6x = 42$) and *F. pratensis* (diploid, $2n = 14$). It is probable that *F. arundinacea* evolved from a natural hybrid between ancestral forms of *F. pratensis* and the tetraploid *F. glaucescens* (= *F. arundinacea* ssp. *fenas*). Evidence that *F. arundinacea* has one genome in common with *F. pratensis* and two genomes in common with tetraploid *F. arundinacea* var. *glaucescens* was provided by genome-specific restriction fragment length polymorphism (RFLP) probes (Xu *et al.*, 1991; Xu and Sleper, 1994). This was confirmed by the use of genomic *in situ* hybridization (GISH) to visualize chromosome identities (Humphreys *et al.*, 1995). More distantly related species of such as *F. mairii* and *F. scariosa* have very hard, fibrous leaves but may contain useful drought tolerance genes. It is possible that the diploid species *F. scariosa* was a progenitor of one of the genomes in the tetraploid species *F. mairii* (Borrill *et al.*, 1977). The distantly related fine-leaved fescues (*F. rubra* and *F. ovina*) are used in high-grade amenity areas such as golf and bowling greens and ornamental lawns. Dwarf forms of *F. arundinacea* are used for coarser amenity turf in adverse environments.

The genus *Lolium* is considerably smaller than *Festuca*, comprising about eight species. It appears to have evolved from an ancestor similar to *Festuca pratensis* about a million years ago. The centre of origin of the most useful diploid *Lolium* species, *L. perenne* (perennial ryegrass) and *L. multiflorum* (Italian ryegrass) appears to be in the Mediterranean Basin from where they spread to north and west Europe following the development of more intensive grazing agriculture. The relative ease of producing intergeneric hybrids between *Festuca* and *Lolium* species confirms the close genetic similarities between the two genera. However, ryegrasses are generally fast growing and digestible but require reasonable fertility levels and lack stress resistance while fescues, although less agronomically desirable in terms of growth rate and nutritive value, are generally more adaptable and resistant to environmental stresses. Specific fescue traits of value include improved drought tolerance, maintenance of colour, reduced protein degradation, desirable fatty acid

content and composition, enhanced mineral uptake, improved cold tolerance, increased leafiness, durable disease resistance and increased persistency.

Utilizing genetic resources

Novel genetic resources are required to assist breeders in meeting new objectives. However, utilizing genetic resources in breeding programmes may be constrained by a widening gap between improved/unimproved material; poor characterization of genetic resources; transfer of adverse traits; genetic disruption of background genotypes; the genetic complexity of some traits; and a slow rate of introgression. Despite these difficulties, ryegrasses from northern and southern Europe have been crossed to produce valuable gene pools for improving seasonal yield and nitrogen-use efficiency (Wilkins and Lovatt, 1989). Gene pools may be extended by hybridization across species boundaries and grass breeders have exploited the potential of allopolyploids to increase adaptive range by creating novel hybrids for new agronomic niches. Identification of useful genes depends on good physiological understanding of adaptive traits. Rates of water use, photosynthesis, leaf conductance, leaf growth, and epidermal and stomatal cell characteristics were found to differ between *Dactylis glomerata* and *D. marina* (Wilson *et al.*, 1980). Better water conservation in *D. marina* than in *D. glomerata* was associated with the presence of large epidermal papilla cells, which was negatively associated with adaxial leaf conductance. In *Dactylis*, naturally occurring and artificially produced triploids demonstrate the feasibility of interploidy hybridization (Zohary and Nur, 1959; Jones and Borrill, 1962). *D. glomerata* has been improved in this way (Casler and Hugessen, 1988) and varieties have been released which contain genes for improved seasonal growth derived from the diploid subspecies *lusitanica* (Borrill *et al.*, 1972; Rumball, 1982). Good agronomic potential has also been demonstrated in hybrids between tetraploid *D. glomerata* and *D. marina* (Borrill *et al.*, 1974).

Successful hybrids among *Lolium* and *Festuca* species include tetraploid hybrids between *L. perenne* and *L. multiflorum* that demonstrate a very flexible response to cutting and grazing (Jones and Humphreys, 1993). Tetraploid hybrids between *F. pratensis* and ryegrasses have extended the ability of grasses with good early growth and high nutritive value to cope with extremes of temperature and moisture (Thomas and Humphreys, 1991; Joks *et al.*, 1995). Good agronomic potential has also been demonstrated in tetraploid hybrids between *L. multiflorum* and *F. arundinacea* var. *glaucescens* (Jadas-Hecart *et al.*, 1992). Octoploid hybrids between

L. multiflorum and *F. gigantea* have potential to improve summer growth (Humphreys *et al.*, 1989). Recent inter-specific grass breeding has concentrated more on the transfer of specific adaptive traits between species rather than combining complete genomes in amphiploid hybrids. Success in this approach depends on the identification of relevant and relatively simple genetic traits as well as on good knowledge of species relationships governing the potential for recombination between genomes. Controlled introgression between ryegrasses and fescues has been successful in transferring drought tolerance from tall fescue into Italian ryegrass (Humphreys and Thomas, 1993). Also a gene that reduces rates of chlorophyll degradation and protein loss during leaf senescence (Thorogood, 1996; Kingston-Smith *et al.*, 2002) has been transferred from meadow fescue into perennial ryegrass.

Progress has also been made in the introgression of traits between *Trifolium* species. Introgression of a rhizomatous trait from Caucasian clover (*T. ambiguum*) into white clover has produced hybrids with growth characteristics of both species (Abberton *et al.*, 1998). Flowering traits from Ball clover (*T. nigrescens*) are also being introgressed into white clover to improve seed yield potential (Marshall *et al.*, 1998).

Value of new technologies

New DNA technologies allow more targeted approaches to the selection and introgression of valuable genes from a range of genetic resources while retaining the integrity of valuable genetic backgrounds. Quantitative trait locus (QTL) analysis based on genetic marker linkage maps is a first step towards dissecting complex traits associated with the growth and persistency of agricultural and amenity grasslands. It depends on detecting DNA variation among individuals using a variety of genetic marker techniques such as RFLPs, randomly amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs—microsatellites), expressed sequence tags (ESTs) and amplified fragment length polymorphisms (AFLPs) (Henry, 2001). Successful breeding using marker-assisted selection (MAS) depends on understanding the genetic architecture of relevant traits and on being able to identify significant QTL with reliable DNA marker tags. Markers such as microsatellites (SSRs) or single nucleotide polymorphisms (SNPs) allow high-throughput procedures to be used with large numbers of plants. Allele-specific markers such as SNPs provide useful tools to explore germplasm collections and monitor the distribution of allelic variance, to optimize germplasm collections in genebanks and to identify varieties for intellectual property rights. Plant breeding primarily acts

at the level of minor allelic variation among wild-type alleles of a given gene. Thus, allele and SNP identification is of crucial importance in effective and competitive plant breeding but little information has been generated so far on naturally occurring allelic sequence variation in crop plants.

Although traits such as resistance/tolerance to abiotic and biotic stresses are common targets for the use of markers in selection or introgression, bias of individual gene effects and risks of identifying 'false-positive' associations can generate disappointing responses (Kearsey and Farquhar, 1998). A novel analytical approach that may be helpful is based upon linkage disequilibrium (LD) in natural populations (Sköt *et al.*, 2002). LD is the non-random distribution of alleles at linked loci. Natural populations rather than mapping populations can be used to exploit this phenomenon for association mapping of phenotypic traits. The advantages are that generic markers rather than cross-specific ones can be identified covering a wide spectrum of genotypes, and that more than one QTL can be studied with the same populations (Rafalski, 2002). For a given recombination frequency, LD is generally smaller in natural populations than in conventional mapping families, so fewer individuals need to be analysed to achieve a given map resolution. Resolving the order of very tightly linked loci is further improved in LD mapping by using older populations in which more recombinants have accumulated. The LD approach has been used successfully for fine mapping and map-based cloning of major human disease genes (Rioux *et al.*, 2001).

Despite the challenges of working with out-breeding forage species, good progress has been made in genetic mapping and QTL analysis in ryegrasses and fescues based on international collaboration. The International Lolium Genome Initiative (ILGI) has produced a reference linkage map of perennial ryegrass that demonstrates highly conserved synteny and colinearity between ryegrass and the Triticeae cereals (Jones *et al.*, 2002). Maps designed specifically to study important traits are being aligned with the reference map. Some of the challenges associated with introgressing genetic variation between species are being alleviated by the use of genetic markers. In forage grasses one of the main targets of introgression is to introduce biotic and abiotic stress tolerance from fescues into ryegrasses (<http://www.iger.bbsrc.ac.uk/igerweb/SAGES2/sages2.html>). DNA markers and GISH are being used to assist in the transfer of genes for crown rust resistance (Roderick *et al.*, 2000) and genes for enhanced winter hardiness through tolerance to either freezing or ice-encasement. Molecular markers are also being located to specific chromosome arms using an introgression approach (King *et al.*, 2002). This helps in gene isolation and is being applied to the

stay-green gene transferred into ryegrass from meadow fescue and other introgressed *Festuca* genes associated with drought resistance and winter survival (Donnison *et al.*, 2002).

In forage legumes most genetic marker work has been carried out in alfalfa (*Medicago sativa*). Genome analysis assists QTL mapping and MAS and there are significant genomics programmes on *Medicago truncatula* and *Lotus japonicus* in the USA, Japan and Europe. The integration of breeding programmes for both grain and forage legumes with these large genomics studies will have considerable value. A genetic map based on AFLPs and SSRs is being developed in white clover using self-compatible inbred lines and targets for MAS include disease resistance and other agronomic, physiological and quality traits (Abberton, personal communication). Work on red clover includes the use of markers for studies of genetic diversity; the identification of RAPD markers associated with disease resistance; isozyme heterozygosity linked to morphological characteristics; and studies of gene expression (Webb *et al.*, 2002). Most work on introgression among forage legumes has been with alfalfa and white clover and some studies are beginning to employ both molecular and cytological markers. In white clover, work has concentrated on introgression from *Trifolium nigrescens* to increase seed production (Marshall *et al.*, 1998) and to improve resistance to clover cyst nematode (Hussain *et al.*, 1997). Bulk segregant AFLP approaches (BSA-AFLP) have been used to identify markers for high seed yield potential in second and third backcross generations derived from hybrids between *T. nigrescens* and white clover as the recurrent parent (Abberton *et al.*, 2000). Chromosome labelling techniques have been used to explore relationships between *T. repens* and related species including *T. nigrescens* (Ansari *et al.*, 1999). Hybrids between white clover and *T. ambiguum* Bieb. (Caucasian clover), which is rhizomatous and very persistent, have been used at IGER to produce drought-resistant material with both stolons and rhizomes (Abberton *et al.*, 2000). They have also been used in New Zealand to transfer virus resistance (Woodfield and Brummer, 2001). BSA-AFLP has been used to identify markers for the rhizomatous habit (Abberton *et al.*, 2000) and backcross material is currently being assessed for cold tolerance at several sites in northern Europe.

Although natural ecotypic variation continues to provide the basic raw material for most forage breeding programmes, developments in cell culture and transformation techniques provide opportunities to access new sources of variation beyond that currently available using conventional techniques. However, use of transformation technology is limited by (i) a lack of precise information on the physiological, morphological and

biochemical consequences of individual gene action as well as their identification and cloning; (ii) 'trade-offs' in whole plant performance that may be alleviated by targeting genes to specific tissues or by using appropriate genetic backgrounds; (iii) problems of stability in the expression and inheritance of transgenes; (iv) a need for specific management practices and variety evaluation systems that recognize the value of novel material; (v) statutory regulation linked to public awareness of benefits and risks; (vi) environmental concerns due to a high probability of gene flow from transgenic plants in out-crossing species, evidence of long-range pollen flow and the existence of feral/wild populations of the same species. Although limited to producing material for contained experimental use in the UK, transformation systems are now well established for all major forage grasses and legumes. Target traits for manipulation relate mainly to improving forage quality characteristics such as protein quality and stability, fermentable carbohydrate content and cell wall digestibility, and to plant performance factors such as resistance to biotic and abiotic stresses and plant developmental processes. Forage crops have also been transformed with a view to bio-farming and there are suggestions that forage species can compete with more 'traditional' industrial crops such as maize, wheat or potatoes. However, further work is required in all the areas of concern before this technology can fulfil its true potential.

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