Physiological and Molecular Approaches to Improve Drought Resistance in Soybean

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Mini Review

Drought stress is a major constraint to the production and yield stability of soybean [*Glycine max* (L.) Merr.]. For developing high yielding varieties under drought conditions, the most widely employed criterion has traditionally been direct selection for yield stability over multiple locations. However, this approach is time consuming and labor intensive, because yield is a highly quantitative trait with low heritability, and influenced by differences arising from soil heterogeneity and environmental factors. The alternative strategy of indirect selection using secondary traits has succeeded only in a few crops, due to problems with repeatability and lack of phenotyping strategies, especially for root-related traits. Considerable efforts have been directed towards identifying traits associated with drought resistance in soybean. With the availability of the whole genome sequence, physical maps, genetics and functional genomics tools, integrated approaches using molecular breeding and genetic engineering offer new opportunities for improving drought resistance in soybean. Genetic engineering for drought resistance with candidate genes has been reported in the major food crops, and efforts for developing drought-resistant soybean lines are in progress. The objective of this review is to consolidate the current knowledge of physiology, molecular breeding and functional genomics which may be influential in integrating breeding and genetic engineering approaches for drought resistance in soybean.

Keywords: Drought resistance • Genetic engineering • Marker-assisted selection • Physiological traits • Soybean [*Glycine max* (L.) Merr].

Abbreviations: AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; CID, carbon isotope discrimination; EST, expressed sequence tag; g_e, leaf epidermal conductance; HI, harvest index; LASH, leaf ash; LG, linkage group; OA, osmotic adjustment; PI line, plant introduction line; QTL, quantitative trait locus; RAPD, random amplified polymorphic DNA; RIL, recombinant inbred line; RFLP, restriction fragment length polymorphism; RWC, relative water content; SSR, simple sequence repeat; STS, sequence-tagged site; TE, transpiration efficiency; TF, transcription factor; WUE, water use efficiency.

Introduction

Soybean is the world's leading economic oilseed crop. Processed soybeans are also the largest source of vegetable oil and protein feed. In addition to being a source of macronutrients and minerals, soybeans contain secondary metabolites such as isoflavones (Sakai and Kogiso 2008), saponins, phytic acid, oligosaccharides, goitrogens (Liener 1994) and phytoestrogens (Ososki and Kennelly 2003). Global production of soybean in 2007 was around 219.8 million metric tonnes (mmt). The country with the greatest output was the USA, producing 70.4 mmt, followed by Brazil (61 mmt), Argentina (47 mmt) and China (14.3 mmt) (Soystats 2008). While soybean has long been important in Japan for the production of traditional foods such as tofu, miso, shoyu and vegetable oil, the consumption of soybean-based products is increasing worldwide because of the reported beneficial effects including lowering of cholesterol, prevention of cancer, diabetes and obesity, and protection against bowel and kidney diseases (Friedman and Brandon 2001). Soybean is also viewed as an attractive crop for the production of biodiesel (Pimentel and Patzek 2008). It also has the ability to fix atmospheric nitrogen (Burris and Roberts 1993) and therefore requires minimal input of nitrogen fertilizer which

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often accounts for the single largest energy input in agriculture.

Plants are subjected to a range of abiotic and biotic stresses that affect their growth and development. In particular, it is predicted that water deficit will continue to be a major abiotic factor affecting global crop yields (Sharma and Lavanya 2002). One-third of the world's population resides in water-stressed regions, and with elevated CO_2 levels in the atmosphere and climatic changes predicted in the future, drought could become more frequent and severe. The resilience of legume crops against present-day weather extremes, such as drought, excess water, heat, cool weather during grain filling, and early frost, is considered to predict their adaptation to future climate change (Cutforth et al. 2007). In soybean, drought reduces yield by about 40% (Specht et al. 1999). Depending on hybrid characteristics, soybeans use about 450–700 mm of water during the growing season (Dogan et al. 2007). The most critical period for water stress in soybean has been reported to be during the flowering stage and the period following flowering (Meckel et al. 1984).

Plants use various mechanisms to cope with drought stress. These may be classified into three groups: drought escape, drought avoidance and drought tolerance (Turner et al. 2001). Drought escape allows the plant to complete its life cycle during the period of sufficient water supply before the onset of drought. Normally the life cycle is shorter and plants set some seeds instead of complete crop failure. An example of drought escape is the Early Soybean Planting System, now used widely in the southern USA. In this system, short season cultivars are planted during March–April in regions where later maturing cultivars have previously been grown. The early maturing cultivars start flowering in late April to early May and set pods in late May, thus completing the reproductive stage before the period of possible drought in July–August (Heatherly and Elmore 2004). The second mechanism, drought avoidance, involves strategies which help the plant maintain high water status during periods of stress, either by efficient water absorption from roots or by reducing evapotranspiration from aerial parts. The third mechanism, drought tolerance, allows the plant to maintain turgor and continue metabolism even at low water potential, e.g. by protoplasmic tolerance or synthesis of osmoprotectants, osmolytes or compatible solutes (Nguyen et al. 1997). In soybean, a widely accepted equation for grain yield (Y) under water-limited conditions is a function of three components, namely the amount of water transpired (T), water-use efficiency (WUE) and harvest index (HI); $Y = T \times WUE \times HI$ (Turner et al. 2001). Maintenance of optimum transpiration, leading to increased WUE, is one of the strategies to improve yield in soybean. Nine secondary traits have been reported to be associated with the likelihood of increasing or maintaining T during drought. These traits are phenology, photoperiod sensitivity, developmental plasticity, leaf area maintenance, heat tolerance, osmotic adjustment, early vigor, rooting depth and rooting density. Additionally, transpiration efficiency and leaf reflectance are the other two traits related to WUE (Purcell and Specht 2004).

Significant progress in breeding for drought resistance cannot be made unless the stress is imposed year after year to allow direct selection for yield. For this reason, a field with poor moisture-holding capacity, soil uniformity and a reasonable drought each year is important, but is unfortunately very difficult to achieve (Pathan et al. 2007). In order to enable high throughput and reliable gravimetric measurements of water relation traits and water use in soybean, it is also important to include modern facilities such as a rainout shelter in a breeding program. By using a rainout shelter with a movable roof, researchers at the National Center for Plant Gene Research (NCPGR) in Wuhan, China, have succeeded in dissecting the genetics of complex traits such as drought resistance (Pennisi 2008). The plant-wise drought treatment strategy developed at the NCPGR provides a useful method for independent evaluation of the individual components of drought resistance (Yue et al. 2006). In addition, current advances, such as automated plant phenotyping platforms to study the plant responses to soil water deficit under controlled conditions (Granier et al. 2006), an automated rotating lysimeter system for evapotranspiration and plant growth measurements (Lazarovitch et al. 2006), non-destructive measurement of plant water status over time using portable unilateral nuclear magnetic resonance (Capitani et al. 2009), and other precision equipments to quantify plant water use should be exploited. Screening of genotypes in such controlled facilities has the advantage of quantifying the morpho-physiological traits that influence drought adaptation, which in turn will complement yield selection criteria.

In this review, we first concentrate on the major physiological traits related to the mechanisms of drought resistance in soybean (**Fig. 1**). Although the emphasis of this article is on soybean, the basic concepts are applicable to drought resistance improvement in other crops as well. Recent advances in marker-assisted selection, functional genomics and genetic engineering approaches to achieve drought resistance in soybean will also be discussed.

Physiological and biochemical responses associated with drought resistance in soybean

Root-related traits

Root morphology and plasticity. Soybean plants often undergo substantial water deficits, even though water is readily available only slightly deeper in the soil. Plants can
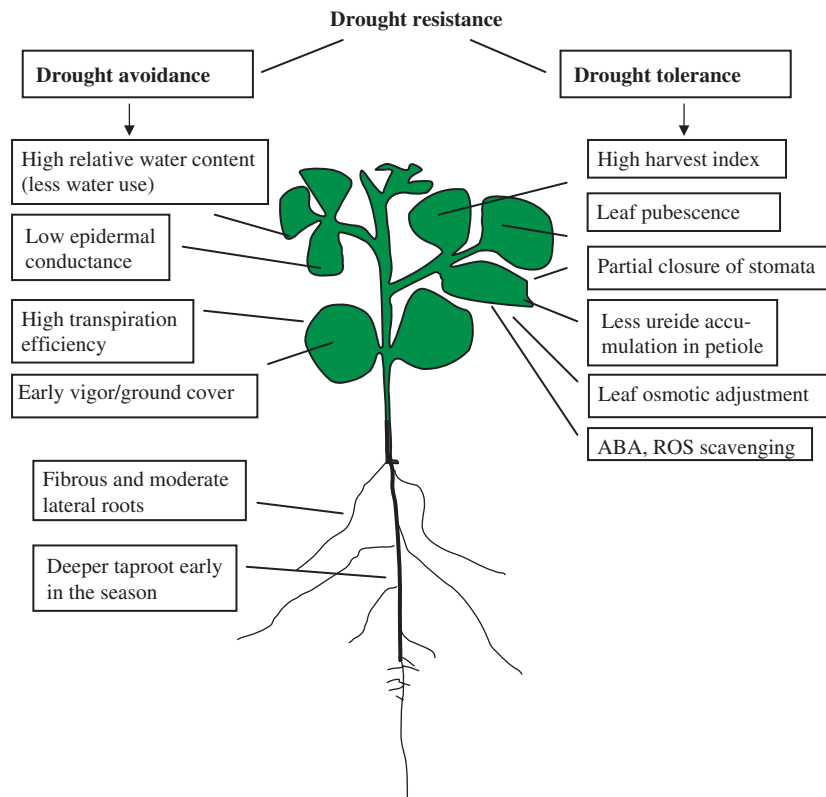
Fig. 1 Traits associated with drought resistance in soybean. ROS, reactive oxygen species.

adapt to drought by developing a longer taproot which helps reach the lower soil layers where water is available (Taylor et al. 1978). In addition, an extensive fibrous root system can be useful for foraging subsoil surface moisture and nutrients such as phosphorus.

One of the major factors influencing soybean rooting depth is taproot elongation rate. As taproots are the first formed roots in soybean, identification of genotypes with rapidly elongating taproots under non-stress conditions may allow the determination of deeper rooting ability. Genotypic variation in the taproot elongation rate in soybean cultivars has been well documented in greenhouse studies. Kaspar et al. (1984) evaluated 105 diverse soybean genotypes from different maturity groups. Taproot elongation within a maturity group varied by as much as 1.3 cm d^{-1}, and contrasting cultivars were selected for field study. The results of these studies indicated that the group of cultivars with faster elongation rates was also more able to deplete soil water 120 cm below the surface. Limited research has been reported regarding genetic variability in producing lateral roots. Under stress conditions, the number of lateral roots per unit of taproot length significantly increased, but no promotion of taproot length or diameter was observed (Read and Bartlett 1972).

Because root growth and water deployment are critical to maintaining function in different environmental conditions, plasticity for root traits is a critical factor to acquire resources. Water stress tends to increase biomass partitioning to roots, increasing the root:shoot ratio. Non-irrigated soybeans showed an increase in root length when compared with irrigated plants, especially in the subsoil (Huck et al. 1983). Significant correlations exist in soybean between drought resistance and various root traits such as dry weight, total length, and volume and number of lateral roots (Read and Bartlett 1972, Liu et al. 2005a). These authors proposed using these traits as indicators for drought resistance. Soybean plant growth and development are classified into vegetative (V) and reproductive (R) stages. The V stage comprises stages VE to Vn, where VE is vegetative cotyledon emergence and Vn are numbered according to how many fully developed trifoliate leaves are present. The R stage comprises flowering in stages R1 and R2, pod formation in stages R3 and R4, and seed filling and maturation development in stages R5–R8. In soybean, when drought was imposed during the later stages of vegetative and/or the early stages of reproductive growth (R1–R2), a large increase in root growth was observed in non-irrigated plants, especially in the deeper soil layers. Root growth was less affected when drought was imposed at

the R4 stage and ceased at the R5 stage (Hoogenboom et al. 1987). In another study, it was demonstrated that soybean plants which experienced drought before flowering had higher seed yields than those stressed after flowering, because they had already developed a large root system before flowering (Hirasawa et al. 1994). This shows that if a plant develops a large root system during its early vegetative growth, it would be in an excellent position to maintain turgor under drought conditions.

All of these early studies were based on popular cultivars/genotypes, and less is known about the rooting ability and/or plasticity of root growth in exotic plant introduction (PIs) lines. The line 'PI416937' has an exceptionally branched fibrous root system (Carter and Rufty 1993, Hudak and Paterson 1996) which has shown drought resistance in the field by limiting transpiration during the high evaporative demand period (Fletcher et al. 2007). The fibrous root trait of 'PI416937' also conferred increased nitrogen fixation through increased root surface area. 'PI416937' possesses the genetic capability to continue root growth in compacted soils (Busscher et al. 2000). However, no correlation was demonstrated between the rooting ability and drought resistance of this PI line, although a positive correlation between rooting ability and seed protein concentration was found (Pantalone et al. 1996).

The vast diversity of PI lines and cultivars worldwide provides valuable resources for identification of germplasm which can be used in breeding for root traits. However, the laborious procedures involved in separation of roots and the lack of rapid screening procedures are the major constraints for genetic improvement of root traits. Since a deep taproot system associated with a moderate number of lateral roots to extract soil water is the most promising genetic mechanism for improving soybean drought resistance, research in this direction is imperative.

Overall, root traits show strong potential for improvement of drought resistance through breeding. However, it would be extremely difficult to perform selection based on measuring the root phenotype. Instead, molecular tagging will facilitate breeding for root-related traits. The candidate gene approach is another alternative methodology, which involves choosing a candidate gene for root traits from public data, obtaining primer sequences to amplify the gene, uncovering polymorphisms, developing a convenient procedure for large-scale genotyping, identifying a population for association studies, carrying out an association study of the candidate gene with trait phenotype and finally verifying the uncovered associations. This approach was successful in finding candidate genes associated with root number in rice under low moisture conditions (Vinod et al. 2006).

Understanding the physiological mechanisms and genetic regulation of root adaptation to drought will help to identify specific genes and metabolic pathways for either gene-based

marker selection or genetic engineering to develop soybean with better root-related traits.

Nitrogen fixation under drought. Nitrogen (N_2) fixation in legumes is very sensitive to soil drying. In drought conditions, soybean not only suffers losses of CO_2 accumulation and reduced leaf area development, but its symbiotic N_2 fixation is also especially vulnerable (Sinclair and Serraj 1995). In dry soils, this results in a reduced supply of N_2 for protein production, which is the critical seed product of the plant, and consequently lower crop yields (Purcell and King 1996). Several factors have been related to the inhibition of N_2 fixation under drought, including reduced oxygen availability, reduction in carbon flux to nodules, decline in nodule sucrose synthase activity and an increase in ureides and free amino acids (Durand et al. 1987, Gonzalez et al. 1995, Arrese-Igor et al. 1999, King and Purcell 2005). In particular, a close relationship was found between leaf and nodule water potential (Durand et al. 1987). Nitrogenase activity showed a 70% decrease during the first 4 d of drought, while photosynthesis only declined by 5%. This implies that water stress exerts an influence on nitrogenase activity that is independent of rate of photosynthesis. It was also found that water deficit directly affected nodule activity through increased resistance to oxygen diffusion to the bacteroid. The increase in oxygen diffusion resistance, decrease in nitrogenase-linked respiration and enzyme activity, accumulation of respiratory substrates and oxidized lipids, and the up-regulation of antioxidant genes together demonstrate that the respiratory activity of bacteroids is impaired in drought conditions, and that oxidative damage occurs in nodules prior to any detectable effect on sucrose synthesis or leghemoglobin (Naya et al. 2007). Soil drying also leads to the accumulation of ureides in soybean leaves and is thought to be an inhibitor of nodulation (Sinclair and Serraj 1995).

Substantial genetic variation was found in the sensitivity of N_2 fixation in response to soil drying (Sall and Sinclair 1991). When comparing soybean germplasm with or without the capacity to maintain tissue turgidity, and therefore leaf and nodule function, under drought conditions at the reproductive stage, the germplasm able to maintain tissue turgity showed the least reduction in grain yield (Patterson and Hudak 1996). Screening for petiole ureide levels is an effective initial approach to identify soybean lines whose N_2 fixation is more tolerant of soil drying. By screening a large collection (> 3,000) of soybean PI lines in this way, eight lines were identified that exhibited N_2 fixation drought tolerance (Sinclair et al. 2000). In a further study, Sinclair et al. (2003) found that ureide catabolism independent of manganese was active in six of the eight PI lines. The variety 'Jackson', which is more tolerant of N_2 fixation in drying soils, has been used as a parent for developing high yielding lines for drought conditions. Two high yielding progeny lines for non-irrigated conditions that exploit the N_2 fixation drought tolerance

trait of 'Jackson' are considered a potential genetic resource for improved symbiotic N_2 fixation and increased yield under rainfed conditions (Sinclair et al. 2007).

It is unclear whether N_2 fixation under drought is only regulated at the whole-plant level (e.g. by a systemic nitrogen feedback mechanism) or whether it can also occur at a local nodule level. There is evidence that signaling for biological N_2 fixation regulation can be provided by an N_2 feedback mechanism involving shoot N_2 status (Serraj et al. 2001). King and Purcell (2005) suggested that a combination of ureide and aspartic acid levels in nodules, plus the transport of several amino acids from the leaves, might be involved in such a feedback inhibition mechanism in soybean. A recent study using a partially droughted split-root system indicated that N_2 fixation activity under drought stress is mainly controlled at the local level rather than by a systemic N_2 signal (Marino et al. 2007). More research should be directed toward understanding the molecular genetics of the factors limiting and regulating the response of N_2 fixation under drought.

Shoot-related traits

Stomatal conductance. One of the shoot-related physiological traits that may affect drought tolerance is the decline in whole-plant water use during a soil water deficit event. As a soil water deficit develops, plants undergo a transition from the water-saturated phase, in which whole-plant water use is not dependent on the soil water content, to a second phase where water use is directly related to the availability of soil water (Sinclair and Ludlow 1986). This transition is associated with a reduction in the average stomatal conductance, and can occur at different soil water contents in different species (Earl 2003). Stomatal conductance is a key variable influencing leaf gas and water vapor exchange. The effects of drought stress on leaf growth, stomatal conductance and plant water relations have been well addressed in soybean (Muchow 1985, Bennett et al. 1987, Ray and Sinclair 1998). It was shown that drought stress decreases relative leaf expansion rate, stomatal conductance and leaf turgor, whereas it increases the content of ABA in the leaf and xylem (Liu et al. 2003). In this study, decreased stomatal conductance coincided with an increase in xylem ABA and occurred before any significant change in leaf turgor was detected, indicating that chemical signals (root-originated ABA) control stomatal behavior at moderate soil water deficit. Relative stomatal conductance decreased exponentially with increasing relative xylem ABA ($r^2 = 0.98$) (Liu et al. 2003). Genotypic differences in the ability to keep stomata open despite internal water stress have been reported in soybean (Vignes et al. 1986). When compared with soybean, other legumes such as green gram (*Vigna radiata*), black gram (*Vigna mungo*) and cowpea (*Vigna unguiculata*) exhibited better stomatal control over water loss (Vignes et al. 1986). Previous research has clearly established that decreases in stomatal conductance are associated with severe plant water deficits in soybean (Bennett et al. 1987, Liu et al. 2005b). Further research is needed to establish the physiological and biochemical basis for stomatal closure during intermittent drought conditions as well as mild soil water deficits, particularly under field conditions. Control of stomatal conductance under drought is a promising physiological trait for developing drought resistance in soybean, considering the non-destructive nature of the measurement and availability of precise instruments for the measurement of conductance. However, a better understanding of the quantitative inheritance of this trait is vital if it is to be used for this purpose.

Epidermal conductance. A second shoot-related physiological trait which may help identify cultivars with increased drought tolerance and prolonged crop survival during severe water stress is low leaf epidermal conductance (g_e). Total leaf conductance to water vapor is the sum of the stomatal and cuticular diffusive pathways acting in parallel (van Gardingen and Grace 1992). When the stomata are open, cuticular conductance is generally a negligible fraction of total conductance. However, in water-stressed or dark-adapted leaves in which the stomata tend to be closed, the cuticular component of leaf epidermal conductance may exceed the stomatal conductance (Boyer et al. 1997). Under severe water deficit when stomatal closure is maximal, g_e (the sum of cuticular conductance and any residual stomatal conductance) determines the rate of water loss from leaf tissues and therefore the rate of progression toward critically low (injurious) leaf water content. Species adapted to arid environments tend to have low g_e (Riederer and Schreiber 2001), and crop species or varieties with low g_e are often those that survive the longest under severe soil water deficits (Sinclair and Ludlow 1986, Jovanovi et al. 1996). Significant variation in epidermal conductance was found among 74 soybean accessions. Expression of genotypic differences in epidermal conductance among the selected accessions was consistent across environments (Paje et al. 1988). Interestingly, these differences in epidermal conductance were not associated with differences in stomatal density. Selection for lower epidermal conductance could allow improved survival of leaves under intermittent water stress, so that sufficient leaf area remains available for growth after the stress is relieved (Paje et al. 1988). Recently, a significant negative correlation ($r = -0.74$) was reported between g_e and WUE under drought, which again supports the hypothesis that lower epidermal conductance is a desirable trait for drought resistance (Hufstetler et al. 2007). Perennial wild-type soybean exhibited lower values of g_e compared with various soybean cultivars and *G. soja*. Among the soybean genotypes, tropically adapted genotypes exhibited the lowest epidermal conductance and more negative osmotic potential when compared with genotypes adapted to temperate regions, suggesting

that the adaptability of tropical genotypes might have resulted from selection for survival under drier environments (James et al. 2008a). Broad-sense heritability for epidermal conductance was significantly different from zero in 10 hybrid populations and ranged from 60 to 93% (James et al. 2008b). The magnitude of the heritability estimates suggested that the trait was highly heritable. However, to validate the usefulness of these traits in breeding for drought resistance, more research is needed to characterize the inter-relationship between g_e, conductance, maintenance of leaf turgor and plant survival.

Leaf pubescence density. Leaf pubescence is a common feature of xerophytic plants, as well as some crop plants including soybean. Generally, leaf pubescence density increases reflectance from the leaf, resulting in lower leaf temperatures under high irradiance. Leaf pubescence density is an important adaptive trait for soybean under water-stressed conditions. Densely pubescent lines had increased vegetative vigor, greater root density and deeper root extension (Garay and Wilhelm 1983). Increased leaf pubescence density may also increase leaf boundary layer resistance by up to 50%. Reduced leaf temperature, restricted transpiration water loss and enhanced photosynthesis due to lower radiation penetration into the canopy were also reported to be associated with the dense pubescence trait (Specht and Williams 1985). Moreover, extra-dense pubescence can significantly reduce the incidence of soybean mosaic virus infection. However, increased pubescence density controlled by the *Pd1* and *Pd2* alleles produces both positive and negative agronomic effects (Pfeiffer et al. 2003). In particular, these alleles have been shown to reduce seed yield, delay maturity and increase plant height, with a concomitant increase in lodging (Specht et al. 1985). Thus, the negative linkage between the leaf pubescence density and seed yield should be considered before introgressing this trait into elite germplasm.

Water use efficiency. The WUE of a plant is generally defined as the amount of biomass accumulated per unit of water used. Genetic variation in WUE has been reported in recent years for many field crops, including soybean (Mian et al. 1996). High WUE can contribute to crop productivity under drought. The positive association between WUE and total biomass yield in a drought environment suggests that improvement of the WUE of a crop plant should result in superior yield performance if a high HI can be maintained (Wright 1996). Soybean cultivar 'Young' exhibited considerably higher WUE (4.4 g DW l^{-1}) than did 'PI416937' (3.7 g DW l^{-1}) in greenhouse conditions (Mian et al. 1996). In another greenhouse study, soybean cultivar 'Jackson' accumulated more biomass and total nitrogen than 'PI416937' with similar transpirational losses (Purcell et al. 1997). Significant genetic differences were observed for β, which is an empirical seed yield based on estimation of genotypic WUE. The close association between genotypic β and genotypic mean yield ($r = 0.71$) provides evidence that soybean WUE can potentially be improved simply by selecting for genotypic mean yield (Specht et al. 2001). In a study using 24 soybean genotypes, WUE ranged from 2.7 g dry matter kg^{-1} water to 3.4 g dry matter kg^{-1} water (Hufstetler et al. 2007). It has been proposed that the aperture of stomata could be regulated in such a way that a partial closure of stomata at a certain level of soil water deficit might lead to an increase in WUE (Liu et al. 2005b). While promising results were observed, many uncertainties still remain regarding the implementation of irrigation techniques in different crop species under varying environmental conditions. Therefore, a deep understanding of the physiological basis for stomatal regulation and improved WUE in drought-stressed plants is required (Liu et al. 2005a).

Another relevant trait related to WUE is transpiration efficiency (TE), which is the assimilation or dry matter accumulation per unit of transpiration (Fischer 1981). TE is under genetic control and excludes amount of water lost by soil evaporation, and hence should be considered as a potential trait.

Osmotic adjustment. Osmotic adjustment (OA) is defined as the active accumulation of solutes that occurs in plant tissues in response to an increasing water deficit. OA is considered a useful measure because it provides a means for maintaining cell turgor when tissue water potential declines. OA has been shown to maintain stomatal conductance and photosynthesis at lower water potentials, delayed leaf senescence and death, reduced flower abortion, improved root growth and increased water extraction from the soil as water deficit develops (Turner et al. 2001). The capacity for OA in soybean ranges from 0.3 to 1.0 MPa, which is not as wide as the ranges observed in chickpea (*Cicer arietinum*) and pigeon pea (*Cajanus cajan*) (0.1–1.3 MPa) (Cortes and Sinclair 1986, Turner et al. 2001). Among six common soybean cultivars tested, leaf OA ranged from 0.3 to 0.5 MPa under prolonged drought control conditions (S. Guttikonda, unpublished). In a recent study, the leaf osmotic potential of cultivated and wild soybean accessions was measured in order to assess the capacity for OA. Leaf osmotic potential at 70% relative water content (RWC) ranged from −2.3 to −3.5 MPa in soybean cultivars and −4.3 MPa in the wild species *G. latifolia* and *G. canescens* (James et al. 2008a). There was a highly significca-tion correlation ($r = 0.98$) between rate of decline in RWC and relative OA among seven different genotypes tested, suggesting that genotypes with higher osmotic potential showed a slower rate of decline in RWC, indicating the ability of these genotypes to maintain turgor for longer periods (James et al. 2008b).

Most of the published studies on OA in crops have used only leaf measurements, and few examined the potential benefits of OA upon crop yield (Serraj and Sinclair 2002).

One study in wheat (Morgan 1995) claimed putative benefits of OA on crop yield. In chickpea, however, the relationship between OA and yield under water-limited conditions was inconsistent (Leport et al. 1999, Moinuddin and Khanna-Chopra 2004, Turner et al. 2007). Turner et al. (2007) studied advanced breeding lines of chickpea over different continents and demonstrated genotypic differences in OA, but the phenotypic expression of OA was not stable and varied from year to year. It was also found that the difference in OA has no effect on yield under terminal drought when grown under rainfed conditions. These inconsistent results could be due to differences in growth habitat, stress level and phenological characteristics of the tested cultivars. An exception where OA may have an important benefit during drought is when it is directed to roots, where turgor maintenance might lead to an increased rooting depth and would thus increase the amount of water available to the crop. Emphasis should be given to studying the role of OA in root tips to improve crop growth under water deficit conditions (Serraj and Sinclair 2002). Another problem with selecting for OA is the limited number of genotypes that could be screened using current water relation traits. Use of multiple psychrometers (Santamaria et al. 1990) and complex relationships between RWC and OA has limited screening to 50 genotypes per study. As suggested by Turner et al (2001), the development and identification of molecular markers, as in rice and wheat, would enable selection for OA in early generation populations of soybean.

Soybean seed and grain filling during drought stress

Yield as measured by the weight of seeds is reduced most by stress occurring during early formation and pod-filling stages. Water stress during early reproductive growth (flowering and pod set) reduces yield, usually as a result of fewer pods and seeds per unit area. It has been shown that root-originated xylem sap ABA can move to reproductive structures causing kernel/pod abortion, presumably via inhibition of cell division in young ovaries (Liu et al. 2003). Stress during seed filling, and after pod and seed numbers are fixed, could affect the seed growth rate by reducing photosynthesis and, therefore, the supply of assimilate available to the seed, ultimately affecting seed size. At the pro-embryo developmental stage, young ovules are undergoing active division, which coincides with rapid pod expansion and is specifically sensitive to water deficit during seed filling (Westgate and Peterson 1993). In a rainout shelter study, the drought tolerance based on seed yield varied among Brazilian soybean cultivars. Cultivars with high drought tolerance maintained a high crop growth rate during the early reproductive stage, associated with high seed yield (Oya et al. 2004). The length of the seed-filling period is another important determinant of yield in all grain crops, including soybean. Seed fill duration is under genetic control (Smith and Nelson 1987) and is sensitive to

water deficit (Meckel et al. 1984). When soybean plants were subjected to continuous water stress from the beginning of growth stage R6 (early in seed filling) until maturity, the plants lost nitrogen and chlorophyll from their leaves more rapidly than non-stressed plants (de Souza et al. 1997). Stress also shortened the seed-filling period (R7 occurred up to 7 d earlier), resulting in smaller seeds (maximum reduction of 32%) and lower yields (up to 44%). Water stress can induce senescence which cannot be stopped by reapplying water, and even short periods of water stress during seed filling might decrease yield by up to 23% (Brevedan and Egli 2003). By applying large quantities of nitrogen fertilizer to supplant the dependency on nitrogen fixation during water deficit, Purcell and King (1996) reported an 18% increase in soybean yield. However, while inherently delayed senescence may be advantageous for yield under optimal growing conditions, it may be of no consequence under post-anthesis stress because then the over-riding stress factor will impose accelerated senescence or leaf killing.

Interestingly, water stress at the flower induction, flowering, pod formation and pod-filling stages of growth did not significantly affect the oil or protein content of soybean seeds (Dornbos and Mullen 1992). Furthermore, drought had little effect on the fatty acid composition of the oil, but high air temperature reduced the proportion of polyunsaturated components. A recent study also showed that irrigation generally had no significant influence on unsaturated fatty acid accumulation in seed oil in soybean genotypes with altered fatty acid profiles (Lee et al. 2008).

In conclusion, the key to a complete understanding of the relationship between water stress and soybean seed growth lies in determining the effect of stress on the supply of assimilate to individual seeds.

Marker-assisted selection for drought resistance

In environments where water is limited, genetic improvement of a crop for drought resistance is an economically attractive option (Blum 2002). However, despite the large resources committed to soybean breeding, progress in improving drought resistance has been slow for a number of reasons. (i) Identifying lines with the highest yield potential under optimum moisture conditions is an important selection criterion in soybean. Conversely, evaluating lines from low yielding environments under drought conditions is often not considered, because small yield differences among lines do not separate high yielding genotypes from low yielding genotypes. (ii) Historically, the emphasis in soybean breeding was upon resistance to biotic stress rather than abiotic stress such as drought, due to the complexity of trait evaluation. This unfortunately resulted in a narrow genetic base for initiating drought resistance breeding programs. (iii) Drought resistance is governed by several complex factors

including genotype, environment and the interaction of the two (Carter et al. 1999). In the future, marker-assisted selection based on genotype will greatly increase breeding efficiency.

Identification of quantitative trait loci (QTLs) related to drought resistance is one of the promising approaches using marker-assisted selection (Pathan et al. 2007). In soybean, a large number of QTLs have been identified for traits related to agronomic, physiological, seed composition, and both biotic and abiotic stress parameters (www.soybase.ncgr.org). However, to date, only a few QTLs have been associated with drought resistance traits (**Table 1**). Reported QTLs also explain <10% of the phenotypic variation for those traits. Most studies for QTL identification have included single and small size populations. The QTL markers identified by a single population may not be automatically applicable to unrelated populations without marker validation and/or fine mapping (Nicholas 2006).

As discussed above, WUE is one of the important traits associated with drought resistance in soybean. Since water consumption and its efficient use by crops are related to yield, carbon isotope discrimination (CID) (Δ, a measure of the $^{13}C/^{12}C$ ratio in plant tissues compared with the air) may provide a useful indirect measure of genetic variation in TE, i.e. the ratio of net assimilation to water transpired, in C3 species (Araus et al. 1998). The expensive equipment and high cost of analyses restrict the use of Δ in breeding programs. However, ash content and K concentration have been proposed as cheap and easily determined surrogates of Δ (Tsialtas et al. 2002). Due to its inherent analytical simplicity, estimation of ash content in vegetative organs became an attractive alternative to Δ for a preliminary screening of large, genetically diverse populations (Masle et al. 1992). Ash concentration (leaf and stem) of crested wheatgrass (*Agropyron desertorum*) was negatively associated with WUE (Mayland et al. 1993). The relationship between ash content and WUE depends on the maintenance of a constant concentration of minerals in the transpiration stream. While environmental conditions could cause variation in the mineral concentration, comparison of different genotypes under the same environmental conditions would nonetheless provide repeatable responses. A molecular investigation may provide some insight into the genetic relationships between WUE and leaf ash (LASH) (Mian et al. 1996).

To identify QTLs associated with WUE (total plant dry weight/amount of water used in liters) and LASH in 36-day-old, greenhouse-grown plants, Mian et al. (1996) constructed a restriction fragment length polymorphism (RFLP) map from a soybean [*G. max* (L.) Merr] population of 120 F_4-derived lines from a cross of' Young×PI416937'. Significant ($P < 0.01$) phenotypic differences were detected among the lines for both traits. A total of four and six independent RFLP markers were associated with WUE and LASH, respectively, and, if combined, each group of markers would explain 38 and 53% of the variability in the respective traits. One marker locus (cr497-1), on USDA Linkage Group (LG) J, explained 13.2% of the variation in WUE, indicating the presence of a major QTL. The authors also found that LASH was negatively correlated with WUE ($r = -0.40$), and two QTLs were associated with both WUE and LASH. For each of these QTLs, the allele for increased WUE was associated with reduced LASH.

To test the consistency of WUE QTLs across populations, Mian et al. (1998) screened another F_2-derived soybean population from the cross of 'S100×PI416937'. They discovered that one QTL linked to the RFLP marker A063E for WUE, was also found in the 'Young ×P1416937' population, but the phenotypic effect was <10%. These two loci, however, have not yet been confirmed in any other genetic background or environmental conditions. To determine the genetic basis of β and CID, 236 recombinant inbred lines (RILs) have been developed from a cross between 'Minsoy' and 'Noir 1'. Only

Table 1 Quantitative trait loci (QTLs) for drought resistance in soybean

Mapping populations	Reported traits (QTL number)	Marker linked to QTL, linkage group (LG) and contribution (R^2)	Reference
Hutcheson×PI471938, 140 F_4 population	Yield (3), wilting (3)	Satt226, LG-D2; Sat_375, LG-F1; Sat_074, LG-F2	Monteros et al. (2006)
Jackson×KS4895, 81 RILs	Leaf wilting (1)	Sat_044, LG-K, $R^2 = 17$	Bhatnagar et al. (2005)
Minsoy×Noir 1,236 RILs	Yield (1)	Satt205-satt489, LG-C2, $R^2 = 7$	Specht et al. (2001)
S-100×Tokyo, 116 F_2 population	Water use efficiency (2)	A489H, LG-L, $R^2 = 14$A063−1, unlinked, $R^2 = 8$	Mian et al. (1998)
Young×PI416937, 120 F_4 population	Water use efficiency (5)	B031-1, LG-G, $R^2 = 8.5$	Mian et al. (1996)
		A089-1, LG-H, $R^2 = 8.7$	
		cr497-1, LG-J, $R^2 = 13.2$	
		K375-1, LG-J, $R^2 = 7.5$	
		A063-1, LG-C1, $R^2 = 5$	

LG, linkage group; R^2, percentage of trait variance explained by a QTL; RIL, recombinant inbred line.

one QTL was identified for CID on LG-C2, with a phenotypic contribution of <10% and with no effect on β (Specht et al. 2001).

So far QTLs in soybean under water deficit conditions have been reported only for WUE and LASH. More studies are needed to identify QTLs that influence root architecture and shoot turgor maintenance. Mapping for new QTL(s)/ gene(s) and determination of gene action under drought will probably provide key resources to improve tolerance to drought stress in soybean. High-density genetic maps and confirmed QTLs/genes, which are screened across the various environments and across genetic backgrounds, are the most important criteria for developing drought-resistant soybeans through marker-assisted selection (Vuong et al. 2007).

Functional genomics resources and tools for drought research in soybean

Plant functional genomics has emerged as a new and rapidly evolving scientific discipline to study the functions of genes. In the last decade, considerable progress has been made in developing genomic resources for soybean, including the sequencing of the entire soybean genome of approximately 975 Mb (http://www.phytozome.net/soybean#C). For the genetic and genomic analyses of the soybean genome, precise genetic and physical maps are important. Various types of physical maps have so far been reported using RFLP, random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers (Shinozaki 2007). A high-density genetic linkage map of soybean using expressed sequence tag (EST)-derived microsatellite markers was generated using a hybrid between the Japanese cultivar 'Misuzudaizu' and the Chinese line 'Moshidou Gong 503' by Japanese researchers (Hisano et al. 2007, Xia et al. 2007). The possibility of segmental duplications in the previously suggested regions of the soybean genome was confirmed by inspecting the co-linearity between the genomes of soybean and *Lotus japonicus*. This comparison, combined with information relating to regulatory networks deduced by transcriptional profiling, may provide clues toward understanding the evolutionary process of the paleopolyploid soybean genome (Hisano et al. 2007). Microsatellite markers have been discovered from bacterial artificial chromosome (BAC) libraries to create detailed genetic and physical maps of the soybean genome, which together cover the soybean genome by >35-fold (Shoemaker et al. 2008). The constructed Williams 82 *Bsty*I library contains 92,160 clones with an average insert size of 150 kb and covers approximately 12 genome equivalents (http://www.soybeangenomics.org/genome_mapping. php). A genetic map was then integrated into this physical map by anchoring approximately 1,000 SSR and sequence

tagged site (STS) markers, developed from ESTs, which are associated with drought responses, disease resistance, seed development and composition traits (Wu et al. 2008). In addition, a six-dimensional pool array has been recently developed from 49,152 *Bsty*I soybean clones (~6×genome equivalents), comprising 208 BAC pools (Wu et al. 2008). The integrated genetic and physical map will be useful for comparative genetic analysis, map-based cloning of QTLs of desired traits, and genomic sequencing. In addition, yeast artificial chromosomes have also been created for the purpose of chromosome walking and in situ hybridization (Santra et al. 2003). Recently, the entire soybean genomic sequence has been released with 66,153 protein-coding loci (http://www.phytozome.net/soybean#C).

EST sequencing projects in soybean have resulted in a wealth of DNA sequence information. A spotted cDNA microarray is available containing 36,000 elements constructed from soybean cDNAs, which were derived from a variety of EST libraries representing a wide source of tissues and organs, developmental stages and stress-related or pathogen-infected plants (Vodkin et al. 2004). The ESTs isolated from the subtracted library of drought-stressed soybean root tips and submitted to GenBank will greatly contribute to the stress-specific unigenes for further functional genomics work aimed at better understanding of the drought stress response of the soybean root system (Valliyodan and Nguyen 2008). This result will provide an important resource for revealing the transcriptional regulatory networks which will illuminate our understanding of the drought stress responses of soybean at different developmental stages.

Full-length cDNAs are important tools for functional analysis of genes, and provide a valuable resource for the improvement of soybean productivity and drought resistance by biotechnology. Umezawa et al (2008) obtained 6570 new full-length sequences of soybean cDNAs derived from tissues treated with different abiotic stresses, in order to analyze gene functions and structures. About 88% of the soybean cDNA clones contained complete coding sequences in addition to 5'- and 3'-untranslated regions.

Although the gene expression profiling data created by microarray analysis have been commonly used as valuable information in functional genomics or in the selection of candidate genes for genetic engineering of soybean plants, the integration of transcriptomics, proteomics and metabolomics represents the frontier of integrated functional genomics. It provides an unprecedented opportunity to gain a whole picture of the physiological and biochemical responses to drought stress. Taking advantage of available proteomic technology, a proteomic analysis of drought-treated soybean roots, leaves and seeds has revealed region-specific regulation of metabolism in the growth zone of water-stressed soybean roots (M. Yamaguchi, unpublished). While transcriptomic data and proteomic analyses do

not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology and biochemical changes in the cell (Morgenthal et al. 2007). It has long been known that in addition to ABA, a variety of osmolytes, including certain sugars (such as raffinose family oligosaccharides, sucrose, trehalose and sorbitol), sugar alcohols (such as mannitol), amino acids (such as proline) and amines (such as glycine betaine and polyamines), accumulate in plants in response to drought stress (Umezawa et al. 2006, Seki et al. 2007). However, the degree to which the metabolome is altered in response to drought stress has remained a mystery. The recent development of highly sensitive metabolite profiling technologies, such as gas chromatography or liquid chromatography coupled with mass spectrometry [GC-time-of-flight (TOF)/MS or UPLC-Q-TOF-MS] makes such comprehensive analysis possible. Recently, Urano et al (2009) used GC-TOF/MS to detail the changes that occur in the metabolome of Arabidopsis in response to drought stress, and to assess the extent to which the dehydrated metabolome is reconfigured by inactivation of the NCED3 gene which is involved in the dehydration-inducible biosynthesis of ABA. Although the role of a number of accumulated metabolites remained unknown, this study has revealed new molecular mechanisms of dynamic metabolic networks in response to drought stress. Additionally, genetic engineering using genes encoding components of drought stress-related metabolic pathways has shown the potential to enhance drought resistance in important crops, including rice, wheat and soybean (de Ronde et al. 2004a, de Ronde et al. 2004b, Umezawa et al. 2006, Seki et al. 2007). Thus, metabolomics, in addition to transcriptomics and proteomics, provides a major tool for characterizing the drought stress response in soybean in the post-genomics era.

Both gain-of-function and loss-of-function approaches can be used to elucidate gene function, as well as provide novel tools and mutant resources for improving drought resistance in plants (Timpte et al. 1994, Klein et al. 2004). Antisense- and RNA interference (RNAi)-mediated transcriptional or post-transcriptional gene silencing has been used to study soybean gene function (Buhr et al. 2002, Nunes et al. 2005, Subramanian et al. 2005). Alternatively, fast neutrons, X-rays, γ-rays and chemical mutagenesis are effective methods to generate mutants in plants for drought research, including legumes such as *Medicago trunculata*, *L. japonicus* and soybean. LegumeBase (http://www.legumebase.agr.miyazaki-u.ac.jp) provides a list of mutant resources in these legumes. Chemical mutagenesis can be applied to soybean followed by screening for mutations in a target of interest, using a strategy known as Targeting Induced Local Lesions IN Genomes (TILLING). Recently, TILLING has been applied to four mutagenized soybean populations in two backgrounds (Cooper et al. 2008). Radiation has been also used to induce

genomic lesions, and deletion mutants have been developed with γ-rays (Bhatia et al. 1999), X-rays and fast neutrons (Men et al. 2002).

Insertional mutagenesis is another method by which gene expression can be modified in plants, creating mutants with either gain or loss of function for drought studies. T-DNA-tagged populations in rice (An et al. 2003) and maize (Cowperthwaite et al. 2002) proved to be very useful resources for functional genomics, including drought functional genomics. However, the development of T-DNA mutants takes a lot of time for soybean transformation (5–7 months), and considerable labor to generate an adequate number of insertions (Parrott and Clemente 2004). To overcome this problem, it has been suggested to couple T-DNA regions with transposon-based elements (Brutnell 2001). Isolation of loss-of-function mutants may be hindered by the lack of an obvious phenotype due to gene redundancy, especially in a paleopolyploid such as soybean (Mathieu et al. 2009). To circumvent this problem, a gain-of-function approach can be adopted to generate mutants by transforming plants with multiple enhancer sequences (Jeong et al. 2006). By using the Ds-transposon system, Mathieu et al. (2009) have created activation-tagging, gene and enhancer trap elements in soybean to establish a transposon-based mutagenesis repository which currently houses around 900 soybean events, with flanking sequence data derived from 200 of these events (http://digbio.missouri.edu/gmgenedb/index.php).

The availability of these functional genomics tools and resources, coupled with the forthcoming completely annotated genome sequence, represents an exciting era in soybean genetics. With the availability of mutant populations and major achievements in marker-assisted selection and soybean transformation, it is now possible to study and characterize the genes related to drought resistance, leading ultimately to better soybean productivity.

Genetic engineering for drought resistance

In response to stress, plants activate a number of defense mechanisms that function to increase tolerance to adverse conditions. A large array of genes is activated and consequently a number of proteins are produced to contribute to the metabolic pathways that lead synergistically to the enhancement of stress tolerance (Shinozaki and Yamaguchi-Shinozaki 2000, Seki et al. 2003, Valliyodan and Nguyen 2006). The response to drought stress, which involves a number of biochemical–molecular mechanisms, is complex, as schematically represented in **Fig. 2**. Although the role of the vast majority of these genes in plant adaptation to environmental stresses remains to be elucidated, the function of some of regulatory genes, which encode regulatory transcription
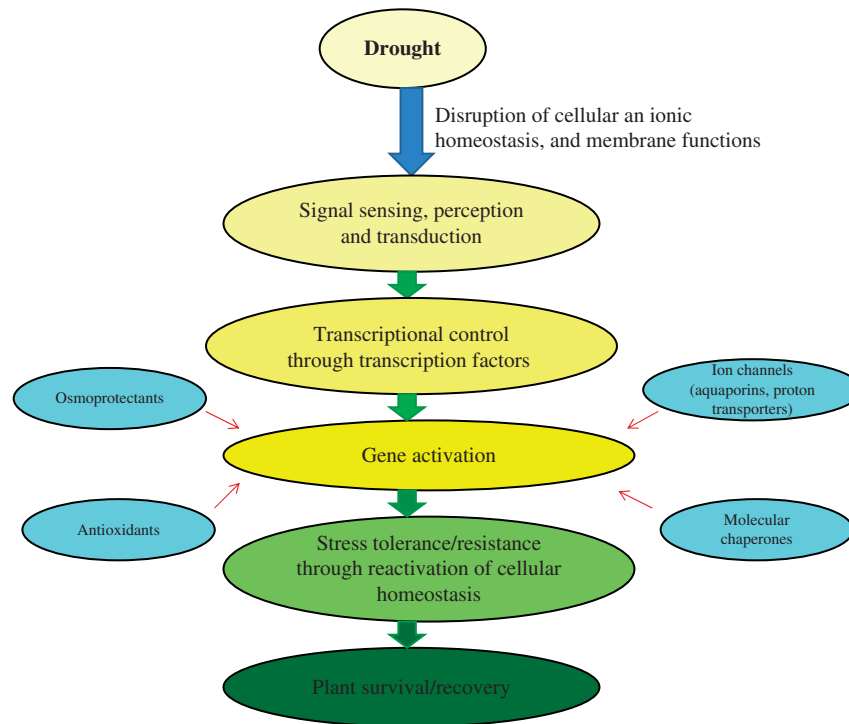
Fig. 2 Molecular mechanisms associated with drought tolerance.

factors (TFs), and that of functional genes, which encode metabolic components, have been described (Yamaguchi-Shinozaki and Shinozaki 2006, Tran et al. 2007a,b).

The application of this emerging understanding to the genetic engineering of food crops has already led to examples of improved drought tolerance and increased yield under drought (Hu et al. 2006, Nakashima et al. 2007, Nelson et al. 2007, Hu et al. 2008). Introduction of the SNAC1 and ZmNF-YB2 TFs into rice and maize, respectively, enhanced the drought tolerance of transgenic plants as demonstrated by field studies. Transgenic rice overexpressing the *SNAC1* gene had 22–34% higher seed setting in the field than the negative control under severe drought stress conditions at the reproductive stage. *SNAC1* transgenic rice plants are drought tolerant not only due to the increased expression of genes encoding proteins functioning in the production of osmolytes, detoxification and redox homeostasis, and in protection of macromolecules, but also because of the increased stomatal closure in transgenic leaves which prevents water loss from the plant (Hu et al. 2006). Similarly, *SNAC2/OsNAC6* transgenic rice plants have improved drought tolerance because of the enhanced expression of a large number of genes encoding proteins with predicted stress tolerance functions such as detoxification, redox homeostasis and proteolytic degradation (Nakashima et al. 2007, Hu et al. 2008). Transgenic maize overexpressing the

ZmNF-YB2 gene produced an ~50% increase in yield relative to controls when water was withheld from the planted field area during the late vegetative stage (Nelson et al. 2007).

A number of important successes have also been reported in creating drought tolerance in economically important plants, by genetic engineering of functional genes. For example, trehalose, a non-reducing disaccharide that functions as a compatible solute to protect biological structures under stress, has been used to increase drought tolerance in rice and tomato (Garg et al. 2002, Cortina and Marcia 2005). Transgenic rice plants were developed with a trehalose-6-phosphate synthase/phosphatase gene using an ABA-inducible promoter. Under drought stress, these transgenic rice plants showed increased amounts of trehalose, leading to higher levels of tolerance not only to drought, but also to salt and low temperature stresses, without any negative effect on plant growth or grain yield (Garg et al. 2002). Transgenic canola was generated using a farnesyltransferase (*ERA1*) antisense construct driven by a drought-inducible *rd29A* promoter. The developed transgenic canola plants showed increased ABA sensitivity, a significant decrease in stomatal conductance and decreased transpiration when tested under drought conditions (Wang et al. 2005). Recently, transgenic tobacco was engineered to overexpress an isopentenyl transferase gene (*IPT*) from *Agrobacterium tumefaciens* driven by the *SARK* promoter, which was isolated from bean

(*Phaseolus vulgaris*) and shown to be induced by both drought stress and maturation. The transgenic plants showed improved drought tolerance by maintaining high water content and photosynthetic activity (Rivero et al. 2007).

Although a considerable body of literature now supports the development of stress-resistant transgenic crops by genetic engineering as a promising approach, only one study has been reported to date in soybean. Transgenic soybean plants overexpressing the *Arabidopsis* Δ^1-pyrroline-5-carboxylate synthase gene, *P5CR*, showed greater tolerance to drought stress due to an increased free proline level and RWC and a reduced level of reactive oxygen species, particularly hydrogen peroxide (de Ronde et al. 2004a, de Ronde et al. 2004b, Kocsy et al. 2005). Since this report, much effort has been directed toward isolating drought stress-related genes in all physiological and biochemical aspects of drought stress, TFs and their respective promoters, which will in turn provide novel tools and resources for the development of engineered soybean with improved drought resistance. The identified soybean candidate genes are usually tested for their ability to enhance drought tolerance in *Arabidopsis* before pursuing their engineering into soybean. Chen et al. (2007) isolated an *AtDREB* homologous gene *GmDREB2* from soybean. Constitutive overexpression of *GmDREB2* increased the survival rate of transgenic plants without growth retardation under water deficit and salinity stress conditions (Chen et al. 2007). In addition, a few basic-leucine zipper genes encoding bZIP TFs were cloned from soybean, and found to be induced by drought and high salt treatments. By overexpressing some of these genes in *Arabidopsis*, the researchers found that transgenic plants have increased freezing and salt tolerance, but no significant difference in drought tolerance (Liao et al. 2008a, Liao et al. 2008b). In two other recent studies it was found that overexpression of a GmERF TF family member, the *GmERF089* gene, and a chilling-inducible *GMCHI* gene promoted enhanced tolerance to drought stress in tobacco and *Arabidopis*, respectively (Zhang et al. 2008, Cheng et al. 2009). Recently, 31 *GmNAC* genes, including the six genes previously identified by Meng et al. (2007), were identified and cloned from soybean. Systematic expression analysis of these 31 *GmNAC* genes demonstrated that nine genes are dehydration inducible (Tran et al. 2009). These nine *GmNAC* genes and their respective promoters are promising tools for genetic engineering to improve drought resistance of soybean, as the NAC family was reported to be a major group of TFs that play a role in root development and stress tolerance in plants (Tran et al. 2004, Hu et al. 2006, Nakashima et al. 2007, Hu et al. 2008).

To develop soybean plants with enhanced tolerance to drought stress, an understanding of the physiological and biochemical responses and gene regulatory networks is essential. Recent advances in soybean research, ranging from breeding programs to genome sequencing and genomics technologies, provide unprecedented opportunities to understand global patterns of gene expression and their association with the development of specific phenotypes, as well as promising tools for the genetic improvement of plants cultivated in adverse environments by molecular breeding or transgenic approaches.

Concluding remarks

Increased demand for soybean products over the past decade has continued to raise global soybean production steadily upward. Indeed, rising demand has prompted producers to shift acres from wheat to soybean. Although there are numerous cultivated and wild soybean accessions in the soybean germplasm collections of the world, only a few germplasm have been screened for drought tolerance. Combining genes from both wild and cultivated species shows promise to obtain genotypes with higher levels of tolerance. During our survey of the literature, we found that there have been only limited efforts to study soybean genotypic variation for important physiological traits related to drought tolerance, such as cell membrane thermostability, osmotic adjustment, canopy temperature depression and metabolic traits such as antioxidants and ABA. Considering the importance of soybean to the world economy, the identification of suitable traits and genotypes associated with drought tolerance in soybean is absolutely necessary. More concentrated efforts are needed to screen for resistant germplasm, discover new candidate genes and combine these genes for higher levels of drought tolerance in soybean. While combining genes/traits, preference should be given to pyramid a set of genes/traits contributing to a whole regulatory pathway. With the recent availability of the entire soybean genome sequence and genome-wide expression profiling data, it is possible to identify key genes regulating drought tolerance. However, the identification and use of traits related to drought tolerance and the development of suitable screening techniques are the prime criteria for cultivar development. Integration of genomics, proteomics and systems biology platforms will undoubtedly lead to the discovery of novel candidate genes and pathways involved in drought resistance.

Transgenic approaches have been shown to be powerful tools to help understand and manipulate the responses of plants to stress, but this can be achieved only when studied by precise physiological and biochemical investigation of transgenic plants under stress conditions. Moreover, to ensure that the responses of the transgenic plants to water stress treatments are comparable with those in field conditions, the plants should be subjected to the same or comparable drought regime that crops experience in the field. Greater emphasis should be placed on the severity and duration of the stress, the method of imposing the stress and the

parameters which will be measured. To facilitate accurate evaluation in the field of the genotypes for drought resistance, screening should be performed under managed drought environments over multiple locations and years. An interdisciplinary approach combining the knowledge of plant breeders, crop physiologists and molecular biologists will be apposite to evaluate the complex response of plants to develop better drought-resistant soybeans in future.

References

An, S., Park, S., Jeong, D.H., Lee, D.Y., Kang, H.G., Yu, J.H., et al. (2003) Generation and analysis of end sequence database for T-DNA tagging lines in rice. *Plant Physiol.* 133: 2040–2047.

Araus, J.L., Amaro, T., Casadesus, J., Asbati, A. and Nachit, M.M. (1998) Relationships between ash content, carbon isotope discrimination and yield in durum wheat. *Aust. J. Plant. Physiol.* 25: 835–842.

Arrese-Igor, C., Gonzalez, E.M., Gordon, A.J., Minchin, F.R., Galvez, L., Royuela, M., et al. (1999) Sucrose synthatse and nodule nitrogen fixation under drought and other environmental stresses. *Symbiosis* 27: 189–212.

Bennett, J.M., Sinclair, T.R., Muchow, R.C. and Costello, S.R. (1987) Dependence of stomatal conductance on leaf water potential, turgor potential, and relative water content in field-grown soybean and maize. *Crop Sci.* 27: 984–990.

Bhatia, C.R., Nichterlein, K. and Maluszynski, M. (1999) Oilseed cultivars developed from induced mutations and mutations altering fatty acid composition. *Mutat. Breed. Rev.* 11: 1–36.

Bhatnagar, S., King, C.A., Purcell, L. and Ray, J.D. (2005) Identification and mapping of quantitative trait loci associated with crop responses to water-deficit stress in soybean [*Glycine max* (L.) Merr.]. The ASA-CSSA-SSSA International annual meeting poster abstract, November 6–10, 2005.

Blum, A. (2002) Drought tolerance—is it a complex trait? *In* Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. Edited by Saxena, N.P. and O'Toole, J.C. pp. 17–22. International Workshop on Field Screening for Drought Tolerance in Rice. ICRISAT, Patancheru, India.

Boyer, J.S., Wong, S.C. and Farquhar G.D. (1997) CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiol.* 114: 185–191.

Brevedan, R.E. and Egli, D.B. (2003) Short periods of water stress during seed filling, leaf senescence, and yield of soybean. *Crop Sci.* 43: 2083–2088.

Brutnell, T.P. (2001) Transposon tagging in maize. *Funct. Integr. Genomics* 2: 4–12.

Buhr, T., Sato, S., Ebrahim, F., Xing, A., Zhou, Y., Mathiesen, M., et al. (2002) Ribozyme termination of RNA transcripts down regulate seed fatty acid genes in transgenic soybean. *Plant J.* 30: 155–163.

Burris, R.H. and Roberts, G.P. (1993) Biological nitrogen fixation. *Annu. Rev. Nutr.* 13: 317–335.

Busscher, W.J., Lipiec, J., Bauer, P.J. and Carter, T.E. (2000) Improved root penetration of soil hard layers by selected genotypes. *Comm. Soil. Sci. Plant Anal.* 31: 19–20.

Capitani, D., Brilli, F., Mannina, L., Proietti, N. and Loreto, F. (2009) In situ investigation of leaf weater status by portable unilateral NMR. *Plant Physiol.* 149: 1648–1647.

Carter, T.E., Jr., D Souza, P.I. and Purcell, L.C. (1999) Recent advances in breeding for drought and aluminum resistance in soybean. *In* Proceedings at the World Soybean Research Conference VI Chicago, IL. pp. 106–125. Superior Printing, Champagne, IL.

Carter, T.E. and Rufty, T.W. (1993) A soybean plant introduction exhibiting drought and aluminum tolerance. *In* Adaptation of Vegetables and other Food Crops to Temperature and Water Stress. Edited by Kuo, G. pp. 335–346. Asian Vegetable Research and Development Center, Publ, Tainan, Taiwan.

Chen, M., Wang, Q.Y., Cheng, X.G., Xu, Z.S., Li, L.C., Ye, X.G., et al. (2007) GmDREB2, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. *Biochem. Biophys. Res. Commun.* 353: 299–305.

Cheng, L., Huan, S., Sheng, Y., Hua, X., Shu, Q., Song, S., et al. (2009) GMCHI, cloned from soybean (*Glycine max* (L.) Merr.) enhances survival in transgenic Arabidopsis under abiotic stress. *Plant Cell Rep.* 28: 145–53.

Cooper, J., Till, B.J., Laport, R.G., Darlow, M.C., Kleffner, J.M., Jamai, A., et al. (2008) TILLING to detect induced mutations in soybean. *BMC Plant Biol.* 8: 9.

Cortes, P.M. and Sinclair, T.R. (1986) Water relations of field-grown soybean under drought. *Crop Sci.* 26: 993–998.

Cortina, C. and Culianez-Macia, F.A. (2005) Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. *Plant Sci.* 169: 75–82.

Cowperthwaite, M., Park, W., Xu, Z., Yan, X., Maurais, S. and Dooner, H. (2002) Use of the transposon Ac as a gene-searching engine in the maize genome. *Plant Cell* 14: 713–726.

Cutforth, H.W., McGinn, S.M., McPhee, K.E. and Miller, P.R. (2007) Adaptation of pulse crops to the changing climate of the Northern Great Plains. *Agron. J.* 99: 1684–1699.

de Ronde, J.A., Cress, W.A., Krüger, G.H.J., Strasser, R.J. and Van Staden, J. (2004a) Photosynthetic response of transgenic soybean plants, containing an Arabidopsis *P5CR* gene during heat and drought stress. *J. Plant Physiol.* 161: 1211–1224.

de Ronde, J.A., Laurie, R.N., Caetano, T., Greyling, M.M. and Kerepesi, I. (2004b) Comparative study between transgenic and non-transgenic soybean lines proved transgenic lines to be more drought tolerant. *Euphytica* 158: 123–132.

de Souza, P.I., Egli, D.B. and Bruening, W.P. (1997) Water stress during seed filling and leaf senescence in soybean. *Agron. J.* 89: 807–812.

Dogan, E., Kirnak, H. and Copur, O. (2007) Deficit irrigations during soybean reproductive stages and CROPGRO-soybean simulations under semi-arid climatic conditions. *Field Crops Res.* 103: 154–159.

Dornbos, D.L., Jr. and Mullen, R.E. (1992) Soybean seed protein and oil contents and fatty acid composition adjustments by drought and temperature. *J. Amer. Oil Chem. Soc.* 69: 228–231.

Durand, J.L., Sheehy, J.E. and Minchin, F.R. (1987) Nitrogenase activity, photosynthesis and nodule water potential in soybean plants experiencing water deprivation. *J. Exp. Bot.* 38: 311–321.

Earl, H.J. (2003) A precise gravimetric method for simulating drought stress in pot experiments. *Crop Sci.* 43: 1868–1873.

Fischer, R.A. (1981) Optimizing the use of water and nitrogen through breeding of crops. *Plant Soil* 58: 249–278.

Fletcher, A.L., Sinclair, T.R. and Allen, L.H. (2007) Transpiration responses to vapour pressure deficit in well watered 'slow-wilting' and commercial soybean. *Environ. Exp. Bot.* 61: 145–151.

Friedman, M. and Brandon, D.L. (2001) Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* 49: 1069–1086.

Garay, A.F. and Wilhelm, W.W. (1983) Root system characteristics of two soybean isolines undergoing water stress conditions. *Agron. J.* 75: 973–977.

Garg, A.K., Kim, K.J., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V., et al. (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl Acad. Sci. USA* 99: 15898–15903.

Gonzalez, E.M., Aparicio-Tejo, P.M., Gordon, A.J., Minchin, F.R., Royuela, M., Cabrerizo, P.M., et al. (1995) The role of sucrose synthase in the response of soybean nodules to drought. *J. Exp. Bot.* 49: 1705–1714.

Granier, C., Aguirrezábal, L.,Chenu, K., Cookson, S.J., Dauzat, M., Hamard, P., et al. (2006) PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in *Arabidopsis thaliana* permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytol.* 169: 623–635.

Heatherly, L.G. and Elmore R.W. (2004) Managing inputs for peak production. *In* Soybeans: Improvement, Production, and Uses. Agronomy Monographs, 3rd edn. No. 16. Edited by Specht, J.E. and Boerma, H.R. pp. 451–536. ASA-CSSA-SSSA, Madison, WI.

Hisano H., Sato, S., Isobe, S., Sasamoto, S., Wada, T., Matsuno A., et al. (2007) Characterization of the soybean genome using EST-derived microsatellite markers. *DNA Res.* 14: 271–281.

Hirasawa, T., Tanaka, K., Miyamoto, D., Takei, M. and Ishihara, K. (1994) Effects of pre-flowering moisture deficits on dry matter production and ecophysiological characteristics in soybean plants under drought conditions during grain filling. *Jpn. J. Crop Sci.* 63: 721–730.

Hoogenboom, G., Peterson, C.M. and Huck, M.G. (1987) Shoot growth rate of soybeans as affected by drought stress. *Agron. J.* 79: 598–607.

Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., et al. (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc. Natl Acad. Sci. USA* 103: 12987–12992.

Hu, H., You, J., Fang, Y., Zhu, X., Qi, Z. and Xiong, L. (2008) Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. *Plant Mol. Biol.* 67: 169–181.

Huck, M.G., Ishihara, K., Peterson, C.M. and Ushijima, T. (1983) Soybean adaptation to water stress at selected stages of growth. *Plant Physiol.* 73: 422–427.

Hudak, C.M. and Patterson, R.P. (1996) Vegetative growth analysis of a drought-resistant soybean plant introduction. *Crop Sci.* 35: 464–471.

Hufstetler, E.V., Boerma, H.R., Carter, T.E. and Earl, H.G. (2007) Genotypic variation for three physiological traits affecting drought tolerance in soybean. *Crop Sci.* 47: 25–35.

James, A.T., Lawn, R.J. and Cooper, M. (2008a) Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild *Glycine spp.* for epidermal conductance, osmotic potential, and relative water content. *Aust. J. Agric. Res.* 59: 656–669.

James, A.T., Lawn, R.J. and Cooper, M. (2008b) Genotypic variation for drought stress response traits in soybean. II. Inter-relations between epidermal conductance, osmotic potential, relative water content, and plant survival. *Aust. J. Agric. Res.* 59: 670–678.

Jeong, D.H., An, S., Park, S., Kang, H.G., Park, G.G., Kim, S.R., et al. (2006) Generation of a flanking sequence-tag database for activation-tagging lines in japonica rice. *Plant J.* 45: 123–132.

Jovanovi, Z., Proki, L., Stiki, R. and Peki, S. (1996) Cuticular characteristics in maize lines differing in drought resistance and maturity grouping. *J. Exp. Bot.* 47: 59–60.

Kaspar, T.C., Taylor, H.M. and Shibles, R.C. (1984) Taproot elongation rates of soybean cultivars in the glasshouse and their relation to filed rooting depth. *Crop Sci.* 24: 916–920.

King, C.A. and Purcell, L.C. (2005) Inhibition of N_2 fixation in soybean is associated with elevated ureides and aminoacids. *Plant Physiol.* 137: 1389–1396.

Klein, M., Geiser, M., Suh, S.J., Kolukisaoglu, H.U., Azevedo, L., Plaza, S., et al. (2004) Disruption of AtMRP4, a guard cell plasma membrane ABCC-type ABC transporter, leads to deregulation of stomatal opening and increased drought susceptibility. *Plant J.* 39: 219–23.

Kocsy, G., Laurie, R., Szalai, G., Szilagyi, V., Simon-Sarkadi, L., Galiba, G. et al. (2005) Genetic manipulation of proline levels affects antioxidants in soybean subjected to simultaneous drought and heat stresses. *Physiol. Plant.* 124: 227–235.

Lazarovitch, N., Ben-Gal, A. and Shani, U. (2006) An automated rotating lysimeter system for greenhouse evapotranspiration studies. *Vadose Zone J.* 5: 801–804.

Lee, J.D., Oliva, M.L., Sleper, D.A and Grover, S. (2008) Irrigation has little effect on unsaturated fatty acid content in soya bean seed oil within genotypes differing in fatty acid profile. *J. Agron. Crop Sci.* 194: 320–324.

Leport, L., Turner, N.C., French, R.J., Barr, M.D., Duda, R., Davies, S.L., et al. (1999) Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *Eur. J. Agron.* 11: 279–291.

Liao, Y., Zhang, J.S., Chen, S.Y. and Zhang, W.K. (2008a) Role of GmbZIP132 under abscisic acid and salt stresses. *J. Integr. Plant Biol.* 50: 221–230.

Liao, Y., Zou, H.F., Wei, W., Hao, Y.J., Tian, A.G., Huang, J., et al. (2008b) Soybean GmbZIP144, GmbZIP162 and GmbZIP178 genes function as negative regulator of ABA signaling and confer salt and freezing tolerance in transgenic Arabidopsis. *Planta* 228: 225–240.

Liener, I.E. (1994) Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr.* 34: 31–67.

Liu, F., Anderson, M.N., Jacobson, S.E. and Jensen, C.R. (2005a) Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. *Environ. Exp. Bot.* 54: 33–40.

Liu, F., Andersen, M.N. and Jensen, C.R. (2003) Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. *Funct. Plant Biol.* 30: 271–280.

Liu, Y., Gai, J.Y., Lu, H.N., Wang, Y.J. and Chen, S.Y. (2005b) Identification of drought tolerant germplasm and inheritance and QTL mapping of related root traits in soybean [*Glycine max* (L.) Merr.]. *Yi Chuan Xue Bao* 32: 855–863.

Marino, D., Frendo, P., Ladrera, R., Zabalza, A., Puppo, A., Arrese-Igor, C., et al. (2007) Nitrogen fixation control under drought stress. Localized or systemic? *Plant Physiol.* 143: 1968–1974.

Masle, J., Farquhar, G.D. and Wong, S.C. (1992) Transpiration ratio and plant mineral content are related among genotypes of a range of species. *Aust. J. Plant. Physiol.* 19: 709–721.

Mathieu, M., Winters, E.K., Kong, F., Wan, J., Wang, S., Eckert, H., et al. (2009) Establishment of a soybean (*Glycine max* (L.) Merr.) transposon-based mutagenesis repository. *Planta* 229: 279–289.

Mayland, H.F., Johnson, D.A., Asay, K.H. and Read, J.J. (1993) Ash, carbon isotope discrimination, and silicon as estimators of transpiration efficiency in crested wheatgrass. *Aust. J. Plant Physiol.* 20: 361–369.

Meckel, L., Egli, D.B., Phillips, R.E., Radcliffe, D. and Leggett, J.E. (1984) Effect of moisture stress on seed growth in soybeans. *Agron. J.* 75: 1027–1031.

Men, A.E., Laniya, T.S., Searle, I.R., Iturbe-Ormaetxe, I., Gresshoff, I., Jiang, Q., et al. (2002) Fast neutron mutagenesis of soybean (*Glycine soja* I.) produces a super nodulating mutant containing a large deletion in linkage group H. *Genome Lett.* 1: 147–155.

Meng, Q., Zhang, C., Cai, J. and Yu, D. (2007) Molecular cloning, sequence characterization and tissue specific expression of six NAC-like genes in soybean (*Glycine max* (L.) Merr.). *J. Plant Physiol.* 164: 1002–1012.

Mian, M.A.R., Ashley, D.A. and Boerma, H.R. (1998) An additional QTL for water use efficiency in soybean. *Crop Sci.* 38: 390–393.

Mian, M.A.R., Mailey, M.A., Ashley, D.A., Wells, R., Carter, T.E., Parrot, W.A., et al. (1996) Molecular markers associated with water use efficiency and leaf ash in soybean. *Crop Sci.* 36: 1252–1257.

Moinuddin, K.H.M. and Khannu-Chopra, R. (2004) Osmotic adjustment in chickpea in relation to seed yield and yield parameters. *Crop Sci.* 44: 449–455.

Monteros, M.J., Lee, G., Missaoui, A.M., Carter, T.E. and Boerma, H.R. (2006) Identification and confirmation of QTL conditioning drought tolerance in Nepalese soybean. The 11th Biennial Conference on the Molecular and Cellular Biology of the Soybean, Abstract PI471938. August 5–8, Lincoln, NE.

Morgan, J.M. (1995) Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in seasons of varying evaporative demand. *Field Crop Res.* 40: 143–152.

Morgenthal, K., Weckwerth, W. and Steuer, R. (2006) Metabolomic networks in plants: transitions from pattern recognition to biological interpretation. *Biosystems* 83: 108–117.

Muchow, R.C. (1985) Stomatal behavior in grain legumes under different soil water regimes in a semi-arid tropical environment. *Field Crop Res.* 11: 291–307.

Nakashima, K., Tran L.-SP., Nguyen, V.D., Fujita, M., Maruyama, K., Todaka, D., et al. (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J.* 51: 617–630.

Naya, L., Ladrera, R., Ramos, J., Gonzalez, E.M., Arrese-Igor, C., Minchin, F.R., et al. (2007) The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to subsequent recovery of plants. *Plant Physiol.* 144: 1104–1114.

Nelson, D.E., Repetti, P.P., Adams, T.R., Creelman, R.A., Wu, J., Warner, D.C., et al. (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc. Natl Acad. Sci. USA* 104: 16450–16455.

Nguyen, H.T., Babu, R.C. and Blum, A. (1997) Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Sci.* 37: 1426–1434.

Nicholas, F.W. (2006) Discovery, validation, and delivery of DNA markers. *Aust. J. Exp. Agric.* 46: 155–158.

Nunes, A.C.S., Vianna, G,R., Cuneo, F., Amaya-Farfan, J., Capdeville, G., Rech, E.L., et al. (2005) RNAi-mediated silencing of myo-inositol-1-phosphate synthase gene (GmMIPS1) in transgenic soybean inhibited seed development and reduced phytate content. *Planta* 224: 125–132.

Ososki, A.L. and Kennelly, E.J. (2003) Phytoestrogens: a review of the present state of research. *Phytother. Res.* 17: 84–869.

Oya, T., Nepomuceno, A.L., Neumaier, N., Farias, J.R.B., Tobita, S. and Ito, O. (2004) Drought tolerance characteristics of Brazilian soybean cultivars—evaluation and characterization of drought tolerance of various Brazilian soybean cultivars in the field. *Plant Prod. Sci.* 7: 129–137.

Paje, M.C.M., Ludlow, M.M. and Lawn, R.J. (1988) Variation among soybean (*Glycine max* (L.) Merr.) accessions in epidermal conductance of leaves. *Aust. J. Agric. Res.* 39: 363–373.

Pantalone, V.R., Burton, J.W. and Carter, T.E. (1996) Soybean fibrous root heritability and genotypic correlations with agronomic and seed quality traits. *Crop Sci.* 36: 1120–1125.

Parrott, W.A. and Clemente, T.E. (2004) Transgenic soybean. *In*: Soybeans: Improvement, Production, and Uses. Agronomy Monographs, 3rd edn. No. 16. Edited by Specht, J.E. and Boerma, H.R. pp. 265–302. ASA-CSSA-SSSA, Madison, WI.

Pathan, M.S., Lee, J.D., Shannon, J.G. and Nguyen, H.T. (2007) Recent advances in breeding for drought and salt stress tolerance in soybean. *In* Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops. Edited by Jenks, M.A., Hasegawa, P.M. and Jain, S.M. pp. 739–773. Springer, New York.

Patterson, P.R. and Hudak, C.M. (1996) Drought-avoidant soybean germplasm maintains nitrogen fixation capacity under water stress. *Plant Soil* 186: 39–43.

Pennisi, E. (2008) The blue revolution drop by drop gene by gene. *Science* 320: 170–173.

Pfeiffer, T.W., Peyyala, R., Ren, Q. and Ghabrial, S.A. (2003) Increased soybean pubescence density yield and soybean mosaic virus resistance effects. *Crop Sci.* 43: 2071–2076.

Pimentel, D. and Patzek, T. (2008) Ethanol production using corn, switchgrass and wood; biodiesel production using soybean. *In* Biofuels, Solar and Wind as Renewable Energy Systems. Edited by Pimentel, D. pp. 373–394. Springer, New York.

Purcell, L.C., DeSilva, M., King, C.A. and Kim, W.H. (1997) Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits. *Plant Soil* 196: 101–113.

Purcell, L.C. and King, C.A. (1996) Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. *J. Plant Nutr.* 19: 969–993.

Purcell, L.C. and Specht, J.E. (2004) Physiological traits for ameliorating drought stress. *In* Soybeans: Improvement, Production, and Uses.

Agronomy Monographs, 3rd edn. No. 16. Edited by Specht, J.E. and Boerma, H.R. pp. 569–620. ASA-CSSA-SSSA, Madison, WI.

Ray, J.D. and Sinclair, T.R. (1998) The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *J. Exp. Bot.* 49: 1381–1386.

Read, D.J. and Bartlett, E.M. (1972) The physiology of drought resistance in soybean plant (*Glycine max*). I. The relationship between drought resistance and growth. *J. Appl. Ecol.* 9: 487–489.

Riederer, M. and Schreiber, L. (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. *J. Exp. Bot.* 52: 2023–2032.

Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., et al. (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proc. Natl Acad. Sci. USA* 4: 19631–19636.

Sakai, T. and Kogiso, M. (2008) Soyisoflavones and immunity. *J. Med. Invest.* 55: 167–173.

Sall, K. and Sinclair, T.R. (1991) Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. *Plant Soil* 133: 31–37.

Santamaria, J.M., Ludlow, M.M. and Fukai, S. (1990) Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) Moench under water-limited conditions. I. Water stress before anthesis. *Aust. J. Agric. Res.* 41: 51–65.

Santra, D.K., Sandhu, D., Tai, T. and Bhattacharyya, M.K. (2003) Construction and characterization of a soybean yeast artificial chromosome library and identification of clones for the *Rps6* region. *Funct. Integr. Genomics* 3: 153–159.

Seki, M., Kamei, A., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2003) Molecular responses to drought, salinity and frost: common and different paths for plant protection. *Curr. Opin. Biotechnol.* 14: 194–199.

Seki, M., Umezawa, T., Urano, K. and Shinozaki, K. (2007) Regulatory metabolic networks in drought stress responses. *Curr. Opin. Plant Biol.,*10: 296–302.

Serraj, R. and Sinclair, T.R. (2002) Osmolyte accumulation: can it really help increase crop under drought conditions? *Plant Cell Environ.* 25: 333–341.

Serraj, R., Vadez, V. and Sinclair, T.R. (2001) Feedback regulation of symbiotic N$_2$ fixation under drought stress. *Agronomie* 21: 621–626.

Sharma, K.K. and Lavanya, M. (2002) Recent developments in transgenics for abiotic stress in legumes of the semi-arid tropics. *In* JIRCAS Working Report pp. 61–73.

Shinozaki, K. (2007) Acceleration of soybean genomics using large collections of DNA markers for gene discovery. *DNA Res.* 14: 235.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* 3: 217–223.

Shoemaker, R.C., Grant, D., Olson, T., Warren, W.C., Wing, R., Yu, Y., et al. (2008) Microsatellite discovery from BAC end sequences and genetic mapping to anchor the soybean physical and genetic maps. *Genome* 51: 294–302.

Sinclair, T.R. and Ludlow, M.M. (1986) Influence of soil water supply on the plant water balance of four tropical grain legumes. *Aust. J. Plant Physiol.* 13: 329–341.

Sinclair, T.R., Purcell, L.C., King, C.A., Sneller, C.H., Chen, P. and Vadez, V. (2007) Drought tolerance and yield increase of soybean resulting from improved symbiotic N fixation. *Field Crops Res.* 101: 68–71.

Sinclair, T.R., Purcell, L.C., Vadez, V., Serraj, R., King, C.A. and Nelson, R. (2000) Identification of soybean genotypes with N$_2$ fixation tolerance to water deficits. *Crop Sci.* 40: 1803–1809.

Sinclair, T.R. and Serraj, R. (1995) Legume nitrogen fixation and drought. *Nature* 378: 344.

Sinclair, T.R., Vadez, V. and Chenu, K. (2003) Ureide accumulation in response to Mn nutrition by eight soybean genotypes with N$_2$ fixation tolerance to soil drying. *Crop Sci.* 43: 592–597.

Smith, J.R. and Nelson, R.L. (1987) Predicting yield from early generation estimates of reproductive growth periods in soybean. *Crop Sci.* 27: 471–474.

Soystats (2008) http://www.soystats.com/

Specht, J.E., Chase, K., Macrander, M., Graef, G.L., Chung, J., Markwell, J.P., et al. (2001) Soybean response to water: a QTL analysis of drought tolerance. *Crop Sci.* 41: 493–509.

Specht, J.E., Hume, D.J. and Kumudini, S.V. (1999) Soybean yield potential—a genetic and physiological perspective. *Crop Sci.* 39: 1560–1570.

Specht, J.E. and Williams, J.H. (1985) Breeding for drought and heat resistance: prerequisites and examples. *In* World Soybean Research Conference III. Proceedings. Edited by Shibles, R. pp. 468–475. Westview Press, Boulder, CO.

Specht, J.E., Williams, J.H. and Pearson, D.R. (1985) Near-isogenic analyses of soybean pubescence genes. *Crop Sci.* 25: 92–96.

Subramanian, S., Graham, M.Y., Yu, O. and Graham, T.L. (2005) RNA interference of soybean isoflavone synthase genes leads to silencing in tissues distal to the transformation site and to enhanced susceptibility to *Phytopthora sojae*. *Plant Physiol.* 137: 1345–1353.

Taylor, H.M., Burnett, E. and Booth, G.D. (1978) Taproot elongation rates of soybeans. Z. *Acker Pflanzenbau Bd.* 146.

Timpte, C., Wilson, A.K. and Estelle, M. (1994) The *axr2*-1 mutation of *Arabidopsis thaliana* is a gain-of-function mutation that disrupts an early step in auxin response. *Genetics* 138: 1239–1249.

Tran, L.-S.P., Nakashima, K., Sakuma, Y., Simpson, S.D., Fujita, Y., Maruyama, K., et al. (2004) Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsive *cis*-element in the early responsive to dehydration stress 1 promoter. *Plant Cell* 16: 2481–2498.

Tran, L.-S.P., Nakashima, K., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2007a) Plant gene networks in osmotic stress response: from genes to regulatory networks. *Methods Enzymol.* 428: 109–128.

Tran, L.-S.P., Quach, T., Guttikonda, S.K., Aldrich, D.L., Rajesh, K., Neelakandan, A., et al. (2009) Molecular characterization of stress-inducible *GmNAC* genes in soybean. *Mol. Gen. Genomics* 281: 647–664.

Tran, L.-S.P., Urao, T., Qin, F., Maruyama, K., Kakimoto, T., Shinozaki, K., et al. (2007b) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* 104: 20623–20628.

Tsialtas, J.T., Kassioumi, M. and Veresoglou, D.S. (2002) Evaluating leaf ash content and potassium concentration as surrogates of carbon isotope discrimination in grassland species. *J. Agron. Crop Sci.* 188: 168–175.

Turner, N.C., Abbo, S., Berger, J.D., Chaturvedi, S.K., French, R.J., Ludwig, C., et al. (2007) Osmotic adjustment in chickpea (*Cicer arietinum* L.) results in no yield benefit under terminal drought. *J. Exp. Bot.* 58: 187–194.

Turner, N.C., Wright, G.C. and Siddique, K.H.M. (2001) Adaptation of grain legumes (pulses) to water limited environments. *Adv. Agron.* 71: 193–123.

Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr. Opin. Biotechnol.* 17: 113–122.

Umezawa, T., Sakurai, T., Totoki, Y., Toyoda, A., Seki, M., Ishiwata, A., et al. (2008) Sequencing and analysis of approximately 40,000 soybean cDNA clones from a full-length-enriched cDNA library. *DNA Res.* 16: 1–14.

Urano, K., Maruyama, K., Ogata, Y., Morishita, Y., Takeda, M., Sakurai, N., et al. (2009) Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. *Plant J.* 57: 1065–1078.

Valliyodan, B. and Nguyen, H.T. (2006) Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol.* 9: 189–195.

Valliyodan, B. and Nguyen, H.T. (2008) Genomics of abiotic stress in soybean. *In* Genetics and Genomics of Soybean. Edited by Stacey, G. pp. 343–372. Springer, New York.

van Gardingen, P.R. and Grace, J. (1992) Vapour pressure deficit response of cuticular conductance in intact leaves of *Fagus sylvatica* L. *J. Exp. Bot.* 43: 1293–1299.

Vignes, D., Djekoun, A. and Planchon, C. (1986) Responses de differents genotypes de soja au deficit hydrique. *Can. J. Plant Sci.* 66: 247–255.

Vinod, M.S., Sharma, N., Manjunatha, K., Kanbar, A., Prakash, N.B. and Shashidar, H.E. (2006) Candidate genes for drought tolerance and improved productivity in rice (*Oryza sativa* L.). *J. Biosci.* 31: 69–74.

Vodkin, L.O., Khanna, A., Shealy, R., Clough, S.J., Gonzalez, D.O., Philip, R., et al. (2004) Microarrays for global expression constructed with a low redundancy set of 27,500 sequenced cDNAs representing an array of developmental stages and physiological conditions of the soybean plant. *BMC Genomics* 5: 73.

Vuong, T.V., Wu, X., Pathan, M.S., Valliyodan, B. and Nguyen. H.T. (2007) Genomics approaches to soybean improvement. *In* Genomics-Assisted Crop Improvement. Edited by Varshney, P.K. and Tuberosa, R. pp. 243–279. Springer, New York.

Wang, Y., Ying, J., Kuzma, M., Chalifoux, M., Sample, A., McArthur, C., et al. (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. *Plant J.* 43: 413–424.

Westgate, M.E. and Peterson, C.M. (1993) Flower and pod development in water deficient soybean. *J. Exp. Bot.* 258: 109–117.

Wright, G. (1996) Review of ACIAR selection for water use efficiency in legumes project recommends further research. *ACIAR Food Legume Newslett.* 2–3.

Wu, X., Zhong, G., Findley, S.D., Cregan, P., Stacey, G. and Nguyen, H.T. (2008) Genetic marker anchoring by six-dimensional pools for development of a soybean physical map. *BMC Genomics* 9: 28.

Xia, Z., Tsubokura, Y., Hoshi, M., Hanawa, M., Yano, C., Okamura, K., et al. (2007) An integrated high-density linkage map of soybean with RFLP, SSR, STS, and AFLP markers using A single F_2 population. *DNA Res.* 14: 257–269.

Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* 57: 781–803.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., et al. (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genetics* 172: 1213–1228.

Zhang, G., Chen, M., Chen, X., Xu, Z., Guan, S., Li, L.C., et al. (2008) Phylogeny, gene structures, and expression patterns of the ERF gene family in soybean (*Glycine max* L.). *J. Exp. Bot.* 59: 4905–4917.