Drought Tolerance in Soybean:
Methods for Improvement

by

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Abstract

As opposed to the past, plant breeders today have access to far greater technology, and far greater obstacles to overcome. One of those challenges is the need for drought tolerance in crops such as soybeans. Soybeans are a major crop, affected by drought due to a lack of tolerance traits in cultivars grown today. Drought tolerant soybeans are important globally, because soybeans are grown in many countries around the world where yield losses from drought are frequent. Therefore, it is essential for farmers to make changes to their practices to help their crops tolerate or avoid drought, and breeding programs should dedicate more effort to develop drought tolerant cultivars. While U.S. plant breeders need to improve soybean cultivars through their own projects, it is also important to help establish programs in countries with the greatest need for drought tolerance traits and fewest resources.

1.0 Introduction

1.1 What is Climate Change

Climate change is “a change in global or regional weather pattern-apparent from the mid to late 20th century onwards and attributed to the increased levels of atmospheric carbon dioxide arising from the use of fossil fuels” (Lineman et al., 2015). This has led to a greater number of extreme weather patterns such as droughts and floods throughout the world (Gray and Merzdorf, 2019).

Farmers face challenges with every crop they grow. Depending upon the location, the challenge may be an insect or disease, and ways to protect plants against such threats. The recent trends in climate indicate that farmers across the globe will continue to struggle to protect their crops from environmental stresses and yield loss, if they are unable to adapt (Reidsma et al., 2010). Therefore, drought is already a major constraint for global agricultural production, and climate change will cause it to worsen (Mir et al., 2012).

One of the many crops that will be affected by these climate changes is soybeans (*Glycine max*) - one of the most important legume crops across the globe. Soybeans are utilized as a protein source for humans and livestock, and as a bio-diesel crop (Zhao et al., 2018). However, soybeans are sensitive to drought. Therefore, it is important to focus on breeding for increased drought tolerance traits.

1.2 Drought Tolerance vs. Resistance

In agriculture, drought is defined as "the shortage of precipitation that causes deficit in soil water and reduction of groundwater levels", resulting in a reduction of crop production (Ku et al., 2013). Drought tolerance and drought resistance are related concepts. The ability of a plant species to survive in an environment with a water deficit is drought resistance; one of the methods of drought resistance is drought tolerance (Mitra, 2001). Plants that are drought tolerant are capable of maintaining the rigidity of cells and continued cellular metabolism (important for plant growth and reproduction), when water availability is low (Manavalan et al., 2009). Figure 1 below illustrates some of the traits of a drought tolerant plant (drought avoidance traits are also

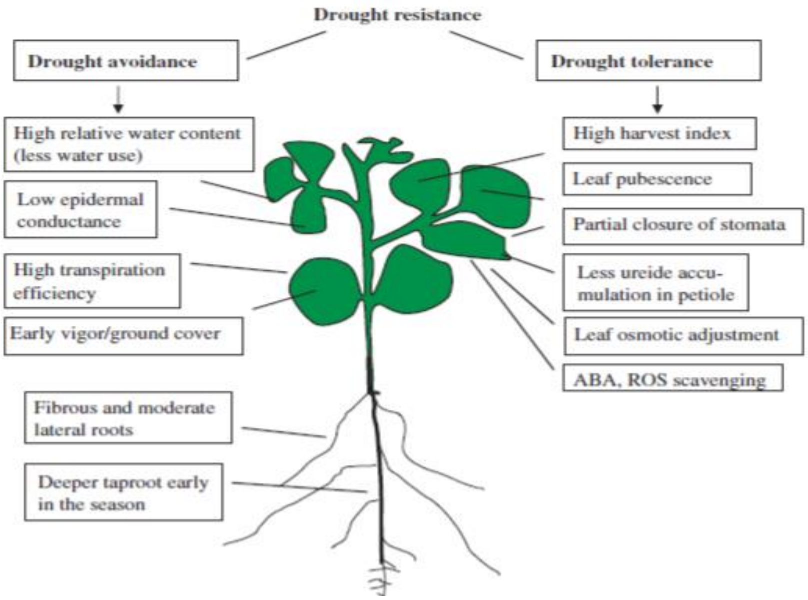

Figure 1 Traits associated with drought resistance in soybean. ROS, reactive oxygen species (Manavalan et al., 2009).

shown, although that will not be discussed). Drought tolerance is a quantitative trait, which means it is a difficult trait to study due to the many contributing genes (Kebede, et al., 2019). The distribution of quantitative traits is continuous and normal when on an appropriate statistical scale (Mackay, 2009), meaning there are different levels of drought tolerance. When a cultivar is being grown where a drought occurs, yield loss will be limited if the plants have high drought tolerance.

1.3 Challenges of Breeding for Drought Tolerance

Breeding for drought tolerance is no easy task, due to the numerous challenges to identify genes that control drought tolerance. Since drought tolerance is a quantitative trait and many genes are involved it is difficult to create a molecular marker for a plant's response to a water-deficit (Sinclair, 2011). A molecular (or genetic) marker is a variation of a gene or DNA sequence with a known location on a chromosome and an association to a particular trait (Al-Samarai and Al-Kazaz, 2015). After a molecular marker is developed, finding tolerant plants can be accomplished through marker-assisted selection (MAS) (He et al., 2014).

The timing, intensity, and duration of a drought varies (Mir et al., 2012), creating a hurdle for properly testing varieties. Therefore, plant breeders typically combine data from multi-location trials to test new varieties, determine how genotypes respond across environments, and minimize effects of genotype by environment interactions (Cross, 1990). However, improving a crop for drought tolerance will not result in an equal yield protection across environments, making it difficult to combine data across environments (Sinclair, 2011).

It is also ideal for drought tolerant varieties to perform well under normal conditions, but it has not been stressed that yield under normal conditions should be improved alongside yield under drought conditions (Basu et al., 2016). There have been suggestions that drought tolerance can cause a yield reduction under normal conditions, making it important to select for high yield potential under normal conditions in combination with good yield under drought stress (Basu et. al., 2016).

This Creative Component will review and analyze various methods used to develop drought tolerant soybeans. Before discussing the methods involved, it is important to investigate the impacts of drought on soybean production nationally and internationally. Some of the methods are conventional and are either achieved by farmers adjusting their practices or breeding without the use of newer technologies. Other methods use molecular tools and require the application of molecular markers and/or transgenes. Programs have been created for areas with high risk of drought, some of which will be discussed. After reviewing all related information, recommendations as to which methods are preferred will be given.

2.0 Impacts of Drought on Soybeans

Drought has negative impacts on soybeans, both biologically and economically. Biologically, the impact on plants varies between below and above ground parts, but generally leads to negative changes in yield. Economically, the impact of drought also depends upon the location where drought occurs.

2.1 Biological Impacts on Below Ground Traits

The plant part essential for obtaining water is the root system. For combating drought, soybean root systems with larger lateral roots, greater amounts of root hairs, and roots whose xylem diameters are large are ideal (Kunert et al., 2016). Improved growth is likely when roots are dense and deeply penetrate the soil (Fenta et al., 2011). When there is a drought, various changes occur in the root architecture, such as the depth, branching, density and angle of roots (Kunert et al., 2016). There is a significant reduction in the growth of lateral roots, caused by a suppression of the activation of their meristems (Basu et al., 2016). Changes also take place in the root to shoot biomass ratio, with an increase in the root mass (Kunert et al., 2016). The changes are caused by chemical signals sent via xylem from root to leaves, which reduces water loss, but also decreases leaf growth (Schachtman and Goodger, 2008). This highlights the sensitivity of roots to water deficits but helps increase the root to leaf surface area ratio, continue the growth of new root tips, and increases the plants abilities for absorbing water to support already existing shoots (Comas et al., 2013).

Soybeans establish a symbiotic relationship with the soil bacterium *Bradyrhizobium* to fix atmospheric nitrogen (N_2) for utilization by the plant (Kunert et al., 2016). In most cases, this fixation of N_2 means there is no need to apply additional nitrogen fertilizer, although this is not enough for some high yielding varieties (Kunert et al., 2016**).** N_2 fixation is highly sensitive to drought (more than above ground processes such as photosynthesis), and the accumulation of nitrogen is limited in a water deficit (Serraj et al., 1999). When under drought conditions, the nodule mass of soybeans is decreased, and a severe drought will limit the nodule number and dry mass (Serraj et al., 1999). If N_2 fixation is limited and an external application of nitrogen cannot be made, a nitrogen deficiency could occur. When soybeans experience nitrogen deficiency, root branching will be limited, creating a short, compact plant; shorter plants with few branches will have fewer pods/seeds than healthy plants, which negatively impacts yield (Casteel, 2018).

The below ground changes that occur in soybeans during time of drought can negatively influence the yield of the crop. The decrease in N_2 fixation can limit the number of seeds that are produced, as well as their size. A study by Purdue Extension (Casteel, 2018) on nitrogen deficiency (due to drought/lack of fixation) saw that healthy plants had a 4.37 t/ha yield in the same field where plants lacking nitrogen had a 2.69 t/ha yield.

2.2 Biological Impacts on Above Ground Traits

Above ground, the canopy of soybeans makes numerous contributions to the plants (such as the interception of light) and is one of the first parts to be negatively affected by drought. Canopies that close earlier in the growing season intercept more light, thereby increasing crop growth rate, dry matter accumulation, and seed yield (De Bruin and Pedersen, 2007). During times of drought, one of the first processes affected is leaf size, due to a reduction in cell development and diminished cell division that eventually lessens the mature cell size in leaves (Anjum et al., 2017). Initially, this defends the plant by limiting the amount of transpiration but decreases the amount of photosynthesis conducted in the long run (Anjum et al., 2017). Plants use photosynthesis to create energy resources for growth and development (Cessna et al., 2010). Therefore, a decrease in photosynthesis means there will be less energy available for the plant to use for growth and reproduction.

When soybean canopies wilt during drought, the onset and severity of the wilting varies between different soybean genotypes (Ye et al., 2019). Field evaluations of different genotypes have occurred in order to determine which ones are slow wilting (improved yield under drought) and which ones are fast wilting (decreased yield under drought) (Ye et al., 2019). The slow canopy-wilting trait in soybeans is associated with drought tolerance (Hwang et al., 2016).

The soybean plant parts that most directly influence yield are the pods and seeds. On average, a soybean plant produces 25-40 pods, and each pod holds an average of 2.5 seeds (Davidson, 2014). When drought occurs at the pod-filling stage, up to a 20% reduction in pod number may occur, likely due to floral abortion (Licht et al., 2013). These effects can negatively impact yield. Consequently, yield loss from drought is predominately from the limited number of seeds, in addition to a reduction in pod numbers (Board and Kahlon, 2011).

Abscisic acid (ABA) is a plant hormone, responsible for signaling plant response to varying stresses, including drought (Sah et al., 2016). ABA-mediated stomatal closure is one of the first responses to drought stress, resulting in the reduction of stomatal conductance and CO_2 availability, thereby minimizing photosynthetic activities (Shukla et al., 2018). As has been discussed, when photosynthetic activities are reduced, overall plant growth and yield will be limited as well.

2.3 Economic Impacts

Soybeans are grown in numerous countries throughout the world. Of all soybean producing countries, the United States has the largest production area, with 34.4 million hectares planted in 2018, and a harvest of 108 million metric tons (MMT) (Karuga, 2018). Other top producing countries (in order of MMT from greatest to least) are Brazil, Argentina, China, India, Paraguay, Canada, Ukraine, Bolivia, and Uruguay (Karuga, 2018). Six of the ten top soybean-producing countries (Brazil, Argentina, Paraguay, Uruguay, Bolivia, and China) are in regions considered chronically drought-prone (Wang et al., 2014). In 2018, the number of hectares of soybeans planted in South America was 57.35 million, while all of Asia had a total of 20 million hectares planted with soybeans (Karuga, 2018). Another region where crops frequently face drought stress is sub-Saharan Africa (Khojely et al., 2018). Slightly more than 2 million hectares were planted in sub-Saharan Africa in 2018 (USDA, 2019). However, the metric ton per hectare production for Africa is less than half of the world's average production (USDA, 2019).

Yield losses in soybeans caused by drought vary based upon the timing and duration of the drought. Therefore, numerous studies have been conducted to determine when in the growing season drought has the most effect on soybeans (Ku et al., 2013). According to a study by Kpoghomou et al (1990) on three different soybean cultivars, yield loss is greatest when there is a water deficit during the R4 (pod-filling) stage. Desclaux et al (2000), conducted experiments of withholding irrigation from the crop for 4-5 days (until water availability was 30-50% of normal conditions) and found the R4 (pod-filling) stage to be the most severely affected. These two studies arrived at the same conclusion, but the R4 stage is not the only stage when drought is detrimental.

According to Mark Licht et al. (2013), water deficits during the germination and reproductive stages are the most detrimental to soybeans, because for a soybean seed to germinate, it needs to absorb water equivalent to 50% of its weight. As for the duration of the drought, soybeans can be resilient if the drought is brief (Licht et al., 2013). When there are short periods during which water is inaccessible during vegetative growth, yield is not typically affected (Licht et al., 2013). However, severe or extended water deficits can cause dehydration of vegetative tissue and plant death (Licht et al., 2013). Therefore, when a drought occurs for an extended period, it is almost expected that there will be a loss in yield and income for farmers.

In recent times, there have been a number of major droughts across the globe that have created economic hardships. For example, in 2018, Argentina faced a drought that caused a 31% decrease in soybean yield, costing the country about 3.4 billion USD (Voiland, 2018). China faced its worst drought in decades from 2010-2011 and it affected 7 million hectares of land, resulting in a loss of 2.3 billion USD (Buckley, 2011). Around the same time, a major drought occurred in East Africa, which was arguably the worst drought in the region in 60 years (Riebeek, 2011). The East African drought resulted in a 20% reduction in crop yield and tens of thousands of human deaths (Riebeek, 2011). The situation is expected to worsen with climate change, resulting in more economic loss. Therefore, there is a need to develop drought tolerant crop varieties, including soybeans to ensure food security in various parts of the world.

3.0 Conventional Methods for Increasing Drought Tolerance

Before resorting to costly molecular methods of plant breeding, there are conventional methods that can be used to increase drought tolerance in soybeans. This is achieved through improvements made by plant breeders and agronomic changes implemented by farmers. These methods include taking advantage of drought escape, using PI (plant introduction) accessions, and applying different plant hormones or extracts.

3.1 Drought Escape

Drought escape is a concept that can be advantageous to farmers and plant breeders as a conventional method for avoiding drought stress. Plants use drought escape to avoid drought stress by rapid development, thus allowing them to complete a full life cycle before the occurrence of a drought event (Shavrukov et al., 2017). Drought escape is commonly used for soybeans in the southern United States and is known as the Early Soybean Planting System (Manavalan et al., 2009). Soybean planting in the southern United States begins as early as March, allowing the crop to complete its reproductive stages (have pods set) before July, when drought is a possible risk (Manavalan et al., 2009). Some of the results of planting early, other than avoiding the effects of a drought during reproductive stages are: more nodes per plant due to the V1 (trifoliate) growth stage being reached sooner, a quicker closing canopy that captures more light during key yield stages, and greater crop transpiration and less soil evaporation (Elmore et al., 2014). Plant breeders can help farmers implement a drought escape planting system by creating varieties better suited for the system.

If a plant breeder wants to develop varieties that are capable of drought escape, a trait to focus on is early flowering. Early flowering (or flowering date) is a secondary trait, because it has the largest impact on yield when plants are stressed (Lafitte et al., 2003). Early flowering can be implemented into a conventional breeding program by making observable selections in the F_2 generation (when all genetic variability is exposed) for plants that flower at the appropriate time for avoiding drought (Richards, 1996). For soybeans in the Early Soybean Planting System, the selection would be for plants that flower earlier in the growing season. Although selections after the F_2 generation would be for other traits, the expression of the early flowering trait would be maintained (Richards, 1996). Maintenance of the early flowering trait could lead to new varieties better suited for escaping drought stress.

Other than breeding for early flowering soybean cultivars, plant breeders can also utilize drought escape to develop earlier maturity groups than would normally be implemented in an area. In a study by Edwards et al. (2003), varieties in maturity groups (MG) 00, 0, I, II, II, and IV were planted in mid-southern United States on different planting dates under both irrigated and non-irrigated conditions. Results from this study showed that under non-irrigation in an early production system, cultivars in MG I and II are best suited for avoiding exposure to drought (Edwards et al., 2003). This means breeders in the southern United States could develop earlier maturity soybeans than are normally planted in the area, providing farmers with more options for drought escape.

3.2 Use of PI Accessions

Another method for plant breeders to increase drought tolerance is through the integration of plant introduction (PI) accessions into their breeding programs. To do so, a breeder must first identify parents with the desired qualities that can be used to develop improved cultivars (Richards, 1996). In this case, one of the parents would be a PI accession that can be introduced into programs by crossing it with an elite cultivar (Byrne et al., 2018). Figure 2 below shows an example of a pedigree breeding program where a PI accession could be utilized. Variety A would be a PI accession and Variety B would be an elite cultivar, and the subsequent generations would be progeny from their crosses.

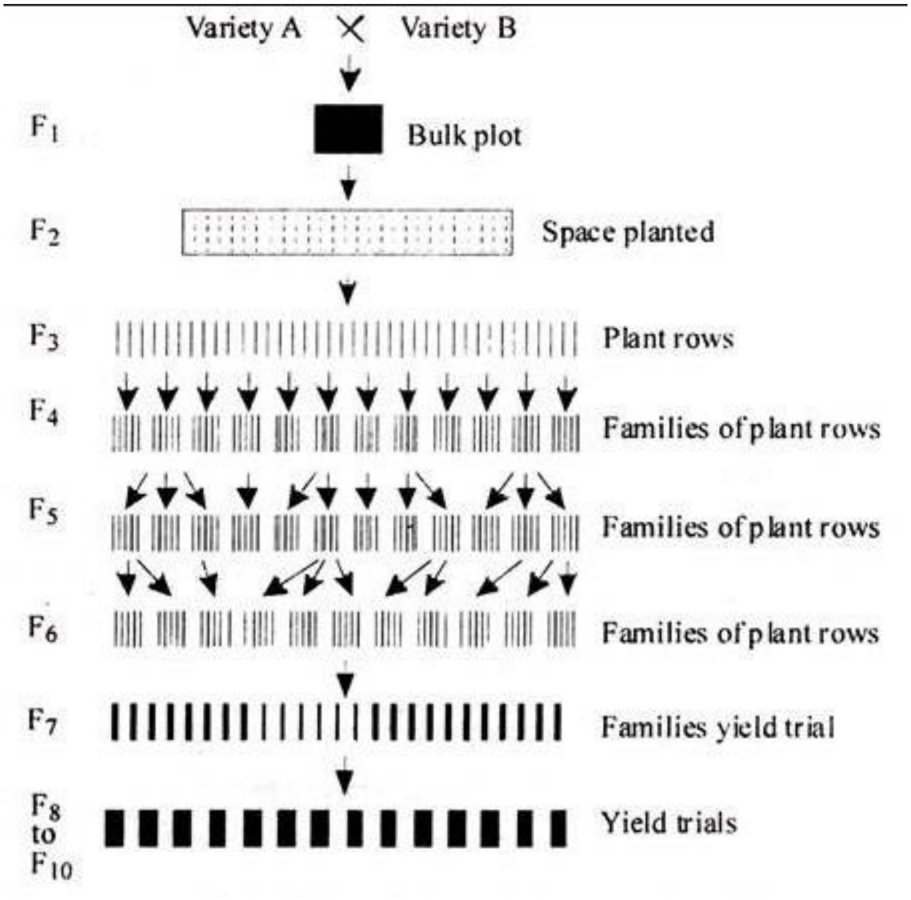

Figure 2 Different steps involved in pedigree method (Sleper and Poehlman, 2006).

As has been previously discussed, breeders would then select for desired traits in the F_2 generation. In this generation selection is not going to be directly used for drought tolerance or other quantitative traits (those traits are selected for in later generations) but will instead be for desirable traits under drought that are easy to select for (Richards, 1996). Marker-assisted backcrossing (MABC) can be utilized to ensure the progeny maintain a majority of the elite cultivar’s genes by selecting for them using background selection (Gupta et al., 2010). A trait of interest for this purpose that can be found in PI accessions is slow canopy-wilting.

Slow canopy-wilting can be used in soybeans to identify and improve drought tolerant varieties (Hwang et al., 2016). Two plant introductions have been identified as slow wilting: PI 416937 and P1 471938 (Sadok et al., 2012). Both have slower canopy-wilting than other lines by several days when in the field under moisture-deficit conditions (Sadok et al., 2012). PI 416937 is a Japanese germplasm accession, and its drought resistance is potentially from multiple mechanisms (Abdel-Haleem et al., 2012). The most noted source of its tolerance to drought is a water-conservation trait that allows the transpiration rate to remain constant, even during atmospheric vapor pressure deficits (Sadok et al., 2012). Another trait associated with the accession is a dense root surface area with a high amount of root tips, in addition to a lower stomatal conductance, which is known to conserve water when there is a deficit (Abdel-Haleem et al. 2012). As for PI 471938, which originates from Nepal, there is no clear explanation for its slow canopy-wilting and resistance to drought (Sadok et al., 2012). Regardless of the source of their slow wilting trait, both accessions have been used in breeding programs for increasing drought tolerance in soybeans.

After accessions with the desired trait have been identified, they can be incorporated into a breeding program. In combined efforts between three different soybean breeding programs (University of Arkansas, Fayetteville, and USDA-ARS in North Carolina), 10 elite breeding lines were developed over 20 years as candidates for germplasm or cultivar release (Devi et al., 2014). Dr. Tommy Carter, a USDA professor at North Carolina State University has used PI 416937 and PI 471938 in a drought resistance breeding program; he has created a commercially available non-GMO cultivar that could also be further used as parental stock for other breeding programs (Carl, 2016). With the progress made by the various soybean breeding programs, more drought tolerant cultivars can be created.

3.3 Application of Plant Hormones

An alternative (or addition) to drought escape or planting drought tolerant varieties, if they are not an option for a farmer, is the application of different plant hormones or extracts. One option is methyl jasmonate (MeJA), which is part of the jasmonate (lipid-based plant hormone) family and works to regulate plant growth (Reyes-Diaz et al., 2016). MeJA is involved in a variety of plant functions, and during times of abiotic or biotic stress, it can help modulate plant defense responses (Reyes-Diaz et al., 2016). There are benefits when a foliar application of MeJA is made to drought-stricken soybeans. Anjum et al. (2011) studied the benefits of MeJA application by withholding irrigation from soybeans grown under normal conditions once they began to bloom, followed by a 50 μM foliar application of MeJA. The application of MeJA resulted in maintenance of relative water content and decreased membrane lipid peroxidation, leading to improved yield under drought. Improved yield occurred because effects of the MeJA application led to an increase in the number of grain per pod, pods per plant, grain per plant, and harvest index. The cost of MeJA is 125 USD for 25ml (Sigma-Aldrich, 2019), which can be expensive to apply depending upon the number of acres it is being utilized for.

Brassinosteroids (BR) are plant steroid hormones, and like MeJA, have a role in numerous plant functions. Some BR roles include regulating the metabolism of plant oxidation radicals, ethylene synthesis, and root gravitropic response (Tang et al., 2016). BR can also be found in a plethora of plants- and are even likely to be widespread in ancient plants (Tang et al., 2016). Zhang et al. (2008) embarked on research to prove the usefulness of brassinolide (BL- the naturally occurring form of BR) for soybeans under water deficits, by applying foliar BL (0.1 mg 1^{-1} BL) once soybeans began to flower. The researchers had two groups- a well-watered control with 80% field capacity and a drought-stricken group with 35% capacity, at pod initiation. In both groups, BL resulted in increased translocation of assimilated carbon and chlorophyll content, thereby increasing the biomass accumulation and seed yield. Both the higher biomass and seed yield seen by drought stressed plants that received BL treatments indicates that it can be used to increase the drought stress tolerance of soybeans (Zhang et al., 2008). Applying BL is not as expensive as applying MeJa, with the cost ranging from 5-10 USD per gram, and a gram being enough to cover up to 5 acres (Vardhini et al., 2006).

In addition to plant hormones, another form of applicants to mitigate the negative impacts of drought are plant extracts. An extract from brown seaweed, *Ascophyllum nodosum* (referred to as ANE) might regulate the ABA-biosynthesis pathway and increase a plant's drought stress tolerance (Shukla et al., 2018). The study was conducted by Shukla et al. (2018) and involved the application of ANE to a soybean variety called Savana, while a control of the same variety received no treatment. ANE was applied through a fertilizer solution after 21 total days of growth. The application of ANE was done twice one week, followed by a third and final application in the next week. Researchers stopped irrigation after the first application of ANE, allowing the onset of drought conditions. The group that was treated with ANE had reduced wilting, 50% higher water content than control plants, and demonstrated an improved ability to recover from drought (Shukla et al., 2018). The plants' ability to recover could be from a 46% increased stomatal conductance compared to the control, and a reduction in the rapid increase of leaf temperature (Shukla et al., 2018). The ability of ANE to regulate the ABA-biosynthesis pathway (as previously mentioned, one of the plant's first responses to drought is ABA-mediated stomatal closure) indicates its usefulness for increasing drought tolerance in soybeans. To use on a large-scale, water soluble seaweed powder can be purchased for around 475 USD per 44 lbs (Growershouse, 2019).

4.0 Molecular Breeding Methods for Increasing Drought Tolerance

There are certain genetic improvements that can be made to soybeans for increasing their drought tolerance that cannot occur by conventional methods alone. The genetic improvement will depend on the application of molecular breeding methods, and highly utilize the application of markers and use of transgenic approaches.

4.1 Application of Molecular Markers to Screen for Root/WUE Traits

A molecular (or genetic) marker is a variation of a gene or DNA sequence with a known location on a chromosome and an association to a particular trait (Al-Samarai and Al-Kazaz, 2015). It is helpful to have molecular markers for a trait to be used to improve a crop. If a molecular marker associated with a trait of interest can be identified, the marker can be utilized later in marker-assisted selection (MAS). Such markers would be useful in screening for root and water use efficiency (WUE) traits that can increase drought tolerance.

Some of the desired root characteristics that improve drought tolerance or resistance are deeper, denser root systems, which are related to improved growth under stress (Fenta et al., 2011). This is because deeper root systems allow the plant to obtain water from greater depths beneath the soil, and as a result, changes in carbon allocation patterns can occur before adverse effects from limited water affect growth (Fenta et al., 2011). Fibrous roots are also an ideal characteristic for root systems, due to their increase in surface area and number of root tips (Abdel-Haleem et al., 2011). Abdel-Haleem et al. (2011) worked to identify QTL for increased fibrous roots in soybean. To identify QTL, a cross was made between PI416937 (an accession with fibrous roots) and 'Benning' (a cultivar with few fibrous roots). From there, a recombinant inbred line (RIL) population was developed and analyzed for two years, under rain fed field conditions. Fibrous root scores were taken in the field (see Figure 3 below for example), and

128 of the 240 RILs were selected in order to reduce QTL analysis costs.

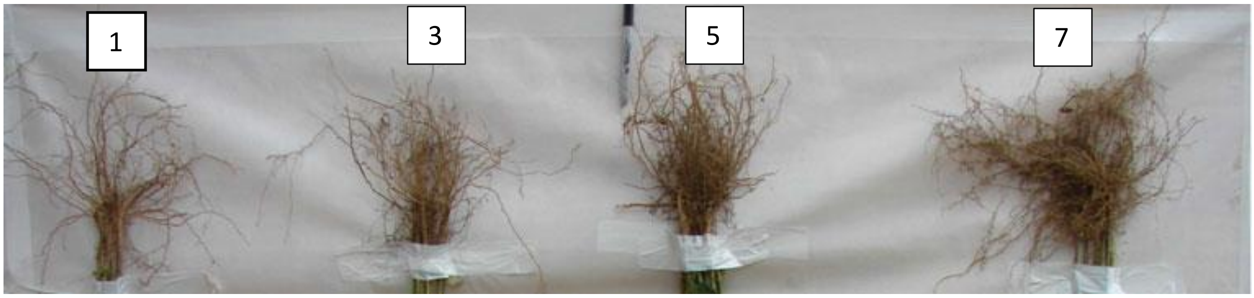

Figure 3: The examples of roots in various root score (white labels) categories; where 1 = few fibrous roots and 7 = many fibrous roots (Abdel-Haleem et al., 2011).

DNA samples were taken from both parents and all chosen RILs, linkage maps were created, and five QTL were identified. Combined, the QTL account for 51% of the root score variation. Although none of the QTL are considered major, they could all be used in MAS to incorporate the fibrous root trait into elite germplasm.

It is preferred that plants have high WUE, because it allows for maximal soil moisture capture for transpiration and minimizes soil loss, thereby enhancing biomass production (Fenta et al., 2011). A study was completed in 1996 to identify QTL associated with WUE in soybeans (Manavalan et al., 2009). In this study, a population of 120 F_4 derived lines from a cross between Young and PI416937 was planted in a greenhouse and phenotypic notes were taken for WUE and leaf ash (LASH). LASH is negatively correlated with WUE. A restriction fragment length polymorphism (RFLP) was also constructed and 4 RFLP markers were found for WUE, and 6 were identified for LASH. It is probable that there is a major QTL for WUE, and the study verified the negative correlation between WUE and LASH. Consistency of the WUE QTL were tested by sampling a separate F_2 population derived from a S100 by PI416937 cross, and one of the QTL was also detected in that population. As more WUE QTL are verified, they can be used to improve soybean cultivars using MAS.

Once markers are identified, they can be used to help obtain desired plant traits in different soybean lines. This is done by utilizing MAS, the method of indirectly selecting for required traits using all identified markers (Fenta et al., 2011). Since drought tolerance is a quantitative trait, the use of MAS would be costly, time consuming, and technically challenging to implement (Fenta et al., 2011). This challenge would be alleviated by creating linkage maps and identifying QTL, which can further refine MAS by completion of the soybean genome sequence (Dubey et al., 2018).

MAS is most effective in conventional soybean breeding programs when it is used for selection in the F_2 or F_3 and the population is segregating (Gupta et al., 2010). The method would be for fingerprinting elite lines and subsequently identifying and selecting for the presence of favorable alleles (Gupta et al., 2010). Since the use of markers is required for the success of MAS, it is important that accurate QTL related to drought tolerant traits are first identified. Consequently, no drought tolerant varieties have yet been released using MAS because of the low percentage of variation accounted for in the traits.

4.2 Transgenic Approaches

A method for improving crops that has many potentials (although it is highly regulated) is the use of transgenes. A transgene is a “foreign” gene that is transferred from one plant species to another (Kebede et al., 2019). One transgene that can be transferred to soybeans is the *Arabidopsis* gene called *AtMYB44*, encoding a transcription factor that induces stomatal closure by positively regulating ABA signaling (Seo et al., 2012). As has been previously discussed, stomatal closure helps increase drought tolerance in plants. Seo et al. (2012) successfully transformed soybean lines with the *AtMYB44* gene from *Arabidopsis* using *Agrobacterium tumefaciens*. Three generations were grown from each plant after they were transformed, creating a stable integration of the transgene. The transgenic plants were then grown in the field and tested for drought tolerance. Compared to the non-transgenic control, the transgenic soybeans were shorter, but maintained similar developmental progression. In addition, the transgenic lines had greater yields than the controls. Testing was carried out in a greenhouse by not watering the plants for 10 days. After four weeks of growth, the transgenic lines displayed much greater drought tolerance than the control lines. While the non-transgenic lines wilted, the transformed plants had little to no wilting (a sign of drought tolerance), indicating the integration of *AtMYB44* into soybeans was successful.

Another gene from *Arabidopsis* that can be used to transform soybeans for the purpose of increasing drought tolerance is the P5CR gene. The P5CR gene encodes L-Δ^1-pyrroline-5-carboxylate reductase, which catalyzes the final step in the proline biosynthesis (NCBI, 2019). The P5CR gene can also enhance the process of proline degradation, which in turn helps maintain $NADP^+$ and allows plants to adapt to environmental stress (De Ronde et al., 2004). De Ronde et al. (2004) transformed soybean with the P5CR gene, using a P5CR-IHSP (inducible heat shock promoter) construct. An inducible heat shock promoter was used because it begins to work once there is an increase in heat. Transgenic plants were created using the *Agrobacterium* transformation procedure. After one generation of selfing, transgenic plants were grown in the greenhouse and subjected to drought stress. The plants were watered three times a week and given a nutrient mix every two weeks. At week 14, just before the flowering stage, water was withheld, and the temperature was increased by 13℃. Samples were taken, and a PCR analysis showed that the P5CR gene had been successfully integrated (6 plants in the sense direction and 6 in the antisense direction). The plants with the gene in the sense direction had full turgor after a period of stress, while the antisense plants almost completely wilted and wild type plants had moderate wilting. The plants with the gene in the sense direction also had much greater regeneration of $NADP^+$ during and after the stress occurred. *Arabidopsis* can clearly be a source of genes for use in engineering drought tolerance in soybeans, but there are possibilities of using other crops as well.

A third trait that has been integrated into soybeans for the purpose of increasing their drought tolerance came from sunflowers (*Helianthus annuus*). It is the Hahb-4 gene, encoding a transcription factor that may bind a dehydration transcription regulating region of genes associated to plant drought stress response (ISAAA, 2019). The company Verdeca LLC has created soybean plants harboring the Hahb-4 gene, to create the "HB4 trait" (Arcadia Biosciences, 2015). This was accomplished by inserting the Hahb-4 gene into soybean plants using the *Agrobacterium tumefaciens*- mediated transformation method (ISAAA, 2019). According to Arcadia Biosciences (2015), Verdeca, (a joint venture between two South American companies, Arcadia Biosciences and Bioceres SA), has extensively tested HB4 soybeans in the field in both Argentina and the United States. This includes six seasons of field trials and two seasons of regulatory trials, all at multiple locations. Results from these trials show a 14% increase in yield under drought stress conditions. Regulatory approval for the HB4 trait for food, feed, and cultivation purposes has been met in Argentina and Brazil, and for only food purposes in the United States (ISAAA, 2019). Now that approval has been granted, the company Tropical Melhoramento e Genetica Ltda (TMG) is working on developing new varieties with the HB4 trait (Arcadia Biosciences, 2015). Currently, the HB4 trait is the only genetically modified trait approved for increasing drought tolerance in soybeans.

A fourth trait that has been integrated into soybeans using transgenic approaches came from a soybean source. The target trait is the ER luminal binding protein (BiP), which delays leaf senescence (Valente et al., 2008). Leaf senescence is associated with tissue deterioration as the plant ages; it is the final stage of leaf development and begins when nutrients are relocated from leaves (source) to reproducing seeds (sink) (Lim et al., 2007). When a plant is drought-stressed, leaf senescence might be prematurely activated in order to decrease canopy size and conserve energy, causing lower yields and economic losses to farmers (Rivero et al., 2007). The BiP gene family in soybeans consists of soyBiPA, soyBiPB, soyBiPC, and soyBiPD (Cascardo et al., 2001). SoyBiPD is expressed in all organs (Cascardo et al., 2001), and was selected for overexpression in soybean. The overexpression of soyBiPD was achieved by creating a plant expression cassette containing the BiP coding sequence under the control of the cauliflower mosaic virus 35S promoter (Valente et al., 2008). Valente et al. (2008) tested successfully transformed lines for drought tolerance against wild type soybeans, which were both planted and grown in the greenhouse. At the V6 developmental stage, 50% of all lines (both transformed and wild type) had their daily water supply reduced by 40% compared to controls. After two weeks, their water supply was increased back to normal levels. The results of this experiment and others showed that the transgenic lines had higher amounts of soyBiPD mRNA and protein than the wild type and greater stomatal conductance and transpiration. There was also significant wilting in the wild type, whereas the transgenic lines experienced less wilting. These results demonstrate that overexpression of BiP in soybeans allows them to withstand drought stress.

Table 1 below provides a summary of all the transgenic approaches discussed above.

Table 1 Summary of Transgenic Approaches for Improvement of Drought Stress in Soybean

Target Trait	**Target Gene**	**Origin of Target Gene**	**Target Tissue**	**Promoter**	**Effect**	**Transfor-mation Method***	**Soybean Genotype**	**Source**
Drought/ salt stress	Transcription factor AtMYB44	A. thaliana	whole plant	CaMV 35S	Tolerance to drought/salt stress	AG	Bert	Seo et al., 2012
Drought stress	L-Δ1-Pyrroline-5-carboxylate reductase gene (P5CR)	A. thaliana	whole plant	soybean heat shock gene	Tolerance to heat/drought stress	AG	Ibis	Yamada et al., 2012
Drought stress	Transcription factor HaHb-4	*Helianthus annuus*	whole plant	Unknown	Tolerance to drought stress	AG	Unknown	ISAAA, 2019
Drought stress	Molecular chaperone BiP (binding protein) gene (soy BiPD)	Soybean	whole plant	CaMV 35S	Tolerance to drought stress	PB	Conquista	Yamada et al., 2012

** AG = Agrobacterium, PB= Particle Bombardment.* Data source: Yamada et al (2012).

5.0 Programs in Countries with High Drought Risk

As has been previously discussed, soybeans grown in sub-Saharan Africa (SSA) countries frequently face drought stress, increasing the importance of drought tolerant soybeans. However, there are programs ran by different organizations that have been established to help SSA expand production of soybeans. Programs to be reviewed are a master's degree program in plant breeding, a soybean innovation lab, and programs for improving germplasm and available varieties.

5.1 Why Plant Breeding/Germplasm Programs are Needed in SSA

Soybeans are not native to Africa, having been introduced to the area in the 19th century by Chinese traders (Khojely et al., 2018). Initially, problems arose when producing soybeans in Africa, including minimal or no germination, low yields, or germination followed by crop failure (Lawrence, 2011). After initial failures, multiple varieties were brought over from the United States and different parts of Asia, but no attempts were successful (possibly due to the change in temperature during shipping that caused the seed to deteriorate) (Lawrence, 2011). According to Khojely et.al (2018), acreage planted of soybeans has exponentially increased in SSA, with 20,000 ha being planted in the early 1970s to 1,500,000 ha being planted in 2016, as has the yield (13,000 t and 2,300,000 t respectively). Although the overall amount harvested has increased, the yield of soybean per hectare (ha) has remained stagnant at 1.1 t ha^{-1} for decades (Khojely et al., 2018). A combination of factors in addition to recurrent droughts contribute to the poor performance of soybeans, including a lack of germplasm and plant breeders in SSA.

An important component of a plant breeding program is the availability of a well-trained plant breeder. Having more plant breeders is important, because the population in African countries is expected to quadruple within the next century and a key to crop improvement and sustainability is education (Feed the Future, 2014). The seed industry has also grown rapidly in some countries (Uganda, Tanzania, and Zambia, for example), and there are not enough plant breeders to keep up with the number required for both private and public breeding programs (Langyintuo, 2010).

However, across SSA, the number of full-time breeders with a PhD is very low (Khojely et al., 2018). One of the reasons for the low number of plant breeders is that African students traditionally participate in graduate programs outside of their home country and may not return home for work after graduating (Shurtleff and Aoyagi, 2019). In fact, in 30 SSA countries, there are only 5 breeders per country to cover all crops (Suza et al., 2016). The shortage of plant breeders requires more training programs within SSA to create advantages such as affordability, relevance, retention, and less disruption to the family and workplace (Suza et al., 2016).

Another important component in a plant breeding program is germplasm. Germplasm is "living tissue from which new plants can be grown" (UCDAVIS, 2019), and it incorporates a range of sources, such as older/current crop varieties, landraces, wild relatives, and specialized breeding lines (Allard et al., 1991). Germplasm can be used for numerous crop improvements, including increased pest and disease resistance, potentially allowing for phenomenal agricultural productivity, as has been witnessed in the United States (Allard et al., 1991). Currently, the germplasm used for soybean varieties in SSA is not adequate. For example, in 2017, germination tests were completed on 15 local varieties in Ghana, and it was determined that many of them did not meet the accepted standard of an 80% germination rate (Awuni and Reynolds, 2017). Numerous programs are working on introducing new soybean germplasm to SSA, in hopes of improving available varieties.

5.2 University of Illinois – Soybean Innovation Lab Programs

A group that is committed to improving soybean production in SSA is the University of Illinois' Soybean Innovation Lab (SIL). The Soybean Innovation Lab spent over 5 years working with the West African Center for Crop Improvement (WACCI) to develop an innovative master's program in plant breeding at the University of Ghana (Shurtleff and Aoyagi, 2019). According to Dr. Peter Goldsmith, the director of SIL, filling the training gap at the master's or technical level provides people who can manage research plots at the region's research stations (Shurtleff and Aoyagi, 2019). SIL and WACCI developed a program specifically for a country that needs a sustainable model for long-term plant breeding and seed system performance (Feed the Future, 2014). SIL's participation has allowed for courses to be developed that were previously unavailable, such as Experimental Design and Statistical Analysis I and II, Population Genetics, and Molecular Marker Analysis, in addition to the creation of summer internships in the United States that connects students with global seed companies (Feed the Future, 2014). By having an improved M.S. Plant Breeding program (which is also a gateway for obtaining a PhD), the number of natives who obtain their degrees outside of African countries and do not return to practice their profession can be lowered.

Another program initiated by the Soybean Innovation Lab is the Soybean Management with Appropriate Research and Technology (SMART) Farm. Established in 2014, it is in Tamale, Ghana (with two field stations in Wa and Bawku), at the Savannah Agricultural Research Institute (SARI) (Goldsmith and Tamimie, 2015). Its purpose is to provide foundational agronomic research for successful soybean production, and it allows for existing knowledge gaps between researchers, NGOs, contractors, farmers, etc. to be filled (Shurtleff and Aoyagi, 2019). This includes needed information about soybean production, such as soil correction and preparation, weed, fungi, and insect management, environmental stewardship, and varietal performance and selection criteria (Goldsmith and Tamimie, 2015). There are issues related to a variety of topics the farm investigates, such as germination, planting dates, soil amendments, planting methods, and varietal performance (Shurtleff and Aoyagi, 2019). Another platform of the SMART farm is germplasm development (Shurtleff and Aoyagi, 2019), which could be beneficial to plant breeders.

5.3 Programs for Improving Germplasm and Varieties

Various organizations are doing work to improve soybean germplasm availability throughout Africa, and one of the key institutions is the International Institute of Tropical Agriculture (IITA). Their efforts are solely for maintaining biodiversity in SSA and one of the ways they are doing so is by maintaining their Genetic Resources Center, or Genebank, where they house thousands of crop varieties that have been made available for research and disaster relief (IITA, 2019). Soybeans are one of the major crops stored at the Genebank, and efforts at the IITA have allowed for the development of varieties that are both rust resistant and high yielding, in addition to varieties with low pod shattering, soil deficiency tolerance, and resistance to frog-eye leaf spot, bacterial pustule, and bacterial blight (IITA, 2019). The IITA has many objectives for soybean improvement, including drought tolerance (Tefera, 2011). Seventeen released varieties have been developed through the IITA for various West and Central African countries (Lawrence, 2011).

The IITA might be the key group for improving and maintaining soybean germplasm in sub-Saharan Africa, but other organizations have made important contributions as well. The Soybean Innovation Lab has introduced elite germplasm from Brazil and the United States (Soybean Innovation Lab, 2014), and over 300 lines from the USDA were evaluated in Ethiopia (Denwar et al., 2016). It was found that many of the lines from the United States have poor adaptability due to low latitude, but some lines were good enough to cross with local adapted varieties (Denwar et al., 2016). Creating crosses between lines from the USDA and local adapted varieties allows for the introduction of new traits, such as non-shattering, higher yields, and earlier maturities (Denwar et al., 2016). Another project SIL contributed to is the expansion of an Integrated Breeding Platform to specifically support soybeans, including access to a database of over 33,000 germplasm accessions (Feed The Future, 2014).

Other organizations, such as the USDA Soybean Germplasm Collection, the Asian Vegetable Research and Development Center, and the Rural Development Administration of the Republic of Korea, India, Brazil, and Argentina helped introduce hundreds of new accessions to more than 25 SSA countries (Khojely et al., 2018). As more work is done with the integration of new germplasm, there is potential to use the germplasm for creating drought tolerant soybean lines, as has been done in the United States.

6.0 Recommendations

As has been discussed, there are numerous options for increasing drought tolerance in soybeans. Plant breeders can use conventional or molecular methods, depending upon the resources provided to their program. Conventional methods tend to take more time to complete but are less costly than transgenic approaches and some of the molecular approaches. Therefore, it is best to recommend a combination of methods, by utilizing molecular markers and MAS to speed up conventional breeding of drought tolerant soybean varieties.

Using MAS in a conventional breeding program is recommended because it would help decrease the time required to see results in a conventional program. At the same time, MAS is not as costly as a program that uses transgenes and requires the additional regulatory approvals. In terms of possibilities, molecular markers have a lot of potential for increasing drought tolerance, from the improvement of root architecture and nitrogen fixation under stress, to increasing water use efficiency.

Although utilizing molecular markers in a conventional breeding program creates many opportunities for improving drought tolerance in soybeans, it is by no means a quick solution. As discussed previously, it takes time for QTL and new genes to be identified and implemented in a soybean improvement program. There are many QTL that can be identified in search for ways to improve drought tolerance, but sometimes not enough QTL are discovered to account for all the variation in the trait. Therefore, it is also recommended that farmers alter some of their agronomic practices (by planting earlier or applying plant hormones) to avoid drought or increase the tolerance in soybeans.

7.0 Conclusions

Soybeans are an important crop globally, and there is a need to improve their tolerance to drought. Climate change is predicted to affect soybean production and lower their yields, especially in sub-Saharan Africa. Therefore, farmers and consumers would be impacted by the negative consequences of climate change and on soybean production, which is why it is essential that numerous approaches be taken to increase drought tolerance in soybeans.

For low-income countries conventional approaches should be considered before resorting to costly molecular methods. This includes essential changes such as planting earlier in the growing season to avoid the effects of drought during crucial times for soybean growth (such as during flowering). Conventional breeding methods using PI accessions are also important, because some PIs might contain traits that can be used to increase drought tolerance.

If resources are available, numerous advancements in technology can be applied. Accessions or other soybean lines can be screened to find key markers for traits related to improving drought tolerance. Soybeans can be genetically modified using transgenes from other species. With the approval of Verdeca’s transgenic soybeans, the door has been opened for more GMO soybeans to be approved.

In certain parts of the world, such as sub-Saharan Africa, there is a comparative lack of progress for drought tolerant crops. Establishing programs similar to those ran by the University of Illinois's Soybean Innovation Lab or the IITA can help provide some of the needed resources and training to increase the advancements made in these countries. Two of the essential ongoing interventions are the training of a new generation of plant breeders and increasing access to superior germplasm. These efforts could one day put sub-Saharan Africa on an equal level as other parts of the world in terms of soybean production.

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