



## Grass and Forage Improvement: Temperate Forages

Chris J. Pollock

*Institute of Grassland and Environmental Research, UK*

M. T. Abberton

*Institute of Grassland and Environmental Research, UK*

M. O. Humphreys

*Institute of Grassland and Environmental Research, UK*

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## Grass and forage improvement: temperate forages

C.J. Pollock, M.T. Abberton and M.O. Humphreys

*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB, Wales, UK*

*Email: chris.pollock@bbsrc.ac.uk*

### Key points

1. Plant breeding has contributed significantly to the development of effective grassland production systems.
2. New technologies offer enhanced precision in breeding and access to wider genetic variation.
3. The requirement for more sustainable production systems will require genetic improvements in complex traits where the use of new technology will be vital.

**Keywords:** selection, genetic mapping, molecular markers, grass breeding, clover

### Introduction

The grazing ruminant animal has been a key element of temperate agriculture since the domestication of sheep and oxen in around 5000 BC (Humphreys, 2003). Ruminant animals provided meat, milk and motive power, and were able to live on plants that humans could not use directly. As agricultural systems developed, their role in recycling nutrients was also recognised, animal production forming a key element in rotational agriculture in the middle ages. Less attention was paid to the forages that ruminants ate, although the value of grasses and clovers in short-term leys was established by the 12th century, and it was well recognised that some permanent pastures were, by nature, more productive than others.

Following the development of practical genetics-based plant breeding in the early 20th century, forage improvement became more formalised. However, the complexities of working with perennial outbreeders in mixed systems that were very sensitive to management meant that the history of true genetic-based breeding of temperate forages is relatively short. The perennial ryegrass variety S 24 for example, was produced in the 1930s based on collected ecotypes from UK pastures and unrestricted crossing between selected plants. This generated a variety that was resilient, persistent and productive (in terms of the norm for UK pastures at the time), but one that was very heterogeneous and where the potential for genuine breeding advances was not fully realised. Since then, for both grasses and legumes, much more attention has been paid to precision crossing and to the incorporation of specific traits from individual genotypes, often collected outside the UK. The results of this process have been a continued improvement in yield and quality with some gains in disease resistance and grass-clover compatibility.

Improved pasture supports the bulk of livestock production in the UK, with grazed forages providing the cheapest feed for ruminant animals, as well as bringing environmental benefits. As we enter the 21st century, both the technology and the targets for temperate forage breeding are changing rapidly. There is increased pressure in many developed countries for sustainable farming systems that yield both a viable economic return and a range of environmental and social goods. This is changing the nature of plant breeding. It is our view that success in breeding for targets such as nutrient use efficiency, palatability and improvements in livestock product quality cannot be done by traditional selection methods,

since the traits are complex, expensive to measure and subject to considerable genotype x environment (GxE) interactions. For this reason we first consider what forage breeders can achieve with the application of such new technology, and then discuss what targets are likely to be important and how the outputs of such programmes will be used.

**Table 1** Breeding advances in forage crops

Species	Average genetic gain (%)			
	Annual yield (dry matter) <sup>1</sup>	Digestibility <sup>2</sup>	Intake <sup>2</sup>	Crude protein <sup>2</sup>
<i>Lolium perenne</i>	3.8	1.0	14.7	6.5
<i>Dactylis glomerata</i>	3.3	2.4		
<i>Trifolium repens</i>	4.9			
<i>Medicago sativa</i>	4.1	3.3		2.7

<sup>1</sup>per decade<sup>-1</sup> (after Humphreys, 1999); <sup>2</sup>per generation cycle<sup>-1</sup> (after Casler, 2001).

### The impact of modern breeding technologies

Over the last twenty-five years, genetic knowledge has expanded extremely rapidly, driven by advances in molecular biology. The sequencing of entire higher plant genomes has generated a huge reference resource, which has proved particularly valuable where close synteny exists between sequenced organisms and those used in agriculture. Among crop plants, *Oryza sativa* (rice) has been sequenced and the degree of alignment between the rice genome and other gramineae is very high. This allows traits to be characterised in non-sequenced organisms and equivalent areas of the sequenced genome in rice to be identified, helping to identify at the sequence level the basis of useable variation for breeding programmes (Armstead *et al.*, 2004). Sequencing work is also under way for the model legumes *Medicago truncatula* and *Lotus japonicus* (Young *et al.*, 2003). This body of information will increasingly influence the way in which other forage legumes will be bred in the future.

#### Marker assisted selection

Of the emerging genetic technologies, marker assisted selection (MAS) is the most likely to have an immediate impact on plant breeding. It depends on the detection of DNA variation among individuals using a variety of techniques (Henry, 2001; Forster *et al.*, 2004). Successful breeding using MAS depends on understanding the genetic architecture of relevant traits. Traditionally, major gene and polygenic variation has been analysed in different ways, but the techniques of Quantitative Trait Locus (QTL) analysis now allow a more integrated approach in dissecting complex traits and assessing gene effects. Quantitative Trait Locus analysis can be used to determine the location or locations of variation for a complex trait directly onto the genetic map of a particular species. At the same time, the density of markers on these maps has increased dramatically associated with the use of the DNA markers. Heterozygous molecular markers that are tightly linked to traits of interest can be used to speed up selection in breeding programmes. As a result of the International *Lolium* Genome Initiative (ILGI; Jones *et al.*, 2002) involving IGER and groups in Australia, Japan and France, a reference linkage map of *L. perenne* has been produced. It comprises 240 loci covering 811cM on seven linkage groups. The map contains 124 co-dominant markers, of which 109 are heterologous anchor restriction fragment length polymorphisms (RFLP) probes from from *Triticum* spp. (wheat), *Hordeum* spp.

(barley), *Avena* spp. (oats) and *O. sativa*, allowing comparative relationships to be investigated between *L. perenne* and other *Poaceae*. The linkage groups of *L. perenne* are numbered to correspond to the homoeologous groups of the *Triticeae* cereals. The genetic maps of *L. perenne* and the *Triticeae* cereals are highly conserved in terms of synteny and colinearity (Jones *et al.*, 2002). There is also general agreement over the syntenic relationships between *L. perenne*, *Festuca pratensis* (meadow fescue), oat and rice, and those between the *Triticeae* and these species.

An 'IGER' linkage map of *L. perenne* based on an F2 mapping population was produced initially for the genetic analysis of water-soluble carbohydrate (WSC) accumulation. This map identified 7 linkage groups covering a total of 515cM and was compared with the ILGI map produced from a BC1-type population (Armstead *et al.*, 2002). The maps could be aligned using 38 common loci, and a marker order for all mapped loci in either population was identified in an integrated map. Using this map, QTL associated with WSC accumulation in the leaves and leaf sheaths of ryegrass have been identified (Humphreys & Turner, 2001; Turner *et al.*, 2001). Interestingly, some QTL overlap or fall close to the location of genes with known function. The genomic region associated with a major flowering time QTL in ryegrass shows a high degree of synteny with the location of genes in rice, having homology with the *Arabidopsis* flowering time gene *CONSTANS* (*CO*) (Donnison *et al.*, 2002; Armstead *et al.*, 2004). Allelic variation in the *CO*-like genes correlate with differences in heading date and may account for up to 70% of the total variation.

In forage legumes, the majority of work has been carried out in *Medicago sativa* (lucerne), primarily in the USA, but with some effort in Europe. In both *M. sativa* and other forage legumes, current effort is directed mainly towards mapping, with little directed MAS except under experimental conditions. The first *Trifolium repens* map has been developed using self-compatible inbred lines in a collaboration between IGER and The Plant Biotechnology Centre, Victoria (Abberton *et al.*, 2000b). This utilises amplified fragment length polymorphisms (AFLPs) and microsatellites. Previous work had used random amplification of polymorphic DNA (RAPDs) to assess the extent of genetic diversity between inbred lines and compare this with the degree of heterosis in crosses between them (Joyce *et al.*, 1999). Quantitative Trait Locus for agronomically important characters in single plants (including seed yield), have been located in a meta-analysis across sites and years. This work is now being extended to studies of plot performance. New mapping families are being used to investigate the genetic basis of stolon morphology and, in collaboration between IGER and Teagasc, Ireland, to locate and clone genes involved in resistance to stem nematode. In parallel with the mapping studies, development of EST collections is being undertaken in a joint programme between PBC and AgResearch, New Zealand (Forster *et al.*, 2001). A *Trifolium pratense* L. (red clover) linkage map has also been published (Isobe *et al.*, 2003).

The use of mapping populations to generate the resources to facilitate MAS poses challenges. There is a need for accurate, rapid and ideally non-invasive phenotyping so that the trait variation can be characterised as well as the marker profiles. High throughput chemometry is being developed for metabolomic studies, and there are real opportunities to use these advances in phenotyping. Near infra-red reflectance spectrometry (NIRS) is an example of one of these unbiased high throughput systems that is already in use as a routine analytical tool (Wilman *et al.*, 2000), and mass spectrometry-based approaches are also in increasing use. For developmental traits, imaging methodologies are being developed, although the challenges are greater. Hand in hand with this type of phenotyping goes the need for good informatics and statistical approaches to ensure that reliable information is derived, analysed

and accurately collated with DNA-based information from the same plants. There is also a growing need for robust process models for land use systems that will allow breeders to estimate the likely significance of potential improvements. Such models, for example of N flows in grassland, do exist, and some of them are scale dependent (Bhogal *et al.*, 2001), but they have rarely been used to identify and validate breeding targets.

### *Introgression*

Introgression has been used as a breeding tool for many years, but the development and employment of molecular markers improves both speed and precision. At IGER, the main targets for introgression have been the introduction of biotic and abiotic stress tolerance from *Festuca* spp. into *Lolium multiflorum* or *L. perenne* (Humphreys *et al.*, 1998, 2003). Introgression into *L. perenne* is necessary following the initial generation of the hybrids in order to ensure that the high nutritive quality of *L. perenne* spp. is retained together with the improved stress tolerance derived from *Festuca*. A number of mapping families have been produced to facilitate marker-assisted introgression. Having identified plants that express introgressed *Festuca*-derived traits, they can also be analysed using *in situ* hybridisation. The close relationship between *L. perenne* and *F pratensis* has allowed work on introgression mapping and alignment of the genetic map with a physical map (Armstead *et al.*, 2001; King *et al.*, 2001). Genomic *in situ* DNA hybridisation can be used to determine the location of alien *Festuca* genes, and genetic markers ascribed to the targeted sequence (Humphreys *et al.*, 1998). *L. perenne* lines carrying different *Festuca* genes that convey a range of adaptations to abiotic stresses are currently being developed alongside 'breeder's toolkits' to aid their commercial exploitation.

Introgression studies have also been carried out on a range of forage legumes, with effort concentrated on *M. sativa* and *T. repens*. Some of these studies are beginning to employ marker-assisted introgression using both molecular and cytological markers. Interspecific hybrids have been produced between *T. pratense* and some of its relatives, but with little impact to date. Significant programmes on *T repens* are in place at IGER (using molecular markers) and Ag Research New Zealand (using cytological markers). Both groups have focused on two introgressions, with *T. nigrescens* and with *T. ambiguum*. *T. nigrescens* is an annual diploid species that is sexually compatible with *T repens*, and is believed to be one of its ancestral genomes. In associated research, fluorescent *in situ* hybridisation of nucleolar organiser regions has been used to explore relationships between *T. repens* and related species including *T. nigrescens* (Ansari *et al.*, 1999). Bulk segregant AFLP approaches (BSA-AFLP) have been used to identify markers for high seed yield potential in second and third generation backcross hybrids, with *T. repens* as the recurrent parent (Abberton *et al.*, 2000a). Hybrids between *T. repens* and the rhizomatous very persistent *T. ambiguum* (Caucasian clover) require ovule culture or embryo rescue, which adds complexity. However, backcross hybrids show considerably enhanced drought tolerance in relation to the *T. repens* parent, together with somewhat reduced protein content, giving the potential to reduce nitrogenous pollution in livestock feeding.

### *Genetic manipulation*

Genetic manipulation (GM) may be an alternative to conventional breeding or an additional element in the programme, and can aid breeding by accessing or creating novel sources of specific plant variation. Genetic manipulation also provides tools to understand basic plant biology and help to identify genes controlling the physiological and biochemical processes

underlying traits of agronomic importance. Transformation systems are now well established for all major forage grasses and legumes. Precision genetic manipulation requires accurate targeting of transgene expression, either to different cell types or to specific intercellular compartments. Some progress has been made using cell specific promoters in grasses and legumes (but many more cell specific promoters are needed), and for compartment targeting using 5' and 3' signal sequences in grasses associated with specific wall degrading enzymes (Morris & Langdon, 2001). Plastid transformation gives higher expression of prokaryotic genes, and may facilitate 'containment' of transgenes within the transformed plants. However, there are marked species-dependent differences in the level of paternal inheritance of plastid DNA. In addition, there are considerable regulatory challenges to be met before GM grasses and legumes are approved for widespread cultivation in areas where compatible wild relatives abound. Currently GM is used predominantly in forage breeding for 'proof of principle' studies, where transgenic material up or down-regulated in an individual gene product, provides a clear approach to establishing gene function in a way that natural variation rarely permits.

### *Genetic resources*

In all cases, variation for traits of interest is being incorporated into potential new varieties. Capturing, curating and maintaining genetic resources that allow access to such variation is central to breeding programmes worldwide. In the case of forages, most of this will be in direct relatives of the agricultural species. Approaches to defining core collections on the basis of allele variation rather than phenotype variation will simplify curation (van Hintum *et al.*, 2001), and the use of molecular markers will speed the process of screening collections for favourable alleles. However, the commitment to collection, characterisation and maintenance of genetic resources is considerable and funding is not easy to obtain. There is a real need to ensure, preferably on an international scale, that funding for this activity is maintained, but also that every opportunity to streamline the process and minimise costs without losing vital material is considered.

### **Current targets for precision breeding in forages**

It has long been known that there are big differences between species in both the suitability of forages for animal production and in their capacity to grow and survive in particular areas. Traditionally, improvement programmes have concentrated on those species that are suited to the locale and that deliver the best return in terms of animal products. Within that, agronomic characters such as yield, flowering date, persistency and compatibility have been priority targets, with less emphasis on improving intake, digestibility, resource use efficiency and end-product quality. There are sound reasons for this approach. Primary agronomic characters can be measured easily, are important in grassland management and show tight genetic control. However, the application of the technological approaches outlined previously allows more complex traits to be manipulated and there is increasing evidence of the relevance of such an approach.

One of the best examples of precision breeding for quality traits in grasses is the ability to manipulate the efficiency of rumen function through altering WSC content. Elevated soluble sugars in grazed herbage provide an accessible carbon source in the early stages of colonisation of fresh feed by rumen microorganisms (Miller *et al.*, 2001; Lee *et al.*, 2003), thus reducing the breakdown of peptides and deamination of amino acids. Table 2 indicates the benefits that accrue in terms of conversion efficiency and reduced environmental

deposition of N. Quantitative Trait Locus analysis has indicated a number of regions of the *L. perenne* genome involved in WSC accumulation. Currently MAS is being used to identify new genotypes with the high sugar trait (Humphreys *et al.*, 2003).

**Table 2** Effects of water-soluble sugar content (WSC) of grass on nitrogen partitioning into milk and urine (after Miller *et al.*, 2001)

	WSC (%)	CP (%)	N intake (g/day)	N output (g/day)	
				Milk	Urine
High sugar ryegrass	20.1	9.2	268	82	71
Normal ryegrass	12.9	10.6	278	69	100

This approach is being applied to a number of quality and efficiency traits that are known to be important in grasses and legumes - including nitrogen and phosphorous use efficiency, mineral composition, and developmental and morphological traits that improve compatibility. In principle, if defined mapping populations exhibiting variation in an important trait can be established, then QTL analysis and MAS can be used, with a concomitant gain in speed and simplicity of selection. Even very complex derivative traits like palatability and grazing preference are susceptible to this approach, although these would require a considerable experimental investment to derive the original mapping populations.

Marker-assisted introgression is also being used to manipulate important traits. Using a range of *Lolium-Festuca* introgressions, variation in drought, heat and cold tolerance, resistance to crown rust and improved protein stability during digestion has been incorporated into a *L. perenne* background and is being assessed. Additionally the stay-green character, which has considerable relevance to turf grasses, has been transferred into *Lolium* and used to generate commercial varieties. Hybrids between *T. repens* and *T. ambiguum* have been used at IGER to produce drought resistant material with both stolons and rhizomes (Abberton *et al.*, 2000a) and in New Zealand primarily for virus resistance (Woodfield & Brummer, 2001). Molecular markers have been used to select for the rhizomatous habit in plants of backcross families (Abberton *et al.*, 2000b) and this material is currently being assessed for cold tolerance at several sites in Northern Europe.

There is increasing interest in improving grass-clover compatibility for mixed swards, and compatibility is now starting to be considered as much from the nutritional point of view as agronomic. The development of marker-based approaches will make this more feasible. It is known, for example that root structures are important determinants of compatibility (Collins *et al.*, 2003), and these are very difficult to select for without good markers. In the future, greater knowledge of rhizosphere processes, including N transfer from clover to grass is also likely to be a key area, particularly since compatibility issues are likely to be affected by climate change.

### Other targets

There are other compositional traits that are known to affect the quality of livestock products. These include lipid composition; particularly the high levels in forages of polyunsaturated fatty acids and the omega-3 fatty acids, which have positive health benefits and also promote

the colour and keeping quality of beef (Wood *et al.*, 2003; Scollan *et al.*, 2005). There are also other approaches to reducing proteolysis within the rumen. Plant-mediated proteolysis is demonstrable in the rumen (Kingston-Smith *et al.*, 2003), opening up the possibility of producing forages with reduced protease contents. There is also evidence that protein protection mediated by polyphenol oxidase is under genetic control (Evans *et al.*, 2002).

Development in *T. repens* of near-isogenic lines (NILS) has allowed study of the expression of recessive genes. For example, one of the lines is deficient in N fixation, permitting the protein content of clover to be manipulated via contrasting levels of fertilizer application. This has been used as ‘proof of principle’ that the lower protein content of legumes during ensiling can result in silage with similar retained protein content, but with significantly lower environmental losses. This is now a target for current precision breeding approaches. The NILS have functioned as a ‘GM analogue’ in terms of trait identification and characterisation. The availability within the NILS of ‘control’ lines that are very closely related to the non-fixing lines has been of great value (Abberton *et al.*, 1998).

There is also physiological evidence for a genetic component affecting tolerance of heavy metals, opening up opportunities for developing grass varieties suitable for managing degraded land. However, the level of investment in basic and strategic science that is required to underpin variety development makes exploitation of the full range of possible targets somewhat unlikely. Reform of the European support system for agriculture will alter the ways in which farmers receive payment from the state, and this in turn is likely to affect their requirements for new forage varieties. However, it is not currently clear what sorts of agricultural systems will prevail and thus what the new targets will be. From an environmental standpoint, the benefits of rotational agriculture, spring cereal growing and hay-making are abundantly clear (Chamberlain *et al.*, 2000). If agricultural support mechanisms end up promoting such practices, then this will stimulate the use of *T. pratense*, cereal/legume mixtures and grass varieties optimised for short-term leys, all of which are relatively minor components of current farming systems. Nevertheless, the generic technologies of genome analysis, marker development and gene identification will continue to influence forage breeding regardless of the specific breeding targets.

### **Opportunities, challenges and constraints**

Within grassland agriculture in developed countries, managing the balance between the natural and the farmed environment is likely to assume increased importance over the next 10-15 years. There are contrasting pressures, with an increased demand for animal products on one hand and evidence of the need for reduction of the impacts of agricultural systems on the other. The Environment Agency in the UK has identified pollution from agriculture as a major issue, with farming being responsible for 27% of major pollution incidents (Anon, 2002). Grassland farming contributes to pollution via losses of nitrate and phosphate into ground water, ammonia and methane into the atmosphere, and by the direct effect of slurry and silage effluent leaking into watercourses. Intensive livestock agriculture involving silage production also has a negative impact on biodiversity, with the declining presence of seed-eating birds in grasslands being significantly correlated with the move away from hay production in the 1960s and 70s (Vickery *et al.*, 2001). Against this must be set the vital role of the grazing animal in preserving key habitats. Unimproved semi-natural pasture requires careful grazing if species diversity and habitat are to be maintained. Such management requires a viable livestock agriculture to achieve this objective, together with a support system that provides incentives for farmers to deliver environmental goods. To achieve this more



broadly based set of objectives, will require a much closer linkage between forage breeding and the development of novel management systems.

It is already known that modelling the processes of nitrogen movement within grassland can identify the optimal times for nitrogen application, leading to significant reductions in both fertiliser application and nitrate leaching without a concomitant reduction in forage yield or animal production (Laws *et al.*, 2000). Linking approaches like this to the genetic improvement of nutrient use efficiency offers further potential gains at the system level. The beneficial effect that *T. repens* has on soil quality in heavy and compacted soils is also a target for genetic improvement, but must be pursued in concert with management systems that are directed towards preserving the improvement attributable to *T. repens*. Current and proposed environmental legislation provides a strong impetus for integrated research of this kind within the EU, and there are welcome signs that research funders are recognising the value of this approach. Increasingly, breeding advances will have to be delineated at the system level, which has consequences for variety assessment. A further approach to improving nitrogen use efficiency at the sward level by increasing protein protection in the rumen involves the presence in a mixed sward of a tannin-containing species such as *Lotus corniculatus* (birdsfoot trefoil). Utilisation of this species can also result in a reduction of the parasite burden in sheep (Marley *et al.*, 2003) although it is not clear whether this effect is mediated through stimulation of the immune response or via a direct effect of tannins.

The Curry Report into UK agriculture argued that the removal of direct production subsidies will broaden the base of UK land use, and it will be important to ensure that these changes feed through into flexible forage improvement programmes. For niche producers, where high on-farm value and product quality are paramount, pasture quality becomes of comparable importance in terms of maximising the contribution of a cheap, traceable and ‘natural’ component of the diet. As well as the direct quality traits indicated above, more complex traits that promote the stability of production over time, particularly in mixed swards, will become important. Again, the challenge here is to define the trait under realistic conditions involving animals, and then to use DNA markers to access and utilise the available genetic variation. A more multifunctional land use base might also generate novel opportunities for forage species. Already sports turf accounts for 30% of UK grass seed sales and the IGER amenity breeding programme has focussed on ‘non-agricultural’ traits such as prostrate habit and wear-resistance (Thorgood, 2003).

There are other opportunities in the development of alternative fibre and biomass crops (J. Valentine, *pers. comm.*). Work is also in progress to assess the available genetic variation in grasses for environmental targets such as C-sequestration in the soil, support for soil biodiversity, bioremediation and flood mitigation (J. Macduff, *pers. comm.*). Candidate traits for C-sequestration include shoot and root turnover rates, rooting depth, lignification and tissue density. Candidate traits impacting on soil microbial biodiversity at the single plant level include C and N root exudation and rhizosphere acidification, as well as those relevant to C-sequestration. Bioremediation targets include (i) heavy metal (Zn, Pb, Fe) uptake/tolerance; (ii) absorption of atmospheric NO<sub>x</sub>; and (iii) buffer zone efficacy with respect to capture of soil nitrate. Flood mitigation involves work on aspects of rooting and canopy architecture that confer increased infiltration rates into the soil profile as well as those conferring increased transpiration rates, such as leaf water conductance, and at a field scale canopy conductance. These novel traits will need to be allied to specified and detailed management systems to deliver benefits.

The challenge involved in getting broader targets incorporated into forage improvement programmes is principally one of cost. It is estimated that the annual wholesale value of forage seed sold to agriculture in the UK is approximately £8M (Caligari, 2002). In contrast, grassland supports approximately 40% of agricultural output, which is estimated to be worth approximately £5billion. Thus the genetic improvement of grassland species has broad social benefits because of its wider impact on agriculture. However the market is capable of supporting only a relatively small research base and needs to ensure a supply of new varieties for existing uses before being able to invest speculatively. For basic and enabling research, collaboration between researchers is recognised as essential, and has led to a number of the advances described above. The technologies involved in basic crop research are likely to become increasingly generic, in turn offering better opportunities for co-ordination. Unfortunately, the need to protect intellectual property and the differing sectoral requirements of different regions mean that downstream improvement programmes remain quite fragmented. This severely restricts the level of effort available to create new niches for forage varieties.

As the targets for forage breeding broaden, and as the need to carry out system studies involving animals increases, the validity of the current variety evaluation systems used for registration become increasingly questionable. Simple determination of the distinctiveness, uniformity and stability (DUS) of varieties remains straightforward and desirable, but the kind of performance measures needed to generate a recommended list add considerably to the costs of variety production. It can be argued that the use of cutting regimes to simulate grazing is not representative of a significant amount of livestock agriculture. The ideal would be to produce data that allowed users to assess the value of the variety within the production systems for which it is intended, and this would have the advantage of continuing to provide robust, experimentally obtained and consistent data. However, it is difficult to see how this ideal can be realised within the current cost structure of grassland farming. The industry may have to rely on data produced directly by breeders and users although it would be beneficial to have some independent monitoring of such information.

## **Conclusions**

The precision breeding and improvement of temperate forage varieties will continue, powered by developments in marker-assisted selection, genome mapping and introgression. Genetic manipulation remains a potentially useful approach to accessing novel genetic variability, but there are operational and public perception issues that need to be resolved first. Changes in temperate agriculture will emphasise a systems approach to both breeding and the development of precision management. There are new opportunities for forages outside the farming sector, but it is not clear whether enabling research in this area would attract enough support to create new markets. Maintenance of current programmes will increasingly come to rely on co-operative approaches to the generation of basic knowledge and to the development of new breeding technologies.

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## References

- Abberton, M.T., T.P.T. Michaelson-Yeates & J.H. Macduff (1998). Characterization of novel inbred lines of white clover (*Trifolium repens* L.) in flowing solution culture. I. Dynamics of plant growth and nodule development in flowing solution culture. *Euphytica*, 103, 35-43.
- Abberton, M.T., A.H. Marshall, T.P.T. Michaelson-Yeates, T.A. Williams, W. Thornley, W. Prewer, C. White & I. Rhodes (2000a). An integrated approach to introgression breeding for stress tolerance in white clover. In: G. Parente & J. Frame (eds.) Crop development for the cool and wet regions of Europe: achievements and future Prospects. *Proceedings of the Final COST Action 814 Conference*, Pordenone, Italy, May 10-13 (EUR 19683) Office for Official Publications of the European Communities. 311-314.
- Abberton, M.T., T.P.T. Michaelson-Yeates & I.A.H. Marshall (2000b). Molecular marker analysis in white clover. In: N.A. Provorov, I.A. Tichonovich & F. Veronesi (eds.) New approaches and techniques in breeding sustainable fodder crops and amenity grasses. All-Russia Institute for Agricultural Microbiology, St Petersburg, 192-195.
- Anonymous (2002). Agriculture and natural resources: benefits, costs and potential solutions. The Environment Agency, U.K. 100pp.
- Ansari, H., N.W. Ellison, S.M. Reader, E.D. Badaeva, B. Friebe, T.E. Miller & W.M. Williams (1999). Molecular cytogenetic organisation of 5S and 18S-26S rDNA loci in white clover (*Trifolium repens* L.) and related species. *Annals of Botany*, 83, 199-206.
- Armstead, I.P., A. Bollard, W.G. Morgan, J.A. Harper, I.P. King & R.N. Jones, (2001). Genetic and physical analysis of a single *Festuca pratensis* chromosome segment substitution in *Lolium perenne*. *Chromosoma*, 110, 52-57.
- Armstead, I.P., L.B. Turner, I.P. King, A.J. Cairns & M.O. Humphreys (2002). Comparison and integration of genetic maps generated from F2 and BC1-type mapping populations in perennial ryegrass (*Lolium perenne* L.). *Plant Breeding*, 121, 501-507.
- Armstead, I.P., L.B. Turner, M. Farrell, L. Skot, P. Gomez, T. Montoya, I.S. Donnison, I.P. King & M.O. Humphreys (2004). Synteny between a major heading-date QTL in perennial ryegrass (*Lolium perenne* L.) and the Hd3 heading-date locus in rice. *Theoretical and Applied Genetics*, 108, 822-828.
- Bhogal, A., M.A. Shepherd, D.J. Hatch, L. Brown & S.C. Jarvis (2001). Evaluation of two N cycle models for the prediction of N mineralization from grassland soils in the UK. *Soil Use and Management*, 17, 163-172.
- Caligari, P.D.S. (2002). The Role of future public research investment in the genetic improvement of UK grown crops. Report to the Department for Environment, Food & Rural Affairs by BioHybrids International Ltd. and ADAS Consulting Ltd. 222pp.
- Casler, M.D. (2001). Breeding forage crops for increased nutritional value. *Advances in Agronomy*, 71, 51-107.
- Chamberlain, D.E., R.J. Fuller, R.J.H. Bunce, J.C. Duckworth & M. Shrubbs (2000). Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. *Journal of Applied Ecology*, 37, 771-788.
- Collins, R.P., M. Fothergill, J.H. Macduff & S. Puzio (2003). Morphological compatibility of white clover and perennial ryegrass cultivars grown under two nitrate levels in flowing solution culture. *Annals of Botany*, 92, 247-258.
- Donnison, I.S., P. Cisneros, T. Montoya, I.P. Armstead, B.J. Thomas, A.M. Thomas, R.N. Jones & P. Morris (2002). The floral transition in model and forage grasses. *Flowering Newsletter*, 32, 42-48.
- Evans, M., S. Thoma, M. Sullivan & R. Hatfield (2002). Isolation and characterisation of polyphenol oxidase genes from alfalfa. *Annual Meeting of the American Society of Plant Biology*, August 2003, Denver, USA, p161.
- Forster, J.W., E.S. Jones, R. Kolliker, M.C. Drayton, J.L. Dumsday, M.P. Dupal, K.M. Guthridge, N.L. Mahoney, E. van Zijl deJong & K.F. Smith (2001). Development and implementation of molecular markers for forage crop improvements. In: G. Spangenberg (ed.) Molecular breeding of forage crops. Kluwer Academic Publishers, Dordrecht, 101-134.
- Forster, J.W., E.S. Jones, J. Batley & K.F. Smith (2004). Molecular marker-based genetic analysis of pasture and turf grasses. Developments in plant breeding. In: A. Hopkins, Z-Y Wang, R. Mian, M. Sledge & R.E. Barker (eds.) Molecular breeding of forage and turf. *Proceedings 3rd International Symposium on Molecular Breeding of Forage and Turf*, Kluwer Academic Publishers, Dordrecht, 197-238
- Henry, R.J. (2001). Plant genotyping - the DNA fingerprinting of plants. CABI Publishing, Wallingford, UK, 325pp.
- van Hintum, Th.J.L., N.R. Sackville-Hamilton, J.M.M. Engels & R. van Treuren (2001). Accession management strategies: splitting and lumping. In: J.M.M. Engels, V.R. Rao, A.H.D. Brown & M.T. Jackson (eds.) Managing plant genetic resources. CABI Publishing, Wallingford, UK, 113-120,

- Humphreys, M.O. (1999). The contribution of conventional plant breeding to forage crop improvement. In: J.G. Buchanan-Smith, L.D. Bailey & P. McCaughey (eds.) *Proceedings of the XVIII International Grassland Congress*, 1997, Vol 3, pp71- 78. Canadian Forage Council, Canadian Society of Agronomy, Canadian Society of Animal Science.
- Humphreys, M.O. & L.B. Turner (2001). Molecular markers for improving nutritive value in perennial ryegrass plant Breeding: sustaining the future. *Abstracts 16th Eucarpia Congress*, Edinburgh, 10-14 September 2001 (Abstract P1.17, p8).
- Humphreys, M.O., L.B. Turner, L. Skot, M.W. Humphreys, I.P. King, I. Armstead & P.W. Wilkins (2003). The use of genetic markers in grass breeding. *Czech Journal of Genetics and Plant Breeding*, 39, 112-119.
- Humphreys, M.W., I. Pašakinskienė, A.R. James & H. Thomas (1998). Physically mapping quantitative traits for stress-resistance in the forage grasses. *Journal of Experimental Botany*, 49, 1611-1618.
- Humphreys, M.W. (2003). The exploitation of chromosome recombination between *Lolium* and *Festuca* to assemble, locate, and identify gene combinations that may underpin future sustainable grassland production. In: Z. Zwierzykowski, M. Surma & P. Kachlicki (eds.) *Proceedings PAGEN International Workshop, application of novel cytogenetic and molecular techniques in genetics and breeding of the grasses*, 21-33.
- Isobe, S., I. Klimentko, S. Ivashuta, M. Gau & N.N. Kozlov (2003). First RFLP linkage map of red clover (*Trifolium pratense* L.) based on cDNA probes and its transferability to other red clover germplasm. *Theoretical and Applied Genetics*, 108, 105-112.
- Joyce, T.A., M.T. Abberton, T.P.T. Michaelson-Yeates, & W.J. Forster (1999). Relationships between genetic distance measured by RAPD-PCR and heterosis in inbred lines of white clover (*Trifolium repens*). *Euphytica*, 107, 159-165.
- Jones, E.S., M.L. Mahoney, M.D. Hayward, I.P. Armstead, J.G. Jones, M.O. Humphreys, I.P. King, T. Kishida, T. Yamada, F. Balfourier, G. Charmet & J.W. Forster (2002). An enhanced molecular marker-based genetic map of perennial ryegrass (*Lolium perenne* L.) reveals comparative relationships with other *Poaceae* genomes. *Genome*, 45, 282-295.
- King, I., H.M. Thomas, I.P. Armstead, L.A. Roberts, W.G. Morgan & M. Kearsey (2001). Introgression mapping elucidates the relationship between chiasma frequency and genetic distance and how genetic distance relates to physical distance. *Journal of Experimental Botany*, 52 (Supplement), C2.13.
- Kingston-Smith, A.H., A.L. Bollard, I.P. Armstead & M.K. Theodorou (2003). Proteolysis and cell death in clover leaves is induced by grazing. *Protoplasma*, 220, 119-129.
- Laws, J.A., B.F. Pain, S.C. Jarvis & D. Scholefield (2000). Comparison of grassland management systems for beef cattle using self-contained farmlets: effects of contrasting nitrogen inputs and management strategies on nitrogen budgets, and herbage and animal production. *Agriculture, Ecosystems and Environment*, 80, 243-254.
- Lee, M.R.F., R.J. Merry, D.R. Davies, J.M. Moorby, M.O. Humphreys, M.K. Theodorou & N.D. Scollan (2003). Effect of increasing availability of water-soluble carbohydrates on *in vitro* rumen fermentation. *Animal Feed Science and Technology*, 104, 59-70.
- Marley, C.L., R. Cook, J. Barrett, R. Keatinge, N.H. Lampkin & S.D. McBride (2003). The effect of dietary forage on the development and survival of helminth parasites in ovine faeces. *Veterinary Parasitology*, 118, 93-107.
- Miller, L.A., J.M. Moorby, D.R. Davies, M.O. Humphreys, N.D. Scollan, J.C. Macrae & M.K. Theodorou (2001). Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Milk production from late-lactation dairy cows. *Grass and Forage Science*, 56, 383-394.
- Morris, P., & T. Langdon (2001). Manipulation of the phenolic acid content and digestibility of forage grass cell walls by targeted expression of a ferulic acid esterase gene. *US Provisional Patent # 249608*.
- Scollan, N.D., R.J. Dewhurst, A. Moloney & J. Murphy (2005). Improving the quality of products from grassland. In: D.A. McGilloway (ed.) *Grassland – a global resource. Proceedings of the XX International Grassland Congress*, Dublin, Ireland, Wageningen Academic Publishers (in press).
- Thorogood, D. (2003). Perennial ryegrass. In: M.D. Casler & R.R. Duncan (eds.) *Turfgrass biology, genetics and breeding*. John Wiley & Sons Ltd., 75-105.
- Turner, L.B., M.O. Humphreys, A.J. Cairns & C.J. Pollock (2001). Comparison of growth and carbohydrate accumulation in seedlings of two varieties of *Lolium perenne*. *Journal of Plant Physiology*, 158, 891-897.
- Vickery, J.A., J.R.B. Tallowin, R.E. Feber, E.J. Asteraki, P.W. Atkinson, R.J. Fuller & V.K. Brown (2001). The management of lowland neutral grasslands in Britain: effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology*, 38, 647-664.
- Wilman, D., M. Field, S.J. Lister & D. I. Givens (2000). The use of near infrared spectroscopy to investigate the composition of silages and the rate and extent of cell-wall degradation. *Animal Feed Science and Technology*, 88, 139-151.
- Wood, J.D., R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard & M. Enser (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, 66, 21-32.

- Woodfield, D.R. & E.C. Brummer (2001). Integrating molecular techniques to maximise the genetic potential of forage legumes. In: G. Spangenberg (ed.) *Molecular breeding of forage crops*, Kluwer Academic Publishers, Dordrecht, 51-65.
- Young, N.D., J. Mudge, & N. Ellis (2003). Legume genomes: more than peas in a pod. *Current Opinion in Plant Biology*, 6, 199-204.

which combine the qualities of three species: the high forage quality and determined flowering cycle of *B. ruziziensis*, the yield and resistance to spittlebug of *B. brizantha* and the vigour and adaptation to acid, infertile soils of *B. decumbens* (Miles & Valle, 1996; Miles, 1999; Valle *et al.*, 2000; Peters & Lascano, 2003). At CIAT the *Brachiaria* breeding program also involves *Rhizoctonia* resistance, tolerance to drought, and increased seed production (Peters & Lascano, 2003).

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

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

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

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