DEVELOPMENT OF DROUGHT-RESILIENT SOYBEAN, A DEEP INSIGHT INTO THE POTENTIAL OF MOLECULAR RESEARCH AND BREEDING TOOLS

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Abstract. Abiotic stresses continuously threaten agricultural crop growth and yield and add risk to food security. Drought stress has significantly reduced the growth and yield of soybean. Soybean is an important crop that offers several nutritional benefits to humans as well as livestock. Breeding of soybean resilient to drought stress is one of the main objectives of soybean breeders to sustain their growth in a water-deficit environment. Genomic regions play a key role in regulating drought tolerance in soybean. Several molecular research and breeding techniques have been used to explore the drought tolerance mechanism in soybean; however, the complexity of the drought tolerance mechanism hindered the large-scale use of breeding tools. Quantitative trait loci (QTL) mapping, genome-wide-association-studies (GWAS), transcriptomes, transcription factors (TFs) analysis, transgenic breeding, and clustered regularly interspaced short palindromic repeats (CRISPR) are being used to identify and transfer the genes regulating drought tolerance in soybean. A lot of QTLs have been used in QTL pyramiding to improve drought tolerance in soybean. GWAS has successfully enhanced the genetic improvement of soybean in response to drought stress. It is important to decipher the genetic control of drought tolerance by recognizing the genes expressed under drought stress using transcriptome and TFs analysis and exploiting these genes *via* genetic engineering and CRISPR/Cas9. The development of drought-tolerant soybean genotypes will

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sustain growth on water-deficit soils and will serve as a reference point for adopting these strategies in other crops. This review will serve as a rich source of information for research breeding that intends to enhance drought tolerance in soybean.

Keyboards: *soybean, drought, yield, molecular tools, genetic breeding*

Introduction

One of the main challenges of the 21st century is to supply food and fuel to the expanding population living under a changing climate. The world population is expected to increase to 10 billion by 2050 (Kaya et al., 2024). The unprecedented increase in population poses a challenge to global food security because more people will need food, fuel, and other resources. Currently, The agricultural sector cannot meet these needs as it faces water scarcity, climate changes, land degradation, loss of biodiversity, and disease outbreaks (Kaya et al., 2024). Soybean is one of the most important crops grown on 125 million hectares worldwide (FAOSTAT, 2023). Soybean provides oil and protein to humans as well as livestock. The increase in rising temperature has severely affected soybean growth and production, as a 17% loss in yield was reported for every 1% increase in temperature (Katam et al., 2020; Vollmann and Škrabišová, 2023). Drought stress affects crops production and causes food insecurity in many countries (*Figure 1*). Drought stress is one of the leading abiotic stresses that affect soybean growth and yield on large areas (Rasheed et al., 2022). Low rainfall, heat stress, low temperature, and salinity are the main reasons for water scarcity in plants. This drought stress type is often called physiological drought (Ashraf and Ozturk, 2008; Lisar et al., 2012; Arbona et al., 2013).

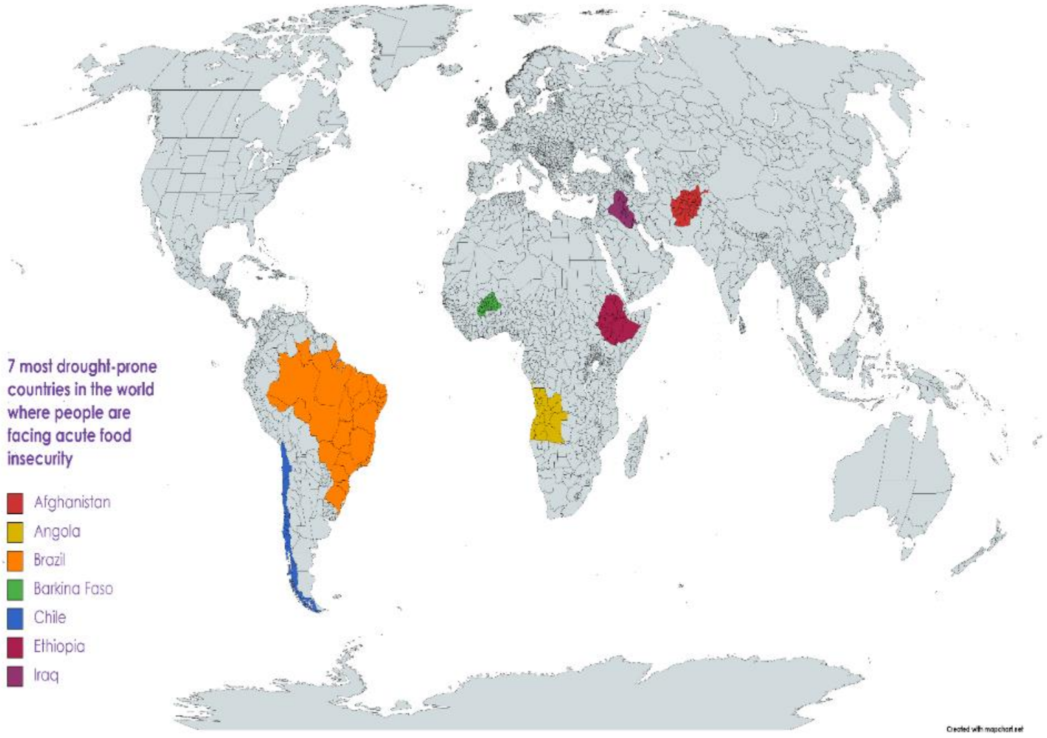

Figure 1. *This map shows the world's most drought-prone countries, where people face serious challenges due to water and food shortages. This map was made with https://www.mapchart.net/world-subdivisions.html*

Drought stress is often associated with high temperatures, as heat stress causes water deficit conditions (Wijewardana et al., 2019; Du et al., 2020). It is critical to understand that drought stress is a serious threat to crops in arid and semi-arid areas of the world (Templer et al., 2017). Many studies showed that drought stress causes deleterious damage to soybeans' yield, harvest index, and seed quality (Cohen et al., 2021). Plants have adopted a lot of defense mechanisms to counter the effects of drought stress. These strategies include stomatal closure, water absorption from deeper soil, and a thick waxy cuticle layer (Sagar et al., 2022; Zahra et al., 2023). One of the main effects of drought stress is the production of reactive oxygen species (ROS), which cause the retardation of growth and yield (Mittler et al., 2022). To counter the ROS, plants used the antioxidant enzymes peroxidase (POD) and ascorbate peroxidase (APX) (Khan et al., 2022). Drought stress reduced seed germination, root length, and shoot length (*Table 1, Figure 2*), as studied by Begum et al. (2022). Drought stress also decreases soybean cultivars' photosynthesis rate and water use efficiency (Zhu et al., 2024). Reduction in water content, pollen germination, proline content, and antioxidant enzyme activity are often lined with an increase in drought stress (Kamrava et al., 2017; Du et al., 2020; Fatema et al., 2023; Poudel et al., 2023).

Table 1. *Effects of drought stress on soybean*

Drought stress treatment	Effects	References
5, 10 and 15% PEG-6000	Drought stress reduced seed germination, root length, and root and shoot total weight.	(Begum et al., 2022)
Severe drought stress (30% moisture content)	Reduced water use efficiency and photosynthesis	(Zhu et al., 2024)
Moderate drought stress) 40% field capacity)	Reduced nodulation, plant growth, and nitrogen fixation	(Lumactud et al., 2023)
Moderate drought stress) 50% field capacity)	Reduced pollen germination, stomatal conductance, and seed number	(Poudel et al., 2023)
Drought stress with 40% field capacity	Reduced water content and dry matter production	(Fatema et al., 2023)
Drought stress with 60, 45, and 30% field capacity	Drought stress reduced the content of organic compounds and damaged the membrane.	(Miranda et al., 2023)
Severe drought stress with 30% moisture content	Drought stress reduced antioxidant activity and nitrogen metabolism in soybeans.	(Du et al., 2020)
5, 10 and 20% PEG-6000	Drought stress reduced the gibberellin (GA) and abscisic acid (ABA), salicylic acid (SA) content in soybean.	(Zhou et al., 2022)

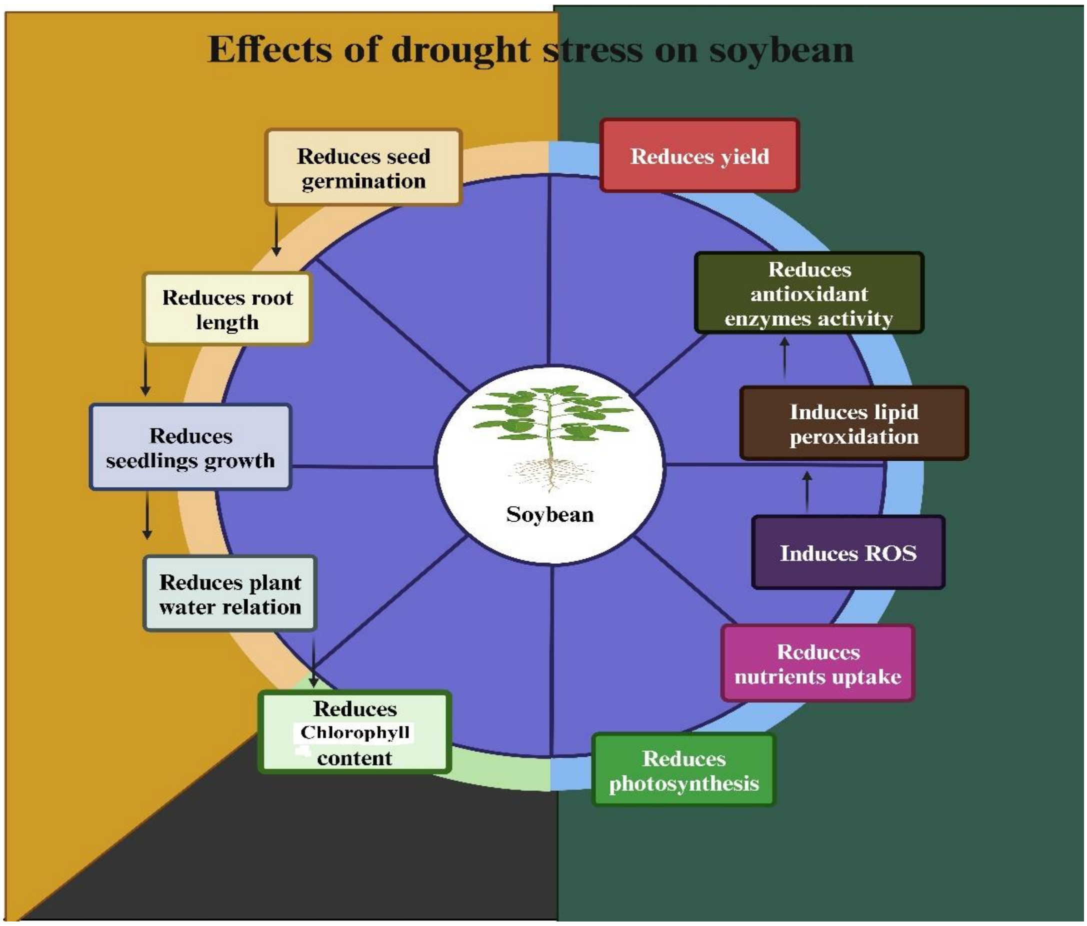

Figure 2. *Drought stress affects seed germination, reduces seedlings' growth, and reduces root length and chlorophyll content. It also reduces the activities of antioxidant enzymes, transpiration rate, and stomatal conductance*

Breeders have been using conventional breeding tools to identify and breed drought-tolerant soybean varieties for decades but they have several limitations. Screening of drought-tolerant soybean seedling in PEG treatment is highly useful. Another screening method is field experiment where soybean plants subjected to moisture deficit conditions and morphological and phycological indications are studies to evaluate degree of drought tolerance (Kim et al., 2001; Kosturkova et al., 2014). Genetic improvement of soybean cultivars with desired traits is the key to solve the above-mentioned challenges (Jiang et al., 2023). With time, research on the molecular regulation of drought tolerance gained more attention, and significant results have been published, as mentioned in a review published by Arya et al. (2021). It is significant to breed the drought-tolerant soybean varieties by identifying the novel genes. Research studies showed that the most significant defense mechanism is the activation of stress-responsive genes.

For this reason, using molecular breeding tools is critical to speed up drought breeding programs (Jiang et al., 2023; Savitri, 2023). Earlier studies proved that the application of molecular breeding tools accelerated the expansion of drought-tolerant soybean. This happens *via* the identification and transfer of tolerant genes. Dozens of drought-resilient genotypes were developed by using the above-mentioned molecular tools (Liu et al., 2020, 2023; Chen et al., 2023; Rahman et al., 2023). QTL mapping, GWAS, transcriptome analysis, TFs analysis, transgenic breeding, and CRISPR/Cas9 have been proven very effective in improving soybean against drought stress (Chen et al., 2013; Fuganti-Pagliarini et al., 2017; Song et al., 2020; Steketee et al., 2020; Saleem, 2022; Liu et al., 2023). The complex nature of drought tolerance mechanism delayed the large-scale use of these breeding methods. There are many review articles on this aspect, however, the latest and updated piece of information needs to be included. This review article has compiled the recent advancements in developing drought-tolerant soybean through molecular breeding tools. We have highlighted some gaps in previous research studies that can be considered in future studies. This comprehensive review paper can help future breeders recognize the mechanism of drought tolerance and implement a more reliable breeding technique to ensure the genetic improvement of soybean.

QTL mapping for drought tolerance in soybean

QTL mapping is one of the most powerful techniques to identify the potential genomic regions controlling abiotic stress tolerance in crops (*Figure 3*) (Dash and Mishra, 2024). Soybean breeders are actively involved in the improvement of drought tolerance in soybean. Previously, a lot of studies have identified the drought-tolerant QTL in soybeans. Li et al. (2023b) identified *qSW2* controlling leaf wilting trait in soybean, which showed that leaf wilting is an important indicator of drought stress and can be chosen as an effective stress tolerance index (Li et al., 2023b).

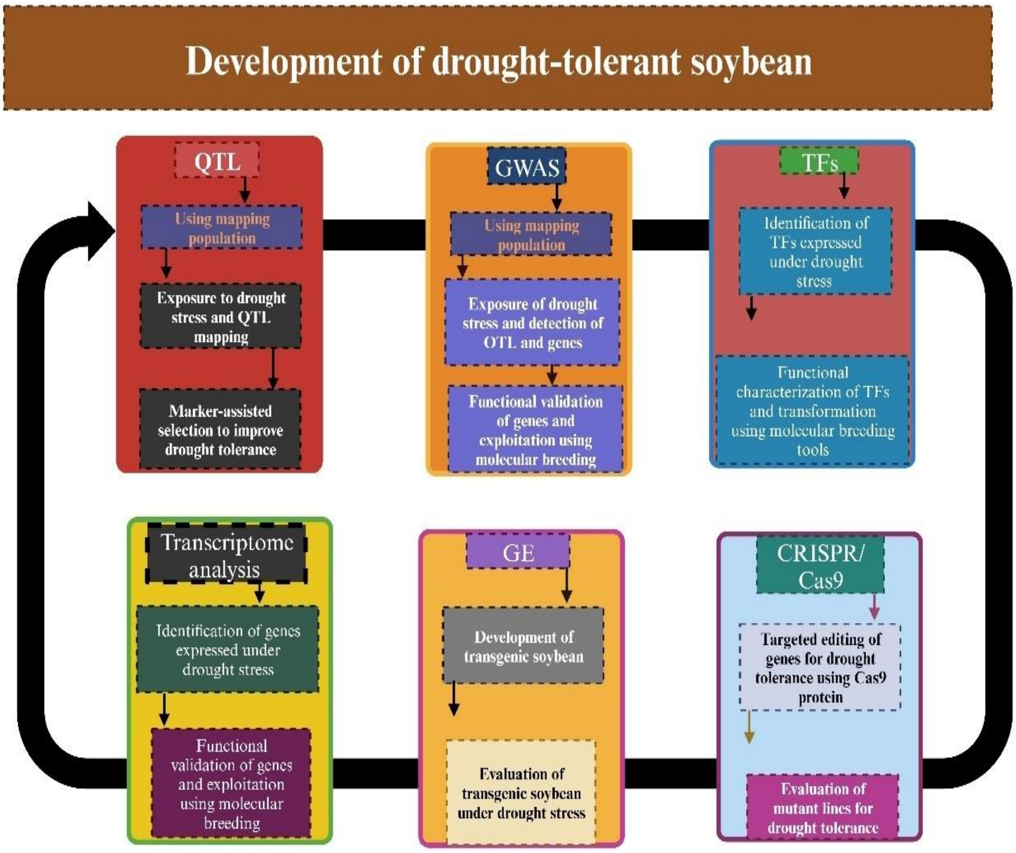

Figure 3. *A schematic way of breeding drought-resilient soybeans includes QTL mapping, GWAS, transcriptomes, TFs analysis, GE, and CRISPR/Cas9. These research and breeding tools have proven effective in identifying, transforming, and editing potential soybean genomic regions*

Improving drought tolerance traits in soybeans can increase the yield and stability of soybeans. In an earlier study, 23 QTLs linked with drought tolerance were identified; among them, seven QTLs were related to plant height. Two pairs of QTL, *qPH2*/*qSWPP2* and *qPH17*/*qSWPP17*, were common for both plant height and seed weight, which indicated that these QTL are closely linked with drought tolerance and can be used to accelerate drought-tolerance breeding (Ren et al., 2020). The choice of mapping population is extremely important in soybeans. The recombinant inbred lines (RIL) population is one of soybeans' most commonly used populations. Dhungana et al. (2021) used the RIL population to detect the QTL linked with drought tolerance in soybeans. A total of 10 QTL were detected on seven chromosomes. Two QTLs, *qWDC2-1* and *qWDC7-1*, were mapped on chromosome 1, linked with drought, and considered stable for drought tolerance (Dhungana et al., 2021). In another study, QTL, which affected drought tolerance, was identified in the RIL population of soybeans. The RIL population was derived from a cross of a tolerant cultivar, Jindou21, and the sensitive cultivar, Zhongdou33. Five QTLs were detected on five chromosomes. One major QTL, *qNNMS-W-16*, was detected under well-watered and stress conditions and considered a key QTL and contributed 17.18% and 15.24% of phenotypic variation (Ouyang et al., 2022).

Identifying QTL at the seedling stage can improve soybeans' drought tolerance. The seedling stage gives an accurate estimation of drought tolerance. Wang et al. (2012) evaluated the soybean under polyethylene (PEG) and well-watered conditions to identify the QTL related to drought tolerance in soybean at the seedling stage. A total of 17 QTLs were detected at the germination stage and were linked with drought tolerance. Four closely linked QTLs were selected as key QTLs associated with drought tolerance in soybeans. *qGR-E-1* and *qGI-A2-1*, representing germination rate and germination index, were tightly linked QTL and can be used for functional characterization in further studies (Wang et al., 2012). Physiological traits also play a key role in drought tolerance in soybeans. Identifying QTL and genetic improvement of physiological traits is key to countering the rising threats of drought stress in soybeans. Hence, drought-tolerant physiological traits can contribute to yield stability in soybeans. Yang et al. (2014) evaluated two soybean cultivars, one drought-tolerant 'SNWS0048' and the sensitive cultivar 'Jinda73', to identify the QTL for physiological traits linked with drought tolerance. Different pairs of QTL were identified, and *qRWC-M-2* and *qCSS-G-5*, representing relative water content and content of soluble sugar, can be further studied for their role in drought tolerance (Yang et al., 2014). Yield is the outcome of any crop, and in soybeans, drought stress significantly reduces the yield and quality of soybeans. Identification of yield QTL in stress and well-watered conditions in soybeans is critical to identifying the genetic regions for this key trait. In order to shed light on this aspect, Lee et al. (2021) evaluated 160 F_4-derived lines of soybean developed by crossing (Hutcheson’ × PI 471938) under drought and well-watered conditions. Two significant QTL, *qIR-YLD-13* and *qRF-YLD-13* (*Table 2*), were identified under irrigation and rainfed conditions, which indicated the differential responses of soybeans under rainfed and well-watered conditions (Lee et al., 2021).

Vu et al. (2015) applied Diversity Array technology (DArT) and soybean DArT libraries to identify the QTL related to drought tolerance in soybeans. Several QTLs identified in this study had a small effect. However, DArT technology enables the simultaneous genotypes of soybean lines, and it can help to manipulate the QTL to increase their cumulative effect (Vu et al., 2015). The above studies showed that QTL mapping is an important technique for identifying the key genomic regions for drought tolerance in soybeans. It is important to conduct more studies to identify the key QTL related to biochemical traits contributing to drought tolerance in soybeans, as earlier studies showed that data is insufficient.

Table 2. *QTLs associated with different traits for drought-tolerance in soybean*

QTL	Trait	Reference
qSW2	Leaf slow wilting	(Li et al., 2023b)
qPH2/qSWPP2 and *qPH17/qSWPP17*	Plant height and seed weight	(Ren et al., 2020)
qWDC2-1 qWDC7-1	Weighted drought coefficient	(Dhungana et al., 2021)
qGR-E-1, *qGI-A2-1*	Germination rate and germination index	(Wang et al., 2012)
qRWC-M-2 and qCSS-G-5	RWC, CSS	(Yang et al., 2014)
qNNMS-W-16	Node number of the main stem	(Ouyang et al., 2022)
qIR-YLD-13, *qRF-YLD-13*	Yield in irrigated and yield in rainfed conditions	(Lee et al., 2021)
qSW-Gm02, *qSW-Gm19*	Slow wilting, canopy wilting	(Abdel-Haleem et al., 2012)

GWAS-based identification of QTL and genes in soybean

GWAS is one of the most powerful tools for detecting the genomic regions controlling drought tolerance in soybeans. Earlier studies identified a lot of QTL and genes linked with drought tolerance in soybeans. However, the molecular mechanism of these traits is still not fully understood. In an earlier study, Liu et al. (2020) studied a diverse panel of 259 Chinese soybean cultivars to investigate drought tolerance at the germination stage. They have identified the 15 SNPs associated with the drought tolerance index. Results indicated that two of these SNPs were positioned upstream of genes, and 11 of them were linked to regions where previously identified QTL were reported. Five of them were drought-tolerant regions (Liu et al., 2020). Likewise, another nested association mapping (NAM) population comprising 403 lines of soybean was tested for drought tolerance using PEG treatment. GWAS has identified 73 and 38 QTL with 174 and 88 alleles. Overall, 134 genes were annotated and involved in nine biological functions. These results provided a new way of molecular breeding for drought tolerance in soybeans (Khan et al., 2018). Different types of mapping populations were used to identify the QTL/genes in soybeans. Mapping populations are the ideal source for dissecting molecular regions governing drought tolerance. Khan et al. (2019) used a half-sib soybean population of 404 lines and tested for drought tolerance under sand culture. GWAS identified 46 novel QTL with 107 alleles representing relative plant length (RPL) and relative plant dry weight (RPDW). Sixty-three genes were annotated for their possible role in drought tolerance and endorsing the genetic complexity of drought tolerance in soybeans (Khan et al., 2019). Yield stability is used as an indicator of drought response in soybeans. However, only a few studies deal with this aspect. Elite germplasm of a locally adapted breeding population was used to determine a yield stability index as an indicator of drought tolerance. Two groups of genotypes showed stable yield under drought stress. Seven QTLs were detected, and their implication on drought tolerance was further studied. Their validation was done by comparing them with previously reported QTL. Exploiting these QTL using marker-assisted selection may help develop drought-tolerant soybean cultivars (Quero et al., 2021).

Recently, GWAS was applied to a soybean germplasm collection of 359 accessions to detect the QTL and genes for short- and long-term drought stress. 17 and 22 significant marker trait associations were detected for four traits under short-term and long-term drought stress. A total of 12 and 16 genes were identified as involved in root formation, stomatal movement, ABA signaling, and cellular repair mechanisms. Further analysis showed that some genomic regions were previously co-localized with QTL for chlorophyll content and photosynthesis. QTL related to water usage efficiency contributed to the growth rate and maintenance of canopy height, and these traits can be used as drought tolerance index in future studies (Saleem et al., 2022). Evaluation of many soybean genotypes under drought stress conditions can be highly useful to identify the best-performing genotypes. Chen et al. (2020) evaluated 136 soybean genotypes for drought tolerance and identified 26 excellent genotypes regarded as drought-stress tolerant. GWAS has identified 422 SNPs and 302 genes for traits like number of seeds per plant, number of seeds per pod, stem diameter (SD), etc. (Chen et al., 2020). The evaluation of drought tolerance at the seedling stage is critical in identifying novel genomic regions. The number of vigor seedlings can affect growth and yield. A mapping population of 234 $F_{6:10}$ lines was used to identify the genomic regions at the seedlings stage. GWAS identified 53 QTL on 19 soybean chromosomes, which included 69 significant SNPs. Two of these QTLs, *qPH7-4* and *qPH7-6*, are linked with PH and can be further validated. A novel gene, *Glyma.07g094200* (*Table 3*) linked with drought stress was reported by association mapping. These results provided ideal genetic material for molecular cloning and marker-assisted selection of drought-tolerant genes in soybeans at the seedling stage (Zhang et al., 2022).

Table 3. *GWAS-based identified QTL and genes for drought tolerance in soybean*

Population	Total QTL/genes	References
NAM) population comprising 403 lines	73 and 38 QTL, 134 genes and 55,936 SNPs	(Khan et al., 2018)
403 soybean lines	46 novel QTL with 107 alleles, 63 genes and 55, 936 SNPs	(Khan et al., 2019)
189 genotypes and five commercial genotypes as check	7 QTL	(Quero et al., 2021)
359 soybean accessions	17 and 22 significant marker-traits association, 12 and 16 genes	(Saleem et al., 2022)
136 soybean genotypes	422 SNPs and 302 genes	(Chen et al., 2020)
234 $F_{6:10}$ lines	53 QTL, *qPH7-4* and *qPH7-6*, *Glyma.07g094200*	(Zhang et al., 2022)
162 diverse soybean lines	45 SNPs associated with 44 loci	(Steketee et al., 2020)
188 soybean germplasm	*qPN-DS8.8*, and *qBM-DS17.4*	(Li et al., 2023a)
149 accessions	19 QTLs were identified, and 8 and 10 QTLs were associated with yield per plant under well-watered and drought-stress conditions. SNP ss246509299 linked with canopy wilting	(Liu et al., 2023)

Canopy wilting leads to the improvement of drought tolerance in soybeans. A group of 162 diverse soybean lines was evaluated using slow canopy wilting as a drought tolerance indicator. GWAS has identified 45 SNPs associated with 44 loci for slow canopy wilting. Several new genotypes were identified, which showed superior slow canopy wilting compared to their checks. Slow canopy wilting can be incorporated into elite germplasm to improve drought tolerance (Steketee et al., 2020). QTL/genes linked with yield and yield-related traits are critical to improving drought tolerance in soybeans. As shown by GWAS mapping, two QTLs, *qPN-DS8.8* and *qBM-DS17.4*, were involved in controlling pod number per plant (PN) and biomass per plant (BM) under drought stress conditions. Further exploitation of these QTLs will help to improve the yield under drought-stress conditions (Li et al., 2023a). The above studies showed that a significant number of genomic regions contributed to drought tolerance in soybeans. More studies should be conducted to identify the QTL/genes linked with biochemical traits contributing to drought tolerance in soybeans.

Transcriptome analysis for drought tolerance in soybean

Omics technologies like transcriptome, proteome, and metabolome have increased our understanding of the genetic mechanism of plant response to abiotic and biotic stresses. RNA sequencing has become one of the most useful strategies for analyzing the interactions between plants and abiotic stresses (Zhang and Song, 2017). Xuan et al. (2022) conducted a comparative transcriptome analysis of two soybean genotypes, drought tolerant (Jindou 21) and drought-sensitive (Tianlong No.1). Transcriptome analysis identified 6038 and 4112 genes in both cultivars. *MAPKs* and *MAP3Ks* of calcium and MAPK signaling pathway (*Table 4*) were recognized in Jindou. Jindou was more tolerant to drought because more genes were expressed and involved in single transduction pathways (Xuan et al., 2022). In another study, transcriptome analysis for canopy wilting was conducted using two contrasting soybean lines. A total of 1940, and 670 genes were identified in the drought-tolerant line (PI 567690) and drought-susceptible line (Pana). SNP variation was observed in aquaporin genes in PI 567690, which are known in some slower canopy wilting lines and can accelerate marker-assisted selection for drought tolerance (Prince et al., 2015). The drought tolerance mechanism during seed development in soybeans is critical for better understanding of genomic regions and their regulatory pathways. Tang et al. (2023) elucidated the molecular mechanism of drought tolerance at seed development stages in soybeans under control and stress conditions. Gene ontology analysis identified genes for drought tolerance at every stage of seed development; however, there were few genes involved in drought tolerance at the primary stage of seed development. These genes were linked with metabolic and other regulatory networks. Hence, seed development stages can be further studied for their role in drought response (Tang et al., 2023).

Integrated transcriptome and metabolome analysis have identified several key genes responsible for the biosynthesis of metabolites involved in drought tolerance in soybeans, mainly in wild soybeans. Wild soybeans can tolerate harsh conditions and can be used as a source of tolerant genes. In an earlier study, photosynthetic physiology and ion physiology of wild soybean was studied. Changes in transcriptome and metabolomes in the leaves of two wild soybeans were analyzed. Results showed a greater root/shoot ratio and efficiency in tolerant soybean. Integrated analysis revealed that the tolerant genotype showed lower hydrogen cyanide (HCN) production to avoid drought stress. The accumulation of GABA is involved in the maintenance of nitrogen balance and the metabolism of amino acids (Zhang et al., 2023). Earlier studies showed that abscisic acid (ABA) played a key in plant response to drought stress. The enrichment of ABA-responsive pathways in soybeans greatly contributed to drought tolerance. ABA-enriched pathways identified in the roots of soybean cultivars were involved in osmoregulation, cross-talk of ABA with ethylene, and sugar biosynthesis (Molinari et al., 2023). The key objective of soybean breeders is to enhance root growth under water-stress conditions. In a recent study, transcriptome analysis of RNA-sequencing data was performed to identify the genes linked with root growth under water deficit conditions. *GmNAC19* and *GmGRAB1* in roots enhanced stress tolerance in transgenic plants. These genes displayed distinct root preferential expression (Mazarei et al., 2023). There are dozens of studies on drought-responsive transcriptomes in soybeans. However, this review highlights only some key findings. These results would be highly useful in understanding the molecular response of soybean to drought stress levels.

Table 4. *Stress-responsive genes identified by transcriptome analysis in soybean*

Genes/metabolites/pathways	Function	References
GmNAC19, *GmGRAB1*	*GmNAC19* enhanced drought tolerance in transgenic plants	(Mazarei et al., 2023)
MAPKs, and *MAP3Ks* of calcium and MAPK signaling pathway	Single transduction pathways	(Xuan et al., 2022)
1940 and 670 genes	SNP variation in aquaporin genes of drought tolerant line that are conserved in known slower canopy wilting lines	(Prince et al., 2015)
1458 and 1818 upregulated and 1582 and 1688 downregulated genes	Downregulation of photosynthesis genes involved in growth retardation, which might be an adaptive strategy for plants survival	(Le et al., 2012)
100 DEGs of all stages of seed development (20 for each)	81 out of 100 showed functional description, and many of them were drought-responsive genes. These include heat shock proteins, proline-rich proteins, etc.	(Tang et al., 2023)
HCN	Lower production of hydrogen cyanide (HCN) to avoid drought stress	(Zhang et al., 2023)
ABA-responsive transcriptome	Osmoregulation, cross-talk of ABA with ethylene and sugar biosynthesis	(Molinari et al., 2023)

TFs analysis for drought tolerance in soybean

TFs are key in increasing plant growth and response to environmental stresses. Earlier studies briefly described the role of TFs in increasing drought tolerance in soybeans. A detailed and updated review of the role of TFs needs to be included. TFs have been categorized into various families, and each family has several TFs with unique roles in abiotic stress tolerance. Earlier, Wang et al. (2017) identified a MYB TF, *GmMYB84*, which had a key role in soybean response to drought stress. The role of MYB TFs in soybeans still needs to be fully understood. Drought stress increased the expression of *GmMYB84*, and mutant lines showed higher survival rates, higher proline content, and increased activities of antioxidant enzymes compared to wild type. Results showed that these TFs, *GmMYB84*, along with antioxidant enzymes, promoted root growth under drought stress in soybean (Wang et al., 2017). Another novel, MYB, TF, *GmMYBJ1*, was isolated from the soybean genome. The expression of *GmMYBJ1* was induced by drought stress, which increased drought tolerance in soybeans (Su et al., 2014).

In the same way, TFs analysis showed different genes involved in drought tolerance in soyeban. Phylogenetic analysis showed that nac, myb, and bzip genes were closely related with nac2, myb78, and bzip48 in soybean, and their expression was enhanced under drought stress (Pereira et al., 2011). WRKY is one of the most significant families of TFs and plays a key role in drought tolerance in soybeans. A WRKY TF, *GmWRKY12* (*Table 5*), was identified in soybean *via* RNA-seq analysis, and its expression was induced by drought stress. Increased expression of *GmWRKY12* enhanced drought tolerance by increasing proline content and reducing malondialdehyde in transgenic soybeans (Shi et al., 2018). In soybeans, TGA, a subgroup of the bZIP family, plays a key role in various biological processes and responses to abiotic stresses. A TGA TF, *GmTGA17*, was identified in soybeans, and its expression was induced by drought stress. *GmTGA17* increased chlorophyll content and proline content under drought-stress conditions. Hence, TGA TFs can be further characterized for their role in drought tolerance (Li et al., 2019).

Table 5. *Role of various TFs in drought tolerance in soybean*

TFs	Role	References
GmZF351	Regulated the expression of *GmCIPK9* and *GmSnRK* genes and led to the closure of stomata	(Wei et al., 2023b)
GmbZIP15	Negatively regulated drought tolerance	(Zhang et al., 2020b)
GmDof41	*GmDof41* increased drought stress by interacting with other *GmDREB1B;1* and *GmDREB2A* genes	(Wei et al., 2023a)
GmEF4	*GmEF4* enhanced biomass, root growth, and delayed wilting in soybean	(Gao et al., 2019)
GmTGA17	Increased chlorophyll content and proline content	(Li et al., 2019)
GmMYB84	Promoted root growth under drought stress	(Wang et al., 2017)
nac2, myb78, bzip48	Higher expression of these genes increased drought tolerance by promoting root growth.	(Pereira et al., 2011)
GmWRKY12	Increased proline content under drought stress in soybean	(Shi et al., 2018)
GmMYBJ1	Increased drought tolerance in soybean	(Su et al., 2014)
GmDREB1B;1	Increased *GmPYL21* expression under drought stress	(Kidokoro et al., 2015)
GmDREB3	Increased drought tolerance in soybean	(Chen et al., 2009)

The DREB family plays a key role in soybean growth and response to abiotic stresses. Kidokoro et al. (2015) identified several DREBI genes, and their expression was induced by drought stress. Results showed that *GmDREB1B;1* increased *GmPYL21* expression under drought stress conditions (Kidokoro et al., 2015). Another study showed that A DREB TF, *GmDREB3* isolated from the soybean genome enhanced drought tolerance in soybean and *Arabidopsis*, which showed the potential role of the DREB TF family (Chen et al., 2009). The calmodulin transcription activators (*CAMTAs*) regulate growth, development, and response to abiotic stresses in soybean. In order to investigate the role of *CAMTA* family members in plant response to drought stress, the expression pattern of 15 *CAMTA* genes was characterized. Results showed that two genes, *GmCAMTA2* and *GmCAMTA8*, negatively regulated the growth and response to drought stress (Baek et al., 2023).

Although Dof TFs play a key role in plant growth and development, their molecular mechanism must be fully understood in many plants. Results showed that *GmDof41* increased drought stress by interacting with other genes *GmDREB1B;1* and *GmDREB2A*. Further studies are required to increase the understanding of molecular mechanism of these TFs and their role in drought tolerance. Detailed functional characterization of Dof TFs is critical for increasing soybean response to drought stress (Wei et al., 2023a). Zinc finger proteins (ZF) play a crucial role in soybean growth and development. However, further studies are still needed to analyze the function of several ZF members in drought tolerance in soybean. In a recent study, Wei et al. (2023b) identified the *GmZF351* gene, and its expression was induced by drought stress. Overexpression of this gene, *GmZF351*, enhanced tolerance in soybeans. The regulation of *GmCIPK9* and *GmSnRK* was increased by *GmZF351*, which led to stomatal closure during the water deficit period. Further manipulation of pathways encoded by these genes can be useful to increase tolerance to abiotic stress in soybean (Wei et al., 2023b).

bZIP is one of the most important families of TFs, and it plays a key role in addressing diverse environmental conditions. A potent bZIP TF, *GmbZIP15*, was characterized for its role in drought tolerance in soybeans. Results showed that *GmbZIP15* negatively regulated drought stress as its activity was suppressed under stress conditions. It is important to conduct more studies to characterize the other members of bZIP for their role in drought tolerance in soybeans (Zhang et al., 2020b). An earlier study indicated that elongation factors also play a key role in plant response to drought stress. Gao et al. (2019) identified and characterized a TF, *GmEF4*, in soybeans under drought-stress conditions. Results indicated that *GmEF4* enhanced biomass, root growth, and delayed wilting in soybeans. More in-depth analyses are needed to identify the pathways regulated by elongation factors under drought stress conditions (Gao et al., 2019). These results showed that TFs have a key role in plant response to abiotic stresses. However, more research studies are needed to fully characterize the potential members of all TF families and investigate their role against drought stress and other abiotic stresses. TF families like WRKY, ZF, and MAC are not fully characterized for their role in drought tolerance. The molecular mechanism of TFs still needs to be explored to develop potential genetic material for future drought-tolerant soybean breeding.

Transgenic breeding in soybean

Transgenic breeding plays a key role in the genetic improvement of soybeans to combat the rising threats of drought stress. Transgenic soybean lines have been successfully developed by targeting several genes/TFs, which showed higher performance under drought stress conditions (Wei et al., 2019). In the last decade, TFs have been extensively used to develop transgenic crops responsive to drought stress (Fang et al., 2024). An *Arabidopsis* TF, *AtDREB1D*, was transformed into soybean using the technique of *Agrobacterium*-mediated gene transfer. Transgenic lines were developed to confirm the integration of transgene. Under water deficit conditions, transgenic lines showed stable growth and utilized less water than wild type. Hence, the feasibility of genetically engineered soybeans is proved in the current experiment (Guttikonda et al., 2014). Shi et al. (2018) used a transgenic hairy root assay to transfer *GmWRKY12* in soybean to develop transgenic lines. Results showed that transgenic lines showed increased content of proline compared to wildtype (Shi et al., 2018). Another WRKY TF, *GmWRKY54*, enhanced drought tolerance in transgenic soybeans by regulating genes of ABA and calcium (Ca^{2+}) pathways. Further manipulation of this gene can increase yield in soybean and other legume crops (Wei et al., 2019).

A TGA TF, *GmTGA17*, was transferred to soybean to develop the transgenic lines, which increased chlorophyll content and proline content under drought stress and proved the effectiveness of genetic engineering (Li et al., 2019). In another study, a member of the MYB family, *GmMYBJ1* increased drought tolerance in transgenic lines of *Arabidopsis* (Su et al., 2014). Recently, Xu et al. (2023) developed a transgenic soybean line showing overexpression of *GmCOL1a*. Transgenic *35S: GmCOL1a* plants showed higher proline and water content in leaves. Further analysis showed that *GmCOL1a* promoted *GmP5CS* protein accumulation in soybeans (Xu et al., 2023). The process of poly (ADP-ribosyl) action is a necessary part of cellular processes, which indicates the tolerance to abiotic stresses. In a recent study, the role of soybean poly (ADP-ribosyl) polymerases, *GmPARPs*, was identified by using *Agrobacterium*-mediated transformation. *GmPARPs* transgenic lines were developed and confirmed by using molecular analyses. Physiological analyses of proline and chlorophyll content showed that transgenic liens had higher tolerance under drought stress. Downregulation of *GmPARPs* effectively improved drought tolerance in soybeans, and its knockdown can only protect soybean plants during drought and not under heat stress. *GmPARPs* can be a key target for the genetic improvement of soybeans, and further studies are needed to cover the gaps in the current study (Tiwari et al., 2023).

Besides genes and TFs mentioned above, emerging evidence showed that long non-coding RNAs (lncRNAs) play a crucial role in various biological and cellular processes. A lncRNA, *lncRNA77580*, was identified in soybean, and transgenic lines were generated, showing the overexpression of *lncRNA77580*. *lncRNA77580* modulated the transcription of several genes during soybean response to drought stress. *lncRNA77580* increased yield by enhancing the number of seeds per plant. Further studies are required to identify the additional pathways regulated by *lncRNA77580* in soybean (Chen et al., 2023). ABA-responsive element-binding (AREB) TF, *AtAREB1*, was used to develop genetically modified soybean lines for drought tolerance. The TF, *AtAREB1* enhanced water use efficiency and leaf area index in transgenic line 1Ea2939. These findings confirmed the importance of AREB TFs in the regulation of drought tolerance in soybeans (Fuganti-Pagliarini et al., 2017). There are some biosafety issues of GM crops. One of the key objections in using GM food is that many of these food items contain genes of resistance to an antibiotic existed in original DNA construction as selectable marker. These genes can pass to human endogenous microflora (pathogenic species) and confer resistance to this antibiotic (Komari et al., 1996). The importance of genetic engineering cannot be denied, but more efforts are needed to strengthen this technique as environmental conditions are changing, which may complicate breeding processes. It is important to do screening of all identified genes and TFs and identify their role in drought tolerance. Other crop genomes can also be targeted to accelerate transgenic breeding.

Application of CRISPR/Cas9 in drought-tolerant breeding of soybean

The use of CRISPR/Cas9 in the genetic improvement of crops is rapidly expanding (Rai et al., 2023). CRISPR/Cas9 is safe to use because it does not involve the introduction of any foreign gene into host cell. It involves targeted editing of genes which are already present in targeted crop genome and hence it’s safe to use this technique (Zhang et al., 2020a). This technique has been used to edit the drought-tolerant genes in soybeans; however, its use is still limited, and expansion of its use is mandatory (Zhong et al., 2022). Zhong et al. (2022) employed a CRISPR/Cas9 mediated gene editing tool to edit the TF, *GmHdz4* belongs to the HD-Zip transcription factors family. Soybean plants overexpressing *GmHdz4* in hairy roots were developed to investigate the role of *GmHdz4* in response to drought stress initiated by polyethylene glycol (PEG). Bioinformatics analysis indicated that *GmHdz4* was closely associated with other HD-Zip I family gens like *Oshox12* and *Gshdz4*. *Gmhdz4* enhanced the growth of above-ground parts and root length and increased antioxidant enzyme activity in mutants. It is concluded that *GmHdz4* can be further used in the enhancement of drought-tolerant breeding in soybeans (Zhong et al., 2022). In another study, NAC TF, *GmNAC8* was studied for its role in the regulation of drought tolerance in soybean. Yang et al. (2020) used CRISPR/Cas9 to generate *GmNAC8* overexpression and knockout lines to investigate the role of *GmNAC8* in drought tolerance. *GmNAC8* overexpressed lines showed higher proline content and antioxidant enzyme activities as compared to wild plants. Besides this, it also interacted with another regulatory protein, GmDi19-3, to regulate drought tolerance (Yang et al., 2020). Recently, Guo et al. (2023) explored the potential role of *GmOTSa* in drought tolerance in soybeans using CRISPR/Cas9. CRISPR/Cas9 edited the *GmOTSa*, and its expression was changed in mutant lines. Results showed that overexpression of *GmOTSa* in soybean lines enhanced the elimination of ROS, increased chlorophyll content, and promoted stomatal closure (Guo et al., 2023). These findings showed the potential of CRISPR/Cas9 mediated gene editing tool in improving drought tolerance in soybeans. Gene editing tools like base editing (BE) and prime editing (PE) should be employed to improve drought tolerance in soybeans. These techniques will accelerate the development of dough-tolerant breeding in soybeans. The application of CRISPR/Cas9 will save time and increase the efficiency of molecular breeding in soybeans and other crops. Overall, molecular contributed greatly to the development of drought-tolerant soybean genotypes. A list of drought-tolerant soybean genotypes is shown in *Table 6*. It is mandatory to note that the specific method used to develop these genotypes is not given here. These are overall best-performing genotypes regardless of the breeding method used to develop this material.

Table 6. *List of some drought-tolerant varieties*

Variety name	Performance	References
EC 538828	Showed drought avoidance mechanism, deep root system, higher chlorophyll content as compared to another sensitive genotype	(Jumrani and Bhatia, 2019)
JS 97−52	Deep root growth and high yield under drought stress	(Tripathi et al., 2023)
TMG1180 RR	Higher performance under drought stress related to increased amino acid content and proline content comparted to genotype AS3810IPRO	(Miranda et al., 2023)
JD36	Showed high tolerance to drought stress linked with super vegetative growth like shoot biomass and leaf area index (LAI)	(Yan et al., 2020)
S14	Showed high drought tolerance in term of yield compared to sensitive genotype, S58	(Chen et al., 2020)
heinong37	Higher survival rate under drought stress compared to sensitive genotype, heinong65	(Wang et al., 2022)

Conclusion

Drought is the most devastating abiotic stress, severely affecting agricultural crop growth and development. Global food security has been severely affected, increasing anger in many parts of the world. Oilseed crops are grown on a large part of agricultural lands and play a key role in human nutrition. Soybean is one of the most important crops for providing oil and protein. Drought stress reduces soybean growth and yields in large areas. For decades, soybean breeders have been trying to develop soybean tolerance to drought stress by using conventional breeding methods and achieved remarkable success in developing tolerant varieties. Soybeans counter drought stress using morphological, physiological, and biochemical defense mechanisms. Conventional breeding methods are laborious and time-consuming. The efficiency of crop improvement could be improved by using conventional breeding tools. Molecular breeding tools like genetic engineering and CRISPR/Cas9 have been successfully used in soybeans to fight against various environmental stresses. A lot of QTLs have been targeted by marker-assisted selection to develop drought tolerance in soybeans, and many of them still need to be characterized for their potential role against drought stress. It is, therefore, necessary to use the QTL in molecular breeding to counter the increasing pressures of water scarcity. In the same way, GWAS has been successfully used to map the novel genes and QTL regulating drought tolerance in soybeans. Some of these QTLs have been successfully used in soybean genotypes to enhance the tolerance to drought stress.

Transcriptome analysis has identified several genes expressed under various levels of drought stress. It is necessary to map the unknown genes and exploit them *via* molecular breeding to develop the genetically modified soybean genotypes. The limitations in transcriptome analysis should be overcome, and new gene mapping tools should be developed. Several families of TFs still need to be fully explored, and additional studies are needed to investigate their role in drought tolerance. The use of genetic engineering should be expanded, keeping in mind the biosafety issues. A newly developed gene editing technique, CRISPR/Cas9, is revolutionizing the field of agriculture by targeting the editing of specific genes of interest without any biological barrios. The above studies showed that CRISPR/Cas9 has great potential to reduce the risk of drought stress by editing the genes without any biosafety issues. It is, therefore, necessary to expand the application of CRISPR/Cas9 to target genes not only in soybeans but also in other crops to improve the genetic makeup of soybeans, which can enable them to thrive under harsh conditions of the environment. It would be more fruitful to ensure the integrated use of several tools like transcriptome, metabolome, proteome, and other molecular tools for the improvement of drought-tolerant breeding. With time, it is necessary to preserve the genetic diversity of soybeans using several molecular tools. Genetic diversity will serve as a treasure trove in future drought-tolerant breeding programs. Overall, in-depth research on drought tolerance mechanisms is critical to identifying the genes and their regulatory pathways involved in drought tolerance in soybeans. Additional studies are urgently needed, keeping in mind the problems mentioned above.

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