

## REVIEW PAPER

# Prospects for crop production under drought: research priorities and future directions

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## Abstract

The efficient use of water supplies requires a systems approach that encompasses all aspects of making water available and its use within society that must recognise global issues. Increasing the efficiency of water use within agricultural systems is an essential priority in many regions including the Mediterranean. This review examines the research priorities, the prospects for crop and soil management and plant breeding and biotechnology that are needed to achieve high stable yield under drought in the Mediterranean. Research must combine the latest genomics resources including quantitative genetics, genomics and biomathematics with an ecophysiological understanding of the interactions between crop plant genotypes and the growing environment to better inform crop improvement.

## Introduction

Water is essential to sustaining human health and environmental quality. Water is in extremely short supply in up to 10 eastern and southern Mediterranean countries (<http://www.mideastnews.com/WaterWars.htm>; <http://www.fao.org/waicent/faoinfo/agricult/agl/aglw/aquastat/neareast.html>; <http://www.nature.com/nsu/000127/000127-11.html>). Although Mediterranean climates, characterised by hot, dry summers and cold, wet winters (when 85% of annual rainfall occurs) are notoriously variable, severe drought is clearly a major problem. Drought may affect entire countries over several years and result in serious social, economic and environmental costs. This situation is compounded by the predicted change in climate with increased temperatures and decreased precipitation as a result of global warming (Mannion, 1995).

Sustainable social and economic development and stability require the efficient and equitable management of water resources. While most aspects of the development of clear water policies and strategic management plans are a national responsibility, there is considerable advantage in developing these for the whole Mediterranean basin,

although the large number of countries involved makes this difficult. However, the Mediterranean Network of Basin Organisations is trying to improve the communication between organisations related to water policy and management (<http://www.remoc.org/DesktopDefault.aspx>). An integrated policy is essential not only to increase the water resources capacity and development of appropriate decision support systems for efficient use but also to ensure the implementation of best practice across the entire region and maintain biodiversity (Firbank, 2005). The 'European Declaration for a New Water Culture' is a foresight document on sustainable water management addressed to the European Commission, European Parliament and National Governments to lobby them to accept and promote such ideals. It is vital that politicians commit to such initiatives.

These conflicting pressures can be resolved by increasing the efficiency of water use within agricultural systems, but this requires an understanding of the interactions between society, agriculture, environment and management (Araus, 2004; Turner, 2004*a,b*). The actual water consumption by the various users is not clear, and research is needed to determine this. Agriculture consumes

large amounts of water for irrigation, so it is important that the analysis fully considers returns from irrigation. The efficient use of water supplies requires an integrated systems approach that embraces all aspects of water supply and utilisation within society and must address conflicts regarding shared international water sources.

In addition, appropriate governmental policies are essential to decrease water use and improve the efficiency of water use. While the use of water meters, charges and taxes together with incentives and penalties are all potential tools to achieve this investment in infrastructure, education and extension activities are also essential Abu-Zeid (2001).

However, in this review, we briefly discuss the prospects, research priorities, both strategic and applied, and future directions for achieving high stable yield under drought (HSYD) to underpin sustainable agricultural production in the Mediterranean basin. HSYD can be achieved either through better crop and soil management or selection of appropriate crops with intrinsically large water-use efficiency and through breeding more efficient genotypes of plants.

### Crop and soil management

The effective management of the available water resource through agronomic practice can play a major role in sustainable production under drought (Peterson & Westfall, 2004). While the traditional systems employed in the Mediterranean countries can give important insights into sustainable production under drought, the most advanced agronomic systems have been developed in Israel, Spain, the USA and Australia and are comprehensively reviewed in <http://www.plantstress.com/Articles/index.asp>

The majority of agricultural production in the Mediterranean region is rain fed (e.g. 60% of the major cereal production) and vital to the water and food security. Rain-fed agriculture is generally characterised by low and uncertain rainfall and crops of limited production potential that are, however, generally more stable in such environments. Despite its scarcity, available rainfall is often poorly managed and much water is lost as run-off and evaporation. In these regions, the collection and efficient use of rainwater is crucial (Araus, 2004). Adoption of simple water-harvesting systems and better agronomic practices can increase water availability. Installation of the simplest water-harvesting systems (e.g. reservoirs) could provide access to water at critical times and minimal irrigation at key growth stages can dramatically improve yield (Oweis *et al.*, 1998; Araus *et al.*, 2002).

### Soil management

The adoption of appropriate sustainable agronomic practices that conserve water, soil and nutrients is essential (Evans, 2005). The detailed practices required are dependant on both the local climate and the soil type. Crop residues are an important resource as soil that is covered evenly with crop residues is not only protected from wind and water erosion but the residue also slows moisture loss during the growing season and ensures that water moves into soil soon after rainfall and will not pond on the surface. In addition, crop residues can decrease disease in subsequent but different crops (Saucke & Doring, 2004). Over time, the organic matter in the crop residue becomes incorporated into the soil, improving soil structure and thereby its manageability. A soil structure with a proper mix of large and small pores favours root growth and the organic matter helps hold moisture and helps to cycle nutrients (Rao & Dao, 1996). Occasionally, the presence of the crop residue can decrease yield, with the yield reduction variously attributed to pests, disease or weeds (Rao & Dao, 1996; Rasmussen *et al.*, 1997). However, the potential benefits of maintaining crop residues appear to greatly outweigh the occasional short-term disadvantages. More research with long-term experiments is needed to demonstrate sustainability of maintaining crop residues.

Tillage practices to conserve water must be adopted; this must take into account local factors including soil texture and drainage characteristics. In general, no tillage with direct drilling into stubble is well suited to many grain crops and reduces wind and water erosion of soil, and in particular maintains a dry mulch, which slows evaporation from the soil surface, and thus retains soil moisture to support crop growth. It also improves soil structure and fertility (Holland, 2004), although it may, in the first seasons, lead to a decrease in yield through decreased N availability (Izumi *et al.*, 2004). No tillage has frequently been shown to improve both seedling establishment and root growth (Klein *et al.*, 2002; Ghosheh & Al-Hajaj, 2004). Studies of minimal tillage under Mediterranean conditions are few and limited. Further research is needed to verify the longer term agronomic, environmental and social benefits of minimum tillage in this region (Holland, 2004).

In hilly areas, contour ploughing is beneficial and creates 'mini terraces' that prevent erosion by slowing or stopping the run-off of rainwater and thereby encouraging percolation into the soil. The same approach on a grander scale, the earth and stone terraces, characteristic of many Mediterranean regions is also a highly effective way of stabilising the soil, minimising run-off and

increasing percolation. This increases the soil moisture available for crop growth.

### Crop management

Water use can be further improved by changing cropping patterns. The choice of crop to be grown is critical and should reflect both the availability of water and socio-economic requirements. Water requirements can be greatly reduced by replacing a crop requiring substantial amounts of water, like cotton, with crops requiring less water, for example, grapevines. Similarly, utilisation of the best-adapted cultivars is important in maximising yield under water stress. Even simple adjustments such as cultivars that can be planted earlier to more closely match crop growth to rainfall distribution can increase productivity (Turner, 2004*a,b*). There may be a compromise between yield and water use as more rapidly maturing varieties may have smaller yield potential. However, breeding combined with agronomy in an optimised system may increase efficiency and productivity. Even if breeding for drought tolerance lowers the yield potential of the crop, it may increase yield stability over seasons, which could still benefit many farmers in drought-prone areas (Araus *et al.*, 2003). However, in many cases, newer improved varieties are accepted slowly by farmers.

Often monoculture rather than polyculture accelerates soil erosion and decreases soil organic matter that further impairs both soil fertility and the capacity of soils for water retention. This can lead to the further depletion of land available for crop production. Crop rotation has major advantages over monocultures and increases the yields from a crop grown as part of a rotation compared to continuous cropping (Pierce & Rice, 1988; Christen & Sieling, 1993; Ghosheh & Al-Hajaj, 2004). Crop rotation reduces the population of pests specific to a crop, and it reduces the risk of crop disease and improves weed control. All these decrease losses and/or requirements for fertilisers, herbicides, insecticides and fungicides. For example, under Mediterranean conditions, growing legumes provided nitrogen for nonlegume crops (Papastylianou, 1993*a,b*; López-Bellido *et al.*, 1996), alternating crops that successfully compete with weeds will reduce pressure on subsequent crops.

Strip farming, in which narrow strips of crops are always present, can also be beneficial, especially in protecting soil from erosion by wind and water. The value of cover crops for their use in reduction of wind and water erosion, and ability to improve soil structure, nutrients and moisture retention, is well established. In the short term, cover crops can increase costs but offer major long-term benefits.

Sociocultural factors may be a major obstacle to optimising sustainable production. For example, overcoming political instability will encourage individual farmers to plan for the future as will individual ownership of land (Abu-Zeid & Hamdy, 2002). Further research is needed to identify ways of moving improved varieties from breeders to their acceptance by farmers.

### Irrigation

Agricultural irrigation is often inefficient, with water losses exceeding 50% in many Middle Eastern countries. This inefficiency can be attributed to technical problems of distribution and on-farm application exacerbated by poor maintenance of irrigation structures. However, it must be remembered that much of the water lost may in fact be recovered by other users in the catchment basin. Rapid evapotranspiration is a major problem especially where spray/overhead irrigation or flood irrigation is used – drip irrigation is much more efficient. Evapotranspiration can also be decreased by scheduling irrigation at night.

Soils in the semiarid and arid regions are particularly vulnerable to soil degradation by erosion and salinisation (Ramoliya *et al.*, 2004). Unsuitable, overintensive agricultural practices have exacerbated this problem and can in the case of severe degradation lead to desertification. The least fertile zones are frequently the most fragile but have been cultivated because of population pressure. This can lead to the further depletion of land available for crop production. These problems are caused mainly by agricultural practices and often occur in areas where irrigation is used to farm infertile lands with sandy and rocky soils but where the water resource is limited. In Egypt, salinity may be responsible for a 30% decline in crop yields (Postel, 1993). Furthermore, the over-exploitation of aquifers, particularly in coastal regions, may lead to the intrusion of salt or brackish water, which reduces their use for irrigation and domestic consumption. This has occurred in the Lebanon (Khair *et al.*, 1994) as well as along the Almería coast (Spain). Mismanaged irrigation systems have led to the loss of some of the most fertile and productive soils in the region (Abu-Zeid & Hamdy, 2002) and more innovative approaches to remediation are essential (Lynch & Moffat, 2005). Agricultural practices, in particular the heavy use of fertilisers and pesticides, can also affect drainage and groundwater quality. This creates problems for domestic, tourism and industrial water supplies as well as impacting on the value of water for irrigation. Furthermore, the sale of pesticide-contaminated products is difficult or impossible, especially to high-value markets.

Optimisation of water use can be achieved by promoting water-efficient irrigation (precision irrigation), water-

efficient agriculture (precision agriculture) and water reuse in agriculture, particularly the recycling of drainage water and reuse of urban wastewater. The options for improving irrigation water productivity, technical, managerial, institutional and agronomic, have been comprehensively reviewed elsewhere (e.g. Postel, 1993; Abu-Zeid & Hamdy, 2002).

In deficit irrigation, limiting irrigation to critical growth stages can improve crop water-use efficiency (Oweis *et al.*, 1998; Araus *et al.*, 2002). The crop requirements for irrigation can be estimated from both soil and plant measurements. Plant-based direct measurements including relative water content, water potential, stomatal conductance, xylem abscisic acid content and xylem pH can all be used to effectively schedule irrigation (Lefi *et al.*, 2004*a,b*). However, newer indirect measurements made on the whole canopy (e.g. infrared thermometry, thermography or chlorophyll fluorescence) are related to transpiration, leaf cooling and stomatal conductance and may give a better measure of canopy water status than individual plant measurements and be more useful in scheduling irrigation than decisions based on individual plant measurements (Jones *et al.*, 2002; Cifre *et al.*, 2005). The development of biosensors, 'smart' plants that report their water physiological status, is a realistic and achievable goal (Day, 2005). However, many of these methods are labour intensive or require equipment and education that may not be available to small farmers who will rely on more empirical approaches. In addition, where water availability is limited, political and social decisions are critical because spreading the water too thinly decreases harvest index and yield. Therefore, for annual crops, focusing the available water supply to adequately supply a restricted area may give the greatest benefit. While this is also true for perennial crops, their long-term survival is a critical consideration in longer term sustainability.

A new irrigation technique where different sides of the root system are irrigated alternately (partial root-zone irrigation or partial root-zone drying) has sometimes improved both the water-use efficiency and the harvest index and yield of important Mediterranean crops, for example, grapevines (Santos *et al.*, 2003; de Souza *et al.*, 2003), orange trees (Loveys & Davies, 2004) and tomato (Davies *et al.*, 2000; Mingo *et al.*, 2003). In addition, in some cases the quality of the harvested product has also improved (Davies *et al.*, 2000; Kang & Zhang, 2004). Further analysis is however necessary to determine the general utility of this approach.

Many of the irrigation technologies and management strategies are already available for sustainable production (Bhattarai *et al.*, 2004). While many components are generic, the most successful solution will take into

account local components (climate, soil types, salt content, crop water requirements, etc.).

However, the adoption of such strategies by farmers is patchy. Further research into the socio-economic factors that hinder the adoption of new methodologies is essential and should be given high priority because failure to adopt best practices will endanger long-term sustainability. This should be linked to the development of multifaceted regional water management models that balance and address the needs of all parties in society (Araus, 2004).

### Plant breeding and biotechnology

Plant breeding aims to ensure that water is used more efficiently by increasing the water-use efficiency (yield of product/water consumed) of crops. Plant breeding has the potential to deliver HSYD but requires the identification of key traits and their incorporation into high-yielding varieties using conventional or biotechnological tools (Slafer, 2003; Reynolds *et al.*, 2005*a*). Conventional plant breeding so far only increased the yield of crops grown under drought at about half the rate achieved for crops grown in temperate regions (Turner, 2004*a,b*). Crop response to stress is dependant on numerous traits many of which are constitutive and expressed irrespective of water availability, but such constitutive traits may also be modified by stress. Directed breeding strategies must focus on the key traits important to performance under drought stress [e.g. phenology, rapid establishment, early vigour, root density and depths, low and high temperature tolerance, <sup>13</sup>C discrimination (a measure of the extent to which photosynthesis is maintained while stomatal conductance decreases), root conductance, osmoregulation, low stomatal conductance, leaf posture, habit, reflectance and duration, sugar accumulation in stems to support later growth of yield components]. Importantly, survival traits as opposed to production traits will only be relevant in perennial crops and some herbaceous annuals growing in the most extreme environments to ensure survival and should not be a major focus unless they have particular socio-economic value (Clary *et al.*, 2004). Survival traits are negatively correlated with productivity and do not provide HSYD (Mitra, 2001). Breeding programmes should focus on integrative traits that impact over a long duration of the cropping period. Some traits for higher yield may in fact decrease yield stability (e.g. longer growth period), whereas others are less likely to have this penalty (e.g. increased harvest index). Attention should focus on traits that will maintain or increase yield stability in addition to yield per se. Full consideration must be given to other traits that limit performance because overcoming losses due to

pests and diseases may offer the greatest return particularly in the short term (Nigam *et al.*, 2005).

In some crops (e.g. potato), natural biodiversity is underexploited (Gur & Zamir, 2004; Latha *et al.*, 2004), but in others (e.g. wheat), prolonged periods of inbreeding may have decreased the available biodiversity. For such major crops with limited variability, attempts to increase the genetic variability (methods for the rapid introgression of traits from diverse germplasm or wild relatives) are needed as well as methods for the generation of novel diversity such as chemical or radiation mutagenesis. One powerful method is TILLING (targeting-induced local lesions in genomes) that allows the generation of targeted mutations, allowing gene candidates discovered by genomics-based experimentation to be used to directly alter trait components (Henikoff & Comai, 2003; Till *et al.*, 2003*a,b*). Because these techniques do not involve the generation of transgenic plants, lines with improved characteristics can be used immediately by plant breeders and adopted by farmers with little regulatory impediment. When applied to elite cultivars, TILLING in particular appears to have great potential, and future research effort should be directed towards undertaking this in important Mediterranean crops. However, biotechnology may also play an important role, particularly in targeting transcription factors that determine gene expression. An oversimplistic appreciation of how growth, metabolism and development in plants are regulated in response to drought has led to unrealistic expectations for crop improvement. Drought tolerance is an extremely complex multigenetic trait. Plants have developed a variety of developmental and metabolic strategies for balancing the needs of growth and reproduction with resistance to diverse stresses (biotic, nutritional and abiotic). It is therefore vital to consider yield performance under drought in this wider context but difficult to identify and target selection criteria for specific drought-tolerance strategies that ensure higher and more stable yields. Attempts to improve crops on the basis of physiological trait analysis criteria have greatly improved our understanding of physiological processes but with few exceptions (e.g. carbon isotope discrimination) thus far contributed little to crop improvement (Araus *et al.*, 2003; Flexas *et al.*, 2004*a,b*; Forster *et al.*, 2004; Lefi *et al.*, 2004*a,b*; Slafer *et al.*, 2005). Nevertheless, there can be no doubt that in the longer term, an improved understanding of the mechanisms (both developmental and metabolic) behind HSYD and the efficient use of water by plants will be an essential prerequisite to crop improvement. Understanding the mechanisms controlling the growth of plants subjected to limited water is essential to achieving stable yield under drought. The identification of xylem pH as an

early signal of water availability together with the inhibition of growth by ethylene should be further examined and exploited in crop improvement programmes. These and other mechanisms have been extensively reviewed elsewhere (Chaves & Oliveira, 2004).

In recent years, the genetic characterisation of segregating populations from various species including maize, sorghum, rice, wheat and barley has enabled the identification and mapping of both individual genes and quantitative trait loci (QTLs) for yield components under drought stress (Hayward *et al.*, 1994; Lebreton *et al.*, 1995; Forster *et al.*, 2004; Lafitte *et al.*, 2004; Talamé *et al.*, 2004). QTLs have now been identified for phenology, plant architecture, plant development, abscisic acid accumulation, photosynthetic parameters, chlorophyll amount, water-use efficiency,  $^{13}\text{C}$  discrimination and water status (Forster *et al.*, 2004). While significant yield QTLs have been identified in various mapping populations, there is not yet strong evidence to show how robust these are when transferred into different genetic backgrounds and in particular elite lines or applied to drought experienced in different environments (Campos *et al.*, 2004). The testing in different environments is critical because QTLs associated with water use often show low heritability. This may reflect inherent variability or that properties that are critical in one drought environment may not be in another. Once a QTL has been unambiguously established, the identification of the genes underlying the QTL is a major step towards understanding the molecular basis for the response (Cattivell *et al.*, 2002). This information allows strategies to be developed that may alter the expression of the gene in the elite line (e.g. by tilling), use of transgenics or marker-assisted integration or even by altering agronomic practices. The transfer of the genes that govern traits to elite lines should not be a technological obstacle (Ye *et al.*, 2000). However, if very large numbers of genes must be introduced, controlling successfully their expression will pose new technical obstacles. Attempts to improve crops by marker-assisted selection for yield, performance under drought, as well as for other QTLs have yet to contribute significantly to crop improvement. However, it is probable that the importance of markers linked to QTLs for HSYD and phenotypic selection will increase in breeding programmes. The similarity in the organisation of genes found in different species, known as synteny (Dicks *et al.*, 2000 (<http://www.nrp.org.uk/enews/edpsyteny.htm>), may also allow knowledge to be transferred between crops. In addition, further advances in bioinformatics are needed to identify and develop linkages between different types of data available (Mayes *et al.*, 2005). However, this will require highly robust and well-tested biological models for the effects of drought to be developed.

Traditionally, the positional cloning of the genes underlying QTLs has been both time consuming and labour intensive. Further genetic analysis of drought tolerance is now feasible using genome-scale transcriptional profiles using microarrays based on plant genomic and cDNA sequences (Seki *et al.*, 2002; Watkinson *et al.*, 2003). These methods allow the identification of candidate genes that respond to drought in a range of environmental conditions and genetic contexts. These drought-responsive genes are candidates that may be involved in the processes producing QTLs (QTL analysis). The rapid mapping of these genes may also identify them as candidates for the specific gene or genes underlying the QTL itself. However, QTLs and molecular markers are not yet available for some crops of regional importance and further research is needed (also genomics resources are not available for many of these crops).

The performance of a genotype often varies widely in different environments (e.g. DeLacy *et al.*, 1990; Annicchiarico, 1997). This variability contributes much to the temporal and spatial instability in crop yields ([http://www.fao.org/documents/show\\_cdr.asp?url\\_file=/DOCREP/005/Y4391E/Y4391E00.HTM](http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/005/Y4391E/Y4391E00.HTM)). While the presence of strong genotype  $\times$  environment interactions can hinder, crop improvement also offers the opportunity to select genotypes specifically adapted to deliver stable yields in specific regions (Ceccarelli, 1996). A more fundamental understanding of genotype  $\times$  environment interactions is necessary to determine the potential opportunities and disadvantages of specific characteristics (Reynolds *et al.*, 2005b). The assessment and detailed analysis of genotypes in multi-environment trials is to be encouraged.

Agricultural biotechnology has yet to produce a commercial cultivar with HSYD. This may reflect the lack of investment by multinational companies who have chosen to focus on pest and disease resistance rather than combating drought stress. Nevertheless, despite the widespread recognition that HSYD is a multigenic trait, the expectations for biotechnological solutions have largely been unrealistic.

Many of the genes that are known to respond to drought stress have been identified and manipulated, the majority of which protect and maintain cellular structure and function. These manipulations have been comprehensively reviewed by Araus *et al.* (2003) and Wang *et al.* (2003). Briefly, there are a wide range of targets including genes that are involved in the biosynthesis of osmolytes [e.g. mannitol, fructans, trehalose, ononitol, proline, glycinebetaine (Ramanjulu & Bartels, 2002)], or related to scavenging active oxygen [e.g. aldehyde dehydrogenase (Sunkar *et al.*, 2003)]. Some genes also encode molecular

chaperones (LEA; Figueras *et al.*, 2004), signalling molecules (MAPKK, SnRK1), or transporters (aquaporins; Aharon *et al.*, 2003; Uehlein *et al.*, 2003) or transcription factors, for example, CBF/DREB (Liu *et al.*, 1998; Hsieh *et al.*, 2002) and ABF/ABAE; (Schöffl *et al.*, 1998; Choi *et al.*, 2000; Shinozaki & Yamaguchi-Shinozaki, 2000). Most of these studies were undertaken in model species; some have now been transferred to crop plants, but few of these have been evaluated in field trials under real drought conditions (Dunwell, 2000). Furthermore, while many authors report substantially increased drought tolerance (Table 1), unfortunately the experimental designs are often flawed and conclusions naive or relate solely to survival traits. Thus, many of the single-gene transformations claimed to improve drought tolerance adversely affected productivity.

In fact, and perhaps with the exception of transporters and transcription factors, all the genes thus far manipulated relate to protection of primary metabolism (i.e. scavenging of oxygen species to prevent oxidation and regulate senescence and programmed cell death, osmolytes to preserve turgor and hence the basic cell structure, chaperones to stabilise vital proteins, etc.). However, eco-physiological studies clearly demonstrate that protection of metabolism is only required under severe drought conditions, in which photosynthesis and growth had already been severely impaired (Flexas *et al.*, 2004b). Therefore, manipulation of such genes, while conferring protection for survival, is unlikely to result in increased yield under drought, except perhaps indirectly, that is, by increasing survival and hence maintaining plants alive at late stages of development, thereby allowing improved allocation to reproductive organs, which are often the harvestable part of the crop.

### Suggested future directions in biotechnology

The major focus should be to improve crop yield by increasing carbon gain during the crop cycle under drought. It is therefore necessary to target genes that increase water-use efficiency without yield penalties. This may be actually difficult because improving water-use efficiency is usually accompanied by decreased photosynthesis (Flexas *et al.*, 2004a) and yield (Condon *et al.*, 2004). This negative relationship is illustrated by Fig. 1. A given genotype (G1) can move 'phenotypically' (i.e. depending on the water availability) along the 'A' curve. Because water-use efficiency is the first derivative of the curve (i.e. the quotient between outputs and inputs), it is clear that moving horizontally (i.e. following the relationship 'A') towards higher photosynthesis results in decreasing efficiency, while moving towards saving

water necessarily results in decreased photosynthesis. Up to now, breeding has been limited to moving plants 'genotypically' along the same curve (Condon *et al.*, 2004). For example, genotypes with early vigour or deeper roots (G2) expend a larger part of their cycle using available water, which results in higher yields (high photosynthesis) but lower leaf-level water-use efficiency. On the contrary, breeding towards higher water-use efficiency (e.g. through screening for low  $^{13}\text{C}$  discrimination) has often lead to constitutively low productivity (G3). Clearly, improving leaf water-use efficiency will only be achieved when a genotype G1 is converted into a G4 genotype with higher photosynthesis at any transpiration rate (i.e. curve 'B'). Thus far, most transformation efforts (Table 1) have targeted genes that operate only in the situation indicated by an asterisk in Fig. 1.

The strength of the relationship between photosynthesis and stomatal conductance has been confirmed by analysing its natural inter- and intraspecific variability (Flexas *et al.*, 2002; Medrano *et al.*, 2002; Hetherington & Woodward, 2003). Nevertheless, there is a substantial opportunity to reach the goal of obtaining genotypes with improved photosynthesis at a given stomatal conductance. This can be achieved by genotypes using  $\text{CO}_2$  at the carboxylation sites more efficiently or able to concentrate  $\text{CO}_2$  at the carboxylation site, or both. The former can be achieved through a Rubisco with increased specificity for  $\text{CO}_2$ . The latter can be met through carbon concentration mechanisms, like those found in  $\text{C}_4$  plants, or improved internal diffusion of  $\text{CO}_2$  (increased mesophyll conductance). The utility of this approach is clearly demonstrated by the greater water-use efficiency found in  $\text{C}_4$  as compared to  $\text{C}_3$  plants. Some elegant biotechnological approaches are seeking to exploit the introduction of genes for all the steps of  $\text{C}_4$  metabolism into single cells within leaves of  $\text{C}_3$  plants (Leegood, 2002). Even if all the steps are introduced and are functional in the leaves of  $\text{C}_3$  plants, it seems unlikely that water-use efficiency will be significantly improved without additional structural changes. Increasing the mesophyll conductance to  $\text{CO}_2$  through biotechnology is not yet achievable because the mechanisms responsible are not fully understood. However, recent reports that suggest the involvement of aquaporins in mesophyll conductance to  $\text{CO}_2$  (Uehlein *et al.*, 2003; Hanba *et al.*, 2004; J. Flexas, unpublished results) may open this possibility in the near future. An alternative strategy to improve water-use efficiency would be to generate  $\text{C}_3$  crop plants expressing an improved form of Rubisco, with high specificity factor and catalytic rate (Spreitzer & Salvucci, 2002; Parry *et al.*, 2003). Exploiting existing variability in Rubisco specificity factor among  $\text{C}_3$  plants appears

a promising way to obtain genetically modified (GM) crops with improved yield under drought (Galmés *et al.*, 2005). It has been argued that because increased specificity factor is usually accompanied by low maximum catalytic rates of the enzyme (Bainbridge *et al.*, 1995), such changes will result in little photosynthetic improvement with increasing specificity (Zhu *et al.*, 2004). While this may be true under conditions where plants can express their maximum catalytic rates, this does not apply under conditions of severely restricted availability of the substrate  $\text{CO}_2$ , such as under drought stress. Thus, increased specificity may result in improved photosynthesis even if maximum catalytic rates are lowered. While these approaches are certainly scientifically sound, there are still numerous obstacles to overcome before they can be exploited (Parry *et al.*, 2003; Mitchell *et al.*, 2004).

A gene that directly regulates plant transpiration efficiency has been recently reported (Masle *et al.*, 2005). This is the *Arabidopsis* *ERECTA* gene, a putative leucine-rich repeat receptor-like kinase. While the mechanisms are not yet fully understood, expression of the *ERECTA* gene, high photosynthetic rate and a low stomatal conductance results in higher water-use efficiency irrespective of water availability.

In addition, to the above-mentioned potential targets, proper consideration must be given to other traits that affect phenology and development and that can be more easily engineered to optimise performance under drought. To date, these have been largely ignored in relation to drought, despite the fact that traditional breeding has actually achieved its best results through selection for phenological rather than physiological traits (Condon *et al.*, 2004).

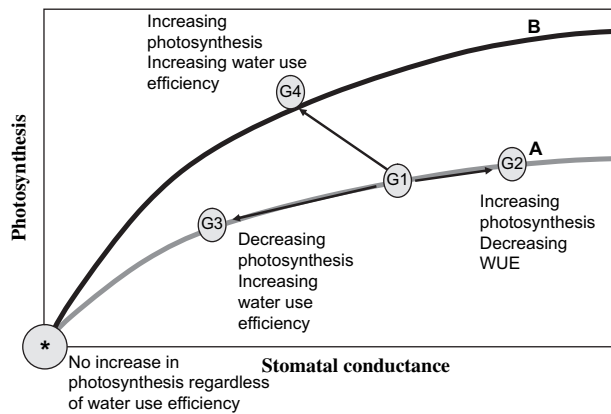
Considerable further research and critical analysis based on more than limited laboratory studies is needed to confirm the promise and utility of the results. In some cases, gene pyramiding may be required (Reynolds *et al.*, 2005a). The exploitation of biotechnology to address this major problem is now approaching a critical phase with respect to practical application. It is vital that the longer term potential of the technology is not oversold, alienating intended beneficiaries (investors, politicians, farmers and consumers). If confirmed, the technology is available to readily transfer genes to elite cultivars of most of the major crops [e.g. maize (Ishida *et al.*, 1996), rice (Hiei *et al.*, 1994) and wheat (Pastori *et al.*, 2001; Sparks *et al.*, 2001)]. However, the technology is not yet available for some crops of regional importance and further underpinning research in this area is needed. But the low acceptability of GM crops to consumers and some politicians in Europe and some developing countries will, at least in the short term, limit the impact that they can have (Halford, 2004).

**Table 1** Examples of transgenic plants reporting effects under drought

Target	Species	Gene	Effect	Reference
Osmolytes	Proline	<i>tomPRO2</i>	Accumulation of proline decreases growth and cell division	Maggio <i>et al.</i> , 2002
		<i>p5cr</i>	Transgenic lines had higher relative water content (RWC) under drought and less pronounced wilting than controls	de Ronde <i>et al.</i> , 2004
	Citrus	<i>p5cr</i>	In pot experiments, the transgenic plants presented superior osmotic adjustment and significantly higher photosynthetic rate than the control plants after 15 days without watering	Molinari <i>et al.</i> , 2004
		<i>p5cr</i>	Transgenic seedlings showed faster growth of shoots and roots in comparison to controls under simulated drought. Stress-inducible expression of the P5CS transgene gave the greatest effect	Su & Wu, 2004
	Fructan	<i>p5cr</i>	Overproduction of proline enhanced root biomass under drought stress	Kavi Kishor <i>et al.</i> , 1995
		<i>p5cr</i>	Drought tolerance	Ishitani <i>et al.</i> , 2004
		<i>oat</i>	Transformants had greater shoot and root biomass than controls under mannitol-simulated drought stress	Roosens <i>et al.</i> , 2002
		<i>SacB</i>	Increased growth and yield under polyethylene glycol (PEG)-simulated drought	Pillon-Smits <i>et al.</i> , 1995
	Galactinol	<i>GolS</i>	Drought-stressed fructan-producing plants attained higher total biomass than controls with no penalty under well-watered conditions	Pillon-Smits <i>et al.</i> , 1999
		<i>lmtI</i>	Increase in desiccation tolerance	Taji <i>et al.</i> , 2002
Ononitol	<i>GolS</i>	Photosynthetic rate recovered faster after drought in transgenic plants than control after severe drought stress	Sheveleva <i>et al.</i> , 1997	
	<i>codA</i>	Majority of transgenics survived an episode of acute drought stress. Under cycles of drought/recovery, the transgenics had higher biomass and were taller than the controls	Sawahel, 2003	
	<i>betA</i>	Slightly faster germination than controls under PEG-simulated drought. In pot experiment after 3 weeks of drought, the grain number and 1000 grain weight all increased in the lines accumulating most glycinebetaine	Quan <i>et al.</i> , 2004	
Mannitol	<i>mtID</i>	PEG-simulated drought transgenic seedlings continued growth while controls wilted and did not grow	Prabhavathi <i>et al.</i> , 2002	
	<i>mtID</i>	In pot experiment after 30 days, drought transgenic line had greater dry weight, plant height and tiller number than control plants	Abebe <i>et al.</i> , 2003	
Trehalose	<i>otsA</i>	Transgenic plants exhibited greater salt and drought tolerance than controls	Serrano <i>et al.</i> , 1999	
	<i>otsA</i> and <i>otsB</i>	Transgenic lines showed more sustained plant growth, less photo-oxidative damage and more favourable mineral balance under drought, than controls	Garg <i>et al.</i> , 2002	
	<i>ots1A</i>	Transgenic lines exhibited less wilting under drought and recovered turgor on rehydration more quickly than the controls	Holmstrom <i>et al.</i> , 1996	
Signalling molecules	<i>otsA</i> and <i>otsB</i>	Transgenic lines exhibited less wilting and increased biomass under drought than the controls	Pillon-Smits <i>et al.</i> , 1998	
	<i>otsA</i> and <i>otsB</i>	Transgenic lines exhibited more drought tolerance than the controls	Kwon <i>et al.</i> , 2004	
	<i>PvNCE1</i>	The transgenic plants had elevated abscisic acid and lower water loss by transpiration (low stomatal conductance) than controls	Qin & Zeevaert 2002	
	<i>AtNCE3</i>	Plants overexpressing <i>AtNCE3</i> showed a reduction in transpiration rate from leaves and an improvement in drought tolerance. By contrast, antisense suppression and disruption of <i>AtNCE3</i> gave a drought-sensitive phenotype	Iuchi <i>et al.</i> , 2001	
Rice	<i>ABF3</i> and <i>ABF4</i>	Lines overexpressing <i>ABF3</i> or <i>ABF4</i> exhibited reduced transpiration and enhanced drought tolerance	Kang <i>et al.</i> , 2002	
	<i>SRK2C</i>	Overexpression of <i>SRK2C</i> increased drought tolerance and enhanced expression of drought response genes	Umezawa <i>et al.</i> , 2004	
	<i>OscDPK7</i>	Overexpression of <i>OscDPK7</i> had less wilting than controls. Transgenic lines had enhanced expression of drought response genes	Sajio <i>et al.</i> , 2000	

Table 1 Continued

Target	Species	Gene	Effect	Reference
Cell cycle	Alfalfa plants	MAPKK	Reduced injury from water-deficit stress	McKersie <i>et al.</i> , 1996
	Maize/tobacco	MAPKKK	Lines expressing MAPKKK produced more kernels than controls under drought stress	Shou <i>et al.</i> , 2004
Membrane	Tobacco	PIPb	Improved growth performance under nonstress conditions, but it was not effective under drought or salt stress	Aharon <i>et al.</i> , 2003
	Tobacco	NtC7	Increase in tolerance to mannitol-induced osmotic stress, with rapid recovery from severe wilting	Tamura <i>et al.</i> , 2003
	Tobacco	BIP	Shoot turgor and RWC was correlated with BIP expression	Alvim <i>et al.</i> , 2001
Transcription factors	Wheat	DREB1A	After water was withheld for 10 days, there was less wilting in transgenic lines than control	Pellegrineschi <i>et al.</i> , 2004
	Tomato	DREB1B	Transgenic lines had greater number of fruits and total biomass under drought stress than controls	Hsieh <i>et al.</i> , 2002
	Arabidopsis	CBF4	Overexpression lines had greater drought and freezing tolerance than controls	Haake <i>et al.</i> , 2002
	Arabidopsis	ALDH3	Improved tolerance and survival when exposed to dehydration and other stress (salt, heavy metals, H <sub>2</sub> O <sub>2</sub> ) compared to controls	Sunkar <i>et al.</i> , 2003
Scavenging active oxygen	Tomato	katE	Expression of the catalase in chloroplasts protected the transgenic plants from the photo-oxidative stress invoked by drought	Mohamed <i>et al.</i> , 2003
	Tobacco	Pssu-ipt	Transgenic plants grew faster than controls under drought, particularly when treatment started in earlier stage of plant development	Synkova & Valcke, 2001
Molecular chaperones	Tobacco	APX3	Under mild water stress and during recovery, transgenic lines had higher photosynthetic activity than controls. However, under moderate and severe water stress, activities were similar. Still, transgenic plants present higher fruit numbers and seed mass than control	Yan <i>et al.</i> , 2003
	Tobacco	Mn-SOD	Better growth under water and salt stress	Kavi Kishor <i>et al.</i> , 1995
	Alfalfa	MnSOD	Under mild water stress, transgenic lines had higher photosynthetic activity than controls. However, under moderate and severe water stress and during recovery, activities were similar	Rubio <i>et al.</i> , 2002
LEA proteins	Rice	HVA1	Transgenic plants maintained higher growth rates than controls under drought	Xu <i>et al.</i> , 1996
	Rice	HVA1	Higher leaf RWC and tolerance to water stress by protecting cell membrane	Babu <i>et al.</i> , 2004
	Rice	HVA1	After salt and drought treatments, transgenic lines showed increased stress tolerance (cell integrity and growth), compared to the control plants	Rohila <i>et al.</i> , 2002
	Wheat	HVA1	Higher root and shoot biomass than controls under drought stress and improved recovery after drought	Sivamani <i>et al.</i> , 2000
	Arabidopsis	Rab17	Transgenic plants have higher sugar and proline contents, and also higher water loss rate under water stress	Figueras <i>et al.</i> , 2004
Rice	PMA80 and PMA1959	Accumulation of either PMA80 or PMA1959 correlates with increased drought tolerance	Cheng <i>et al.</i> , 2002	



**Figure 1** The relationship between photosynthesis and stomatal conductance in plants. Converting a genotype G1 into G2 will increase photosynthesis but decrease water-use efficiency. Conversely, converting a genotype G1 into G3 will increase water-use efficiency but decrease photosynthesis. Achieving a G4 genotype with higher water-use efficiency and high photosynthesis requires ‘moving’ curve ‘A’ towards curve ‘B’. This can only be achieved through (a) CO<sub>2</sub> concentrating mechanisms, (b) increased mesophyll conductance to CO<sub>2</sub> or (c) increased Rubisco specificity factor (see text for details).

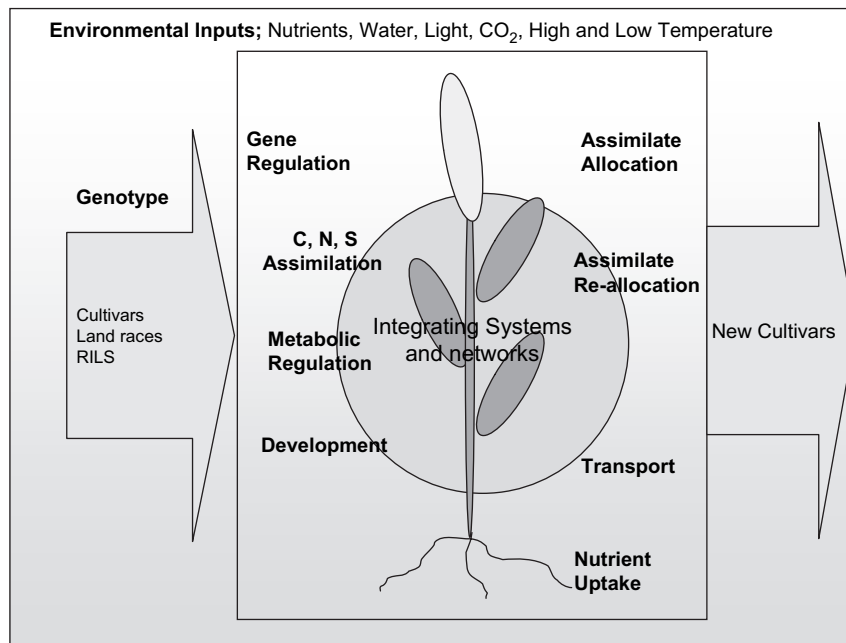
**Conclusions**

An integrated systems approach is essential in the study of complex quantitative traits like yield stability under drought stress in plants (Fig. 2). The research must use

the latest genomics resources combining the new technologies in quantitative genetics, genomics and bio-mathematics with an ecophysiological understanding of the interactions between crop plant genotypes and the growing environment and thus better inform crop improvement (Hawkesford & Buchner, 2001; Araus *et al.*, 2003; Araus, 2004). Most current research programmes lack this necessary interdisciplinary approach. Such co-ordination of the various concepts and use of methods is particularly appropriate for the European Union (EU) projects, and the clustering of these projects should yield substantial added value. In addition, the researchers need to engage more effectively with policy makers and socio-economists to explain the importance and urgency of research on this topic.

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**Figure 2** Plant responses to drought stress are complex and determined by genetic and environmental factors. Optimisation of water use in the field requires an integrative systems approach that considers crop management, environmental and genetic factors. RILs, recombinant inbred lines.

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