**Breeding for abiotic stress resistance**

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**Abstract**

Abiotic stresses are the main causes of yield losses in plants, with yields being reduced by up to 50%. Abiotic stresses, which include low or high temperatures, drought, floods, high salinity, heavy metals, and UV radiation, are harmful to plant growth and development and have a significant negative impact on agriculture production globally. The era of genomics research has emerged with new and promising opportunities in breeding superior varieties against abiotic challenges as conventional approaches for crop improvement approach their limits. The molecular-assisted selection method predicts a quick advancement in selecting crop plants that have been adapted to stress with increasing selection precision. Molecular-aided selection is promising can target traits in a single progeny more simply and accurately in a few chosen generations with minimal unintended consequences. This chapter represents an overview about the basics of advanced technologies *viz.*, molecular mapping, molecular-assisted mapping, transcriptomic, metabolomic, transgenics and genome editing and its utilization in improving crop plants with special focus on stress tolerance.

Keywords: - Abiotic stresses; Drought; Flood; metabolomic; transcriptomic

1. **Introduction**

Stress is a external factor that has a negative impact on a plant's growth, development, or productivity. (1). Broad collection of plant responses reported due to stress such as changes in crop yields, growth rates, altered gene expression, cellular metabolism etc. A plant stress generally reflects some sudden changes in environmental condition. Before the year 2100, atmospheric CO2 level are predicted to climb to 800 ppm as a result of deforestation and excessive fossil fuel consumption (2, 3). Furthermore, climate change causes extreme weather, such as drought, severe rainfall, and temperature fluctuations, among other effects.

In the current climate scenario, abiotic stresses are the important factors contributing for potential yields in farmers' fields. Abiotic stress represents one of the most significant issues facing agriculture today. A serious loss is caused in crop production at a worldwide level and reduces the planted acreage impact of the abiotic stress. The scenario gets more complicated as a result of population growth and climate change. By 2050, the world population is probable to rise from seven to 10 billion people, necessitating a 60 to 110 percent increase in global food supply (4).

In future, it is predict that ultimately intensity of abiotic stresses will increase because of fresh water scarcity will increase. To maintain food security and safety in the upcoming years, it is urgent to develop crop varieties that are resistant to abiotic stresses. The roots of a plant act as its first line of defence against abiotic stress. If there are healthy and biologically diverse crop plants, the chances of surviving stressful conditions will be high. Disruption of the Na+/K+ ratio in the plant cell's cytoplasm is one of the main reactions to abiotic stress, such as excessive salinity. The phytohormone abscisic acid (ABA) plays an important role for the duration of plant adaptation to abiotic stress such as drought, high salinity, low temperature or mechanical wounding (5).

It is essential to adapt food security and sustainable agriculture to develop advanced breeding strategy that effectively mitigates abiotic stress. Additionally, a multifaceted approach involving hormone modulation, plant enzymatic system activation, and stress gene expression is necessary for crops to improve their capacity to adapt to abiotic stress. Determining how cereal crops respond to abiotic stress is therefore essential. More research is needed to understand the properties of abiotic stress and how their physiological, biochemical, and molecular (genetic, epigenetic, transcriptomic, and metabolomic) basis might contribute in breeding attempts to develop abiotic stress-resistant crops.

At present there are several methodology to enhance abiotic stress tolerance in crop species: (1) Genetic engineering including GMOs, cisgenic, transgenic, and intragenic plants; (2) the appliance of plant bio-stimulants (3) advanced breeding technology, including the utilize of the (CRISPR/Cas) gene editing tool; and (4) conventional breeding methods. Bio-stimulants are defined as plant fertilizers that improve nutrient use efficiency, resistance to abiotic stress, quality character or availability of micronutrients in the rhizosphere or soil (6).

**Why we need to study about abiotic stress in crop plants?**

1. Abiotic stressors, such as high or low temperature, drought, and salt, are the main characteristics that affect plant diversity.
2. Abiotic stresses have an impact on the world's crop production system and threaten human food security.
3. Abiotic stresses generally lead to a significant loss in agricultural production.
4. **Crop plants and Abiotic stresses**

Crop plants suffer biotic and abiotic environmental stresses. The biggest factor affecting agricultural production globally is abiotic stress, which lowers yields for the main crop plants. These abiotic stresses interact to generate osmotic stress, which disturbs the cell's homeostasis and ion distribution. It mostly results from changes in the gene expression patterns of a particular group, which causes reactions that impact growth rates and productivity. Therefore, it is s crucial to identify some genes that are susceptible to abiotic stress in order to understand the stress response mechanism in plants.

1. **Drought**

Increases in atmospheric CO2 levels and temperature, as well as changes in rainfall patterns, are all results of global climate change. Drought is caused by extended periods of inappropriate rainfall. Severe drought conditions reduce soil water availability to plants and induce early plant death, whereas intermittent drought conditions influence plant growth and development but are seldom deadly.

The severity of the drought depends on the occurrence and distribution of rainfall, evaporative demands, and moisture storing capacity of soils, all of which are unpredictable (7). In the modern age, climate has altered due to steadily rising temperatures and atmospheric CO2 levels around the world. Climate change causes uneven distribution of rainfall, which acts as a significant factor for emerging drought. Extreme drought conditions are gradually reducing the quantity of soil water available to plants, which causes plants to die early stage. Growth arrest is a plant's initial response to drought stress. Shoot growth reduction under drought reduced metabolic demands of the plant and mobilizes metabolites for the production of defensive chemicals required for osmotic adjustment.

1. **Salinity**

Soil salinity is a global issue that poses a severe danger to global agriculture since it affects crop output in afflicted areas. Crop development and production are impacted by salinity stress in various ways. Plants are adversely impacted by salt in two ways: ionic toxicity and osmotic stress (8).

The osmotic pressure in the soil solution during salinity stress surpasses the osmotic pressure in plant cells owing to the excessive levels of salt, limiting plants' capacity to uptake water and minerals such as K+ and Ca2+. These main consequences of salt stress cause a number of indirect effects, such as assimilate synthesis, cytosolic metabolism, and ROS generation, as well as reduced cell growth and membrane function.

Salt stress tolerance level ranged from intra-specifically. The most resistant cereal crop, barley (H. *vulgare*), can withstand up to 250 mM NaCl (equal to 50% saltwater) in soil and moderate salt-tolerant crop is a bread wheat, whereas rice, durum wheat, sorghum and maize are less tolerant to salinity (9).

1. **Heat tolerance**

High temperatures have become a universal problem, drastically affecting plant development and productivity, particularly crops. Heat stress has emerged as the most significant limiting factor to agricultural growth and, eventually, food security (10).

Plants are more susceptible to temperature changes; in severe circumstances, an adverse change in temperature causes plant mortality. Plant growth and function would usually be better at optimal temperature; situations below and above optimum temperature have a detrimental impact on plant growth and productivity. Most biochemical and enzymatic processes double in rate for every 10°C increase flanked by 20 and 30°C (11). In various agricultural crops, including rice, maize, barley, sorghum, wheat, and chickpea, the early stage of the male gametophyte is adversely affected by abiotic stresses, particularly high and low temperatures (12).

The activities of the tapetal cells are lost throughout the reproductive development phase due to heat stress, and the anther is dysplastic. Increased temperatures prevent pollen grains from swelling during lowering, which causes poor pollen release and anther indehiscence. To withstand heat stress, plants have developed a different type of molecular and physiological responses (8).

1. **Cold**

One of the primary abiotic factors that reduces the quality and post-harvest life of agricultural crops is cold stress. Cold stress, which includes chilling (0-15°C) and freezing (0°C), is an abiotic stress that has a negative impact on plant development and agricultural production (13, 14).

Cold-induced abiotic stress has a profound impact on all cellular processes in plants. The cold stressors are transduced by a number of signal transduction pathways, including components of ROS, protein kinase, protein phosphate, ABA, and Ca2+, among others, and ABA emerges as the most effective of these (15). Maize, tomato, rice, cotton, and soybean lack the ability to acclimate to cold temperatures and can only grow in tropical or subtropical regions (16). Thus, cold stress badly affects plant development and growth, limits the ecological distribution of plant species and decrease crop yields globally (17).

1. **Toxins**

Increased level of chemical fertilizers and sewage waste water irrigation as well as increased industrialization, toxic metals have been introduced to agricultural soils, having negative effects on the soil-plant environment system (18). Cadmium (Cd) is one example, which is the principal metal pollution and is regarded as a major environmental hazard for the agricultural system due to its long residence period in soil. Over time, these metals enter the food chain and gradually poison plants through the air, water, and food chain (19).

# Table 1. Physiological and biochemical changes in plants caused by abiotic stresses.

|  |  |  |
| --- | --- | --- |
| Abiotic stress | Primary effects | Secondary effects |
| Drought | * Water potential reduction * Cellular dehydration | * Reduced cell/leaf expansion * Reduced cellular and metabolic activities * Stomatal closure * Photosynthesis inhibition * Leaf abscission * Membrane and protein destabilization * Ion cytotoxicity * Cell death |
| Salinity | * Cellular dehydration * Water potential reduction | * Reduced cell/leaf expansion * Reduced cellular and metabolic activities * Stomatal closure * Photosynthesis inhibition * Membrane and protein destabilization * Ion toxicity * Cell death |
| Metal toxicity | * Damage in protein and DNA * ROS production | * Disruption in metabolism |
| Heat | * Membrane and protein destabilization | * Photosynthesis and respiration inhibition * ROS production * Cell death |
| Cold | * Membrane destabilization * Water potential reduction * Cellular dehydration * Ice crystal formation in cells | * Reduced cell/leaf expansion * Reduced cellular and metabolic activities * Stomatal closure * Photosynthesis inhibition * Membrane and protein destabilization * Cell death * Physical destruction |

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# Fig 1. Some biotic stress affecting crop plants productivity and development

1. **Conventional breeding tactics for abiotic stress tolerance**

The sources of abiotic tolerance in agricultural plants are well recognized. Land races, wild relatives, high-yielding varieties, initial breeding stock, and advanced breeding material can all exhibit tolerance. Land racesfrom arid regions have been effectively employed in breeding to create open-pollinated varieties or hybrids for environments with limited water resources. The wild species and progenitors of our cultivated crops were constantly used as a rich source of abiotic stress donors. The choice of genetic resource to utilize as donor for abiotic stress tolerance depends on the probability of discovering the required genes as well as the expected difficulties and projected success in introgression of these genes into the chosen recurrent cultivar.

In breeding materials for drought and salinity tolerance, as well as in certain improved cultivars of other crop species, there is a lot of genetic variations. Since these sources of drought resistance are the easiest to employ, finding and using them should be a breeder's first priority.

Land races (old or desi varieties) that have developed in and adapted to drought and saline conditions are great sources of traits for drought and salinity tolerance. Due of undesirable linkages, using these materials in breeding programmes may be challenging. The efforts should be focused on using wild relatives only when the variability present in the elite breeding materials and land races is exhausted.

Wheat, sugarcane, tomatoes, and other crops have all been discovered to include wild sources of drought and salinity resistance. When a wild species provides drought and salinity tolerance, transfer presents several difficulties, including cross-incompatibility, hybrid sterility, inviability, and the coupling of several undesirable genes with beneficial ones.

When choosing a breeding technique for any crop, the species' mode of reproduction, whether self-pollinating, cross-pollinating, or asexual, should be taken into consideration. The kind of cultivar and the genetic regulation of characters. Breeding a species for more than one resistant characteristic at once can be difficult since different plants respond to the same abiotic stress factors in different ways.

The same breeding techniques used for yield and other economic characteristics also apply to drought resistance. In self-pollinated crops, the pedigree and bulk methods might be employed, while for cross-pollinated crops, recurrent selection could be utilized. However, backcross is the recommended technique if the goal is to transfer a small number of traits related to drought tolerance to a genotype that has yields well. Breeding method for drought resistance has been discussed by Hurd 1969 and 1976 (20, 21), Rao *et al*. 1971 (22). Crop cultivars resistant to drought can be developed by selection, hybridization, introduction, and mutation. Salinity-tolerant cultivars were developed using pedigree, modified bulk pedigree, and a different culture-based method. Genetic modification for effective stress tolerance is challenging because of the complex characteristics of abiotic stress occurrences in plants (23). The stage, duration, and severity of the stress are three critical factors of drought characterisation for successful stress resistance breeding. Drought tolerant lines of crops such as peanut, common bean, safflower, chickpea, wheat, tall fescue, soybean, wheatgrass, barley and maize have been developed using conventional breeding techniques.

Breeding for any trait starts with the integration of genetic variation through the collection and evaluation of the available germplasm. If the desirable variability is not available within a locality or species, introduction of the exotic germplasm can be resorted to. This classical approach is still very relevant in all breeding strategies. Few varieties with salinity tolerance have been developed worldwide using selection and introduction approaches. The rice varieties Jaladhi-1 (a selection from Kalakhersail), Jaladhi-2 (a selection from Baku), Jalaprabha (a selection from composite), Neeraja (a selection from a landrace), Dinesh [CN-570-652-39-2(Jaladhi-2/Pankaj)], and Hangseswari (a pure line selection) were developed through selection for tolerant to deepwater (24).

Pedigree selection method can be used to identify superior genotypes for grain yield in a cultivar development program. Pedigree selection for grain yield/plant needs to evaluate selections under a series of environments such as different planting dates (25,26), different water stress (27). The modified pedigree method (single panicle selection) was adopted by breeders at Narendra Deva University of Agriculture and Technology (NDUAT), Faizabad, Uttar Pradesh (28). Pedigree selection method was fund effective to produce new lines tolerant to drought stress (29). CSR0-10, CSR-13, CSR-23, CSR-27, CSR-30, CSR-36, CR Dhan-402, CR Dhan-403, TRY-1, TRY-2, TRY3, White ponni, CO-43 etc. were developed through recombinant varieties (30).

The backcross method is a form of recurrent hybridization by which a gene for a superior characteristic may be added to desirable variety. To transfer of traits relating to drought tolerance to a high yielding cultivar, backcross method of breeding is appropriate (31). However, transferring drought tolerance in high yielding genotypes is complicated due to lack of understanding of physiological and genetic basis of adaptation in drought condition. A comprehensive scheme for breeding for drought resistance and high yield potential has been proposed, which takes advantages of recurrent selection procedure (32). This method of breeding is presently used by International Rice Research Institute (IRRI), for improving drought tolerance in rice (33). Large number of introgression lines with enhanced abiotic stress tolerance has been developed in a massive backcross breeding program using three recurrent parents and 203 donor lines with tolerance to several abiotic stresses in rice (34).

1. **Advanced breeding technologies for crop improvement against abiotic stress**

High-throughput methods for DNA and protein analysis have transformed the way in which cells can be examined compared to previous generations. To strengthen stress tolerance, modern transgenic technology introduces novel foreign genes or modifies the levels of endogenous gene expression. Due to the complexity of stress tolerance traits and the limited success of conventional breeding methods, the transgenic technique is now widely used to produce transgenic crops that are resistant to abiotic stresses (35). Therefore, interpreting the molecular mechanisms by which plants observe and transduce stress signals to cellular machinery to initiate adaptive responses is an essential prerequisite for identification of the key genes and pathways to engineer stress-tolerant crop plants (36, 37, 38).

1. **Marker assist selection**

In the last few decades, significant efforts have been made to improve the stress tolerance of cereal crops when challenged with stress. However, problems including increased environmental stresses, inadequate moisture, and unpredictable climatic changes created significant obstacles for breeders to create new crop varieties (39). The use of modern technology, such as molecular assisted breeding to increase the yield of cash crops, is crucial in current era to address these problems. It offers the opportunity to choose crop plants that can withstand stress with great accuracy (40). For several traits, such as abiotic and biotic stress tolerance, yield, and quality traits, molecular-assisted breeding techniques have become two times faster than phenotypic-based breeding. (41). Additionally, MAS has enhanced crop plants' agronomic characteristics (42). DNA marker maps are developed using the molecular markers.

Based on the degree of relationship between the marker and target trait, these marker maps are used to identify the presence of candidate genes that influence the desirable characteristics. With abiotic stresses such water logging, salinity, and desiccation in mind, the targeted alleles and their loci have been identified and utilized in the MAS (43,44,45). The conventional, molecular-assisted, and DNA markers can be used in new problematic areas for generating new multi-abiotic-resistant lines (46). Genetic engineering has recently been used to improve the salt and drought resistance of various crops, including Arabidopsis (47), rice (48), maize (49) and Brassica (50). Similarly, waterlogged-tolerant lines in different crop plants also developed by MAS (51). Marker-assisted selection can improve efficiency, however so far, selection for markers related to low heritability component traits has not produced expected results. In pearl millet, QTL introgression for yield under stress is proving to be a more successful technique (52). QTL fine-mapping and map-based cloning will allow them to be employed more efficiently in breeding by removing the impacts of undesirable linked alleles (53). MAS must be fine-tuned so that precise allele combinations can be combined for optimal effect.

1. **Maker Assist Backcross Selection (MABC)**

One of the most promising methods for using molecular markers to find and choose the genes influencing resistance is marker assisted backcrossing (MABC). MABC is a recently enhanced as an effective technology that makes use of large number of population (400 or more plants) for the backcross F1generations, with just two or three backcrosses, the recurring parent genotype can be recovered.

Through MABC, a number of high yielding, quality, and stress-tolerant varieties have so far been created in a number of development countries in the smallest amount of time. Modern plant breeding programmes frequently use MABC to invent different varieties and lines. Backcrossing is a common method in rice breeding for introducing or replacing a target gene from the donor parent to the recipient. South and Southeast Asia have adopted backcross breeding as a breeding technique to increase the blast resistance of elite types including KDML105, Basmati, and Manawthukha (54, 55). New genes for resistance to biotic and abiotic stress have been developed because of advances in rice biotechnology and genomics, and MABC has already demonstrated its ability to transfer important genes into elite rice parents utilising both foreground and background selection. To introduce gress three major genes, namely Pi9 for blast, Xa21 for bacterial blight (BB), and Gm8 for gall midge (GM), as well as three major QTLs, namely qDTY1.1, qDTY2.2, and qDTY4.1, conferring increased yield under drought in the background of Naveen, a combined approach of marker-assisted forward (MAFB) and back cross (MABC) breeding was followed. With improved yield performance compared to Naveen, a group of 8 MAFB lines and 12 MABC lines with 3 to 6 genes/QTLs and resistance/tolerance against biotic stresses and reproductive stage drought stress were developed (56). With the use of the marker-assisted backcross breeding (MABB) method and three linked quantitative trait loci (QTLs), five wheat lines with inherent resistance to drought stress were developed from an initial population of 516 BC1F1 plants. The high-yielding wheat cultivar HD2733, which has been widely cultivated over the past few years in India's eastern plains and is utilised as the recurrent parent, is very drought-sensitive. The donor parent was "HI1500", it was developed for water-stressed areas and contains drought-tolerant QTLs (57).

1. **Omic methods**

Functional genomics approaches use methods like quantitative real-time PCR, microarray, and high-throughput RNAseq to analyse the transcriptome of plants under abiotic environmental stress. Expressed Sequence Tags (ESTs) that have already been submitted in a variety of cDNA libraries can be used to search for the gene expression patterns of certain tissues at a chosen stage of development. Abiotic stress responsive genes in particular abiotic stress tolerance mechanisms can be evaluated before progressing further in experimental design (58).

Using high throughput sequencing and functional genomics methods, significant progress has been achieved in accepting the molecular mechanisms of abiotic stress responses in plants. A number of essential genes concerned in abiotic stress tolerance have been found and verified recently, and they can be divided into two categories: functional genes and regulatory genes. The former actively functions to protect cells from stresses by encoding essential metabolic proteins (functional proteins) and enzymes including detoxifying enzyme, water channel, ion transporter, heat shock protein (HSP), and late LEA protein. The latter regulates gene expression and signal transduction in the stress responses by encoding a variety of regulatory proteins, such as TFs, protein kinases, and protein phosphatases.

In Arabidopsis, expression studies have discovery numerous genes that are responsive to abiotic stresses (59, 60). Such study yields a large number of candidate genes and their associated functions, for example, identified 53 genes induced by cold stress, 277 by drought stress, and 194 by salt stress. Through expression analyses, the separation of component genes for root QTLs could be sped up. There were 66 transcripts in rice that were differentially expressed in various root tissues of the upland variety Azucena when there was a severe drought (61).

Recently, Hasegawa and Wang (62, 63) addressed the metabolic mechanisms of salt adaptation. However, only a small number of studies have identified transcripts clearly associated to salt tolerance (64). Often, investigations are not designed to identify the genes that provide tolerance in natural conditions: for example, high NaCl concentrations provided in a single dose without Ca2+ to decrease shock make it unlikely that genes controlling Na+ or K+ transport would be discovered. Because of this disregard for tolerance mechanisms, much research has focused on tolerance to osmotic stress rather than salinity.

1. **Transgenic approaches**

A transgenic approach to increasing endogenous defence mechanisms, which typically involves the production of antioxidants, compatible osmolytes and polyamines, alteration of transporters and regulatory proteins, the maintenance of hormone homeostasis, and counting transcription factors and alternative splicing events, is capable of creating crops resistant to abiotic stress. The production of certain proteins and metabolites due to over expression of some genes might occasionally affect normal metabolism, decreasing yield.

The transfer of genes that are either involved in signalling and regulatory pathways, encode enzymes found in pathways that result in the synthesis of structural and functional protectants like antioxidants and osmolytes or encode proteins that associated with stress tolerance is the basis of current engineering approaches [63]. To develop crops that can survive in abiotic stress. Genetic modification primarily targets phytohormones such as ABA.

The dehydration-responsive element-binding (DREB) transcription factors of the ABA-dependent pathway IV, which were first linked to increased cold tolerance, have been used in a large portion of this research in Arabidopsis (65). These have been incoprated together with constitutive promoters and are being controlled by the Arabidopsis dehydration-responsive promoter rd29A. Crop plants such as rice (66), maize (67), and soybean (68) have shared DREB genes and promoters. In tobacco (69) and wheat (70), the stress inducible promoter rd29A in combination with DREB1 may improve drought tolerance.  In Mexico, researchers are conducting experiments on transgenic wheat using Arabidopsis DREB1A under the control of rd29A, which has a more branching root phenotype and generated more ears under drought stress than control lines under greenhouse conditions. The transgenic slows germination, as noticed by Pellegrineschi et al. (2004), although it is unknown whether the gene expression can be "switched-off" when the plants return to non-stress conditions.

Genes encoding aquaporins (water channel proteins) and late embryogenesis abundant proteins (LEA; proteins with several putative roles) have been investigated in transgenic experiments addressing drought and salt. However, much more research is required before we can determine whether these genes will be helpful to breeders. According to Figueras 2004 (71), a maize LEA that was over expressed in Arabidopsis has improved salinity tolerance, and a barley LEA expressed in two rice varieties may promote growth under either salt or drought stress (72, 73).

Much has been written about improving salt tolerance through gene over expression (74, 75). However, there has been no effect on performance in saline field circumstances thus far, and articles reporting on increases in salt tolerance are mainly limited to glasshouse trials (e.g. improved salinity tolerance in cotton (76).  Although candidate genes for tolerance features are listed and over 700 patents citing salt tolerance have been awarded (64), their use to develop cultivars for farmers has been relatively slow.

1. **TILLING (Targeting induced local lesions in genomes)**

The best tools for finding candidate genes for desirable qualities, such stress tolerance, are genome-wide expression profiles. A portion of functional studies uses these candidate genes' inactivation or over expression for further characterization and application. Targeting Induced Local Lesions IN Genomes (TILLING), one of these, allows for high-throughput study of many mutants (77). TILLING has been reported in various crop species, including hexaploid wheat, and is applicable to almost all genes in all species where mutations may be generated (78). Sorghum (79), maize (80), barley (81), soybean (82), rice (83), and other crops have all been reported to have TILLING mutants. The use of several TILLING mutants in the explanation of stress responses has been demonstrated, despite the fact that TILLING populations are often screened by phenotypic or genotypic variations. In this study, the response of various legume species to salt stress was evaluated using TILLING mutants for a particular kinase.

1. **Genome editing**

The success rate of achieving a desired genotype is very high because genome editing modifies DNA at particular sites by using sequence-specific nucleases that cause double-stranded breaks in the target genomic loci selected for editing. Zinc finger (ZF) nucleases, transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats are the main genome editing tools (CRISPR) (84). C-repeat binding factors (CBFs) are in control of plants' adaptability to extreme cold. It is extremely challenging to produce triple mutant CBF1,2,3 lines through conventional genetic crossing since the CBF1-3 loci are all on the same chromosome. Therefore, it was successful to develop single, double, and triple mutants of the CBF genes using the genome editing technology CRISPR/Cas9. Under cold-acclimation treatment, the cbfs triple mutants are the ones that are most susceptible to freezing stress among these various mutants. According to RNA-seq analysis of the triple mutants, c. 10–20% of COR genes' expression is CBF dependent (85, 86). These studies provide support for the theory that CBFs are important regulators that perform redundant functions in plants' cold adaptation. Different crops' drought tolerance has been increased by the use of genome editing.Proton gradients are generated in plant cells by the H+-ATPase encoded by the OPEN STOMATA 2 *(OST2)* gene. Drought stress resistance has been reported to be conferred by precise alteration of this gene using CRISPR/Cas9. This modification modifies stomatal closing in response to water scarcity environments. The OsOREB1, OsRab21, OsRab16b, OsLEA3, OsbZIP23, OsSLAC1 and OsSLAC7 genes, which act downstream of SAPK2, were modulated in expression in the loss-of-function SAPK2 mutant rice plants produced using CRISPR/Cas, increasing their tolerance to drought stress (87). Another gene susceptible to drought stress that has been altered through genome editing is *ARGOS8.*It is a negative regulator of the ethylene signalling system, and plants are known to be more resistant to drought stress when its expression is increased (88, 89). Similarly, CRISPR-based microRNA editing has been successfully used in plants (90) modified miR169a and MIR827a for Arabidopsis thaliana drought stress tolerance. The well-known function of ABA-responsive element-binding protein (AREB) genes in controlling ABA-dependent drought stress responses is well identified.For drought stress tolerance, Roca Paixao, et al (91) used CRISPR-mediated genetic editing to modify the Arabidopsis AREB1 gene. Additional examples of CRISPR-edited genes for regulating drought stress tolerance in various plant species include the ARGOS8 (92), AGO18A and AGO18B (93), MS8 (94), DMC1 (95), ZB7 (96), DREB and ERF3 (97), LOX and UBIL1 (98), MS1 (99).

**Conclusion**

Abiotic stresses, which result in more than 50% harvest losses, are the primary cause of crop losses. Numerous studies have shown that salt and drought conditions have a detrimental impact on plant growth, development, physiology, and yield. It's important to develop crop varieties, lines, and hybrids that are more resistant to drought, salinity, heat, high temperatures, and nutrient scarcity using both conventional and molecular breeding techniques and genetic engineering in to meet the world's food demands while utilizing less water. It is quicker and more effective to induce drought resistance through the genotypic data in agricultural plants to increase and sustain productivity in drought-prone regions when traditional breeding skills and marker-assisted selection are combined. Future plant breeders will need a combination of skills in a variety of fields, including plant breeding,  genomics, genetic diversity, statistics, genetics, experimental design, and germplasm management, in order to achieve success.

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