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A Review of Biotechnology Techniques in Genetic Modification of Crop Plants to Cope with Drought Stress

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ABSTRACT

Drought stress is one of the most critical limiting factors affecting the growth and productivity of crop plants in arid and semi-arid regions worldwide. Due to its polygenic nature, complex interaction with the environment, and low heritability, genetic improvement for drought tolerance has consistently posed a major challenge in plant breeding programs. In this context, modern biotechnological tools have provided exceptional opportunities for identifying, selecting, and transferring key genes associated with drought resistance. This review article explores the most prominent biotechnological approaches employed in enhancing drought tolerance in crops. These methods include marker-assisted selection (MAS), tissue culture and somaclonal variation, in vitro selection under stress conditions, haploid production and chromosome doubling, and regeneration under stress conditions. Each technique, with its specific potentials and limitations, contributes significantly to optimizing the genetic improvement process in response to drought stress. Furthermore, key challenges such as the genetic complexity of the trait, genotype × environment interactions, lack of sufficient infrastructure, and socio-economic concerns are discussed. Ultimately, this study emphasizes that integrating biotechnological strategies with conventional breeding, alongside the use of advanced phenotyping tools and genomic analyses, can pave the way for developing drought-resilient crop varieties in the near future.

Keywords: Plant biotechnology, Genetic improvement, Drought tolerance Molecular markers, Tissue culture, Haploid, Stress-induced regeneration, Somaclonal variation.

INTRODUCTION

Drought stress is one of the major global challenges in agriculture, exerting profound impacts on crop production. With the intensification of climate change and increasing scarcity of water resources, drought has emerged as a significant barrier to global food security(Mehdiniya Afra *et al.*, 2020b). This issue is particularly acute in arid and semi-arid regions, where it can lead to substantial yield reductions, diminished crop quality, and even complete crop failure (Zhang *et al.*, 2022). Addressing this challenge requires the application of novel and effective strategies in the genetic improvement of plants for drought tolerance. In recent decades, biotechnology has been recognized as one of the most advanced and efficient tools for enhancing agricultural traits (Mehdiniya Afra *et al.*, 2020a). Through techniques such as genetic engineering, marker-assisted selection (MAS), and genome editing tools like CRISPR-Cas9, biotechnology offers farmers the ability to develop drought-tolerant crops (Gupta & Kumar, 2021). Especially when traditional breeding methods such as grafting or random selection are time-consuming and inefficient, biotechnological approaches provide alternative pathways to accelerate crop improvement. Biotechnology enables the identification and transfer of key genes involved in drought tolerance into high-yielding and economically important crop species. In this regard, numerous studies have focused on the molecular mechanisms that help plants withstand drought stress. These include cellular water regulation, activation of antioxidant systems, and adaptive responses to environmental stress. For instance, genes such as DREB (Dehydration-Responsive Element-Binding Protein) and LEA (Late Embryogenesis Abundant proteins) have been identified as key regulators in drought response and have been successfully introduced into crop plants using genetic engineering methods (Munns & Tester, 2008).

These techniques enable the development of drought-resistant cultivars in a much shorter timeframe compared to conventional methods. One of the most notable advancements in plant genetic improvement is the use of genome-editing technologies like CRISPR-Cas9, which allows precise and targeted modification of genes associated with traits such as drought tolerance. For example, this approach can facilitate the transfer of genes responsible for tolerance to water scarcity and salinity into a wide range of plant species (Singh *et al.*, 2020). Despite significant progress in applying biotechnology to mitigate drought stress, several challenges remain. A major barrier is the limited acceptance and application of these technologies in commercial agriculture(Mehdiniya Afra *et al.*, 2023). Additionally, concerns regarding the long-term environmental and genetic impacts of such modifications must be addressed. Strengthening biotechnological capacities for drought-tolerant crop production also requires enhanced collaboration among researchers, industry stakeholders, and farmers. Therefore, the development of drought-tolerant cultivars that not only maintain high yields but also require fewer environmental inputs is essential. Such cultivars can contribute to reduced water consumption and improved food security in water-limited regions (Zhang *et al.*, 2022). Ultimately, the application of modern biotechnological tools in crop improvement holds significant potential to enhance agricultural resilience to climate change and serve as a sustainable solution for global food security.

PHYSIOLOGICAL RESPONSES OF PLANTS TO DROUGHT STRESS

Drought is one of the most critical environmental stresses that limits plant growth and productivity. To cope with water scarcity, plants employ various physiological mechanisms, among which osmotic adjustment plays a central role. This process enhances cellular water retention and uptake, thereby maintaining cell turgor and structural stability under drought conditions (Farooq *et al*., 2009;Mehdiniya Afra *et al*., 2019).

MECHANISMS OF OSMOTIC ADJUSTMENT IN RESPONSE TO DROUGHT STRESS

Osmotic Adjustment through Accumulation of Proline, Betaine, and Other Osmolytes

Osmotic adjustment is a key adaptive strategy that plants utilize to withstand drought stress. It involves the accumulation of organic osmolytes, regulation of mineral ions, and enhanced activity of related enzymes (Mehdiniya Afra *et al*., 2021). This mechanism improves water uptake capacity, maintains cell turgor, and sustains physiological processes such as growth and photosynthesis under water-limited conditions. Plants accumulate soluble organic compounds such as proline, glycine betaine, polyols, and soluble sugars within their cells(Mehdiniya Afra *et al*., 2017). These osmolytes contribute to osmotic balance and protect cellular structures from oxidative damage (Ashraf and Foolad, 2007). Among them, proline is particularly important, as its accumulation under drought helps preserve turgor pressure and stabilize membranes and proteins (Szabados & Savouré, 2010). Soluble sugars such as sucrose and fructans also contribute to osmotic regulation. In addition to their role in osmotic balance, they protect enzymes and stabilize protein structures under drought stress (Hoekstra *et al*., 2001). Some plants also use inorganic ions such as potassium (K^+), calcium (Ca^{2+}), and sodium (Na^+) to modulate osmotic potential. Accumulation of these ions in vacuoles lowers osmotic potential, facilitating water absorption from the environment (Munns & Tester, 2008). Enzymes involved in osmolyte biosynthesis, such as pyrroline-5-carboxylate synthase (P5CS) and sucrose phosphate synthase (SPS), show increased activity under drought, leading to elevated osmolyte production (Verbruggen & Hermans, 2008). Osmotic adjustment plays a vital role in maintaining cell expansion, photosynthesis, and membrane stability during drought. Plants with higher capacity for osmotic adjustment are better able to maintain water uptake and resist reductions in turgor (Chaves *et al*., 2003). In crops like wheat and maize, increased proline and soluble sugars under drought enhance water absorption and reduce the negative impact of drought on photosynthesis (Seki *et al*., 2007). Similarly, in rice and soybean, osmotic adjustment via potassium ion accumulation and organic osmolytes has been linked to improved water use efficiency and reduced transpiration losses (Zhang *et al*., 2006).

Alterations in Photosynthesis and Stomatal Closure under Drought Stress

Drought stress is one of the main limiting factors for plant productivity in arid and semi-arid regions. This stress leads to significant physiological and biochemical changes in plants, including osmotic adjustment, alterations in photosynthetic activity, and regulation of stomatal movement (Ghasemi *et al*., 2020). Osmotic potential is a major determinant of plant cell water status. Under drought stress, a decrease in water potential leads to reduced turgor pressure and cellular expansion. In response, plants reduce their osmotic potential by accumulating solutes such as proline, soluble sugars, and inorganic ions, thereby enhancing water uptake capacity (Moradi *et al*., 2021). One of the earliest plant responses to drought stress is stomatal closure. A decrease in water potential reduces turgor in guard cells, causing stomata to close. This mechanism prevents excessive water loss but also restricts CO_2 entry into the leaf mesophyll, leading to a reduction in photosynthetic rate (Ahmadi and Rezaei, 2019). In addition to stomatal effects, drought stress alters the activity of key photosynthetic enzymes such as Rubisco. Reduced water potential may also damage chloroplasts and suppress chlorophyll synthesis, further impairing photosynthetic efficiency (Nemati *et al*., 2018). Stomatal closure is a key adaptive response to reduce transpiration under drought. Osmotic regulation in guard cells, via the accumulation of potassium ions and other solutes, reduces turgor pressure and leads to stomatal closure (Hosseini *et al*., 2022). While osmotic adjustment enhances water uptake, drought-induced stomatal closure limits CO_2 assimilation, ultimately restricting plant growth and productivity. Understanding these mechanisms can aid in developing strategies for enhancing drought tolerance in plants. Recent studies have shown that reduced water potential leads to stomatal closure and decreased photosynthesis. In one study, potassium nanochelates were applied as a drought-mitigating agent, and results indicated improved gas exchange and reduced drought damage via enhanced osmotic adjustment (Hosseini *et al*., 2022). Moreover, stomatal closure due to reduced leaf water potential restricts CO_2 entry, limiting photosynthesis (Lawlor and Cornic, 2002). Recent findings indicate that compounds such as proline, trehalose, mannitol, and glycine betaine act as osmoprotectants. In drought-stressed plants, they contribute to water balance and help maintain turgor. These compounds also increase the activity of antioxidant enzymes, protecting chloroplasts from oxidative damage and preserving photosynthetic processes (Rahimi *et al*., 2023). International studies have also confirmed the role of these osmolytes in mitigating drought effects (Morgan, 1984). Under drought conditions, osmotic potential reduction and membrane damage lead to increased electrolyte leakage and loss of cellular integrity. In a study on quinoa, drought stress induced stomatal closure, reduced photosynthetic rate, and increased electrolyte leakage, indicating membrane destabilization (Farooq *et al*., 2020). These results align with previous findings confirming drought's detrimental impact on membrane integrity (Tardieu & Davies, 1993). A study on two potato cultivars revealed that reduced water potential during drought stress led to osmolyte accumulation and stomatal closure, resulting in reduced photosynthesis (Heidari *et al*., 2022). Furthermore, drought stress alters protein expression patterns and decreases photosystem II activity. These effects are attributed to stomatal closure, reduced CO_2 uptake, and elevated reactive oxygen species (ROS) production, which damages cellular membranes and chloroplast proteins (Flexas *et al*., 2018).

Reduction in Vegetative Growth for Water Conservation

Reduction in vegetative growth is one of the effective strategies that plants adopt in response to drought and salinity stresses, aiming to decrease water consumption and improve water use efficiency. Under stress conditions, plants restrict cell division and elongation in vegetative organs to reduce transpiration surface area. This process is largely regulated by stress hormones such as abscisic acid (ABA), which induces stomatal closure and reduces photosynthesis, ultimately leading to a slowdown in vegetative growth (Sharp *et al*., 2004). Moreover, the downregulation of genes involved in cell growth, such as EXPANSINs, and the upregulation of stress-responsive genes like RD29A and NCED, contribute to a growth reprogramming strategy focused on survival rather than expansion (Skirycz & Inzé, 2010). Studies have shown that, during early stages of drought stress, root growth is maintained or even enhanced in some plant species to allow access to deeper water sources, while shoot growth is restricted. This shift in growth pattern is part of a water-saving mechanism (Chaves *et al*., 2003). Therefore, the reduction in vegetative growth, along with physiological changes such as increased proline accumulation and reduced transpiration, represents a crucial adaptation mechanism that enables plants to cope with water scarcity(Mehdiniya Afra *et al*., 2023).

BIOCHEMICAL AND MOLECULAR RESPONSES

Plants deploy a wide range of biochemical and molecular responses to cope with biotic and abiotic stresses such as drought, salinity, heat, cold, and pathogen attacks. These responses aim to prevent cellular and molecular damage and ensure survival. They include changes in gene expression, production of secondary metabolites, regulation of antioxidant systems, and activation of hormonal and ion signaling pathways (Zhu, 2016). At the biochemical level, the increased production of osmolytes such as proline, glycine betaine, soluble sugars, and polyols helps maintain osmotic balance. In addition, the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) is enhanced to counteract the harmful effects of reactive oxygen species (ROS) (Gill & Tuteja, 2010). At the molecular level, signaling pathways such as Mitogen-Activated Protein Kinases (MAPKs) and Calcium-Dependent Protein Kinases (CDPKs) are activated. These pathways phosphorylate specific transcription factors that induce the expression of defense-related genes, including DREB, NAC, MYB, and WRKY (Shinozaki and Yamaguchi-Shinozaki, 2007). Furthermore, phytohormones such as abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and ethylene play critical roles in modulating plant defense responses (Verma *et al*., 2016).

Upregulation of Stress-Responsive Genes: DREB, HSP, and LEA

DREB (Dehydration-Responsive Element-Binding proteins) belong to the larger AP2/ERF transcription factor family and play a central role in regulating plant responses to abiotic stresses like drought, salinity, and cold. These genes bind to DRE/CRT elements in the promoter regions of target genes, activating them under stress conditions. Enhanced expression of DREB1/CBF and DREB2 promotes the synthesis of proteins such as LEA, antioxidants, and osmolytes that contribute to osmotic balance and protection against oxidative stress (Nakashima *et al*., 2009; Lata & Prasad, 2011). Transgenic studies have demonstrated that overexpression of DREB genes significantly improves plant tolerance to drought and salinity. Heat shock proteins (HSPs) are categorized into families including HSP100, HSP90, HSP70, HSP60, and small HSPs (sHSPs), and they are crucial for maintaining protein structural integrity, assisting in proper folding, and preventing aggregation of denatured proteins (Wang *et al*., 2004). The expression of HSP genes rapidly increases under heat stress and is regulated through heat shock factors (HSFs). In combined stress conditions, such as heat and drought, HSPs are often co-induced with other stress-responsive genes like DREB, indicating complex regulatory interactions (Mittler *et al*., 2012). LEA (Late Embryogenesis Abundant) proteins, which are highly hydrophilic, typically accumulate in the late stages of embryogenesis and under dehydration and salinity stresses. These proteins help stabilize cellular membranes, protect proteins and enzymes, and prevent ice crystallization, thereby enhancing cellular tolerance to stress (Tunnacliffe and Wise, 2007). LEA genes are commonly induced through ABA-dependent pathways, and their synergistic activity with DREB and HSP genes is a key area of research in genetic engineering and plant breeding for stress tolerance (Hundertmark and Hincha, 2008).

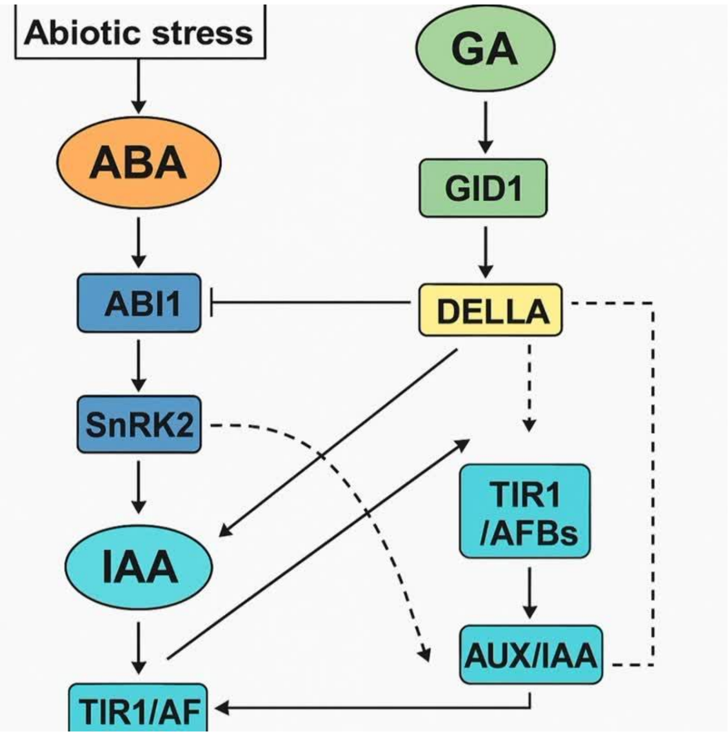

Figure 1. Expression Pathways of Stress-Responsive Genes such as DREB, HSP, and LEA

The Role of Hormonal Signaling Pathways: ABA, Gibberellins, and Auxins

Plant hormones play a pivotal role in regulating growth, development, and defensive responses to biotic and abiotic stresses(Mehdiniya Afra *et al*., 2023). Among them, abscisic acid (ABA), gibberellins (GA), and auxins (IAA) are key hormones that activate complex signaling pathways, especially under stress conditions(Mehdiniya Afra *et al*., 2020b). ABA, known as the “stress hormone,” rapidly accumulates in response to drought, salinity, cold, and nutrient deficiency. It induces stomatal closure, reduces transpiration, and activates stress-responsive genes such as RD29A, NCED3, SnRK2, and ABI5, providing protective functions. The ABA signaling pathway begins with the PYR/PYL/RCAR receptors and proceeds via inhibition of PP2C and activation of SnRK2s, leading to phosphorylation of specific transcription factors (Cutler *et al*., 2010; Fujita *et al*., 2011). Gibberellins are primarily known for their role in promoting growth and stem elongation. However, under stress conditions, their levels decrease, and growth suppression occurs to limit resource consumption. GA signaling functions through the GID1 receptor and degradation of growth-repressing DELLA proteins. Under stress, DELLA stability increases, which helps suppress growth pathways and enhances stress tolerance (Achard *et al*., 2006). The interaction between DELLA proteins and ABA-related transcription factors like ABI3 plays a significant role in hormonal pathway crosstalk. Auxins are critical in regulating developmental responses such as tissue differentiation, cell elongation, and root growth. The auxin signaling pathway involves TIR1/AFB receptors and the degradation of AUX/IAA repressors, releasing ARFs (Auxin Response Factors) to activate target gene expression. Under stress, auxin distribution within tissues is altered, resulting in root architecture reprogramming and shoot growth reduction to conserve water (Shani *et al*., 2017). Auxin also interacts with ABA and ethylene pathways to coordinate growth and survival simultaneously.

Epigenetic Regulation in Response to Drought Stress

Epigenetic regulation is one of the key mechanisms enabling plants to adapt to environmental stresses such as drought. These modifications influence gene expression without changing the DNA sequence, through chromatin remodeling, DNA methylation, and RNA-level regulation. One of the primary epigenetic mechanisms is cytosine DNA methylation, which can lead to gene silencing or alter transcription factor accessibility to promoters. Studies in rice and Arabidopsis have shown that drought stress causes significant changes in methylation patterns, which regulate the expression of stress-related genes such as DREB, RD29A, and NCED3 (Wang *et al*., 2016). Post-translational modifications of histones—such as acetylation, methylation, and phosphorylation—alter chromatin structure and modulate gene expression. For instance, H3K4me3 is recognized as a marker of active gene expression under drought conditions, while H3K9me2 is associated with silencing non-essential genes during stress (Kim *et al*., 2015). MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) also play a role in post-transcriptional regulation of genes. miR398 and miR169 are examples of small RNAs that modulate the expression of antioxidant and stress-adaptive genes during drought (Sunkar *et al*., 2007). These regulatory processes help maintain a balance between growth and survival during water deficit conditions. Some epigenetic changes can be stable and even passed on to the next generation—a phenomenon known as stress memory. This feature allows plants to respond more rapidly and effectively upon re-exposure to the same stress (Crisp *et al*., 2016).

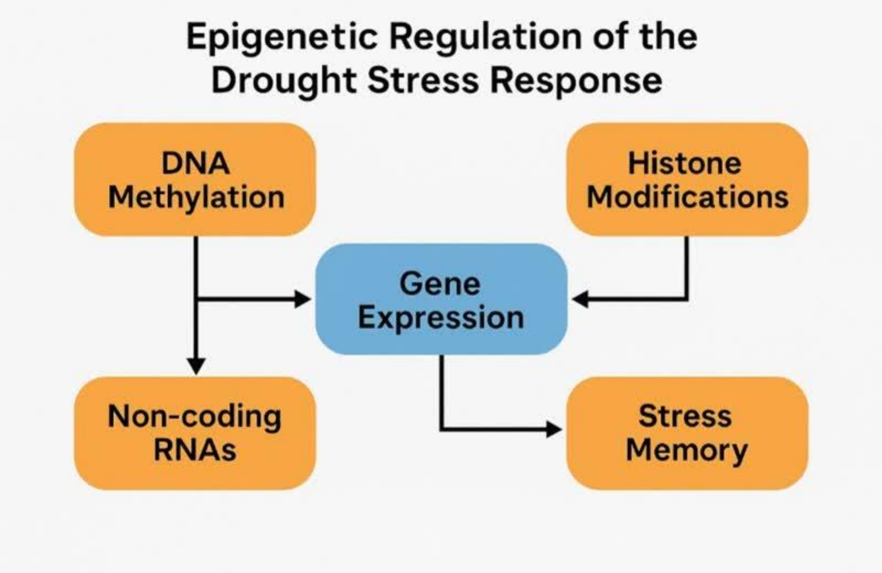

Figure 2 . Epigenetic regulation of plant response to drought stress

DROUGHT RESISTANCE

Genetic Engineering and Gene Transfer

Genetic engineering is considered one of the most important modern biotechnology tools in plant breeding. In this approach, specific genes identified in drought-tolerant species or wild relatives are transferred into the genome of drought-sensitive crop plants to help them maintain their performance and survival under water-deficit conditions (Kasuga *et al*., 1999). In this method, the Ti plasmid from the bacterium Agrobacterium tumefaciens acts as a vector for the desired gene. This technique is particularly effective in dicotyledonous plants such as soybean, cotton, and tomato, where gene insertion into the genome usually occurs with high stability and efficiency (Gelvin, 2003). For plants that do not respond well to Agrobacterium—such as maize and wheat—the gene gun method is applied. In this technique, DNA is coated onto gold or tungsten particles and is shot into plant cells using high pressure (Sanford, 2000). Another technique involves electroporation, where an electric field is used to create temporary pores in the membranes of protoplasts, allowing foreign DNA to enter. This method is primarily used in laboratory settings and for certain specific species (Fromm *et al*., 1985).

- ***Introduction of Drought-Tolerance Genes***

- The genes introduced into transgenic plants for drought resistance typically serve one or more of the following functions:
- Regulating osmotic pressure, such as the P5CS gene which increases proline synthesis
- Regulating expression of stress-responsive genes, such as the transcription factor DREB1A
- Enhancing protein and membrane stability, through genes like LEA or HVA1
- Regulating ionic and water balance, such as NHX1, which facilitates sodium antiport activity

- ***Examples of Drought-Resistant Transgenic Plants***

- Transgenic rice with the HVA1 gene from barley: Shows higher drought tolerance and improved water balance (Xu *et al*., 1996;Mehdiniya Afra *et al*., 2020a)
- Transgenic maize with the P5CS gene: Enhances proline content and osmotic stability under stress (Kishor *et al*., 1995)
- Wheat expressing the DREB1A gene: Enhances expression of drought-response genes and improves performance under water-limited conditions (Kasuga *et al*., 1999)

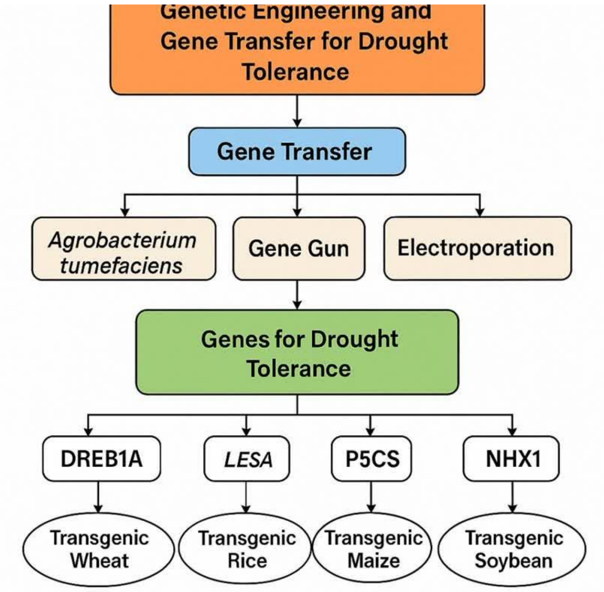

Figure 3 .Biotechnology techniques in genetic modification of plants for drought resistance

Genome Editing Using CRISPR-Cas9

The CRISPR-Cas9 system (Clustered Regularly Interspaced Short Palindromic Repeats) is one of the most advanced biotechnology tools for precise genome editing in plants. This technology enables targeted DNA cleavage and allows for the mutation, deletion, or insertion of specific nucleotides, proving highly effective in improving complex traits such as drought tolerance (Jaganathan *et al*., 2018).

Key Components of the CRISPR-Cas9 System

Guide RNA (gRNA): A sequence that identifies the specific genomic target.

Cas9 Enzyme: A nuclease that cuts the DNA at the desired location.

DNA Repair System: After the DNA is cleaved, the plant repairs the site via either non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways.

Applications of CRISPR-Cas9 in Enhancing Drought Tolerance

There are two main strategies for increasing drought tolerance using CRISPR:

A) Inactivating Negative Regulators

This involves deleting or suppressing genes that increase drought sensitivity.

Example: Silencing genes that negatively regulate ABA signaling in rice and tomato (Li *et al*., 2017).

B) Upregulating Positive Stress-Response Genes

This involves enhancing the expression of key stress-responsive genes like DREB, NAC, or NCED using artificial enhancers.

In tomato, for instance, the SlMAPK3 gene was edited using CRISPR, resulting in improved drought tolerance (Wang *et al*., 2017).

Advantages of CRISPR over Traditional Methods

High precision in editing specific genomic sites

No need for foreign genes in some applications, improving environmental and consumer acceptance

Faster development of improved plant varieties compared to traditional transgenic methods

Marker-Assisted Selection (MAS) in Drought Tolerance Improvement

Drought tolerance is one of the most important goals of breeding programs in dry and semi-dry regions of the world. The use of advanced breeding methods like Marker-Assisted Selection (MAS) facilitates the identification and transfer of genes associated with drought tolerance with greater precision and speed. MAS is an effective tool for accelerating the plant breeding process, especially for traits with complex genetic control and low heritability, such as drought tolerance (Collard & Mackill, 2008; Gupta *et al*., 2010). In MAS, QTLs (Quantitative Trait Loci) or genes associated with the trait of interest are first identified. Then, markers closely linked to these QTLs are used to select desirable individuals in breeding populations. Multiple markers related to drought tolerance have been identified and applied in breeding programs for crops like wheat, rice, corn, soybean, and barley (Varshney *et al*., 2012). For example, in wheat, SSR and SNP markers linked to traits such as root depth, relative leaf water content (RWC), water use efficiency (WUE), and photosynthesis under drought stress have been identified. Similarly, in rice, MAS-based breeding projects have led to the development of drought-resistant lines like DRR Dhan 42 and Sahbhagi Dhan (Xu *et al*., 2020).

Key Advantages of MAS

Eliminates the need for time-consuming phenotyping in variable environmental conditions
Effective selection during early growth stages
High precision in the transfer of target genes
Ability to pyramid desirable genes (Pyramiding) (Collard & Mackill, 2008)

In recent years, combining MAS with advanced technologies such as Genotyping-By-Sequencing (GBS), CRISPR/Cas9 genome editing, and Genomic Selection has opened new horizons in breeding for drought tolerance (Xu *et al*., 2020).

Tissue Culture and Somaclonal Variation

Plant tissue culture is an efficient method for producing uniform plants and studying genetic variation under controlled laboratory conditions. One significant outcome of tissue culture is the emergence of somaclonal variation, which arises from genetic and epigenetic changes during dedifferentiation and redifferentiation processes. This variation can serve as a valuable source for identifying genotypes tolerant to drought stress (Jain, 2001). By evaluating traits such as chlorophyll content, antioxidant enzyme activity, ion leakage, leaf water potential, and seedling survival in media containing polyethylene glycol (PEG), it is possible to select drought-tolerant genotypes (Rai *et al*., 2011).

In Vitro Selection of Drought-Tolerant Plants

In vitro selection using stress agents like mannitol, PEG, or NaCl as drought simulators enables the identification of resistant clones. In this method, cells or calli that can grow in stress conditions are selected and regenerated into whole plants. This approach saves time and resources while increasing selection accuracy (Sairam *et al*., 2002).

Production of Haploids and Chromosome Doubling for Accelerated Breeding

Haploid production via androgenesis or gynogenesis, followed by chromosome doubling using colchicine, is an effective method for obtaining homozygous pure lines in one generation. Such lines, which would take years to develop using traditional breeding, can be rapidly produced and evaluated under drought stress. The best-performing lines are then used in breeding programs (Germanà, 2011). This technique has been successfully applied in rice, barley, wheat, and tobacco (Malik *et al*., 2021).

Stress-Induced Regeneration for Selecting Resistant Genotypes

Direct or indirect regeneration under stress is a powerful strategy to identify genotypes capable of cell division, differentiation, and growth under stressful conditions. Genotypes that can regenerate in media with higher levels of stress agents (like PEG or mannitol) are considered drought-tolerant. This method not only offers precise genotype selection but also enhances understanding of molecular mechanisms of resistance (Zhao *et al*., 2000).

CHALLEnGES AND LIMITATIONS OF GENETIC IMPROVEMENT FOR DROUGHT RESISTANCE

Despite significant advances in the field of genetic improvement for drought tolerance, several challenges and limitations still hinder its widespread application and development:

1. Genetic complexity of the trait

Drought resistance is a polygenic trait with low heritability, influenced by complex interactions between genes and environmental factors. This complexity makes it difficult to accurately identify and transfer effective genes (Tuberosa and Salvi, 2006).

2. High dependency on environmental conditions

The phenotypic expression of drought resistance is highly influenced by environmental conditions, which reduces the reproducibility of results across different locations and seasons (Blum, 2011).

3. Lack of precise markers and major QTLs

In many crop species, information on effective QTLs and related genes under drought conditions is still incomplete. Moreover, many identified QTLs have small effects or are unstable across populations (Collins *et al*., 2008).

4. Genotype × Environment interaction (G×E)

The complex interaction between genotype and environment makes it difficult to identify stable and resistant genotypes. A genotype may perform well in one environment but poorly in another (Passioura, 2007).

5. Technological and infrastructural barriers

Effective use of genetic improvement techniques requires advanced lab facilities, access to diverse germplasm, and precise genotyping and phenotyping tools, which are often lacking in many developing countries (Fita *et al*., 2015).

6. Ethical issues and public acceptance

In some societies, genetically modified or edited crops face cultural, ethical, or legal resistance, which limits the application of modern tools like gene editing (CRISPR/Cas9) or gene transfer (Qaim, 2009).

Future Perspectives and Research Recommendations

Given the intensifying climate-related stresses and water scarcity, the development and application of advanced biotechnological tools for improving drought tolerance in crops will play a crucial role in global food security. Emerging technologies such as gene editing (CRISPR/Cas9), genome-wide association studies (GWAS), precision phenotyping using artificial intelligence, and advanced bioinformatics offer new opportunities to deepen our understanding of molecular and genetic mechanisms of drought resistance.

- ***Key future research recommendations include:***

❖ Identifying and functionally characterizing key genes involved in drought tolerance through transcriptomic, proteomic, and metabolomic studies in native and wild crop species.

- Developing accurate gene editing approaches that target specific traits without introducing foreign genes, especially in crops where transgenic acceptance is limited.
- Establishing comprehensive genomic and phenotypic databases for drought-resistant species under various climatic conditions in Iran, with a focus on native species.
- Integrating biotechnological methods with conventional breeding in the form of integrated breeding strategies to improve yield stability.
- Investing in the development of rapid and precise field phenotyping systems using remote sensing and machine learning.
- Conducting multi-location and multi-year trials to assess the stability of improved genotypes under real-world climatic conditions.
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CONCLUSION

The increasing frequency and severity of drought stress across various global climates—particularly in arid and semi-arid regions—has underscored the urgent need to adopt modern breeding approaches. Plant biotechnology, including marker-assisted selection (MAS), tissue culture and somaclonal variation, in vitro selection, haploid plant production, and regeneration under stress, provides innovative tools that enable more rapid and precise genetic improvement of crop plants. These techniques allow for the accurate identification of resistant genotypes, the transfer of effective genes, the development of pure homozygous lines, and ultimately, improved yield stability under drought conditions. However, challenges such as the genetic complexity of the trait, genotype × environment interactions, limited access to precise genetic resources, and social and economic barriers still hinder the widespread application of these technologies. Overall, integrating biotechnological methods with conventional breeding, along with the use of advanced phenotyping tools and predictive modeling, can open new horizons for enhancing agricultural sustainability in the face of water scarcity and climate change. Investment in research, infrastructure development, and the training of skilled personnel are essential prerequisites for effectively harnessing these technologies in the future.

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