The genetic improvement of forage grasses and legumes to enhance adaptation of grasslands to climate change

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Michael T. Abberton, James H. MacDuff, Athole H. Marshall and Mike W. Humphreys from the Plant Breeding and Genetics Programme, Institute of Grassland and Environmental Research, Aberystwyth, United Kingdom, in collaboration with Plant Production and Protection Division Crop and Grassland Service of the Food and Agriculture Organization of the United Nations.

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Summary for policymakers

Grasslands cover about 70% of the world's agricultural area. They have a crucial role in terms of food production and in the delivery of ecosystem services such as water supplies, biodiversity and carbon sequestration.

The grasslands of the world face a range of challenges from climate change including the effects of elevated atmospheric carbon dioxide, increasing temperatures, changes in precipitation regime and higher concentrations of ground level ozone. These factors threaten productivity, species composition and quality, with potential impacts not only on livestock production but also on other aspects of the multifunctional role of grasslands. In a previous work we considered the contribution grasslands make to greenhouse gas emissions and the potential of genetic improvement of key grassland species to reduce these emissions and enhance carbon sequestration in grassland soils. In this paper we summarize the targets and approaches plant breeding programmes should adopt to enable grasslands to adapt to climate change whilst realizing their potential contributions to food security and reducing the environmental impact of livestock agriculture.

We focus on the following major challenges:

(i) Developing grassland crops with improved drought tolerance and enhanced water use efficiency.

(ii) Improving tolerance of saline soils

(iii) Tolerance of floods and related consequences of changes in rainfall patterns

(iv) Maintaining nutrient use efficiency and forage quality

In general the most advanced examples are from work carried out on the key species of temperate grasslands. State of the art genomic approaches are beginning to be deployed in these crops. However, there is an urgent need for increased public sector resources to be dedicated to the development of new varieties of grassland crops for the tropics and sub-tropics. Genetic improvement approaches could be complemented by research to explore the potential of introduced species and ecotypes and allied with modeling of climate change scenarios to facilitate breeding targeted to the needs of regions most affected.

Introduction

The anticipated impacts of climate change on grassland systems and appropriate management responses have been reviewed extensively, though the emphasis has been on European temperate and North American rangeland systems (Cambell *et al*., 2000; Nösberger *et al.,* 2000; Polley *et al*., 2000; Lüscher *et al*., 2005; Morgan, 2005; Hopkins and Del Prado, 2006; Baron and Bélanger, 2007). Options for mitigation and adaptation have also been discussed in a European context (Humphreys and Humphreys, 2005; Humphreys *et al.,* 2006). Global climate change is likely to shift the adaptive regions of most forage species in the long term. Hence there is a need to identify and incorporate the relevant adaptive traits into existing and new forage species in order to maintain and, where feasible, enhance productivity of grassland systems in the face of the changing environmental constraints imposed upon them.

In this paper we firstly briefly summarize the relevant impacts of climate change and then discuss the opportunities for genetic improvement of forage species with respect to:-

(i) Drought tolerance and water use efficiency
(ii) Salinity tolerance
(iii) Flood tolerance
(iv) Tolerance to elevated ground level ozone
(v) Nutrient dynamics
(vi) Forage quality
(vii) Potential role for the introduction of new species or ecotypes

We also consider future opportunities with respect to grassland design utilizing state of the art approaches in the context of the broad systems level understanding.

A. Impacts of climate change

(i) Elevated carbon dioxide

Carbon dioxide (CO_2) enrichment and global warming are predicted to increase net primary production (NPP) on most temperate pastures and rangelands, slow canopy-level evapotranspiration as a result of reduced stomatal conductance, and hence reduce the rate and extent of soil water depletion (Cambell *et al.,* 2000, Nösberger *et al*., 2000; Polley *et al*., 2000, Morgan, 2005; Baron and Bélanger, 2007). Drake *et al*. (1997) reported an average increase in photosynthesis of 58% over 60 experiments conducted under elevated CO_2. However, the average increase in sward productivity across the grassland ecosystems studied under the Global Change and Terrestrial Ecosystem research project network was only 15% (Lüscher *et al*., 2005). Most measurements have been made under rangeland or cool/temperate climates; corresponding information on other climate zones is very sparse. Studies on Africa are fewer than for any other continent. Hely *et al*. (2006) focused on large scale biomes and their responses to changes in precipitation patterns. Likewise, responses of species mixtures other than perennial ryegrass and white clover have received relatively less attention. However, the available evidence suggests that forage legumes in general show higher responses than grasses to elevated CO_2 (Lüscher *et al.*, 1998).

Increases in canopy dark respiration and soil respiration under elevated CO_2 are highly correlated with the changes in canopy gross assimilation (Casella and Sousanna, 1997).Growth under elevated CO_2 generally increases carbon (C) allocation to root biomass and other below ground processes (e.g. Rogers *et al*., 1996), and in perennial ryegrass to an extent that may exceed the observed yield increases (Soussana *et al*., 1996; Schapendonk *et al*., 1997). Purple Moor Grass (*Molinia caerulea* (L.) Moench) plants exposed to elevated CO_2 showed a reduced specific leaf area (SLA), an increased number of senescent leaves and an increased rootstock growth (Franzaring *et al.* 2008). Higher C inputs to the soil (e.g. Xiao *et al*., 2007) might be expected to increase competition for nitrogen (N) between soil microbial community and plant roots, and in low N status soils there is some evidence for a decrease in plant available forms of N (Gill *et* al., 2002). However, changes in the structure and composition of soil microbial populations under elevated CO_2 may, in the long run, increase plant available N (Lüscher *et al*., 2004). Li *et al*. (2004) reported a model characterizing ungrazed semi-arid grassland in Canada which showed a balance of effects under a regional climate change scenario leading to little change in carbon sequestration. Although the evidence is inconsistent with respect to the impact of elevated CO_2 on litter quality, amount, and the impact of higher C:N ratios on mineralization rates (O'Neill and Norby, 1996), gross increases in litter and root litter, and hence in decomposition, are expected to result in increased release of nutrients (Soussana *et al*., 1996). A doubling in the concentration of CO_2 increased tissue C: N ratio by 15% on average in a study by Gifford *et al*. (2000), mainly reflecting the increase in carbohydrate content. The situation is further complicated by the interactions between litter quantity, quality and decomposition with respect to temperature and the other environmental variables associated with climate change.

(ii) Elevated temperatures

Simulation of the potential temperature-induced changes in global productivity range from significant increases in northern temperate zones to significant decreases in drier zones (Ojima *et al*. 1996). Warmer temperatures are likely to enhance the growth response of most C_3-dominated grasslands to higher CO_2 (Polley *et al*., 2000) and hence their productivity, particularly where water is not limiting, as in North and North West Europe (Hopkins and Del Prado, 2006). In high- and mid-latitude rangelands, currently subject to severe cold-temperature restriction on growth rate and duration, warmer temperatures alone are likely to enhance production (Polley *et al*., 2000). In these zones substantial increases in the number of growing degree days may translate into opportunities for extra harvests (Bootsma *et al*., 2001). According to Baron and Bélanger (2007), effects in continental America include (a) a limited northward shift in production areas in US and Canada arising from higher temperatures and the frost-free season extending by 1-9 weeks, and (b) subtropical conditions extending further north with changes in relative distribution of C_3 and C_4 species. However, in arid and semi-arid rangelands of Central and South America, Africa, Middle east, Asia and Australia, positive effects of temperature may be lessened or negated by accompanying increases in evapotranspiration (Polley *et al*., 2000) and water deficit, leading to reductions in photosynthesis (Nösberger *et al*., 2000). In a European context, the vulnerability of grassland to negative temperature-related impacts of climate change is likely to be greatest in Mediterranean and southern Europe (Schroter *et al.,* 2005), due to summer heat and drought, and also at the highest latitudes where natural ecosystems are threatened.

Effects on winter survival of perennial crops are difficult to predict, in spite of anticipated increases in mean annual temperatures, as a number of factors are involved, including changes in length of winter hardening period. To survive the winter, a plant requires mechanisms whereby sensitive tissues can avoid freezing or for warmer climates chilling, or alternatively undergo cold hardening, coordinate the induction of the tolerance at the appropriate time, maintain adequate tolerance during times of risk, and time the loss of tolerance and resume growth when the risk of freezing has passed (Guy, 1990). Development of winter hardiness requires a set exposure (its length dependent on the species and its level of winter hardiness) to low non-freezing temperatures, typically 0-10ºC, and shortened photoperiod (Humphreys *et al*. 2006).

(iii) Changes to precipitation patterns

Significant changes in seasonality of rainfall, combined with warming and increased intensity of rainfall events, and increased severity of summer droughts are predicted for many parts of the world. Given that the productivity in most rangelands is limited by water in a near linear manner (Cambell *et al.*,2000; Baron and Bélanger, 2007), changes in the total annual rainfall alone will significantly impact these systems, particularly in arid and semi-arid lands, although probably less so than the alterations in seasonal patterns storm intensity (Giorgi *et al.*, 1998). However, increased water use efficiency (WUE) under elevated CO_2 may in part compensate for reduced summer precipitation and increased potential evapotranspiration (Lüscher *et al.*, 2005). Species composition will also be affected given that the primary environmental control on species distribution is water balance, especially in rangeland (Stephenson, 1990).

(iv) Increased environmental variability

Increased environmental variability, for example in terms of increased frequency of episodes of environmental stress outside the range associated with adaptation, will have ecological significance. For example, inter-annual temperature variability is predicted to increase by 100% for Central Europe by 2071-2100; with winter rainfall increasing and summer rainfall decreasing (Schär *et al*., 2004).Besides increasing the frequency of episodes of environmental stress beyond the adaptive capacity of the pasture, increased variation may exacerbate problems associated with 'false breaks' (e.g. germination-inducing rainfall events followed by death from drought); a phenomenon factored into the selection criteria employed in the appraisal of new legumes for Australian systems (Nichols *et al.,* 2007).

Increased gap formation and subsequent species invasions, resulting from extreme environmental conditions such as summer droughts, could be a serious aspect of future climatic conditions (Lüscher *et al.*, 2005), making management of desired species mixture problematic.

(v) Changes in species distribution

According to Lüscher *et al*.(2005), C_4 species tend to be favoured over C_3 species in warm humid climates, the reverse being so in cool climates. Global warming is likely to favour C_4 plants through its impact on increasing minimum daily temperature of a region, increasing photorespiration and reducing quantum yield in C_3 plants (Polley *et al*., 2000). However, elevated CO_2 levels may provide a partial offset, favouring C_3 grasses (Polley *et al.*, 1993) as well as broadleaved species (Byrne and Jones, 2002). Nevertheless, predictive analysis suggests that relative abundance of C_4 grasses in temperate grasslands will increase throughout most regions of North and South America (Epstein *et al.*, 2002). A similar trend is anticipated in New Zealand where exposure to extreme heating events increases in frequency (White *et al.*, 2000).

B. Potential for improved crop adaptation

The adaptation potential for most natural ecosystems is regarded as low, whilst that of many managed land-use systems is relatively high (IPCC, 2001). Forage species are generally adapted to specific climatic regions and, at the centre of their adaptive zone, may regularly survive extremes of temperature and moisture as well as stress of lax management (Nelson and Moser, 1994). Both genotypic and phenotypic plasticity influence adaptation (see Nelson, 2000); the former depends upon survival of genotypes making up the population and the latter results from interaction between the genotype and the environment. Alfalfa (*Medicago sativa* L.) is an example of a forage species with high genotypic and phenotypic plasticity enabling its adaptation to many ecoregions (Baron and Bélanger, 2007).

Based upon experience in Australia, Nichols *et al*. (2007) have highlighted the need to distinguish between the adaptive requirements of short-term pasture/ley-farming systems (i.e. annual legume based pastures used in rotation to restore fertility) and those of more permanent pasture, requiring perennial species to support meat, wool and dairy production. Whilst some selection targets will be common to both, there will be a number specific to each type of system.

Given the range and complexity of likely climate change impacts, it is clear that forage breeding faces a number of significant adaptive challenges. These include:

(a) The consequences of elevated CO_2 in terms of the increased inputs of nutrients and water required to support the increased yield potential.

(b) The impact of increasing variability in temperature and rainfall distribution/intensity on plant development and reproduction.

(c) Changes in the frequency with which pastures are resown, particularly if the successive generations of varieties fail to sustain their performance under progressively changing climatic conditions.

(d) Increasing the genetic variation present in varieties and cultivars.

(e) Undesirable changes in the species composition of pastures.

(f) Changes in pasture management (i.e. cutting frequency, irrigation, grazing rotations, managed sward heights), implemented in part to mitigate undesirable changes in species composition.

(g) Maintaining sward composition and output in the face of the increasing use of species mixtures and combinations of cultivars adapted to different environmental stresses.

(h) Accommodating adaptive trade-offs.

(i) Enhanced drought tolerance and increased water use efficiency

Drought is an important environmental factor limiting the productivity of crops worldwide. Climate change models predict greater variability in rainfall patterns and increased periods of summer drought will affect many regions including temperate grasslands. Predicted population growth will also require that more of the available water is used for domestic and industrial use, rather than irrigation (Condon *et al*., 2004). The growing demand for crops exhibiting greater drought tolerance and water use efficiency is reflected in the increasing emphasis within forage plant breeding programmes on selection of varieties better able to tolerate prolonged periods of water deficit.

In recent years, developments in molecular marker technologies and their use in quantitative trait loci (QTL) analysis have provided effective new opportunities for the study of plant responses to environments including complex traits such as tolerance of drought (Prioul *et al.,* 1997; Ribaut *et al.,* 1997; Courtois *et al.,* 2000; Yadav *et al.,* 2002, 2004). Molecular marker technology provides opportunities not only to identify QTL that determine complex phenotypes such as drought tolerance (Prioul *et al.,* 1997), but also to greatly improve the efficiency of genetic improvement by facilitating introgression of desirable traits through the use of linked markers (Tanksley 1993, Mohan *et al.,* 1997). Once a marker-trait association has been established, the linked markers can be used in selection of drought tolerance or yield under drought reducing (or even eliminating) the reliance on specific environmental conditions during the selection phase, a major problem in the conventional breeding of traits influenced by drought *(*Tuberosa and Salvi 2006)*.*

A considerable body of knowledge has accumulated on the QTL and genes involved in the response to drought and other abiotic stresses (e.g. Babu *et al*., 2003; Cattivelli *et al*., 2008). A number of well characterized pathways have been established and their interactions analysed (Chavez *et al*., 2003). Genomics approaches to crop improvement in general (Varshney *et al.,* 2005) and drought tolerance in particular (Tuberosa and Salvi, 2006) have also been recently reviewed.

The ryegrasses, especially perennial ryegrass (*L. perenne*) are widely considered to offer the optimal forage for livestock agriculture in many temperate areas. However, both the productivity and persistence of perennial ryegrass, and to an even greater extent of Italian ryegrass (*L. multiflorum*), are reduced by incidences of summer droughts (Humphreys *et al.,* 1998). One of most promising approaches to reducing the impacts of drought on high quality ryegrass pastures involves the introduction of alleles from a range of closely related and more resilient fescue species (Humphreys *et al*., 1998). This was highlighted in a recent European Union funded Framework V project SAGES[1]), the outcomes of which included novel genotypes of ryegrass with enhanced drought resistance transferred from related fescue species adapted to drought stressed conditions.

The large and complex range of adaptive traits for drought resistance in perennial ryegrass outlined in Humphreys and Humphreys (2005) was reduced by the SAGES consortium to a small number of key targets for future crop improvements with adaptations for grasses suited for moderate or more extreme drought stress typical of European growth conditions (Table 1). Drought resistance may be categorized into (i) drought avoidance, (ii) desiccation delay and (iii) desiccation tolerance. Drought avoidance includes traits such as summer quiescence, and early flowering; desiccation delay, the maintenance of high relative water content, or the presence of dense surface roots. Desiccation tolerance is more of an adaptive response to soil water deficit and includes production of osmoprotectants and changes to cell membranes to reduce cell water loss.

Yield stability of a genotype or cultivar over a range of environments has been suggested as one effective measure of drought resistance (Finlay and Wilkinson, 1963; Thomas, 1997). Flowering phenology interacts significantly with a forage grass plants' capability to withstand drought stress, and if flowering is completed early in the summer before the onset of any severe soil water deficit will provide an effective avoidance mechanism to the most severe detrimental effects on forage production, survival, and recovery (Thomas, 1997).

In addition to rapid establishment, increased growth vigour and more concentrated and early flowering (Table 1), the capability to extract water from deep in the soil during periods of water deficit is considered as a major factor in determining a grass species drought resistance, at least in temperate locations (Humphreys *et al.,* 2006; Durand *et al*., 2007). Ryegrasses' comparatively poor drought resistance compared with certain fescue species is in large part a reflection of their differing floral phenology and their inferior rooting capabilities and inability to extract water from deep in the soil (Garwood and Sinclair, 1979; Thomas, 1997; Durand *et al.,* 2007). Humphreys *et al.,* (1997) described how differences in stomatal distribution and leaf water conductance and in root depth and thickness explained, at least in part, the greater drought resistance found in fescue species such as tall fescue (*Festuca arundinacea* Schreb.) compared with that found in ryegrass (*Lolium spp.*).

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[1] http://www.iger.bbsrc.ac.uk/SAGES2/sages2.html

Table 1: Traits that were confirmed or re-assessed as essential to resistance to water deficits of perennial forage grasses in Western and Continental Europe. The maritime climate is characterized by moderate drought. Drought resistance (DR) traits may be grouped into strategies: DA drought avoidance, DD desiccation delay (or avoidance), DT desiccation tolerance. Symbols: √ advantageous, X deleterious.

			Importance in different climatic zones of Europe	
Trait	New ranking of traits	Strategy	Maritime (e.g. UK)	Continental (e.g. France)
Ontogenetic/ developmental Rapid establishment	1	DR	✓	✓
Concentrated flowering period	2	DA	✓	✓
Roots At least some deep roots	3	DA DD	✓	✓
Ability to extract nutrients from soil	?	DR	✓	✓
Leaves Continued leaf growth in drought	4	DR	✓	✓
Stomata close at low Relative Water Content (RWC)	5	DD DT	✓	✓ X
Maintenance of green leaf area	6	DR	✓	✓
Metabolism/Anatomy Osmotic adjustment, hydration, solute accumulation	7	DD DT	✓ ?	✓

Interspecific hybrids between ryegrass and fescue species, such as Festuca arundinacea and F. glaucescens are produced quite readily, and at meiosis homoeologous chromosomes pair and recombine at high frequencies enabling gene transfer between ryegrass and fescue chromosomes to proceed effectively with progeny in backcross breeding programmes having increased production under mild drought and survival under extreme drought stresses (Humphreys and Thomas, 1993; Humphreys et al., 1998; Humphreys J. et al., 2005).

The Lolium and Festuca genomes share considerable synteny and homology with rice, and the Triticeae (Alm et al., 2003; Armstead et al., 2005). In a pilot study, Bacterial Artificial Chromosomes (BACs) of rice chromosome 1 were mapped by fluorescence in situ hybridization (FISH) and by use of orthologous markers onto Lolium/Festuca chromosome 3 sites and were found in the same order as they occur in rice along the entire chromosome length (King pers. comm.). Rice chromosome 1 contains major QTL for resistance against severe drought stress (Zhi-Kang et al., 2005), and equivalent loci might be expected in the same order at locations on chromosome 3 of Lolium and Festuca. Indeed, introgression mapping studies identified different loci of F. arundinacea (Humphreys and Thomas, 1993; Humphreys and Pašakinskienė, 1996) and F. arundinacea var glaucescens (Humphreys et al., 2005) that had subsequent to their transfer to chromosome 3 of L. multiflorum provided the capability to resist and recover the onset of 3 consecutive months without irrigation in glasshouse conditions by which time the Lolium parental genotypes had died. Alm et al. (2003) produced the first molecular map of F. pratensis and subsequently (Alm et al. in press), the first QTL map in forage grasses for resistance against abiotic stresses. Alm et al. (in press) found QTL for resistance against severe drought stress along the entirety of F. pratensis chromosome 3 under the same droughting conditions used in the introgression-mapping studies (Humphreys and Thomas 1993; Humphreys et al., 2005). Rice chromosome 1 also carries QTL for root length, thickness and root/shoot ratio (Ikeda et al., 2007) and this may help explain how introgressed fescue genes from chromosome 3 may impact on the drought resistance of ryegrass.

Water use efficiency (WUE), defined as the ratio between plant (usually shoot) production and transpiration is one measure of the ability of a plant to perform well under incipient drought (Thomas, 1997). This can also be defined as the yield of product/water consumed. The potential of breeding crop species with higher WUE to reduce water usage and the approaches used have been reviewed by Condon et al. (2004). They identified three key processes that can be exploited in breeding for higher water-use efficiency; (i) moving more of the available water through the crop rather than it being wasted as evaporation from the soil surface or drainage beyond the root zone or being left behind in the root zone at harvest; (ii) acquiring more carbon (biomass) in exchange for the water transpired by the crop; (iii) partitioning more of the achieved biomass into the harvested product. These processes are not independent and targeting specific traits to improve one of the processes may have detrimental effects on the other two. A number of studies have quantified differences in WUE between temperate forage grass species (Johnson and Bassett, 1991; Jensen et al., 2002). Cocksfoot (Dactylis glomerata L.) was found to have a greater WUE than the ryegrasses and a Festulolium hybrid (Jensen et al., 2002).

Alm et al. (in press) described how several QTL for stress tolerance in Festuca were co-located with loci of dehydrin genes (responsible for protecting cells and cell membranes from desiccation). They provide evidence that tolerance to severe drought stress may well be associated with an abscisic acid (ABA) induced expression of a dehydrin gene Dhn5 on chromosome 4F. A QTL for growth under moderate drought (QDtm6F) mapped onto 6F with the dehydrin gene Dhn6 as the peak marker. Dhn6 is known in barley to be only induced by drought and ABA (Choi et al., 1999).

As safeguards to extreme drought stress, genes for ryegrass breeding programmes are being sought currently from fescue species adapted to prolonged and intense summer droughts and high temperatures. Recently Festuca species with extreme xerophytic traits were sourced from the Atlas mountains of North Africa as new donors of drought resistance and rooting traits for Lolium. These include the tetraploid species F. mairei (Atlas fescue) (2n=4x=28, M1M1M2M2)) which has an exceptional drought tolerance. The M genome in Festuca is associated with xerophytic adaptation allowing the plant to survive long summers under severe drought stress, and may be derived from the diploid species F. scariosa (Borrill et al., 1971; Harper et al., 2004). It has higher leaf photosynthesis and a larger root system than conventional hexaploid Festuca (Nelson, 1988) and has better drought tolerance than other Festuca grasses (Wang and Bughrara, in press). Chen et al., (1997) described a backcross breeding procedure aimed at the introgression of F. mairei genes for drought resistance into perennial ryegrass and Wang et al. (2003) using molecular markers confirmed the efficacy of their introgression procedures.

As all the major grass crops derive from a common ancestor, much work on the genetics of drought resistance and WUE can be achieved in parallel with advances in one crop having benefit for all. Major advances in genome sequencing and genomics will pave the way for the identification of the major determinants of drought resistance and these will be selected as required suitable for adaptations specific to the stress level in any selected environment and tailored for the specific crop. Targets for alternative crops will differ, at least in part, especially when crop yield is represented either in terms of seed production at one specific time in the year for cereals, and as continued supplies of foliar growth for livestock feed or indeed as new sources of bioenergy in grasses such as Miscanthus over a prolonged period.

White clover (Trifolium repens L.) and red clover (T. pratense L.) are the most important legumes of temperate pastures. The former is used largely in systems based around sheep or cattle grazing and is grown together with a companion grass species. Red clover is typically cut three or more times in a season and often used to make silage or hay for winter feeding. It is less persistent than white clover and grows from a central crown rather than a spreading network of central stems, or stolons. In temperate forage production systems where mixtures of grasses and legume species are grown, white clover (T. repens L.) is generally considered to be more drought resistant than perennial ryegrass. This may be due in part to differences in plant morphology. White clover spreads by means of stolons (horizontal stems) from the nodes of which adventitious roots are produced. Stoloniferous spread and adventitious rooting combine to give individual genotypes the ability to tap water resources across a considerable area. However, within the white clover gene pool and particularly within plant germplasm well adapted to the other multiple stresses of UK agriculture (winter, grazing, pests and diseases) genetic variation for drought tolerance is not great (Abberton and Marshall, 2005).

Classical approaches to selection of white clover for drought resistance were summarised by Williams (1987). The importance of both plant morphology and seasonal growth patterns was highlighted and progress to that date in different parts of the world reviewed. Barbour et al. (1996) showed differences between ten white clover cultivars with respect to their response to water stress but Brink & Pederson (1998) found little variation in response to a water gradient between six lines of white clover. Field studies have also shown that drought, in combination with other stresses and influenced by management, can have marked effects on plant survival and these effects differentiate between plant populations (Jahufer et al., 1995).

Phenotypic selection for improved drought tolerance, or for yield under drought stress conditions, is widely accepted as difficult. This is because occurrences of drought stress in natural environments are highly variable in their timing, duration, and severity, making it difficult to identify traits that confer a predictable advantage across stress environments (Passioura, 1996). Direct selection for drought tolerance has been carried out in the field and indirect methods have also been used, but success has been limited. Ourcival et al. (1992) showed that chlorophyll fluorescence methods can be used to give early indications of drought stress and offers one approach to the ranking of genotypes with respect to their response to water deficit.

Where insufficient genetic variation is available to achieve any significant improvements in drought resistance from within a species, increasingly new allelic variants are being sought from wild relatives adapted to drier environments. Backcross hybrids have been produced between white clover and the more drought tolerant Kura or Caucasian clover (T. ambiguum M. Bieb.) with white clover as the recurrent parent and show considerably enhanced drought resistance compared to the white clover parent (Marshall et al., 2001). The basis of this enhanced drought resistance is not clear, however differences in stomatal density and in root density throughout the soil profile have been identified between parental species and hybrids.

An alternative approach is to broaden the range of species used and include those that are more suited to drought prone environments. Use of drought tolerant alfalfa (Medicago sativa) in the UK could offer potential benefits provided it was agronomically suited to UK conditions. Bell et al. (2007) compared drought response of alfalfa with the potential alternative forage of Mediterranean environments-Dorycinium hirsutum and D. rectum, the former showing considerable promise for dry environments. Increased drought tolerance and adaptation to semi arid environments were highlighted by Williams et al. (2007) in a review of the future of pasture plant breeding in New Zealand. Acharya et al. (2006) reviewed progress in the genetic improvement of cicer milkvetch (Astragalus cicer) a forage legume which may become more important in temperate environments since it is relatively drought tolerant.

Genetic resource collection and characterization can have an important role in identifying germplasm adapted to drought prone environments. In alfalfa, for example Prosperi et al. (2006) have demonstrated the diversity in uncultivated alfalfa in Spain with respect to adaptation to drought environments. Alfalfa is widely used in temperate, Mediterranean and sub-tropical environments. Collino et al. (2005) reported studies concerning the effects of temperature and water stress on this species in Argentina and Aranjuelo et al. (2007) explored possible impacts of change in Mediterranean context on photosynthesis and N fixation in alfalfa. The biochemical basis of the response of alfalfa nodules was explored by Naya et al. (2007) and the wider impact on N allocation and storage by Erice et al. (2007). Ainsworth et al. (2003) showed that for legumes the ability to fix nitrogen contributes to the maintenance of significant increases in photosynthesis at elevated carbon dioxide levels.

In tropical systems, most emphasis has been placed on quantifying the tolerance of different forage species to drought and identifying species most appropriate for drought conditions rather than improving drought tolerance per se. Comparisons of the response to moderate drought of the perennial tropical grasses Andropogon gayanus, Hyparrhenia rufa, Echinochloa polystachya and Brachiaria mutica have been carried out under controlled conditions (Baruch, 1994). The tolerance of a number of Brachiaria species to drought has been the focus of extensive studies in both glasshouse (de Mattos et al., 2005a) and field (de Mattos et al., 2005b). Tolerance of accessions of the tropical pasture legume Centrosema brasilianum to drought has been studied to identify accessions which combine good agronomic production and good drought tolerance (Peters et al., 1998).

Ghannoum et al. (2003) studied the biochemical and physiological basis of the response to drought stress in tropical grasses (e.g. Panicum coloratum, Cenchrus ciliari) and Guenni et al. (2002) analysed response to drought in five Brachiaria species. Ohashi et al. (1999) studied water stress effects on three tropical legumes –compared with pasture legumes Siratro and Desmodium with soybean. Clearly, in legumes the effects of drought or other stresses on nitrogen fixation needs to be quantified alongside the effects on the plant itself. This was considered by Pitman and Lai (1998) with respect to the perennial pasture legume Desmodium and more broadly by Sicardi de Mallorca and Izaguirre-Mayoral (1994) who considered a very wide range of legumes native to tropical savannas. A similar exercise was conducted by Pandey et al. (1984) for a number of tropical grain legumes.

Studies have quantified differences in WUE in the temperate forage legume alfalfa (Medicago sativa) (Johnson and Tieszen, 1994) and between tropical legumes (Wang et al., 2006). In recent studies, ninety six genotypes in a white clover family used to study stolon morphology and produce a genetic map of this species (Jones and Abberton, 2007), were characterized with respect to WUE and preliminary QTL identified. This suggests that this is a feasible route to marker identification and marker assisted selection strategies in this species.

Direct evaluation of WUE, which requires precise measurement of individual plant growth and water consumption, is not feasible in the field, making selection for WUE difficult within a breeding programme (Barker et al., 1989). However, carbon isotope discrimination provides a robust, if indirect, method of identifying variation in WUE and is increasingly used in breeding programmes (Condon et al., 2004; Rytter, 2005), including forage grasses (Jensen et al., 2002) and alfalfa (Johnson and Tieszen, 1994), although not as yet in clovers.

Progress is also being made in the development of new molecular tools. In alfalfa, for example, an expressed sequence tag simple sequence repeat genetic map of alfalfa has recently been described (Sledge et al., 2005). Although not extensively discussed in this paper, transgenic approaches have also been used to enhance drought tolerance and in alfalfa; Zhang et al. (2005) reported enhanced drought tolerance from increased cuticular wax deposition resulting from over-expression of a transcription factor gene.

In recent years considerable gains have been made in our understanding of plant genome organization and gene expression. In large part this has been achieved through the study of ‘model species’ i.e. species in which genetics and genomics are more tractable than in many crop plants. The best known and most developed of these models is Arabidopsis thaliana the DNA sequence of which was published in 2000. Subsequently a number of different model species have been developed. Most relevant to grassland crops are the legumes Lotus japonicus and Medicago truncatula, the latter being particularly closely related to the clovers. In the monocots the major crop rice has also been developed as a model due to the intensive effort in generating genomics tools and resources in this species.

One way of searching for novel gene expression changes in response to drought is to use a transcriptomics approach i.e. a global survey usually based on a gene chip where a significant proportion of the expressed genome can be interrogated. At IGER, in collaboration with Teagasc, Oak Park, Ireland and the University of Bristol an experiment has been carried out using the recently developed M. truncatula Affymetrix GeneChip to explore changes in gene expression in droughted (vs. control) plants of both white clover and M. truncatula. A number of genes showed up-regulation in response to drought and these are ‘candidates’ with respect to a role in drought tolerance. In tropical systems the molecular basis of drought tolerance has received little attention.

(ii)Increased tolerance of saline soils

Problems of soil salinity are a major constraint to crop development in many other drier areas of the world, and these are frequently a consequence of mismanaged irrigation practices (Malhotra and Blake, 2005). In 1980, in excess of 3 x 106 ha of arable land were considered saline (Brinkman, 1980). This area has more than doubled in the past two decades (Malhotra and Blake, 2005) and it is estimated that salinity in soil affects about 7% of the land’s surface (Humphreys and Humphreys, 2005). For many regions, the design of salt-tolerant crops is considered a priority. Unfortunately, there are few naturally occurring salt-tolerant higher plant species but amongst the grasses, the temperate species Agrostis stolonifera and Festuca rubra have excellent salt tolerance, whereas other grass species such as Holcus lanatus, Dactylis glomerata and L. perenne display considerable adaptive variation (Ashraf et al., 1986). Rising sea-levels, and increased wind speeds both a likely consequence of climate change will also induce more instance of coastal flooding and increased salinity and desiccation stresses affecting particularly low-lying locations and coastal areas found typically in the UK and elsewhere.

New sources of salt tolerance are required as are more efficient techniques for identifying salt-tolerant germplasm so that new genes for salt tolerance can be introduced into crop cultivars by marker-assisted-selection.

The mechanisms of salt-tolerance remain poorly understood, despite salinity being studied in a range of glycophytic and halophytic plants (Hasegawa et al., 2000). It is associated with a range of physiological adaptations, including ion compartmentation and the production of compatible solutes. Salt tolerant plants may use vacuolar sodium storage and synthesize organic osmotic protectants including carbohydrates such as trehalose and fructans, and protein protectors such as glycine betaine and various compounds capable of scavenging reactive oxygen species like superoxide dismutase and glutathione peroxidise (Malhotra and Blake, 2005).

Growth stage is also important in relation to salt tolerance. As is the case for most legumes, germination of chickpea is affected less by salinity than is subsequent growth (Yadav et al., 1989). Kumar (1985) and Siddiqui and Kumar (1985) reported that salinity tolerance decreased with age in chickpea and in pea. For legumes, an important consideration is the impact of salt on symbiotic nitrogen fixation. Free living rhizobia are frequently more salt-tolerant than their host, although the symbiotic process itself is sensitive to salinity

New genetic and biochemical approaches are assisting development of crops with improved salt-tolerance. In citrus crops, 17 QTL were identified connected directly with salinity-tolerance (Tozlu et al., 1999). Genome studies using model species such as Arabidopsis have also revealed important regulatory genes and pathways, such as a calcium-regulated protein kinase pathway (Zhu, 2001). Duncan and Carrow (1999) list a number of specific genes controlling osmoprotectants such as glycine betaine, dehydrins and K-Na selectivity. The first plant Na+/H+ antiporter (responsible for extruding or compartmentalizing Na+ ions) gene to be fully characterized was AtNHX1 which confers if over-expressed, salt tolerance in transgenic Arabidopsis (Apse et al., 1999). The antiporter salt overly sensitive gene SOS1encoders a plasma membrane-localized Na+/H+ antiporter which functions in excluding Na+ from the cytoplasm (Shi et al., 2000).

With respect to the genetic control of salt tolerance, Gregorio and Senadhira (1993) demonstrated in rice that both additive and dominance gene effects govern salinity. Prasad et al. (2000) mapped QTL in rice seedlings and identified 7 QTL, with 4 identified on chromosome 6. A major gene for salinity tolerance has been mapped on rice chromosome 1 (Malhotra and Blake, 2005), a chromosome implicated strongly in drought resistance and syntenic to chromosome 3 of Lolium and Festuca (Humphreys et al., 2006).

Many genes involved in plant adaptations to drought stress and desiccation-tolerance also confer improved salinity tolerance. This has been observed in the Lolium-Festuca complex, where Festuca-derived genes for drought resistance (Humphreys et al., 2005; Humphreys et al., 2006) also conferred tolerance to prolonged exposure to saline conditions in concentrations of 150-300mM NaCl (Latorre, unpublished results). Seki et al. (2002) used cDNA microarray analysis in Arabidopsis thaliana to identify genes increased after cold, drought, and high-salinity, respectively. These authors identified 22 stress-inducible genes that responded to all three stresses. However, overall many more genes were co-expressed during drought and salinity stress than were involved when plants were exposed only to low temperatures.

The transfer of gene(s) for salt tolerance into wheat following hybridisation with its relative (salt tolerant) species Thinopyrum bessarabicum (King et al., 1997) demonstrates the potential of introgression as a breeders' tool for transferring salt tolerance from wild into a crop species and as demonstrated above (Latorre et al. (unpublished) can also be achieved in forage grasses as demonstrated by the transfer of Festuca genes into Lolium.

In Argentina, the Flooding Pampa, is a component of the biological area that is called Pampa Argentina and occupies six million hectares in the Province of Buenos Aires. Within this important agricultural region the "Saline River" (Rio del Salado) has a big influence on the soil-hydrology and as a regulator of various natural factors (Latorre, pers comm.). Salt-tolerant genotypes of Lolium and natural Festulolium hybrids have been identified recently (Latorre et al., 2007) as potential sources of novel genes for salinity tolerance which could if required be used in *Lolium* crop improvement programmes.

(vi) Improved tolerance of flooding

Whilst water deficit is referred to frequently as an outcome of climate change, episodes of intense rainfall leading to flooding now occur more regularly in many countries and are likely to increase in frequency bringing other major challenges to national economies. Heavily populated industrial nations with land increasingly encased by impermeable surfaces incapable of water absorption will only exacerbate instances of urban flooding. In addition, prolonged summer droughts due to climate change will increase soil compaction and as a consequence will later increase opportunities for surface flooding. Soil compaction decreases yields of grassland species and cultivars. In addition, it is likely that in a number of temperate regions the change in rainfall distribution through the year will have a significant impact on the ability of animals to over-winter outside or undertake extended grazing in the spring and autumn increasing the risk of poaching and damage to pasture and soil.

Detailed studies have illustrated the importance of rooting depth and the vertical variability of root function on soil water uptake. They have also highlighted that the porosity of soil should not be considered a fixed parameter, but is actually under the influence of the vegetation (Macleod *et al.,* 2007). Recently there have been a number of laboratory studies published which describe how roots do change soil hydraulic properties (Whalley *et al*., 2005; Macleod *et al*., 2007). The studies have demonstrated a change to the water release characteristics which tend to be associated with an increased number of larger pores in the rhizosphere and an increase in water repellence. This follows from the observation that root activity tends to increase the number of large pores at the root-soil interface. The generation of soil structure by roots has been widely reported in the literature and there is evidence that this phenomenon is influenced by plant species (Materechera *et al*., 1994).

Recent developments that have led to grasses with improved and more extensive rooting systems designed initially for better soil water uptake during periods of water deficit (e.g. as in Humphreys *et al.,* 2005; Humphreys and Thomas, 1993), may also contribute to improved soil porosity and thereby improve soil water holding capacity during times when water is in surplus and soils saturated with potential to flood (Macleod *et al*., 2007). These research programmes describe transfer of traits from *Festuca* species into *Lolium*. In pilot studies at Lancaster University an increase of >1% soil porosity was found in the top 10cm from soil cores with three months growth of root systems of *Festuca arundincea* compared with equivalent soil cores containing *Lolium multiflorum* root systems (Papadopoulos and Binley, pers. comm.)

Although water stress is the most intensively-researched physical stress to root growth, field data show that it may not always be the most critical. Various physical stresses may act in combination to limit root elongation. Hypoxia, water stress and mechanical impedance to root growth will change with the water content of the soil and their relative importance will depend upon the degree of soil compaction.

Genes for the capability to penetrate compact soils have been identified in rice. The close genetic conservation of gene order between related species between ryegrass and rice should aid targeting and transfer of fescue genes for penetration of compact soils into ryegrass, and pilot studies at IGER have already identified putative QTL for root development on ryegrass chromosome 3 (= rice chromosome 1) (Humphreys *et al.,* 2006). Rice chromosome 1 is known to carry QTL for a number of important rooting characters (root length and soil-penetration) and for drought resistance (Zhi-Kang *et al*., 2005; Zhang *et al.,* 2001).

In many crops, including *Lolium,* waterlogging due to flooding will induce development of aerenchyma. This helps to alleviate hypoxic effects through cell collapse and development of channels for air conductance between the shoots and roots. An important adaptation to hypoxic conditions requires a metabolic adjustment towards alcoholic fermentation. The two principal enzymes involved are alcohol pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH).

Until recently crop improvement programmes aimed at improved flood tolerance for grass and legume species have not been viewed as a priority. However, efforts to isolate genes governing flood tolerance have been reported in other crop species such as soybean (Van Toai *et al*., 2001) and rice (Xu and Mackill, 1996; Nandi *et al*.,1997; Xu *et al.,* 2000; Siangliw *et al*., 2003; and Toojinda *et al.,* 2003). Genes for flooding-tolerance were reviewed by Vanavichit *et al*. (2005).

One option for grass and legume breeding is to seek genes for flood tolerance from related species or ecotypes adapted to flood conditions for use in crop improvement programmes. Whilst synthetic *Lolium x Festuca* species hybrids are being created at IGER and elsewhere in Europe (Canter *et al*., 1999; Zwierzykowski *et al.,* 1998) with the objective of increasing grassland sustainability, nature has already taken a hand in the form of the little studied natural hybrid of *Lolium perenne* and *Festuca pratensis c*alled *Festulolium loliaceum.* The natural hybrid species is found especially in mature water and flood meadows where they concentrate on the areas of most waterlogged and anaerobic soils. Despite their widespread occurrence in old permanent grasslands, little is known of the origins, age, and genetic composition of *Festulolium loliaceum*, but the species may serve as a new source of genes for flood-tolerance for use in *Lolium* crop improvement programmes.

The hybrids had very low fertility but backcrosses involving both diploid and triploid hybrids yielded a few progeny and intercross with their parental or related species (Gymer and Whittington, 1975; Harper (unpublished results). As the parental species *L. perenne* and *F. pratensis* are diploids it was considered highly likely that the triploid hybrids resulted from unreduced gametes produced by one or both the parent species (Essad, 1966).

With the confirmation that *F. loliaceum* is fertile comes the opportunity for introgression of genes for improved submergence tolerance in *Lolium* and is now a target for crop improvement programmes at IGER. Synteny with rice should provide candidate QTL and genes for selection and transfer from *F. loliaceum* into *L. perenne.*

(vii) Selection for enhanced tolerance to elevated ground level ozone

During the last two decades, damage to crops by ground level (tropospheric) ozone has become an issue of increasing concern leading to ongoing international effort to quantify the impacts of ozone on vegetation across Europe. "Critical levels" of ozone, above which adverse effects on crops (e.g. yield reduction, visible injury) occur, have been established and are used to map those areas of Europe where the critical levels have been exceeded in previous years, or are predicted to exceed in future years, and ozone injury or reductions in yield can be expected.

Ozone episodes often last for a few days, and concentrations are generally highest in rural areas that are downwind of major cities, industrial areas and areas with high traffic density. Ambient ozone has long been established to cause visual injury to sensitive species (e.g. Ashmore *et al.,* 1980) and if warmer summers become more frequent, it is to be expected that the frequency of episodes which can cause visible ozone damage to sensitive species will increase. Forage legumes such as white clover, red clover and *Lotus corniculatus* are particularly sensitive to ozone (Warwick and Taylor, 1995; Bungener *et al*., 1999), and grassland containing a high content of these species is potentially sensitive to ozone. There is also good evidence that ozone sensitive material exhibits greater sensitivity to water deficit and consequently the physiological effect of ozone and relationship between ozone and other traits has been the subject of extensive research (Bernejo *et al*., (2006).

If periods of elevated ozone are predicted to increase, selection of improved forage varieties with greater ability to tolerate elevated ozone will be important. Although there has been considerable research to quantify variation in response to ozone between temperate forage species, research to quantify genotypic variation to ozone is limited. This is in contrast to other species eg. tree species where a number of studies to quantify genotypic variation in response to ozone levels have been undertaken (Paludan-Muller *et al*., 1999). There are no published studies that have quantified genotypic variation in response to ozone within temperate grasses. In white clover intra-specific variation in ozone-response is well established in commercial varieties, largely due to the fact that the differential sensitivity within *T. repens* cv Regal is exploited as a biomonitoring system (Heagle *et al.*, 1995). Recent work has shown genotypic variation in response to ozone extends to elite UK adapted varieties and also hybrids between white clover and related species (Marshall, unpublished data) which could be used to develop varieties with greater tolerance to elevated ozone. Studies to quantify genotypic variation to elevated ozone of a number of forage species are therefore required to develop improved varieties and tolerant species mixtures. In tropical forage systems there is no published information on selection for increased tolerance of tropospheric ozone in either grasses or legumes.

(viii) Maintaining or increasing nutrient use efficiency

A number of significant impacts of global climate change on nutrient availability and plant demand are anticipated in grasslands across the world. Predicted outcomes from the interactions between elevated temperatures and CO_2, changing patterns of precipitation and multiple nutrient dynamics range from positive to negative with respect to the productivity and species composition of pastures (e.g. Grünzweig and Körner, 2003). However, many of these interactions are complex and remain difficult to quantify using current models (Lynch and St Clair, 2004). Little is known, for example, about the impact of temperature on plant-nutrient interactions in changing environments. Nevertheless, the balance of evidence suggests that nutritional limitations on global grassland productivity are likely to become more pervasive both spatially and temporally, all the more so if the potential increases in net primary productivity arising from elevated CO_2 levels are factored in. Furthermore, changes in rainfall patterns (i.e. episodes of drought and storm events) are likely to intensify removal of nutrients from soil profiles and increase the frequency of episodes during which nutrient availability and crop demand are significantly and deleteriously imbalanced.

Whilst inherent nutrient use efficiencies may increase under elevated CO_2 (Drake *et al.*, 1997), potential gains in net primary productivity may be limited by nutrient availability and/or uptake (see Menge and Field, 2007), both of which may be affected in contradictory ways, depending on the climate variable considered. The reduction in transpiration by C_3 plants under elevated CO_2 may reduce transpiration-driven mass flow of (mobile) nutrients such as Ca to roots. On the other hand the increased C allocation to below ground processes, including root biomass (e.g. Rogers et al., 1996), may have a positive impact on productivity depending on nutrient availability. The poor understanding of how many aspects of root morphology and function, including root architecture and exudation, are likely to respond to climate change means that the identification and selection of adaptive traits remains difficult. Furthermore, traits conferring adaptation to one stress may incur negative 'trade- offs' with respect to other stresses (Lynch and St Clair, 2004). This is particularly so for root adaptations. For example, low P induces alterations in root architecture that enhance top soil foraging (Lynch and Brown, 2001), but may consequently result in greater susceptibility to drought stress (Ho *et al.,* 2004). Likewise, increased specific root length may result in decreased root life-span (Eissenstat, 1997).

The assumption that increased nutrient input will be necessary to sustain the increases in net primary productivity (NPP) predicted for many temperate grassland systems under elevated CO_2 (Daepp *et al.*, 2001; Lüscher *et al.*, 2005; Morgan, 2005) argues for targeting macronutrient use efficiencies (i.e. N, P, K) in future adaptive breeding programmes, with additional economic and environmental benefits accruing from limiting further increases in fertilizer use. Greater reliance upon grass-legume mixtures clearly offers an alternative approach to increasing the N status of pastures. In temperate systems, a growing body of evidence suggests that elevated CO_2 appears to favour the legume component, at least in the case of white clover/perennial ryegrass mixtures (Hartwig *et al.,* 2002). Unfortunately, little comparable evidence is available for other legume/grass systems, although the importance of N_2 fixation in determining NPP of lucerne (*Medicago sativa*. L.) under elevated CO_2 has been demonstrated (Lüscher *et al.*, 2000).

Many of the world's ecosystems are characterized either by sub-optimal nutrient availability, ion toxicities, or both (Lynch and St Clair, 2004) and the majority of world agriculture is conducted with low fertility inputs on soils with poor availability of P and other nutrients. Xiao *et al*. (2007) concluded that NPP of temperate grasslands of northern China is primarily limited by nutrient as opposed and water availability. Globally, large areas of low input grassland, in particular, are currently or potentially phosphorus-deficient. Consequently, the extent of any adaptive increases in the legume content of swards in response to elevated CO_2, and hence increased N inputs to the sward, may depend on the availability of P (Grünzweig and Körner, 2003). In the case of white clover, P limitation significantly reduces its response to elevated CO_2 under growth room conditions and in micro-swards (Stöcklin *et al*., 1998; Almeida *et al.*, 1999). Taken in conjunction with the economic and environmental pressures to reduce P fertilizer consumption, not least because of the finite nature of P fertilizer reserves (USGS, 2002; Orris and Chernoff, 2002), this underlines the adaptive importance of breeding for enhanced phosphorous use efficiency (acquisition, utilization and retention components) in both legume and grass species.

Prioritization of breeding objectives is complicated by the gaps in our understanding of the range and magnitude of nutritional adaptations likely to be required in grassland systems as the impacts of climate change become more acute,. However, there are reasonable grounds for focusing on (a) optimizing nutrient availability and nutrient use efficiencies under increasingly variable environmental conditions, in order to realize and sustain, where feasible, the potential gains in productivity under elevated temperatures and $CO_{2,}$ and (b) minimizing the impact of current and emerging mineral deficiencies. Whilst plenty of genetic variation for adaptation to mineral stress is available, its characterization and exploitation will require development of appropriate screens for evaluating performance under repeated episodes of multiple environmental stresses. The facility for rapid appraisal of rooting traits, under quantitative genetic control, will also be of central importance, as will novel approaches for accommodating, or circumventing, 'trade-offs' in adaptations to different edaphic stresses (see Lynch and St Clair, 2004). Selection criteria meeting these two breeding objectives include:

a) Enhanced nutrient use efficiencies, particularly N and P, in terms of all three major components: acquisition (i.e. both higher V_{max} lower K_m values), utilization and retention efficiencies (e.g. Garnier and Aronson, 1998), with the former aimed at enhancing competition for nutrients with the soil microbial biomass and the latter at increasing remobilization of nutrients from senescent tissue.

b) Higher N_2 fixation by annual and perennial legume species under conditions of mineral (i.e. P) and water stress.

c) Deeper rooting systems to increase capture of nutrient leached down the profile and increased uptake of nutrients and water from subsoil under drought conditions. Consideration should be given to transferring rooting traits associated with perennial species into annual species (e.g. Roumet *et al*., 2006).

d) Proliferation and turnover of fine roots; this is relevant to nutrient capture, particularly of P, as well as to C sequestration (Strand *et al.,* 2008).

e) Compositional stability of species and cultivars in legume-grass mixtures in order to sustain and, where possible, increase N fertility and soil quality; hence assisting in supporting potential net primary productivity gains under elevated CO_2.

f) Preferential uptake of ammonium over nitrate, particularly at low soil temperatures when ground is likely to be saturated. Developed in conjunction with greater use of ammonium-based N fertilisers (Dobbie and Smith, 2003) this will assist in countering impacts of high rainfall events on nitrate leaching and generation of N_2O by denitrification.

In the context of maintaining N fertility, Nichols *et al*. (2007) have called for greater efforts to improve annual tropical legumes to complement species such as lablab (*Lablab purpureus* L.) and cowpea (*Vigna unguiculate* L.). Historically, well-adapted tropical legumes for cropped soils have been unavailable and were perceived as unnecessary for maintaining grain yield, and because animal production was not as profitable as grain production (Pengelly and Conway, 2001). However, this is likely to change due to increasing agricultural commodity prices and demand for fertilizer N inputs.

(ix) Maintenance and improvement of forage quality

Whilst the effects of climatic variables on forage quality (e.g. Raymond, 1969; Seligman and Sinclair, 1995; Polley *et al.*, 2000; Fales, 2007) and the role of genetic improvement (e.g. Hanna, 1993; Vogel and Sleper, 1994; Casler, 2001; 2006) have been well documented, the merits of targeting adaptation to climate change in forage quality breeding programmes have received little attention. There are various reasons for this. Firstly, explicit breeding for improved forage quality is a relatively new objective (Cherney and Hall, 2007; Fales, 2007). Until recently very few cultivars possessing improved forage quality, with notable exceptions such as the high water soluble carbohydrate cultivars of *L. perenne* had been fully evaluated in terms of improved animal performance (Casler, 2006). Secondly, the main factors affecting quality are (Cherney and Hall, 2007), in decreasing order of importance: (1) maturity, (2) crop species, (3) harvest and storage, (4) environment (climate), (5) soil fertility, and (6) variety (cultivar); this suggests relatively little incentive to pursue breeding rather than management strategies (i.e. manipulating plant development) to improve forage quality. Thirdly, the predicted impacts of climate change on quality are complex, including positive, neutral and negative responses to elevated CO_2 (Polley *et al.,* 2000), with different aspects of quality influenced differently, and not uniformly so across different regions of the globe (Seligman and Sinclair, 1995). Fourthly, identification of appropriate 'adaptation' traits and selection criteria is not straightforward, in so far as the three main components of forage quality - digestibility, intake potential and energetic efficiency (Raymond, 1969) – cannot strictly be defined in the absence of the animal and are therefore not plant traits (Casler, 2006), requiring, instead, evaluation as proxy traits like *in vitro* dry matter digestibility (IVDMD), acid detergent lignin (ADL) and neutral detergent fibre (NDF). Further, most measures of forage quality are influenced by growth stage/ reproductive maturity and by the relative plant part composition (i.e. leaf: stem ratio) at time of sampling (Casler, 2006), leading the confounding of intrinsic differences in quality with those attributable to differences in maturity.

The high genotypic heterogeneity present within populations of grass species has been interpreted as indicating little selection pressure has been applied for quality traits during their evolution and domestication (Fales, 2007) and a number of studies have suggested that QTL-marker associations are highly population specific and cannot be generalized across populations (Casler 2001). Transgenic technology offers the means of creating additional genetic variability for certain traits, and attention so far has focused on down-regulating the lignin biosynthesis pathway and upregulating protein synthesis (Casler, 2006).

The combined effects of elevated CO_2 and temperature on forage quality are likely to be complex, ranging from positive, neutral to negative impacts, depending upon, for example, the accompanying changes in rainfall patterns (Seligman and Sinclair, 1995; Polley *et al.,* 2000). Interestingly, results from Free Air Carbon Dioxide Enrichment (FACE) experiments in New Zealand showed digestibility of seven species including C_3 and C_4 grasses and legumes to be unaffected by elevated CO_2 (Allard *et al.*, 2003). Nevertheless, a number of general trends in forage quality under elevated CO_2 are discernible. Negative effects are likely to include (a) reductions in crude protein content (hence in digestibility), given the observed declines in plant N concentrations, increased concentrations of total non-structural carbohydrates and C:N ratios of litter (Owensby *et al.*, 1994; Soussana *et al.,* 1996; Cotrufo *et al.*, 1998; Körner, 2000), particularly under conditions of low soil N availability (Bowler and Press, 1996); (b) increased fibre content (hence reduced intake), and (c) increases in a range of plant secondary compounds with toxic and/or anti-nutritional properties (i.e. phyto-oestrogens, cyanogenic glycosides, coumarin, hypercin, condensed tannins). Positive effects may include (a) increased non-structural carbohydrates at elevated CO_2 (Read *et al.*, 1997) and (b) increases in crude protein levels under conditions of reduced rainfall. Impacts of elevated temperatures *per se* are difficult to assess due to inevitable confounding of effects with those of altered plant ontogeny and maturation (Fales, 2007). However, higher ambient temperatures during growth are associated with decreased digestibility, attributable to higher concentrations of cell wall components and lignin (Ford *et al.*, 1979; Cherney and Hall, 2007). The increasing frequency and severity of drought predicted for many regions may have less impact on forage quality than on growth and development, primarily because it delays maturity. According to Fales (2007), the compositional trait exhibiting greatest sensitivity to water stress is NDF which decreases due to reduction in amount of fixed C incorporated into cell walls. A study of ryegrass and orchardgrass cultivars (Jensen *et al.*, 2003) indicated the primary effect of water stress was to increase nutritional value by increasing concentrations of crude protein and digestible NDF. At the other end of the spectrum, the increasing frequency of storm events, rain damage and climate induced delays in harvesting may result in greater maturity and hence lower quality (Cherney and Hall, 2007).

Climate also exerts a major influence on forage quality through its effects on species distribution and abundance (Woodward, 1987). Wide differences in quality occur between grasses and legumes, and between cool- and warm-season grasses (Fales, 2007), due primarily to variation in total fibre and lignin, the primary inhibitor of fibrer digestion (Moore and Jung, 2001). Grasses contain higher fibre concentrations and lower concentrations of readily fermentable cell solubles compared with legumes. Similarly, warm-season grasses are generally less digestible than cool-season grasses at comparable growth stages, their higher fibre levels partly due to lower leaf:stem ratios (Fales, 2007). Consequently, shifts in the composition of pastures caused by climate change may have significant implications for quality, both positively and negatively, depending upon region and predominant environmental factor. For example, under conditions of increased temperature, where adapted C_4 grasses are favoured over C_3 grasses (Baron and Bélanger, 2007), there is likely to be a reduction in quality (Barbehenn *et* al., 2004). However, both warming and elevated CO_2 increase clover content of temperate mixed swards (Lüscher *et* al., 2005). Evidence of adaptive increases in the legume component of mixed grass-clover pastures compensating for a decline in forage quality at the sward level is provided by Schenk *et al.*, (1997), where CO_2 enrichment initially reduced the crude protein content of both species, but eventually increased the crude protein content on a whole sward level as the content of clover increased.

Compositional traits may be categorized into those associated with characteristics of individual plants and those associated with composition at the sward level (i.e. affecting species composition of pasture). Amongst the former, digestibility is perhaps the most approachable target in that it is a repeatable and heritable trait, as measured in terms of genetic variation in IVDMD (Casler, 2006), and its improvement is highly geared to changes in profitability of livestock enterprises (Vogel and Sleper, 1994). Selection criteria including stability in ratios of soluble carbohydrate:structural carbohydrate concentrations, leaf:stem and in lignin contents under fluctuating environmental conditions are worth evaluating in the context of maintaining overall digestibility. Lignin, measured as acid detergent lignin (ADL) has accounted for up to 80% of variation in IVDMD in some studies (Casler, 2001). However, breeding explicitly for lower lignin contents as an adaptive measure may have negative knock-on effects on traits such as yield and drought tolerance, particularly if realized through increases in leaf:stem ratios (Clark and Wilson, 1993).

According to Casler (2006), intake has a greater impact on animal performance than digestibility, but cannot be maximized for most forage diets due to limitations in feed quality (Van Soest, 1994). Intake is limited by rumen fill which in turn is limited by digestibility of the feed, with fibrous bulk, (i.e. plant cell walls) generally considered to be the main factor. Selectable traits include NDF, providing a direct measure of fibrous bulk, and alkaloid (plus other anti-nutritional compounds) content, affecting intake through reducing palatability (Casler, 2006).

With respect to quality at the sward level, selection for increased resilience and competitive fitness in the legume components of mixtures, notwithstanding the likelihood that elevated CO_2 may act in concert, should be beneficial in terms of protein and fibre content. Selection for lower fibre content in warm-season grasses, although not necessarily via higher leaf: stem ratios, might also be construed as adaptive for regions where these species are likely to displace cool-season grasses.

(x) The potential of introduced species or ecotypes

The introduction of new species offers a valuable alternative and/or complementary approach to increasing the adaptive capacity of existing species and cultivars through selective breeding, provided the appropriate ‘duty of care’ obligations (see Revell and Revell, 2007) are met. The need to develop more robust pastures, in terms of stable long term performance, buffered against greater variation in growing season, timing and intensity of rainfall, soil fertility, pests, diseases and management decisions associated with climate change and other emerging environmental threats has been highlighted (i.e. Francis, 1999; Nichols et al. (2007). Nichols et al. (2007) advocate a strategy of increasing biodiversity, citing observations that well-managed native pastures in the Mediterranean basin have a higher diversity of annual legumes (Cocks and Bennett, 1999). This approach has been implemented in pasture legume breeding programmes in Australia, where the drivers - reflecting a number of problems likely to be exacerbated by climate change, both in temperate and tropical regions - include poor adaptation to (1) water-logged and saline soils, (2) ‘false breaks’, and (3) longer growing seasons; together with the need for deeper rooting plants to reduce groundwater recharge and potential for dry-land salinity and greater annual legume diversity to stabilize productivity within and between seasons. In order to fill a larger proportion of these problematic agro-ecological niches, a generation of new annual legume species possessing a suite of 13 desirable characteristics, including traits incorporated in legumes of Mediterranean basin origin, are being developed (Loi et al., 2005), complementing/replacing current temperate ley-farming systems based on subterranean clover and annual Medicago species. The genetic resources waiting to be exploited in this kind of approach are potentially huge. For example, over 100 grass, legume, herb and shrub species have been identified as high priority research targets for salt-affected areas (Rogers *et al*., 2005), most of which are yet to be domesticated.

C. New opportunities and potential targets for future multifunctional grassland design

Whilst very substantial progress has been made over recent years in terms of identifying and understanding the adaptive pressures facing grasslands across temperate and semiarid regions, the same cannot be said for tropical grasslands. Equally important is the provision of more cost-benefit information, especially with respect to the outcomes of multiple interactions and the agronomic consequences of significant adaptive trade-offs, if informed decisions are to be made regarding intervention, including: (i) whether attempts should be made to maintain, increase or accept declines in the productivity of grassland systems in different climatic zones, and (ii) whether to pursue adaptive breeding strategies with current economically important forage species, or to introduce new species with greater inherent adaptive fitness. Decision-making in the latter case, together with subsequent ‘cultivar design’, may be assisted by the application of multidimensional stress matrices (Nichols *et al.*, 2007) to identify and prioritize the problematic agroecological niches to be filled.

Numerous uncertainties surround the question of how much genetic variation is desirable in pastures (both intra- and inter-specific) in order to achieve and sustain the optimal economic balance between adaptation and productivity. This has a significant bearing on (i) the genetic variation to be included in future cultivars, with implications for statutory evaluation procedures in terms of uniformity, (ii) the development of mixtures of complementary cultivars, with each cultivar differentially adapted to environmental stress, as a means of improving the overall pasture resilience, and (iii) commercial lifetime of varieties (before they lose fitness) and hence frequency of re-sowing.

In mixtures and more complex ecosystems, a wide variety of factors may be important in affecting responses to changing climate. For example, differential responses to altered water availability may be important determinants of changes in species composition, as suggested by Swemmer *et al.* (2006), with respect to the mesic grasslands of the central plains of North America. McCulley *et al*. (2007) suggested that soil respiration may be water limited and this sensitivity increases with increasing woody plant abundance.

A major tool of these studies has been free-air carbon dioxide enrichment (FACE), the use of which was reviewed by McLeod and Long (1999). This complexity was underlined by Duckworth *et al*. (2000) in a study of calcareous grasslands of Atlantic Europe. Subtle shifts were seen from a 2 degree warming due to interaction of soils and management of multiple species. Thornley and Cannell (1997) stressed the importance of grazing in affecting response to climate change and also the site specific nature of such impacts. Harmens *et al.* (2004) also showed that defoliation, in this case cutting frequency, could play a major role in determining responses. Baruch and Jackson (2005) showed that in a tropical situation also changes in the competitive balance between grasses in response to elevated carbon dioxide and water stress was likely. In this case the introduced African grasses *Hyparrhenia ruta* and *Melinis minutiflora* are likely to gain an enhanced advantage over the native grass of South American savannas *Trachypogon plumosus*. Swemmer *et al*. (2007) studied three perennial grasslands of southern African temperate areas and showed that whilst precipitation patterns were important a range of other factors, some of them local, also affected ecosystem function.

The review by Luscher *et al*. (2005) also emphasized the difference between the certainty with which we can predict the physiological responses of individual plants and the difficulty of predicting responses at the community level. Zavaleta *et al*. (2003) described changes in the biodiversity of Californian grassland with forb diversity particularly affected by climate change. Higher carbon dioxide and N deposition reduced biodiversity, elevated precipitation increased it and warming alone showed no change. Increased carbon dioxide may drive the incursion of woody plants into grasslands since they are more responsive to carbon dioxide and may be more effective at reaching deep soil water. Morgan *et al*. (2007) presented some experimental evidence for this from the Colorado short grass steppe. The wider impacts on biodiversity may also be difficult to predict. For example Morecroft *et al*. (2002) showed that impacts on insect populations may also be difficult to predict e.g. the extent to which drought would lead to gap colonization.

The likelihood of greater seasonal and annual variation in climate will require greater emphasis to be placed upon minimizing the susceptibility of new cultivars to environmentally induced ‘tipping points’ that precipitate subsequent catastrophic decline in their abundance in swards.

A key consideration for the future will be the harnessing of the power of genomics to the adaptation of crops to climate change. Edmeades *et al*, (2004) reviewed the linking of physiology and genomics in the context of crop improvement and Ishitani *et al* (2004) gave an example of the integration of genomics with classical breeding in the development of aluminium tolerance in *Brachiaria* and drought tolerance in common bean. Such approaches to crop improvement, combined with appropriate phenotypic analysis of key traits will be essential for the development of improved forage cultivars that will enable grasslands to adapt to climate change.

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