Enhancing drought tolerance in C₄ crops

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Received 20 December 2010; Revised 2 March 2011; Accepted 15 March 2011

Abstract

Adaptation to abiotic stresses is a quantitative trait controlled by many different genes. Enhancing the tolerance of crop plants to abiotic stresses such as drought has therefore proved to be somewhat elusive in terms of plant breeding. While many C₄ species have significant agronomic importance, most of the research effort on improving drought tolerance has focused on maize. Ideally, drought tolerance has to be achieved without penalties in yield potential. Possibilities for success in this regard are highlighted by studies on maize hybrids performed over the last 70 years that have demonstrated that yield potential and enhanced stress tolerance are associated traits. However, while our understanding of the molecular mechanisms that enable plants to tolerate drought has increased considerably in recent years, there have been relatively few applications of DNA marker technologies in practical C₄ breeding programmes for improved stress tolerance. Moreover, until recently, targeted approaches to drought tolerance have concentrated largely on shoot parameters, particularly those associated with photosynthesis and stay green phenotypes, rather than on root traits such as soil moisture capture for transpiration, root architecture, and improvement of effective use of water. These root traits are now increasingly considered as important targets for yield improvement in C₄ plants under drought stress. Similarly, the molecular mechanisms underpinning heterosis have considerable potential for exploitation in enhancing drought stress tolerance. While current evidence points to the crucial importance of root traits in drought tolerance in C₄ plants, shoot traits may also be important in maintaining high yields during drought.

Key words: Effective use of water, heterosis, maize, photosynthesis, root mass at depth, stay green, sugarcane.

Introduction

Water is already a scarce commodity in many parts of the world, and predicted climate changes will aggravate the situation in future. In addition to decreases in the amount and frequency of precipitation, global increases in temperature are predicted to enhance water losses further through evapo-transpiration (Intergovernmental Panel on Climate Change, 2007). The increased irrigation required to meet the needs of crop growth is expected to lead to groundwater depletion (Konikow and Kendy, 2005; Oki and Shinjiro, 2006).

The availability of water (in addition to atmospheric CO₂) is considered to have been a major driving force for the evolution and the ecological success of C₄ plants (Edwards et al., 2010; Westhoff and Gowik, 2010). C₄ plants are often considered to have mastered the art of drought control particularly as they are able to maintain leaf photosynthesis with closed stomata. Sorghum, for example, is considered to be better adapted to water-limiting environments than most other crops (Ludlow and Muchow, 1990; Sanchez et al., 2002). Even though photosynthesis can be decreased under drought conditions (Carmo-Silva et al., 2008), C₄ grasses such as Panicum are often classified as drought tolerant as they are able to grow through the dry seasons. Within the context of an increasingly water-poor world, the exploitation of C₄ photosynthesis has great potential for the enhancement of crop production and food security. Moreover, although C₄
plants comprise <4% of global terrestrial plant species, they are important from an ecological, agricultural, and atmospheric perspective, and contribute ~20% to global primary productivity (Ehleringer et al., 1997). Moreover, the C₄ plant maize (Zea mays L.) is the most important cereal crop in the world in terms of annual metric tonnes of production (FAOSTAT, 2009).

C₄ plants have high water use efficiencies (WUEs), and the presence of the CO₂-concentrating mechanisms makes C₄ photosynthesis more competitive in conditions that promote carbon loss through photorespiration, such as high temperatures, high light intensities, and decreased water availability (Edwards et al., 2004). C₄ photosynthesis is characterized by the presence of a metabolic CO₂ pump that concentrates CO₂ in the vicinity of the main enzyme of carbon dioxide fixation, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Edwards et al., 2001, 2004). This confers a number of important advantages in terms of WUE because it allows high rates of photosynthesis to occur even when stomata are closed while limiting flux through the photorespiratory pathway (Edwards et al., 2001). Although only a limited number of studies have been undertaken to date in C₄ species, available data suggest that the enzymes that comprise the metabolic CO₂ pump are more resistant to water deficits than the enzymes of C₃ photosynthesis (Ghannoum, 2009).

The identification of trends in the responses of C₄ plants to drought is complicated by the occurrence of different C₄ subtypes [including NADP-malic enzyme (NADP-ME), NAD-ME, and phosphoenolpyruvate carboxykinase (PEPCK)] (Carmo-Silva et al., 2007) which can exhibit different strategies for coping with water deficits. Literature evidence suggests that there is considerable diversity in the responses to drought of different C₄ subtypes (Ripley et al., 2010). For example, Paspalum dilatatum (NADP-ME) and Zoysia japonica (PEPCK) respond to water deficit by early stomatal closure in order to conserve leaf water status, whereas this response was not apparent in Cynodon dactylon (NAD-ME), which lost water rapidly when deprived of water (Carmo-Silva et al., 2007). Of these three species, C. dactylon was classed as the most resistant to the rapid imposition of water deficits while P. dilatatum was the least resistant (Carmo-Silva et al., 2007). Panicoid grasses of the C₄ NADP-ME type were found to be metabolically more sensitive to drought than C₃ species and they recover more slowly (Ripley et al., 2010). The following discussion will focus on the main traits that are considered to be useful in the selection of C₄ varieties with improved drought tolerance characteristics. The genetic variability in the responses of C₄ plants to drought is readily demonstrated, as illustrated in Fig. 1. Moreover, the models used for the integration of complex gene–phenotype relationships in breeding C₄ plants such as maize are becoming increasingly effective (Messina et al., 2011).

**Adaptation to drought**

The ability to escape from drought by accelerating the life cycle or, alternatively, the evolution of avoidance and tolerance mechanisms have long been considered to be important strategies in drought adaptation (Levitt, 1972; Chaves et al., 2003). Escape strategies often rely on successful reproduction before the onset of severe stress (Campos et al., 2004) using a short life cycle with high growth rates together with the efficient storage and use of reserves for seed production. Dehydration avoidance mechanisms involve the maintenance of a high (favourable) plant water status during stress. Such strategies include minimized water loss (e.g. stomatal closure, reduced leaf area, and senescence of older leaves) or maximal water loss afforded by increased root proliferation at depths where the water is available. However, there is inherent conflict between biomass accumulation and stress avoidance via a reduction in transpiration rates because the acquisition of photoassimilates is dependent on stomatal aperture and leaf area (Araus et al., 2008; Blum, 2009, 2011). Tolerance to low water potentials requires the maintenance of plant functions under conditions of limited water availability and/or the rapid recovery of plant water status and plant function after stress. This requires precise osmotic adjustments and may also involve the rigidity of cell walls and/or small cells (reviewed in Chaves et al., 2003).
Responses to drought are species specific and often genotype specific (Hall et al., 1982; Campos et al., 2004). Moreover, the nature of drought responses of plants is influenced by the duration and severity of water loss (Yang et al., 1993; Pinheiro and Chaves, 2011), the age and stage of development at the point of drought exposure (Chimenti et al., 2006), as well as the organ and cell type experiencing water deficits (Pastori and Foyer, 2002). Plants subjected to soil water deficits typically exhibit characteristic losses in leaf water content leading to decreased growth and leaf elongation. This is accompanied by decreases in leaf osmotic potential (Westgate and Boyer, 1985), stomatal conductance, photosynthesis, and transpiration rates (Ripley et al., 2007, 2010). The abundance of reactive oxygen species (ROS) tends to increase in the tissues of plants exposed to drought, as do the activities of antioxidant enzymes (Jiang and Zhang, 2002). Hormone signalling pathways are highly responsive to drought, particularly those associated with increased synthesis and cycling of the stress hormone abscisic acid (ABA; Bray, 2002; Pinheiro and Chaves, 2011).

The responses of plants to drought observed under field conditions are generally much more complex than those measured under controlled environment conditions because of other factors accompanying water deficits which influence the nature of the stress response. Water deficits lead not only to low tissue water status and cell dehydration but also to nutrient deprivation and osmotic stress, and often additionally heat stress linked to decreased transpiration. This complicates the definition of target stress for plant breeders as well as the environmental conditions required for evaluation and selection of improved varieties. Moreover, periods of soil water deficits can occur at any time during the growing season, and not all stages of plant development are equally sensitive in terms of impact on ultimate crop yield. For example, crop species such as maize are particularly sensitive to drought at the heading stage, which is just before tassel flowering. In sugarcane, drought stress occurring when the leaf canopy is already well established has more serious impacts on final yield in terms of total biomass, stalk biomass, and stalk sucrose than when drought occurs earlier during the season (Robertson et al., 1999a). For simplicity, much of the following discussion will focus on the traits and mechanisms that have the potential to confer increased performance in maize under mild or moderate water deficits (Araus et al., 2011). Moreover, because maize is a cross-pollinating species that is prone to failure of grain set under stressful conditions, traits that serve to prevent seed abortion will be considered.

Strategies for enhancing yield potential and stress adaptation

The breeding value of any trait is intrinsically dependent on the prevailing growing conditions (such as soil type and climate) in the target regions in which genotypes will be grown (Tardieu and Tuberosa, 2010; Araus et al., 2011).

The classical approach to breeding varieties with improved drought tolerance traits has involved multilocation progeny testing, in which variations in water availability across the different sites are sufficient to facilitate random selection on the basis of high and stable yield. While breeding for high yield in drought-prone environments using multilocation testing is inherently complicated by the year to year variability in the amount and temporal distribution of available soil water and the low heritability of drought resistance traits under these conditions, selection for drought tolerance in routine breeding programmes with adequate weighting has yielded significant increases in maize yields (Banziger et al., 2004). Higher shoot biomass can be achieved through different, and in many cases conflicting, strategies (Collins et al., 2008). If this is accompanied by higher root biomass and improved root architecture, it should facilitate better water extraction capabilities under field conditions. However, while improved yield potential can translate into better performance under stress, it also places a greater demand on water and other resources. Thus in conditions of limited soil water availability, a higher capacity for water uptake by the plant can sometimes result in an increased frequency of stress experiences. Thus, it is preferable that a higher yield potential is accompanied by enhanced stress tolerance.

For moderate stresses that cause up to a 70% reduction in potential yield, there are complementary strategies which can be employed to optimize total yield, although this sometimes occurs at the expense of yield stability. In the absence of stress, the main yield optimization strategies are as follows.

(i) Increasing yield potential through shoot mechanisms such as increased transpiration rates or ‘stay green’ characteristic (i.e. the ability to retain chlorophyll pigments in photosynthetic tissues and maintain a green leaf area at the end of the crop cycle). Increases in yield may translate to a higher biomass accumulation and grain yield under mild to moderate water deficit.

(ii) Maintaining growth and thus biomass accumulation under decreasing water status. This strategy is not without risk as crop failure may occur as a consequence of soil dehydration, as discussed in detail below.

For more severe water deficit scenarios, breeding strategies tend to focus on reducing the risk of total yield loss. This can be achieved by decreasing cumulative transpiration, which often incurs a penalty in terms of yield potential and performance under mild or moderate water deficit conditions. Such strategies therefore often increase yield stability at the expense of yield potential. These strategies include, for example, the following.

(iii) Shortening the duration of the crop cycle (i.e. phenological adjustment) to escape from the drought period. This strategy has proved to be most successful for C₃ cereals such as wheat or barley in environments, such as those of the Mediterranean region, which experience drought at the
end of the growing season, here referred to as ‘terminal drought’ (Araus et al., 2002).

(iv) Reducing leaf area or stomatal conductance, traits which frequently increase the WUE (i.e. the amount of harvested biomass per unit transpired water).

Any trait can thus have different (positive, negative, or neutral) impacts on yield, depending on the drought scenario. Water conservation traits have beneficial effects in the most severe drought conditions or in terminal drought scenarios, in which it is useful to save water for the end of the crop cycle and to decrease the numbers of sinks for assimilates in order to obtain larger seeds. Traits that serve to conserve water (conservative traits) include low stomatal conductance, low leaf growth rate, high WUE, or deep but sparse root systems. Conversely, growth ‘maintenance’ traits have beneficial effects under milder drought conditions, in which the soil profile is periodically re-watered.

Biomass production is tightly linked to transpiration, WUE, and nitrogen accumulation. Blum (2011) has argued that breeding for high WUE under drought conditions will ultimately result in low-yielding genotypes with reduced drought tolerance because WUE is increased by reduced transpiration and water use. Therefore, biomass production under most drought conditions (i.e. except for marginal drought-prone areas) can only be enhanced by maximizing soil moisture capture for transpiration, which also involves minimized water loss by soil evaporation. Collectively, this property has been called effective use of water (EUW; Blum, 2011).

Biomass production is tightly linked to transpiration, and hence breeding for maximal soil moisture capture for transpiration is perhaps the most important target for yield improvement under drought stress conditions. In addition, breeding for EUW in order to improve plant water status helps to sustain assimilate partitioning and reproductive success, resulting in increased harvest indices. This is particularly important in maize, because the reproductive stage is the most susceptible to drought.

Use of stable isotopes to screen for water use efficiency

WUE, defined as the ratio of biomass accumulated to water transpired, has been traditionally considered to be the key determinant of drought tolerance (Tambussi et al., 2007). In C3 cereals, carbon isotope discrimination (Δ13C) has been used to screen for genotypes with higher WUE (Araus et al., 2002). In contrast, few reports are available concerning the use of Δ18O in C4 crops such as maize (Monneveux et al., 2008; Cabrera-Bosquet et al., 2009c). Moreover, the results obtained to date are not encouraging, mainly because of the nature of C4 photosynthesis.

Much attention has focused on improving WUE when breeding for drought adaptation. However, it would now appear that EUW is a more important adaptive trait than WUE except in severe drought conditions (Araus et al., 2008; Blum, 2009). EUW is related to the genotypic capacity to use available water and, therefore, to sustain transpiration under unfavourable environmental conditions.

Oxygen isotope enrichment (Δ18O) can also be useful in selecting for drought adaptation in both C3 and C4 grasses. Such measurements are independent of the photosynthetic process and have therefore been proposed as a ‘time-integrative’ indicator of transpiration and EUW in different plant species (Barbour, 2007; Farquhar et al., 2007; Cabrera-Bosquet et al., 2011a) including maize (Cabrera-Bosquet et al., 2009b; Araus et al., 2010). Moreover, associated genotypic differences in Δ18O and grain yield have been reported in cereals such as wheat (Cabrera-Bosquet et al., 2009a) and maize (Cabrera-Bosquet et al., 2009b). Negative relationships between Δ18O and grain yield have been observed under both well-watered and moderate water deficit conditions (Cabrera-Bosquet et al., 2009b). These findings would suggest that genotypes with higher transpiration traits were the most productive. Heterosis in maize, as discussed in more detail later, appears to confer higher transpiration. Maize hybrids have lower Δ18O values and higher ash contents compared with the inbred lines regardless the growing conditions (Araus et al., 2010).

Given the cost, technical skills, and facilities involved in oxygen isotope analysis, its large-scale application to breeding programmes may not be feasible for small/medium breeding programmes and therefore other alternatives for assessing plant transpiration are often required. Thus, the accumulation of mineral or ash content in vegetative tissues may be an inexpensive and easy way to predict yield and genotypic adaptation to drought in different cereals (Araus et al., 1998). Mineral accumulation in vegetative tissues can be explained through the passive transport of minerals via the xylem driven by transpiration (Araus et al., 2002). Leaf ash has been correlated with yield in barley (Voltas et al., 1998) and wheat (Araus et al., 1998) plants grown under different water regimes. While this approach can be used successfully in both C3 and C4 crops, to our knowledge there is only one report of the application of leaf ash measurements in maize and that is a recent study from the CIMMYT where a close relationship between leaf ash content and isotope enrichment was observed (Cabrera-Bosquet et al., 2009c). Based on these results, however, it is possible to conclude that leaf ash content is a viable alternative criterion to Δ18O for assessing yield performance in maize grown under drought conditions. Moreover, the analysis of ash (or total mineral) content is not costly and it does not require extensive facilities or highly qualified technical staff. Hence, this trait is ideal for breeding programmes in developing countries.

Studies on maize conducted at the CIMMYT (Cabrera-Bosquet et al., 2009c; Araus et al., 2010) and other unpublished results using CIMMYT germplasm support the potential usefulness of both Δ18O measurements (particularly in kernels) and ash content in leaves as secondary traits for direct selection of maize genotypes with improved performance under drought through higher EUW. While further studies are needed to match both traits to usefulness...
criteria, specifically with respect to the extent of genotypic variance, heritability, and genetic correlation of both $\Delta^{18}O$ and ash content with grain yield, the findings to date clearly indicate the potential value of these techniques. In addition these two traits may be estimated in a fast and low-cost manner by near-infrared reflectance spectroscopy (Cabrera-Bosquet et al., 2011b). Moreover, it will be equally important to characterize what root traits, as discussed in detail below, might be responsible for the observed genotypic variation in $\Delta^{18}O$ and mineral contents.

An additional tool for the assessment of water uptake capacity by the root system is the isotope composition of oxygen ($\delta^{18}O$) and hydrogen ($\delta^2H$) in stem water (Ehleringer and Dawson, 1992). This technique, which has largely been developed in trees to date, is based on the existence of evaporative gradients in the isotope composition of soil water that tends to be more enriched in the heavier isotope at the soil surface than in deeper layers because of evaporation. A comparison of the isotopic composition of stem water (i.e. the water taken up by the plant) with that of soil water at different depths has shown that it is possible to determine which parts of the root system contribute most to water uptake (see, for example, Ferrio et al., 2005; Patz, 2009; Holst et al., 2010). In addition, genetic differences in water uptake parameters are significant and may be partly responsible for the observed genetic variability in $\delta^{18}O$ data (Retzlaff et al., 2001; Ferrio et al., 2007; Voltas et al., 2008). Traditionally, stable isotopes have been determined by isotope ratio mass spectrometry (IRMS), which is an expensive technique requiring costly equipment and a complex laboratory infrastructure. Moreover, standard IRMS devices cannot be used to analyse $\delta^{18}O$ and $\delta^2H$ simultaneously. This means that the time and cost of the analyses are effectively doubled. However, commercial alternatives based on high precision infrared laser spectroscopy have recently emerged, providing cheap, fast, and accurate determinations of both $\delta^{18}O$ and $\delta^2H$ in a single measurement (Aggarwal et al., 2006; Lis et al., 2008).

Root system architecture and root mass at depth

Plants use different strategies, ranging from avoidance to desiccation tolerance, to cope with dry soils. Desiccation avoidance is associated with the minimization of water loss and the maximization of water uptake (Ludlow and Muchow, 1990). Evidence suggests that the adverse effects of drought can be successfully avoided by changing carbon allocation patterns to allow the formation of a deep root system before the onset of a growth-limiting water shortage (Jordan et al., 1983; Jones and Zur, 1984; Blum, 1985; O'Toole and Bland, 1987; Sponchiado et al., 1989; Sinclair and Muchow, 2001; Campos et al., 2004; Manschadi et al., 2006 2008; Reynolds et al., 2007; Lopes and Reynolds, 2010, 2011). Deep root systems have a proven advantage when water is available deep in the soil profile but not in shallow soils (Sponchiado et al., 1989). Positive relationships between root ramification and yield under drought have not always been found and, moreover, a significant association between a lower root mass and increased ear growth was observed under drought in a tropical maize population (Bolan˜os and Edmeades, 1993). However, the latter results are possibly a rather exceptional response to specific environmental conditions (Bolan˜os and Edmeades, 1993).

Root system architecture, which is defined as the spatial configuration of a root system in the soil, is used to describe the shape and structure of root systems (Dorlodot et al., 2007). This parameter is important because major soil resources are heterogeneously distributed in the soil. The spatial deployment of roots determines the ability of a plant to secure edaphic resources (Dorlodot et al., 2007). There is overlap between quantitative trait loci (QTLs) for root features and plant productivity in terms of yield, water use, or nutrient capture (Tuberosa et al., 2002; Steele et al., 2007). In maize, for example, QTLs for the elongation rate of axile roots, the number of axile roots, and yield were co-localized (Trachsel et al., 2009). Such evidence indicates that root architecture is an important determinant of yield.

Root traits are notoriously difficult to measure under field conditions. Of the root traits that can be easily measured in such circumstances, few are positively related to yield. The ‘root mass at depth’ parameter but not total root mass has been shown to correlate with yield under drought conditions. Of no yield penalty under full irrigation (Lopes and Reynolds, 2011). Genetic variation in root mass at depth has been shown in C3 plants such as wheat (Lopes and Reynolds, 2010) and C4 plants such as maize (Hund et al., 2009) and sorghum (Ludlow et al., 1990; Santamaria et al., 1990). Moreover, remote sensing techniques might be useful indirect indicators of the root mass at depth parameter. For example, canopy temperature has been suggested as a possible marker, cooler canopy temperatures indicating genotypes that have the ability to maintain transpiration because the roots can access water deep in the soil profile. Negative correlations between canopy temperatures at grain filling and root dry weight at depth have been observed (Lopes and Reynolds, 2010). Similar associations under drought have been found in four bean cultivars, where genotypes with deeper roots showed better seed yield, crop growth, lower canopy temperature, and lower soil moisture extraction, in all but poor soils where root variations were no longer observed (Sponchiado et al., 1989).

Taken together, such findings suggest that the spatial distribution of the roots in the soil, and not total root mass per se, defines the ability of a root system to take up water. An ‘ideal’ root system, with an even distribution of ~1 cm root m⁻³ of soil (root length density or RLD) is considered to be the most suitable under field conditions (Tardieu et al., 1992). Higher RLDS are adequate when evaporative demand increases and soil water reserve decreases. However high RLD values might ultimately prove to be wasteful of photoassimilates and other resources without giving appreciable increases in water uptake. The conductivity...
properties and water retention capacity of the soil have to be taken into account when defining the required hydraulic properties of the roots, and target types for root architecture and efficiency (Javaux et al., 2008). Root angles and branching are considered to play a basic role in improving water acquisition by plants. Novel approaches have been applied to improve our current understanding of the complexities of maize root architecture, together with the use of fractals and associate derivatives (Bohn et al., 2006; Zhong et al., 2009). However, it is important to note that many of the root architecture traits described above have only been assessed in controlled environment studies. Unfortunately, conditions in controlled environment chambers or cabinets are not always representative of the stresses experienced by plants in the field. For instance, root mass at depth, which is expected to improve yields under post-anthesis drought (Lopes and Reynolds 2010), cannot be determined in gel chambers or growth columns without root growth restrictions. Thus, fine mapping of genes and markers related to root traits has to be assessed in the field, and only then can the data obtained be effectively incorporated in breeding programmes.

Exploitation of heterosis to enhance drought tolerance in maize

Heterosis in maize is associated with higher yield potential and it confers adaptation to a wide range of growing conditions that might be successfully exploited to enhance drought tolerance. Traits associated with heterosis have potential to reveal new mechanisms that contribute to drought tolerance. A recent study undertaken at the CIMMYT revealed that maize hybrids have better water use than inbred lines, regardless of the water regime during cultivation (Araus et al., 2010). Thus, the effects of heterosis on growth and yield parameters in maize may, at least in part, be explained in terms of variations in water use and status, factors that are also present under well-watered conditions. Therefore, the study of root characteristics associated with heterosis is promising in terms of determining traits conferring improved EUW. Heterosis in root architecture can already be seen at the very early stages of root development, a few days after germination. Thus lateral root density shows the highest degree of heterosis (Hoecker et al., 2006), and maize hybrid seedlings develop a greater number of fine roots than their parents (Li et al., 2008). Similarly, heterosis in the total length of lateral roots, together with a higher ratio of lateral root length to that of the axial root, was already found in 20-day-old maize seedlings grown under different N conditions (Chun et al., 2005). Together with the post-embryonic shoot-borne root system, lateral roots, which become dominant later in root development, are responsible for most of the water and nutrient uptake by the root system (Hochholdinger et al., 2004). Moreover, heterosis exists for water uptake ability at the root cell level under well-watered conditions (Liu et al., 2009). However, to date, the above studies on roots have been performed only on seedlings under controlled growth conditions. The difficulties associated with performing systematic studies of root traits in heterosis on adult plants in the field should not be underestimated.

Shoot architecture and stay green phenotypes

Using a set of historical yield trends in maize, Hammer et al. (2009) demonstrated that changes in root architecture and water capture are associated with biomass accumulation. While changes in canopy architecture are likely to have little direct effect on water capture, they may have important indirect effects via leaf area retention and partitioning of carbohydrate to the ear (Hammer et al., 2009). Stay green phenotypes in C4 crops such as sorghum are often a consequence of other earlier traits, such as a slow leaf growth rate. Such genotypes tend to save water for the end of the crop cycle and have a deep root system (Harris et al., 2007). Strong evidence in support of the value of stay green characteristics in the adaptation to abiotic stresses comes from retrospective comparisons of the performance of temperate maize hybrids produced over the last 70 years in the USA (Tollenaar and Lee, 2006). A high level of association between yield stability and general stress tolerance is observed in new temperate maize hybrids, where yield stability does not appear to have declined with increasing yield potential (Tollenaar and Lee, 2002; Duvick et al., 2004). Higher dry matter accumulation during grain filling in newer compared with the older hybrids can be attributed, at least in part, to a longer duration of the grain-filling period in the newer hybrids which are adapted to higher planting densities (Tollenaar and Lee, 2006). Therefore, the higher yield potential of the newest hybrids is related to a higher expression of stay green characteristics, together with the ability to grow well under higher planting densities. These findings suggest that as well as having a positive role in increasing the amount of light used effectively in photosynthesis for the production of assimilates to drive growth and biomass production, stay green is a consequence of the requirement of the newest hybrids to cope with growth conditions where nutrient and water resources are limiting (Blum, 2011). Water deficits will tend to be higher when plants are subjected to high density propagation, and thus tolerance of high plant densities must inherently involve a high resistance to drought stress (Tollenaar and Wu, 1999).

The benefits of stay green phenotypes have been described in sorghum (Borrel et al., 2000), where the rate of leaf senescence was shown to be negatively correlated with yield under conditions of late (pre- and post-flowering) water deficits (Borrel et al., 2000). It has been claimed that stay green is a constitutive trait in sorghum, with important contributions to yield that are expressed under well-watered conditions. However, the expression effects of stay green relative to the faster senescence of genotypes with wild-type patterns of senescence become more prominent under severe
drought stress (Blum, 2011). Moreover, the utilization of stay green expression as a secondary trait can incur problems related to interactions with other variables. For example, stay green can result from late flowering or low reproductive sink strength, and stay green phenotypes are highly prone to variation in response to environmental factors (Rosenow and Clark, 1995). Taken together, these findings indicate that while stay green shows potential for routine use in breeding programmes seeking to enhance abiotic stress tolerance, any interactions of stay green expression with other complicating factors that might adversely affect yield should first be eliminated.

Growth regulation and stress tolerance

A decrease in leaf growth occurs rapidly in plants exposed to water deficits, occurring even before stomatal closure (Saab and Sharp, 1989). There is considerable genetic variability in the sensitivity of leaf expansion to water deficits in temperate and tropical maize varieties (Welcker et al., 2007). The high sensitivity of leaf growth to water deficits is considered to be a ‘stress avoidance’ mechanism, which enables plants to tolerate severe drought scenarios by saving soil water. Leaf rolling or epinasty is a short-term, reversible mechanism that fulfils essentially similar functions by decreasing the active leaf area. However, high sensitivity of growth and leaf movements to water availability has several potential drawbacks. First, the genetic determinism of leaf growth is partially linked to reproductive sink strength, and stay green expression as a secondary trait can incur problems related to interactions with other variables. For example, stay green can result from late flowering or low reproductive sink strength, and stay green phenotypes are highly prone to variation in response to environmental factors (Rosenow and Clark, 1995). Taken together, these findings indicate that while stay green shows potential for routine use in breeding programmes seeking to enhance abiotic stress tolerance, any interactions of stay green expression with other complicating factors that might adversely affect yield should first be eliminated.

Drought-triggered seed abortion and early seed growth

Seed number, which is a major component of yield potential, is mainly determined at flowering and slightly afterwards. While the number of ovules greatly exceeds the number of seeds that are finally produced in most species, exposure to water deficits decreases the seed/ovule ratio even more because of flower abortion (Barnabas et al., 2008). Flower abortion is considered to be an adaptive mechanism, which allows the remaining seeds to be filled appropriately in relation to the availability of fixed carbon provided by photosynthesis. Thus, flower abortion can have either a positive or a negative effect on yield, depending on the drought scenario. However, from a breeding perspective, flower abortion is considered as a negative trait for both tropical and temperate maize.

Sucrose feeding experiments and metabolite measurements have shown that carbon metabolism and transport make a significant contribution to early seed growth and that the absence of an appropriate carbon supply leads to flower abortion (Zinselmeier et al., 1995; McLaughlin and Boyer, 2004). However, the relationships between sugar content and flower viability are complex and so flower abortion does not result solely from limitations in sugar supply, because sugar levels are usually maintained in ovules even in plants experiencing water deficits. Therefore, flower abortion may result from a combination of drought-mediated disruption of metabolic pathways and changes in ear development (Carcova and Otegui, 2007). Other processes, such as a delay in silk growth (anthesis-silking interval; ASI) also have a major impact on seed number and the susceptibility of maize to stress-induced flower abortion (Bolaños and Edmeades, 1996). Over the last 50 years the ASI has been successfully modified by genetic improvement.
Drought-induced limitations on photosynthesis

The reduction in plant growth resulting from the imposition of drought is in part related to changes in whole-plant carbon status, which in turn depends on changes in the partitioning of assimilate between different organs and also the balance between photosynthesis and respiration (Flexas et al., 2006a). While drought-induced inhibition of photosynthesis has been extensively studied (e.g. Cornic, 2000; Lawlor, 2002; Chaves et al., 2003; Flexas et al., 2004; Pinheiro and Chaves, 2011) and there has been an extensive characterization of drought-induced changes in plant physiology, metabolism, and gene expression patterns (Chaves et al., 2003; Huang et al., 2008; Ghannoum, 2009; Hayano-Kanashiro et al., 2009), a recent literature review concluded that relatively few general trends were apparent in the metabolic and molecular responses of photosynthesis to water stress (Pinheiro and Chaves, 2011). The photosynthetic systems of C4 plants are as sensitive to drought-induced inhibition as they are in C3 plants (Ripley et al., 2010). Both stomatal and metabolic limitations on photosynthesis occur during drought stress (Tezara et al., 1999; Lawlor and Cornic, 2002; Medrano et al., 2002; Flexas et al., 2006a; Lawlor and Tezara, 2009). While there is ongoing debate as to which of these processes plays the more dominant role, there is a general consensus that reduced movement of CO2 from the atmosphere to the site of carboxylation within the chloroplast is a major cause for decreases in CO2 assimilation capacity under most drought stress situations (Chaves and Oliveira, 2004; Flexas et al., 2006b; Warren, 2008). The reduced movement of CO2 involves two important components, namely stomatal closure, which restricts CO2 uptake from the atmosphere into the leaf, and decreased internal conductance (g), which restricts CO2 movement from the intercellular air spaces within the leaf to the site of carboxylation. Although decreased stomatal conductance (g) during drought stress is a very well known phenomenon, it has only been suggested fairly recently that decreased g also represents an important limitation to CO2 movement (Flexas and Medrano, 2002; Warren et al., 2004; Warren, 2008). As a consequence the actual chloroplastic CO2 concentration (C) could be substantially lower than the measured intercellular CO2 concentration (Ci) even under conditions of mild drought stress. A noteworthy implication of reduced g is that it may, at least in part, explain reduced CO2 assimilation rates at common Ci, or reduced apparent carboxylation efficiencies (slope of the A–Ci curve), symptoms of drought stress that are normally regarded as indicative of metabolic limitation.

The relative effects of stomatal and metabolic limitations are species dependent and that they are also influenced by growth and experimental conditions (Lawlor and Tezara, 2009). A conceptual model was therefore proposed in which mild drought stress leads to decreased g, with CO2 assimilation rates falling in the presence of only minor reductions in Ci and C (Lawlor and Tezara, 2009). Lawlor and Tezara (2009) suggested that the magnitude of changes in C might often be overestimated in the literature because of assumptions and potential errors in the estimation of g.

While the responses of C4 photosynthesis to drought have been much less studied than those of C3 photosynthesis, literature evidence suggests that drought-treated C4 plants also show stomatal and metabolic limitations on photosynthesis (Ghannoum, 2009). Photosynthesis is decreased in the leaves of C4 plants subjected to drought through stomatal and non-stomatal limitations (Foyer et al., 1998; Ghannoum et al., 2003; Carmo-Silva et al., 2007; Ripley et al., 2007). Recent evidence suggests that C4 photosynthesis is prone to metabolic limitations on photosynthesis and has poor rates of recovery following drought (Ripley et al., 2010). The available evidence suggests that there is preferential inhibition of the activity of C3 cycle enzymes during drought (Ghannoum, 2009). The differential inhibition of the C3 and C4 cycles could lead to a rise in the CO2 concentration inside the bundle sheath cells and a build-up of a CO2 gradient across the bundle sheath membrane that would increase CO2 leakage (Bowman et al., 1989; Saliendra et al., 1996).

The pathway of photorespiration is much reduced as a result of the C4 pathway of photosynthesis. However, photorespiration is still required in C4 plants as it plays important roles in cellular redox homeostasis and nitrogen metabolism (Foyer et al., 2009). Photorespiration rates do not increase markedly in C4 plants subjected to drought stress (Carmo-Silva et al., 2008). The mesophyll chloroplasts of C4 plants have a high capacity for the Mehler reaction, which generates hydrogen peroxide (Siebke et al., 2003), and this pathway may provide an alternative sink for electrons during drought stress, particularly if it becomes partially uncoupled from ATP synthesis. The C4 pathway enzymes located in the mesophyll chloroplasts of plants could be inactivated by enhanced oxidation during drought stress, and this might also explain why recovery of photosynthesis after drought is more delayed in C4 than in C3 plants (Ibrahim et al., 2008). Impaired metabolism is a particularly important limitation on photosynthesis in C4 crops such as sorghum and sugarcane (Countour-Ansel et al., 1996; Du et al., 1996).
Drought and the production of sugarcane

Sugarcane is important in tropical and subtropical agriculture in >80 countries (Molinari et al., 2007), but unfortunately our understanding of sugarcane physiology has lagged behind that of other major food and fibre crops (Innam-Bamber et al., 2005). Drought stress is common in dryland sugarcane production (Innam-Bamber and Smith, 2005; Smit and Singels, 2006). Drying-off prior to harvest through the withholding of irrigation is an important strategy in irrigated sugarcane production, as it serves to enhance stalk sucrose content and to facilitate infield operations during harvesting (Robertson et al., 1999b; Innam-Bamber, 2004). With the gradual development of water deficit during drying-off, stalk elongation declines rapidly along with lower rates of leaf development. Reduced stalk and leaf growth lowers the demand for photoassimilate and, because photosynthesis is less sensitive to water deficit than growth, more sucrose thus becomes available for storage in millable stalks (Robertson et al., 1999c). However, there are certain risks associated with drying-off because if the water deficit becomes too severe, photosynthesis is inhibited with an associated lowering of cane and sucrose yields (Robertson et al., 1999b). Increases in stalk sucrose content during periods of low rainfall under dryland production of sugarcane occur naturally during a process known as natural ripening, but excessive drought stress, which lowers photosynthetic capacity, frequently has large negative impacts on cane and sucrose yields depending on the timing of the stress (Robertson et al., 1999a).

The effects of drought stress on sugarcane growth and photosynthesis were measured (Koonjah et al., 2006) under rainshelter conditions as illustrated in Fig. 2. Plant extension rates were the first process to be affected by drought stress when the midday leaf water potential decreased below –0.4 MPa. Photosynthetic rates were then reduced when the midday leaf water potential reached –0.7 MPa. Ultimately radiation use efficiency was decreased by 50% when water potential reached –1.5 MPa. A similar sequence of events relative to midday leaf water potentials was observed in potted sugarcane plants subjected to drought stress, (Innam-Bamber and de Jager, 1986) and under field conditions (Koonjah et al., 2006). At midday leaf water potentials of –1.0 MPa to –1.7 MPa green leaf area was reduced, plant extension ceased completely, and gs reached a minimum. The higher drought sensitivity of growth processes compared with photosynthesis was clearly demonstrated by Innam-Bamber et al. (2008), who found that only when stalk extension rate was reduced by 33% did drought stress also cause a reduction in photosynthetic rates. Besides slower growth rates, shoot and leaf senescence is also accelerated by severe drought stress in sugarcane (Fig. 3) and, with the concomitant slower development of new leaves, the reduction in radiation use efficiency is further aggravated (Inman-Bamber and de Jager, 1986; Koonjah et al., 2006; Smit and Singels, 2006). The combined effects of drought stress on the extent of the green leaf canopy, and thus whole-plant photosynthetic capacity, and reduced photosynthetic rates on a leaf area basis per se explain why severe drought stress can have substantial negative effects on sugarcane yield components. It has been shown that drought stress that occurs when the leaf canopy is already well established will have more serious impacts on final yield in terms of total biomass, stalk biomass, and stalk sucrose than when drought stress occurs earlier during the season in a younger crop (Robertson et al., 1999a). The reduction in biomass production was mainly due to lower photosynthetic rates of the leaves, which decreased sharply because of rapid development of drought stress in the crop with the larger leaf area index.
Based on an analysis of available evidence it was proposed that with declining leaf water status, C₄ photosynthesis goes through three successive stages (Ghanoum, 2009). The initial stage is mainly characterized by a decline in stomatal conductance, which may or may not be accompanied by reduced rates of CO₂ assimilation. This is because the CO₂-concentrating mechanism in C₄ plants is capable of saturating photosynthesis even at relatively low Cᵢ. As leaf water status drops further, a combination of stomatal and non-stomatal factors are introduced, while during the third stage of photosynthetic decline at very low leaf water status, mainly non-stomatal factors are involved in severe inhibition of photosynthesis. A survey of the limited evidence available suggests that photosynthetic decline in sugarcane during drought stress generally follows this pattern. At midday leaf water potentials above ~0.85 MPa the decline in CO₂ assimilation rates in sugarcane was mainly caused by stomatal closure, while at leaf water potentials below ~0.85 MPa, non-stomatal components became increasingly important (Du et al., 1996). In these experiments the CO₂ assimilation rate and gs were already reduced by 50% when midday leaf water potential reached approximately ~0.7 MPa, while the activities of five key photosynthetic enzymes only reached the 50% inhibition level at a leaf water potential of approximately ~1.5 MPa (Du et al., 1996).

When considering the non-stomatal factors involved, the response in sugarcane during drought stress appears to be as diverse as in C₃ plants. Du et al. (1996) demonstrated that severe drought stress in sugarcane inhibited the activities of Rubisco, fructose bisphosphatase (FBPase), phosphoenolpyruvate carboxylase (PEPcase), NADP-ME, and pyruvate,orthophosphate dikinase (PPDK). In particular, PPDK activity correlated very closely with CO₂ assimilation rates during the latter stages of drought stress, and it was concluded that PPDK was very likely the limiting factor in non-stomatal components in sugarcane during drought stress (Du et al., 1996). However, Saliendra et al. (1996) noted a decline in the Rubisco:PEPCase activity in sugarcane during drought stress, mainly because Rubisco activity was not inhibited while PEPCase activity actually increased. In another study on sugarcane, drought stress caused inhibition of Rubisco activity but not PEPCase activity (Vu and Allen, 2009), which fits the general idea of preferential inhibition of key C₃ cycle enzymes by drought stress, rather than key C₄ cycle enzymes (Ghanoum, 2009). Differences in drought sensitivity between sugarcane cultivars (e.g. Innam-Bamber and de Jager, 1986; Smit and Singels, 2006; Silva et al., 2007; Rodrigues et al., 2009), the duration and intensity of the drought treatments, and various other factors could perhaps explain these differences in findings. Clearly, further research is required to advance our understanding of the complex and interacting mechanisms involved in the drought response of photosynthesis in sugarcane and why cultivars differ in their sensitivity.

Looking to the future: increased atmospheric CO₂ and drought adaptation in C₄ plants

Atmospheric CO₂ concentrations have risen from ~270 µl l⁻¹ in pre-industrial times to close to 390 µl l⁻¹ at present, and values could reach between 530 µl l⁻¹ and 970 µl l⁻¹ by the end of this century (Intergovernmental Panel on Climate Change, 2007). Growth of maize under CO₂ enrichment has an impact on the transpiration rates and water status of C₄ plants even under well-watered conditions that is particularly evident in the older leaf ranks (Prins et al., 2011). Under well-watered conditions, elevated CO₂ has little effect on the photosynthesis or growth of C₄ plants in either controlled environments (Ziska and Bunce, 1999; Driscoll et al., 2006; Soares et al., 2007; Prins et al., 2010) or in the field free-air concentration enrichment experiments (FACE) studies (Leakey et al. 2009a, b). However, the negative effects of drought on yield and biomass are attenuated at high CO₂ in both C₃ and C₄ plants because of stomatal closure (Conley et al., 2001; Wullschleger et al., 2002; Wu et al., 2004). The combined effects of high elevated CO₂ and drought were assessed by comparing the performance of two CIMMYT tropical maize hybrids under conditions of optimal water supply, moderate water stress, and severe water stress (Table 1 and Figs 4–7). Both maize genotypes accumulated more shoot biomass under drought conditions at elevated CO₂ compared with ambient CO₂ conditions (Table 1). This finding is consistent with previous observations of higher biomass production in water-stressed maize plants at elevated CO₂ compared with ambient CO₂ conditions (Table 1). Water loss was slower when maize plants were grown at elevated CO₂ compared with ambient CO₂ conditions (Fig. 4). Drought-induced decreases in CO₂ assimilation rate and in stomatal conductance were more rapid in maize plants grown under ambient CO₂ conditions compared with elevated CO₂ conditions (Figs 5, 6). Moreover, transpiration efficiencies were significantly higher at elevated CO₂ (Fig. 7). These observations clearly illustrate the better performance of maize leaf photosynthesis under conditions of drought when plants are grown at high CO₂. This may be due to a combination of factors including enhanced WUE, lower stomatal conductance rates, and possibly enhanced root proliferation (Wullschleger et al., 2002).

Drought stress may also be triggered in leaves by increased temperatures. While growth under elevated CO₂ can partially ameliorate the negative effects of drought in both C₃ and C₄ species, it is unlikely that high CO₂ will offer any protection from heat stress (D. Wang et al., 2008). Rather, high CO₂ may exacerbate the negative effects of high temperatures. Plants grown under drought stress and/or elevated CO₂ tend to exhibit higher leaf temperatures because transpiration rates are decreased and thus less latent heat is lost. Photosynthesis may therefore be more sensitive to acute heat stress under conditions of drought and/or elevated CO₂. High temperatures often occur along with drought, and little is known about the interaction of
the two stress factors—because most studies concentrate on one of them at a time (due to the inherent complexity). So, basically, more studies on this interaction are needed.

**Final remarks and perspectives**

In previous decades, the increases in the yield potential in C₃ crops such as wheat were largely achieved through improvements in harvest index. While further large increases in harvest index are considered to be unlikely, it may be possible to increase productive biomass through enhanced photosynthetic capacity and efficiency (Parry et al., 2011). The yield potential of maize hybrids has increased steadily over the past 70 years in the USA (Tollenaar and Lee, 2002). However, major advances, if not breakthroughs, in breeding strategies are required for further increases in the yield potential of C₄ crops such as maize (Duvick and Cassman, 1999). Moreover, enhanced drought tolerance must be achieved without creating penalties in yield potential (van Ginkel et al., 1998). While genotypes with high yield potential generally perform well under rain-fed and irrigated treatments (Tollenaar and Wu, 1999; Tollenaar and Lee, 2002), the selection for high yielding varieties under largely stress-free conditions has to a certain extent improved yield under water-limiting conditions (Cattivelli et al., 2008). In contrast, the selection for drought adaptation has often resulted in lower genetic gains

### Table 1. A comparison of the effects of drought on the growth of two CIMMYT maize genotypes (14 and 9) that were selected on the basis of differing ASI (anthesis–silking interval) values, under ambient CO₂ levels (350 µl l⁻¹) or with CO₂ enrichment (700 µl l⁻¹).

<table>
<thead>
<tr>
<th>Maize genotype</th>
<th>Growth CO₂</th>
<th>WR</th>
<th>Shoot biomass (g FW)</th>
<th>Shoot biomass (g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 9</td>
<td>Air</td>
<td>MS</td>
<td>136.9±26.6</td>
<td>11.6±0.9</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>96.9±8.4</td>
<td>8.9±1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>MS</td>
<td>162.4±16.0</td>
<td>13.3±2.3</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>101.1±10.7</td>
<td>9.8±2.2</td>
<td></td>
</tr>
<tr>
<td>Genotype 14</td>
<td>Air</td>
<td>MS</td>
<td>125.9±35.3</td>
<td>9.6±3.9</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>92.1±12.0</td>
<td>6.8±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>MS</td>
<td>151.2±27.3</td>
<td>12.0±1.1</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>109.4±15.9</td>
<td>9.7±1.4</td>
<td></td>
</tr>
</tbody>
</table>

CO₂, <0.05; genotype, NS; CO₂×genotype, NS; WR, <0.0001; CO₂×WR, NS; CO₂×genotype×WR, NS.

NS, not significant.

**Fig. 4.** A comparison of the effects of drought on soil water content in the pots in two CIMMYT maize genotypes (14, top panel, and 9, bottom panel) that were selected on the basis of differing ASI (anthesis–silking interval) values, under ambient CO₂ levels (350 µl l⁻¹) or with CO₂ enrichment (700 µl l⁻¹). Plants were grown for 5 weeks under an optimal watering regime (WW), and two additional water regimes (WR) were then applied for 7 d, involving either a moderate drought stress (MS) where water was provided every other day or severe stress (SS) where water was completely withheld for the whole 7 d period of the experiment. The total fresh weight (FW) and dry weight (DW) values of the shoots were determined on day 7 of the drought treatment experiment. The analysis of variance and probabilities were then calculated in relation to CO₂, genotype, and WR effects. (Campos et al., 2006). Despite the lower genetic gains obtained by the slower conventional breeding approaches, some literature examples bear testimony to the fact that selection for drought tolerance in routine maize breeding programmes with adequate weighting can result in significant increases in yield (Banziger et al., 2004).

Breeding for high yield in drought-prone environments using multilocation testing remains inherently complicated by the year to year variability in the amount and temporal distribution of available soil water and the low heritability of drought resistance traits under these conditions. An increasing array of useful molecular tools that can be applied in classical breeding approaches is now available to plant breeders, together with a greater knowledge of the traits that sustain yield under drought. Transcript profiling studies on maize leaves exposed to drought has revealed an
Fig. 5. A comparison of the effects of drought on photosynthesis (A) in two CIMMYT maize genotypes [genotype 9 corresponds to (a) and (c) and genotype 14 corresponds to (b) and (d)] that were selected on the basis of differing ASI (anthesis–silking interval) values, under ambient CO₂ levels (350 μl l⁻¹) in (a) and (b) or with CO₂ enrichment (700 μl l⁻¹) in (c) and (d). Plants were grown for 5 weeks under an optimal watering regime (WW), and two additional water regimes (WR) were then applied for 7 days, involving either a moderate drought stress (MS) where water was provided every other day or severe stress (SS) where water was completely withheld for the whole 7 d period of the experiment. Photosynthetic gas exchange measurements were performed daily throughout the course of the drought treatment. Measurements were performed at an irradiance of 1000 μmol m⁻² s⁻¹ and with either ambient (350 μl l⁻¹) or high (700 μl l⁻¹) CO₂ levels.

Fig. 6. A comparison of the effects of drought on stomatal conductance (gₛ) in two CIMMYT maize genotypes [genotype 9 corresponds to (a) and (c) and genotype 14 corresponds to (b) and (d)] that were selected on the basis of differing ASI (anthesis–silking interval) values, under ambient CO₂ levels (350 μl l⁻¹) in (a) and (b) or with CO₂ enrichment (700 μl l⁻¹) in (c) and (d). Plants were grown for 5 weeks under an optimal watering regime (WW), and two additional water regimes (WR) were then applied for 7 days, involving either a moderate drought stress (MS) where water was provided every other day or severe stress (SS) where water was completely withheld for the whole 7 d period of the experiment. Photosynthetic gas exchange measurements were performed daily throughout the course of the drought treatment. Measurements were performed at an irradiance of 1000 μmol m⁻² s⁻¹ and with either ambient (350 μl l⁻¹) or high (700 μl l⁻¹) CO₂ levels.
induction of stress-associated genes (Zheng et al., 2004; Jia et al., 2006; Yue et al., 2008). Such molecular approaches provide essential candidate gene sequences that allow dissection of QTLs or transgenic approaches to drought tolerance (Shou et al., 2004; Cattivelli et al., 2008; Wang et al., 2008). The most effective QTLs and/or transgenes can be accumulated in elite genotypes, parental inbreds, and hybrids without detrimental effects on yield potential. Prediction models are becoming more accurate as genetic analyses are effectively combined with high throughput phenotyping in the field (Collins et al., 2008; Tardieu and Tuberosa, 2010; Araus et al., 2011). Molecular genetic approaches are increasingly used to characterize the complex network of drought-related traits in C₄ plants (Bolanos and Edmeades, 1993; Bruce et al., 2002), and traits such as stay green are also proving to be useful in selection for enhanced drought tolerance in maize (Haussman et al., 2002). To date, however, relatively few applications for DNA marker technologies have emerged in practical maize breeding programmes, presumably because of the complexities associated with genetic background, developmental stage, and environment on QTL effects, as well as inadequate phenotyping, gene-by-gene effects and the time and cost considerations in developing these tools. Since the costs of applying marker-assisted selection or genomics for many QTLs/markers with small effects are often greater than those of conventional cross-breeding, it may be preferable to target only major QTLs/markers with a considerable effect that is consistent across germplasm. Unfortunately, to date, studies have not identified a QTL/marker in maize with sufficiently large effects to be used effectively. Thus, an improved conceptual framework for the molecular breeding of drought tolerance in C₄ plants is required, together with its implementation in future QTL and -omics studies.

Acknowledgements

JLA acknowledges the support of the DTMA project (Bill and Melinda Gates Foundation) and the 08.7860.3-001.00 project (BMZ, Germany). We thank Dr M. A. Smit (SASRI) for Figs 2 and 3. CHF acknowledges the support of the European Union (Projects ITN: PITN-GA-2008-215174 Chloroplast Signals and FP7- PIRSES-GA-2008-230830 Legume Improvement). We thank J.P. Ferrio (UdL, Spain) and L. Cabrera-Bosquet (UB, Spain) for critical reading of the manuscript.

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