

## Plant Responses to Drought Stress: A Review

### Authors' name:

<sup>1\*</sup>Dr Suchinnata S. Sardar

<sup>1</sup>Sudha Suman

<sup>2</sup>Smruti Smaranika Nayak

<sup>1\*,1</sup>Dept of Botany, Rama Devi Women's University, Bhubaneswar, Odisha, India

<sup>2</sup>Dept of Industrial Microbiology, Rama Devi Women's University, Bhubaneswar, Odisha, India

Orcid ID: <https://orcid.org/0000-0002-7255-4577>

### Abstract

Drought resistance in plants is one of the most important world constraints of plant production and food security with the enhanced impact of climate change. In the present study, a review was conducted with minute attention given to plant mechanisms of drought resistance with primary emphasis on prominent morphological and physiological characteristics ensuring acclimation in plants in conditions with restricted water availability. Major morphological adaptations like modified root anatomy, curled leaves, and lowered stomatal density are dealt with in addition to physiological ones like osmotic adjustment, stomatal regulation, and detoxification of ROS. Additionally, the review describes the signal transduction mechanisms including calcium signalling, MAPK cascades, receptor kinases, and ABA-dependent as well as independent gene control networks. Transcription factors, cis-regulatory modules, and post-transcriptional regulators are evaluated for the part they play in coordinating drought-responsive gene expression. The article further gives an overview of the current progress in genetic dissecting using genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, and RNA sequencing (RNA-seq), and followed by functional validation by overexpression and knockout strategies. Particular focus is given to identifying elite alleles and their inclusion in molecular breeding pipelines. Lastly, the review shows adopted methods like marker-assisted selection, genomic selection, and gene-editing technologies in major cereal crops intending to serve as a working companion for plant biologists and breeders who aim to acquire details on the grounds of drought reaction and how it can be adopted to implement similar to introduce resilient crop cultivars against climate stress.

**Keywords:** Drought resistance; Morphological traits; Physiological responses; Gene regulation; Signal transduction; Climate resilience

### 1. Introduction

Plant water is required for plant growth and sustenance of photosynthesis, nutrient transport, and cellular metabolism. Climatic unpredictability and worldwide water deficiency have, however, rendered drought an essential plant growth and crop productivity constraint, causing 34% yearly crop yield loss (Farooq *et al.*, 2009). Drought is not a deficiency of rain but a consequence of intricate interactions of climate, soil, and hydrologic factors. It may happen at any stage of development, and reproductive ones are most vulnerable (Passioura, 2007). As future droughts were projected to be more severe and occur more often by climate models one must revisit plant response mechanisms to drought, they have developed sophisticated regulation mechanisms to perceive, signal, and react to drought at the cellular, physiological, and molecular levels. Plants have drought avoidance such as deep root growth, tolerance (e.g., osmo-protectant). They display hormonal regulation-mediated, gene expression-mediated, signal transduction-mediated, and metabolic change-mediated responses (Zhu, 2002). Master regulator is abscisic acid (ABA), which is involved in stomatal closure, other hormone regulation, and drought stress gene initiation by the PYR/PYL/RCAR-PP2C-SnRK2 signalling module (Cutler *et al.*, 2010; Umezawa *et al.*, 2010).

Transcription factors like DREB, NAC, bZIP, WRKY, and MYB families control the key genes involved in osmotic adjustment and stress tolerance (Nakashima *et al.*, 2012). Gene expression is also controlled by non-coding RNAs like

miRNAs and lncRNAs during drought (Sunkar *et al.*, 2007). Drought is sensed at the cellular level by changes in membrane integrity and water potential that leads to the activation of secondary messengers such as calcium ions, ROS, and phospholipids (Mahajan & Tuteja, 2005). These trigger MAPK signal cascades that control stress-responsive genes. Epigenetic changes like histone modification and DNA methylation also control the accessibility of genes to establish stress memory. SOD, HSPs, LEA, and CAT cause detoxification and protection through proteins (Kosová *et al.*, 2011). Proline and trehalose maintain membrane and protein stability (Obata & Fernie, 2012). Root structure is changed to increase water uptake, controlled by auxins and TFs (Uga *et al.*, 2013). New progress in genomics, GWAS, and CRISPR/Cas9 enabled the identification and editing of genes responding to drought. DREB1A or SNAC1-overexpressing transgenic plants exhibited improved drought resistance (Hu *et al.*, 2006; Zhang *et al.*, 2018).

The review considers the major regulatory pathways of drought response and how they are being applied to breeding climate-resilient crops.

## 2. Morpho-Physiological Adaptations to Drought Resistance

Drought is the most severe abiotic stress occurring in plants, especially under arid and semi-arid conditions. Plants make use of a synergy of morphological and physiological mechanisms to resist drought by stimulating water influx and minimizing water loss. These reactants adapt through the following mechanisms of adaptation in root system architecture, leaf anatomy, stomatal regulation, osmotic adjustment, and enhancement in water-use efficiency (WUE).

### 2.1. Root, Leaf and Stomatal Adaptations:

Deep roots allow the utilization of water in the subsoil, which is required during a long drought. Thick and deep drought tolerance genotypes in wheat and maize slow down transpiration and do not wilt (Comas *et al.*, 2013; Blum, 2011). Drought-tolerant rice maintains thin lateral roots to enhance topsoil water uptake after drought starts (Henry *et al.*, 2011). Longitudinal growth of lateral roots provides better anchorage and nutrient uptake. Hydraulic conductance, which is usually regulated by aquaporins, also facilitates water uptake. Overexpression of aquaporin facilitates water transport even in the absence of water potential in the soil (Vandeleur *et al.*, 2009; Pou *et al.*, 2013).

Leaf rolling in rice and sorghum minimizes exposed surface area and cuts transpiration by the formation of a humid microclimate (Sharma *et al.*, 2015). Enhanced deposition of wax lowers cuticular water loss, for example, in wheat and barley that are tolerant to drought (Shepherd & Griffiths, 2006). Leaf area decreases in certain species due to senescence of older leaves to conserve water. Others leaf features such as stomata sunken, trichomes, and low stomatal density (Xu & Zhou, 2008) also minimize water loss. Reduced stomatal density has been associated with drought tolerance in barley by QTL mapping (Franks *et al.*, 2015).

ABA, calcium, and ROS signalling rapidly open stomata. Overexpression of Arabidopsis OST1 is heightened under ABA-mediated stomatal regulation, leading to promotion of WUE (Umezawa *et al.*, 2009). Other species prefer more but narrower stomata, aiming for a trade-off between CO<sub>2</sub> entry and water loss.

### 2.2. Osmotic Adjustment and Cell-Water Relations

Under drought stress, plants also accumulate compatible solutes such as proline, glycine betaine, and sugars that contribute to turgor and cell function protection (Ashraf & Foolad, 2007; Yancey, 2005). Rice is induced by root elongation through solute storage expression of inducible tonoplast transporters (Martinoia *et al.*, 2007). Relative water content (RWC) is one of the most significant indicators of drought and is a measure of yield under water stress (Barrs & Weatherley, 1962; Blum, 2011).

### 2.3. Water-Use Efficiency (WUE)

Increased WUE is expressed as greater carbon fixed per water lost. Methers' drought sorghum has desirable A/e ratios and Rubisco activation under stress maintains photosynthesis. Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) estimates long-term WUE and is heritable and thus ideal for breeding. Phenotyping of WUE traits can be done now using hyperspectral imaging (Cobb *et al.*, 2013).

Nerica, drought-tolerant rice and wheat landraces have traits like root depth, enhanced stomatal control, and osmolyte concentration. Yield and root depth are controlled by genes like DRO1 and QTLs in 7A and 2B chromosomes (Yuga *et al.*, 2013). In maize, the "stay-green" trait maintains photosynthesis under stress (Borrell *et al.*, 2014).

Together, morpho-physiological traits impart essential drought resistance in the form of water uptake facilitation and loss reduction both areas of future crop improvement.

## 3. Biochemical parameter dynamics under drought

Plant drought stress induces extensive arrays of biochemical processes of vital significance for plant survival and adaptation. These biochemical indicators are also widely applied to determine drought tolerance and direct breeding activities. Some of the most vital parameters are osmolyte accumulation, antioxidant activity (enzymatic and non-enzymatic), and reactive oxygen species (ROS) metabolism.

Proline is the best-studied osmo-protectant. It accumulates in drought-resistant genotypes of barley and wheat at high concentrations and is involved in osmotic adjustment, membrane stabilization, as well as ROS detoxification (Ashraf & Foolad, 2007). Proline synthesis takes place via the glutamate pathway by utilizing P5CS, an enzyme regulated by drought (Verslues & Bray, 2006). Glycine betaine, another osmolyte, membrane and protein stabilizer and is biosynthesized in crops like sugarcane and maize when waterlogged (Chen & Murata, 2008). Drought tolerance has been enhanced by genetic engineering through an increase in glycine betaine synthesis (Sakamoto & Murata, 2002). Osmotic adjustment and stress signalling are also facilitated by soluble carbohydrates like trehalose, sucrose, and raffinose (Ruan *et al.*, 2010).

Drought-induced stomatal closure limits CO<sub>2</sub>, and ROS is accumulated in the form of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>•-</sup> and may initiate oxidative damage (Mittler, 2002). Lipid peroxidation is a byproduct that is represented by malondialdehyde (MDA), and it is an indicator of oxidative stress and increases in sensitive genotypes (Gill & Tuteja, 2010). Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) scavenge ROS and maintain redox balance (Alscher *et al.*, 2002). Ascorbate, glutathione,  $\alpha$ -tocopherol, and carotenoids are some of the non-enzymatic antioxidants that also play a role in ROS scavenging as well as stress protection (Smirnoff, 2000).

Polyamines like putrescine and spermidine, which have the role of stabilizing membranes and stress signalling, are induced under drought stress (Alcázar *et al.*, 2010). Biochemical traits like proline accumulation and antioxidant activities are suitable markers for the screening of drought tolerance in breeding (Silva *et al.*, 2010). These traits have been localized to QTLs and utilized in marker-assisted selection (Tuberosa *et al.*, 2002).

#### 4. Secondary Metabolite Modulations Under Drought Stress

Plants developed sophisticated defence systems, such as secondary metabolite modulation, in order to acclimatize under drought stress. Although the metabolites are not directly implicated in growth and reproduction processes, they have pivotal roles in stress acclimation by stabilizing the cell structure, detoxifying ROS, and modulating the stress signalling (Selmar, 2013).

Flavonoids such as flavonols and anthocyanins are accumulated in drought-stressed plants such as *Sorghum bicolor* and *Oryza sativa*, providing antioxidant defence and auxin transport regulation (Nakabayashi *et al.*, 2014). Anthocyanins are also naked eye-visible stress markers, helping to relieve photooxidative stress (Chaves *et al.*, 2009). Tannins as antioxidants are induced during drought stress in legumes (Nguyen *et al.*, 2016). Lignin biosynthesis is also initiated to enhance cell walls and lower water loss (Lee *et al.*, 2017). Phenylpropanoid pathway is also controlled by drought stress that suppresses major enzymes such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and cinnamate-4-hydroxylase (C4H) (Dixon & Paiva, 1995). The enzymes are controlled by transcription factors such as MYB and bHLH, and overexpression has enhanced drought tolerance in plants (Stracke *et al.*, 2001). Carotenoids such as drought-tolerant maize and tomato function in photoprotection and as an abscisic acid (ABA) precursor responsible for regulating stomatal closure and stress signalling (Dall'Acqua *et al.*, 2019).

Alkaloids such as tobacco's nicotine and lupins perform osmotic regulation and stress signalling roles (Facchini, 2001; Zhao *et al.*, 2006). Glucosinolates in *Brassica napus* are broken down to bioactive metabolites with antioxidant and antimicrobial properties under stress (Hasegawa *et al.*, 2000). More contemporary transcriptomic and metabolomic information has outlined drought-controlled metabolites like saponins and coumarins in *Medicago truncatula* and tomato (Urano *et al.*, 2009). Few of these genes involved in the pathways, i.e., PAL, NCED, and CHS, are associated with drought tolerance by marker-assisted selection and GWAS (Des Marais *et al.*, 2012). The secondary metabolites are therefore efficient markers to be used for crop plant breeding for drought tolerance and candidate genes for genetic improvement.

#### 5. Genomic modulations throughout Drought

The plants respond with complex genomic modulations under drought stress by activating genes and reprogramming the transcriptional networks for survival. These changes control water perception, signalling, and adaptive metabolism. Genomic tools like transcriptomics, GWAS, and gene editing have enabled the possibility of delivering information regarding drought-responsive genes and control factors to a very large extent.

In water stress, genes belonging to two large categories are induced:

- (i) Biosynthetic genes encoding proteins like LEA proteins, aquaporins, and enzymes involved in osmolyte biosynthesis
- (ii) Regulatory genes like transcription factors (TFs), kinases, and ABA pathway components

DREBs (DREB2A, DREB2B) regulate drought stress via DRE/CRT elements, and overexpression increases the drought tolerance of crops (Xu *et al.*, 2011). SNAC1 and OsNAC6, among the NAC TFs, promote stress-induced gene expression and increase drought yield (Hu *et al.*, 2006). WRKY, MYB, and bZIPs like ABFs are some other TFs playing a role in ABA-dependent transcription (Fujita *et al.*, 2005; Golldack *et al.*, 2011).

RNA-seq detects thousands of drought-response genes like RD29A, COR15A, and KIN1 in Arabidopsis (Seki *et al.*, 2002). Transcription-associated gene expression is higher and ABA is higher in drought-tolerant lines of *Oryza sativa* (Lenka *et al.*, 2011). ROS detoxification genes and water transport genes are induced in Zea mays and Glycine max (Zhou *et al.*, 2020; Li *et al.*, 2013). Promoters have motifs like DRE, ABRE, MYB/MYC, controlled by some TFs (Nakashima *et al.*, 2006). Epigenetic control by histone acetylation (H3K9) and DNA methylation are involved in drought memory (Kim *et al.*, 2015).

CRISPR-Cas9 editing, i.e., OsSAPK2 knockout, improves drought tolerance in rice (Miao *et al.*, 2020). QTLs qDTY12.1 (OsNAM12.1 gene) are applied for yield improvement by drought (Vikram *et al.*, 2011). GWAS identified drought-tolerant SNPs like ZmNAC111 in maize (Mao *et al.*, 2015), and genomic selection (GS) decreases breeding cycles (Cooper *et al.*, 2014).

Network analysis and systems biology have recognized some of the major regulators such as DREB2A and NAC072 (Maruyama *et al.*, 2012). Transgenics with improved water efficiency and yield have been developed using genes such as AtDREB1A, AVP1, and SNAC1 (Park *et al.*, 2005). Genomics in combination with physiology and quantitative breeding has the ability to produce stress-tolerant crops.

## 6. Proteomic Alterations Under Drought Stress

Plant proteomes are drastically affected by drought stress, and modifications in the level of proteins, post-translational changes, and degradation pathways are controlled in the plant to enable it to perceive water-deficit conditions. The proteomic changes comprise several functional classes such as stress protectant proteins, enzymes of energy metabolism, antioxidant defence system, signalling proteins, and those related to cellular homeostasis. In contrast to transcriptomic adaptation, proteomic adaptation encompasses both transcriptional as well as overall post-transcriptional control and thus accurately represents drought adaptation at the molecular level (Kosová *et al.*, 2011).

Plants accumulate various classes of proteins in response to drought stress:

- Stress-protection proteins: LEA proteins, dehydrins, HSPs
- Antioxidant enzymes: SOD, APX, CAT
- Signalling molecules: kinases, phosphatases
- Metabolic enzymes: glycolysis, photosynthesis, lipid metabolism
- Transport proteins: aquaporins, ion channels
- Proteolysis proteins: proteases, ubiquitin ligases

### 6.1. Regulation of Functional Proteins Expressed for Energy Metabolism Under Drought Stress

Drought stress suppresses photosynthesis through stomatal closure chiefly and oxidative degradation of photosynthetic proteins. Drought-resistant plants, nonetheless, do not down-regulate the activity of essential photosynthetic proteins like Rubisco activase, chlorophyll a/b-binding proteins, and photosystem II subunits to enable carbon fixation during drought (Sharma and Dubey, 2005; Xu *et al.*, 2017). Glycolytic enzymes like glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enolase, and pyruvate kinase are up-regulated to supply energy and metabolic intermediates (Wang *et al.*, 2019). Tricarboxylic acid cycle enzymes isocitrate dehydrogenase and malate dehydrogenase are further upregulated in abundance levels to enhance respiratory metabolism for the upkeep of cellular energy demands (Li *et al.*, 2018). ATP synthase and cytochrome c oxidase are further regulated to maximize ATP in stress conditions. Proteins that regulate redox like thioredoxins and peroxiredoxins regulate the production of reactive oxygen species (ROS) in energy metabolism to avoid oxidative damage (Mittler, 2002).

### 6.2. Regulations of Functional Proteins responsible for non- energy metabolism

Proteins of non-energy metabolism deal with structural support, cell defence, and osmotic adjustment. LEA proteins and dehydrins accumulate to stabilize proteins and membranes during dehydration stress. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione-S-transferase (GST)

neutralize excess ROS produced under drought, preventing oxidative stress (Foyer and Noctor, 2005). Cell wall-mediating proteins such as expansins and xyloglucan endotrans-glycosylases (XTHs) ensure cell wall extensibility and structural integrity (Tenhaken, 2015).

Protein chaperones such as heat shock proteins (HSPs) promote protein folding and prevention of aggregation caused by protein denaturation during drought stress (Wang *et al.*, 2004). Proteolytic enzymes and ubiquitin-proteasome pathway degrade damaged proteins, providing proteome quality control during stress (Vierstra, 2009). Proteins are typically abscisic acid (ABA) signal-regulated and specific transcription factor-regulated, integrating drought response networks (Cutler *et al.*, 2010).

In summary, proteomic profiling indicates drought tolerance needs a delicate balance between energy metabolism preservation and cell component acquisition, and it designates the supreme molecular targets for improvement of crops.

## 7. Non-Enzymic Antioxidants in Drought Stress Response

Drought stress diminishes water availability and leads to the over-production of reactive oxygen species (ROS) like hydrogen peroxide ( $H_2O_2$ ), superoxide anion ( $O_2^-$ ), and hydroxyl radicals ( $\bullet OH$ ), which harm cellular constituents. Plants counteract this by using non-enzymic antioxidants low molecular weight molecules that detoxify ROS directly or recycle other antioxidants. The major non-enzymic antioxidants are ascorbic acid (vitamin C), glutathione (GSH), tocopherols (vitamin E), carotenoids, and phenolic compounds (Gallie, 2013).

Ascorbic acid detoxifies ROS in the cytosol and organelles, most notably by the ascorbate–glutathione cycle where ascorbate peroxidase (APX) utilizes ascorbate in reducing  $H_2O_2$  to  $H_2O$ . Its biosynthesis utilizes GDP-mannose pyrophosphorylase and L-galactono-1,4-lactone dehydrogenase (GLDH) enzymes that are induced by drought (Smirnoff, 2018). Glutathione is a direct ROS scavenger and substrate for glutathione peroxidase and glutathione-S-transferase, and helps regulate redox balance by protecting the GSH/GSSG ratio, which is very important in stress tolerance (Noctor *et al.*, 2012).

Tocopherols, lipid-soluble antioxidants, defend membranes against lipid peroxidation and preserve thylakoid membrane integrity under drought. They are synthesized at higher levels in response to drought-dependent genotypes like sunflower and soybean (Munne-Bosch & Alegre, 2002; Kruk & Strzalka, 2001). Carotenoids such as  $\beta$ -carotene and lutein scavenge singlet oxygen and guard chlorophyll, while drought-dependent species keep carotenoids at equivalent concentration or even elevate carotenoid concentrations, controlled by genes like phytoene synthase (Dall'Acqua *et al.*, 2019).

Phenolic compounds such as flavonoids and tannins detoxify free radicals and metal ions. Phenylpropanoid pathway enzymes chalcone synthase (CHS) and flavonol synthase (FLS) control biosynthesis of flavonoids, which is elevated in drought plants, and boosts oxidative stress resistance (Nakabayashi *et al.*, 2014). Tannins and lignin enhance resistance against pathogens and water loss by making the cell wall thicker. (Lee *et al.*, 2017).

Other metabolites, such as alkaloids, glucosinolates, and terpenoids, are also involved in osmo-protection, antioxidant function, and membrane stabilization in drought (Facchini, 2001). Such antioxidants may be assayed by spectrophotometry or chromatography for quick screening in breeding schemes (Silva *et al.*, 2010).

Genes involved in antioxidant biosynthesis (e.g., VTC1, GSH1, VTE1, CHS) have been associated with drought tolerance based on QTL mapping and GWAS, and those SNP markers have been employed in marker-assisted selection. Transgenic and CRISPR strategies altering those pathways increase drought resistance without cost of yield, thereby rendering non-enzymic antioxidants as putative targets to enhance drought tolerance of crops (Des Marais *et al.*, 2012; Welsch *et al.*, 2008).

## 8. Plant Hormone Control of Drought Response in Plants

Plant hormones, or phytohormones, have important roles in drought stress adaptation of plants by regulating growth, development, and stress tolerance by modulating signal networks. Phytohormones regulate stomatal closure, osmotic adjustment, antioxidant defence, and gene expression and would thus be key to explaining crop drought tolerance improvement through breeding.

Abscisic acid (ABA) is the major drought hormone, accumulating quickly in response to water stress and activating tolerance responses such as stomata closure via cytosolic  $Ca^{2+}$  elevation in guard cells to minimize water loss. ABA signalling is regulated by receptors (PYR/PYL/RCAR), phosphatases (PP2C), and kinases (SnRK2), which manage drought-responsive genes (Cutler *et al.*, 2005). ABA also has opposing effects to such growth hormones as gibberellins (GAs) and auxin (IAA) but is synergistic with jasmonic acid (JA) in regulating drought response transcription factors. JA, similarly, was traditionally known to respond for defence against biotic stress but also is responsible for increasing drought tolerance by triggering gene action through the MYC2 pathway and ABA signal crosstalk (Danquah *et al.*, 2014). Genes controlling JA and ABA pathways are gene breeding and editing targets.



Salicylic acid (SA) controls ROS scavenging and osmolyte accumulation under drought through high concentrations of antioxidant enzymes and proline, although in a general counteractive relationship with ABA and JA, reflecting hormonal complexity (Miura & Ohta, 2010). Auxin regulates the root morphology by facilitating increased water uptake through the re-organization of its transport by regulation via PIN and AUX1 carriers; ABA stress signalling during drought suppresses auxin transport in order to restore the growth balance similarly (Farooq *et al.*, 2009).

Gibberellins are usually downregulated in drought, where DELLA proteins build up to suppress growth and conserve energy, and cytokinins decrease in a bid to limit cell growth and division, regulating resources (Zwack & Rashotte, 2015). The action of ethylene is not only multi-scalar but also utilizes multifaceted mechanisms whereby it induces stress responses or senescence, differentially affecting ABA and JA pathways according to the intensity of drought (Anderson *et al.*, 2004).

Brassinosteroids (BRs) control cell enlargement and drought tolerance by activating drought-responsive genes like DREB and RD29, enhancing water use through BRI1 and BIN2 receptor-mediated signalling (Zhou *et al.*, 2006). All of these hormones form an interaction network controlling morphological and molecular drought adaptation. All of these pathways are potential targets for marker-assisted selection, gene editing, and genetic engineering to develop enhanced drought-tolerant crops for production in water-limited environments.

### 9. Mechanism of Gene Regulation During Drought Stress

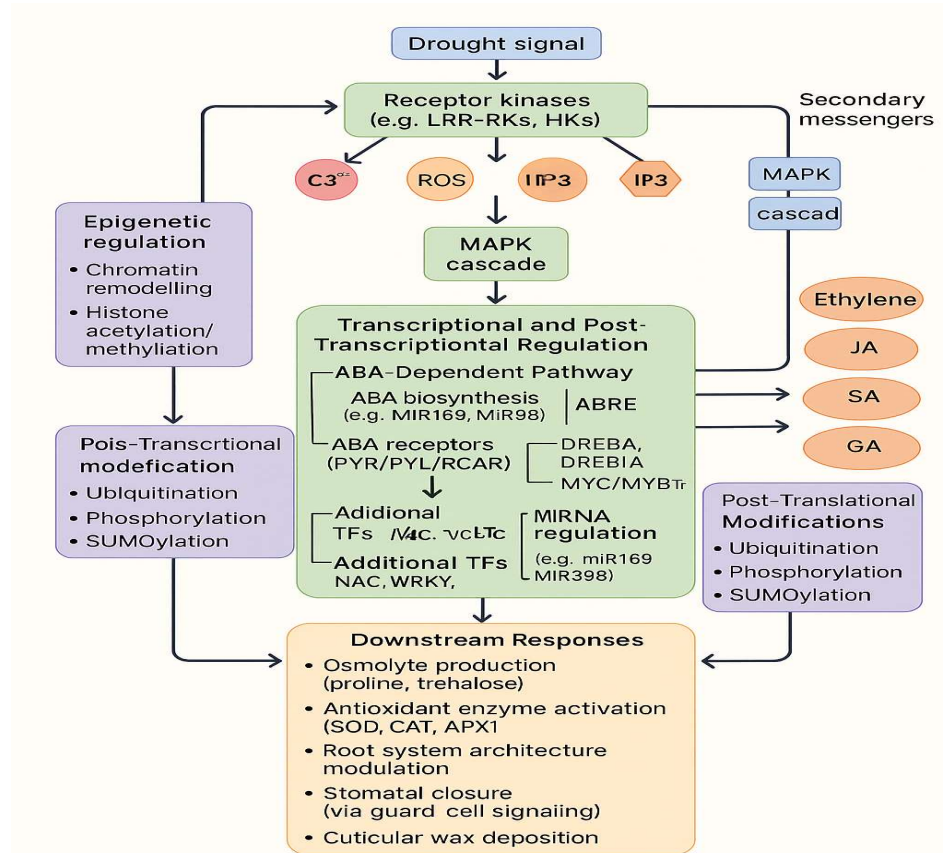
Drought stress is a significant abiotic factor globally repressing plant growth and yield. Elucidation of drought-affected gene expression pathways is significant in improving plant tolerance. Plants evolved through adaptation to drought involve gene expression changes, where adaptive processes like stomatal closure, osmotic adjustment, and antioxidant defence are involved. Two significant pathways control drought-affected gene expression: ABA-independent and ABA-dependent.

In **ABA-dependent pathway**, abscisic acid (ABA) is built up in response to water loss and initiates signalling after it binds to PYR/PYL/RCAR receptors. Inhibition of type 2C protein phosphatase (PP2C) facilitates SnRK2 kinases to turn on transcription factors like ABFs and AREBs. They interact with ABA-responsive elements (ABREs) of gene promoters to control drought tolerance genes for stomatal closure, osmoprotectant biosynthesis, and antioxidant defence (Cutler *et al.*, 2010). ABRE element regulation has breeding potential in the production of drought-resistant crop plants (Kim *et al.*, 2010).

**ABA-independent** pathway includes transcription factors such as DREB and MYC, which interact with dehydration-responsive elements (DREs) of drought-responsive gene promoters that regulate biosynthesis of osmolytes, ion homeostasis, and oxidative stress responses. Overexpression of DREB1A increases drought tolerance in some plants (Shinozaki *et al.*, 2003). The pathway is typically regulated by other hormones and reactive oxygen species produced during drought.

**Table:1- Key transcription factors (TFs) involved in drought stress responses in plants, their families, functions, and representative roles in gene regulation**

<b>Transcription Factor Family</b>	<b>Representative TFs</b>	<b>Function in Drought Stress</b>	<b>Regulatory Role</b>
AP2/ERF (including DREB)	DREB1A, DREB2A	Induce expression of drought-responsive genes in ABA-independent pathways	Bind to dehydration-responsive elements (DRE/CRT) in promoters of target genes
bZIP	ABF1, ABF2, ABI5	ABA signalling; regulate late embryogenesis and stress-inducible gene expression	Bind to ABA-responsive elements (ABREs) and modulate gene expression during water deficit
NAC	SNAC1, OsNAC10, ANAC019	Regulate osmotic balance, stomatal function, and root development	Activate drought-inducible genes such as LEA and RD genes; enhance root growth for water uptake
MYB	AtMYB2, OsMYB2, TaMYB30-B	Control proline biosynthesis, ROS detoxification, and stomatal closure	Bind MYB recognition elements (MRE) and modulate transcription of antioxidant and stress genes
WRKY	WRKY33, WRKY40, WRKY46	Modulate ABA signalling, oxidative stress, and pathogen interaction	Bind to W-box elements in promoters and act as transcriptional activators or repressors
bHLH	bHLH122, ICE1	Control stomatal regulation and drought recovery	Interact with ABA and JA pathways, regulate stress-responsive and development genes
HD-ZIP	ATHB7, ATHB12	ABA-induced expression under drought stress	Regulate genes related to water retention and stress adaptation



**Fig.1- Mechanism of Gene Regulation During Drought stress**

### 10.1. Upregulation of Genes under Drought Stress

Drought stress triggers the expression of some plant genes that allow them to survive and resist water-limited conditions. They code for the drought-induced proteins, osmo-protectants, antioxidant enzymes, and regulatory transcription factors that regulate the drought response. These mechanisms have applications in producing drought-resistant crops through molecular breeding.

Among the transcription factors, DREB (Dehydration Responsive Element Binding) family is important. DREB1A/CBF3 is inducible by DRE and acts on DRE/CRT elements in gene promoter regions to induce stress tolerance by activation of defence genes (Yamaguchi & Shinozaki, 2006; Kasuga *et al.*, 1999). DREB2A is induced under extreme dehydration conditions and regulates osmotic adjustment and protein stabilization genes (Sakuma *et al.*, 2006). The NAC family also should be added; Arabidopsis RD26 and rice SNAC1 are drought- and ABA-induced and play roles in root elongation and water storage (Hu *et al.*, 2006; Fujita *et al.*, 2004). Drought tolerance roles of MYB transcription factors like AtMYB2 and OsMYB4 include regulation of proline accumulation and antioxidant defence (Vannini *et al.*, 2004; Abe *et al.*, 2003). WRKY transcription factors such as WRKY30 and WRKY57 induce ABA signalling and produce stress-inducible genes accountable for drought resistance (Jiang & Deyholos, 2006; Jiang *et al.*, 2012).

Osmo-protectant genes such as proline, glycine betaine, and sugar are also induced by plants to stabilize proteins and allow homeostasis within the cell during drought. Proline accumulation is needed, which is regulated by P5CS enzyme catalysing its biosynthesis whereas proline degradation by Proline Dehydrogenase (ProDH) is suppressed in an attempt to sustain high levels of proline. Drought-induced Late Embryogenesis Abundant (LEA) proteins like Dehydrins (DHNs) are overexpressed upon drought to shield cells from dehydration damage through protein and membrane stabilization (Tunnacliffe & Wise, 2007; Close, 1996).



**Table:2- Up-Regulatory Genes in Drought Stress Tolerance**

Gene Name	Pathway / Function	Role in Drought Tolerance
<b>DREB1A (CBF3)</b>	ABA-independent, transcription regulation	Activates expression of stress-inducible genes; enhances tolerance to dehydration
<b>RD29A (LTI78)</b>	late embryogenesis abundant (LEA) protein	Protects cellular structures during dehydration; maintains osmotic balance
<b>NCED3</b>	ABA biosynthesis	Increases ABA levels under drought stress; triggers ABA-dependent responses
<b>P5CS1</b>	Proline biosynthesis	Promotes osmolyte accumulation to stabilize proteins and membranes
<b>LEA14</b>	LEA protein synthesis	Stabilizes proteins and membranes under water-deficit conditions
<b>SNAC1</b>	NAC transcription factor	Regulates stomatal closure and root development
<b>AREB1/ABF2</b>	ABA-responsive bZIP transcription factor	Enhances ABA signalling and expression of stress-responsive genes
<b>RAB18</b>	ABA-responsive gene	Involved in dehydration and osmotic stress responses
<b>ERD1</b>	Early response to dehydration	Involved in protein turnover and protection during drought
<b>MYB2</b>	MYB transcription factor	Regulates proline biosynthesis and stress-responsive genes
<b>TPS1</b>	Trehalose biosynthesis	Enhances osmotic stress tolerance through sugar signalling and stabilization

### 10.2. Downregulation of Genes Under Drought Stress

During drought stress, in addition to expression of the drought-responsive genes, other growth genes, photosynthesis genes, metabolism genes, and cell division genes are repressed in order to conserve energy and resources for survival. Stress tolerance at the expense of growth is achieved by specific repression inhibiting cell division, elongation, and biomass production. For instance, Expansin coding genes (EXPA1, EXPA2, EXPB1, EXPB2) that relax the cell walls in order to trigger growth in the plant are down-regulated to produce the low leaf area and cell growth. Low leaf area is an indicator of protection against water loss through transpiration in order to enable better survival under drought tolerance (Sade *et al.*, 2011). Thus, gibberellin biosynthesis genes like GA20-oxidase and GA3-oxidase are suppressed to minimize development in number, while GA-inactivating genes like GA2-oxidase are activated to retard development further (Achard *et al.*, 2006).

Photosynthesis gene are also downregulated to save energy and water loss, leading to senescence of leaves (Chaves *et al.*, 2009). Photosynthetic enzyme gene like RuBisCO subunits (RBCS, RBCL) and chlorophyll biosynthesis enzymes (CAO, POR) downregulate, leading to reduced carbon fixation, chlorophyll degradation, and yellowing of the leaf. Light-harvesting complex proteins (Lhcb1, Lhca4) also downregulate, which reduces photosynthetic electron transport (Zhou *et al.*, 2019).

Being one of the measures to conserve water, stomatal opening genes such as Plasma Membrane H<sup>+</sup>-ATPase (AHA), Aquaporins (PIP1, PIP2), and Guard Cell K<sup>+</sup> channels (KAT1) are repressed to repress stomatal aperture and also transpiration (Merlot *et al.*, 2007).

Genes involved in cell wall synthesis like nitrogen assimilation metabolic pathways (NIA1, NIA2), cellulose and lignin biosynthesis (CesA, PAL), and flavonoid and anthocyanin biosynthesis (FLS, CHS) are downregulated to conserve energy to divert resources towards stress response rather than growth or pigmentation (Cabane *et al.*, 2012).

Drought tolerance is dependent on selective gene downregulation and could be a master key for improving drought-resistant crops through genetic modification.

### 10.3. Other Regulatory Mechanisms of Gene expressions under Drought Stress

Gene expression regulation in drought stress is ABA-dependent because it is also ABA-independent. In the ABA-independent pathway, cis-regulatory elements such as CCA-TCC are involved in the regulation of drought-responsive genes. Cis-regulatory elements are sequestered by DREB transcription factors that are involved in improving drought tolerance and thus are good candidates for genetic improvement.

Alternative splicing offers the possibility of generating multiple isoforms of proteins from a single gene, and thus transcript diversity is increased for the sake of stress adaptation. Alternative splicing of ion transporter genes such as NHX offers the plant a strategy to respond to excess or deficiency of ions in order to shed water-limited states (Vensel *et al.*, 2013).

MicroRNAs (miRNAs), or small non-coding RNAs, are involved in gene expression regulation by inhibiting translation or causing mRNA degradation. A number of miRNAs are also known to be involved in drought response; for instance, miR169 inhibits NF-YA transcription factors in the ABA signalling pathway, which regulates stomatal closure and osmotic adjustment in drought tolerance (Wu *et al.*, 2009).

Advances in the discovery of these regulating mechanisms have enabled improving drought tolerance in crops by marker-assisted selection and CRISPR/Cas9 gene editing technology. CRISPR/Cas9 is today a handy tool for specific editing of drought-regulated genes, for example, DREB and ABF transcription factors, to improve drought tolerance in major crops like rice, wheat, and maize (Bortesi *et al.*, 2016). More understanding of these molecular processes will speed up the development of drought-resistant crop varieties to sustain food production under progressively deteriorating water scarcity conditions triggered by climate change.

**Table:3- Down-Regulatory Genes in Drought Stress**

Gene Name	Pathway / Function	Role in Drought Stress
<b>CAB1 (LHCB1)</b>	Light-harvesting chlorophyll-binding protein	Down-regulated to reduce photosynthesis and limit ROS production under drought
<b>RBCS</b>	Small subunit of Rubisco enzyme	Suppressed to minimize carbon fixation and conserve energy
<b>EXP1</b>	Expansin protein	Repression reduces cell wall loosening and growth during water deficit
<b>XET</b>	Xyloglucan endotransglucosylase	Down-regulated to inhibit cell expansion under drought stress
<b>TUB6</b>	$\beta$ -Tubulin	Repression affects cytoskeleton dynamics and growth inhibition
<b>CYCD3</b>	Cyclin D3	Reduced expression slows down cell cycle progression under stress
<b>GA20ox1</b>	Gibberellin biosynthesis enzyme	Down-regulated to suppress growth-promoting gibberellin signalling
<b>SUS1</b>	Sucrose synthase	Repression limits sucrose breakdown, conserving carbohydrate reserves
<b>PEPC</b>	Phosphoenolpyruvate carboxylase	Reduced activity slows metabolism under dehydration
<b>TPS7</b>	Trehalose-6-phosphate synthase	Suppressed in some species to limit sugar signalling under severe drought

### 11. Drought Stress-Induced Signal Transduction

Plant drought stress responses trigger complex cascades of signal transduction that convey extracellular water scarcity signals to adaptation-critical cellular and molecular responses. Important signalling pathways include calcium signalling, reactive oxygen species (ROS), phospholipid signalling, mitogen-activated protein kinase (MAPK) cascades, and receptor-like kinases (RLKs).

Calcium ions ( $\text{Ca}^{2+}$ ) are the pivotal secondary messengers in drought and produce characteristic "calcium signatures" in cytosol and organelles. They are detected by calcium-binding proteins such as calmodulin (CaM), calcium-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs), that stimulate the drought response pathways downstream (Dodd *et al.*, 2010). ROS molecules such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide ( $\text{O}_2^{\bullet-}$ ) act as signalling molecules at low concentration, which influence ion channels, transcription factors, and protein kinases.  $\text{Ca}^{2+}$ -controlled NADPH oxidases (RBOHs) produce ROS under drought, forming a feedback mechanism where ROS induces  $\text{Ca}^{2+}$  entry, controlling processes like stomatal closure and antioxidant defence (Mittler *et al.*, 2011).

MAPK cascades convey signals through serial MAPKKK and MAPKK and MAPK phosphorylation. MAPK cascades translate upstream ROS,  $\text{Ca}^{2+}$ , and receptor kinase signals into modulating gene expression. Arabidopsis MAPK6 phosphorylates WRKYs and MYBs stress-related transcription factors, and rice MAPKs OsMAPK5 and OsMAPK33 are implicated in ABA-dependent and independent drought responses (Danquah *et al.*, 2014).

Phospholipid signalling is regulated by phospholipase C (PLC) and phospholipase D (PLD) enzymes that generate second messengers as inositol trisphosphate ( $\text{IP}_3$ ) and phosphatidic acid (PA).  $\text{IP}_3$  activates the release of  $\text{Ca}^{2+}$  from within the cell, enhancing calcium signalling, and PA controls kinases and protein recruitment during drought (Zhu, 2016).

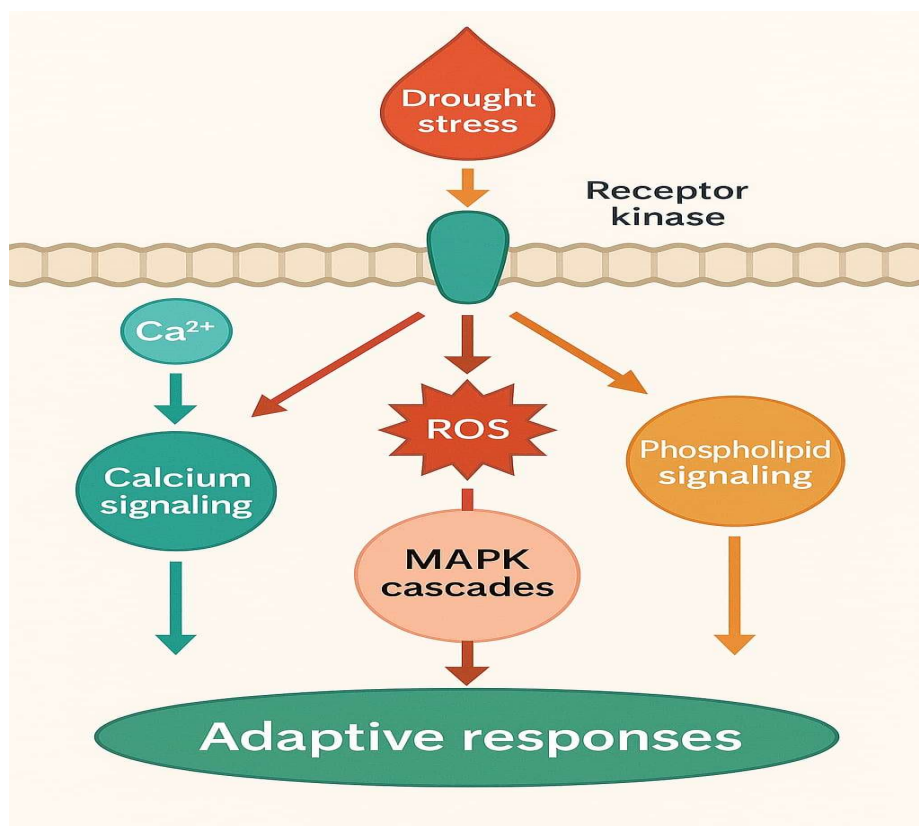


Fig.2- Illustrating signal transduction in plants under drought stress

Plasma membrane receptor-like kinases (RLKs) sense drought-induced signals and initiate cascades of signals. Wall kinases (WAKs), leucine-rich repeat RLKs (LRR-RLKs), and some receptors like FERONIA (FER) sense cell wall integrity and pass the information to ROS and MAPKs (Stegmann *et al.*, 2017). Histidine kinase AHK1 is an osmosensor that triggers phospho-relay signalling to transcription factors like ARR, linking osmotic stress to gene expression (Tran *et al.*, 2007). Receptor-like cytoplasmic kinases (RLCKs) enable biotic stress response interaction with drought signal with cross-talk being necessary for integrative adaptation.

Signal transduction cascades all form an integrative network that perceives drought and triggers adaptive gene expression and the adaptive physiological response. Upstream mechanisms are of great importance as a target for genetic improvement and crop breeding towards improved drought tolerance.

## 12. Recent Advances in Genetic Analysis of Drought-Responsive Genes

Plant drought tolerance is controlled by complex networks of genes and regulatory pathways, and genomics has made great leaps of progress in unravelling these processes over the past decade. Genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, and RNA sequencing (RNA-seq) are some of the technologies employed which are used to map candidate genes linked to drought response.

GWAS is used to screen natural genetic variation to link single nucleotide polymorphisms (SNPs) with drought traits. GWAS has identified root depth loci and leaf rolling loci in rice, and in maize, SNPs near ZmDREB2A and ZmNAC111 are linked with stomatal control and root architecture (Kumar *et al.*, 2014; Xiang *et al.*, 2017). The technique was also applied to soybean, wheat, and chickpea to detect SNPs linked with physiological drought response.

Bi-parental populations are employed for use in QTL mapping in order to associate genetic markers with phenotypic variation. QTLs for water use efficiency and yield have been mapped within wheat, deep rooting in rice drought avoidance being controlled by the QTL DRO1. These loci are valuable targets for MAS. RNA-seq enables the detection of drought-responsive expression profiles and genes. Thousands of differentially expressed genes that include protective proteins and transcription factors have been detected in Arabidopsis and crops like maize and soybean (Harb *et al.*, 2010; Liu *et al.*, 2020). Combining RNA-seq with GWAS and QTL information strengthens candidate gene selection.

Functional validation through overexpression or knock-out has worked. Arabidopsis DREB1A overexpression in wheat increased drought resistance, and repressor knock-out like OsERF922 in rice enhanced resistance (Du *et al.*, 2018). High-performance alleles discovered with natural variation or genome editing, such as maize ARGOS8 base editing, are excellent tools for developing drought-tolerant cultivars with no yield penalty (Shi *et al.*, 2017).

## 13. Emerging Technologies in Enhancement of Drought Tolerance in Crops

Breeding drought resistance is necessary due to climate change and water limitations. Recent advances in molecular breeding marker-assisted selection (MAS), genomic selection (GS), transgenic methodologies, and CRISPR/Cas9 genome editing have driven the production of drought-resistant cultivars in rice, wheat, maize, and sorghum.

MAS links the characters correlated with drought with molecular markers and enables precise introgression of tolerance alleles. For example, rice has been improved by introducing the Deeper rooting 1 gene, and wheat has been improved by canopy temperature and water use efficiency QTLs (Uga *et al.*, 2013; Simmonds *et al.*, 2014). GS provides drought tolerance prediction using genome-wide marker information and performs better than traditional approaches for maize and sorghum (Crossa *et al.*, 2017; Santantonio *et al.*, 2022).

Genetic engineering has also improved drought tolerance via overexpression of stress-inducible genes such as DREB1A and OsNAC6, causing biomass and root growth in rice and wheat (Pellegrineschi *et al.*, 2004). CRISPR/Cas9 facilitates direct editing of endogenous genes without going around transgenic problems. Edited rice OsERA1 knockout and maize ARGOS8 promoter improved drought tolerance and yield with no yield penalty (Shi *et al.*, 2017; Yu *et al.*, 2022). The TaDREB2 and TaMYB13 genes for wheat have been also edited to enhance stress responses (Zhang *et al.*, 2022).

MAS has introduced drought yield QTLs like qDTY12.1 in rice; CRISPR has modified genes for stomatal response and root control. Maize uses GS and transgenic techniques like MON87460 for drought resistance (Crossa *et al.*, 2017). Such innovations offer the promising paths for breeding climate-tolerant crops. Such innovations offer the promising paths for breeding climate-tolerant crops.

## 14. Future Challenges and Opportunities

As climatic stress increases, enhanced drought tolerance in crops is critical for sustainable farming. The future hinges on the integration of multi-omics data such as genomics, transcriptomics, proteomics, metabolomics, and epigenomics



to procure an integrated understanding of plant drought stress response. Integrative analysis has the potential to expose intricate gene networks, regulatory networks, and metabolic pathways obscured by using single-omics platforms, facilitating biomarkers and molecular targets to be identified for breeding or gene editing (Varshney *et al.*, 2021).

The most difficult problem is to overcome the multigenic and multifactorial character of environmental stresses. Field crops are frequently exposed to combined stresses like drought, heat, salinity, and nutrient stress, which interact synergistically with one another and induce different physiological responses than those of single stresses. Such future research will be compelled to break the one-stress tradition of controlled experiments to simulate more realistic field conditions in an attempt to render drought tolerance screening and resistance breeding to wide-range stress more relevant and effective (Mittler, 2006).

Extrapolation of results obtained in the laboratory to field conditions is rendered challenging by genotype-environment interaction and heterogeneity of the environment. There should be an interface between molecular biologists, plant breeders, and agronomists. Farmer participatory breeding, precision agriculture, and phenotyping are crucial in linking scientific innovation and successful crop improvement (Furbank and Tester, 2011). Breeding schemes also need to take socio-economics and farmer acceptability and adoption needs into consideration.

## 15. Conclusion

With increasing global climate change, drought itself becomes increasingly dangerous to crop yields and global food security, particularly for agro-dominant countries. The review provided an overview of the major morphological, physiological, and genetic processes underlying plant drought tolerance. Plants morphologically defend themselves against water loss and regulate water uptake through structures such as deep root, low leaf area, hard cuticle, and rolling of the leaves. Physiological processes include stomata closure for the reduction of transpiration as well as the induction of stress signalling pathways, e.g., abscisic acid (ABA), which control water balance and tolerance to stress. Genetically, unprecedented breakthroughs in the identification of drought tolerance genes and QTLs have occurred. Genomic, transcriptomic, and proteomic technologies complemented the information on the molecular mechanism of drought resistance and facilitated better cultivar enhancement. Application of laboratory data to field-effective drought-resistant crops remains difficult under the scenario of environmental and genotype-by-environment heterogeneity. These molecular breeding tools like marker-assisted selection (MAS), genomic selection (GS), and CRISPR/Cas9 gene editing have been successful in drought tolerance crop variety development in rice, wheat, and maize. It can help to have effective regulation of drought-responsive genes and speed up the breeding process. It also relies on the bridging of molecular information with real field phenotyping and agronomic management. Participatory breeding involving farmers is also necessary for the achievement of compatibility of the new varieties with farmer acceptability and local environments.

Future studies must be focused on multi-omics integration to reveal intricate gene networks and disentangle the crop reaction to poly-stress such as heat and drought. Precision agriculture and high-throughput phenotyping will assist in the identification of tolerant genotypes. Finally, drought tolerance breeding must be done with interdisciplinarity from the molecular sciences to agriculture practice. Breaking this barrier is most vital for food production security because of the rising instances of drought as well as climatic uncertainty.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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