

## Genetic improvement of drought stress tolerance in maize, recent advancements and future research direction

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### Abstract

Maize is an imperative crop around the globe, and it provides several essential nutrients to humans and animals. Environmental changes seriously affect growth and productivity. Drought stress is one of the most important abiotic stresses, reducing maize growth and yield and threatening global food security. For decades, breeders have been trying to improve maize's ability to counter the toxic effects of drought stress. Drought tolerance is controlled by many genes and it complicates molecular breeding. The use of conventional breeding methods limited the development of drought tolerance in maize because of the complex nature of this trait. Hence, maize breeders have shifted their focus towards improvement of drought tolerance in maize at molecular level. Different molecular tools like quantitative trait loci (QTL) mapping, genome-wide-association-studies (GWAS), transcriptome analysis, transcription factor (TFs) analysis, and CRISPR/Cas9 have played a vital role in gene's identification and their use in molecular breeding. These genomic regions have been proven very effective, and more studies are being conducted to increase their efficiency; however, the improvement level is limited because of the complex genetic mechanism of drought tolerance. Different review articles have been published on this aspect; however, a comprehensive and updated overview of drought tolerance needs to be included. The current review highlights the role of diverse molecular techniques to improve drought tolerance in maize. This review article will enhance the interest of researchers working on the genetic improvement of maize.

**Keywords:** CRISPR/Cas9; drought; maize; QTL; transcriptome

### Introduction

Global warming resulted in human activities like the emission of greenhouse gases, increasing the temperature by 1.1 °C between 1850, 1900, 2011 and 2020 (Aliniaiefard *et al.*, 2023). Abiotic stresses are rapidly decreasing the crop's growth and yield in large areas and increasing the risk of food deficiency, which can affect food security (Gupta *et al.*, 2020; Kopecká *et al.*, 2023; Zhang *et al.*, 2023b; Bahadur *et al.*, 2024;

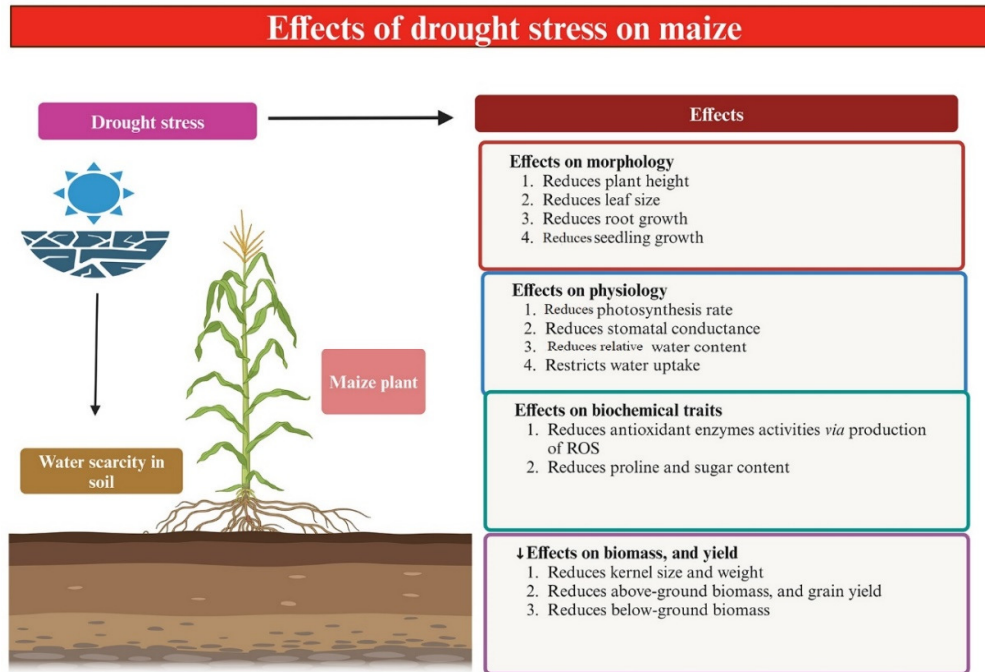
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Hostetler *et al.*, 2024). Maize is an important crop grown worldwide as it is a source of calories, particularly for people in developing nations (Hoisington *et al.*, 1999; Von Braun *et al.*, 2010; Maazou *et al.*, 2016). Maize is an important crop consumed as a staple food by more than 900 million people globally, and it is also used for biofuel (Adebayo and Menkir, 2015; Jiang *et al.*, 2017; Pei *et al.*, 2023). By 2050, the need for maize will be doubled due to the increasing population of human beings. Like other crops, maize is also exposed to various abiotic stresses throughout its life cycle (Mittler, 2006). Recent estimates showed that drought stress causes 15% to 20% yield loss in maize each year, and these losses will be more severe due to an increase in episodes of drought stress triggered by climate change (FAOSTAT, 2010). The problem of drought stress cannot be solved by irrigation because of the limited possibilities of expansion of irrigation systems in developing countries (Bank, 2005). Lack of water in the soil can affect maize growth and yield due to reduced seedling size (Nuccio *et al.*, 2015). In China, 60% of maize-grown areas are affected by seasonal or long-term drought, which may decrease yield by up to 30% (Gong *et al.*, 2015).

Prolonged water shortage conditions may reduce leaves size and promotes premature flowering, which can reduce yield potential (Campos *et al.*, 2004). Drought stress damages morphological, physiological and biochemical processes by affecting stomatal closure and cellular dehydration. Drought stress severely affects the photosynthesis of maize by reducing chlorophyll content (Khayatnezhad and Gholamin, 2012). Drought stress also reduces the activities of antioxidant enzymes and endogenous hormones and causes lipid peroxidation (Figure 1) (Gao *et al.*, 2018; Huang *et al.*, 2019). Drought stress reduced maize yield as reported by Khalili *et al.* (2013). Maize plants adopt several survival techniques, such as avoidance, escape, and tolerance mechanisms, to sustain their growth under water shortage conditions. The drought avoidance mechanism involves the maintenance of water status by absorbing more water from the soil. Drought escape involves completion of life cycle before start of drought conditions, and the drought tolerance mechanism involves the activation of genes/enzymes to tolerate drought stress. The plant also maintains turgor pressure *via* osmotic adjustment (Maiti and Satya, 2014; Gong *et al.*, 2015; Song *et al.*, 2016). Water management is a worthy task to ensure food security and zero hunger worldwide. Researchers have adopted many ways to counter drought stress; however, the complex genetic mechanism hindered the efficiency of scientific tools (Turan *et al.*, 2014; Zhou *et al.*, 2015). With the advancement in genetic research, plant breeders are identifying different genomic regions to ensure drought tolerance in maize (He *et al.*, 2023; Sarkar *et al.*, 2023). Molecular tools like QTL mapping and GWAS have successfully identified genomic regions (Ningning *et al.*, 2023; Sarkar *et al.*, 2023). Earlier studies showed that potential QTL increased the maize tolerance to drought (Sarkar *et al.*, 2023). In the same way, genetic regulation of maize tolerance to drought stress has been investigated using GWAS (Ningning *et al.*, 2023).

The use of TFs analysis has allowed to increase tolerance to drought stress in maize, and many genes have been reported to improve drought tolerance in maize (Ying *et al.*, 2012; Min *et al.*, 2016; Wang *et al.*, 2018a; Zheng *et al.*, 2020). CRSSIPR/Cas9 is a novel gene-editing tool that has been effectively employed to enhance drought tolerance in many crops, tomatoes (Wang *et al.*, 2017), soybean (Zhong *et al.*, 2022), rice (Liao *et al.*, 2019) and maize (Shi *et al.*, 2017). Despite these studies, the drought tolerance mechanism must be fully understood. Hence, an updated and more detailed review article dealing with the latest advancement in drought tolerance is critical. This review highlights the potential of diverse molecular techniques to improve drought tolerance in maize. This information will provide guidelines for readers on how to develop drought tolerance in maize.



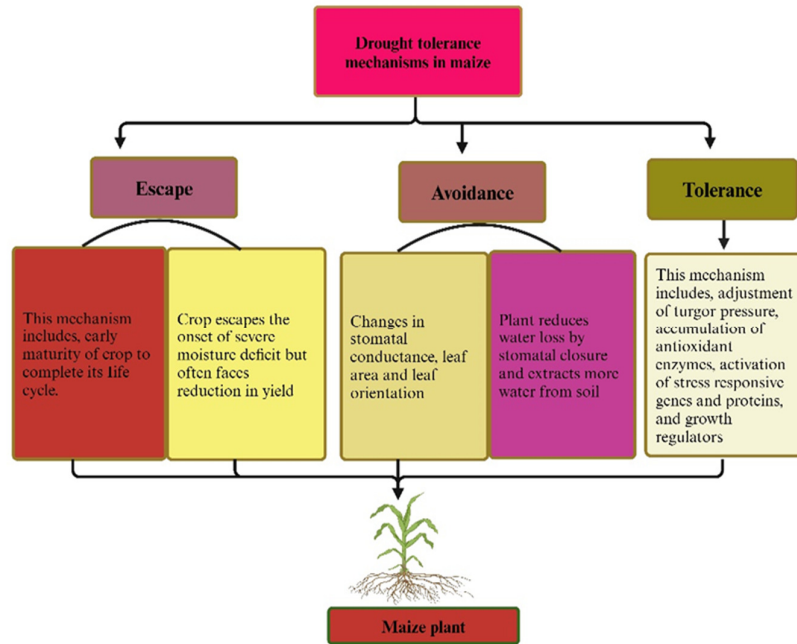
**Figure 1.** Drought stress affects plant height, leaf size, and seedling growth. It also reduces leaf water content and photosynthetic efficiency. Drought stress also reduces antioxidant enzyme activities and grain yield. This Figure was created with BioRender.com

### Drought tolerance mechanism in maize

The plant adopts diverse mechanisms like escape, avoidance and tolerance to counter drought stress (Arshad *et al.*, 2022; McMillen *et al.*, 2022). The drought escape mechanism involves the completion of life cycle before drought stress starts (Ribaut *et al.*, 2009). The drought avoidance mechanism involves decreased water loss by stomatal closure and more water uptake by an extensive root system. This shows that developing an extensive root system is a drought-avoidance strategy in many plants (Kavar *et al.*, 2008; Ribaut *et al.*, 2009). The drought tolerance mechanism involves the activation of genes and enzymes and the maintenance of turgor pressure. The plant accumulates various organic and inorganic substances (sugars and amino acids) to maintain the cell turgor potential. The plant also reduces oxidative stress by activating antioxidant enzymes (Figure 2) (Du *et al.*, 2004; Ribaut *et al.*, 2009).

These strategies have been developed in plants over time *via* natural or artificial selection. Plant response to drought stress depends on the plant stage at which drought stress occurs, and plant response varies among species (Arshad *et al.*, 2022). Plants' ability to withstand low water levels for extended periods is drought tolerance (Arshad *et al.*, 2022). Drought stress is a severe abiotic stress, and plants have a series of complex responses to counter its toxic effects. Drought causes a substantial reduction in nutrients uptake, which can cause a noticeable reduction in maize growth (Jurgens *et al.*, 1978). The abscisic acid (ABA) acts at this stage and often interacts with other growth hormones such as ethylene and also interacts with reactive oxygen species (ROS) (Kim and Lee, 2023; Ribaut *et al.*, 2009). Maize response to drought stress includes redirecting growth and shifting dry matter away from root and shoot (Hsiao and Xu, 2000). The capability of roots to extract more water from the soil profile under drought stress is one of the significant traits in drought tolerance (Robertson *et al.*, 1993). The active accumulation of valuable solutes in the cells is called the osmotic

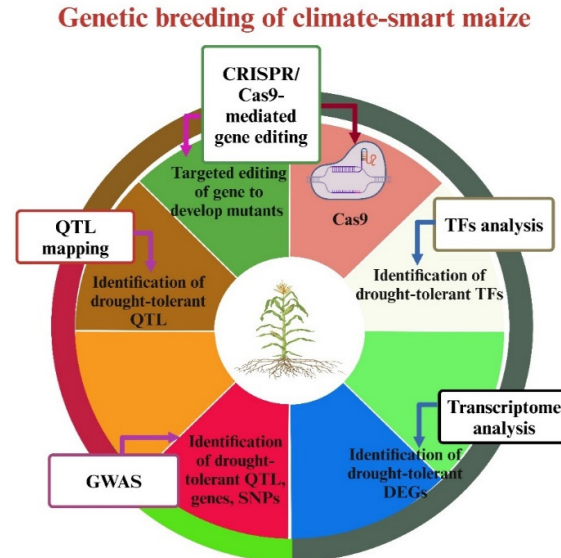
adjustment (OA). This enables the plants to minimize the loss of turgor pressure and damage, resulting in cell shrinking (Ribaut *et al.*, 2009). In physiological response to drought stress, plants also close stomata to reduce water losses, and it is an important technique used by plants to counter the toxic effects of drought stress. Plants also increase water use efficiency and photosynthetic rate to maintain growth under water shortage conditions. (Tardieu and Simonneau, 1998; Kim and Lee, 2023). Multidisciplinary techniques like breeding, genetics and physiology can help to understand the maize tolerance to water deficit conditions. (Maazou *et al.*, 2016). Despite all the efforts, it is still a challenging task for breeders. Thus, developing drought tolerant varieties is crucial to satisfy the hunger of the world population. More studies are needed to deeply understand the plant response to drought stress and adopt a more reliable way to enhance drought tolerance in maize.



**Figure 2.** Maize plants use three types of mechanisms under drought stress. Plants complete their lifecycle early to escape from the onset of drought. Secondly, plants prevent water loss by stomatal closure and uptake of water from the deep soil layer, called the avoidance mechanism. The third mechanism called the tolerance mechanism, includes activating genes, proteins, and antioxidant enzymes under drought stress. This Figure is created with BioRender.com.

### Genetic improvement of drought tolerance in maize

Plants have complex responses against drought stress which involves diverse genes and regulatory pathways (Manjunath *et al.*, 2024; Yang *et al.*, 2024). The employment of genetic manipulation techniques has enabled the development of tolerant maize cultivars. Molecular breeding involves genetically improving crop traits to survive in changing environments. An overview of molecular research and breeding tools is given in Figure 3.



**Figure 3.** The successful development of drought-tolerant maize depends on identifying potential stress-responsive genes and their use in molecular breeding *via* genetic engineering or CRISPR/Cas9

### QTLs mapping for maize tolerance to drought stress

Achieving higher yield and productivity of maize under drought stress is critical, so mapping genetic regions is crucial to accelerate genetic breeding. Recently, Sarkar *et al.* (2023) used a mapping population of recombinant inbred lines (RILs) under drought stress and normal conditions, and they identified diverse genomic regions linked with drought tolerance. They identified 27 diverse QTL for yield and morphological traits and out of these 27 QTL, 12 were mapped for drought tolerance while 13 were mapped for control conditions. Two significant and common QTL *qCW2-1* and *qCHI-1* (Table 1) were detected for cob weight and height under both water regimes (Sarkar *et al.*, 2023). Kaur *et al.* (2023) evaluated 135 F<sub>8</sub> RILs under well-watered and water-stress conditions. Results showed that 12 QTL were detected on chromosomes 1, 3, 4, 6, 7, and 9 and related to drought tolerance. The potential QTL was identified for the number of kernels per year. Thus, these QTLs must be used in future studies to analyze their role (Kaur *et al.*, 2023). QTL mapping is an essential tool used globally for the identification of genomic regions that can control drought tolerance in crops (Hu *et al.*, 2021; McMillen *et al.*, 2022; Sheoran *et al.*, 2022; Manjunath *et al.*, 2024). Many QTL mapping studies yielded significant results dealing with drought tolerance in maize (Hu *et al.*, 2021). An updated review of recently conducted studies dealing with QTL mapping is critical. Hao *et al.* (2010) identified a network of 239 genes under drought stress and 160 under normal water conditions. The finding can help to construct the QTL networking and decipher the drought tolerance in plants (Hao *et al.*, 2010). Likewise, Almeida *et al.* (2013) also identified 62 and 83 QTL for anthesis-silking interval (ASI) and grain yield of maize. In another meta-analysis, authors identified 7 QTL for kernel yield and 1 QTL for ASI (Almeida *et al.*, 2013). These QTLs can be used in molecular breeding of maize and lay the foundation for additional research studies focusing on unfolding the complexity of drought tolerance. In a recent study a double haploid (DH) population of maize consisting of 217 lines was evaluated to recognize the QTLs controlling maize tolerance to drought stress. A total of 18 QTLs were mapped; 9 were linked with well-watered conditions, and 9 were linked with water stress conditions (Hu *et al.*, 2021). Another study developed a group of DH inbred lines using parents, LPSC7F64 × CML495, to detect the drought-tolerant QTL. These DH inbred lines were evaluated under dry and irrigated environments. QTLs were detected for early vigour under drought

conditions. Several QTLs for yield were also identified, which can help improve yield under dry conditions (Vazquez-Pozos *et al.*, 2020). Grain yield is a primary interest in maize breeding for drought tolerance. In order to map the yield-related QTLs under drought stress, 116 F<sub>3</sub> families of DTP79 × B73 cross were grown in fields under drought and well-watered conditions. QTL mapping was done using composite interval mapping. Results showed that 5 QTLs were identified for grain yield per plant and 40 QTLs were identified for eight yield-related traits (Nikolić *et al.*, 2013). Rahman *et al.* (2011) identified 22 QTL for several drought-tolerance traits. Three potential QTLs were detected for grain yield on chromosomes 1, 5 and 9 (Rahman *et al.*, 2011). These findings revealed the importance of QTLs for grain yield and their possible breeding applications. In any drought-breeding program, grain yield should be a primary objective of maize breeders (Rahman *et al.*, 2011). In a recent study, Wu *et al.* (2021) evaluated the population of 368 maize genotypes with or without drought stress for 98 days and identified 1529 QTLs that were aligned with previously reported QTLs for drought tolerance. These findings demonstrated the value of genomic regions in molecular breeding in future studies (Wu *et al.*, 2021). Phenotyping of a large set of maize populations reveals significant differences among genotypes and helps to choose a suitable breeding approach (Wu *et al.*, 2021). Flowering time is very critical under drought stress and recently, QTLs were identified in maize-teosinte population under both normal and drought stress conditions. 16 QTLs were identified for three flowering-time traits namely days to anthesis, days to silk, and anthesis-silking interval (ASI), and these QTLs were sensitive to drought stress (Tang *et al.*, 2023). He *et al.* (2023) studied the RILs population for grain yield under drought stress and normal conditions and identified 31 QTLs. QTL, *qGYP1-1*, and *qLAI-1* were detected for grain yield per plant and leaf area under water stress conditions and could be further studied to validate their role in drought tolerance (He *et al.*, 2023). These QTLs should be validated in additional research studies for marker-assisted selection. Near isogenic lines (NIL) should be used to validate the QTL detected in RILs and other populations.

**Table 1.** QTL for drought tolerance in maize

Population/parents	QTL	Chromosome	Traits	Reference
218 F <sub>8</sub> RIL population	<i>qGYP1-1</i> , and <i>qLAI-1</i>	1	GYP and leaf area	(He <i>et al.</i> , 2023)
BC <sub>2</sub> F <sub>5</sub> Population of 191 lines	<i>qASI-WS2</i>	2	ASI	(Tang <i>et al.</i> , 2023)
135 F <sub>8</sub> RILs	<i>qKPE-9</i>	9	Kernel per year	(Kaur <i>et al.</i> , 2023)
RILs	<i>qCW2-1</i> , and <i>qCH1-1</i>	2, 1	Cob weight and cob height	(Sarkar <i>et al.</i> , 2023)
DH 217 lines	<i>qWS-GY3-1</i> , <i>qWS-ESP3-1</i>	3	Grain yield and ear-setting percentage	(Hu <i>et al.</i> , 2021)
DH/ LPSC7F64 × CML495	<i>qGY-4</i>	4	Grain yield	(Vazquez-Pozos <i>et al.</i> , 2020)
116 F <sub>3</sub> families of DTP79xB73 cross	<i>qGY-2</i> , <i>qGY-10</i>	2, 10	Yield per plant	(Nikolić <i>et al.</i> , 2013)
Three tropical maize populations	<i>qGY-7</i> , <i>qASI-3</i>	7, 3	Grain yield and anthesis silking interval	(Almeida <i>et al.</i> , 2013)
210 RILs	<i>qPH-1</i> , <i>qASI-1</i>	1	Plant height and ASI	(Prasanna <i>et al.</i> , 2009)
F <sub>23</sub> families/DTP79 and B73	<i>qGY-1</i> , <i>qGY-5</i> , <i>qGY-9</i>	1, 5, 9	Grain yield	(Rahman <i>et al.</i> , 2011)

## GWAS analysis of drought tolerance in maize

GWAS is an essential and reliable technique for identifying markers linked with targeted traits. This technique is used to identify QTL to understand the complex nature of diverse traits (Fofana *et al.*, 2024; Liu and Qin, 2021; Ren *et al.*, 2021; Yang *et al.*, 2024). Compared to traditional QTL mapping, GWAS has the advantage of identifying the genetic mechanism of complex traits and does not involve screening large parental populations (Dinka *et al.*, 2007). Chen *et al.* (2023) used the panel of 379 diverse inbred lines and investigated the genetic makeup of drought tolerance at the seedling stage. A total of 15 genes were involved in drought tolerance at the seedling stage. The gene *Zm00001d012176* was involved in metabolism, and *Zm00001d017495* (Table 2) was involved in plant growth, and these results helped to understand the drought tolerance in maize (Chen *et al.*, 2023).

**Table 2.** GWAS studies for identification of genes/QTLs regulating maize tolerance to drought stress

Population	QT/SNP/genes	Role	References
210 ILs	<i>ZmADFS</i>	<i>ZmADFS</i> overexpressing plants were sensitive to ABA	(Liu <i>et al.</i> , 2024a)
324 DH lines	475 QTL	GLAI is a promising grain yield secondary trait	(Blancon <i>et al.</i> , 2024)
379 inbred lines (ILs)	15 candidate genes	<i>Zm00001d012176</i> for metabolism, and <i>Zm00001d017495</i> for cell growth and development	(Chen <i>et al.</i> , 2023)
210 ILs	413 and 696 SNPs identified under WW and WS conditions	Results provide valuable information for the drought tolerance mechanism	(Wang <i>et al.</i> , 2019)
201 ILs	147 loci	<i>ZmNAC49</i> responded to drought stress by regulating stomatal density	(Ningning <i>et al.</i> , 2023)
279 ILs	32, 59 and 12 genes	Genes are linked with ASI, PH and EBM	(Khan <i>et al.</i> , 2022)
68 ILs	Eight SNPs and eight genes	Genes are linked with the drought resistance index of SOD, POD and REC	(Liu <i>et al.</i> , 2021)
209 diverse maize accessions	7 candidate genes	These genes are related to seminal root length (SRL)	(Guo <i>et al.</i> , 2020)
240 subtropical lines	1053 SNPs	77 SNPs related to drought tolerance	(Shikha <i>et al.</i> , 2017)
201 maize ILs	115 genes and nine QTL	Genes were linked with traits like grain yield, ASI, and number of ears per plot.	(Wang <i>et al.</i> , 2016)
350 maize ILs	42 SNPs, 33 genes	<i>GRMZM2G125777</i> linked with 100 kernel weight	(Xue <i>et al.</i> , 2013)

In another investigation, the authors investigated the drought-tolerant QTLs using GWAS. They identified 62 QTLs under normal and drought stress conditions. Further, they also selected 7 different genes for normal and drought stress conditions (Guo *et al.*, 2020). Earlier study also evaluated the panel of 201 diverse maize inbred lines. Researchers identified 206 single nucleotide polymorphisms (SNPs) linked with 115 genes for drought tolerance in maize (Wang *et al.*, 2016).

Different studies used different types of mapping populations to analyze the genetic mechanism of drought tolerance in maize. Shikha *et al.* (2017) used 240 maize subtropical lines to analyze the genetic background of drought tolerance using the GWAS technique. A set of 1053 SNPs was selected across all databases to validate the genes and QTLs. Out of 1053 SNPs, 77 SNPs were linked with drought tolerance. These results are valuable for future breeding of drought tolerance in maize (Shikha *et al.*, 2017). The flowering

stage is severely influenced by drought stress. Analysis of flowering traits under drought stress conditions is critical to safeguard the yield of maize and avoid economic loss. Khan *et al.* (2022) used a panel of 279 inbred lines of maize to study the anthesis-silking interval (ASI), plant height (PH) and ear biomass (EBM) at the silking stage. GWAS has identified 71, 159, and 21 SNPs in 32, 59 and 12 genes linked with ASI, PH and EBM. These genes can be used in breeding for drought tolerance in future research studies (Khan *et al.*, 2022). Candidate genes associated with drought tolerance traits at the seedling stage give valuable insights into molecular breeding. Screening for drought tolerance at the seedling stage and identifying genes is essential for exploring drought tolerance in maize. Liu *et al.* (2021) identified 8 different SNPs linked with 8 genes for drought tolerance in maize. These genes belonged to malondialdehyde (MDA), superoxide dismutase (SOD) and relative conductivity (REC) (Liu *et al.*, 2021).

Ningning *et al.* (2023) identified 147 loci controlling drought tolerance in maize using GWAS. These loci were linked with grain yield or drought resistance index. Out of these QTLs, 54 were linked with drought resistance index. Molecular analysis showed that 22 genes were candidate genes involved in drought tolerance. The cloned genes, *ZmNAC49*, responded to drought stress by regulating stomatal density. Further characterizing these genes is crucial to accelerate the drought breeding program (Ningning *et al.*, 2023). Wang *et al.* (2019) evaluated 210 maize ILs under well-watered (WW) and water-stress (WS) conditions to study drought tolerance using GWAS. GWAS identified 413 and 696 traits-associated SNPs under two water regimes. These results can be precious to improve drought tolerance in maize and need additional studies (Wang *et al.*, 2019). Xue *et al.* (2013) evaluated 350 ILs under normal and drought stress conditions and detected 42 SNPs for 33 genes. Gene *GRMZM2G125777* was associated with the timing of male and female flowering and 100 kernel weight. This gene can help maize cultivars grow in drought-stress environments (Xue *et al.*, 2013). In a newly published study, 475 QTL were detected for green leaf area index (GLAI) traits of maize evaluated under moisture deficit conditions. Results showed that the genetic mechanism of GLAI traits is less complex than yield traits. GLAI is a promising grain yield secondary character in drought conditions. Hence, GLAI traits should be prioritized in future drought-breeding research studies to enhance the maize yield in changing environments (Blancon *et al.*, 2024). In another study, authors found that *ZmADF5* was strongly linked with drought tolerance and drought-induced osmotic stress increased the expression of this gene (Liu *et al.*, 2024a). These results showed that drought tolerance is a complex mechanism that involves many genes. GWAS has been proven to be an effective and influential technique to unfold the mystery of complex polygenic traits. Despite this work, the drought tolerance mechanism must be completely understood and it demands additional studies. Integration of GWAS with other molecular research tools like transcriptome would be beneficial in identifying potential candidate genes that can be used in future breeding programs.

### **Transcriptome analysis for drought tolerance in maize**

Genes regulation is essential for drought tolerance, and gene expression determines the plant responses against drought stress. Water deficiency affects the maize physiology and biochemistry. Zou *et al.* (2024) induced drought stress in maize root during the seedling stage using poly-ethylene glycol (PEG) and identified (597, 2748, 6588, and 5410) differentially expressed genes (DEGs) under 5%, 10%, 15% and 20% PEG levels. Remarkably, two transcription factors (TFs), bHLH 35 and bHLH 96, were significantly upregulated under drought stress and considered as potential genomic regions for breeding drought-tolerant maize (Zou *et al.*, 2024). In another research investigation, two different maize ILs (si287, drought-tolerant, and X178, drought-sensitive) were subjected to drought stress for 7 days. These authors reported that different genes like flavin-containing monooxygenase 6 (*ZmYUC6*, 103,629,142), indole-3-acetaldehyde oxidase (*ZmA01*, 542,228), and catalase 1 (*ZmCAT1*, 542,369), appreciably improved drought tolerance in maize (Li *et al.*, 2024b).

*ZmC2H2-149* gene significantly suppressed the expression of *ZmHSD1* (hydroxysteroid dehydrogenase 1), a drought tolerance regulator. *ZmC2H2-149* can regulate *ZmHSD1* (Table 3) to enhance drought tolerance and can be useful for molecular breeding (Liu *et al.*, 2024b). The other findings showed that the gene (*ZmEREB24*) appreciably increases the drought tolerance in maize (Ren *et al.*, 2024). Gillani *et al.* (2022) also studied the comparative transcriptome analysis of two contrasting maize genotypes under drought stress conditions (Gillani *et al.*, 2022). This research study concluded that the expression of stress-responsive genes was more dominant in drought-tolerant maize genotype 478, and these findings can lead to the selection of novel genes for future breeding programs (Gillani *et al.*, 2022).

**Table 3.** Transcriptome analysis for drought tolerance in maize

Maize genotypes	Genes	Role	References
Inbred line Q901	597, 2748, 6588, and 5410 DEGs	bHLH 35 and bHLH 96 upregulated under drought stress and involved in plant hormone pathways	(Zou <i>et al.</i> , 2024)
ILs, si287 and X178	( <i>ZmA01</i> , 542,228), ( <i>ZmCAT1</i> , 542,369) and ( <i>ZmYUC6</i> , 103,629,142)	Tryptophan biosynthesis	(Li <i>et al.</i> , 2024b)
IL W22 maize, 150 mutant lines	<i>ZmC2H2-149</i>	<i>ZmC2H2-149</i> regulates <i>ZmHSD1</i> to enhance drought tolerance	(Liu <i>et al.</i> , 2024b)
IL, Yu882	<i>ZmEREB24</i>	<i>ZmEREB24</i> plays a key biological role in maize	(Ren <i>et al.</i> , 2024)
W9706-drought tolerant, and B73-drought sensitive	3147 DEGs in both lines	DEGs in W9706 regulated ABA signaling and wax biosynthesis	(Jiang <i>et al.</i> , 2023)
Germplasm, CIMBL55	65 alleles	The function of <i>ZmRtn16</i> was verified at the molecular level	(Tian and Qin, 2023)
ILs CML69 and LX9801	10,084 DEGs	Regulation of osmotic adjustment and carbon fixation pathways	(Waititu <i>et al.</i> , 2021)
YE8112 and drought-sensitive MO17 IL	10,612 DEGs	Genes identified in IL, YE8112 were involved in carbohydrate biosynthesis, and nitrogen metabolism	(Zenda <i>et al.</i> , 2019)
Drought-sensitive RIL93, and drought-tolerant RIL70 and	9 vs. 358, 477 vs. 103, and 5207 vs. 152 DEGs, respectively in DT1, DT2, and DT5 (drought at day 1st, second and 5th)	Expression of genes related to ABA and programmed cell death was stable in RIL70	(Min <i>et al.</i> , 2016)

The recent findings showed the transcriptome analysis of two maize ILs (drought-tolerant YE8112 and drought-sensitive MO17). The authors identified 10612 genes, and the expression of genes was more in the drought tolerant line. The identified genes in YE8112 were involved in synthesis of amino acids, carbohydrates and nitrogen metabolism. Hence, these genes should be validated for their use in molecular breeding (Zenda *et al.*, 2019). Tian and Qin (2023) performed the de novo assembly of drought-tolerant maize germplasm, CIMBL55 and identified the drought-tolerant genes *via* transcriptome analysis. Three essential genes, *ZmABF4*, *ZmNAC075*, and *ZmRtn16*, were identified for their role in drought tolerance and function of *ZmRtn16* was verified at the molecular level (Table 3) (Tian and Qin, 2023). Two maize ILs, CML69, drought tolerant, and LX9801, drought susceptible, were subjected to drought stress and transcriptome analysis of maize leaves was performed to identify the stress-responsive genes. A total of 10,084 DEGs were identified. DEGs in the tolerant line, CML69, were associated with osmoregulation, drought avoidance and carbon

fixation pathways. These genes can be used for drought-tolerant breeding programs (Waititu *et al.*, 2021). It is essential to validate the functions of these genes to choose the potential candidates for future breeding programs. However, more studies are needed to speed up the breeding of drought-tolerant maize. The integrated use of metabolome and transcriptome can be useful for identifying genes that can control drought tolerance in maize.

### Transcription factors analysis for drought tolerance in maize

Identifying a network of drought response genes is critical to improving the genetic makeup of maize. Different TFs like WRKY, bZIP MYB, and HSP induce drought tolerance in maize. TFs regulate the growth of plants by regulating gene expression (Jiang *et al.*, 2018; Leng and Zhao, 2020; Mao *et al.*, 2016; Wu *et al.*, 2019). Nuclear factors (NY-Ys) are also essential TFs; however, their role in drought tolerance still needs to be fully discovered. Results showed that overexpression of *ZmNF-YA1* enhanced drought tolerance in maize by promoting root development. *ZmNF-YA1* mediated jasmonic acid (JA) signaling and histone modification. However, additional studies are necessary to deeply explore the role of other members of this TFs family in enhancing drought tolerance in maize (Table 4) (Yang *et al.*, 2022). Another family of TFs is MYB proteins, which play a crucial role in plant response to several stresses. However, more data is needed about stress-related MYB genes. A MYB gene, *ZmMYB3R*, was identified and functionally characterized in maize and was located in the nucleus. Transgenic *Arabidopsis* plants with overexpression of *ZmMYB3R* showed enhanced tolerance to drought stress. *ZmMYB3R* increased the expression of catalase (CAT) and superoxide dismutase (SOD) (Wu *et al.*, 2019). Likewise, another member of MYB, *ZmMYB56*, enhanced drought tolerance in maize seedlings by stomatal closure and activation of *ZmTOM7*, which is an essential target gene of *ZmMYB56* (Li *et al.*, 2024a).

**Table 4.** TF analysis for drought tolerance in maize

TFs	Role	References
<i>ZmDof22</i>	Increased drought tolerance by enhancing antioxidant enzyme activities	(Cao <i>et al.</i> , 2024)
<i>Zmbdz9</i>	Promoted direct transcription of <i>ZmTCP9</i> gene, which enhanced lignin accumulation	(Jiao <i>et al.</i> , 2024)
<i>ZmMYB56</i>	Promoted stomatal closure and activated the expression of <i>ZmTOM7</i>	(Li <i>et al.</i> , 2024a)
<i>ZmEREB24</i>	Increased the expression of genes <i>ZmSUS4</i> and <i>ZmSASA3</i>	(Ren <i>et al.</i> , 2024)
<i>ZmCCT</i>	Enhanced the expression of genes, <i>ZmRD17</i> , and <i>ZmAFP3</i> under drought stress	(Zhang <i>et al.</i> , 2024)
<i>ZmTIFY16</i>	Increased root growth under drought stress in maize plants	(Zhang <i>et al.</i> , 2023a)
<i>ZmNAC20</i>	Promoted stomatal closure under drought stress	(Liu <i>et al.</i> , 2023a)
<i>ZmHsf28</i>	Increased JA and ABA signaling	(Liu <i>et al.</i> , 2023b)
<i>ZmNF-YA1</i>	Promoted root development and mediated jasmonic acid (JA) signaling	(Yang <i>et al.</i> , 2022)
<i>ZmMYB3R</i>	Increased activity of CAT and SOD enzymes	(Wu <i>et al.</i> , 2019)
<i>ZmNAC55</i>	Enhanced drought tolerance at the seedling stage	(Mao <i>et al.</i> , 2016)
<i>ZmWRKY40</i>	Increased the expression of stress-responsive genes and activities of POD and CAT	(Wang <i>et al.</i> , 2018b)

NAC is an important TFs family, and plays an important role in inducing drought tolerance in maize. Mao *et al.* (2016) examined the cloning of maize NAC, TF, and gene *ZmNAC55* and identified their essential role in drought tolerance. Likewise, transgenic *Arabidopsis* with increased expression of *ZmNAC55* depicted a substantial increase in drought tolerance (Mao *et al.*, 2016). Recently, another NAC TF, *ZmNAC20*, was

studied for its role in drought tolerance in maize. *ZmNAC20* promoted stomatal closure and enhanced the activities of stress-responsive genes. These findings showed the significance of NAC members for their role in drought tolerance in maize (Liu *et al.*, 2023a). Heat shock factors are a TFs family which plays a crucial role in abiotic stress response in maize. A recent study showed that *ZmHsf28*, a member of the HSF family, increased the drought tolerance in maize by increasing jasmonate (JA) and abscisic acid (ABA) in transgenic plants compared to wild type (Liu *et al.*, 2023b). WRKY TFs are plant-specific TFs that can be essential in plant responses against stress conditions. For instance, *ZmWRKY40* increased drought tolerance in transgenic plants through an increase in antioxidant activities (Wang *et al.*, 2018b).

The findings of other authors showed that ZmCCT-TF played an essential role in regulating drought tolerance in maize, however more studies are needed to explore the potential of CCT-TF (Zhang *et al.*, 2024). Another family of TFs is TIFY, a plant-specific TF that governs several abiotic stress responses in maize. A TIFY member, *ZmTIFY16*, was identified and cloned in maize for its role in drought tolerance. *ZmTIFY16* increased the expression of gene *ZmMYC2* responsible for JA signaling, increased root growth under drought stress in maize plants and hence enhanced drought tolerance. This TF may have more diverse functions, which can be explored in future research studies (Zhang *et al.*, 2023a). The *ZmEREB24* gene belongs to the AP2/ERF protein family and increases drought tolerance in transgenic maize plants at the seedling stage, as Ren *et al.* (2024) studied. *ZmEREB24* regulated the expression of key genes *ZmSUS4* and *ZmSASA3* under drought stress in maize. However, the interaction of this TF with other genes and enzymes needs to be clarified, and more studies are needed (Ren *et al.*, 2024). HD-Zip TFs family plays a crucial role in maize drought tolerance. A recent study revealed that *Zmhdz9*, a member of the HD-Zip protein family, increased drought tolerance in maize by promoting direct transcription of *ZmTCP9*, which increased lignin accumulation (Jiao *et al.*, 2024). There are many more studies on TFs and their role in drought tolerance in maize. However, there is still a need to deeply explore the role of WRKY, DREB and HD-Bzip TF families in regulating drought tolerance in maize. It is important to note that previously reported TFs have yet to be fully utilized in drought-breeding programs, which emphasizes on more studies.

### CRISPR/Cas9 applications for drought tolerance

CRISPR/Cas9 is one of the most powerful tools for editing a gene of interest without any biological barrier. CRISPR/Cas9 has revolutionized the field of plant biology because it offers simplicity, cost-effectiveness and high efficiency in gene editing. CRISPR/Cas9 has been successfully used to knock out the stress-responsive genes in many plants like *Arabidopsis*, rice, wheat, and maize (Abdallah *et al.*, 2022; Cong *et al.*, 2013; Li *et al.*, 2013; Liu *et al.*, 2023c). Maize has been given considerable importance for its great economic value. Liu *et al.* (2023c) employed the CRISPR/Cas9 tool to edit the function of the enzyme *ZmGA20ox3*, responsible for gibberellin (GA) biosynthesis. Results showed that editing of *ZmGA20ox3* resulted in a semi-dwarf phenotype of mutants with reduced content of GA and no yield loss compared to wild plants. *ZmGA20ox3* enhanced drought tolerance by improving plant height, and therefore, CRISPR/Cas9 can be expanded as a novel way to breed drought-tolerant genotypes (Liu *et al.*, 2023c). Wang *et al.* (2024) used CRISPR/Cas9 to manipulate the *ZmHDT103* gene to improve drought tolerance in maize. They noted that plants with this gene showed lower ROS production and higher antioxidant activities (Wang *et al.*, 2024). An increase in grain yield of CRISPR/Cas9-based generated maize mutants reveals the efficiency of genome editing. An earlier research study showed that transgenic maize plants with overexpression of *ARGOS8* showed higher grain yield and decreased sensitivity to ethylene under drought-stress conditions. CRISPR/Cas9 was used to edit the gene *ARGOS8* to generate its novel variants. Compared to the wild type, *ARGOS8* variants showed higher grain yield and no water loss under drought-stress conditions (Shi *et al.*, 2017). These findings

showed that CRISPR/Cas9-based gene editing is very effective and reliable. There is an urgent need of time to increase the use of CRISPR/Cas9 to increase the crop's performance under changing environments.

## Conclusions

Human activities have severely impacted the environment in which we are living. With the increase in human population, the need for food is increasing, which puts pressure on available resources. Abiotic stresses continuously damage the crop's growth and yield on large areas. Abiotic stresses like drought and heat are among the global issues that cause crops to suffer in various parts of the world. Crops play a crucial role in meeting the nutritional needs of human beings. Due to rapid industrialization and deforestation, the issue of global warming is becoming severe. The crops are facing serious challenges of drought stress, reducing crop productivity and causing serious threats to food security. Maize is an important crop for feed and food purposes; however, it is seriously affected by drought stress. Maize breeders are engaged in improving maize performance in drought-prone environments. Traditional breeding techniques allowed the development of drought tolerant maize; however, these techniques are costly and laborious. With the advancement in molecular biology, use of molecular breeding tools has rapidly increased in recent years, increasing the efficiency of maize breeders and saving time and cost. Molecular research tools like QTL, GWAS, transcriptome analysis, TFs analysis, and CRISPR/Cas9 identify genome regions to induce drought tolerance in maize.

Furthermore, many QTLs were also identified, and marker-assisted selection increased drought tolerance in maize. GWAS is also important for identifying the diverse traits that induce drought tolerance. However, it must be studied in depth to explore the molecular mechanism of drought tolerance. Recently, transcriptomic investigations also identified the DEGs that can regulate drought tolerance in maize. Studying the use of DEGs in genetic engineering and CRISPR/Cas9 is also essential.

Although several TFs families have been studied for their role in drought tolerance, it is evident from the above findings that their detailed mechanism in regulating drought tolerance in maize still needs to be fully understood. Hence, it is essential to conduct more studies to explore the possible role of TFs genes, which still need to be fully explored. Use of CRISPR/Cas9 should be expanded to expedite drought-breeding in maize and increase the efficiency of molecular breeding. The combined use of different techniques like GWAS and transcriptome direly needs more attention. This will help us understand the molecular mechanisms that induce DS tolerance in maize crops.

## Authors' Contributions

ZWG prepared the draft. YHC, XNL, MYB and ZJW involved in writing. TAA and MH reviewed and improved the manuscript. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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