Chapter 19
Molecular Biology for Stress Management

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19.1 Introduction to the Genomics of Drought Tolerance in Legumes

Drought is a meteorological term and an environmental event, defined as a water stress due to lack of or insufficient rainfall and/or inadequate water supply (Toker et al., 2007). The impact of drought and importance of drought tolerance to legume crops has been discussed in other sections of this book (Chapters 8, 9, 10, 19, 20, etc.). Drought tolerance is a complex trait associated with several physiological attributes. In the agronomical sense, drought resistance refers to the ability of a plant to produce its economical product with minimum loss in a water-deficit environment, relative to normal water conditions. In a genetic sense, the mechanisms of drought resistance can be grouped into three categories, viz., escape, avoidance and tolerance (Turner et al., 2001; Malhotra and Saxena, 2002). These mechanisms are inter-related and there is no fixed line of demarcation.

Drought escape: Drought escape can be defined as the ability of a plant to complete its life cycle before serious soil and water deficits develop. Plants facing terminal drought at the end of the growing season may escape drought by early vigour, early flowering and maturity. The two usual approaches toward drought escape are by using early maturing (short-duration) varieties (Kumar et al., 1996) or early sowing (Toker et al., 2007), which depends on the prevalent cropping system. In chickpea, a shift of growing season from spring to winter to efficiently utilise available soil moisture was suggested (Singh, 1990) and has become the norm for southern Australia.

Drought avoidance: Drought avoidance is the ability of a plant to maintain relatively high tissue water potential in a water-stressed environment. Drought stress can be avoided by maintaining water uptake and reducing the water lost by the plant. The two important traits conferring drought avoidance are a larger/deeper root

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system (allowing greater water extraction) (Saxena et al., 1993) and a smaller leaf area (reducing transpirational loss) (Saxena, 2003; Toker et al., 2007). Other traits that allow drought avoidance or turgor maintenance are increased hydraulic conductance, reduced epidermal (stomatal and lenticular) conductance, leaf movement (like folding and rolling) and phenological plasticity (Mitra, 2001).

Drought tolerance: Drought tolerance is the ability of cells to metabolise at low leaf water status (Toker et al., 2007). Turgor maintenance is achieved through osmotic adjustment (accumulation of solutes in the cell), increase in cell elasticity, decrease in cell size, and protoplasmic resistance (including stabilising cell proteins) (Mitra, 2001). Membrane stability is achieved by reducing the leakage of solutes from the cell (Nayyar et al., 2005). The cell water content is maintained by accumulating compatible solutes like fructan, trehalose, polyols, glycine betaine, proline and polyamines that are non-toxic and do not interfere with cellular activities (Mitra, 2001). Degradation of cellular proteins due to the stress is stabilised by amino acids like proline (Munns, 2005).

Information regarding the types, interactions and levels of expressions of genes involved in these drought-tolerant mechanisms would potentially lead to selective development of elite varieties. Recently, Australian wheat geneticists have developed transgenic wheat lines with a 20% higher yield increase under drought conditions (Spangenberg, 2008 pers. comm.). Additionally, it was demonstrated that expression of related cold shock proteins (CSPs) from bacteria, CspA from *Escherichia coli* and CspB from *Bacillus subtilis*, promotes water stress adaptation in multiple plant species (Castiglioni et al., 2008). Expression of CSP proteins in maize was not associated with negative pleiotropic effects, indicating that stress tolerance does not come at a cost to crop productivity under well-watered conditions. Although release of genetically modified wheat/maize is currently not possible due to strong resistance from consumer and environmental groups worldwide, this may change as world food shortages grow and grain prices remain high. The need to develop varieties able to grow with minimal inputs in more hostile, drought environments is likely to put further pressure for the development and release of genetically manipulated food sources, with the implicated drought-tolerance genes coming from wider germplasm than is currently available in breeding programs.

19.2 The Currently Available Germplasm Resources and Phenotypic Responses to Drought Worldwide

The following section outlines the germplasm sources and methods used to select drought tolerant genotypes of several legume species. The identified genotypes will be pivotal for use in future molecular studies to identify the underlying drought tolerance mechanisms and associated genetic components. Once identified, they may potentially be selected to introgress through breeding or to artificially move into elite genetic backgrounds.

19.2.1 Common Bean (Phaseolus vulgaris *L.*)

The common bean is the most important grain legume and about 60% is cultivated under drought stress conditions in the developing world (Graham and Ranalli, 1997). A common bean germplasm collection comprising over 40,000 accessions is maintained at the *Centro Intemacional de Agricultura Tropical* (CIAT) in Cali, Colombia, and includes indigenous wild and weedy specimens, unimproved landraces, and pure lines of *Phaseolus vulgaris,* as well as numerous related species (Hidalgo, 1991). However, the breeding material of common bean has been mostly grouped into landraces and utilised likewise because of the potential incompatibility between different landraces, e.g. Andean and Mesoamerican germplasm are incompatible (Singh and Gutiérrez, 1984; Gepts and Bliss, 1985). In fact different core collections have been reported for different landraces; e.g., common bean core collections have been reported for the Netherlands (Zeven et al., 1999), the Iberian peninsula (Rodiño et al., 2003) and Mexico (Skroch et al., 1998). Further, genotypes selected for drought tolerance in the Mexican highlands and Columbia did not perform well in other environments and it was suggested that the importance of a particular genotype as a drought tolerant parent depends on its yield in that environment (White et al., 1994).

As seen for other legumes, common bean germplasm has been screened for root and shoot characters that confer drought tolerance. Evaluation of root growth of two drought tolerant and two drought sensitive bean cultivars in different soil types suggested that drought avoidance through greater root growth and extraction of soil moisture are important traits but limited where soil conditions restrict root growth (Sponchiado et al., 1989). Further, Boutraa and Sanders (2001) interrogated the influence of water stress on grain yield and vegetative growth of indeterminate and determinate bean cultivars that were stressed at flowering and pod-filling stages. They found that water stress during both phenological stages affected the seed weight, number of seeds per plant and number of pods per plant, and delayed maturity specially when stressed at flowering stage. Water stress also reduced vegetative growth parameters like the number of trifoliate leaves, stem height, number of main branches and number of nodes on the main stem.

Ramirez-Vallejo and Kelly (1998) studied the association of specific phenological and physiological traits with drought resistance in common bean. The study of five genotypes and 16 progeny lines concluded that the most effective approach in breeding for drought resistance in common bean would be based first on selection for high geometric yield followed by selection among the high-yielding individuals for low to moderate levels of the drought susceptibility index. Previously, the effect of water stress on diverse shoot genotypes that were grafted on selected root genotypes concluded that the effect of shoot genotype on growth and yield under water deficits was small compared with that of root genotype (White and Castillo, 1992). Studies at the cellular level have also been conducted to identify drought tolerant bean cultivars. On the basis of evaluation of oxidative stress and the plant antioxidant system of three contrasting bean cultivars, Zlatev et al. (2006)

identified "Plovdiv 10" and "Prelom" as drought tolerant and "Dobrudjanski" as drought sensitive.

Different screening methods for drought tolerance in common bean have identified SEA 5, SEA 10, SEA 13, San Cristobal 83, ICA Palmar, LEF 2RB, AC 1028, Matterhorn and Pinto Villa as drought tolerant genotypes (White and Singh, 1991; Acosta-Gallegos et al., 1995; Schneider et al., 1997a; Kelly et al., 1999; Singh et al., 2001; Rosales-Serna et al., 2004). In an attempt to develop a line resistant to terminal drought and diseases, Frahm et al. (2004) screened RILs from crosses between a drought tolerant line and two disease resistant lines. They identified line L88-63 as the one with highest yield potential and broad adaptation to all four locations tested. Subsequently, Munoz-Perea et al. (2006) recorded the response of three dry bean landraces and 13 cultivars evaluated under non-stressed and intermittent drought-stressed environments over two years. They found that "Common Red Mexican" and "CO 46348" had high seed yield in both non-stressed and drought-stressed environments, whereas "Matterhorn" and "Othello" yielded comparatively high under a drought-stressed but moderately in a non-stressed environment. Not surprisingly, recent evaluation of common bean landraces and cultivars to identify those with greatest water use efficiency (WUE) showed that the cultivar "Othello" and the landrace "Common Red Mexican" had the highest WUE in the *pinto* and *red* market classes, respectively (Munoz-Perea et al., 2007).

19.2.2 Soybean [Glycine max *(L.) Merr.*]

Soybean is our primary source of protein and oil and its genetic diversity has been reviewed in detail by Carter et al. (2004). The USDA has the most extensive soybean germplasm collection consisting of 18,603 cultivated and 1,116 wild soybean accessions (http://www.ars.usda.gov). Most of the early breeding efforts in soybean were directed at shatter resistance, disease and pest resistance, whilst abiotic stresses received little importance. One of the first methods to screen soybean germplasm for drought tolerance involved the use of growth chamber (Sammons et al., 1978) and drought box (Sammons et al., 1979) methods. However, both these methods failed to characterise genotypes as drought tolerant or sensitive by themselves and further testing in the field was recommended. Later, Bouslama and Schapaugh Jr. (1984) evaluated three different techniques to screen 20 soybean genotypes for drought and heat tolerance. Of the three methods, the hydroponic seedling test (subjecting seedlings to –0.6 MPa osmotic pressure in hydroponic solution for 14 days) was found to be most reliable and recommended for screening soybean for drought tolerance. Kpoghomou et al. (1990a) tested 17 determinate soybean cultivars for drought tolerance at germination and seedling stages. They observed that the cultivars that grew taller under drought stress conditions had greater dry matter accumulation and higher germination stress indices, indicating the reliability of height to predict cultivar performance under such conditions. They further tested three cultivars at vegetative, flowering, and pod-filling stages under stressed and well-watered conditions in glasshouse and field. They found reproductive stages

were more sensitive to drought than vegetative stages and identified Lee 74 as the most drought tolerant cultivar (Kpoghomou et al., 1990b).

In another study, several soybean genotypes from maturity groups III through VII were evaluated at three osmotic potential levels (–0.017, 0.3 and –0.5 MPa) using polyethylene glycol M.W. 8000. This study identified the lines PI 408.155, PI 423.827B, PI 423.759 and Pershing as drought tolerant (Sapra and Anaele, 1991). Further, Djekoun and Planchon (1991) interrogated the use of photosynthetic activity of water stressed plants to assess drought tolerance in soybean. A PAM modulation fluorometer was used to measure the effects of dehydration on the activity of the photosystems of detached or attached leaves at ambient CO_2. They concluded that the readily and rapidly measurable fluorescence parameter such as Rfd (that is associated with photosystem activity and CO_2 exchanges) is a valuable selection criteria for drought tolerance in soybean. Moreover, cultivars with higher drought tolerance were shown to have high crop growth rate and larger leaf area under drought stress (Oya et al., 2004).

Symbiotic N_2 fixation in soybean is very sensitive to drying soil and has a negative impact on crop yield under many cropping situations (Purcell and King, 1996). In an effort to identify soybean germplasm that might have substantially decreased sensitivity of N_2 fixation to water deficits, 3,000 plant introduction lines were screened in a three-stage screening process. Eight lines having substantial tolerance of N_2 fixation to soil drying were identified (Sinclair et al., 2000). Recently, 100 progeny lines from a cross between Jackson, a cultivar proven to have N_2 fixation tolerance to drought, and KS4895, a high-yielding line were screened. Two lines with higher yields than commercial checks in low-yielding environments were identified. These lines also had nitrogen fixation activity at lower soil water contents than exhibited by the sensitive parent (Sinclair et al., 2007).

19.2.3 Chickpea (Cicer arietinum *L.*)

Chickpea is the second most important legume in the world with 11.6 million ha under cultivation (http://faostat.fao.org). The most extensive chickpea germplasm collection is at ICRISAT, India containing 16,992 accessions from 44 countries (Upadhyaya, 2003). Extensive and deep root systems have been recognized as one of the most important traits for improving chickpea productivity under progressively receding soil moisture conditions. Genetic variability for the root traits in the mini-core germplasm collection of ICRISAT and several wild relatives of chickpea was evaluated using a cylinder culture system during two consecutive growth seasons (Kashiwagi et al., 2005). The largest genetic variability was observed at 35 days after sowing for root length density (RLD) (heritability, $h2 = 0.51$ and 0.54) across seasons, and followed by the ratio of plant dry weight to root length density with $h2$ of 0.37 and 0.47 for the first and second seasons, respectively. Accession ICC 8261 had the largest RLD and one of the deepest root systems, whilst ICC 4958 had one of the most prolific and deep root systems.

Since a direct relationship exists between transpiration efficiency and SPAD Chlorophyll Meter Readings (SCMR, Nageswara Rao et al., 2001), the mini core subset of chickpea was evaluated for SCMR (Kashiwagi et al., 2006). Accession ICCV 2 had the highest SCMR reading (55.5), under rainfed conditions. Also, regardless of the irrigation schemes, ICC 16374 had a superior SCMR with 66.4 (1st rank) in irrigated conditions and its rank was 4th in a rainfed environment. Moreover, ICC 4958 also had a better SCMR irrespective of the irrigation schemes (11th rank in a rainfed environment, 3rd in an irrigated one).

Previously, more than 1,500 chickpea lines were screened for drought tolerance and the genotype ICC 4958 was the most promising (Saxena et al., 1993). Subsequently, ICC 4958 was used in a three-way cross with *cv*. Annigeri and the *Fusarium* wilt resistant genotype ICC 12237. The progeny were selected for high yield and drought tolerance traits (Saxena, 2003). Several lines combining the large root trait of ICC 4958 and the small leaf area trait of ICC 5680 were reported to be more drought tolerant and yielded similarly to the high-yielding parent (Saxena, 2003). Also, genotypic variation for osmotic adjustment in chickpea has been reported but its correlation with yield under drought stress is unclear and the heritability was low ($h^2 = 0.20$–0.33) (Morgan et al., 1991; Turner et al., 2001; Moinuddin and Khanna-Chopra, 2004). Recently, a chickpea collection of 1,600 *desi* and 1,400 *kabuli* lines was evaluated for drought resistance (Yadav et al., 2007). From detailed evaluation of 82 lines, terminal drought was shown to reduce yields by 13–37%, depending on plant type. The yields in the *kabuli* types were more severely reduced than the *desi* types, due to a greater reduction in the number of branches and pods per plant.

19.2.4 Peanut (Arachis hypogea *L.*)

Peanut is a widely used oilseed crop around the world with the greatest variation found in Brazil, where the species *Arachis* originally evolved (Gregory et al., 1980). The largest collection of cultivated peanut is at ICRISAT, consisting of 14,310 accessions from 92 countries in addition to 413 accessions of other *Arachis* spp. (Upadhyaya et al., 2001). The USDA collection has over 8,000 accessions of *A. hypogea* (Holbrook, 2001) and ~800 other *Arachis* spp. (Stalker and Simpson, 1995).

The peanut germplasm has been screened using various methods to identify drought tolerant genotypes that have high soil water extraction capacity and/or greater water use efficiency. Del Rosario and Fajardo (1988) conducted greenhouse tests to evaluate four high-yielding, five intermediates, and one low-yielding genotype under water-stress and well-watered conditions. Genotypes Acc 847, 55-437 and GNP 1157 performed better than others in morphophysiological characters and seed yield. High-yield correlated to high leaf area, high leaf water potential, high shoot weight and plant height. Further, drought tolerant cultivars were identified by evaluating leaf transpiration efficiency (Wright et al., 1994) and rooting habit (Rucker et al., 1995; Songsri et al., 2008). Later, Nageshwara Rao et al. (2001) successfully used the ratio of leaf area to dry weight for large-scale screening of

drought tolerant genotypes in Australia. The SPAD chlorophyll meter was used to identify genotypes with high transpiration efficiency and thus drought tolerance (Nageshwara Rao et al., 2001).

Recently, a detailed study to investigate the effect of drought stress on Total Dry Matter (TDM), pod yield, Water Use Efficiency (WUE), harvest index (HI), SPAD Chlorophyll Meter Reading (SCMR), Specific Leaf Area (SLA) and canopy temperature was conducted in Thailand to identify drought tolerant genotypes and establish relationships among drought resistance traits (Jongrungklang et al., 2008). Drought was reported to reduce TDM, pod dry weight, HI, WUE and SLA, but increased SCMR and canopy temperature. Also, WUE was positively related to SCMR under water-limited conditions and could be useful for selection of drought tolerance. Genotypes Tifton-8, 14 PI 430238 and 205 PI 442925 possessed high WUE at all drought levels, whilst KK 60-3, 101 PI 268659 had high WUE only under severe drought conditions.

19.2.5 Cowpea [Vigna unguiculata *(L.) Walp.*]

Cowpea is one of the most important food and forage legumes in the semi-arid tropics including Asia, Africa, Southern Europe, Southern United States, and Central and South America (Singh, 2005). The International Institute for Tropical Agriculture (IITA, Nigeria) has the most extensive collection of cowpea germplasm including over 15,000 cultivated and 2,000 wild genotypes. Also, USDA has about 7,000 accessions collected from IITA and around the world (www.isp.msu.edu).

Cowpea is relatively drought tolerant compared to other legumes; and earliness, delayed leaf senescence, and indeterminate growth habit are the traits being combined to improve drought adaptation (Ehlers and Hall, 1997). The accession "CB5" was identified to have one of the best indeterminate growth habits, being able to survive vegetative stage drought and recover after subsequent irrigation to produce yields of about 4,000 kg/ha (Turk et al., 1980). Also, two accessions, "Ein El Gazal" and "Melakh" that had erect growth habit and flowered early (30–35 days after sowing) were released in Sahel (Cisse et al., 1997; Elawad and Hall, 2002) to help plants escape terminal drought. Singh et al. (1999) evaluated a wooden box screening method to select drought tolerant plants in cowpea at the seedling stage. This method showed good correlation with drought tolerance at vegetative and reproductive stages. The accessions Kanannado, Dan Ila, IT88D-867-11, and IT90K-59-2 were rated as highly drought tolerant; TVu 11986, TVu 13464, IT89KD-288, and TVu 11979 as moderately drought tolerant; TVu 12349 and TVu 12348 as slightly drought tolerant, and TVu 8256 and TVu 7778 as susceptible to drought stress. Later, Matsui and Singh (2003) used a pin-board root-box method to identify the role of root characteristics in drought tolerance. By comparing rooting patterns of drought tolerant and susceptible genotypes they observed that drought tolerance was associated with an increase in root dry matter per leaf area under mild water-stress conditions, and downward movement of roots (increasing access and use of soil moisture in deep soil layers) under mild and severe water stress conditions.

19.3 Mapping Populations, Identification of Drought Tolerance QTL and MAS

Breeding for drought tolerance is hampered by our limited knowledge about the genetic basis of drought resistance and negative correlations of drought resistance traits with productivity (Mitra, 2001). Moreover, selection for yields in legumes like chickpea is not effective in early segregating generations because of their indeterminate growth habit (Ahmad et al., 2005). Traditional selection for drought tolerance is also difficult because the timing and severity of episodes of drought in the field are extremely variable (Passioura et al., 2007).

Drought tolerance is a complex trait governed quantitatively by many genes. In contrast to conventional breeding, the advent of molecular markers has allowed us to dissect quantitative traits into their single genetic components, called the quantitative trait loci (QTLs; Dudley, 1993; Tanksley, 1993). Further, molecular markers tightly linked to a QTL could assist the selection and pyramiding of the beneficial QTL alleles through marker-assisted breeding (MAB; Ribaut et al., 2002). The use of molecular markers for the indirect selection of improved crops speeds up the selection process by alleviating time-consuming approaches of direct screening under greenhouse and field conditions. Thus, QTLs linked to different root, shoot and physiological traits that confer drought tolerance can be transferred into an elite cultivar using associated markers.

Although linkage maps and molecular markers have been published for all important legume crops, most of the work on QTL mapping and molecular breeding has been done to address disease and insect resistance. The status of molecular breeding of pulse crops has been recently reviewed in detail in books published by Springer (edited by Kole, 2007; edited by Moore and Ming, 2008). Here we outline the work addressing QTLs and molecular markers for drought tolerance in legumes and discuss the future perspectives.

In common bean, Schneider et al. (1997b) evaluated the potential to select drought tolerance with QTL analysis and MAS in seven environments in Michigan, USA and highland Mexico. Using RAPD analysis, four markers for drought tolerance QTL were identified in one population and five in a second population. Selection based on MAS was effective under severe drought in Michigan but not for moderate drought in Mexico. It was suggested that the Genotype $\times$ Environment (G $\times$ E) interaction affected the expression of QTL, the genome coverage was incomplete and some unidentified QTL might have determined yield in the Mexican environments. Further, drought tolerance QTL have been identified for the BAT 477 source under non-irrigated conditions at *Centro Internacional de Agricultura Tropical*, Colombia (Blair et al., 2002).

In soybean, Mian et al. (1996, 1998) mapped seven QTL for WUE in two mapping populations; Young $\times$ PI416937 and S-100 $\times$ Tokyo. Among them, two QTL linked to RFLP markers cr392-1 of linkage group (LG)-J and A489H of LG-L explained 13 and 14% phenotypic variation, respectively. Another QTL linked to RFLP marker A063E for WUE was common in both the populations, but the

phenotypic effect was less than 10%. Later, Specht et al. (2001) determined the genetic basis of beta and carbon isotope discrimination using a population of 236 RILs developed from a cross between Minsoy × Noir 1. They reported one QTL on LG C2 with a phenotypic contribution of <10% and with no effect on beta. Subsequently, Bhatnagar et al. (2005) identified a major QTL linked to SSR marker Sat_044 on LG K that explained 17% of the phenotypic variation for slow wilting. Recently, Wood et al. (2006) documented a number of QTL related to water stress tolerance in soybean. They reported three QTL for root architecture of basal root, Satt509 (LG A2), Sat_083 (LG B2), and Satt316 (LG 316); one QTL for root dry weight, Satt554-CAA19 (LG F); and one QTL Satt214 in (LG G) for root and shoot dry weight ratio. In the same year, Monteros et al. (2006) identified three QTL associated with seed yield and slow wilting in a mapping population of 140 F4 lines from the cross Hutcheson × PI471938 (drought tolerant). One QTL from the PI471938 mapped to LG D2 near the SSR marker Satt226 and two QTL were located on LG F1 near the markers Sat_375 and Sat_074. The PI471938 QTL on LG D2 and LG F1 were associated with yield and linked with slow wilting.

In Chickpea, in the hope to help plants escape terminal drought by early flowering, two QTL for days to 50% flowering have been located on LG 3 (Cho et al., 2002; Cobos et al., 2004). Since selection of root traits is very laborious, it was suggested that molecular tagging of major genes for these traits may enable MAS and greatly improve the precision and efficiency of breeding. Serraj et al. (2004) evaluated the root traits of 257 RIL derived from a cross between a breeding line with a large root system (ICC 4958) and an agronomically preferred variety (Annigeri) to assess the potential for identifying QTL for desirable root traits and to investigate the relationship between root traits, plant growth and seed yield under terminal drought stress. The existence of large variability among RILs justified their use towards efforts for the identification of molecular markers for root traits. Over 250 STMS markers were initially screened on parents of the RILs (Chandra et al., 2004). Fifty-seven STMS markers detected polymorphisms and were mapped on the RIL population. A QTL flanked by the STMS markers TAA 170 and TR 55 on LG 4A accounted for maximal phenotypic variation in root length ($R_a^2 = 33.1\%$), root weight ($R_a^2 = 33.1\%$) and shoot weight ($R_a^2 = 54.2\%$), where R_a^2 was the adjusted coefficient of determination (Chandra et al., 2004). This locus also accounted for substantial variation observed in these traits under simulated and actual field conditions. Subsequently four accessions that contrasted extremely in rooting depth and total root biomass (ICC 8261 and ICC 4968 with large roots; ICC 283 and ICC 1882 with small roots) were selected for development of new mapping populations. Two crosses were made (ICC 4958 × ICC 1882 and ICC 8261 × ICC 283) and about 260 RIL developed from each cross. These two mapping populations have been phenotyped in 2005 and 2006, and genotyping is underway to generate sufficient markers to identify QTL for drought avoidance traits (Gaur et al., 2008).

Research focussed on identifying QTL for drought tolerance and associated markers in legume crops is very much in its infancy. Although MAS is yet to be

exploited to produce drought tolerant legumes, it has been used to generate disease and insect resistant varieties. MAS has been successfully used for the breeding soybean resistant to cyst nematode (Diers, 2004), common bean resistant to common bacterial blight (Mutlu et al., 2005) and lupin resistant to phomopsis stem blight and anthracnose (Yang et al., 2002; You et al., 2005). The results obtained in other crops to introgress drought tolerance traits using MAS are also encouraging enough to explore MAS for this trait in legumes. MAS has been used to introgress QTL alleles for reducing the anthesis-silking interval in maize as it is negatively associated with grain yield under drought (Ribaut et al., 2002). The molecular markers linked to five QTLs for anthesis-silking interval were used to select lines that outperformed unselected controls under severe drought stress (Ribaut et al., 2004). In Rice, marker-assisted back-cross (MABC) programme was used to introgress four QTL from the tropical *japonica* rice variety "Azucena" into the Indian upland rice variety, "Kalinga III". The introgressed QTL9 (on chromosome 9) significantly increased root length in the new genetic background. The field testing for agronomic traits in near-isogenic lines (NILs) revealed that NILs out-performed "Kalinga III" for grain and straw yield (Steele et al., 2007).

The quantitative complexity of the drought tolerance trait that has been approached by scientists by breaking it into major components that increase yield under stress, such as larger roots, smaller leaves, and greater water use efficiency. Plants need a different combination of these and other traits to perform well under drought in a given environment. For example, common bean cultivars selected for drought tolerance in one environment did not perform well in another environment (White et al., 1994). The genetic background and particular environment in which a plant is growing both have significant influence on the types and locations of the quantitatively inherited and expressed genes (Flowers, 2004). Moreover, the fact that a single QTL may represent many, perhaps, hundreds of genes, poses a problem in finding the key loci that actually govern tolerance (Flowers, 2004). Sometimes it is difficult to find a marker tightly linked to a QTL and there is always a chance of identifying a false positive marker. These factors greatly hinder marker-assisted breeding, causing "linkage drag" of undesirable traits due to the large regions of chromosomes identified by the QTL (Asins, 2002). The logical way forward is to identify specific and individual candidate gene sequences that may account for the QTL effects. This would require validating the function or role of the genes associated with the QTL individually. The identification of candidate genes and elucidation of their role can be facilitated by combining QTL analysis with different sources of information and technological platforms (Wayne and McIntyre, 2002). The recent progress in genome sequencing and mass-scale profiling of the transcriptome, proteome and metabolome facilitates investigation of concerted responses of thousands of genes to a particular stress. This area of study known as "functional genomics" involves development and application of global (genome-wide or system-wide) experimental approaches to assess gene function by making use of the information provided by genetic, physical and transcript maps of an organism.

19.4 Transcriptomics and Expression of Candidate Drought Tolerance Genes

In order to dissect the complex genetic basis of drought tolerance, the diversity of stress responses and their contributions to the survival of plant needs to be investigated. Such studies on diversity of stress responses and identification of candidate drought tolerance genes have been facilitated by the analyses of gene expression products at transcriptomic, proteomic and metabolomic levels.

Transcriptomics involves comparison of changes in gene expression profiles between treated and untreated plants, allowing the association of a particular set of genes with the treatment under investigation and hence suggesting gene function (Alba et al., 2004). A variety of tools are available to study changes in gene expression in response to the treatment of drought stress in plants. These include RNA gel blot (Hauser et al., 1997), differential display (Liang and Pardee, 1992), cDNA amplified fragment length polymorphism (cDNA AFLP; Bachem et al., 1998), microarrays (Schena et al., 1995), serial analysis of gene expression (SAGE; Velculescu et al., 1995), massively parallel signature sequencing (MPSS; Brenner et al., 2000), real time polymerase chain reaction (RT-PCR), suppression subtractive hybridization (SSH), and cDNA fingerprinting. Some of these methods are suitable for the intensive study of a relatively few number of genes and others allow genome-wide analysis of gene expression in response to the particular stimulus.

Most of the gene expression studies in response to drought in legumes have been limited to cloning of homologous known drought responsive genes using sequence information available from other species, and interrogating their expression levels in response to specific drought treatments. Here, we present a review on our current understanding of drought tolerance responses in legumes based on gene expression studies. First, we provide a crop wise update on work reported to date, followed by a discussion on how to utilise the current knowledge and develop future resources for better understanding of drought stress responses in legumes.

19.4.1 Common Bean (Phaseolus vulgaris *L.*)

Common bean is susceptible to drought compared to other bean species (Vasquez-Tello et al., 1990). A comparative study on aspartic protease (AP) activity in common bean (drought susceptible) and cowpea (drought tolerant) under drought stress found AP to be strongly induced in common bean (Cruz de Carvalho et al., 2001). They concluded that induction of AP only in common bean plants under drought stress may contribute toward its susceptibility.

Colmenero-Flores et al. (1997) identified several cDNA clones that were induced in common bean under drought stress. The expression of these clones encoding one lipid transfer protein (LTP), two late-embryogenesis abundant (LEA) proteins and two proline-rich proteins (PRP), was further characterised in different plant organs (Verdoy et al., 2004). The LTP transcript was highly induced only in the leaves of

drought stressed plants. Whilst, both LEA transcripts were induced in the roots, one transcript, *Pvlea-18*, was highly induced in the nodules. The PRP transcripts were induced in roots and nodules under drought stress. The LTP, LEA and PRP have been shown to be induced under drought stress in other crops and are thought to help in drought stress adaptation (as reviewed by Bartels and Sunkar, 2005).

Among the transport facilitators, a putative organic cation transporter (OCT) transcript, located in the phloem, was shown to be transitorily induced in the roots of common bean plants an hour after dehydration stress (Torres et al., 2003). Furthermore, the effects of drought, abscisic acid (ABA) and transpiration rate on the regulation of PIP aquaporin gene expression has been reported (Aroca et al., 2006). Drought and ABA caused decline in transpiration rate and induced the expression of two PIP transcripts in the leaves. Whilst, in the roots, only drought stress raised the expression of three PIP genes examined. Interestingly, the expression of PIP genes in the roots under drought stress differed by the presence or absence of arbuscular mycorrhizal fungus *Glomus intraradices* (Aroca et al., 2007).

Among the regulatory genes, a root-specific bZIP transcription factor that is transcribed only under water deficit conditions has been reported (Rodriguez-Uribe and O'Connell, 2006). In situ hybridisation and immuno-localisation revealed that this protein accumulated in the epidermis and phloem of the roots. The bZIP transcription factors were shown to mediate the abscisic acid regulated dehydration response in the vegetative tissues of *Arabidopsis* and barley (Uno et al., 2005; Xue and Loveridge, 2004).

Further, Torres et al. (2006) used differential display RT-PCR to identify 42 clones responsive to dehydration in common bean roots. These clones were identified to encode genes involved in signalling, protein-turnover and translocation, and root modulations. Differential display has also been recently used to identify genes responsive to drought in the leaves (Kavar et al., 2008). Fifteen transcripts were significantly expressed in all the eight cultivars assessed. All these were different to those reported for roots by Torres et al. (2006).

Microarrays have recently been employed to study drought stress response in common bean. Comparative transcription profiling in roots of common bean and its drought tolerant relative *P. acutifolius* identified 64 and 488 genes to be drought responsive, respectively (Micheletto et al., 2007). Most of the drought responsive genes in *P. vulgaris* were in the functional class of stress responsive genes, while the largest functional class in *P. acutifolius* was populated with unannotated or novel genes.

19.4.2 Cowpea (Vigna unguiculata *L. Walp.*)

Cowpea is a drought tolerant legume (Summerfield et al., 1985) and has been therefore utilised to study molecular mechanisms of drought tolerance. Ten cDNAs induced by drought stress were cloned from cowpea using differential display. Nine of these were induced by drought stress at different time points (Iuchi et al., 1996). Three genes were further characterised out of which two (old yellow enzyme and LEA protein) were also induced by ABA but one (dihydroflavonol-4-reductase)

was not; indicating the presence of both ABA-dependent and ABA-independent pathways. The involvement of ABA-dependent gene regulation under drought stress was reinforced by isolation and characterisation of a clone related to 9-cis-epoxycarotenoid dioxygenase (*VuNCED1*) from drought stressed cowpea plants (Iuchi et al., 2000). Both, the accumulation of ABA and expression of *VuNCED1* were strongly induced by drought stress.

Most of the gene expression studies in cowpea have been done by cloning genes from drought stressed plants and studying their expression in tolerant vs. susceptible cultivars. These include expression profiling of transcripts related to phospholipase D (PLD), patatin and multicystatin, all of which showed varied responses among tolerant and susceptible cultivars (El-Maarouf et al., 1999; Matos et al., 2001; Diop et al., 2004). The maintenance of steady levels of PLD and multicystatin, and low levels of patatin in the drought tolerant cultivar compared to the susceptible cultivar, have been proposed to contribute towards tolerance/susceptibility.

Further, gene expression of clones related to two key detoxification enzymes, ascorbate peroxidase and glutathione reductase have been studied (Contour-Ansel et al., 2006; D'Arcy-Lameta et al., 2006). Comparison of gene expression of four different ascorbate peroxidase genes among tolerant and sensitive cultivars revealed early expression in the tolerant cultivar suggesting a capacity to efficiently detoxify active oxygen species at their production site. Similarly, comparison of gene expression of two glutathione reductases among tolerant and susceptible cultivars revealed a quicker response by the tolerant cultivar to fast desiccation, suggesting a better ability to respond. Recently, a drought expression cDNA library was constructed in South Africa using SSH (Gazendam and Oelofse, 2007). Clones from this library are being employed to interrogate their differential expression under drought stress using microarray.

19.4.3 Soybean [Glycine max *(L.) Merr.*]

The characterisation of a cDNA clone related to proline-rich protein revealed its expression to be induced under drought stress (He et al., 2002). Two studies on gene expression of transcripts related to pyrroline-5-carboxylate synthetase (P5CS), and late embryogenesis abundant (LEA) protein in the roots of mycorrhizal and non-mycorrhizal plants under drought stress showed lower transcript accumulation in non-mycorrhizal plants for both (Porcel et al., 2004, 2005). Therefore, it was proposed that the induction of P5CS and LEA is not the mechanism by which mycorrhizal symbiosis protects soybean against drought.

Among the regulatory genes, three dehydration-responsive element-binding (DREB) genes were cloned from soybean and their response to abiotic stresses was characterised (Li et al., 2005). The transcriptions of *GmDREBa* and *GmDREBb* were significantly induced in the leaves, whilst *GmDREBc* was significantly induced in the roots under drought stress, revealing the specificity of gene functions. Interestingly, a study of DREB gene expression in a drought tolerant soybean cultivar and a more drought tolerant wild relative (*G. soja*) showed that the expression of DREB was rapid and higher in the wild relative (Chen et al., 2006). These results

reinforced the involvement of DREB genes in drought tolerance, while highlighting the importance of a quick response by the plant for drought stress tolerance.

A few studies have reported changes in protein/metabolite abundance under drought stress. One such study concluded that sucrose synthase may play a key role in the regulation of nodule carbon metabolism and, therefore, of nitrogen fixation under drought stress conditions (Gonzalez et al., 1995). Another study indicated that under drought stress, the accumulation of pinitol was more pronounced than that of proline or other sugars and may be linked to drought tolerance in soybean (Streeter et al., 2001). Further, consistently higher glutathione reductase activity in the roots and nodules of mycorrhizal soybean plants than the roots of non-mycorrhizal plants has been observed under drought stress (Porcel et al., 2003). It was proposed that this high GR activity protects the roots and nodules against oxidative damage to biomolecules under drought stress.

Microarray resources like ESTs and oligonucleotides have recently become available for soybean. About 300,000 ESTs and 38,000 unique oligos have become available due to a community based program at University of Illinois, USA (URL: www.soygenetics.org). This resource will be highly valuable to study the genetic response to drought, however, to date these microarrays have only been used to investigate the response to pathogens, CO_2 atmospheric conditions, seed development and germination (Zou et al., 2005; Ainsworth et al., 2006; Gonzales and Vodkin, 2006).

19.4.4 Peanut (Arachis hypogea *L.*)

Drought tolerance-related gene expression studies in peanut include cloning and characterisation of 9-cis-epoxycarotenoid dioxygenase (NCED) that is believed to catalyse a rate-limiting step in ABA biosynthesis. The *AhNCED1* was induced by drought but repressed upon rehydration, suggesting its involvement in ABA biosynthesis under drought stress (Wan and Li, 2005). Another study involved cloning of a lipid degrading enzyme, phospholipase D (PLD), and studying its expression in peanut cultivars differing in ability to tolerate drought (Guo et al., 2006). The PLD gene was expressed faster in sensitive cultivars than in tolerant ones and may be indicative of sensitivity/tolerance to drought as PLD hydrolyses phospholipids to produce phosphatidic acid which acts as signalling messenger.

Luo et al. (2005a) developed two EST libraries for peanut after challenging *Tomato spotted wilt virus* (*TSWV*) and drought resistant cultivars with the respective stresses. A cDNA microarray containing 386 unigenes from these libraries was used to generate gene expression profiles in response to drought in a tolerant cultivar (Luo et al., 2005b). A total of 52 genes belonging to secondary metabolism, detoxification, heat shock proteins, ion transporters, defence, and signalling were induced in response to drought. Recently, cDNA clones related to membrane phospholipid, proteases and LEA protein were employed to study comparative expression in drought tolerant and sensitive cultivars under drought stress and rehydration. A good

correspondence between molecular responses of these cultivars and their physiological responses previously defined in field and greenhouse experiments was observed (Dramé et al., 2007). Such molecular characters if well established can be integrated into the peanut breeding programmes.

19.4.5 Chickpea (Cicer arietinum *L.*)

Gene expression studies in response to drought in chickpea include construction of a cDNA library to investigate drought response in seedlings and plants (Romo et al., 2001). Genes coding for LTP and LEA proteins were found to be important in chickpea water stress response. The expression of LTP was higher in young than mature tissues, and its transcript level decreased gradually as the age of epicotyls increased. Another study identified 101 dehydration-inducible transcripts by repetitive rounds of cDNA subtraction and investigated the steady state level of these transcripts during the recovery period between consecutive dehydration stresses (Boominathan et al., 2004). Seven transcripts maintained threefold expression after 24 h and more than twofold expression even at 72 h after removal of the stress. A correlation between the longer period of abundance of these transcripts in the recovery period and improved adaptation of the plants to subsequent dehydration was observed and suggested their role in maintenance of messages from previous stress experiences.

Recently, a 768-feature cDNA microarray was used to compare the expression profiles of two drought tolerant (BG 1103 and BG 362) and two drought susceptible (Kaniva and Genesis 508) chickpea cultivars (Mantri et al., 2007). Significant differences were shown to exist between the responses of drought tolerant and susceptible genotypes, and highlighted the multiple gene control and complexity of the drought tolerance mechanism(s). The key findings included repression of the transcripts associated with senescence like auxin-responsive protein IAA9, magnesium chelatase, phosphate-induced protein, and senescence-associated protein in the tolerant genotypes. Further, the induction of a protein-transport protein and a lipid-transfer protein, that facilitate solute transport, may be essential for drought tolerance. Subsequently, the induction of RAC-GTP binding protein that facilitates pollen tube growth may contribute towards drought tolerance by promoting successful fertilisation and seed production.

19.4.6 Alfalfa (Medicago sativa *L.*)

Gene expression studies of drought tolerance in alfalfa have identified an ankyrin protein kinase (*Msapk1*) under osmotic stress. The *Msapk1* expression was induced in roots starting from 3 h up to two days of osmotic stress (Chinchilla et al., 2003). Gene expression was also studied with respect to involvement of carbon metabolism and oxidative stress in the decline of nitrogenase activity in nodules of drought stressed alfalfa (Naya et al., 2007). Under drought stress, oxidative stress occurred

in nodules prior to any detectable effect on sucrose synthetase or leghemoglobin. A limitation in metabolic capacity of bacteroids and oxidative damage of cellular components was concluded to contribute towards inhibition of nitrogenase activity in alfalfa nodules.

Recently, a 16K *Medicago truncatula* microarray was used for gene expression profiling of two non-nodulated alfalfa cultivars in response to drought stress (Chen et al., 2008). Many known drought-responsive genes were induced in the shoots and roots, including up-regulation of heat shock-related protein, dehydrin and LEA after 3 and 8 h of drought stress. Interestingly, the genes encoding caffeoyl-CoA *O*-methyl transferase and dirigent were induced in 3 h stressed roots, while two aquaporin genes were repressed, suggesting that lignification and prevention of water loss in roots in initial dehydration stress was a common strategy for both the cultivars.

19.4.7 Pea (Pisum sativum *L.*)*, Mung Bean* [Vigna radiata (*L.*) *Wilczek*] *and Faba Bean* (Vicia faba *L.*)

A single study of gene expression related to drought stress has been reported for each of pea, mung bean and faba bean. The expression of cytokinin oxidase/dehydrogenase (CKX) was monitored in drought stressed leaves of pea and showed an unexpectedly low level of transcription (Vaseva-Gemisheva et al., 2005). In mung bean, the expression of phosphoinositide-specific phospholipase (PI-PLC) was characterised in response to drought stress. Among the three PI-PLC clones studied, *VrPLC1* and *VrPLC2* were observed to be constitutively expressed to varying degrees in all the tissues examined, while *VrPLC3* expression was rapidly increased in an ABA-independent manner only under drought and high-salinity stresses (Kim et al., 2004). PI-PLC catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate two secondary messengers (inositol 1,4,5-trisphosphate and diacyglycerol), which may serve in drought stress signalling. In faba bean, a novel calcium-dependent protein kinase (CDPK) was cloned and its expression in response to drought stress was interrogated (Liu et al., 2006). The *VfCPK1* was induced in the leaves submitted to drought stress or ABA. The CDPKs are predominant Ca^{2+}-regulated protein kinases and play an important role in calcium signal transduction in plants subjected to drought stress (Yamaguchi-Shinozaki and Shinozaki, 2006).

19.4.8 Model Legumes: Barrel Medic (Medicago truncatula) *and Lotus* (Lotus japonicus)

M. truncatula and *L. japonicus* have been proposed as model legumes to investigate various growth and developmental processes, and resistance and tolerance to stresses (Harrison, 2000; Dita et al., 2006). However, to date, there are no published

reports involving gene expression studies of drought tolerance in shoots or roots in these species. Although one related study reports transcriptional profiling of processes leading to desiccation tolerance in seeds using the 16K *M. truncatula* microarray (Buitink et al., 2006). More than 1,300 genes were differentially expressed during the re-establishment of desiccation tolerance in germinated seeds, most of them belonging to carbon metabolism. The genes induced at later stages of re-establishment of desiccation tolerance were comparable to those involved in late seed maturation. This coincided with the repression of large number of genes related to cell cycle, biogenesis, primary and energy metabolism. The re-establishment of desiccation tolerance in germinated radicles was concluded to agree with a partial return to the dormant state prior to germination.

19.4.9 Perspectives

Studies on the transcriptomics for drought tolerance in general has led to the identification of a number of associated genes including osmosensors (SLN1 and SHO1), Ca^{2+} signalling cascades, various transcription factors (including MYC, MYB, NAC), regulatory elements (DREB, zinc-finger proteins, PKS5, bHLH, AP2/ERF), and response proteins (e.g. osmoprotectants like proline, trehalose, etc.) that function in a ABA-dependent or ABA-independent manner (Bartels and Sunkar, 2005). The ABA-independent gene expression functions through a drought-responsive element binding (DREB) protein that binds to a drought-responsive element (DRE) motif of the effector gene. One of the genes induced by drought, cold and ABA in *Arabidopsis* is *RD29A/COR78/LTI78* (Kreps et al., 2002).

Most of this information about drought stress response comes from study of the model species *Arabidopsis thaliana*. As seen above, most of the studies in legumes have involved utilising the information generated from studying other crops (mainly *Arabidopsis*), to identify and clone corresponding genes, and study their expression under drought stress. Even though these studies have been done in bits and pieces in different legume crops, a good thing about some of these studies is that gene expression data was matched to physiological changes like transpiration rate, root hydraulic conductance, osmotic potential, and accumulation of proteins, enzymes, or metabolites. These kind of studies are key to link the genome to phenome.

19.5 Transgenic Approaches to Overcome Drought Stress

As discussed in the previous section, a number of drought-inducible "tolerance" genes have been identified, mainly in *Arabidopsis*, and some of them have been cloned and characterised in legumes. The functional analysis of these genes is critical to our further understanding of the molecular mechanisms governing drought stress responses and tolerances. One mode of establishing this 'proof-of-function' is by overexpressing these genes by genetic manipulation and observing the phenotype for desired changes.

The genes induced in response to drought stress can be classified into two main types, functional and regulatory proteins. The functional proteins include protectors of macromolecules (e.g., chaperones, LEAs, LTPs), detoxification enzymes (e.g., GST, peroxidases), and osmoprotectants (e.g., proline, glycine betaine, sucrose, mannitol). Regulatory proteins include transcription factors (e.g., DREB, ERF, MYB, MYC, bZIP), protein kinases (e.g., MAPK, MAPKKK, CDPK), protein phosphatases, and calmodulin-binding proteins (Seki et al., 2003). Most of these genes have been transformed into plants to interrogate their contribution towards drought tolerance. Again, these studies have predominantly been performed in the model plant *Arabdiopsis* (as reviewed by Umezawa et al., 2006). A limited number of these studies have been done in crop plants including legumes. Here we review the current progress in understanding the mechanisms of gene regulation and roles of protective metabolites in drought stress tolerance of legumes by genetic or metabolic engineering. First we provide a status update of work done in legumes followed by an outline of how to harness the information available from other species.

Legumes are generally recalcitrant to transformation (Somers et al., 2003). However, significant progress has been made to develop efficient transformation system. The reproducibility, robustness and efficiency of these methods have been evaluated by Popelka et al. (2004). The various genes introduced into legumes to assess their role in drought tolerance are listed in Table 19.1. All these studies used the *Agrobacterium*-mediated transformation method.

19.5.1 Transgenics Involving Functional Proteins

Among the functional proteins, the L-Δ^1-pyrroline-5-carboxylate synthetase (P5CS) that controls a common step for both the proline synthesis pathways (glutamic acid and ornithine) has been intensively studied in soybean. The *P5CS* gene from *Arabidopsis* was cloned and its antisense version was introduced into soybean under the control of a heat shock-inducible promoter (*IHSP*). Under drought stress at high temperature, the *IHSP* was activated to induce the production of the antisense transcript, blocking proline synthesis (de Ronde et al., 2000). This confirmed an association between *P5CS* translation and proline accumulation. However, the transformants failed to survive a 6-day drought stress at 37 °C, indicating that proline plays a definitive role in survival of soybean plants under drought stress.

Further, a comparative study was performed between transgenic soybean containing the *P5CS* gene in sense or antisense orientation and an untransformed control under drought stress. The sense plants showed significantly higher relative water content (RWC), particularly after eight days of stress, that coincided with much higher free proline levels compared to control and antisense plants (de Ronde et al., 2004). The proline dehydrogenase activity was highest in antisense plants, followed by control plants, and least in sense plants, confirming that some of the proline measured in antisense plants was degradation products. The sense plants were more drought tolerant than control or antisense plants, again reinforcing the involvement of *P5CS* and thus proline in drought tolerance reaction of soybean.

Table 19.1 Use of transgenics to study role of functional and regulatory proteins in conferring drought tolerance to legumes

Gene name	Gene function	Gene origin	Promoter	Transgenic plant	Performance of transgenic plants	References
Functional proteins						
P5CR – antisense	Block proline biosynthesis	*Arabidopsis thaliana*	*IHSP*	*Glycine max*	Failed to survive a 6-day drought stress at 37 °C	de Ronde et al. (2000)
P5CR – sense/antisense	Induce/block proline synthesis	*Arabidopsis thaliana*	*IHSP*	*Glycine max*	Sense plants were more tolerant to drought, had highest RWC and proline levels than antisense and control plants	de Ronde et al. (2004)
P5CR – sense/antisense	Induce/ block proline synthesis	*Arabidopsis thaliana*	*IHSP*	*Glycine max*	Manipulation of proline affected the GSH concentration and antioxidant levels	Kocsy et al. (2005)
P5CR	Proline synthesis	*Arabidopsis thaliana*	*IHSP*	*Glycine max*	Accumulated 6-times more proline than control. Level of proline accumulation affected levels of other amina acids	Simon-Sarkadi et al. (2006)
MnSOD, FeSOD	Antioxidant	*Nicotiana plumbaginifolia*	*CaMV 35S*	Alfalfa	Higher photosynthetic activity at mild stress; performed similar to non-transformed plant at moderate and severe stress	Rubio et al. (2002)
LEA		*Brassica napus*	*CaMV 35S*	Kidney bean	Enhanced tolerance; delay in development of drought stress sympotoms	Liu et al. (2005)

Table 19.1 (continued)

Gene name	Gene function	Gene origin	Promoter	Transgenic plant	Performance of transgenic plants	References
Regulatory proteins						
WXP1	Wax production	*Medicago truncatula*	*CaMV 35S*	*Medicago sativa*	Wax accumalation led to reduced water loss and chlorophyll leaching, and enhanced drought tolerance	Zhang et al. (2005)
DREB1A	Transcription factor	*Arabidopsis thaliana*	*rd29A*	*Arachis hypogea*	Maintained transpiration rate equivalent to well watered controls and showed higher transpiration efficiency	Bhatnagar-Mathur et al. (2007)
NTR1	Methyl jasmonate synthesis	*Brassica campestris*	*CaMV 35S*	*Glycine max*	Better dehydration tolerance during seed germination and seedling growth	Xue et al. (2007)

Koscy et al. (2005) studied the effects of different proline accumulation levels in sense, antisense, and control plants, on antioxidant activities under drought stress. The antisense plants had highest H_2O_2 and lipid hydroperoxide levels and greatest injury, whilst the opposite was true for sense plants. Moreover, during stress treatment, the highest proline and ascorbate levels were detected in sense plants, whilst the highest recuced/oxidised glutathione ratio, ascorbate/dehydroascorbate ratio, and ascorbate peroxidase activity, was detected in antisense plants. This indicated that manipulation of proline affects not only the (homo)glutathione concentrations, but also the levels of other antioxidants. Similar study to question the effect of proline accumulation on free amino acid concentrations revealed that manipulating the content of a single amino acid influences the whole free amino acid composition in soybean (Simon-Sarkadi et al., 2006).

Among other functional proteins, antioxidant enzymes like Mn-containing superoxide dismutase (MnSOD) and Fe-containing-superoxide dismutase (FeSOD) were constitutively overexpressed in alfalfa under the control of the *Cauliflower mosaic virus* 35S (*CaMV35S*) promoter. Three types of transgenic lines that overproduced MnSOD in mitochondria of leaves and nodules, MnSOD in chloroplasts, and FeSOD in chloroplasts were generated. Under mild water stress, transgenic lines displayed 20% higher photosynthetic activity than untransformed lines. However, both untransformed and transgenic lines performed similarly during moderate and severe water stress, and recovery with respect to important markers of metabolic activity and oxidative stress in leaves and nodules (Rubio et al., 2002). The base genotype used for transformation and background SOD isozymic composition was concluded to potentially intensely effect the relative tolerance of transgenic lines.

Subsequently, late embryogenesis abundant (LEA) protein that is reported to have a protective role enabling cells to survive protoplasmic water depletion was constitutively overexpressed in kidney bean. Transgenic kidney bean containing a group 3 *lea* gene from *Brassica napus* demonstrated enhanced growth ability under salt and water deficit conditions (Liu et al., 2005). The increased tolerance was reflected by delayed stress damage in the transformants. The role of *lea* in drought tolerance was reinforced by the observation that higher stress tolerance was seen in the transformants with higher levels of *lea* gene expression and lower tolerance was seen in lines with lower expression levels.

19.5.2 Transgenics Involving Regulatory Proteins

Recent progress in engineering for drought tolerance has involved manipulation of transcription factors believed to confer tolerance. Among them, transcriptional activators of wax production (*WXP1*), floral nectary-specific protein (*NTR1*), and dehydration-responsive element-binding protein (*DREB2*) have been transformed in legumes. The *WXP1*, a transcription factor belonging to the AP2/ERF family is able to activate wax production. Overexpression of *WXP1* in alfalfa under the control of *CaMV35S* led to significant increase in cuticular wax loading on leaves (Zhang et al., 2005). Transgenic plants showed reduced water loss and chlorophyll

leaching, and enhanced drought tolerance as demonstrated by delayed wilting under stress and faster recovery after rehydration (Zhang et al., 2005).

Another transcription factor, *DREB1A*, belonging to the AP2/EREBP family was overexpressed in a drought-sensitive peanut cultivar under control of the stress inducible promoter from *rd29A*. Under drought stress, the transgenic plants maintained a transpiration rate equivalent to well watered controls. The transformants also displayed higher transpiration efficiency than untransformed controls under well watered conditions, which was explained by a lower stomatal conductance (Bhatnagar-Mathur et al., 2007).

Further, the *NTR1* from *Brassica campestris* that is involved in methyl jasmonate (MeJA) production was constitutively overexpressed in soybean under control of *CaMV35S* (Xue et al., 2007). MeJA is a signalling molecule involved in plant development and regulates gene expression in response to environmental stresses (Creelman and Mullet, 1995). The transgenic soybean plants accumulated more MeJA than untransformed controls which conferred dehydration tolerance during seed germination and seedling growth as reflected by percentage of fresh weight of the seedlings (Xue et al., 2007). Detached leaf testing also showed superior water retention ability of the transgenic plants.

19.5.3 Perspectives

Transgenic approaches offer a powerful means to acquire important knowledge that will lead to a better understanding of drought tolerance mechanisms. As reported above, scientists can introduce a single gene into plants that lack or do not express them, and observe physiological and biochemical changes under drought stress at different stages of plant growth. This preliminary investigation of overexpressing functional and regulatory proteins in legumes has produced promising results. These studies have reinforced the fact that knowledge gained from studying model species and other crops is transferable across species (e.g., overexpression of *DREB1A* in peanut that was originally studied in *Arabidopsis*).

Most of the known candidate drought tolerance genes have been transformed and their importance under stress interrogated (as reviewed by Umezawa et al., 2006). Although manipulation of most of these genes has proven their possible contribution in conferring drought tolerance, none of these can be pronounced as a key to tolerance. Following a focus on downstream genes producing osmoprotectants or antioxidants there is a growing realisation that it would be more beneficial to manipulate the key genes that govern the production of all these molecules under stress. Hence, the focus has shifted towards manipulating regulatory genes like transcription factors. Recently, in an attempt to find those key genes, researchers have manipulated various signal transduction systems (like those involving protein phosphorylation/dephosphorylation, phospholipid metabolism, and calcium sensing) that function upstream of transcription factors (Bartels and Sunkar, 2005; Boudsocq and Lauriere, 2005). Some of the problems of using a constitutive promoter (like *CaMV35S*) to express these genes were overcome by exploiting stress

inducible promoters (like *IHSP* or *rd29A*). However, the major breakthrough discovery of what key genes control drought tolerance response pathways under different conditions and across different environments is yet to be made.

19.6 Future Molecular Approaches for Drought Tolerance

The mechanisms through which plants perceive environmental signals and transmit them to cellular machinery to generate adaptive response is of fundamental importance to biology (Xiong et al., 2002). Plants sense a change in environmental condition and the signal is relayed through signalling cascades that amplify the signal and notify parallel pathways resulting in the production of effector molecules that mitigate stress (Vij and Tyagi, 2007). Drought stress response is complex and diverse, and every gene involved in the tolerance response, from perception to signalling to direct involvement, forms part of a coordinated response network. It is interesting to note that varieties of single plant species exhibit a high degree of variation in salt and drought tolerance suggesting that only a few key genes might enhance plant adaptation to adverse growth conditions (Crespi, 2007).

Functional genomics research of drought tolerance over the past decade has significantly enhanced our knowledge on the molecular control of the tolerance mechanism. Compared to the information available for molecular mechanisms of drought tolerance in *Arabidopsis* and some cereal crops, the research in legume crops is much in its infancy. The *Arabidopsis* model is likely to be different from legumes in the responses to stress in relation to grain filling, nitrogen utilisation, fixation and transport, root architecture and interactions – all physiological processes that are fundamentally different in legumes (Crespi, 2007). Therefore, there is a need to develop molecular resources (like gene/EST sequences, oligonucleotides, cDNA libraries and BACs) for legumes so that large scale gene expression profiling experiments can be performed. Since genome sequencing of all legumes is a costly affair (some legumes have huge genomes, e.g. faba bean = 13.06 pg), selection and sequencing of a model legume was proposed. Leguminous species *Medicago truncatula* and *Lotus japonicus* were chosen mainly because of their compact genome. Recent sequencing initiatives have led to the *M. truncatula* genome being completely sequenced and *L. japonicus* sequencing is nearly finished (URL: http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html). However, their microsynteny and thus functional homology and usefulness to studying the crop legume species is debatable.

As the importance of understanding the molecular bases of physiological responses continues to rise, hundreds of thousands of gene/EST sequences have become available for a wide range of legume crops, which are being used to investigate stress tolerance mechanisms. To facilitate efficient utilisation of the large amount of information being generated, the Legume Information System (URL: http://www.comparative-legumes.org), has been developed by the National Centre for Genome Resource in collaboration with the USDA Agricultural Research Service (ARS). This is a comparative genome resource that integrates genomic and

molecular data from multiple legume species allowing cross-species genomic and transcript comparison.

The availability of such resources has set an excellent platform, which shall greatly aid in enhancing knowledge about gene expression required to effect drought tolerance in legumes. The candidate genes identified through gene expression studies can be validated for "proof-of-function" using reverse genetic approaches like knockouts/TILLING-mutants/overexpressing-transgenics. The identification of novel genes, determination of their expression patterns in response to drought stress conditions, and an improved understanding of their functions in stress adaptation will provide basic knowledge to design effective engineering strategies for enhancement of drought tolerance in legumes.

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