



Breeding and Molecular Approaches for Evolving Drought-Tolerant Soybeans

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Abstract

Soybean [*Glycine max* (L.) Merr] is an agronomically important oilseed crop in the world and an important source of protein and oil for both humans and animals. In addition, soybean is also becoming a major crop for bio-diesel production. Therefore, demand for soybean is increasing continuously worldwide. Soybean enriches the soil by fixing atmospheric nitrogen through symbiotic interaction with Rhizobia. With increasing challenges posed by climate change, it is predicted that incidents of drought will be more frequent and severe and it will further reduce crop yields. Abiotic stresses such as drought cause severe losses to soybean productivity worldwide by adversely affecting the plant growth, development, and yield. Introgression of genes controlling drought adaptive traits,

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yields related traits and root system architecture traits by breeding and molecular approaches will be very useful for enhancing drought stress tolerance in soybean, leading to cultivar development. Elucidation of function of genes and their integration in soybean genotypes by molecular breeding and genomic approaches and utilizing robust phenotyping tools to evaluate drought adaptive traits will be crucial for understanding response of soybean plants to drought stress. Recent advances in genomics lead identification, functional characterization, and introgression of genes associated with adaptation of soybean plants to drought stress. In order to perform genetic and genomic analysis, molecular markers have been employed on RIL or F₂ populations. In addition, the genome typified with single nucleotide polymorphisms (SNPs) and its utilization in molecular breeding applications like QTL mapping, positional cloning, association mapping studies, genomic selection and genome editing is gaining impetus. Thus, the rapid development of soybean genomics and transcriptomics has provided tremendous opportunity for the genetic improvement of soybean for drought tolerance with yield stability.

Keywords

Drought tolerance · Abiotic stress · Quantitative trait loci · Breeding · Genetic engineering · Signal transduction · Transcriptomic approaches

4.1 Introduction

Soybean is one of the most important crops in the world and provides oil and protein for both humans and livestock. Global food security is a major challenging task for world agricultural community since the world population is growing exponentially and crop cultivable land is decreasing due to adverse climatic conditions (Foley et al. 2011). The USA, Brazil, Argentina, China, and India account for about 93% of global soybean production. It is one of the most economical sources of good quality protein (40%), edible oil (20%), essential amino acids, dietary minerals, vitamins, and nutraceuticals like isoflavones, tocopherols, etc. of immense health benefits. Such diverse uses of soybean make it a wildly desired crop, and demand for soybean is rapidly increasing (Ray et al. 2013; Deshmukh et al. 2014). However, soybean yield is threatened by various abiotic stresses mainly drought (Phang et al. 2008; Manavalan et al. 2009). Adverse environmental factor mainly drought leads to morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Wang et al. 2001). Thus, understanding responses of soybean plants to drought stress and enhancing abiotic stress resilience to maintain genetic yield potential are extremely demanding areas in agricultural research. To improve drought stress tolerance in soybean, a wide range of approaches, including gene discovery, QTL mapping, genome-wide association studies (GWAS), and biotechnological approaches such as genomic selection and

gene editing can be used to develop soybean varieties with improved drought tolerance (Deshmukh et al. 2014; Phang et al. 2008; Manavalan et al. 2009).

Climate models have indicated that drought episodes will become more frequent because of the long-term effects of global warming (Salinger et al. 2005; Cook et al. 2007) and may significantly affect soybean yield in many countries (Long et al. 2005; Easterling et al. 2007). While increase in CO₂ under climate change might be expected to increase soybean productivity, vicious effects of frequent droughts and associated diseases and herbivores infestation may counteract such benefits. Drought is considered one of the most devastating among abiotic stress factors (Manavalan et al. 2009; Tran and Mochida 2010) reducing about 40% soybean yield annually (Specht et al. 1999) and depending upon the intensity of its occurrence at critical growth stage, the losses could be as high as 80% (Oya et al. 2004; Dias et al. 2012). Efficient resource acquisition and remobilization are challenges for soybean productivity under water-limited scenario. The hydrostatic pressure created by transpiration from the shoot is transmitted to the xylem vessels of the shoot and the roots, which drives water in the root cylinder toward the xylem vessels (Steudle 1995; Tyree 1997) and are governed by hydraulic mechanisms such as leaf conductance (Sinclair et al. 2010), leaf canopy size (Ratnakumar and Vadez 2011; Vadez et al. 2011), control of leaf expansion (Simonneau et al. 2009), and transpiration response in soybean to high vapor pressure deficit (VPD) (Sinclair et al. 2008; Ocheltree et al. 2014). In soybean, vegetative growth is sensitive to water deficits. Besides, usual inhibitory effects on leaf expansion, transpiration and photosynthesis, water deficit also inhibit nitrogen fixation in soybean. Water stress, occurring during the beginning of pod setting and full seed-filling, has a greater negative irreversible impact on yield through reducing seed size considerably (Doss and Thurlow 1974), as compared to other stages, and thus invite designing of breeding strategy targeting the final expression of yield under drought, i.e. seed size by integrating constitutive plant traits and stress-responsive processes (Blum 2011). A drought resistance index in terms of yield can be developed by comparing yield between stress and non-stress conditions, which is gaining popularity as a useful criterion in selection for drought resistance (Fukai et al. 1999). Drought resistance is measured by phenotyping the specific and relevant attributes of dehydration tolerance and dehydration avoidance, where a concrete breeding program integrates robust, reliable, relatively fast, and economical phenotyping facilities. Dehydration tolerance, phenotyped only on the basis of similar plant water status in all the genotypes, includes the most prominent feature of whole plant dehydration tolerance assessing the capacity for stem reserve utilization for seed filling by chemical desiccation method. Protocols for dehydration avoidance include measuring plant water status, in terms of visual symptoms of leaf senescence, relative water content, and constitutive traits without exposure to drought stress such as root system architecture traits. (Blum 2011).

An important component of reproductive success of the crop under drought stress is the capacity for seed filling from stem reserve, when transient photosynthesis is inhibited by stress. This is a dehydration tolerance mechanism since the transport of reserves from stem to seed takes place in dehydrated plants, in the case of severe drought in the field. It can be phenotyped in large populations by the chemical

desiccation method. The method was developed by Blum et al. (1983a, b) as a fast and relatively simple field assay for revealing the capacity for seed filling from stem reserves. The method is based on the application of a chemical desiccant to plant canopies after flowering as a means for inhibiting plant photosynthesis and thus revealing the capacity for seed filling by stem reserves. The treatment does not simulate drought stress. It simulates the effect of stress by inhibiting current assimilation. With this method a chemical desiccant (potassium iodide; 0.2% w/v) is sprayed to complete wetting over the whole canopy, at seed filling stage (R5 plus 8–10 days) in soybean (Bhatia et al. 2014), which mainly destroys chlorophyll and simulates natural senescence. Chemical desiccation can be incorporated into breeding programs in two ways: (1) it can be used to assess responses of individual advanced lines or families, always compared with non-treated controls under non-stress conditions and (2) the method can be used for early generation advancement through mass selection where F_2 bulks are chemically desiccated and selections are made for seed size divergently by mechanical sieving. After two cycles of early generation selection, vigorous lines were selected and tested for their response to chemical desiccation stress. Mass selection for large grains under chemical desiccation significantly improved grain weight and grain yield of the population under desiccation stress, as compared to control where selection for grain size was performed without chemical desiccation (Blum et al. 1991; Haley and Quick 1993; Annual Report 2019).

Physiological processes such as delayed leaf senescence, water status, and canopy temperature are very crucial for drought stress tolerance capability in plants. Delayed leaf senescence in a flowering plant induces extreme drought tolerance and evidenced by suppression of drought-induced leaf senescence in transgenic tobacco plants expressing *isopentenyltransferase* (IPT), an enzyme that catalyzes the rate-limiting step in cytokinin synthesis, resulted in outstanding drought tolerance as shown by vigorous growth after a long drought period, among other responses of high water contents, retained photosynthetic activity albeit at a reduced level, and displayed minimal yield loss when watered with only 30% of the amount of water used under control conditions during the drought (Rivero et al. 2007). A “slow-wilting” line has been recognized in soybean (Fletcher et al. 2007). The visual scoring of delayed leaf senescence of a given genotype must be based on an integrated impression of the symptom in the whole plant or even the whole plot. Scoring is performed on 1–5 arbitrary rating scale with 1 being sensitive with dried leaves chaffy matured pods and 5 being delayed leaf senescence with well-filled matured pods and green leaves. Very small variations in leaf senescence score, even if they are statistically significant, are of no real consequence in breeding for dehydration avoidance and large and prominent differences are sought (Blum 2011).

Leaf relative water content (RWC) is a simple, standard, and effective estimate and a reliable indicator of water status in plants (Sinclair and Ludlow 1985) with respect to dehydration avoidance. Usually the top-most fully expanded sun-lit leaf must be sampled to determine leaf RWC as per Blum (2011). Boyer et al. (2008) cautioned against excessive rehydration of samples which can result in excessive absorption of water by the leaf sample, beyond its normal full turgor capacity. This

would bias estimated RWC downward. In order to control the severity and timing in the field, drought stress is affected by stopping water supply, be it by terminating irrigation or by activating the rainout shelter. Stress will then develop gradually and it is crucial to be able to translate the number of days without watering into the desired level of plant stress. When grown on deep soil of good water holding capacity soybean may take around 2–3 weeks to reach midday RWC of about 60–70%. In phenotyping and selection work, it is not absolutely necessary to measure all relevant atmospheric and soil variables, in order to estimate daily crop water-use, and thus to predict the timing and rate of the planned imposed drought, where one can access the plant and estimate directly or indirectly its water status with only a minimal reference to environmental variables. Since drought phenotyping is usually repeated in the same location during the course of breeding, experience gained can be an important lead for gauging stress treatments (Blum 2011).

Canopy temperature depression, an indirect measure of plant-water status of the crop, is the difference between air temperature and plant canopy temperature (Tuberosa 2012). Genotypes use more available soil moisture to cool the canopy by transpiration under drought stress (Reynolds et al. 2009). Further, plant leaves emit long-wave infrared radiation according to their temperature. Low water status in stressed plants leading to reduced transpiration raises canopy temperature. The infrared thermometer is designed to sense long-wave infrared radiation emitted from its target, converting it to an average temperature display which can be related to transpiration and to the genetic potential of roots in exploring soil moisture (Pinto and Reynolds 2015) and drought susceptibility index in stressed environments (Blum 1989).

4.2 Exploring Roots of Drought Tolerance

The food security in the 21st century will rely increasingly on the release of cultivars with improved resistance to drought conditions and with high yield stability (Swaminathan 2005; Borlaug 2007; Pennisi 2008) and demands attention of plant scientists belowground (Bishopp and Lynch 2015). The development of crops with root systems that can capture water and nutrients efficiently would contribute in improving the economic development in poor nations and the sustainability of agriculture in rich nations by reducing reliance on intensive fertilization and irrigation (Bishopp and Lynch 2015). Carbon through CO₂ from air is stored in the roots and leaves. Varieties having root systems extended around one meter limit their access to available water in deeper layers, particularly during drought stress at pod filling stage. Doubling root biomass to a nominal two meters would lock away more carbon in soil down to 2 meters in the croplands which could reduce the annual rise in global CO₂ levels in the atmosphere helping fight global warming (Kell 2011) and develop drought resilient varieties. Root system architecture traits are measured in soybean, morphologically, to capture narrow root angle to the soil surface, which promotes lateral root development in the upper root regions where light penetration is the greatest and at the same time number of forks and number of crossings could

be significantly important traits for soil penetration, enhancing rooting depth away from the soil surface and root length density (RLD), with profuse fine roots accommodating large surface area and root volume (Satpute et al. unpublished), the traits deemed essential for water extraction during soil moisture stress. However, deep or profuse rooting would have no effect in shallow soil, in soil where there is no water at depth, or under conditions of mild water stress (Vadez 2014). A modeling study in soybean has shown that increasing the rate of rooting depth would lead to faster soil depletion and yield penalties, especially in the driest quartile of the years, and there would be no benefit, but even a penalty from faster and deeper rooting (Sinclair et al. 2010). A relationship between water extraction and RLD could be resolved to some extent, using root development model that is capable of reconstituting root system architecture in a 3-D context (de Dorlodot et al. 2007; Draye et al. 2010; Pages et al. 2010; Lobet et al. 2011), which gives power to interpret water extraction data for harnessing the genetics of the components of this architecture such as root angles, different types of roots, branching patterns, etc. (Draye et al. 2010; Lobet et al. 2011; Lynch and Brown 2012). The conditions to the success of this breeding strategy are that water would be available at depth (deep soil and water available at depth); deep-water extraction would have an increased benefit if it took place during the grain filling period and that might imply searching for genetic material capable of sustaining root growth during reproductive development; and cropping conditions of moderate VPD in crops where this potential extra water uptake from deep rooting would represent a large proportion of the total transpirational water needs (Vadez 2014).

An alternative way to approach the role of root for water stress adaptation, using a lysimetric system, is assessing water extraction by roots as a way to harness the functionality of root systems. Roots need to be looked at with a view to the whole plant (Comas et al. 2013) and resource availability in time and space (Lynch 2013). Programs need to focus on traits regulating the rate at which plants use the available water before and during stress, involving roots in the sensing mechanism of water stress. The capacity to extract the available water at depth is probably critical and may come from deep roots having a high hydraulic conductivity (Vadez 2014). Certain root anatomical traits, including xylem vessel size and abundance, root cortical aerenchyma, the number of root cells or the number of root cell files, contributing to drought adaptation as the building blocks of its hydraulic properties, eventually affect critical plant water-budget traits.

The current model of water uptake through the root cylinder to the xylem, the composite transport model, is such that water is taken up via two major pathways. In an apoplastic pathway, a large part of that water travels across the intercellular space between cells (apoplast) in the root cortex, toward the endodermis and the xylem vessels. The exodermis could represent a variable apoplastic barrier that plants could use to modulate their water transport characteristics (Hose et al. 2001). The resistance to water flow usually increases under water deficit (Steudle 2000). Most of that resistance is located in the root cylinder (radial resistance), whereas xylem vessels normally offer much less resistance (axial resistance) (Steudle 2000). Another pathway is symplastic water transfer. During the night in the absence of transpiration,

water can be taken up by roots through an osmotic gradient (Steudle 2000) and has high resistance because water goes through cells, traveling in the membrane continuum (endoplasmic reticulum and plasmodesmata) using membrane transporters (aquaporins—AQPs) (Steudle and Peterson 1998; Steudle 2000) highlighting a possible role of AQPs to alter the hydraulic properties of the roots (Tyerman et al. 2002; Maurel et al. 2009). AQPs are integral membrane proteins that increase membrane permeability to water and other small molecules (Kaldenhoff and Fischer 2006). Root water uptake in soybean can be enhanced or reduced by the over-expression or loss of one or more PIP genes, respectively (Javot 2003; Zhou et al. 2014b). In addition to water transport in roots, a variety of AQPs are expressed in the coats of developing seeds (Schuurmans et al. 2003). Nutrient and water transport across plasma membranes (PMs) in seed coats is highly coordinated by regulatory mechanisms and integrates the activities of many nutrient transporters and facilitators. Thus, it is expected that plasma membrane intrinsic proteins (PIPs) that are specifically expressed in native PMs of seed coats are important for seed filling (Zhou et al. 2007). A soybean GmPIP2 subfamily member, GmPIP2;9, was found predominately expressed in roots and developing seeds (Lu et al. 2018). The soybean genome contains a total of 22 PIP genes (Sakurai et al. 2005). A recent study showed that altered plant transpiration led to rapid changes in root expression of soybean PIP1;6 (GmPIP1;6) that correlated with changes in root hydraulic conductance (Vandeleur et al. 2014). Notwithstanding evidence for importance of root traits in drought tolerance (Garay and Wilhelm 1982; Chen et al. 2007b; Manavalan et al. 2010; Sinclair et al. 2010; Fenta et al. 2014; Fried et al. 2018), little work has been done in breeding for drought-tolerant soybean varieties.

4.3 Breeding Approaches for Drought Tolerance in Soybean

Drought-tolerant traits, introduced through breeding approaches, resulted in soybean transpiration rates that plateau at VPD levels above 1.4–2.1 kPa (Mourtzinis et al. 2019). Developing high productivity genotypes under water-limited scenario by introgressing traits explaining plant water relations and hydraulic processes into a single genetic background either through breeding and/or genomic approaches is a way forward in realizing genetic combinations supported by plant genetic resource activities identifying candidate drought-tolerant parental lines and genomic resources (Satpute et al. 2020). Advance phenotyping-based breeding approaches are pre-requisite and being adopted systematically by developing early generation biparental, backcross, or multi-parent intercross populations (Shivakumar et al. 2018) using identified candidate drought-tolerant exotic and/or indigenous parental lines and wider-adaptability high yielding variety(-ies). The populations are advanced through F₂ generation by mass selection where bulks are chemically desiccated with potassium iodide 0.2% (Blum et al. 1991; Bhatia et al. 2014; Satpute et al. 2019b) followed by selections made divergently for seeds size by mechanical sieving. Mass selection for large seeds under chemical desiccation significantly improved seed weight and grain yield under chemical desiccation

stress, as compared to controls where selection was performed without chemical desiccation (Blum 2011). After two cycles of selection, intensive selection of candidate genotypes for multiple drought tolerance related traits is practiced in advance generations using three-tier selection scheme (Satpute et al. 2018) followed by multi-traits indexing (Satpute et al. 2020) for analyzing traits function.

4.3.1 Three-Tier Selection Scheme

Selection for yield per se under water-limited environment confounding the complexity of breeding implores identifying less complex traits related to the drought tolerance and separating them into major components which may ease further understanding of the genetic basis. A large-scale three-tier sequence of selection scheme, which utilizes a set of drought related traits, has previously been employed by a research group (Sinclair 2011) in recognizing drought-tolerant soybean lines. From a screen of about 3500 well-watered soybean plant introduction (PI) lines, about 250 lines (slightly <10%) with low petiole ureide concentrations were selected. High leaf ureide concentrations had been shown to be associated with sensitivity to nitrogen fixation to drought. The lines in the second-tier screen were grown for a field test of nitrogen accumulation under dry conditions on a sandy soil, which had both a low nitrogen content and low water holding capacity. The lines were lightly irrigated approximately every other day to maintain them for about 3 weeks at or slightly above the soil water content resulting in slight leaf wilting. Selected 10% of these field-tested lines (24 lines) were screened in the third tier for intensive measurement of nitrogen fixation response to soil drying in the greenhouse test. Ultimately, 11 PI lines were identified that had substantial superiority in nitrogen fixation tolerance to soil drying. The group identified candidate parental soybean lines for use in breeding efforts to sustain nitrogen fixation activity during soil water deficit.

On the similar line, a three-tier selection scheme has been reported in order to evaluate large number of germplasm accessions (Fig. 4.1) and breeding populations (Satpute et al. 2019a, 2020). Unlike previous approach, with choice of traits, viz. low petiole ureide concentrations under well-watered condition, field test of nitrogen accumulation and greenhouse test of intensive nitrogen fixation response under dry conditions, 1000 soybean germplasm accessions, procured from Medium Term Storage (MTS) facility at ICAR-Indian Institute of Soybean Research, Indore, India were evaluated for delayed leaf senescence trait, stem reserve mobilization, drought tolerance related above-ground traits, viz. drought tolerance index, canopy temperature depression, relative water content, which are reliable and speedy in measurement in large breeding populations, and root system architecture traits (Fig. 4.1).

In the first tier of three-tier selection scheme, about 86 accessions (slightly less than 10%) with 4-5 scores were selected for delayed leaf senescence, in a field trial during summer season. Accessions were sown in early, medium and late flowering groups and irrigations were withheld at pod initiation stage for imposing 21 days

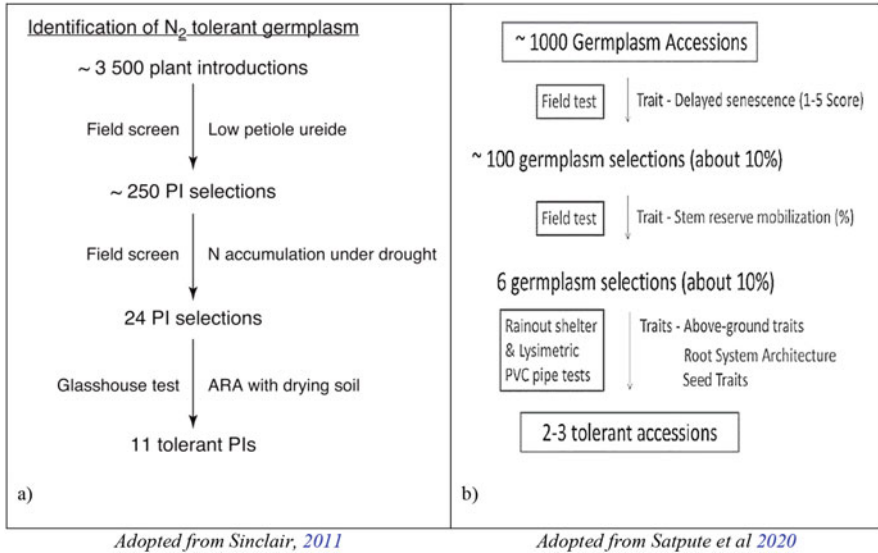


Fig. 4.1 Three-tier selection scheme is applicable for different sets of drought tolerance related traits and was developed to identify candidate soybean lines with (a) high tolerance of nitrogen fixation (Adopted from Sinclair 2011) or (b) high productivity under drought conditions (Adopted from Satpute et al. 2020). The broadest and the least accurate screen was based on (a) concentration of ureides in the petiole or (b) delayed leaf senescence trait; and from a large number of germplasm accessions ~10% accessions were selected in the first tier. In the second-tier screen about 10% of these field-tested accessions were selected for (a) N accumulation under drought or (b) stem reserve mobilization trait. The third tier of screening was performed for intensive measurement of (a) nitrogen fixation response to soil drying in the greenhouse or (b) above-ground plant traits and seed traits in rainout-shelters and root system architecture traits in lysimetric PVC pipes for identifying drought-tolerant germplasm. Ultimately, novel accessions were identified that had substantial superiority in (a) nitrogen fixation tolerance or in (b) productivity traits to soil drying

stress and inducing plant level stress of relative water content (RWC) $\leq 70\%$ arriving at pod filling stage (R5 plus 8–10 days) (Blum 2011; Bhatia et al. 2014). In the semi-arid tropics, setting up the managed stress environment out of the normal season i.e. during the dry off-season is a common approach to avoid unpredictable seasonal rainfall (Mahalakshmi and Blum 2006) with due care to all possible off-season effects for effective drought phenotyping. When planted off-season, the humidity, temperature and photoperiod may not allow reasonably normal crop growth and development, crop cycle, phenology. Besides, certain diseases and pests like YMV and white flies can be highly prevalent and birds are attracted to grains outside its normal season which require protection measures. In such a case data on plant water status and plant responses to stress like delayed leaf senescence may be targeted while actual selection of seed is performed from the preferred lines grown also during the normal season (Blum 2011). In the second tier, accessions were evaluated for stem reserve mobilization trait (SRM) during rainy season in a field test conducted in sets of: (1) sprayed chemical desiccant, potassium iodide

(KI) (0.2%), at 8–10 days after R₅ plus 8–10 days stage and (2) unsprayed control (Bhatia et al. 2014) and ~10% of the accessions (six accessions) were selected for the trait. These promising six accessions were evaluated in the third tier for a number of above-ground plant traits at soil drying plant stress of $\leq 70\%$ RWC at pod filling stage in the rainout shelter test and below-ground traits by morphologically measuring root system architecture traits, adopting standard lysimetric procedure under well-watered condition using PVC pipes (Vadez 2014) and *WinRhizo* Arabidopsis (Regents, Canada) root image analysis. The approach of lysimetric system for root studies is a set of long and large PVC tubes, in which plants are grown individually and have plant spacing and soil volume available for soil exploration close to what is practiced under field conditions (Vadez et al. 2008, 2013). Root growth is known to stop its downward movement around anthesis (Robertson et al. 1993), although maintenance of growth can be found (e.g., Hafner et al. 1993), a trait worthy of screening, provided water is available at depth. The importance of deep-water extraction would be more if its timing coincided with the time of most critical water demand, i.e. reproduction and grain filling. Water extraction after anthesis with restricted vegetative growth completely contributes to grain development leading to high water use efficiency (kg grain mm^{-1}).

In breeding populations, the three-tier selection scheme is applicable in advance generations where approximately half of the seeds from selected F₄ generation single plants are used for screening lines for delayed senescence trait in F₅ generation during summer field trial in the first tier. The remaining half of the seeds from those F₄ single plants, of which lines were selected for delayed senescence trait, serves as a population for selecting lines for SRM trait during rainy season in the second tier. Thus, the scheme offers selection for delayed senescence trait followed by SRM trait in consecutive summer and rainy seasons, respectively, in the same year and selection can be practiced effectively for both the traits at 10% intensity in each selection cycle. Selected lines are evaluated for above-ground plant traits in rainout shelter-induced water stress condition vis-à-vis root architecture traits in the PVC pipes. Multiplexing drought tolerance related traits, using principal component analysis for correlation matrix in SAS (Version 3.0), provides a powerful multi-trait index which helps in identifying a set of drought-tolerant accessions or elite breeding lines (Satpute et al. 2020) for developing climate-smart drought resilient soybean varieties, understanding the role of surrogate traits and discovering unique drought tolerance related genes/QTLs.

4.3.2 Genetic and Genomic Resources

In crops such as soybean, drought resistance is translated to several related traits enhancing yield stability rather than that increasing survivability under drought (Blum 2009; Passioura 2010; Sinclair 2011; Valliyodan et al. 2016). These related traits are correlated with yield under drought and have no yield penalty under non-stress conditions. The success of soybean improvement through molecular approaches under drought stress depends on the discovery of genetic variations for

Table 4.1 Genetic resource for drought tolerance

Tolerant genetic resource/genotype	Basis of drought tolerance	Source	References
PI 416937	High Relative Water Content (RWC), higher lateral root spreading, increased Water Use Efficiency (WUE), low leaf hydraulic conductance, slow wilting	USDA, ARS	Sloane et al. (1990), Patterson and Hudak (1996), Sinclair et al. (2007), King et al. (2009)
Young	High WUE	USDA, ARS	Mian et al. (1996)
Jackson	High biomass and total N ₂	–	Purcell et al. (1997)
PI 407155	Low electrolyte leakage, high biomass accumulation, high root moisture content	–	Chen et al. (2006)
R01-416F and R01-581F	Higher N ₂ fixation	USA	Chen et al. (2007a, b)
93705-36 and PI 471938	Slow wilting	USDA	King et al. (2009)
PI 468917	Lower transpiration efficiency, greater root length	–	Seversike et al. (2014)
C12 and W05	Long root and high biomass, high leaf expansion	CUHK, China	Hossain et al. (2014)
PI 567690 and PI 567731	Leaf wilting	China	Pathan et al. (2014)
EC 538828, JS 9752 and EC 602288	Remobilization of stem reserve at terminal drought, drought resistance index based on yield	India	Bhatia et al. (2014), Bhatia and Jumrani (2016)
NTCPR94-5157, N09-13890, NC-Raleigh, SC07-1518RR	High WUE and greater root penetration	USA	Fried et al. (2019)
PK 1180 and SL 46	Seedling survivability	India	Sreenivasa et al. (2020)

drought related traits present in the germplasm and efficient utilization of available genomic resources. Identification of genetic diversities for traits related to drought tolerance, viz. root system architecture (RSA), water use efficiency, canopy wilting, and sustained N-fixation under drought have helped in discovery of genetic resources (Table 4.1) in soybean which are routinely being used as donor/check genotypes for deciphering soybean genetic response for drought stress through marker and genomics assisted strategies.

In the past decades, accelerated development in genetics, genomics, and soybean genome sequence information has resulted in the identification of SNPs, copy number variation, and structural variation in soybean germplasm (Kim et al. 2010; Schmutz et al. 2010; Ratnaparkhe et al. 2014) (Table 4.2). The advancement in next-generation sequencing approaches (NGS) and cheap sequencing cost have

Table 4.2 Details of whole-genome sequencing efforts in soybean (Chaudhary et al. 2019)

Genotype(s)/no.	Sequencing depth	Method	No. of SNPs	References
<i>G. max</i> var. Williams 82 (1)	–	De novo sequencing and assembly	–	Schmutz et al. (2010)
<i>G. soja</i> var. IT182932 (1)	~52.07×	Resequencing De novo sequencing and assembly	~2.5 Million	Kim et al. (2010)
17 <i>G. soja</i> and 14 <i>G. max</i> (31)	×5 depth	Resequencing	6,318,109	Lam et al. (2010)
8 <i>G. soja</i> , 17 <i>G. max</i> (8 landraces, 9 cultivars) (25)	–	SOAP	5,102,244	Li et al. (2013a, b)
10 <i>G. Max</i> , 6 <i>G. soja</i> (16)	>14×	Resequencing	3,871,469	Chung et al. (2014)
<i>G. soja</i> (7)	~111.9×	De novo sequencing and assembly	3.62–4.72 M SNP per line	Li et al. (2014)
10 Semi-wild, 1 <i>G. soja</i> (11)	9 Semi-wild at ~3× while 1 Semi-wild at ~41×, and 1 Wild at ~55×	Resequencing De novo sequencing and assembly	7,704,637	Qiu et al. (2014)
<i>G. soja</i> W05 (1)	~1×	De novo sequencing and Assembly	1,798,504	Qi et al. (2014)
62 <i>G. soja</i> , 240 <i>G. max</i> (130) landraces, 110 improved cultivars) (302)	>11×	Resequencing	9,790,744	Zhou et al. (2015a, b, c)
<i>G. max</i> cv. Enrei (1)	22.2×	Reference-based assembly	1659,041	Shimomura et al. (2015)
Wild, Landraces, Elite Lines (106)	17×	Resequencing	10,417,285	Valliyodan et al. (2016)

revolutionized soybean research in various forms of molecular tools, viz. de novo sequencing, whole-genome resequencing (WGR), genotyping by sequencing (GBS), and transcriptomic analysis (Liu et al. 2020b). These advances have made a significant impact on molecular breeding strategies through marker development such as SSRs (Hwang et al. 2009), SNPs (Kim et al. 2010; Lam et al. 2010; Chung et al. 2014; Zhou et al. 2015a; Valliyodan et al. 2016; Ratnaparkhe et al. 2020), insertion/deletion (INDEL) markers (Song et al. 2015), specific-locus amplified fragment (SLAF) markers (Zhang et al. 2016). Furthermore, the technical progress and availability of millions of SNPs have facilitated the development of high-density array-based genotyping chips such as Illumina Infinium array (SoySNP50 K iSelect Bead Chip) for ~50,000 SNPs (Song et al. 2013), Soy SNP 6 K Infinium Bead Chip

(Akond et al. 2013), and the Axiom Soya SNP array for approximately 180,000 SNPs (Lee et al. 2015), which are being used for the genotyping of soybean lines (Chaudhary et al. 2019). GBS is becoming one of the popular sequencing-based genotyping approaches which has significantly reduced labor and time and improved precision in the identification of key genes as compared to the conventional PCR-based genotyping methods and being utilized in several crop species and soybean (Poland and Rife 2012; Sonah et al. 2013). Additionally, GBS also allows the detection of new variants in the population of interest, which can be utilized in future breeding programs (Chaudhary et al. 2019).

4.4 Quantitative Trait Loci for Drought Tolerance Related Traits

Different type of molecular markers has been used to map genomic location of major genes and quantitative trait loci (QTLs) for many traits in soybean. More than thousand QTLs representing more than 100 agronomically important traits have been mapped in soybean (Grant et al. 2010). Current information on all mapped QTLs in soybean is available on the USDA-ARS soybean genetic database *SoyBase* (<http://soybase.org>). Although a number of QTLs were mapped in the soybean but introgression and pyramiding of genes or QTLs affecting the same trait is a great challenge to breeding programs. Due to increasing necessity to develop drought-tolerant soybean with enhanced yield, breeding strategies with molecular tools have progressed at a massive rate in the past decade. Since molecular markers identified genetic variants for different drought related traits more precisely, markers are important in developing genetic linkage maps, genetic resource evaluation, and selection of desired alleles and mapping of genes/QTLs. Since microsatellites (SSRs) are less abundant in the genome, SNP markers became more popular and facilitated QTL analysis for nearly every agronomic trait in soybean (<https://soybase.org>, <http://soykb.org>). Gene/QTLs mapping in soybean has become more standard with the availability of whole-genome sequence (WGS) (Schmutz et al. 2010). This ground-breaking change in genome sequencing made available of the development of thousands of SSRs and millions of SNP markers. QTL analysis plays a significant role in identifying genomic regions which control over phenotypic variation and it requires a large segregating population (biparental mapping population) such as an F₂ population or recombinant inbred lines (RILs). In general, QTL mapping uses a large number of RILs, which are established for at least several generations of Selfing (typically up to F₆ or F₇) (Takuno et al. 2012). However, RILs are helpful for the QTL detection, but it estimates the influence of single QTL depending on population size. Moreover, the results are highly population specific for multigenic traits like drought tolerance traits (Deshmukh et al. 2014). On the other hand, plants that are homozygous for the unfavorable allele are eliminated in an F₂ population and frequencies of favorable alleles increase during inbred development (Bernardo 2010). There are a number of important QTL studies (Table 4.3) for traits related to drought tolerance reported in the past three decades. Although QTL mapping has

Table 4.3 List of QTLs related to drought tolerance traits

Parents involved	Population/size	Traits related to drought tolerance	Linked marker/s	Type of marker	LG/Ch No.	QTLs	PVE (%)	References
Young/ PI416937	F ₄ (120)	Water use efficiency (WUE), leaf ash	cr497-1	RFLP	J	3	13.2	Mian et al. (1996)
S100/Tokyo	F ₂ (116)	WUE	A489H	RFLP	L	2	14	Mian et al. (1998)
Minsoy/Noir 1	RIL (236)	Transpiration efficiency (TE), carbon isotope discrimination (CID), yield	Satt205, Satt489	SSR	C2	3	7-12.8	Specht et al. (2001)
Kefeng I/ Nannong 1138-2	RIL	Dry root weight, total root length and root volume, canopy wilting	-	-	N6-C2 N8- D1b + WN11-E, N18-K	13	-	Liu et al. (2005)
Jackson/ KS4895	RIL (81)	Leaf wilting	Sat_044	SSR	K	1	17	Bhatnagar et al. (2005)
Hutcheson/ PI471938	F ₄ (140)	Yield and wilting	Satt226, Sat_375, Sat_074	SSR	D2, F1, F2	6	-	Monteros et al. (2006)
KS 4895/ Jackson	RIL (92)	Canopy wilting	Satt177, Satt362	SSR	A2, B2, D2, F	4	47	Charlson et al. (2009)
Kefeng I/ Nannong 1138-2	RIL (184)	Seed yield per plant, drought susceptibility index	-	-	C2, H	10	-	Du et al. (2009a)
Kefeng I/ Nannong 1138-2	RIL (184)	Leaf water, seed yield	-	-	C2, D1b, H, A2	17	-	Du et al. (2009b)

PI 416937/ Benning	RIL (128)	Root traits	Satt383, Satt339, Set_191, Satt429 Sat_299	SSR	1, 3, 4, 8, 20	5	51	Abdel- Haleem et al. (2011)
Hongfeng 11/Clark	BC	Relative water content (RWC), water holding capacity, root traits, canopy wilting	Sat_136, Satt167, Satt398, Satt694, GMSL514	SSR	A1,A2, B1, B2, D1a,C2, E, F, K, G, I, L, M, N, O	40	–	Li et al. (2011)
Hongfeng 11/Clark	BC (46)	Germination rate and seedling stage drought	Satt449, Satt499, Satt440, Sat_180	SSR	A1, K I, H	31	–	Qiu et al. (2011)
PI 416937/ Benning	RIL (150)	Canopy wilting	Satt 302	SSR	2, 4, 5, 12, 14, 17,19	7	75	Abdel- Haleem et al. (2012)
Hongfeng 11/Harosoy	BC2F3 (95)	–	Satt253, Satt513, Satt693, Satt240, Satt323, Satt255	SSR	–	18	–	Zhang et al. (2012)
SNWS 0048/ Jinda73	BIL	Physiological trait	–	–	D2, G, M, N	9	–	Yang et al. (2014)
Jingdou 23/ZDD 2315	RIL (447)	Root and shoot traits of seedlings	Satt333, Satt327, Satt519, Satt597	SSR	–	24	7.05–38.91	Liang et al. (2014)
373 genotypes	–	WUE	39 MTAs	SNP	–	21	–	Dhanapal et al. (2015)
93,705 KS4895/ Jackson, 08705 KS4895/ Jackson	–	Canopy-wilting trait	–	–	2, 5, 11, 14,17, 19	13	–	Hwang et al. (2015)

(continued)

Table 4.3 (continued)

Parents involved	Population/size	Traits related to drought tolerance	Linked marker/s	Type of marker	LG/Ch No.	QTLs	PVE (%)	References
KS4895/PI 424140, A5959/PI 416937, Benning/PI 416937								
Dunbar/PI 326582A,	BIL (251)	Root and shoot traits	Satt315, Satt253, Satt142	SSR	8, 12	4	7.7–20.8	Manavalan et al. (2015)
CPI 26671/G 2120; Valder/G 2120	RIL	Epidermal conductance, RWC, plant survival after stress	soPt-856,602	DArT	6	106 34	–	Vu et al. (2015)
M8206/ TongShan, Zheng Yang/ M8206	–	Drought tolerance	–	SNP	Chromosomes from 01 to 20	111	–	Khan et al. (2019)
PK 1180/ UPSL 298	F ₂ /BSA	Seedling survivability	Satt277	SSR	6	One gene	–	Sreenivasa et al. (2020)
PI 416997/PI 567201D	RIL (196)	CID	–	SNP	6,7,10, 11, 15, 17, 20	16	2.5–29.9	Bazzer et al. (2020)

advanced swiftly in the past few years, a large number of mapped QTLs cannot be utilized in the breeding program because of false-positive QTLs and low accuracy. However, the accuracy can be enhanced by adapting different QTL mapping methods and effective statistical analysis such as single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping (MIM), and Bayesian interval mapping (BIM). Also, a number of QTL mapping software have been developed such as Mapmaker/QTL, QTL Cartographer, MapQTL, PLABQTL, PGRI, MapManager, QTLMAPPER, QGene, QTLSTA, Ici Mapping, and QTL network. Further utilization of QTL information for marker-assisted breeding has become challenging due to complex inheritance of unstable QTLs (Deshmukh et al. 2014). Statistical tools such as “Meta-QTL analysis” have been advanced that compile QTL data from different reports together on the same map for identification of precise QTL region (Deshmukh et al. 2012; Sosnowski et al. 2012). Hwang et al. (2015) identified various QTLs related to canopy wilting, during “Meta-QTL” study on five different populations (RILs), among identified QTLs, one QTL on chromosome 8 in the 93,705 KS4895 \times Jackson population co-segregated with a QTL for wilting published previously in a Kefeng1 \times Nannong 1138-2 population. The advances in sequencing technologies, statistical approaches, and software resulted in exponential intensification in soybean studies to understand plants response to extreme climatic conditions importantly drought stress.

Identification of genes underlying root system architecture and canopy characteristics is critical to develop soybean that is suited to water-limited environments. Prince et al. (2015a) identified four significant QTLs associated with different root architectural traits on Gm06 and Gm 07 in an interspecific RILs population of *G. max* (V71–370) \times *G. soja* (PI407162). In an another study, Manavalan et al. (2015) identified a major QTL on Gm08 that governed root traits (tap root length and lateral root number) and shoot length and identified six transcription factors (MYBHD, TPR, C2H2 Zn, bZIP, GRAS, and Ring finger) and two key cell wall expansion-related genes which encode xyloglucan endo-transglycosylases as candidate genes in the confidence interval of the QTL. These are key candidate genes for validation and to develop a better root ideotype in soybean.

4.5 Genome-Wide Association Studies for Drought Tolerance Related Traits

QTL mapping using biparental populations has limitations because of restricted allelic diversity and genomic resolution. The allelic diversity can be increased to some extent by using populations derived from multi-parental crosses (Deshmukh et al. 2014). Recently, Multi-parent Advanced Generation Inter-Cross populations (MAGIC) has been used to identify QTL for blast and bacterial blight resistance, salinity and submergence tolerance, and grain quality traits in rice (Bandillo et al. 2013). Such multi-parental populations have mapping resolution limitations since it

depends on meiotic events (crossing-over) (Kover et al. 2009). In contrast, the genome-wide association study (GWAS) approach provides opportunities to explore the tremendous allelic diversity existing in natural soybean accessions. Mapping resolution of GWAS is also higher since millions of crossing events have been accumulated in the germplasm during evolution. The recent advances in high-throughput genotyping have played important role in the genome-wide association studies in soybean. The large data sets generated from NGS and high-density genotyping require sound computational algorithms for detection of minor QTLs as well as rare alleles with major effect phenotype. GWAS is routinely being used in many plant species, but only a few studies have been reported in soybean in regard to drought tolerance. GWAS for quantitative traits like drought tolerance is predictable to be affected by a confounding population. Different models have been developed for population stratification and spurious allelic associations like MLM and CMLM which takes into account the population structure and kinship (Deshmukh et al. 2014). Development in statistical tools, genotyping approaches, and studies involving larger set will definitely improve GWAS power. Recently, a large number of QTLs associated with shoot ureide were mapped in both biparental populations and genome-wide association studies (GWAS) in diverse lines (Hwang et al. 2013; Ray et al. 2015), which indicated the complexity of N-fixation under drought and suggested that genomic selection should be better suited to improve such complex trait. Dhanapal et al. (2015) analyzed a population of 373 genotypes with 12,347 single nucleotide polymorphisms (SNPs) in four environments for carbon isotope ratio ($\delta^{13}\text{C}$), an important physiological trait acting as surrogate for water use efficiency (WUE) and found association of 39 SNPs, which are likely tagged to 21 different loci with this drought-tolerant trait (Table 4.4).

Likewise Kaler et al. (2017) also reported 54 environment-specific SNPs associated with $\delta^{13}\text{C}$ and 47 SNPs associated with $\delta^{18}\text{O}$, which are tagged with 46 putative loci and 21 putative loci for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively. There are many loci reported for several drought related traits, viz. chlorophyll fluorescence (Hao et al. 2012; Herritt et al. 2018), canopy temperature (Kaler et al. 2018), delayed canopy wilting (Steketee et al. 2020; Ye et al. 2020), and drought susceptibility index (Chen et al. 2020) (Table 4.4). Latest updates on GWAS in soybean for drought tolerance were reported in germplasm association panel containing 259 soybean released Chinese cultivars for drought related traits based on germinating soybean seeds. The enquiry was based on a total of 4616 SNPs, and 15 SNP trait associations were identified by GWAS, among which three SNPs were suggestively linked with two of the drought-tolerance indices (Liu et al. 2020a).

4.6 Transcriptomic Approaches

Transcriptome analysis provides gene function information under various conditions, which differs in dissimilar environments, cell types, developmental stages, and cell states. There has been a tremendous progress in the application of transcriptome analysis for the abiotic stress tolerance. During abiotic stresses, a wide

Table 4.4 Details of genome-wide association studies (GWAS) performed for traits related to drought tolerance in soybean

Trait	GWAS loci	Markers	Genotypes	Method	References
Chlorophyll and chlorophyll fluorescence parameters	51	1536 SNP	168	MLM	Hao et al. (2012)
Ureide concentration	53	33,957SNP	374	PROC GLIMMIX	Ray et al. (2015)
Carbon isotope ratio ($\delta^{13}C$)	21	12,347 SNP	373	GLM and MLM	Dhanapal et al. (2015)
Chlorophyll contents	27	31,253 SNP	332	MLM	Dhanapal et al. (2016)
Carbon isotope ratio ($\delta^{13}C$) Oxygen isotope ratio ($\delta^{13}C$)	46 21	31,260 SNP	373	Farm-CPU	Kaler et al. (2017)
Canopy temperature	34	31,260 SNP	345	Farm-CPU	Kaler et al. (2018)
Chlorophyll fluorescence	53	32,453 SNP	189	CMLM	Herritt et al. (2018)
Delayed canopy wilting	44	34,379 SNP	162	MLM	Steketee et al. (2020)
Germination under drought	15	4616 SNP	259	MLM	Liu et al. (2020)
Drought susceptibility index and yield traits	302	105,970 SNP	136	MLM	Chen et al. (2020)

range of defense mechanisms are activated that increase tolerance against adverse situations in order to avoid damage caused by abiotic stresses such as drought. The first step toward stress response is stress signal recognition and subsequent molecular, biochemical, and physiological reactions activated through signal transduction (Le et al. 2012). Earlier, strategies using expressed sequence tags (ESTs) and techniques, i.e. suppression subtractive hybridization (SSH), have been extensively used for transcriptome profiling of soybean under abiotic stress conditions (Clement et al. 2008). These techniques are competent but do not give analysis of entire genes in the soybean genome. Several high-throughput techniques have been developed for transcriptome investigation due to the advancement in sequencing technology and the availability of the whole soybean genome sequence (Schmutz et al. 2010; Cheng et al. 2013). These platforms have been extensively used for transcriptome profiling to understand drought stress tolerance mechanisms in soybean (Table 4.5).

Microarray is a high-throughput technology where thousands of probes representing different genes are hybridized with RNA samples. The Affymetrix

Table 4.5 List of differentially expressed genes (DEGs) related to drought tolerance in soybean

Genotype name	Number of DEGs/ candidate genes	Putative functions	Platform used	References
<i>Glycine max</i> L. Merr	9148 genes	Genes related to membrane transport, defense signaling, metabolism associated with roots	Affymetrix chips containing 37,500 probe sets	Haerizadeh et al. (2011)
Williams 82	3276 for V ₆ 3270 for R ₂	Expression of many <i>GmNAC</i> and hormone-related genes	61 K Affymetrix Soybean Array GeneChip	Le et al. (2012)
DT 2008 and W82	822 and 632 genes	Genes related to osmoprotectant biosynthesis cell wall-related proteins phosphatase 2C proteins TFs (NAC, AP2)	66 K Affymetrix, Soybean Array GeneChip	Ha et al. (2015)
Pana and PI 567690	1914 and 670	Hydrolase activity carbohydrate/lipid metabolism apoplast, and chlorophyll a/b binding proteins xyloglucan endo-transglycosylases	–	Prince et al. (2015b)
Williams 82	6609	Genes related to cell wall modification, lipid metabolism, carbohydrate metabolism, hormonal pathways, and TFs	Illumina HiSeq 2000	Song et al. (2016)
<i>Glycine max</i> L. Merr	49, 148, and 1576 genes, respectively	RNASeq analysis was performed on seed coat which plays a crucial role in controlling carbon and nitrogen transfer to developing seed set	RNASeq	Leisner et al. (2017)
Williams 82	<i>GmWRKY12</i>	Increase in proline (Pro) content and decreased malondialdehyde content	RNA-Seq, qRT-PCR	Shi et al. (2018)
Williams	<i>NAC4</i> , <i>NAC29</i> , <i>NAC25</i> , <i>NAC72</i>	2771 DEGs 1798 genes were upregulated and 973 were downregulated and related to ABA biogenesis, secondary metabolite synthesis, etc.	RNA-Seq, HiSeq4000	Xu et al. (2018)

Gene Chip representing 61K probe sets is frequently being used for transcriptome analysis of soybean RNA samples under drought stress (Haerizadeh et al. 2011; Le et al. 2012). The normalized expression data generated using the Affymetrix Gene Chip can be utilized to compare soybean experiments performed across the world. RNA-seq, another cost-effective and high-throughput technique, analyzes transcriptome by sequencing. The RNA-seq approach has several advantages over the microarray technology where available genomic information is used. For

instance, RNA-seq is being used for transcription start site mapping, strand-specific measurements, gene fusion detection, small RNA characterization, and detection of alternative splicing events (Ozsolak and Milos 2010). Transcriptome profiling revealed through massively parallel RNA sequencing has offered new insights into gene networks that respond to drought stress (Table 4.5), such as NAC, etc. (Xu et al. 2018). These efforts can be used to generate an expression atlas for soybean genes related to drought tolerance which may serve as a useful genomic resource.

4.7 Molecular Events During Drought Stress in Soybean

Understanding the molecular mechanism of stress tolerance and developing stress-tolerant cultivars is important to achieve optimal yield from soybean crop. Modulations in gene expression are the earliest responses in plants, and a number of stress-responsive genes have been noted to have important functions in drought and salt resistance. Drought tolerance property of soybean involves complex network of genes and metabolites. Calcium channels, calcium binding proteins, receptor like protein kinases (RLKs), G-protein coupled receptors, histidine kinases are proposed to act as potential osmosensors in plants. Expression of several transcription factors (TFs), receptor like kinases (RLKs), calcium signaling components were upregulated in roots under drought stress (Tripathi et al. 2016a). It was shown that the genes involved in hormone, carbohydrate, and cell wall metabolism were differentially regulated in soybean roots under water stress. In another study, the level of expression of two auxin-responsive factors (ARFs), GmARF3 and GmARF50, was increased in roots and shoots under dehydration stress (Ha et al. 2015). Proteomic analysis of root suggested the involvement of osmoprotectants, kinases, and transcription factors in drought response (Mohammadi et al. 2012).

4.7.1 Signal Transduction Under Drought Stress

Abscisic acid (ABA) biosynthesis and accumulation in response to drought is reported in several plants (Sachdeva et al. 2020). ABA regulates the stomatal closure and other metabolic pathways during abiotic stress. Ca^{2+} and ROS are reported to participate in ABA mediated signal transduction pathway. Increased cytosolic Ca^{2+} level induces several Ca^{2+} binding proteins, viz. calmodulins (CaMs), calmodulin like (CML), calcium-dependent protein kinases (CDPKs), and calcineurin B like proteins (CBLs). Ca^{2+} application affects the nodulation process in soybean. Under drought stress, ROS generation is enhanced leading to accumulation of H_2O_2 , which activates ROS scavenging mechanism. Mitogen activated protein (MAP) kinase cascade is involved in signaling pathway of many TFs under both biotic and abiotic stresses (Fujita et al. 2006). Degradation of proteins mediated by ubiquitination is another pathway involved in abiotic stress tolerance (Lyzenga and Stone 2012). Metabolic engineering using TFs may regulate several genes of the downstream pathway leading to improved tolerance to abiotic stresses. To reduce the undesired

phenotypes of constitutively expressed TFs, tissue and developmental stage specific promoters may be used (Kasuga et al. 2004). In soybean, the expression level of various calcium-dependent protein kinases (GmCDPKs) was studied in response to wounding, stimulated herbivory, aphid feeding, treatment of jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) and in response to drought and abscisic acid (ABA). Also, many GmCDPKs were induced after drought or ABA treatment (Hettenhausen et al. 2016).

4.7.2 Role of Transcription Factors in Drought Tolerance

Transcription factors (Tfs) are DNA-binding proteins that interact with other transcriptional regulators, including chromatin remodeling/modifying proteins, to initiate or inhibit the transcription by RNA polymerase. TFs have the capacity to act as a tool to improve the multigenic traits like drought tolerance (Rabara et al. 2014). Multigenic control makes the development of drought-tolerant varieties a difficult task using conventional breeding methods. TFs act as master regulators of many physiological processes and play an important role in the regulation of gene expression under abiotic stress. Alteration in expression levels of TFs regulates the expression of many downstream genes resulting in several different phenotypic effects. A particular TF can control the expression of several target genes of a specific pathway, thereby have the potential to modulate multigenic traits, such as drought tolerance. Forty genes belonging to six major families of TFs were upregulated during various abiotic stresses (Seki et al. 2002).

WRKY proteins are involved in the signal transduction of plant hormones, like abscisic acid (ABA), jasmonic acid (JA), and gibberellin (GA). Expression levels of four WRKY family of transcription factor (GmWRKY2, GmWRKY15, GmWRKY50, and GmWRKY55) were induced in drought treatment. In this regard, several reports strongly establish the potential of WRKY TFs as effective tool to engineer abiotic stress tolerance, such as drought in plants (Rushton et al. 2010; Chen et al. 2012; Rabara et al. 2013; Tripathi et al. 2013).

The potential of basic leucine zipper (bZIP) family of TFs as tools to improve crop responses to drought was shown by heterologous expression of soybean GmbZIP1 in *Arabidopsis* (Gao et al. 2011). Soybean bZIP (GmbZIP1) over-expression was shown to increase drought tolerance in *Arabidopsis* (Gao et al. 2011). Fifteen bZIP genes were induced by drought and salt stress (Yang et al. 2020). Among these, the expression of GmbZIP2 was significantly induced under stress conditions. It was shown to improve drought and salt tolerance upon over-expression by enhancing the expression of stress-responsive genes, such as GmMYB48, GmWD40, GmDHN15, GmGST1, and GmLEA. Another bZIP transcription factor, GmFDL19 has also been reported to enhance drought tolerance in soybean (Li et al. 2017b). Chlorophyll content and activities of several antioxidant enzymes were more in over-expressors while malonaldehyde content was lower than wild-type plants.

Four NAC family TFs (GmNAC4, GmNAC25, GmNAC29, and GmNAC72) were reported to be increased significantly in drought stress. In another study, 28 dehydration-responsive GmNAC genes were analyzed and it was revealed that eight of these genes were found to be induced in drought-tolerant soybean varieties under drought conditions (Hussain et al. 2017). Also, four of these (GmNAC4, GmNAC5, GmNAC20, and GmNAC21) were more dehydration resistant than others. The NAC family TFs are also proposed to be master regulators of various metabolic pathways in plants and have potential to manipulate the drought tolerance in transgenic plants.

ERF TFs are plant-specific TFs regulating a number of developmental and stress-related processes (Dietz et al. 2010). The AP2/ERF family consists of several subfamilies: the AP2, ERF, dehydration responsive element binding protein (DREB), and RAV (Mizoi et al. 2012). Several studies have reported that DREB TFs have potential to engineer drought tolerance. GmDREB2 improved salt and drought tolerance in *Arabidopsis* (Chen et al. 2007a). Two different types of transgenic soybean plants over-expressing AtDREB1D gene under constitutive and ABA-inducible promoters were raised. The transgenic plants showed increased drought tolerance by maintaining higher membrane stability (Guttikonda et al. 2014).

Transgenic soybean harboring GmDREB6 transcription factor was raised and the expression of P5CS gene and proline content was studied (Nguyen et al. 2019). Under normal condition, proline content was slightly higher in the transgenic plants. However, under salt stress the proline content increased to a large extent in transgenic plants. GmDREB6 has been proposed to bind GT-1 region in the promoter of P5CS gene and activate its expression (Zhang et al. 2013). Expression level of soybean DREB TF was studied in drought-sensitive and drought-tolerant cultivars. Also, the expression level of some known DREB regulated target genes were also investigated under water stress conditions (Stolf-Moreira et al. 2010). The drought-tolerant genotype had increased expression of aquaporin (Gmpip1), defensin (Gmdefensin), and galactinol synthase (Gmgols) under drought stress conditions.

In soybean, an R1 MYB transcription factor, GmMYB176, is reported to regulate isoflavone synthesis by affecting the expression of GmCHS8. GmMYB118 was significantly regulated by salt and drought treatment, and over-expression of GmMYB118 improved tolerance to drought and salt in both *Arabidopsis* and soybean. GmMYB expression was induced by drought, salt, ABA, and H₂O₂. The transgenic lines over-expressing GmMYB84 exhibited enhanced drought tolerance than WT plants. The over-expressors have longer primary root length, greater proline and ROS content, higher antioxidant enzyme activity, lower dehydration rate, and reduced MDA content (Wang et al. 2017). The activities of antioxidant enzymes were induced by ROS in over-expressor lines. The GmMYB84 was shown to bind the *cis* elements in the promoter of GmRBOHB1 and GmRBOHB2 which results in increased ROS levels leading to increased root growth under drought stress conditions (Wang et al. 2017). In addition, several members of bHLH, SRS, VOZ, NFYA family of transcription factors are shown to be involved in regulation of expression of various abiotic stress-related genes in soybean.

4.8 Genomics Assisted Breeding

Marker-assisted selection (MAS) is the indirect selection method where the linked molecular marker is used to transfer important agronomical traits from one genotype to another genotype. Marker-assisted backcrossing is an important approach in soybean for transferring trait of interest (Lee et al. 2006). The high-throughput genotyping technologies eased the process of marker identification and QTL mapping for different traits in soybean. The molecular breeding approaches such as marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) aided in the introgression of the trait of interest in soybean. The soybean cyst nematode-resistant line, LDX01-1-65(PI636464) was developed using MABC (Chaudhary et al. 2019). Gene pyramiding involves combining favorable alleles controlling the same attribute from more than two parental lines (Melchinger 1990). Marker-assisted gene pyramiding was successfully carried out to develop durable resistance to several pathogens causing diseases in soybean (Walker et al. 2010). Although drought tolerance is accompanied by many traits which governed by mainly polygenes/QTLs, introgression of minor QTLs from donor to recipient cultivar is not an easy task. In QTL mapping in five biparental populations, a total of ten genomic regions or QTLs (Table 4.3) were mapped to be associated with canopy wilting under drought, with varied phenotypic contributions, and the majority of these QTLs (9/10) have donor alleles for slow wilting phenotypes from PI 416937, Jackson, or both (Charlson et al. 2009; Abdel-Haleem et al. 2012; Hwang et al. 2015). Molecular markers associated with these QTLs (identified in Meta-QTL studies) can be used to perform MAS to introgress the slow canopy wilting phenotypes from the donor in elite backgrounds. However, transferring these QTLs is a challenging job for breeders due to complex, quantitative nature and sensitivity to environmental factors of canopy-wilting trait under drought. Most of the mapped minor QTLs were found to be unstable across independent environments and populations. For instance, even major QTLs on chromosome 12 ($R^2 = 0.27$) were identified in all five environments from Benning \times PI 416937 (Abdel-Haleem et al. 2012) but were not detected in any populations or site-years, including the Benning \times PI 416937 cross reported by Hwang et al. (2015). For this reason, QTL confirmation in more advanced generations should be performed to validate each individual QTL. This also indicates that stacking all confirmed QTLs in the same elite background by MAS is necessary to build the drought tolerance shown in the donor (Valliyodan et al. 2016).

Marker-assisted breeding for simple Mendelian traits is effective, but it can be challenging for complex traits such as drought stresses that are generally polygenic. Even major QTLs linked to drought tolerance traits can explain only a small fraction of phenotypic variation and may show unexpected trait expression in new genetic backgrounds because of epistatic interactions or GE interaction. These limitations can be effectively overcome by the use of strategy called “Genomic-selection” (GS). GS is a relatively simple and more powerful approach since it uses all marker information simultaneously to develop a prediction model avoiding biased marker effects (Heffner et al. 2009). In soybean, some efforts have been made to evaluate

GS using different models. A GS study conducted in soybean has used a panel of 288 accessions and 79 SCAR markers to predict 100 seed weight (Shu et al. 2012). In this report, high correlation ($r^2 = 0.9$) has been observed among the genomic estimated breeding value (GEBV) and the phenotypic value. Predicting the precision of GS will need more investigations involving high-throughput genotyping of larger populations evaluated with multi-environment. These multi-environmental trials not only include the effect of $G \times E$ but also increase the number of breeding cycles per year. The challenge for GS is to get accurate GEBV with respect to the $G \times E$ effect. Improved factorial regression models have been proposed for GS that consider stress covariates derived from daily weather data, which revealed increased accuracy by 11.1% for predicting GEBV in unobserved environments where weather data is available (Heslot et al. 2014). This study suggests possible utilization of phenotypic data and historical data of weather conditions accumulated over decades in different soybean breeding programs. Similar information can be used for drought tolerance improvement in soybean (Deshmukh et al. 2014). Most of the GS studies have used RIL populations to train the prediction model. Therefore, GS and QTL mapping can be performed simultaneously. A set of diverse cultivars can be used for GS and GWAS altogether, so GWAS, GS, and QTL mapping can be combined together for marker-assisted breeding for drought tolerance related traits (Deshmukh et al. 2014). QTL or GWAS loci possess hundreds of genes which make the identification of candidate genes difficult (Sonah et al. 2012). This is similar in transcriptome profiling where thousands of genes have been found to be differentially expressed even with genetically similar isogenic lines (Table 4.5). Therefore, combining QTL mapping or GWAS with transcriptome profiling can complement each other (Deshmukh et al. 2014). Recently, several sequences based data sets have been generated by resequencing efforts (Lam et al. 2010; Li et al. 2013b, 2014; Chung et al. 2014; Qiu et al. 2014; Zhou et al. 2015c; Valliyodan et al. 2016). The availability of well-annotated soybean genome sequence and resequencing based data sets also facilitates development of large number of SNP and Indel markers which are being utilized in QTL mapping and molecular breeding for drought tolerance in soybean.

4.9 Genetic Engineering Approaches for Developing Drought Tolerance in Soybean

The complexities of mechanisms controlling drought adaptive traits and the limited availability of germplasm for tolerance to drought stress have restricted genetic advances in soybean for increase in yield and improvement of other traits associated with drought stress tolerance. Understanding the mechanisms by which plants perceive and transduce the stress signals to initiate adaptive responses and their engineering using molecular biology and genomic approaches is essential for improving drought stress tolerance in soybean crops. Attempts have been made to enhance drought stress tolerance through biotechnological approaches and drought-tolerant varieties of soybean have been produced. Zhang et al. (2019) reported that

over-expression of C-5 sterol desaturase gene from *Flammulina velutipes* (*FvC5SD*) improves drought tolerance in soybean. In this study, *FvC5SD* gene was introduced into the soybean variety Shennong9 through the *Agrobacterium*-mediated transformation in order to enhance drought stress tolerance. Under drought conditions, the transgenic soybean plants accumulated lower levels of reactive oxygen species and exhibited higher activities and expression levels of enzymes than wild-type soybean. The basic leucine zipper (bZIP) family of transcription factors plays an important role in the growth and developmental process as well as responds to various abiotic stresses, such as drought. Li et al. (2017b) demonstrated that *GmFDL19* also enhances tolerance to drought and salt stress in soybean at the seedling stage. Wei et al. (2019) generated transgenic soybean plants and further investigated roles and biological mechanisms of *GmWRKY54* in response to drought stress. Wei demonstrated that expression of *GmWRKY54*, driven by either a constitutive promoter (pCm) or a drought-induced promoter (RD29a), confers drought tolerance. *GmWRKY54* is a transcriptional activator and affects a large number of stress-related genes as revealed by RNA sequencing.

Efforts have been made to study response of soybean plants to drought stress using advanced genetic engineering and genomic approaches like high-throughput sequencing technologies, chip-based analysis, RNA seq, etc., which enabled researchers to utilize enormous nucleotide database to find genes involved in various metabolic pathways dealing with abiotic stress tolerance. But still, there are many drought-responsive genes that have been identified but their function is still unknown. Therefore, genetic engineering through reverse genetics approaches could be useful for identification and functional elucidation of drought stress tolerance related genes (Azevedo et al. 2011). Identification, a functional characterization and introgression of stress-related genes through advanced genetic engineering techniques are important to provide long-term tolerance against drought stress (Jan et al. 2016, 2017).

The first commercial cultivation of genetically engineered soybean was started in 1996 which has spread in the area of 95.9 million hectares (mHa) till 2018. This area covers about 78% of the global soybean cultivated area, i.e., 123.5 mHa. The USA is the world's top producer of soybean whereas Brazil is the top exporter of soybean in the world. To bring suitable changes into the molecular structure of soybean, 38 transgenic events were approved in around 31 countries, mostly in the North and South American continent. These events were attributed to traits like herbicide resistance, insect resistance, drought tolerance, and pyramiding of two or more gene traits. All these events account for 50% of the world's biotech crop area. Among them, there are two specific transgenic events, commercially available for farmers to grow drought-tolerant soybeans, namely HB4 (popular as trade name Verdeca HB4 Soybean) and HB4 × GTS 40-3-2. In these events, gene Hahb, isolated from *Helianthus annuus*, has been genetically engineered to produce transcription factor Hahb-4 which binds to a dehydration transcription regulating region of the plant responsible for better performance under drought condition. In addition, new advances in functional genomics studies in soybean using VIGS, RNAi and genome

editing approaches has played important role in soybean improvement and gene function studies.

4.9.1 Virus-Induced Gene Silencing: A Potential Biotechnological Tool for Rapid Elucidation of Genes Function

Although comparative and functional genomic strategies have provided initial clues about function of abiotic stress-responsive genes in soybean and many other crop species (Gorantla et al. 2007; Tran and Mochida 2010; Soares-Cavalcanti et al. 2012), comprehensive functional characterization tools are necessary for understanding the precise role of these genes in combating drought stress. One such tool is virus induced gene silencing (VIGS) which has emerged as a potential gene knock-down technique in several crop species because it does not require transformation (Baulcombe 1999; Burch-Smith et al. 2004; Senthil-Kumar and Mysore 2011). Virus-induced gene silencing (VIGS) is a reverse genetic tool for functional elucidation of genes involving gene transcript suppression. In VIGS system, recombinant virus carrying a partial sequence of a host gene is used to infect the plant. When the virus spreads systemically, the endogenous gene transcripts, which are homologous to the insert in the VIGS vector, are degraded by post-transcriptional gene silencing (PTGS) (Baulcombe 1999).

With increased identification of differentially expressed genes employing high-throughput transcript profiling under various abiotic stresses including drought stress, functional elucidation of stress-responsive genes is crucial to understand their role in stress tolerance. In recent years, VIGS has been successfully used as a versatile tool for gene function analysis in various model plants and also in crop plants like soybean. Viral vector-based silencing of gene of interest and studying the gene knock-down in plants under stress can be one of the potential options for assessing functional significance of stress-responsive genes in soybean. Analysis of stress downregulated as well as stress upregulated genes is crucial for understanding molecular responses of crop plants to abiotic stresses. A large number of genes whose expression altered during various abiotic stresses have been identified through expression profiling, expressed sequence tags (ESTs), and cDNA library generated from various plant species (Seki et al. 2002; Govind et al. 2009; Marques et al. 2009; Bohnert et al. 2006; Becker and Lange 2010; Chen et al. 2015; Ramegowda et al. 2017; Abd El-Daim et al. 2018). However, identifying the functional significance of individual differentially expressed genes during drought stress is a challenging task. It is utmost important to elucidate the function of these stress-responsive genes to understand the mechanism of stress tolerance and also for characterizing candidate genes contributing tolerance of susceptible species by genetic engineering. An inventory of genes showing altered expression under several abiotic stresses has been established for many crop species employing expressed sequence tag (EST) analysis (Gorantla et al. 2007; Wani et al. 2010; Blair et al. 2011). In contrast to the enormous progress made in generating sequence information, functional analysis of stress-responsive genes is lagging behind.

VIGS technology has been extensively used to investigate function of genes responsive to various kinds of abiotic stresses (Senthil-Kumar et al. 2007; Cho et al. 2008; Govind et al. 2009; Kuzuoglu-Ozturk et al. 2012; Manmathan et al. 2013; Bao et al. 2015; Wang et al. 2016; Li et al. 2017b; Park et al. 2017; Ramegowda et al. 2017; Ullah et al. 2018). Recent development in VIGS vectors has extended the application of VIGS for functional characterization of abiotic stress-responsive genes and also enhancing abiotic stress tolerance in several crops including soybean. Rao et al. (2014) studied functional relevance of *GmCam4* (*Calmodulin*) gene by silencing and over-expression using Bean Pod Mottle Virus (BPMV)-based vector. Silencing of *GmCam4* resulted susceptible response to salt stress while over-expression resulted salinity tolerance in soybean plants at 200 mM NaCl level (Rao et al. 2014). In the recent past, VIGS has been successfully used to unravel the abiotic stress tolerance mechanisms in crop plants (Senthil-Kumar and Udayakumar 2006; Senthil-Kumar et al. 2008; Manmathan et al. 2013). Zhou et al. (2020) found that over-expressing gma-miR398c in soybean decreased *GmCSD1a/b*, *GmCSD2a/b/c*, and *GmCCS* expression, which weakened the ability to scavenge O_2^- and by their means negatively regulates drought tolerance in soybean.

4.9.2 RNAi Approach: A Powerful Tool for Gene Function Studies and Enhancing Drought Tolerance in Soybean

RNA interference (RNAi) is a versatile tool frequently used for gene function studies in soybean. RNAi phenomenon involves small interfering RNA (siRNA) or short hairpin or microRNA (miRNA) to suppress the expression of sequence-specific gene at post-transcriptional or translational level. This technology has been extensively used to study functional relevance of genes, enhancing crop yield, improvement of nutritional quality, and increasing crop productivity through suppression of expression of genes responsive to abiotic stress, involved in biomass and grain yield.

In future, there will be huge demand for genetically improved crops with ability to maintain yield stability under adverse environmental conditions. Drought stress tolerance and adaptation of crop plants to drought stress have been improved through RNAi approach for manipulating expression of transcription factor genes, genes associated with signaling and biosynthetic pathways, and accumulation of antioxidants (Gupta et al. 2014; Wang et al. 2015; Pradhan et al. 2015; Meena et al. 2017; Li et al. 2017a). Several genes associated with drought stress-related physiologies and pathways have been functionally characterized to understand stress tolerance mechanisms and for improving abiotic stress tolerance in crop plants (Zhou et al. 2015b; Guo et al. 2016; Ji et al. 2016; Ma et al. 2017; Li et al. 2017a; Cai et al. 2018). It is utmost important to elucidate the role of transcription factors or genes by genetic manipulation for higher yield and also yield stability under various abiotic stress conditions. Several researchers tried to identify and characterize various genes responsive to drought and salinity stress by using genomics, transcriptomics, proteomics, and metabolomics approaches (Wang et al. 2015; Tripathi et al. 2016b; Ji et al. 2016; Qin et al. 2016; Li et al. 2017a). Therefore, it

is essential to know the role of specific small RNA followed by genetic manipulation for improvement of drought stress tolerance in soybean crop. The RNAi technology has been successfully used for improvement of soybean crop in terms of enhancing abiotic stress tolerance (Wang et al. 2015; Srivastava et al. 2017; Li et al. 2017a; Mao et al. 2018). RNAi has been effectively utilized for incorporating desired traits for abiotic stress tolerance in various plant species (Jagtap et al. 2011; Pradhan et al. 2015; Meena et al. 2017; Li et al. 2017a). Wang et al. (2015) studied the interaction of *GmWRKY27* with *GmMYB174* and reported that these two cooperatively inhibit transcription of *GmNAC29* by binding to the core sequences in its promoter. The downregulation of expression of *GmNAC29* leads to reduced intracellular ROS levels. The *GmWRKY27* may also increase proline content by indirectly suppressing the transcription of PDH which ultimately led to improvement in stress tolerance in soybean (Wang et al. 2015). Li constructed soybean *GmRACK1* silenced (RNA interference, RNAi) and over-expressing plants. The *GmRACK1*-RNAi lines showed significantly improved drought stress tolerance while the over-expressing seedlings were hypersensitive to drought stress when compared to wild-type in terms of plant survival rates after 10 days of drought. *GmRACK1*-RNAi plants were found to be more sensitive to ABA when seeds germinated and root grew.

4.9.3 Genome Editing Based Techniques

The availability of soybean wild species and genetic variations in soybean germplasm is crucial for soybean improvement programs targeting drought tolerance. However, the lack of enough natural germplasm, genetic diversity, and mutant collections limits both basic and applied research, particularly in soybean. The genome editing tools provide opportunity to overcome these limitations via creation of such variations in the genome of crop plants. Such approaches can reduce breeding or gene transformation time greatly for production of new varieties/transgenic plants with desired traits, such as abiotic stress tolerance. The CRISPR technology is being seen as an advancement of plant breeding technologies. Non-transgenic approaches are also available for delivery of such nucleases to produce mutant plants (Marton et al. 2010). As a result, crop varieties produced using these technologies may qualify as non-GM and would have enormous impact on plant biotechnology and breeding. There are four genome editing tools, meganucleases, zinc-finger nucleases (ZFN), Transcription Activator-like Effector Nucleases (TALEN) and the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-Associated nuclease protein (Cas) system, which have provided targeted gene modification in plants (Cermak et al. 2015; Gao et al. 2010; Li et al. 2012, 2013a, b; Shukla et al. 2009). Among these, the CRISPR-Cas9 system is the easiest to implement and is highly efficient. The system consists of a Cas9 endonuclease derived from *Streptococcus pyogenes* and a chimeric single guide RNA that directs Cas9 to a target DNA sequence in the genome. The CRISPR-Cas9 genome editing is accomplished by introducing a DNA double-strand break in the target locus by nuclease enzyme named Cas9, followed by DNA repair through

either the endogenous imprecise Nonhomologous End-Joining (NHEJ) or the high-fidelity Homology-Directed Repair (HDR) pathways. NHEJ can induce small insertions or deletions at the repair junction while HDR stimulates precise sequence alterations, including programmed sequence correction as well as DNA fragment insertion, when a DNA repair template is exogenously supplied. The system has been successfully tested in staple crops, such as maize, wheat, rice, and soybean (Cai et al. 2015; Du et al. 2016; Sun et al. 2016; Svitashv et al. 2015; Wang et al. 2014; Zhou et al. 2014a, 2015c).

The recent availability of genome editing tools provides ample opportunity to introduce targeted modifications in the genome efficiently to study the functional aspects of various components of the genome in diverse plants and offers potential avenues for production of drought-tolerant soybeans. Genome editing tools provide a method for introducing targeted mutation, insertion/deletion (indel), and precise sequence modification using customized nucleases in a wide variety of organisms. CRISPR-Cas9 mediated genome engineering can enable manipulation of nearly any sequence in the genome. Abiotic stress is a complex trait, which is governed by multiple genes. There is a substantial interaction between components of several signaling, regulatory and metabolic pathways, which lead to abiotic stress response/adaptation (Nakashima et al. 2009; Garg et al. 2014; Mickelbart et al. 2015). Further, plants have undergone whole genome duplication events and a large fraction of genes are represented by multi-gene families with functional redundancy. Many times knock-out of a single gene may not produce desired phenotype, thus making it difficult to reveal its function. Due to ease of design and high efficiency of sgRNAs, multiple genes can be targeted simultaneously using CRISPR-Cas9 system, which can overcome the problem posed by functional redundancy of genes. Multiplex genome editing has been successfully implemented in model and crop plants (Li et al. 2013a; Mao et al. 2013; Zhou et al. 2014a). Such approaches can allow deciphering the role of multiple and functionally redundant genes involved in the same biological process such as drought stress response. Another approach could be the pyramiding/stacking of multiple genes involved in a stress response pathway or regulatory network via HDR-mediated gene targeting. The genes involved in drought stress-related gene regulatory network, signal transduction, and metabolite production may be targeted via CRISPR-Cas9 technologies for production of drought tolerant soybeans.

4.9.4 Rhizobial Inoculation to Enhance The Drought Stress Tolerance in Soybean

Symbiotic association between legume plants and N₂ fixing microbes like rhizobium has always been one of the fascinating areas for the researchers across the world since decades. Soybean, a legume plant makes the symbiotic association with soil bacterium, Rhizobium species by which plant can harness the benefit of biologically fixed nitrogen which helps the host plant for achieving growth and development. In return, rhizobia species get food and shelter inside the root nodules of a legume

plant. In this way, both the participating species get mutually benefitted from each other. In the recent studies, it is also found that such symbiotic association not only contributes to the growth and vigor development of the host plant but it can also enhance the drought stress tolerance capacity. The bacterial endophytes producing high trehalose inside the nodules or assimilating trehalose in bacteria could be potential strategy to enhanced survival and stability (Sharma et al., 2020). Bradyrhizobia imparts growth and drought alleviation in soybean through various direct and indirect mechanisms (Bharti et al., 2018). Soybean plants inoculated with Bradyrhizobium improved plant fitness and physiological parameters besides improving the plant rhizospheric health has been reported (Sharma et al., 2012). This opens the door for various possible opportunities where host specific rhizobium strains can be identified and can be used to study the host–microbe interactions at the physiological and molecular level to understand the mechanism of enhanced capacity of plant against the abiotic stresses. A similar kind of study has been conducted at Helsinki Institute of Sustainability Science (HELSUS), University of Helsinki, Helsinki, Finland, and Southern Agricultural Research Institute, Hawassa, Ethiopia where Aserse et al. (2019) came up with the identification of some elite rhizobial strains (HAMBI3562 and HAMBI3570 for common bean and HAMBI3513 for soybean) that significantly increased shoot dry length and nitrogen content in common bean and soybean (Table 4.6).

In another study conducted differently by Bado et al. (2013) and Zerihun and Haile (2017) they mentioned the other benefits like improvement of N and C content of the soil which in turn benefit the sustainability of the soil fertility and enhance subsequent cereal production cultivated in rotation with legumes.

Table 4.6 List of rhizobium strains specific for common bean and soybean to enhance drought tolerance

Sr. no.	Host plant	HAMBI code for <i>rhizobium</i> strain	<i>Rhizobium</i> strain
1	Common bean	HAMBI3562	<i>Rhizobium phaseoli</i> HBR10
2	Common bean	HAMBI3570	<i>R. phaseoli</i> HBR53
3	Common bean	HAMBI3556	<i>R. Etili</i> HBR5
4	Soybean	HAMBI3524	<i>Bradyrhizobium japonicum</i> TAL379
5	Soybean	HAMBI3520	<i>B. elkanni</i> SBR2B
6	Soybean	HAMBI3513	<i>B. elkanni</i> SBR8B

4.9.5 Application of Nanotechnology to Increase Drought Tolerance in Soybean

Nanotechnology is one of the promising approaches that has been extensively studied as biotechnological tools where metal-based nanoparticles are being applied in the crop system to study the effectiveness and targeted delivery of the molecular product stimulating the regulation pathways (Tripathi et al. 2018). Nanoparticles (nano-scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Roco 2003) and are used in low quantity as the replacement of plant mineral nutrients. As compared to conventional fertilization the amendment of nanoparticles improved the plant response to drought stress (Saxena et al. 2016). The application of these micronutrient-based nanoparticles, such as copper, iron, cobalt, manganese, magnesium, nickel, and zinc, helps to increase the crop yield, even under environmental stress conditions (Ashraf et al. 2012).

ZnO nanoparticles (NP) application increased seed germination of soybean under water stress (Sedghi et al. 2013). The use of extremely low concentrations of ZnO NP, lower than 500 ppm, can guarantee the enhancement of the Zn content in the seed without toxicity to plant cells (Hossain et al. 2016). The physiological traits, viz. drought tolerance index, relative water content, and biomass reduction rate, were significantly improved, especially in Fe NP-treated plants. Fe and Cu NP-treated plants maintained relative water content (RWC) at 71%, which was significantly higher than the RWC of control plants (64%) (Linh et al. 2020). Root architecture plays an important role in resistance to drought. Iron oxide NPs at the concentration of 50 to 2000 mgL⁻¹ increased root growth by 6–40% in soybean (Alidoust and Isoda 2013). The efficacy of iron oxide NPs was higher due to higher solubility, higher surface area, higher penetration through seed coat, and subsequently emerging roots and better availability to root radicals (Denher et al. 2010). Quantitative PCR analysis of drought-responsive genes showed a gene-, tissue-, and nanoparticle-dependent upregulation of gene expression. In addition to this the expression of three drought-responsive genes promoted in leaves OD nanoparticle-treated plants. The Fe NP triggered the expression of all tested genes in roots. The expression of the selected drought tolerance marker genes, GmRD20A, GmDREB2, GmERD1, GmFDL19, GmNAC11, GmWRKY27, GmMYB118, and GmMYB174, was found to be upregulated in roots or shoots (or both) of NP-treated plants under drought. This suggests that nanoparticle application can increase drought tolerance of soybean by promoting the expression of genes associated with drought (Linh et al. 2020).

4.10 Conclusions and Future Perspectives

In just the past few years we have witnessed tremendous progress in soybean genomics and an explosive expansion of new resources. We have seen the development of high-density soybean genetic maps, construction of physical and transcript maps, EST sequencing and analysis, development of high-density cDNA and oligo

arrays, and large scale re-sequencing of soybean genomes and comparison of homologous segments. These resources and the resultant studies have shed much light on the structure, organization, and evolution of the soybean genome. Tremendous progress has been made in the mapping and molecular breeding for various abiotic stresses in soybean. However, genes for many quantitative traits of economic interest are yet to be identified. A lot of genotypic and phenotypic information needs to be generated for identification and characterization of gene associated with abiotic stresses. Once the underlying gene sequence is fully characterized, haplotype analysis of the structural variants identified in the underlying genes for quantitative trait of interest could discover novel and useful alleles. The knowledge of gene function generated through QTL mapping, gene identification and characterization and resulted development of functional markers through allele mining may be translated in to a useful product using genomics assisted breeding approaches for drought tolerance. The latest genome editing tools provide opportunity to overcome certain limitations via creation of variations in the soybean genome. Such approaches can reduce soybean breeding time greatly for production of new varieties/ transgenic plants with desired traits such as abiotic stress tolerance. Due to these advances, we will be able to further explore genomic approaches to the elucidation of key genes or functional components that control complex drought related agronomical and physiological traits in soybean. Breeding approaches with potentially effective plant genetic resources (PGR), high hybridization efficiencies, and precise phenotyping facilities help realize climate-smart drought-tolerant varieties with adaptability to the Target Environment, sustaining long-term profitability of farmers in soybean cultivation, facing worse drought-trodden situation globally.

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