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COMBATING SOIL DROUGHT IN MAIZE (*Zea mays* L.): GENETIC-ENGINEERING STRATEGIES FOR DROUGHT TOLERANT VARIETIES

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Abstract. Drought is the global challenge of crop agriculture across the globe. The detrimental effect of drought hinders a significant growth and development impairment leading to devastating harvest losses. Maize, a vital staple for food faces future challenges with a rapidly expanding drought area. To address this challenge, scientists and breeders are urged to develop new varieties that are not only resistant or tolerant but also even potentially thrive under drought planting conditions. New breeding technologies involving molecular biology and biotechnology have been developed and implemented thus offering a promising solution. Genetic engineering has allowed humans to straightforwardly transfer beneficial genes across species and varieties, thus deliberating gene pool transfer of similar species and varieties. Furthermore, this technology has evolved to the level of creation, deletion, or modification of existing alleles with high precision through CRISPR/Cas9-mediated genome editing. This review article delves into the morpho-physiology, biochemical, and molecular responses of maize varieties against drought stress. It subsequently explores how genetic engineering has been utilized to optimize the selected genes underlying those responses. By exploring the current progress of genetic engineering, this article aims to prepare the ground for future advancement in combating drought through drought-tolerant maize varieties. Thus, this review article has the potential to contribute to improving food security in the increasing drought challenges.

Keywords: *Breeding, CRISPR/Cas9, drought, maize, soil, transgenic*

INTRODUCTION

Cereal crops play an important role in feeding the globe as their seed content is dominated by carbohydrates, protein, lipids, minerals, and other nutritional substances (Arefin et al., 2022). Maize serves as one of important cereal crops with nearly 1 billion people across the globe depending on maize as third primary staple after rice and wheat (McMillen et al., 2022). The carotenoid content in maize seeds is higher than that of wheat and rice (Trono, 2019). As a result, maize seeds can be described as nutritious and beneficial seeds for health. This fact is unfortunately accompanied by the sensitivity of maize plant to soil drought. A study by (Gupta et al., 2020) predicted that the escalating drought area will be worsened by the rapid human population growth by 2050. Thus, the limited water availability in soil will be forced to suffice a growing number of crop production in future, including maize.

Soil drought is the condition by which water level status in soil becomes scarce and is not able to support various life forms. This condition has been accelerated in recent decades by the global increase in temperature imposed by global warming and fluctuation in the earth's climate (Chávez-Arias et al., 2021).

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Notwithstanding the stage of growth, water deficit in soil affects growth performance of several maize genotype performance (Ali et al., 2022). There are three physiological modes of maize varieties to fight against soil drought including escape, avoidance, and tolerance (Aslam et al., 2015). The escape strategy involves the early entrance of certain growth stages to ensure soil's water availability suffices proper main developmental stages. The avoidance strategy works by alleviating the effect of water deficit through an increase of water uptake and water use efficiency. The last strategy, which mostly took breeders' and scientists' attention, enables plants to tolerate water shortage conditions and maintain physiological processes working properly under such water-limiting conditions. Therefore, various breeding approaches have been implemented to achieve the goal of maize breeding under drought conditions, i.e. to produce optimum yield in water scarcity without yield penalty in normal conditions (Chávez-Arias et al., 2021).

Recently, core traditional breeding had been focused on extensive screening and selection of maize genotypes to be adapted in drought conditions, followed by intensive crossing and backcrossing activities to combine beneficial character of each yield and plant vigour component (Gazal et al., 2018). Unfortunately, such approach has progressed slower than the drought areas expansion across the globe and rapid growth of human consumption rate. Therefore, new breeding approach is vital to accelerate the creation of new varieties, one of approach is through biotechnology.

Thanks to the advent of biotechnology, through genetic engineering approaches, scientists have enabled the creation of genetically modified plants (GMPs) with specific desired traits. Despite its profound breakthrough, transgenic approaches encounter challenges related to market acceptance and the concern about the foreign genetic materials existing in the genetically modified crops and their potential impact on the environment. Therefore, a more precise and efficient breeding approach for maize improvement under drought stress becomes imperative (Aslam et al., 2015; S. Liu & Qin, 2021).

CRISPR/Cas9 is a revolutionary biotechnology that allows precise maize breeding. This robust and versatile tool allows scientists and breeders to accurately manipulate specific genes with unprecedented accuracy, unlocking the potential for enhancing maize quality, yield, and resilience (Jiang et al., 2022). The technology works by making targeted changes to the specific DNA strands of living organisms. The targeted changes of DNA strands, called 'edit', were subsequently inherited throughout generations, leaving off foreign DNA materials used to construct the edited plant through a natural segregation process. Exceeding former transgenic approaches, this approach allows 'edited crop' to be a-transgene-free which substantially offers a wider market acceptability to its product (Muha-Ud-Din et al., 2024). The advance of CRISPR/Cas9 genome editing technology has allowed a more precise breeding of crops with desirable traits with potentially enhancing market reception and consumer assurance compared to the former transgenic approach (Cardi et al., 2023). Nevertheless, the number of released maize varieties produced by this technology is still underway. Therefore, both transgenic approaches and genome editing approaches are still applicable.

The application of genetic engineering in maize breeding has shown a promising step forward, including the development of an ideal architecture plant, increased yield, improved grain quality, a shorter life cycle, and a better ability to withstand various pests and diseases as well as environmental stresses (Dong et al., 2019; Muntean et al., 2022). New breeding techniques (NBT) encompass various genetic engineering approaches that have been used to modify the genetic composition of plants to develop new varieties. It includes transgenic approach and the newcomer CRISPR/Cas9 technique (Muntean et al., 2022) with various purposes including mutagenesis (Sauer et al., 2016), knockdown (Kausch et al., 2021), knockout (Doll et al., 2019), overexpression (Gillani et al., 2021), epigenetic modification (Dalakouras & Vlachostergios, 2021), as well as creating new alleles (Shi et al., 2017a; Y. Wang et al., 2022).

In this mini-review, we explore the exciting advancements of genetic engineering (GE), -with emphasis on transgenesis and CRISPR-mediated GE, used for the development of tolerant maize varieties against stress impacted by soil drought. We discuss the advance of genetic engineering to accelerate maize improvement for soil drought-resistant varieties and its implications for global food security. By providing a general overview, we hope to inspire further research and innovation in the field of maize breeding and contribute to the sustainable development of our global food system.

MATERIALs AND METHODS

A literature review was conducted by searching scholar databases for relevant research A literature review was conducted by searching scholar databases (Google Scholar (https://scholar.google.co.id/), PubMed (https://pubmed.ncbi.nlm.nih.gov/), bioRxiv (https://www.biorxiv.org/) for relevant research publications focused on maize genome editing for drought tolerance and resistance. The search employed targeted keywords, including *maize, drought*, *genes, breeding, genetic engineering, transgenic, genome editing*, and other relevant keywords published within past decade (2014-2024).

RESULTS AND DISCUSSION

Maize Responses to Soil Drought

Maize plantations are sensitive to drought with almost up to 20 % yield reduction were observed under drought conditions. The common responses of maize to water deficit in soil will depend on the intensity, frequency, and duration of drought stress. Maize response to drought stress encompasses early transition to mature stage, accumulation of drought-protectant substance, and adjustment of physiology and morphology responses (Chávez-Arias et al., 2021). The typical maize morphology at the mature stage (**Fig. 1)**. Various biological processes are adversely affected by drought stress such as plant growth, ear size, pollination and fertilization, and kernel number (K.-H. Kim & Lee, 2023). Consequently, the utilization of drought- tolerant maize varieties is indispensable.

Figure 1. Mature corn (Sweet corn) cv Bimmo in an early reproductive phase at 70 DAP. White bar = 0,88 cm

Maize response towards water depletion in soil or planting medium can be categorized into four types: morphological, physiological, biochemical, and molecular responses (**Fig. 2**). Morphological response represents the observable physical effects indicating the severity of drought stress experienced by plants, encompassing the changes in height, length, and organ size. Upon drought stress, maize leaves tend to shrink in size and number as a response to reducing excessive transpiration via leaves, hence limiting water loss (Aslam et al., 2015). Additionally, the opening and closing of stomata, which come as an entry port for carbon dioxide in photosynthesis also play an immense role in addressing drought in soil.

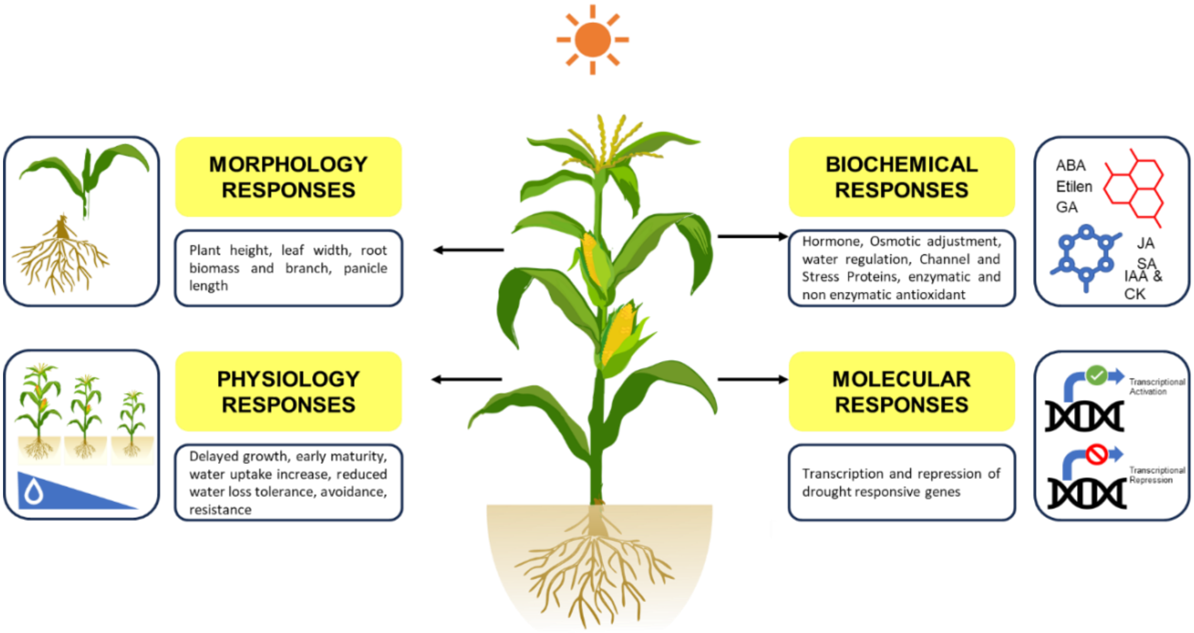

Figure 2. Graphical representation of maize responses to soil drought

Water deficit in soil induces water status rebalancing in the plant tissue which afterward impacts the changes in leaves and root turgor. Since the turgor pressure of leaves gradually decreases in line with the severity of drought, this induces the closes of stomata which can further help plants to minimize the water loss.

Nevertheless, the prolonged stomatal closure under severe drought could also make a drawback by limiting the amount of CO_2 plants can uptake from the air, thus reducing the rate of photosynthesis which is fundamental to plant growth and development (Guo et al., 2023a).

The physiological response of plants towards drought in soil involves fundamental changes in plant functions and processes in plants including growth, developmental stages, metabolism rate, transportation of assimilates (Taiz et al., 2015). Drought in soil occurs at early planting stage is likely to fail or impair the germination process which then affects the post-germination growth performance (Aslam et al., 2015; Radić et al., 2018). A comprehensive report by (Aslam et al., 2015) describe the physiological response of maize to drought stress across various growth stages. Vegetative stage is the process when the plants develop most of the photosynthetic organs and gather most of their biomass. Drought at vegetative stage affects the rate of growth, the ability of maize to absorb nutrients in soil, and early transition to reproductive stage. Drought at reproductive stage can potentially affect pollen viability and fertility and thus impact the success of pollination. Drought at endosperm and embryo development also impact water content of maize's kernel. Upon a series of biological processes of pollination and fertilization, embryo development is subsequently proceeded into kernel development. During kernel development, grain water content gradually increases its capacity to allow subsequent maximum biomass deposition. Biomass deposition determines the size of individual kernels which ultimately determines the final yield after reaching its physiological maturity. The adequate physiological maturity allows plant to accumulate various beneficial biochemical substances, preparing the maize seeds to continue the next life cycle.

Biochemical responses involve the changes of important osmolytes, accumulation of protective substances, as well as balancing of enzymes and phytohormones. Essential osmolytes such as proline, glycine betaine, sucrose, and other oligosaccharides play crucial roles in adjusting osmotic potential in cells. Living plant cells consist of 85-90% water build up their cell mass. Water is contained mostly in cell's vacuole and cytoplasm parts. These molecules act as osmolytes that protect cells from death caused by dehydration (Taiz et al., 2015).

Molecular responses include the perception of external drought signals by cells' receptors machinery, the transmission of drought signals from receptors into nucleus, and transcription of drought- responsive genes. The expression of drought- responsive genes may subsequently induce adjustments to morphology, physiology, and biochemical properties within cells, tissue, and organs to balance the water, assimilate, and respond to the coming drought stress (Aslam et al., 2015; Wahab et al., 2022).

Genetically Engineered Genes Used for Development of Maize Tolerance to Drought

The selection of genes for drought resistance improvement encompasses transcription factors, cell membrane proteins, and some enzymes. Various genetic engineering approaches such as overexpression, knock-out, and generation of new allelic variants are implemented to use genes for drought resistance improvements in maize. We list a few selected genes used in maize genetic engineering to design drought tolerance (Table 1). Among the listed genes, *ARGOS* (*Auxin-Regulated Gene Involved In Organ Size*) is a protein-encoding gene situated on plasma membrane of maize cells that was hypothesized to act as a negative regulator of ethylene signal transduction (Shi et al., 2015, 2017a). Ethylene signal transduction is one of the key pathways that receive drought cues from environment and subsequently deliver water scarcity signals to plant cells. The plant cells subsequently responded to the signal by up and downregulation of ethylene-mediated drought-responsive genes (Qi et al., 2023).

In plants, gene expression is started by the binding of RNA polymerase to a specific upstream region of gene which is called a promoter. This promoter determines the time, location, frequency, and the strength of certain gene expression. In genetic engineering, the use of constitutive promoters to constantly boost gene expression enables researchers to observe the impact of excessive gene expression above the normal ones on the phenotypes (Villao-Uzho et al., 2023). In one report, Shi and co-workers use 35S CaMV (Cauliflower Mozaic Virus Promoter) and Ubi (Maize Ubiquitin) promoter to drive *ARGOS1a* and *ARGOS8* overexpression which results in an increase in yield under drought stress (Shi et al., 2015). Additionally, the generation of new variants of *ARGOS8* sequence using CRISPR/Cas9 also results in an improved yield under drought conditions (Shi et al., 2017b).

Under drought conditions, Abscisic Acid (ABA) also plays an important role in mediating drought responses in plants. Plant receives water level dropping in the soil as an early signal of drought that is sensed by root organs. From root, the signal was subsequently spread throughout plant body, mainly leaves which harbor stomata. Stomata opening and closure are regulated by the accumulation of ABA. Under drought conditions, ABA is highly accumulated in the vasculature of leaves. This stimulates the stomata to close its aperture hence mitigating water loss by transpiration via stomata (Takahashi et al., 2020). Among ABA signal transduction pathways, kinases are enzymes that are involved in a series of phosphate groups addition to protein or sugar molecules. In maize, among the important kinases are *ZmMPKL1* which encodes MAPK (Mitogen-activated protein kinase) Like Protein 1 and *ZmPP84* which endodes Protein Phosphatase Clade C 84. While ZmMPKL1 protein possesses autophosphorylation and phosphorylation activity on specific proteins (Myelin Basic Protein), ZmPP84 dephosphorylates other proteins involved in drought response (ZmMEK1). ZmMPKL1 and ZmPP84 are known to negatively regulate drought responses in maize. In response to drought conditions, the increase in *ZmMPKL1* expression grants the maize seedlings a more sensitive phenotype to drought stress, including increased stomata opening, water loss, and wilting. Thus, in his research (D. Zhu et al., 2020) they introduced deletion in 65 base pairs of *ZmMPKL1* coding sequence.

Table 1. List of selected maize genes modified by genetic-engineering for development drought tolerance

Types	Gene and its Abbreviation	Type of Modification	Gene Function	Result	Reference
Morphology, Physiology, Biochemical, and Molecular Response	*ZmARGOS8* *Auxin-Regulated Gene Involved In Organ Size 8*	Overexpression and Cas9-mediated allelic variants generation	Encodes a putative transmembrane protein acting as negative regulators of ethylene signal transduction	Increased grain yield under drought stress with no loss in normal conditions	(Shi *et al.*, 2015, 2017)
Morphology, Physiology, and Biochemical, and Molecular Response	*ARGOS1a* *Auxin-Regulated Gene Involved In Organ Size 1a*	Overexpression	Encodes a putative transmembrane protein acting as negative regulators of ethylene signal transduction	Improved yield under drought and well-watered stress	(Shi *et al.*, 2015)
Physiology, Biochemical, and Molecular Response	*ZmMPKL1* *(MAPL-Like) Proteins 1*	Cas9-knockout	Encodes a kinase protein involved in ABA-signaling transduction pathway	Reduced drought sensitivity in seedling stage	(Zhu *et al.*, 2020)
Physiology and Biochemical response	*ZmPP84* *A clade F PP2C Phosphatase*	Cas9 knockout	Encodes A clade F PP2C Phosphatase	Regulate drought tolerance through inhibition of stomatal closure	(Guo *et al.*, 2023)
Morphology, Biochemical, and Molecular Response	*ZmLBD5* *Lateral organ Binding Domain protein 5*	Cas9-knockout	Encodes Class II LBD Domain plant-specific transcription factor involved in GA and ABA Biosynthesis	Knockout lines showed dwarfism but with higher drought tolerance	(Feng *et al.*, 2022)

Physiology, Biochemical, and Molecular response	*ZmASR1* *Abscisic acid-, stress-, and ripening-induced (ASR) proteins 1*	Cas9-knockout	Encodes absisic acid, stress, ripening-induced (ASR) proteins with possible function as transcription factor or chaperone like activity	Transgenic lines showed improved drought tolerance	(Yang *et al*., 2024)
Morphology, Physiological, and Molecular Response	*ZmSRL5* *Semi-Roll Leaf 5*	Cas9-knockout	Encodes a plasma membrane protein type Casparian-Strip-Membrane-Domain-Like (CASPL) for cuticular Wax formation	Increased sensitivity towards drought	(Pan *et al*., 2020)
Physiology and Biochemical response	*ZmAhb2* *Abscisic acid 8′-hydroxylase*	Cas9-knockout	Encodes an abscisic acid 8′-hydroxylase (ABAox), involved in ABA catabolism	Knockout lines in seedling stages showed higher survival rate under drought conditions	(Liu *et al*., 2020)
Morphology, Physiology, and Biochemical response	*ZmTIP1* *Tip Growth Defective 1 (TIP1)*	Overexpression	Encodes a functional S-acyltransferase, involved in posttranslational modification of cysteine residue of polypeptide	Overexpression lines increased hair root length and drought tolerance level	(Zhang *et al*., 2020)
Physiology and Molecular response	*ZmLAZ1-3* *Lazarus 1*	Cas9-knockout	Encodes a transmembrane protein involved in negative regulation of drought	Knock out lines improved drought tolerance	(Yu *et al*., 2024)
Morphology and physiology response	*ZmVPP1* *Vacuolar-type H+ pyrophosphatase 1*	Overexpression	Encodes a vacuolar-type H+ pyrophosphatase	Improvement of drought tolerance through root development and water use efficiency enhancement	(Liu *et al*., 2022)

Morphology and physiology response	*ZmNac111* *NAM, ATAF, and CUC (NAC)-type transcription factor 111*	Overexpression	Encodes NAM, ATAF, and CUC (NAC)-type transcription factor	Improvement of water use efficiency and survival rate at seedling stage	(Liu *et al.*, 2022)
Physiology and Biochemical response	*ZmTPP1* *Trehalose-6-phosphate phosphatase 1*	Overexpression in Maize ear	Encodes Trehalose-6-phosphate phosphatase, involved in sugar metabolism	An improved expression in maize ear increase yield in watered and drought conditions	(Nuccio *et al.*, 2015)
Physiology and Biochemical response	*Zmnced* *9-cis-epoxycarotenoid dioxygenase*	*salT* promoter-driven constitutive expression	Encodes a 9-cis-epoxycarotenoid dioxygenase, involved in ABA biosynthesis	Improved drought resistance under 14-day stress treatment.	(Muppala *et al.*, 2021)
Physiology and Biochemical response	*Zmrpk* *Receptor-like protein Kinase*	*lea* promoter-driven constitutive expression	Encodes a plasma membrane-localized Receptor-like Kinase, involved in stress response	Improved drought resistance under 14-day stress treatment.	(Muppala *et al.*, 2021)
Morphology, Physiology, and Molecular response	*ZmC2H2-149* *Cys2 /His2 zinc-finger-proteins 149*	Overexpression	Encodes Zinc finger protein transcription factor, positive regulator of drought tolerance	Decreased drought tolerance in overexpression lines	(Liu *et al.*, 2024)
Physiology and Biochemical response	*ZmSUS1* *Sucrose Synthase 1*	Overexpression	Encodes Sucrose Synthase	Increased drought resistance by modulating sucrose metabolism and soluble sugar content	(Xiao *et al.*, 2024)
Morphology, Physiology, Biochemical, and Molecular response	*ZmPTF1* *Phosphate starvation-induced basic helix-loop-helix (bHLH) transcription factor*	Overexpression	Encodes phosphate starvation - induced transcription factor	Increased drought tolerance of overexpression lines	(Li *et al.*, 2019)

Morphology, Physiology, Biochemical, and Molecular response	*ZmEREBP60* *Ethylene-responsive element binding protein (AP2/EREBP)*	Overexpression	Encodes Ethylene-responsive element binding protein (AP2/EREBP) transcription factor	Enhanced tolerance to drought	(Zhu *et al.*, 2022)
Morphology, Physiology, Biochemical, and Molecular response	*ZmPMP3g* *Plasma membrane proteolipid 3 protein g*	Overexpression	Encodes plasma membrane proteolipid 3 protein, responsive to abiotic stresses	Enhanced drought tolerance, increases in total root length, and antioxidative system	(Lei *et al.*, 2023)

This deletion produced an early stop codon in *ZmMPKL1* transcript which resulted in lower expression of *ZmMPKL1* in *mpkl1* maize mutant. Under drought stress, *mpkl1* seedlings exhibited fresh weight retention of 36% and 32% dry weight retention higher than wild type B73. Similarly, the elevation of ZmPP84 increased maize seedlings' sensitivity to water deficit stress. Therefore, in the research by (Guo et al., 2023b) *Zmpp84* mutant generated by CRISPR/Cas9 showed an improved relative water content and tolerance against drought in seedling stage with up to 80 % survival rate in *pp84* mutant that is 4 times higher than wild-type (20% survival rate) under soil drought treatments. This indicated that the mutation in *ZmMPKL1* and *ZmPP84* genes may be utilized for the development of tolerant maize against soil drought stress.

Besides ABA, Gibberellic Acid (GA) also plays an important role in drought response in plants. ABA and GA can be synthesized from a common precursor which is Geranylgeranyl pyrophosphate (GGPP) (Chen et al., 2020; Du et al., 2015). ABA biosynthesis process takes place at plastids and cytosol which involves the conversion of carotenoid substances into active ABA. Meanwhile, GA biosynthesis takes place in 3 different cell parts; plastids, endoplasmic reticulum, and cytosol which involves conversion of terpenoid substances into bioactive GA (Taiz et al., 2015). A transcription factor namely *ZmLBD5* encodes a transcription factor harbouring specific motifs of cysteine c-block, Gly-Ala-Ser (GAS) block, and leucine-zipper-like coiled-coil motifs. These transcription factors are responsible for various fundamental processes of maize growth and development, one of them is TPS-KS-GA2ox pathway which controls the availability and biosynthesis of both GA and ABA. The seedling of *Zmlbd5* mutant generated by CRISPR/Cas9 showed reduced plant height but enhancement of drought tolerance in comparison to wild type. Additionally, yield also increases in *lbd5* mutant under drought conditions with no significant yield loss under normal conditions compared to wild-type maize (Feng et al., 2022).

Another important ABA-mediated drought-responsive gene is *ZmASR1. ASR1* in maize is known as "ABA, Stress, And Ripening-induced (ASR) transcription factor" Differentiating from its counterpart *ZmASR2* which are positive regulator of drought tolerance in maize (Liang et al., 2019), knock-out of a negative regulator of *ZmASR1* caused expression decline of *ZmASR1* transcripts which subsequently conferred the maize lower reactive oxygen species (ROS), higher ABA content and a higher rate of stomatal closure, contributing important component of soil drought tolerance in maize (Yang et al., 2024). Thus, genome editing techniques such as CRISPR/Cas9 could be utilized to introduce deletion of *ZmASR1* in other maize varieties or create new variants of *ZmASR1* to increase maize tolerance against soil drought.

One of morphology responses shown by maize grown in soil drought conditions is the retardation of plant growth and development, including the delay of leaf expansion to mitigate water loss (Gupta et al., 2020). Leaves are the main photosynthetic organs of plants, producing carbohydrates and oxygen through light energy from water and carbon dioxide molecules. In a process of carbon dioxide uptake, leaves' stomata also lose a certain amount of water through transpiration. Therefore, in addition to hormonal control of stomatal aperture, maize leaves are morphologically equipped with cuticles. The cuticle is a waxy layer coating the epidermal tissue of leaves which prevents excessive water loss from leaves.

ZmSRL5 is a protein-encoding-gene for the formation of cuticular wax. *ZmSRL5* got its name "Semi Roll Leaf" as the phenotypes of adult maize harbouring a mutation in this gene have semi-rolled leaves similar to maize grown under water soil scarcity. Unlike its wild-type, *Zmsrl5* mutant was unable to recover after 9 days of drought treatment followed by 7 days of re-watering. The water content of *Zmsrl5* is significantly lower after drought treatments which reflected the inability to maintain water status in plant tissue. This highlights the main feature of tolerant or resistant varieties to soil drought by maintaining certain level of water in tissue and ability to utilize the remaining resources to maintain growth and development (Yan et al., 2023). The expression analysis confirmed that *ZmSRL5* is an essential gene for wax formation in maize leaves' cuticles, maintaining proper structure of cuticle and helping to provide maize plants protection against drought conditions (Pan et al., 2020). Therefore, maintaining proper function of this gene may be a great consideration for drought-tolerant maize varieties breeding.

Since maize is cultivated around the globe across the continent over time, the diversity of maize genetic background also diverges since then and offers a possibility to develop new maize varieties in response to coming drought stress in future. In a collection of 224 maize accessions from diverse environments, a genetic variation study using genome-wide single nucleotide polymorphism analysis was performed. This study discovered Abscisic Acid 8′-Hydroxylase encoded by *ZmAbh8* gene as a candidate for negative regulator of drought tolerance in maize (S. Liu et al., 2020). Therefore, the mutation of *ZmAbh8* may provide a new allele for increasing maize tolerance to soil drought.

Soil drought imposed a variety of growth limitations on plant cells to normally divide, elongate, and expand which commonly manifests in shrinkage of leaf expansion in maize under water deficit circumstances. This phenomenon was responded physiologically by maize cells via an increase in chlorophyll content, antioxidant enzyme activity, and some important metabolites (Avramova et al., 2015). One of the adaptations of drought stress in plants is usually attained by increasing water uptake from roots (Gupta et al., 2020). Although drought stress also negatively influences optimal root growth, the plant is still able to maintain certain parts to extend deeper into soil layer to collect available water resources and to subsidize major water loss (Kang et al., 2022). The increasing water uptake can be obtained via elongation of root and the extension of root surface through root hair growth.

A genetic and functional study by (X. Zhang et al., 2020) revealed *ZmTIP1* (*Tip Growth Defective 1*), an S-acyltransferase encoding gene in maize, plays a role in maize drought response via maintenance of root hair growth. An elevated expression of *ZmTIP1* in overexpressor maize showed better tolerance against water deficit conditions at seedling stages. In contrast, the lower expression of *Zmtip1* in *tip1* mutant caused a severe wilting response after dehydration treatments. The importance of roots in alleviating the effect of drought is also demonstrated by a study of (X. Wang et al., 2016). Wang and his group used drought-inducible promoter (MYB) and constitutive promoter (Ubiquitin) to induce expression of *ZmTIP1* and discovered that elevated expression of *TIP1* enhance higher photosynthetic rate along with a better root development with higher lateral root number and relatively higher root dry mass in comparison to wild type control.

A plasma membrane in cell plant is responsible for molecule exchanges between plant cells and surrounding environments. In maize plasma membrane, harboured various proteins such as intrinsic and extrinsic functioning at plant cell communication, adherence, transportation, and enzyme activities. *ZmLAZ1 (Lazarus 1), ZmPMP3g (Plasma membrane Proteolipid 3 Protein g),* and *ZmRPK (Receptor-like Protein Kinase)* are genes encoding intrinsic plasma membrane protein or transmembrane protein which has an impact on drought response of maize plants. LAZ1 is a member of DUF300 protein, a plasma membrane protein responsible for organic molecules transport. The study using ectopic expression of maize *LAZ1* in *Arabidopsis* revealed that the overexpression led to reduced germination ratio, root length, accumulated biomass, and relative water content. Thus, using a genetic engineering approach such as CRISPR/Cas9, it is suggested that the mutation or knockout of this gene could positively contribute to improving drought resistance in maize (Yu et al., 2024). PMP3g is a transmembrane lipid-containing protein that is widely associated with various abiotic stress responses such as cold, salt, and drought. In many plants such as Alfalfa (*Medicago sativa*) (D. Zhang et al., 2022), *Camelina sativa* (H.-S. Kim et al., 2022), and Tobacco (*Nicotiana tabacum*) (Ben Romdhane et al., 2017) the overexpression of PMP3 family confer the plants a higher degree of resistance in the salt excessive environment. In maize, the overexpression of *ZmPMP3g is* characterized by enhanced drought tolerance, elevated activity of antioxidants enzymes system, and increased total root length (Lei et al., 2023). Therefore, the genetic engineering of *ZmPMP3g* regulatory factors to increase its expression could be applied to develop drought tolerance of maize varieties.

Zmrpk is a gene encoding Receptor-like Protein Kinases from which its name refers. Located on plants' plasma membrane, these receptor molecules are involved in transmitting stress cues perceived initially by ABA receptors and work downstream of ABA receptors. Upon perception of stress signals, downstream signal-transmitting proteins continue message delivery from the membrane to the cytosol to the nuclei. In the nucleus, gene expression is primarily controlled in accordance with cells' necessity from which various stress-related genes are enhanced and repressed (Muppala et al., 2021). The increase of drought stress intensity is usually adjusted by the plants by increasing the biosynthesis of ABA. A gene namely *9-cis-epoxycarotenoid dioxygenase (nced)* is responsible for the cleavage of carotenoids for ABA biosynthesis. Increasing its expression could improve plants' tolerance to drought as observed in rice (Sultana et al., 2014) and tobacco (Pedrosa et al., 2017). The co-overexpression of *Zmnced* along with *Zmrpk* was observed to equip the maize plants with a better tolerance against drought stress, maintain a low level of reactive oxygen species (ROS), and enhance water and oxidative stress tolerance. Therefore, the incorporation of *Nced* and *Rpk* genes could be important factors that aid breeders and scientists in developing maize that performs better in response to stress (Muppala et al., 2021).

Maize cells comprise the majority of cell biomass with water. To properly function, the cells and tissues require ample amounts of water to survive especially in drought conditions. Drought stress imposes a dehydrating effect on plant metabolism, growth, and development. Thus, to alleviate the devastating effects of drought that can damage plant cells' protein and cellular membrane, plant cells accumulate some osmoprotectants (Aslam et al., 2015; Kerbler et al., 2023) such as Trehalose and Sucrose. Trehalose is carbohydrate molecule formed from two glucose molecules linked with α,α-1,1-glucosidic bond which has been associated with various stress tolerance. One of the important genes for trehalose biosynthesis is *ZmTPP1*. *ZmTPP1* encodes Trehalose-6-Phosphate (T6P) Phosphatase which dephosphorylates T6P into available Trehalose (Acosta-Pérez et al., 2020). Biotechnology approaches taken by (Nuccio et al., 2015) have successfully created maize lines that overexpressed *ZmTPP1* gene. His research group observed in multiple sites throughout seasons at least 9-49% of kernel set and harvest index increment in transgenic lines than in non-transgenic lines grown under mild drought stress. Furthermore, under severe drought conditions, the transgenic lines even reach up to 31-123% increment relative to non-transgenic lines. Similar to trehalose, Sucrose is composed of two monomers of simple sugar molecules linked by a glycosidic bond. Sucrose is also linked to various processes of plant physiology such as pollen formation and stress protection (Li et al., 2022). Sucrose Biosynthesis is controlled by numerous enzymes with complex interactions, one of which is *ZmSUS1*. *ZmSUS1* is a gene encoding a glycosyl transferase enzyme for catalysing the formation of Sucrose from UDP-Glucose and Fructose as well as the degrading sucrose into the composing monomers (Stein & Granot, 2019). Using transgenic maize, (Xiao et al., 2024) and his groups discovered that the elevation of *ZmSUS1* expression significantly increased various soluble sugar contents and achieved higher relative water content. This physiological response promoted better adaptation in a drought environment. These results highlight the strength of genetic engineering to select potential maize genes and modify their expressions to not only increase the plant survival under drought conditions but also achieve the optimum yield under such harsh environments.

Complex regulatory networks are involved in orchestrating morphological, physiological, and biochemical responses at the molecular level. The perception and transmission of drought signals from the environment to the cell via intriguing signalling pathways bring about the alteration of gene expression level. This gene expression is dictated by specific proteins namely transcription factors. Transcription factor works by selecting some of target genes, binding to their promoter, and inducing RNA transcription so their expression is increased (Leng & Zhao, 2020). Transcription factors are classified based on structure and specific motifs they have (Hrmova & Hussain, 2021). Given that drought-responsive genes are numerous the transcription factors involved in their regulation. In this study, few transcription factor genes are used by genetic engineers to modify maize so that they will have a better performance under drought conditions. *ZmC2H2-149* encodes Cys2 /His2 zinc-finger-proteins 149, a transcription factor known for its involvement in stress tolerance. *Zmc2h2-149* mutant made by transposon insertion showed an increased tolerance against drought stress while the overexpressor lines showed the opposite effect (H. Liu et al., 2024). With the advance of genome editing technology, it is highly possible to use this gene to be targeted by CRISPR/Cas9 mediated gene silencing to produce maize varieties that are more tolerant against soil drought stress.

Other study using transcription factors related to drought stress revealed that co-expression of *ZmNAC111* (encoding NAM, ATAF, and CUC transcription factor 111) with *ZmVPP1* (encoding Vacuolar H^+ Pyrophosphatase), with a transgenic approach provided the plant a higher survival rate up to 62-66% in comparison to wild type and individual overexpression of *ZmVPP1* and *ZmNAC111* which only retain survival rate around 23% and 37-42% under drought stress (S. Liu et al., 2022). The function of *VPP1* in maize is well explored by (X. Wang et al., 2016) that use diverse genetic variants in a genome-wide association study (GWAS) a gene related to Vacuolar H^+ Pyrophosphatase that catalyses pyrophosphate hydrolysis and pumps the released proton (H^+) from the cytosol into vacuoles, lowering pH of the vacuoles in comparison to wild type counterparts. The expression of *VPP1* is subsequently linked with the alteration of auxin transport and auxin-mediated plant growth and development. Thus, the transgenic lines overexpressing ZmVPP1 demonstrated higher photosynthesis rates, stomatal conductance, water use efficiency, and root development.

ZmPTF1 is a family member of basic helix-loop-helix (bHLH) transcription factors. ZmPTF1 got its name from the nature of Phosphate starvation-inducible Transcription Factors. This *ZmEREBP60* encodes maize transcription factor Apetala2/Ethylene Response Factor (AP2/ERF) which plays fundamental roles in the growth, development, and stress responses (Qi et al., 2023). Belong to AP2/ERF transcription factor family, *ZmEREBP60* overexpression in maize caused an improvement in maize tolerance to drought stress and lowered the accumulation of malondialdehyde and hydrogen peroxide, leading to an enhanced survival towards drought (Zhu et al., 2022). The result presented in this paper highlights the promising potential of genetic engineering strategies for development of drought-tolerant maize varieties. By discovering new pathway of drought tolerance mechanisms, scientists and breeders can utilize them to equip maize with better tolerance to answer future drought challenges and securing global food securities.

CONCLUSION

Drought stress in maize is one of the constant challenges to be addressed for maintaining global food supply. Therefore, modification of morpho-physiology, biochemical, and molecular responses through a biotechnology approach could optimize genes to enhance drought tolerance in maize. By using genetic engineering, scientists and breeders could accelerate the development of drought-tolerant varieties that help establish food security and answer global drought challenges.

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