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Molecular Insights to Comprehend Maize's Resistance to Drought and Nitrogen Deficiency

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Abstract

Maize is vulnerable to various abiotic stresses. Drought and low nitrogen stress are the two major constraints that hinder maize growth, development, and productivity. Severe drought stress leads to stomatal closure, restricting CO_2 intake, decreasing chlorophyll content, and ultimately reducing photosynthesis. Nitrogen (N) is the essential macronutrient required for proper growth and development of crop. N is a vital component of many biomolecules. N deficiency affects many biological processes in plants, such as, development, architecture, flowering, senescence, photosynthesis and photosynthates allocation. Thus, drought and nitrogen stress change the physiological and developmental process in plants, at molecular level plant respond by changing and reprogramming of genes and metabolism. The purpose of the review is to gain insight molecular aspects of plants stress response, to understand the role of transcription factors, and miRNA, and to enumerate latest developments in the transgenic approach to intercept drought and nitrogen deficiency. Also discusses how plants respond to signaling networks in drought and nitrogen deficiency. To create drought-tolerant germplasm, recent techniques and tools such as transcriptomic analysis, plant transformation, manipulation of TF, and genome editing tools might play a crucial role.

1. Introduction

Environmental abiotic stresses are the major challenges for crop production and productivity. Rising population has increased the food demand further, and saturation in productivity of the major crops like wheat, rice and maize have threaten the food security. Drought and Nitrogen are two main abiotic stresses for maize production, particularly, in the tropical region of the globe (Parajuli *et al.*, 2018). Drought stress affects maize plants at every stage, including germination, emergence, seedling establishment, and vegetative and reproductive growth phases (Liu & Qin 2021). Plants under drought stress experienced a range of physiological reactions, including wilting, restricted growth, metabolic alterations, stomatal cell closure, and in extreme cases, death (Shanker *et al.*, 2014). Drought during the seedling or growing period causes leaf curling and stunted growth (Song *et al.*, 2015; Effendi *et al.*, 2019). The flowering stage is most critical stage, during this period it may have a greater effect on the development of female flowers (silks) than male flowers, slow growth of silk increases ASI, significantly reducing kernel set. Therefore, water scarcity before and after flowering drastically reduced maize yield (Lu *et al.*, 2011; Edmeades, 2013). N is a vital component of chlorophyll, amino acids, proteins, nucleic acids, and various plant hormones, and a major regulator of numerous biological processes, including protein synthesis, carbon metabolism, and amino acid metabolism (Frink *et al.*, 1999). Since, more than 50% N is present in leaf as an enzyme or chlorophyll molecules. Nitrogen deficiency reduced leaf area development, photosynthesis rate by

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accelerating early leaf senescence. Hence reduces source strength which led to kernel and ear abortion as a result decline in yield (Kaur *et al.*, 2015). In cereal crop nitrogen use efficiency (NUE) ranges from 40 to 50% (Raun and Johnson 1999). Enhancing NUE (nitrogen usage efficiency) and N balance is crucial for modern agriculture's sustainable growth (Yang *et al.*, 2019). Drought stress reduces nitrogen uptake due to reduction in soil moisture content that slow down the diffusion rate of the nitrogen from soil matrix to root surface (Hu *et al.*, 2007). Water and N uptake by maize are positively correlated (Kim *et al.*, 2008). Nitrogen availability increases WUE and adequate water increases N use efficiency. These two stresses show the complex interaction and together led to 80% reduction in maize yield (Hambert *et al.*, 2013; He and Dijkstra, 2014; Benziger *et al.*, 2006). Nitrogen is taken up by crop plants in a form of nitrate, its deficiency leads to growth retardation and yield loss. Plants can overcome their nitrogen deficiency by improving the efficiency of the nitrate uptake system in the root and by improving the N utilization efficiency within the plant (Hirel *et al.*, 2001; 2007). Hence, developing crop genotypes with improved NUE would be of prime importance to achieve sustainable agriculture and high productivity under low resources. These physiological and developmental responses of drought and low-N stress are caused by reprogramming of metabolism and gene expression (Reddy *et al.*, 2004; Chaves *et al.*, 2009; Hayano-Kanashiro *et al.*, 2009; Zhang *et al.*, 2014). This review has been done to understand molecular mechanism of drought and low-N tolerance mechanism, the role of miRNA and transcription factors in drought and low-N stress, also discuss the recent advances in the transgenic approach to alleviates drought and nitrogen deficiency and plant response to signalling network under drought and in N deficiency.

2. Molecular mechanism of drought tolerance

Recent models of global climate change predict an increase in global temperatures as well as more frequent and severe drought episodes (IPCC, 2014). Water shortage is therefore expected to worsen, which will significantly impact the physiological status and productivity of the maize crop (Andjelkovic, 2018). Therefore, maintaining agricultural yields during drought stress requires an understanding of the molecular mechanisms underlying drought tolerance and to mine candidate genes that would help to increase drought resistance in the diverse maize germplasm, to increase yield. A key component of adaptation and stress response is through regulation of gene expression. Thus, a transcriptomic approach on the drought stress response in maize has been frequently employed (Thatcher *et al.*, 2014; Thatcher *et al.*, 2016; Danilevskaya *et al.*, 2019). The nature of the response to drought stress is complex, even while the expression of multiple genes vulnerable to drought is high. The molecular mechanism behind drought tolerance is not completely well understood.

3. Comparison of tolerant mechanism of tolerant and sensitive genotypes

To survive abiotic stress, maize plants have complex adaptation mechanisms that cause the expression of several genes that are translated in response to drought stress (Harb, 2010). In physiological comparison between drought-tolerant YE8112 lines, shows increased leaf RWC, proline content, and stronger peroxidase activity, and higher malondialdehyde concentration as compare to sensitive lines (MO17). However, malondialdehyde concentration was lower in drought-sensitive lines (MO17). Whereas comparative transcriptome analysis showed that the DEGs in drought-tolerant YE8112 were primarily linked to pathways involved in amino acid biosynthesis, carbon metabolism, and nitrogen metabolism. Conversely, DEGs of susceptible drought line (MO17) were more abundant in the ribosome pathway (Zenda *et al.*, 2019). Similarly, Zheng *et al.*, (2020) examined the two inbred lines of maize, the transcriptome data showed that distinct ROS scavenging capacities, signal interaction networks, and certain transcription factor pathways may be linked to the variations in drought tolerance between 287M and 753F. Further, comparative transcriptome and physiological analyses of drought-tolerant (CML69) and susceptible (LX9801) inbred lines showed that tolerant line had significantly higher relative water content in the leaves, as well as lower electrolyte leakage and malondialdehyde levels, than the susceptible line. The tolerant-line DEGs were predominantly associated with the cytoskeleton, cell wall modification, glycolysis/gluconeogenesis, transport, osmotic regulation, drought avoidance, ROS scavengers, defense, and transcription factors. For the susceptible line, the DEGs were highly enriched in the photosynthesis, histone, and carbon fixation pathways (Waititu *et al.*, 2021). Transcriptional and metabolic profile of two maize inbred lines, a drought-tolerant line (si287) and a drought-sensitive line (X178) revealed significant differences in pathways related to

glycolysis/gluconeogenesis, flavonoid biosynthesis, starch and sucrose metabolism, and biosynthesis of amino acids. However, tryptophan (Trp), was found to enhance tolerance via IAA-ABA signaling, as well as SA and nicotinamide adenine dinucleotide (NAD) consequent reactive oxygen species (ROS) scavenging (Li *et al.*, 2024) In another study drought tolerant line (W9706), upregulated DEGs were enriched in specific processes, including ABA signaling, wax biosynthesis, CHO metabolism, signal transduction and brassinosteroid biosynthesis-related processes, while the downregulated DEGs were enriched in specific processes, such as stomatal movement. Drought tolerance of W9706 is due to the more rapid response to stimulus, higher water retention capacity and stable cellular under drought stress (Jiyang *et al.*, 2023). Furthermore, maize inbred line ChangC7-2 (C7-2) was irradiated with 60Co-γ to create a drought-tolerant maize mutant (C7-2t). Transcriptome profiling, shows genes linked to photosynthetic processes were suppressed in the wild-type (C7-2) but not in the mutant (C7-2t) under drought conditions. On the other hand, cell components linked to membrane systems and cell wall biosynthesis were enriched in C7-2t DEGs linked to taurine (hypotaurine) metabolism and phenylpropanoid production. (Zhang Q *et al.*, 2020). On comparing gene expression profile between the drought-tolerant line Han21 and the drought-sensitive line Ye478. shows a common probe set of the abscisic acid signaling pathway components (Zheng *et al.*, 2010). Thus, it has been concluded that maize tolerant lines overcome the drought conditions by improving the antioxidant system and maintaining photosynthesis, primarily linked to pathways involved in amino acid biosynthesis, carbon metabolism, and nitrogen metabolism, but signaling network pathways linked through ABA signaling were is common in both, tolerant and susceptible genotypes.

3.1. Role of Hormones Associated genes in drought stress

Phytohormones plays central role in all the biological process, during adverse environmental conditions, phytohormones have a regulatory role in the expression of genes. They modulate the plants physiological and biochemical responses under stressful conditions and prepare the plants to sustain in unpredicted situation (Hirsch *et al.*, 1997; Iqbal *et al.*, 2014). According to Yang *et al.*, (2019), during post-silking, drought stress accelerates leaf senescence and downregulates gene expression profiles of photosynthesis. While genes involved in pectin and sucrose production are up-regulated. Drought also increases two asparagine synthetase genes (ZmAS3 and ZmAS4) (Yang *et al.*, 2019). Although genes linked to inflorescence development are found to be associated with hormones. Similar findings reported by Ning *et al.* (2021) They found decreased ZmACO2 expression that in turn decreased ethylene emission in vivo and increased the number of viable florets and kernels. Ethylene has a crucial role in the growth of inflorescences by influencing the number of spikelets, floral fertility, length of the ear, and number of kernels. Therefore, yield can be raised by maximizing the ethylene levels in maize or in other grains. (Nelson *et al.*, 2007). In addition, using quantitative trait loci (QTL) (Capelle *et al.*, 2010) and oligo-microarray (Luo *et al.*, 2010) analyses, several QTLs or genes associated with kernel desiccation were found to be involved in ABA synthesis. Other studies also found that many droughts response-related proteins or genes, such as ZmTPA (Huang *et al.*, 2012), ZmRFP1 (Xia *et al.*, 2012), and ZmCPK4 (Jiang *et al.*, 2013) were induced by ABA or in an ABA-dependent manner. Maize genotypes under prolonged drought (20-day interval) at the vegetative stage up regulated the genes involved in molybdenum cofactor sulfurase (LOS5), checkpoint clamp loader component (Rad17), 9-cis-epoxycarotenoid dioxygenase (NCED1), cationic amino acid transporter 1 (CAT1), and delta1-pyrroline- 5-carboxylate synthase 1 (ZmP5CS1). Moreover, on the transcriptional level, prolonged water stress shows the higher expression of drought-responsive genes (LOS5, Rad17, NCED1, CAT1, and ZmP5CS1 (Saad-Allah *et al.*, 2021).

3.2. Drought and Re-watering mechanism in tolerance

According to Kamoshita *et al.*, (2004) In drought stress, plants recovering ability after rewatering and drought resistance is more important than drought tolerance. Although, drought tolerance mechanism is the result of a plant's attempts to survive or recover from stress and allows plants to grow and maintain relatively high yields even in presence of drought (Bu *et al.*, 2009), and many genes were involved in the process. Leaf growth is inhibited in drought stress in maize inbred line B73 32 of the 54-cell division cell cycle genes in the leaf growth zone were down-regulated during drought stress. However, after re-watering in the recovery period, photosynthesis was up-regulated, and in the

proliferative zone, antioxidant enzyme activity and metabolite levels were elevated. All these shows faster leaf growth and more cell division (Avramova *et al.*, 2015). In a study, the response mechanism of maize evaluated in drought and re-watering. Results shows that maize response altered significantly in response to drought for 60and 96 h and re-watering for 3 days. DEGs mainly involve in photosynthesis, proline metabolism, ABA signaling, and oxidative stress. Also, indicating that DNA methylation is involved in responses under drought stress (Cao *et al.*, 2021). Gene expression profile of maize during drought/re-watering showed the common DEGs (differently expressed genes) between drought-treated and control plants were involved in cellular process, metabolic process, cell part, and binding and catalytic activity. Moreover, analyses of gene expression profiles revealed that 26 of the DEGs under drought encoded 10 enzymes involved in proline synthesis, suggesting that increased proline synthesis was a key part of the drought response. Also investigated cell wall-related genes and transcription factors regulating abscisic acid-dependent and -independent pathways (Cao *et al.*, 2018). Hence, we can say that these results provide new insights into the molecular mechanisms of drought tolerance, and may identify new targets for breeding drought-tolerant maize lines.

3.3. Molecular mechanism Low-N stress tolerance

Nitrogen is considered the most important nutrient in maize production. The main target is to develop maize genotypes with combined high nitrogen, use efficiency and high yield potential., There are various approaches used to select genotypes with a greater ability to utilized applied or soil available N, transcriptional study provides a better understanding of the physiological and molecular basis of plant NUE. The comparative transcriptome analysis of early vegetative stage of maize leaf zone revealed that most of the LN-responsive genes were developmental stage-specific (SGs). They are involved in plant growth and development under N deficiency. While, the common genes (CGs) rebalance carbon and N metabolism in maize leaves under LN condition (Guo *et al.*, 2022). Further transcriptomic analysis of leaf photosynthesis under low nitrogen supply in maize shows the down-regulated expression of genes related to the antenna system, reduced light absorption, light transport, and electron transport in maize (Mu *et al.*, 2017). A wide-ranging transcript profile of maize seedling roots grown under different nitrate conditions reveals that more than half of the genes were respond to protracted nitrate shortage, and some genes were part of the early nitrate signaling pathways. Nonetheless, genes presumably involved in scavenging and nitric oxide generation were present in root epidermal cells. The results also showed the existence of a novel complex signaling framework in which the responses of maize to nitrate may be significantly influenced by brassinosteroids (BRI1), the MKK2-MAPK6 module, and the fine regulation of nitric oxide homeostasis through the co-expression of synthetic (nitrate reductase) and scavenging (haemoglobin) components (Trevisan *et al.*, 2011). The leaf and root tissues of two inbred lines: DMI 56 (resistant to N stress) and DMI 81 (sensitive to N stress) their comparative transcriptomic profile under low-N shows that majority of the DEGs belongs into several metabolic pathways, including signal transduction, amino acid metabolism, N-assimilation and metabolism, and starch metabolism. Further analysis, it was discovered that the tolerant genotype had higher expression levels of several important genes related to N uptake (high-affinity nitrate transporter 2.2 and 2.5), N assimilation and metabolism (glutamine synthetase, asparagine synthetase), redox homeostasis (SOD, POX), and transcription factors (MYB36, AP2-EREBP) (Singh *et al.*, 2022). Nazir *et al.* (2016) observed an increased expression of a few defense proteins in the low N-tolerant genotype, which could potentially contribute to the genotype's tolerance to N famine. Whereas, pathways for the production of reducing equivalents (the pentose phosphate pathway and glycolytic pathway) and genes involved in carbon (C) metabolism were also affected by N supply. Numerous additional nitrogen response genes were also discovered, including sesquiterpene cyclase, phosphoenolpyruvate carboxylase, uroporphyrinogen methyltransferase, tonoplast intrinsic protein, and early light-inducible protein (Nazir *et al.*, 2016). Jiang *et al.* (2018) indicated nitrate responsive genes which were repressed under low nitrogen conditions GLK5, GLK8 and NLP15 as candidate regulators of genes. While, the transcription factor bZIP108 might play a role in gene activation. In low-N condition, two unrelated bi-parental double haploid populations (DHpop1 and DHpop2) reveal five candidate genes associated with chlorophyll fluorescence (pet1, hcf102, and spt2), SEN (sweet15a), and sugar metabolism (CBF3) were present in bins 2.07, 4.05, and 8.05. A QTL for SEN was located in bin 4.05 in both populations. Sweet15a and/or spt2 affecting chlorophyll content, auxin levels, and SEN are candidate genes at this

locus (Liu *et al.*, 2020). Gibberellins hormones play important role in nitrogen uptake and improve NUE of the crops. GA synthesis-deficient mutant zmga3ox grown under low (LN) and sufficient (SN) N condition. LN significantly suppressed the production of GA1, GA3, and GA4, lead to higher anthocyanin accumulation and the decrease in chlorophyll content. The transcript levels of ZmNRT2.1/2.2 were downregulated in zmga3ox plants, while the GA3 application enhanced the expression level of the gene. Furthermore, NRTs related genes were influence by several transcription factors that are involved in the GA-mediated transcriptional operation. The study suggested that GAs influenced N uptake involved in the transcriptional regulation of NRTs and physiological responses in maize responding to nitrogen supply (Wang *et al.*, 2020). BRs (Brassino steroids) modulate the physiological response to nitrogen (N) supply in maize. In BR signaling deficient mutant zmbri1-RNAi lines, low-N induced the expression of the BR signaling-associated genes ZmDWF4, ZmCPD, ZmDET2, and ZmBZR1 and the production of longer primary roots than normal N (NN) treatment. The expression of nitrate transporter (NRT) genes (ZmNPF6.4, ZmNPF6.6, ZmNRT2.1, ZmNRT2.2) was lower in zmbri1 than in wild-type roots. But exogenous application of 2,4-epibrassinolide (eBL) treatments up-regulated the transcript expression of NRT genes. Thus, BRs modulated N physiological response and regulated the transcript expression of NRT genes to promote N uptake in maize (Xing *et al.*, 2021).

3.4. Maize root response under N-deficiency

Nitrogen deficiency is one of the important constraints that limits maize productivity. Under optimal conditions, maize root system architecture is capable to absorption of nutrient from the soil. However, in N deficiency, larger root system helps the plant to explore a larger soil volume to increase total N uptake (Wiesler *et al.*, 1992). To improve the utilization efficiency of mineral elements in maize, key genes that regulates the growth of maize roots under N deficient conditions were identified by Ma *et al.*, (2020). Results indicate, MRP2, bZIP77, and bZIP53 play a positive regulatory role in maize root growth in an environment suffering from nutrient deficiency. Evaluation of 213 maize inbred lines for natural variation root traits and shoot traits using hydroponic systems under normal (2 mM nitrate) and low-N (0 mM nitrate) conditions. Total of 51 candidate genes with amino acid variations in coding regions were identified under low nitrogen conditions. A total of 38 single nucleotide polymorphisms and 12 insertions and deletions were significantly associated with lateral root length of primary root, primary root length between 0 and 0.5 mm in diameter, primary root surface area, and total length of primary root under a low-N condition (Wang *et al.*, 2022). Maize inbred lines were assayed for root growth at seedling emergence under high-nitrate and low-nitrate conditions. Under LN conditions, the protoporphyrinogen IX oxidase 2 gene was associated with total root surface area and the DELLA protein-encoding gene was associated with the length of the visible lateral root zone of the primary root. Under HN conditions, a histone deacetylase gene was associated with plant height. Under both LN and HN conditions, the gene encoding MA3 domain-containing protein was associated with the first whorl crown root number. The phenotypic and genetic information from this study may be used for genetic improvement of root traits with the aim to increase NUE in maize (Sun *et al.*, 2020).

3.5. Response of sensitive and tolerant maize lines in N-stress

Under field conditions, 304 maize accessions for low-N tolerance were evaluated. Their transcriptome analysis revealed that in the root of Qi319 (low- N tolerant) the DEGs up regulated in N deficiency were mainly enriched in metabolic energy related pathway. At 5th days after low-N treatment, older leaves become yellow in Ye478 but not in Qi319. The N deficiency-responsive DEGs related to thylakoid, chloroplast, photosynthetic membrane, and chloroplast stroma pathways were repressed by low-N stress in Ye478(low-N sensitive). Furthermore, the net photosynthetic rate was significantly decreased in Ye478 after 5 days of low-N stress. Thus, the improved root system and integrity of photosynthesis apparatus in Qi319 were the main factors for the different tolerance to low-N stress between Qi319 and Ye478. (Du *et al.*, 2021). In response to low nitrogen stress, two inbred lines of maize (Mo17 and Hz4) showed changes in global gene expression in their leaf tissues. Nitrogen stress for longer duration resulted in good stimulation of genes related to nitrate transport and assimilation (Chen *et al.*, 2011). The key gene (ZmTGA), in maize mutant respond to low nitrogen stress, the root length of the mutants under low nitrogen treatment increased by 10.1%, while that of the wild-type increased by 14.8%. While, root surface area of the wild type (low nitrogen

treatment) increased by 9.6%, whereas that of the mutants decreased by 5.2%. The activities of glutathione and guaiacol peroxidase enzymes in the mutant were lower than those in the wild-type under low nitrogen treatment. Hence, the mutant was less adaptable to a low nitrogen environment than the wild type (Wang *et al.*, 2022). On examining the transcriptome of two maize genotypes that differed in NUE at maturity under low-N demonstrate that the SRG- 200 genotype retained a greater sugar content in leaves and that a higher NUE because this genotype was linked to higher transcript levels for the genes involved in nitrate transport, N assimilation, and GS. These maize lines may serve as helpful markers, may aid in the development of elite varieties with improved NUE (El-kereamy *et al.*, 2011).

4. Role of Transcription factors in drought and Low-N stress resistance

TFs are proteins usually consisting of two domains, namely (1) the DNA binding domain (DB) and (2) an activation domain (AD). A TF binds to the cis-acting element (TF binding site) located in the promoter region of a stress-induced gene (Yamasaki *et al.*, 2013). Networks of TFs together with transcription factor binding sites (TFBS) directly control transcriptional regulation of plant genes (Chaves & Oliveira, 2004). The response to various abiotic stress is through ABA-dependent gene activation pathways. Molecular analyses showed that ABA regulates the expression of many genes under osmotic stress conditions, and that the ABRE is the major cis-element for ABA-responsive gene expression (Maruyama *et al.*, 2012). In various abiotic stresses transcription factors (TFs) regulate multiple genes by specifically binding to cis-elements in their promoter sequences (Hirayama & Shinozaki, 2010; Han *et al.*, 2020; Santos *et al.*, 2011).

4.1. Gene encoded transcription factors and their role in drought and Low-N stress

Many studies indicated drought-induced TFs (MYB, WRKY, AP2, NAC, SAP, and bZIP families) plays significant roles in the transcriptional reprogramming in drought tolerance (Jung *et al.*, 2017; Nakashima *et al.*, 2007; Yamaguchi-Shinozaki and Shinozaki, 2006; Zheng *et al.*, 2009). In maize, Mittal *et al.*, (2017) discovered ten drought-responsive TF families (AP2, ERF, MYB, bZIP, bHLH, GRAS, WRKY, NAC, NF-YA, and NF-YB) that control several physiological and molecular processes, including osmoregulation, hormone signalling, stomatal regulation, and root growth. Later, Mittal *et al.* (2018) describe the main TF families linked to drought that are encoded by 1,436 genes in maize. Ren *et al.* (2020) demonstrated that the transcriptional factor ZmNST3 improves maize drought resistance by regulating the expression of genes involved in cell wall formation, antioxidant enzymes, and secondary metabolites. Using drought-resistant maize inbred line Y882 as experimental material under PEG stress and re-watering treatment. The ZmNAC genes expression showed that ZmNAC118, ZmNAC25, ZmNAC49, ZmNAC74, ZmNAC122, and ZmNAC20 were up-regulated during PEG treatment under drought stress. After re-watering on 3 days, the transcript levels drastically decreased (Wang *et al.*, 2020). Additionally, a putative transcription factor known as Asr1 is genetically linked to drought tolerance and changed CO_2 fixation rates in leaves as a result of changed activity of the enzyme C4 phosphoenolpyruvate carboxylase (C4-PEPC). Under mild drought conditions, the highest C4-PEPC overexpressing line demonstrated a +20% increase in dry weight and a +30% increase in intrinsic water use efficiency (WUE) (Zheng *et al.*, 2010). On performing RNA-seq after drought treatment on leaves of drought-tolerant line (W9706) and drought-susceptible line (B73), Shows the upregulated genes in W9706 were enriched in ABA signaling, wax biosynthesis, CHO metabolism, signal transduction, and brassinosteroid biosynthesis. While downregulating DEGs were enriched in stomatal movements. The tolerance mechanism of drought tolerance line (W9706) because of the more rapid response to stimulus, higher water retention capacity and stable cellular environment under water deficiency (Jiang *et al.*, 2023).

4.2. Functionally validated TFs and their role in drought stress

On overexpression of OsMYB55, a rice MYB encoding gene in transgenic maize resulted in improved plant growth as well as decreased negative effects of drought and high temperature (Casaretto *et al.*, 2016). The ZmBES1/BZR1-1 gene, localized in the nucleus, and showed transcription activation activity and drought-inhibitory expression in maize. The gene (ZmBES1/BZR1-1) was cloned from maizeB73 and functionally characterized in transgenic Arabidopsis and rice by Feng *et al.*, (2023). Moreover, under drought stress, overexpression of ZmBES1/BZR1-1 resulted in the enhancement of drought sensitivity of transgenic

Arabidopsis and rice with a lower survival rate, reactive oxygen species (ROS) level and relative water content (RWC) and a higher stomatal aperture and relative electrolyte leakage (REL). Similarly, a maize NAC transcription factor, ZmNAC49, was cloned and identified its function in response to drought stress. The study showed that ZmNAC49 overexpression affects the expression of genes related to stomatal development, namely ZmTMM, ZmSDD1, ZmMUTE, and ZmFAMA. The results show that the transcription factor ZmNAC49 represses ZmMUTE expression, reduces stomatal density, and thereby enhances drought tolerance in maize (Xiang *et al.*, 2021). A novel transcription factor encoding gene, APETALA2 (AP2)/Ethylene response factor (ERF), which is tightly associated with drought tolerance in maize seedlings. ZmERF21 is mainly expressed in the root and leaf. Overexpression of ZmERF21 in maize significantly increased the chlorophyll content and activities of antioxidant enzymes under drought conditions. While mutant plants (zmerf21) displayed a reduced drought tolerance phenotype. RNA-Seq and DNA affinity purification sequencing analysis further revealed that ZmERF21 may directly regulate the expression of genes related to hormone (ethylene, abscisic acid) and Ca signaling, as well as other stress-response genes, through binding to the promoters of potential target genes (Wang *et al.*, 2021)

4.3. Role of Transcription Factors under Low-N stress

Some genomic studies have indicated that overexpression of transcription factor DOF1 under low-N conditions results in increased plant growth and N content (Yanagisawa, 2004). A total of 216 transcription factors (TFs), including ZmNLP5, were identified as N deficiency responsive experiment. The expression levels of ZmNLP5 were much higher in low-N tolerant line (Qi319) than in low-N sensitive line (Ye478), the findings were coherent with the increasing N content in low-N tolerant lines. Also indicating the importance of transcriptional regulation of N stress-responsive pathway in different tolerance to low-N stress between crop genotypes (Du *et al.*, 2021). On overexpressing the Zmm28 (MADS-box transcription factor) under the control of a moderate-constitutive promoter enhances photosynthesis capacity and N utilization (Wu *et al.*, 2019). NIN-like protein ZmNLP5 directly regulated the expression of nitrite reductase 1.1 by binding to the nitrate-responsive cis-element, whereas, natural loss-of-function allele of ZmNLP5 conferred less N accumulation in Mo17 (Ge *et al.*, 2019). Transcription factor ZmCCT function in high salt and low-nitrogen stress. RNA-Seq analysis indicated that ZmCCT can directly activate these salt inducible genes of ZmNADP, ZmPP2C, ZmbHLH55, ZmPIP1-1, ZmPIP2-4 and some low nitrogen involved genes of ZmWRKY47, ZmMYB44, ZmMYB36, ZmPIN10 and ZmbHLH83 when respond to high salt and low nitrogen tolerance. It further highlights that ZmCCT has multiple roles in abiotic stress tolerance (Zhang *et al.*, 2025). Thus, to improve the plant performance under various abiotic stresses particularly in drought and low-N stress transcription factors participate in the regulation of plant growth and development, Physiological, biochemical, and morphogenesis, and various environmental stress responses via regulating the expression level of their target stress-responsive genes independently or through cross talk with other transcription factors, thereby provide the comprehensive resistance to crops under stress conditions. Table 1 shows some recent TF's and their role in drought and N deficiency tolerance.

5. Drought and N-deficiency stress and transgenic plants

Transgenic approaches are used to exploit the target genes with appropriate modifications to improve drought and low-N stress tolerance (Bartels & Nelson 1994; Bruce *et al.*, 2000). Elite maize inbred line DH4866 was inserted beta gene for choline dehydrogenase (an essential enzyme in the production of glycine betaine from choline). Transgenic maize seedlings accumulated higher levels of glycine betaine during germination and the early seedling stage and demonstrated greater tolerance to drought stress compared to wild-type (non-transgenic) plants. Furthermore, following drought treatment, the transgenic plants' grain output was noticeably higher than that of the wild-type plants. Also, transgenic maize's increased glycine betaine buildup offers superior protection for the integrity of the cell membrane and increased enzyme activity in comparison to wild-type plants (Jeanneau *et al.*, 2002). Drought tolerance of four transgenic inbred maize lines overexpressing ZmVPP1 (PH4CV-T, PH6WC-T, Chang7-2-T, and Zheng58-T) and their transgenic hybrids was evaluated at various stages. Transgenic inbred maize lines exhibited improved growth and drought tolerance as well as enhanced photosynthesis. In addition, the crosses of the transgenic inbred lines (PH 4CV-T × PH 6WC-W and Chang7-2-T × Zheng 58-W) produced.

Table 1. Some recent TF's and their role in drought tolerance

Transcription Factor	Activity	References
ZmE2F	The overexpression of ZmE2F6 enhanced drought tolerance in transgenic *Arabidopsis* with longer root length, higher survival rate, and biomass by upregulating stress-related gene transcription.	Yang Cao *et al.*, 2024
Transcription factor ZmHsf28	Positive regulator of plant drought responses; e. Overexpression of ZmHsf28 increased jasmonate (JA) and abscisic acid (ABA) production in transgenic maize and *Arabidopsis;* It decreased reactive oxygen species (ROS) accumulation and elevated stomatal sensitivity significantly.	Lijun Liu *et al.*, 2023
ZmPMP3g	Affects drought signaling genes, help in survival under drought conditions.	Lei *et al.*, 2023
ZmWRKY79	The overexpression of ZmWRKY79 in *Arabidopsis* improved the survival rate under drought stress; More lateral roots, lower stomatal aperture, and water loss. ROS scavenging was also boosted by ZmWRKY79 to result in less H2O2 and MDA accumulation and increased antioxidant enzyme activities; ZmWRKY79 overexpression lines under drought stress, shows higher ABA production, which was consistent with up-regulated ABA biosynthetic gene expression.	Gulzar *et al.*, 2022
HD ZIP TF (ZmHDZ9gene)	overexpressing transgenic plants exhibited higher SOD and POD activities and higher accumulation of soluble proteins, which resulted in enhanced developed phenotype and improved resistance.	Qiu *et al.*, 2022
ZmLBD5	Negatively regulates drought tolerance by impairing ABA synthesis.	Feng *et al.*, 2022a, b
*ZmVPP1*and *ZmNAC111*	Enhance photosynthesis, antioxidant activity, and grain yields.	Liu *et al.*, 2022
ZmNAP, ZmNAC19, ZmNAC4 ZmJUB1, ZmNAC87	Improve drought tolerance in transgenic *Arabidopsis.*	Ding *et al.*, 2022
ZmNF-YA1	Enhances drought tolerance in transgenic maize.	Yang *et al.*, 2022
Heat shock TF ZmHSf08	The overexpression of *ZmHsf08* in maize resulted in enhanced sensitivity to salt and drought stresses, displaying lower survival rates, higher reactive oxygen species (ROS) levels, and increased malondialdehyde (MDA) contents compared with wildtype (WT) plants. Furthermore, *ZmHsf08* negatively regulates a number of stress/ABA-responsive genes under salt and drought stress conditions.	Wang *et al.*, 2021
ZmNAC49	Overexpression significant decreases the transpiration rate, stomatal conductance, and stomatal density in maize. ZmNAC49 overexpression affects the expression of genes related to stomatal development.	Xiang et al (2020)
NAC Transcription Factor 87 ZmNAC	Exhibited different expression levels at (3 treatment points), indicating different response to drought stress.	Wang *et al.*, 2020
WRKY TF (ZmWRKY40 gene)	Overexpression of ZmWRKY40 improved drought tolerance in *transgenic Arabidopsis* by regulating stress-related genes, and the reactive oxygen species (ROS) content in transgenic lines was reduced by enhancing the activities of peroxide dismutase (POD) and catalase (CAT) under drought stress.	Wang *et al.*, 2019

Table 2. Some recent TF's and their role in N deficiency tolerance

Transcription Factor	Activity	References
MADS26 is a unique TF	Overexpression of MADS26 enhances the sensitivity to chlorate and the utilization of nitrate in maize.	Zhang *et al.*, 2024
NIN-like protein 3.2	ZmAux/IAA14 molecular module is responsible for regulating maize root biomass in response to nitrogen limitation.	Wang *et al.*, 2024
Transcription factor ZmEREB97	Positively respond to Nitrate. Important regulator in the N signaling network in maize; it plays an important role in optimizing nitrate uptake.	Wu *et al.*, 2024
ZmNRT1.1B	Modulate high affinity NO^{3-} uptake and signaling. Enhance NUE and grain yield.	Cao *et al.*, 2023
ZmNLP5	acts as a central hub in the regulation network of N metabolism and directly regulates the expression of ZmNIR1.1 via binding the NO^{3-} responsive cis-element.	Ge *et al.*, 2020
ZmTMM1	Modulates lateral root development	Liu *et al.*, 2020
BZR TF; *ZmBZR*	Function as a Growth regulator during maize development; Stress signaling activity; Regulate plant physiology and morphology.	Manoli *et al.*, 2018
AtNRT1.1homologs ZmNPF6.4, Zm NPF6.6	It displays distinct substrate affinity for $NO3^{-}$and chloride in regulation of N transport.	Wen *et al.*, 2017

higher yielding hybrids under drought conditions (Jing *et al.*, 2020). Two shikimate kinase-like genes, SKL1 and SKL2, were cloned from maize, compared to the wild-type, transgenic Arabidopsis plants overexpressing ZmSKL1 or ZmSKL2 exhibited improved drought stress tolerance through increases in relative water content and stomatal closure. Besides, transgenic lines showed reduced accumulation of reactive oxygen species as a result of increased antioxidant enzyme activity (Liu *et al.*, 2022). Transgenic lineY7-1 overexpressing ZmPMP3g were shown to enhance drought tolerance by harmonizing ABA-GA1-GA3 homeostasis/balance, improving root growth, enhancing antioxidant capacity, maintaining membrane lipid integrity, and regulating intracellular osmotic pressure (Liu *et al.*, 2023). The overexpression of ZmPP2C55 positively enhanced tolerance to drought stress. The transgenic plants exhibited higher RWC, proline content, POD and SOD activities, GSH content and GSH/GSSG ratio and lower MDA content, electrolyte leakage and GSSG content compared with WT plants under natural stress treatment (Zhang *et al.*, 2021). Complex regulatory systems, including those at the biochemical, physiological, and molecular levels, are triggered in plants during water deficiency stress (P Rampino *et al.*, 2017). Additionally, plants are more resilient to stress recovery. Transgenic plants can be used to manipulate gene functions with the advancements in genetic transformation technologies.

5.1. Role of Transgenic approach to alleviate N deficiency

Recent advances in genetic manipulation techniques, in which isolated genes are introduced into crops, offer the potential for the engineering of metabolic pathways, and use of plant characteristics linked to the utilization of nitrogen have been discuss in this section. Although there are limitations to this technology due to inaccessibility of tissue-specific promoters, variability in transgene expression, but they can be overcome by careful selection of transgenic with desirable expression level and through backcrossing to breeding populations. Robson *et al.*, (2004), reported that promoter of maize protease gene (senescence enhancer) transformed by agrobacterium mediated gene "isopentenyl transferase" shows increased cytokinin levels and conferred a "staygreen" phenotype, delaying senescence, loss of photosynthetic activity, and days to flowering. Many genes encoding enzymes involved in nitrogen metabolism in cereals have been successively identified and cloned using genetic engineering for example, nitrate reductase, nitrite reductase, glutamate synthase (GOGAT), and glutamine synthetase (GS, EC 6.3.1.2) (Martin et al, 2006; Bernard et al, 2008; Tanaka et al, 2009). The cDNAs of Gln1-3 and Gln1-4 genes, encoding glutamine synthetase isoenzymes (GS1), were fused to the rice actin1 promoter and over-expressed in the inbred maize line DH9632 by Agrobacterium-mediated genetic transformation. Over-

expression of these two genes led to increased chlorophyll content and improved photosynthesis after 14 days. In addition, yield-related traits such as ear length, ear diameter, ear weight, grain weight per ear, and hundred-kernel weight were improved in the transgenic lines (He *et al.*, 2014). In another study, constitutive overexpression of a cytosolic GS isoform increased kernel number and grain yield approximately 30% relative to non-transgenic sibling plants (Martin *et al.*, 2006). Transgenic DP202216 hybrids have an improved N uptake during late-vegetative stages (inducing N storage in lower leaves of the canopy) and, thus, N uptake efficiency relative to WT. Transgenic plants were able to achieve better N utilization efficiency. (Javier *et al.*, 2022). The key gene ZmTGA respond to low nitrogen stress, and its function was characterized in maize mutants. The results showed that with normal nitrogen treatment, the root length of the mutants under low nitrogen treatment increased by 10.1%, while that of the wild-type increased by 14.8%; the root surface area of the wild type under low nitrogen treatment increased by 9.6%, while that of the mutants decreased by 5.2%; the root surface area of the wild type was higher than that of the mutant at both nitrogen levels; and the activities of glutathione and guaiacol peroxidase enzymes in the mutant were lower than those in the wild-type under low nitrogen treatment In summary, the mutant was less adaptable to a low nitrogen environment than the wild type (Wang *et al.*, 2022). Therefore, advances in genetic transformation technologies allow for the alteration of gene functions in transgenic plants.

6. Role of miRNA in drought and low-N tolerance

Many aspects of plant biology are impacted by mi-RNAs, which are endogenous, short, noncoding RNA molecules with a length of about 22 nucleotides (nt) (Kidner & Martienssen, 2005). Additionally, maize miRNAs that control processes associated to abiotic stress have been identified and linked to gene networks; several researchers have also suggested a gene model that illustrates their work (Ding *et al.*, 2009; Xu *et al.*, 2010). miRNAs can regulate gene expression by degrading target RNAs at the post-transcriptional level or inhibiting translation at the translation level (Bartel, 2004; Humphreys & Preiss, 2005). According to a number of studies, posttranscriptional miRNA-mediated gene expression may enhance key biological pathways for NUE, including higher plants' defense response and laccase, which is involved in catalytic oxidation to increase plant stress tolerance (Xu *et al.*, 2019). The nitrogen limitation adaptation (nla) mutant could not accumulate anthocyanins and instead produced an N limitation- induced early senescence phenotype (Peng *et al.*, 2008). In drought stress, the majority of miRNA-mediated target genes had a cis-acting region that responded to phytohormone (ABA) stimuli. In a miRNA microarray hybridization investigation reveals that 34 miRNAs showed markedly altered expression in drought stress. Target gene PDH, a negative regulator of proline buildup is downregulated in drought stress due to increased miR474, as a result improves osmo-protective reactions to drought stress (Wei *et al.*, 2009). In a study, ZmCIPK8 was cloned from maize. According to the research, ZmCIPK8 controls other genes linked to stress, which influences the way maize reacts to drought stress (Tai *et al.*, 2015). The response mechanism of maize during drought and re-watering, shows that maize response altered significantly in response to drought for 60and 96 h and re-watering for 3 days. Four miRNAs were down-regulated under drought stress; (Zma-miR529-5p, miR5072-3p, zma-miR167f-5p, ma-miR167j-5p, and zma-miR167e-5p). These miRNAs have potential roles in cell division, plant growth, stomatal closure, and the stress response. After re-watering, zma-miR167f-5p, miR5072-3p, zma-miR167j-5p, and zma-miR167e-5p were up-regulated. Among them, up-regulated miRNAs (novel-m0414-5p) targeted eight genes associated with the drought response and tissue development. These genes encoded proteins related to carotenoids (which are involved in ABA synthesis), protein phosphatase (PP2C), plant-specific serine/threonine kinase, and the ABA-responsive element binding factor (Cao *et al.*, 2021).

6.1. Role of miRNA in Nitrogen deficiency

Nitrogen metabolism in plants is controlled by several processes which are regulated at the molecular and genetic level. The miRNAs were identified by sRNA sequencing in response to nitrogen stress and understand relevant physiological changes in nitrogen-deficient maize after nitrogen resupply. The results reveal a total of 226 known miRNAs, among them 106 miRNAs were differentially expressed between the control and N-deficient groups, and 103 were differentially expressed between the N-deficient and N-resupply groups. Some differentially expressed miRNAs were predicted to target transcription factors and functional proteins. Gene Ontology (GO) analysis shows the biological function of these targets and revealed that some

miRNAs, such as miR169, miR1214, miR2199, miR398, miR408 and miR827 might be involved in nitrogen metabolism regulation (Yang *et al.*, 2019). For low-N tolerance, 304 maize accessions under field conditions were evaluated, and select the low-N sensitive Ye478 and low-N tolerant Qi319 inbred for further investigations. After a 5-day low-N treatment, yellowing of older leaves was observed in N-deficient phenotype (Ye478) but not in N-tolerant inbred (Qi319). Besides, N deficiency-responsive miRNAs were also identified a total of 15 miRNAs belonging to 10 families in root and 11 miRNAs belonging to six families in shoot. The expression level of miRNA398 was lower in Ye478 shoot than in Qi319 shoot, indicating that the oxidative stress originated from low-N is more serious in Ye478 than in Qi319 (Du *et al.*, 2021). Maize seedlings exposed to N deficiency identified eight miRNA families (five conserved and three newly identified) differentially expressed under the N-deficient condition. Predicted and degradome-validated targets of the newly identified miRNAs suggest their involvement in a broad range of cellular responses and metabolic processes. This research contributes to the understanding of the regulatory roles of miRNAs in plant adaption to N-deficiency stress (Zhao *et al.*, 2011). Therefore, most of the miRNAs either directly or indirectly affects the genes either down-regulating them or upregulate them in various biological process involve in plant growth and abiotic response.

7. Signaling network in response to drought and Nitrogen stress:

Plants are sessile organisms, they are in stress due to environmental changes, however, plants are capable to adapts in changing conditions. They balance growth, reproduction, and defense in order to survive in harsh or stressful conditions. The plants under stress conditions allocates more of its nutrients and energy to defense mechanisms rather than development processes. (Sonwald and Prasch, 2014). This intricate equilibrium of nutrition and energy allocation between growth, reproduction and defense maintained by the complex multicomponent signaling network, that integrates stress adaptive metabolic and structural changes into endogenous developmental programs and maintains the complicated balance (Krasensky and Jonak, 2012; Cramer *et al.*, 2011). Plant growth and productivity significantly limited by water deficiency in many agricultural areas of the world. Plant loss cell water in drought stress through the stomata which are present on leaf surface, consequently decreasing the cell osmotic potential., To cope with drought stress, plants close their stomata to maintain tolerable cell turgor and to endure cellular metabolism. The phytohormone abscisic acid (ABA) functions as a key regulator in the activation of plant cellular adaptation to drought stress. ABA plays crucial role in regulation of stress sensing and signaling by closing the stomata to reduce the water loss from the plants and adjust the stomatal conductance so that allow sufficient CO2 uptake for photosynthesis. Abscisic acid signals are perceived by different cellular receptors. In maize, ZmPYLs played important roles in responding to multiple abiotic stresses (He *et al.*, 2018). Transgenic lines overexpressing ZmPYL8, ZmPYL9, and ZmPYL12 were more resistant to drought. Accumulation of proline and enhanced expression of drought-related marker genes in transgenic lines further confirmed the positive roles. Baxter *et al.* (2014) emphasize the role of reactive oxygen species (ROS) as a signalling molecule in response to abiotic and biotic stress. Maize plants have enzymatic and non-enzymatic antioxidants, like GPX, APX, CAT, and SOD, to regulate and scavenge ROS generated under drought. ROS signalling and antioxidant pathway are important in maize drought tolerance (Talaat *et al.*, 2022). Nutrient signaling is influence on external and internal concentration of nutrients, plants growing in nutrient deprivation senses and adjust their growth according to the resource availability (Schachtman and Shin, 2007). It also depends on environment they evolve, as well as timings like, early and late signals. The roots are the primary sight of sensing the nitrogen deprivation. Nitrate transporter Nrt2.1 was reported to involve nitrogen sensing or transduction (Little *et al.*, 2005). Particularly in maize, several studies indicated the possible role of cytokinin in nitrogen signaling from root to shoot (Takei *et al.*, 2002; Sakakibara *et al.*, 1998; Sakakibara, 2003). On investigating the functions of NRG2 family members of *Arabidopsis thaliana* and maize showed that both AtNRG2.10 and AtNRG2.15 regulated nitrate signaling and metabolism. In addition, the maize genome harbors 23 ZmNRG2 members, the expression of these genes treated with nitrate and the highest expression of four genes was strongly induced with ZmNRG2.7. Furthermore, overexpression of ZmNRG2.7 in the atnrg2 mutant could restore the defects of atnrg2, suggesting that ZmNRG2.7 is involved in nitrate signaling and metabolism. Also overexpressed lines of ZmNRG2.7 showed increased biomass and NUE (Li *et al.*, 2024). Therefore, signaling

network is complicated and specific for each stress. However, crosstalk is common between various signaling network.

8. Conclusions and Future prospects

Maize is an important cereal crop worldwide that extensively consumed as food, fodder and fuel. Water and nitrogen deficiencies are the two main abiotic constraint which limits the high yield potential of maize crop. Current global prediction model on climate change have projected more intense and frequent episodes of drought. Maize is sensitive to drought stress, under drought stress, stomata close, reduction in CO_2 uptake, and inhibition of carbohydrate assimilated in leaves that may cause increase in ROS, which induces alterations in physiological status and disturbances in metabolic homeostasis, which deleteriously influence plant growth and development, including a marked suppression in the photosynthetic efficiencies of plants. Nitrogen (N) is an essential component for synthesizing chlorophyll and photosynthetic enzymes in plants. Its presence or absence directly influences crop photosynthesis and overall yields. Nearly every physiological activity in maize plants, including photosynthesis, transpiration, and leaf and root growth during the vegetative and reproductive stages, is impacted by drought, and low-N stressors. Physiology plays a crucial role in the identification and analysis of traits as well as the understanding of the genetic regulation of different physiological processes. In conventional breeding, the process involves selecting superior plants, crossing them with different genotypes, and then testing the progeny over several years to enhance yield under drought stress. However, this approach for developing drought-resistant varieties is time-consuming and can take many years to achieve results. In order to improve selection efficiency, new methods have been employed, such as marker-assisted selection (MAS), which maps quantitative trait loci (QTL) to examine the genetic basis of complex traits. A chromosomal fragment between two distinct breakpoints can be checked for association with a given phenotype via QTL mapping. An even more potent genetic tool is now available to thoroughly analyse the molecular and physiological causes of NUE in maize. To increase our understanding of NUE in crops by incorporating data from whole plant physiological and molecular research into this genetic tool. Recent biotechnological techniques such as genetic modification, genome-wide marker-assisted selection, transcriptome analysis, and gene editing technologies allow direct, efficient and accurate approaches to improve the traits. Transcriptional analysis is frequently used to determine gene expression for adaptation and stress response, aid in genetic dissection, and investigate the regulation of gene expression in response to abiotic stressors. Plant transformation studies can also be used to transfer beneficial genes to other crops and are helpful for functional research of genes involved in different stress responses and adaptations. In order to create drought-tolerant germplasm, genome editing tools have recently been created to quickly and precisely alter DNA sequences by modifying important genes. The genes that help to cope with drought stress are regulated by various transcriptional factors. It is important to genetically manipulate the combination of drought responsive TF genes instead each functional gene singly. Therefore, to understand the molecular mechanism of drought tolerance mining candidate genes, pathways, and interaction of genes in different pathways is more feasible approach.

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11. Conflict of Interest

The authors declare no conflict of interest.

12. Authors' Contribution

Suphia Rafique wrote this manuscript.

13. SDGs Addressed

This review addresses SDG 2: Zero Hunger by exploring molecular strategies to improve maize resilience under drought and nitrogen stress, ensuring sustainable food production, and SDG 13: Climate Action by developing stress-tolerant crops that can adapt to climate variability.

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