Breeding for Abiotic Stress Resistance

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Abstract

Abiotic stresses, responsible for yield reduction by up to 50%, are the primary drivers of yield losses in crop plants. Abiotic stress, which damage plant growth and development and have a large detrimental effect on agriculture production globally, include low or high temperatures, droughts, floods, high salinity, heavy metals, and UV radiation. As traditional crop development approaches reach their limits, the age of genomics research has come, bringing with it new and interesting possibilities for breeding superior cultivars against abiotic challenges. With improving selection accuracy, the molecular-assisted selection method anticipates rapid improvement in the selection of agricultural plants that have adapted to stress. It is encouraging that molecular-assisted selection can target features in a single progeny more quickly and precisely in a few selected generations with few unexpected consequences. This chapter provides an overview of the fundamentals of cutting-edge technologies, including molecular mapping, molecular-assisted mapping, transcriptomic, metabolomic, transgenic, and genome editing, and how they are used to enhance crop plants with a focus on stress tolerance.

Keywords: Abiotic; Drought; Flood; Metabolomic; Stress; Transcriptomic

I. INTRODUCTION

Climatic conditions play major role in the development and growth of crops (1). Growth and development has their impact on the crop yield. Environmentalists have predicted an increase in average surface temperature by 0.2 ° C per decade. The increased temperature may affect the availability of fresh water, crop yield as well as loss of biodiversity. It means the altered climatic conditions may have their effects on global food security. Global grain output was claimed to have decreased by 6.2% between 2000 and 2007 based on the results of many research looking at the impact of rising temperature on crop productivity (2).

Stress is an unfavourable condition that hinders a plant's development, growth and metabolism (3). Numerous plant reactions to stress have been documented, including crop yields (4), growth rates (5), changed gene expression (6), cellular metabolism, etc. A plant under stress typically exhibits some abrupt alterations in the environment (7). Deforestation and excessive fossil fuel use are expected to cause the atmospheric CO_2 level to reach 800 ppm before the year 2100 (8, 9). In addition, among other effects, climate change brings about extreme weather, including drought, heavy rain, and temperature swings (10).

Abiotic stressors are significant contributors to reduce yields in farmers' crops in the current climate situation. Currently, abiotic stresses are the biggest problems faced by farmers in the whole world (11). The poor have very little power to respond to the consequences of climate change and adapt to a shifting hazard burden because of their inadequate human and financial resources (12). Population increase and climate change make the situation more challenging. By 2050, the population of the globe is predicted to rise from seven to ten billion, necessitating a 60–110 percent increase in the global food supply. (13).

Future predictions indicate that abiotic stress may eventually become more severe due to a rise in the scarcity of fresh water (14). Crop cultivars that are resistant to abiotic stress must be developed immediately if food safety and security are to be maintained in the coming years (15). The roots of a plant are its first line of defence against abiotic stresses. The likelihood of crop plants surviving adverse circumstances will be greater if they are robust and biologically varied. Abiotic stress, such as excessive salt, causes plant cells to respond in part by disrupting the Na+/K+ ratio in the cytoplasm. For the length of plant adaptation to abiotic stress, such as drought, excessive salinity, low temperature, or mechanical injury, abscisic acid (ABA) is crucial (16).

To create an improved breeding strategy that successfully reduces abiotic stress, it is crucial to adapt food security and sustainable agriculture (17). Additionally, for crops to increase their capacity to adapt to abiotic stress, a multimodal strategy including hormone modulation, plant enzymatic system activation, and stress gene expression is required (18). Therefore, it is crucial to understand how cereal crops respond to abiotic stress. More study is required to fully comprehend the characteristics of abiotic stress and how its physiological, biochemical, and molecular (genetic, epigenetic, transcriptomic, and metabolomic) underpinnings might aid in efforts to breed crops that are resistant to abiotic stress (19).

Currently, a number of methodologies exist to improve crop species' abiotic stress tolerance. (1) the use of plant bio-stimulants; (2) the creation of genetically modified organisms (GMOs), cisgenic, trans-genic, and intra-genic plants (4). Old breeding techniques (3) sophisticated breeding technology, including the use of the (CRISPR/Cas) gene editing tool. Bio-stimulants are characterised as plant fertilizers that increase the availability of

micronutrients in the soil or rhizosphere, tolerance to abiotic stress, and nutrient utilisation efficiency (20).

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

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

II. CROP PLANTS AND ABIOTIC STRESSES

The concept of stress in plants has been established since last 50 years. Both biotic and abiotic factors, which affect crop plants are considered as stresses. Between both of these stresses, abiotic stress reduces yields of the major crop plants. Now a day it is the key factor affecting agricultural productivity globally. Osmotic stress, which upsets the cell's homeostasis and ion distribution, is created when these abiotic stimuli interact. It mostly results from modifications to a group's gene expression patterns, which have an effect on growth rates and productivity. In order to comprehend how plants respond to abiotic stress, it is essential to identify those genes that are susceptible to it.

1. Drought

Global climate change has an impact on everything from temperature and CO_2 levels in the atmosphere to rainfall patterns. Extended periods of inappropriate rainfall are the main cause of drought. While intermittent drought circumstances can affect plant growth and development but are rarely fatal to plants, severe drought conditions drastically reduce the amount of soil water available to plants and cause early plant death.

The intensity of the drought is influenced by a variety of factors, including soil moisture storage capacity, evaporative demands and rainfall distribution and amount (21). Due to global warming and continually increasing CO_2 levels, the climate has changed in the contemporary era. Uneven rainfall distribution brought on by climate change is a major contributor to the emergence of drought. Extreme drought conditions are slowly decreasing the amount of soil water that is available to plants, which results in early plant death. A plant's initial reaction to drought stress is growth halt. Reduced shoot growth caused by dryness decreased the plant's metabolic demands and mobilised metabolites for the production of protective compounds needed for osmotic adjustment.

2. Salinity

Global soil salinity is a problem that poses a serious threat to world agriculture since it reduces crop production in affected regions. Salinity stress has a variety of effects on crop development and productivity. Ionic toxicity and osmotic stress are two ways that salt negatively affects plants (22).

The capacity of plants to absorb water and minerals like K+ and Ca2+ is hampered by salinity stress because the osmotic pressure in the soil solution is greater than the osmotic pressure in plant cells. In addition to reducing cell growth and membrane function, these primary impacts of salt stress also have a number of secondary implications, including assimilate synthesis, cytosolic metabolism, and ROS production.

There are different levels of salt stress tolerance. Rice, durum wheat, sorghum, and maize are less tolerant of salinity than barley, which is the most resilient cereal crop and can endure up to 250 mM NaCl in soil (23).

3. Heat tolerance

High temperature is now a global issue that significantly hinders plant growth and output, especially in crops. The most important issue restricting agricultural productivity and, eventually, food security, has been identified as heat stress. (24).

In extreme cases, a bad change in temperature results in plant mortality since plants are more sensitive to temperature changes. The ideal temperature would typically be better for plant growth and function; conditions below and above the ideal temperature have a negative effect on plant growth and productivity. For every 10°C increment followed by 20°C and 30°C, the majority of biochemical and enzymatic processes double in speed (25). Abiotic stressors, particularly high and low temperatures, have a negative impact on the early stage of the male gametophyte in a variety of agricultural crops, including rice, maize, barley, sorghum, wheat, and chickpea (26).

Due to heat stress, the tapetal cells' functions are lost during the reproductive development period, and the anther is dysplastic. Pollen release is inadequate and indehiscence occurs as a result of increased temperatures preventing pollen grains from expanding during lowering. Plants have evolved specific chemical and physiological reactions to cope with heat stress (21).

4. Cold

One of the primary abiotic factors that affects agricultural crops' quality and post-harvest life is cold stress. On plant development and agricultural productivity, cold stress, which includes freezing and chilling, has negative consequences (27, 28).

All cellular functions in plants are significantly impacted by cold-induced abiotic stress. Then, a variety of signal transduction pathways, including those involving ROS, protein kinase, protein phosphate, ABA, and Ca2+, among others, are used to translate cold stressors, with ABA emerging as the most efficient of them (29). Only tropical or subtropical regions are suitable for the growth of maize, tomato, rice, cotton, and soybean due to their inability to adapt to low temperatures (30). Thus, cold stress hinders the ecological spread of plant species, has a detrimental effect on plant growth and development, and decreases world food production (31).

5. Toxins

Due to increased industrialisation, toxic metals have been introduced to agricultural soils, increasing the quantity of chemical fertilisers and sewage wastewater irrigation, and negatively affecting the soil-plant environment system (32). One such instance is cadmium (Cd), which is the main metal pollutant and is regarded as a significant environmental risk for the agricultural system due to its protracted residence time in soil. These metals gradually poison plants through the air, water, and food chain when they enter the food chain over time (33).

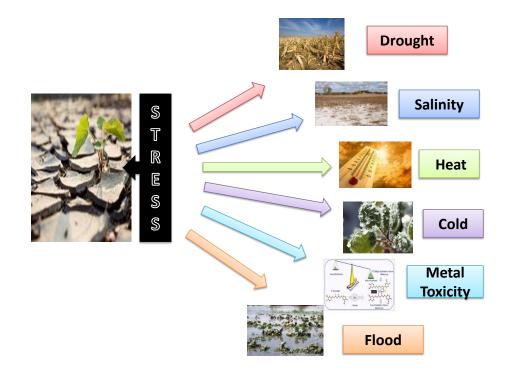


Figure 1. Some biotic stress affecting crop plants productivity and development

III. CONVENTIONAL BREEDING FOR ABIOTIC STRESS TOLERANCE

Abiotic tolerance in agricultural plants has well-established origins. Tolerance can be found in land races, wild cousins, high-yielding cultivars, original breeding stock, and advanced breeding material. Landraces from arid areas have been successfully used in breeding to produce open-pollinated types or hybrids for water-scarce situations. Breeders frequently employed the wild species and ancestors of our farmed crops as a rich source of abiotic stress donors. The likelihood of finding the necessary genes, along with the anticipated challenges and likelihood of success in introducing these genes into the selected recurrent cultivar, all influence the genetic resource to be used as a donor for abiotic stress tolerance.

There are several genetic variants in better cultivars of other crop species as well as in breeding materials for drought and salinity resistance. Finding and utilising these sources of drought resistance should be a breeder's first focus since they are the most straightforward to use. Land races (old or desi variations) are excellent sources of characteristics for drought and salinity resistance since they have evolved in and adapted to arid and saline circumstances. It may be difficult to use these materials in breeding programmes because of unfavourable links.

Wheat, sugarcane, tomatoes, and other crops have been identified to include wild donors of drought and salinity resistance. Transfer presents a number of challenges, including cross-incompatibility, hybrid sterility, inviability, and the coupling of multiple unfavourable genes with positive ones, when a wild species provides drought and salinity resistance. The mode of reproduction of the species, whether it be self-pollinating, cross-pollinating, or asexual, should be taken into account while selecting a breeding approach for any crop. the type of cultivar and character genetic regulation. It might be challenging to breed a species for many

resistance traits at once since various plants have diverse responses to the same abiotic stress stimuli.

Drought resistance can be bred for using the same breeding methods as for yield and other economic traits. The pedigree and bulk approaches can be used with self-pollinated plants, whereas recurrent selection can be used with cross-pollinated plants. However, backcrossing is the suggested method if the objective is to add a few drought-related characteristics to a genotype with high yields. Hurd (34, 35) and others have discussed breeding techniques for drought resistance (36). Crop cultivars that can withstand droughts can be developed by introduction, hybridization, selection, and mutation. To develop cultivars that can withstand salt, pedigree, modified bulk pedigree, and an alternative culture-based method were all applied. The complexity of abiotic stress events in plants makes genetic modification for efficient stress resistance problematic (37). Drought characterization for successful stress resistance breeding depends on the stage, duration, and severity of the stress. Using traditional breeding methods, drought-tolerant varieties of crops such, safflower wheatgrass, chickpea, barley, tall fescue, soybean, wheat, peanut and maize have been created.

The first step in breeding for any trait is incorporating genetic variation through the collection and assessment of the available germplasm. The introduction of the foreign germplasm might be used if a locality or species lacks the desired variability. All breeding tactics still rely heavily on this traditional strategy. Only a few salinity-tolerant cultivars have been created using selection and introduction techniques worldwide. The rice cultivars Jaladhi-1 (a Kalakhersail selection), Jaladhi-2 (a Baku selection), Jalaprabha (a composite selection), Neeraja (a landrace selection), Dinesh [CN-570-652-39-2(Jaladhi-2/Pankaj)], and Hangseswari (a pure line selection) were created through selection for tolerance to deepwater (38).

In a cultivar development programme, the pedigree selection approach can be employed to find superior genotypes for grain yield. Pedigree selection for grain yield/plant must assess choices under a range of conditions, such as various planting dates (39, 40), various water stress, etc (41). Breeders at NDUAT, Faizabad, Uttar Pradesh, employed the modified pedigree approach (single panicle selection) (42). The use of the pedigree selection strategy to create new lines tolerant of drought stress was quite successful (43). Recombinant variations were used to create White ponni, CR Dhan-403, TRY-1, TRY-2, TRY3, CO-43, CSR-30 CSR0-10, CSR-23, CSR-13, CSR-36, CSR-27, and other plants (44).

The backcross method is a type of recurrent hybridization that allows for the addition of a gene for a superior trait to a desirable variety. Backcross breeding is the best technique to add drought tolerance features to a high yielding cultivar (45). However, propagating drought tolerance in high yielding genotypes is difficult because the physiological and genetic bases of adaptation to drought conditions are not well known. A complete strategy for breeding for drought resistance and high yield potential that benefit from repeated selection method has been developed (46). The International Rice Research Institute (IRRI) is now using this breeding technique to increase rice's tolerance to drought (47). In rice, a considerable number of introgression lines with better abiotic stress tolerance have been developed using three recurrent parents and 203 donor lines with different abiotic stress tolerance (48).

IV. ADVANCED TECHNOLOGIES FOR CROP IMPROVEMENT AGAINST ABIOTIC STRESS

In comparison to earlier generations, high-throughput techniques for DNA and protein analysis have revolutionised how cells may be studied. Modern transgenic technology inserts novel foreign genes or alters the amount of endogenous gene expression to increase stress tolerance. In order to create transgenic crops that are resistant to abiotic stresses, the transgenic technique is increasingly frequently used. Assorted breeding methods, including hybridization, mutation, and marker-aided breeding, genome sequencing along with omics approaches, could be used to improve the chickpea germplasm lines(s) against drought stress (49). Determining the relevant genes and pathways to engineering stress-tolerant crop plants requires an understanding of the molecular mechanisms by which plants observe stress signals and translate them to cellular machinery to begin adaptive responses (50,51,52).

1. Marker Assist Selection

In recent years, significant efforts have been undertaken to improve the capacity of grain crops to endure stress. However, issues including increased environmental stress, insufficient moisture, and unpredictably changing climatic conditions made it difficult for breeders to develop new crop kinds (53). In the present period, it is essential to apply technology to solve these issues, such as molecular aided breeding to boost the production of cash crops. It provides the chance to precisely choose crop plants that can endure stress (54). In comparison to phenotypic-based breeding, molecular-assisted breeding approaches have become two times faster for a number of traits, including yield and quality traits, resistance to biotic and abiotic stress, and yield traits. (55). Additionally, MAS has enhanced crop plants' agronomic characteristics (56). DNA marker maps are developed using the molecular markers.

Based on the degree of relationship between the marker and target characteristic, these marker maps are used to identify candidate genes that impact the desired attributes. The targeted alleles and their loci have been identified and used in the MAS with regard to abiotic stressors such water logging, salinity, and desiccation (57,58,59). In order to create new multi-abiotic-resistant lines, conventional, molecular-assisted, and DNA markers can be used to newly challenged locations (60). Various crops, including Arabidopsis (61), rice (62), maize (63) and Brassica (64), have lately benefited from genetic engineering to increase their salt and drought resistance (64). Similarly, MAS developed waterlogged-tolerant lines in other crop plants (65). Marker-assisted selection can increase effectiveness, however thus far, findings have not been as anticipated when looking for markers associated with low heritability component traits. QTL introgression for yield under stress is proving to be a more effective method in pearl millet (66). By eliminating the effects of unwanted linked alleles, QTL fine-mapping and map-based cloning will enable them to be used more effectively in breeding (67). To combine specific allele combinations for the best result, MAS needs to be fine-tuned.

2. Maker Assist Backcross Selection (MABC)

Marker-assisted backcrossing is most promising techniques for employing molecular markers to identify and select the genes impacting resistance (MABC). With merely two or three backcrosses, the recurrent parent genotype can be recovered using MABC, a recently improved approach that uses a huge number of populations (400 or more plants) for the backcross F1 generations.

Several high yielding, high-quality, and stress-tolerant cultivars have so far been developed by MABC in a number of developing nations in the shortest amount of time. The creation of new varieties and lines occurs often in modern plant breeding programmes using MABC. Rice breeding frequently uses backcrossing to transfer a specific gene from the donor parent to the recipient plant. Backcross breeding has been used as a breeding technique in South and Southeast Asia to improve the blast resistance of premier kinds including KDML105, Basmati, and Manawthukha (68, 69). Advances in rice biotechnology and genomics have led to the development of new genes for resistance to biotic and abiotic stress. MABC has previously shown that it is capable of introducing critical genes into superior rice parents by using both foreground and background selection. Eight MAFB lines and twelve MABC lines with superior yield performance compared to Naveen and three to six genes/QTLs for resistance/tolerance to biotic stressors and reproductive stage drought stress were created (70). Five wheat lines were created from a starting population of 516 BC1F1 plants using the marker-assisted backcross breeding (MABB) technique and three linked quantitative trait loci (QTLs). These lines all naturally tolerate drought stress. The high-yielding wheat cultivar HD2733, which is used as the recurrent parent and has been widely grown in India's eastern plains during the past few years, is extremely drought-sensitive. The donor parent, "HI1500," was created for water-stressed locations and contains QTLs that are resistant to drought (71).

3. Omics Methods

Functional genomics methodologies analyse the transcriptome of plants under abiotic environmental stress using techniques including quantitative RT-PCR, microarray, and high-throughput RNAseq. In various cDNA libraries, expressed sequence tags (ESTs) that have already been submitted can be utilised to look for the gene expression patterns of certain tissues at a particular developmental stage. Before moving on with the design of an experiment, abiotic stress responsive genes, in particular abiotic stress tolerance mechanisms, might be assessed (72).

In establishing the molecular underpinnings of abiotic stress responses in plants, great progress has been made using high throughput sequencing and functional genomics techniques. Recently discovered and confirmed critical genes involved in abiotic stress tolerance can be split into two groups: functional genes and regulatory genes. By expressing crucial metabolic proteins (functional proteins) and enzymes as detoxifying enzyme, water channel, ion transporter, heat shock protein (HSP), and late LEA protein, the former actively works to protect cells from stressors. By encoding a range of regulatory proteins including TFs, protein kinases and protein phosphatases the latter controls gene expression and signal transmission in the stress responses.

Numerous genes in Arabidopsis have been shown to respond to abiotic stressors as a result of expression investigations (73, 74). Such research produces a significant number of candidate genes and the associated biological processes. There are 53 genes that are activated by cold stress, 277 by drought stress, and 194 by salt stress, for instance. The disentanglement of root QTL component genes could be accelerated using expression analysis. When there was a severe drought, the upland variety Azucena's root tissues displayed differential expression of 66 transcripts (75).

Recently, the metabolic processes of salt adaptation were discussed by Hasegawa and Wang (76, 77). However, only a few studies have convincingly identified transcripts linked to salt tolerance (78). When high NaCl concentrations are given in a single dosage without Ca2+ to

lessen shock, it is unlikely that genes governing Na+ or K+ transport would be found. Often, investigations are not designed to identify the genes that confer tolerance in normal situations. Due to this disregard for the principles underlying tolerance, many studies have concentrated on tolerance to osmotic stress rather than salinity.

4. Transgenic Approaches

Crops can be made resistant to abiotic stress by using a transgenic approach to increasing endogenous defence mechanisms, which typically involves the production of antioxidants, compatible osmolytes, and polyamines, altering transporters and regulatory proteins, maintaining hormone homeostasis, and counting transcription factors and alternative splicing events. When specific genes are over expressed, various proteins and metabolites are produced that occasionally interfere with normal metabolism and reduce yield.

The foundation of current engineering approaches is 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 associated with stress tolerance (77). to create plants that can withstand abiotic stress. ABA and other phytohormones are the main targets of genetic alteration.

A significant component of this work in Arabidopsis has utilised the dehydration-responsive element-binding (DREB) transcription factors of the ABA-dependent pathway IV, which were first associated with enhanced cold tolerance (79). These have been combined with constitutive promoters and are regulated by the rd29A promoter of Arabidopsis, which responds to dehydration. Crop plants with DREB genes and promoters in common include rice (80), maize (81), and soybean (82). The stress inducible promoter rd29A in conjunction with DREB1 may increase drought tolerance in tobacco (83) and wheat (84). Arabidopsis DREB1A, which has a more branching root phenotype and produced more ears under drought stress than control lines under greenhouse conditions, is being used in tests on transgenic wheat in Mexico under the control of rd29A. It was observed that the transgenic slows germination, but it is unknown whether the gene expression can be "switched-off" when the plants return to non-stress settings.

In transgenic studies addressing drought and salt, genes encoding aquaporins (water channel proteins) and late embryogenesis abundant proteins (LEA; proteins with multiple possible roles) have been examined. To find out whether these genes will be advantageous to breeders, however, requires conducting a great deal more research. A maize LEA that was over expressed in Arabidopsis has improved salinity tolerance, and a barley LEA expressed in two rice types may promote growth under either salt or drought stress (85, 86, 87).

Increasing salt tolerance through gene expression has been the subject of numerous articles (88, 89). However, there has not yet been a change in performance in salty field conditions, and studies reporting on increased salt tolerance are generally restricted to glasshouse trials (for example, better salinity tolerance in cotton) (90). The application of these genes to create cultivars for farmers has been very gradual, despite the fact that they are identified as candidate genes for tolerance traits and that more than 700 patents stating salt tolerance have been granted (78).

5. Tilling (Targeting induced local lesions in genome)

The best tools for finding candidate genes for desirable qualities, such stress tolerance, are genome-wide expression patterns. Inactivation or over-expression of these potential genes is used in some functional investigations for further characterisation and applicability. TILLING, one of them, permits high-throughput investigation of many mutations (91). TILLING has been observed in numerous crop species, including hexaploid wheat, and is relevant to practically all genes in all species where mutations may arise (92). There have been reports of TILLING mutants in sorghum (93), maize (94), barley (95), soybean (96), rice (97) and other crops. It has been established that several TILLING mutants can be used to explain stress responses, despite the fact that TILLING populations are regularly screened for phenotypic or genotypic abnormalities.

6. Genome Editing

The success rate of achieving a desired genotype is fairly high because genome editing modifies DNA at exact sites by using sequence-specific nucleases that create double-stranded breaks in the target genomic loci chosen for editing. The primary genome editing tools (CRISPR) are zinc finger (ZF) nucleases, transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (98). C-repeat binding factors (CBFs) regulate how well plants can withstand extremely low temperatures. Since the CBF1-3 loci are all on the same chromosome, it is extremely difficult to develop triple mutant CBF1,2,3 lines through traditional genetic crossing. Therefore, using the genome editing technique CRISPR/Cas9, it was possible to create single, double, and triple mutants of the CBF genes. Among these several mutants, the cbfs triple mutants are the ones that are most vulnerable to freezing stress after cold-acclimation treatment. The triple mutants' RNA-seq research revealed that approximately 10-20% of COR genes' expression is CBF dependent (99, 100). The hypothesis that CBFs are crucial regulators that carry out redundant tasks in plants' cold adaptation is supported by these researches. Genome editing has improved the ability of several crops to withstand drought. The H+-ATPase, which is expressed by the OST2 gene, produces proton gradients in plant cells. It has been shown that precise CRISPR/Cas9 gene modification of this gene confers drought stress resistance. Stomatal closure is altered by this alteration in circumstances where there is a lack of water. In the loss-of-function SAPK2 mutant rice plants created using CRISPR/Cas, the expression of the OsSLAC7, OsRab21, OsLEA3, OsOREB1, OsSLAC1, OsbZIP23 and OsRab16b genes was altered, enhancing their tolerance to drought stress (101). ARGOS8 is another gene that has been modified through genome editing and is vulnerable to drought stress. When its expression is elevated, plants are known to be more resilient to drought stress because it is a negative regulator of the ethylene signaling pathway (102, 103). Similar to this, plants have successfully used CRISPR-based microRNA editing to modify miR169a and MIR827a for Arabidopsis thaliana drought stress tolerance (104). The Arabidopsis AREB1 gene was modified by Roca Paixao, et al. (105) using CRISPR-mediated genetic editing to increase drought stress tolerance. ARGOS8, AGO18A and AGO18B, MS8, DMC1, ZB7, DREB and ERF3, LOX and UBIL1, MS1, and MS1 are other examples of CRISPR-edited genes for modulating drought stress tolerance in various plant species (106-113).

Conclusion

Agricultural crops losses are mostly caused by abiotic stresses, which account for more than half of all harvest losses. Numerous studies have demonstrated the negative effects of salt and drought on plant growth, development, physiology, and yield. In order to meet the world's food demands while using less water, it is crucial to develop crop varieties, lines, and hybrids that are stronger against drought, salinity, heat, high temperatures, and nutrient scarcity. When traditional breeding techniques and marker-assisted selection are coupled, it is quicker and more efficient to instill drought resistance through the genotypic data in agricultural plants to boost and sustain productivity in drought-prone locations. In order to be successful, future plant breeders will need a number of abilities in a range of areas, such as plant breeding, genomics, genetic diversity, statistics, genetics, experimental design, and germplasm management.

References

- [1] Costa MD, Farrant JM. Plant Resistance to Abiotic Stresses. Plants (Basel). 2019 Nov 28;8(12):553. doi: 10.3390/plants8120553.
- [2] Lesk C., Rowhani P., Ramankutty N. Influence of extreme weather disasters on global crop production. *Nature*. 2016;529:84–87. doi: 10.1038/nature16467.
- [3] Choudhary, ML, Tripathi, MK Gupta, N, Tiwari, S, Tripathi, N, Parihar, P and Pandya, RK Screening of pearl millet [*Pennisetum glaucum* [L] R Br] germplasm lines against drought tolerance based on biochemical traits. Current Journal of Applied Science & Technology. 2021;40(23):1-12.
- [4] Mishra N., Tripathi M.K., Tiwari S., Tripathi N., Gupta N., Sharma A. (2021a) Morphological and physiological performance of Indian soybean [*Glycine max* (L.) Merrill.] genotypes in respect to drought. Legume Research. DOI: 10.18805/LR-4550.
- [5] Mishra N., Tripathi M.K., Tiwari S., Tripathi N., Sapre S., Ahuja A., Tiwari S. (2021b) Cell suspension cultures and *in vitro* selection for drought tolerance in soybean (*Glycine max* (L) Merr.) using Poly-Ethylene Glycol. *Plants* https://doi.org/10.3390/plants10030517.
- [6] Mishra N., Tripathi M.K., Tiwari S., Tripathi N., Trivedi H.K. (2020) Morphological and molecular screening of soybean genotypes against yellow mosaic virus disease. *Legume Research*. *DOI:* 10.18805/LR-4240.
- [7] Mishra N., Tripathi M.K., Tripathi N., Tiwari S., Gupta N. and Sharma A. (2022b). Screening of soybean genotypes against drought on the basis of gene-linked microsatellite markers. In Innovations in Science and Technology Vol. 3, Chapter 4, Print ISBN: 978-93-5547-469-8, eBook ISBN: 978-93-5547-470-4. DOI: 10.9734/bpi/ist/v3/2454C.
- [8] Mishra N., Tripathi M.K., Tripathi N., Tiwari S., Gupta N., Sharma A. and Shrivastav M.K. (2021c). Role of biochemical and antioxidant enzymes activities in drought tolerance in soybean: A Recent Study. In Current Topics in Agricultural Sciences vol 3. Print ISBN: 978-93-5547-186-4, eBook ISBN: 978-93-5547-187-1. DOI: 10.9734/bpi/ctas/v3/2117C.
- [9] Mishra N., Tripathi M.K., Tripathi N., Tiwari S., Gupta N., Sharma A., Shrivastav M.K. (2021d). Changes in biochemical and antioxidant enzymes activities play significant role in drought tolerance in soybean. *International Journal of Agricultural Technology* 17(4):1425-1446.
- [10] Mishra N., Tripathi M.K., Tripathi N., Tiwari S., Gupta N., Sharma A. (2021e). Validation of drought tolerance gene-linked microsatellite markers and their efficiency for diversity assessment in a set of soybean genotypes. *Current Journal of Applied Science and Technology* 40(25):48-57.
- [11] Sharma A., Tripathi M.K., Tiwari S., Gupta N., Tripathi N., Mishra N. (2021). Evaluation of soybean (*Glycine max* L.) genotypes on the basis of biochemical contents and anti-oxidant enzyme activities. *Legume Research* DOI: 10.18805/LR-4678.
- [12] Tiwari, H., Naresh, R. K., Kumar, L., Kataria, S. K., Tewari, S., Saini, A., Yadav, R. K., & Asati, R. (2022). Millets for Food and Nutritional Security for Small and Marginal Farmers of North West India in the Context of Climate Change: A Review. *International Journal of Plant & Soil Science*, 34(23), 1694-1705. https://doi.org/10.9734/ijpss/2022/v34i232594
- [13] Rockström J, Williams J, Daily G, Noble A, Matthews N, Gordon L et al. Sustainable intensification of agriculture for human prosperity and global sustainability. Ambio. 2017; 46:4–17. https://doi.org/10.1007/s13280-016-0793-6.

- [14] Upadhyay S., Singh A.K., Tripathi M.K., Tiwari S., Tripathi N., Patel R.P. (2020) In vitro selection for resistance against charcoal rot disease of soybean [Glycine max (L.) Merrill] caused by Macrophomina phaseolina (Tassi) Goid. Legume Research. DOI: 10.18805/LR-4440.
- [15] Verma S, Nizam S, Verma PK. Biotic and abiotic stress signalling in plants. Stress Signaling in Plants: Genomics and Proteomics Perspective. 2013; 1:25-49.
- [16] Hofmann DJ, Butler JH, Tans PP. A new look at atmospheric carbon dioxide. Atmos Environ 2009; 43:2084–2086. doi: 10.1016/j.atmosenv.2008.12.028.
- [17] U.S. Global Change Research Program. Global Climate Change Impacts in the United States: A State of Knowledge Report. Cambridge University Press; Cambridge, UK; New York, NY, USA: 2009.
- [18] Rockström J, Williams J, Daily G, Noble A, Matthews N, Gordon L et al. Sustainable intensification of agriculture for human prosperity and global sustainability. Ambio. 2017; 46:4–17. doi: 10.1007/s13280-016-0793-6.
- [19] Seki SK, Reddy KR, Li J. Abscisic acid and abiotic stress tolerance in crop plants. Frontiers in Plant Science. 2007; 7:571
- [20] Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 Laying Down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003 (Text with EEA Relevance) Vol. 170. European Parliament, Council of the European Union; Luxembourg: 2019. pp. 1– 114.
- [21] Hazel JR. Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation?. Annual Review of Physiology. 1995; 57:19-42
- [22] Kumar M. Crop Plants and Abiotic Stresses. J Biomol Res Ther. 2013, 3:1. http://dx.doi.org/10.4172/2167-7956.1000e125.
- [23] Maas EV, Hoffman GJ. Crop salt tolerance Current assessment. Journal of the Irrigation and Drainage Division. 1977;103:115-134
- [24] Liliane TN, Charles MS. Factors affecting yield of crops. In: Amanullah, editor. Agronomy Climate Change & Food Security. IntechOpen. 2020. DOI: 10.5772/intechopen.90672
- [25] Mathivanan S. Abiotic Stress-Induced Molecular and Physiological Changes and Adaptive Mechanisms in Plants. In S. Fahad, S. Saud, Y. Chen, C. Wu, & D. Wang (Eds.), Abiotic Stress in Plants. IntechOpen. 2020. https://doi.org/10.5772/intechopen.93367
- [26] Boyer JS, Westgate ME. Grain yields with limited water. Journal of Experimental Botany. 2004; 55:2385-2394
- [27] Guo XY, Liu DF, Chong K. Cold signaling in plants: Insights into mechanisms and regulation. Journal of Integrative Plant Biology. 2018; 60:745-756
- [28] Liu H, Yu C, Li H, Ouyang B, Wang T, Zhang J, et al. Over expression of ShDHN, a dehydrin gene from Solanum habrochaites enhances tolerance to multiple abiotic stresses in tomato. Plant Science. 2015; 231:198-211
- [29] Gull A, Lone A. A., & Wani, N. U. I. Biotic and Abiotic Stresses in Plants. In (Ed.), Abiotic and Biotic Stress in Plants. IntechOpen. 2019
- [30] https://doi.org/10.5772/intechopen.85832
- [31] Chinnusamy V, Zhu J, Zhu JK. Cold stress regulation of gene expression in plants. Trends in Plant Science. 2007; 12(10):444-451
- [32] Pearce RS. Plant freezing and damage. Annals of Botany. 2001; 87:417-424
- [33] Nievola CC, Carvalho CP, Carvalho V, Rodrigues E. Rapid response of plants to temperature changes. Temperature. 2017;4(4):371-405
- [34] Rauwane M, Ntushelo K. Understanding biotic stress and hormone signalling in cassava (Manhot esculenta): Potential for using hyphenated analytical techniques. Applied Sciences. 2020;20:8152. DOI: 10.3390/app10228152
- [35] Hurd EA. A method of breeding for yield of wheat in semi-arid climates. Euphytica. 1969; 18:217-226.
- [36] Hurd EA. Can we breed for drought resistance ? In : Larson, K. L. and Eastin, J. D. (Eds) : Drought injury and resistance in crops. CSSA Special Publication 2. Crop Science Society of America, Madison, Wisconsin. 1971; 77-88.
- [37] Rao MJB, Murth KS. Choudhury D. Srinivasulu K. and Gangacharan G. Breeding for drought and upland conditions in rice. Oryza,1971; 8(2):75-84.
- [38] Wang JP. Raman H. Zhou MX. Ryan PR. Delhaize E. Hebb DM et al. High-resolution mapping of the Alp locus and identification of a candidate gene HvMATE controlling aluminium tolerance in barley (Hordeum vulgare L.). Theoretical and Applied Genetics. 2007; 115: 265–276.
- [39] Indrani Dana, Sitesh Chatterjee and Chinmoy Kundu. Twenty years of achievements of the EIRLSBN at the Rice Research Station, Chinsurah. Collard, B.C.Y., Ismail, I.S. and Hardy, B. (eds.), IRRI. EIRLSBN Twenty years of achievements in rice breeding. 2013; Pp. 53-64.

- [40] Zakaria MM. Selection for earliness and grain yield in bread wheat (Triticm aestivum L.) under different environments. Ph.D Thesis, Assiut University, Egypt. 2004.
- [41] Ali MA. and Abo-El-Wafa A.M. Inheritance and selection for earliness in spring wheat under heat stress. Assiut J. Agric. Sci. 2006; 37: 77-94.
- [42] El-Morshidy MA, Kheiralla KA, Ali MA. and Ahmed AAS. Efficiency of pedigree selection for earliness and grain yield in two what populations under water stress conditions. Assiut J. Agric. Sci. 2010; 37: 77-94.
- [43] Mishra B, Akbar M, Seshu DV and Senadhira D. Genetics of salinity tolerance and ionic uptake in rice. Int. Rice Res. Newsl. 1996; 21: 38-39.
- [44] Tammam AM, El-Ashmoony MSF, El-Sherbeny AA. and Amin AL. Selection responses for drought tolerance in two bread wheat crosses. Egypt J.Agric. Res. 2004; 82: 1213-1226.
- [45] Reddy MA, Francies RM, Rasool Sk. and Reddy VRP. Breeding for tolerance to stress triggered by salinity in rice. Internat. J. Appl. Biol. AndPharmaceutical Tech. 2014; 5(1): 167-176.
- [46] Edmeades GO, Bolanos J. and Lafitte HR. Progress in breeding for drought tolerance in maize proceedings of the Fourthy-Seventh Annual Corn and Sorghum Industry Research Conference. 1992; Pp. 93-111.
- [47] Paroda RS. Breeding approaches for drought resistance in crop plants. In: Approaches for Incorporating Drought and Salinity Resistance in Crop Plants, Chopra VL, Paroda RS (eds.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 1986; pp. 87-107.
- [48] Lafitte HR, Li ZK, Vijayakumar C.H.M, Gao YM, Shi Y, Xu JL et al. Improvement of rice drought tolerance through backcross breeding: Evaluation of donors and selection in drought nurseries. Field Crops Research. 2006; 97: 77–86
- [49] Ali AJ, Xu JL, Ismail AM, Fu BY, Vijayakumar C.H.M, Gao YM et al. Hidden diversity for abiotic and biotic stress tolerances in the primary gene pool of rice revealed by a large backcross breeding program. Field Crops Res. 2006; 97: 66–76.
- [50] Asati, R.; Tripathi, M.K.; Tiwari, S.; Yadav, R.K.; Tripathi, N. Molecular Breeding and Drought Tolerance in Chickpea. *Life* 2022, *12*, 1846. https://doi.org/10.3390/life12111846
- [51] Ray S, Dansana PK, Bhaskar A, Giri J, Kapoor S, Khurana, JP, et al. Emerging trends in functional genomics for stress tolerance in crop plants. Plant Stress Biology, ed H. Hirt (Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA). 2009; 37–63.
- [52] Heidarvand L, and Amiri RM. What happens in plant molecular responses to cold stress? Acta Physiol. Plant. 2010; 32, 419–431. doi: 10.1007/s11738-009-0451-8
- [53] Sanchez DH, Pieckenstain FL, Szymanski J, Erban A, Bromke M, Hannah MA, et al. Comparative functional genomics of salt stress in related model and cultivated plants identifies and overcomes limitations to translational genomics. PLoS ONE. 2011; 6:e17094. doi: 10.1371/journal.pone.0017094
- [54] Lateef DD. DNA marker technologies in plants and applications for crop improvements. J Biosci Med. 2015; 3:7–18
- [55] Kiriga WJ, Yu Q, Bill R. Breeding and genetic engineering of drought-resistant crops. Int J Agric Crop. 2016; 9(1):7–12
- [56] Oliveira LK, Melo LC, Brondani C, Peloso MJD, Brondani RPV. Backcross assisted by microsatellite markers in common bean. Genet Mol Res. 2008; 7(4):1000–1010
- [57] Rozema J, Flowers T. Crops for a salinized world. Science. 2008; 322:1478–1480
- [58] Baltazar MD, Ignacio JCI, Thomson MJ, Ismail AM, Mendioro MS, Septiningsih EM. QTL mapping for tolerance of anaerobic germination from IR64 and the aus landrace Nanhi using SNP genotyping. Euphytica. 2014; 197:251–260
- [59] Kretzschmar T, Pelayo MA, Trijatmiko KR, Gabunada LF, Alam R, Jimenez R et al. A trehalose-6phosphate phosphatase enhances anaerobic germination tolerance in rice. Nat Plants. 2015; 1:15124. https://doi.org/10.1038/nplants.2015.124
- [60] Gonzaga ZJC, Carandang J, Sanchez DL. Mapping additional QTLs from FR13A to increase submergence tolerance in rice beyond SUB1. Euphytica. 2016; 209:627. https://doi.org/10.1007/s10681-016-1636-z
- [61] Rumanti IA, Nugraha Y, Wening RH, Gonzaga ZJC, Nasution A, Kusdiaman D, et al. Development of high-yielding rice varieties suitable for swampy lands in Indonesia. Plant Breed Biotechnol. 2016; 4(4):413–425
- [62] Nakashima K, Ito Y, Yamaguchi-Shinozaki K. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol. 2009; 149:88–95
- [63] Fukao T, Xiong L. Genetic mechanisms conferring adaptation to submergence and drought in rice: simple or complex?. CurrOpin Plant Biol. 2013; 16:196–204
- [64] Tollefson J. Drought-tolerant maize gets US debut. Nature. 2011; 469:144
- [65] Zhang X, Lu G, Long W, Zou X, Li F, Nishio T. Recent progress in drought and salt tolerance studies in Brassica crops. Breed Sci. 2014; 64(1):60–73

- [66] Devi EL, Devi CP, Kumar S, Sharma S, Beemrote A, Chongtham SK, Akoijam R. Marker assisted selection (MAS) towards generating stress tolerant crop plants. Plant Gene. 2017; 11:205–218
- [67] Serraj R, Hash C.T, Rizvi S.M.H, Sharma A, Yadav R.S, Bidinger F.R. Recent advances in markerassisted selection for drought tolerance in pearl millet. Plant Prod. Sci. 2005; 8:331– 337. doi:10.1626/pps.8.334
- [68] Price A.H. Believe it or not, QTLs are accurate. Trends Plant Sci. 2006; 11:213– 216. doi:10.1016/j.tplants.2006.03.006
- [69] Joseph M, Gopalakrishnan S, Sharma RK, Singh VP, Singh AK, Mohapatra T. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Mol Breed. 2004; 13:377387.
- [70] Sreewongchai T, Toojinda T, Thanintorn N, Kosawang C, Vanavichit A, Tharreau D, Sirithunya P. Development of elite indica rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. Plant Breed. 2010; 129:176-180.
- [71] Janaki Ramayya P, Vinukonda VP, Singh UM, Alam S, Venkateshwarlu C, Vipparla AK, et al. Markerassisted forward and backcross breeding for improvement of elite Indian rice variety Naveen for multiple biotic and abiotic stress tolerance. PLoS ONE. 2021; 16(9): e0256721. https://doi.org/10.1371/journal.pone.0256721
- [72] Rai, Neha & Bellundagi, Amasiddha& Kumar, Prashant & Thimmappa, Ramya & Rani, Sushma & Sinha, Nivedita & Krishna, Hari & Jain, Neelu& Singh, Gyanendra & Singh, Pradeep & Chand, Suresh & Prabhu, Kumble. Marker-assisted backcross breeding for improvement of drought tolerance in bread wheat (Triticum aestivum L. emThell). Plant Breeding. 2018; 137. 10.1111/pbr.12605.
- [73] Sreenivasulu N, Sopory SK, Kishor PBK. Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches, Gene. 2007; 388:1-13. doi:10.1016/j.gene.2006.10.009.
- [74] Seki M, et al. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and highsalinity stresses using a full-length cDNA microarray. Plant J. 2002; 31:279–292. doi:10.1046/j.1365-313X.2002.01359.x
- [75] Bray E.A. Genes commonly regulated by water-deficient stress in Arabidopsis thaliana. J. Exp. Bot. 2004; 55:2331–2341. doi:10.1093/jxb/erh270
- [76] Yang L, Zheng B, Mao C, Qi X, Liu F, Wu P. Analysis of transcripts that are differentially expressed in three sectors of the rice root system under water deficit. Mol. Genet. Gen. 2004; 272:433–442.
- [77] Hasegawa P.M, Bressan R.A, Zhu J.K, Bohnert H.J. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 2000; 51:463–499. doi:10.1146/annurev.arplant.51.1.463
- [78] Wang W, Vinocur B, Altman A. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta. 2003; 2181-14.
- [79] Munns R. Genes and salt tolerance: bringing them together. New Phytol. 2005; 167:645–663. doi:10.1111/j.1469-8137.2005.01487.x
- [80] Gilmour S.J, Fowler SG, Thomashow MF. Arabidopsis transcriptional activators CBF1, CBF2, and CBF3 have matching functional activities. Plant Mol. Biol. 2004; 54:767– 781. doi:10.1023/B:PLAN.0000040902.06881.d4
- [81] Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet E.G, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. OsDREB genes in rice *Oryza sativa* L. encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 2003;33:751–763. doi:10.1046/j.1365-313X.2003.01661.x
- [82] Qin F, Sakuma Y, Li J, Liu Q, Li Y.-Q, Shinozaki K, Yamaguchi-Shinozaki K. Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in Zea mays L. Plant Cell Physiol. 2004; 45:1042–1052.
- [83] Li XP, Tian AG, Luo GZ, Gong ZZ, Zhang SS, Chen SY. Soybean DRE-binding transcription factors that are responsive to abiotic stresses. Theor. Appl. Genet. 2005 ;110:1355–1362. doi:10.1007/s00122-004-1867-6
- [84] Kasuga M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K. A combination of the Arabidopsis DREB1A gene and stress-inducible rd29A promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. Plant Cell Physiol. 2004;45:346–350
- [85] Pellegrineschi A, Reynolds M, Pacheco M, Brito R.M, Almeraya R, Yamaguchi-Shinozaki K, Hoisington D. Stress-induced expression in wheat of the *Arabidopsis thaliana* DREB1A gene delays water stress symptoms under greenhouse conditions. Genome. 2004; 47:493–500.
- [86] Figueras M, Pujal J, Saleh A, Savé R, Pagès M, Goday A. Maize Rabi17 overexpression in Arabidopsis plants promotes osmotic stress tolerance. Ann. Appl. Biol. 2004; 144:251– 257. doi:10.1111/j.1744-7348.2004.tb00341.x

- [87] Rohila JS, Jain RK, Wu R. Genetic improvement of Basmati rice for salt and drought tolerance by regulated expression of a barley Hva1 cDNA. Plant Sci. 2002; 163:525–532. doi:10.1016/S0168-9452(02)00155-3
- [88] Babu RC, Zhang JX, Blum A, Ho THD, Wu R, Nguyen HT. HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. Plant Sci. 2004; 166:855862. doi:10.1016/j.plantsci.2003.11.023
- [89] Winicov I. New molecular approaches to improving salt tolerance in crop plants. Ann. Bot. 1998; 82:703– 710. doi:10.1006/anbo.1998.0731
- [90] Bohnert H.J, et al. A genomics approach towards plant salt stress tolerance. Plant Physiol. Biochem. 2001; 39:295–311. doi:10.1016/S0981-9428(00)01237-7
- [91] He C, Yan J, Shen G, Fu L, Scott Holaday A, Auld D, Blumwald E, Zhang H. Expression of an Arabidopsis vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fibre yield in the field. Plant cell physiol. 2005; 46:1848– 1854. doi:10.1093/pcp/pci201
- [92] McCallum CM, Comai L, Greene EA, and Henikoff S. Targeting induced local lesions IN genomes (TILLING) for plant functional genomics. Plant Physiology, vol. 123, no. 2. 2000; pp. 439–442.
- [93] Chen L, Huang L, Min D et al. Development and characterization of a new TILLING population of common bread wheat (Triticum aestivum L.). PLoS ONE, vol. 7, no. 7. 2012; Article ID e41570.
- [94] Xin Z., M. Li Wang, N. A. Barkley et al. Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biology, vol. 8, article 103, 2008.
- [95] Till B. J., S. H. Reynolds, C. Weil et al. Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biology, vol. 4, article 12, 2004.
- [96] Caldwell D. G., N. McCallum, P. Shaw, G. J. Muehlbauer, D. F. Marshall, and R. Waugh. A structured mutant population for forward and reverse genetics in Barley (Hordeum vulgare L.). Plant Journal, vol. 40, no. 1, pp. 143–150, 2004.
- [97] Cooper J. L., B. J. Till, R. G. Lapor et al. TILLING to detect induced mutations in soybean. BMC Plant Biology, vol. 8, article 9, 2008.
- [98] de Lorenzo L., F. Merchan, P. Laporte et al. A novel plant leucine-rich repeat receptor kinase regulates the response of Medicago truncatula roots to salt stress. Plant Cell, vol. 21, no. 2, pp. 668–680, 2009.
- [99] Voytas DF. Plant genome engineering with sequence specific nucleases. Annual Review of Plant Biology. 2013; 64:327-350. DOI: 10.1146/annurev-arplant-042811-105552
- [100] Jia YX, Ding YL, Shi YT, Zhang XY, Gong ZZ, Yang SH. The cbfs triple mutants reveal the essential functions of CBFs in cold acclimation and allow the definition of CBF regulons in Arabidopsis. The New Phytologist. 2016; 212:345-353
- [101] Zhao CZ, Zhang ZJ, Xie SJ, Si T, Li YY, Zhu JK. Mutational evidence for the critical role of CBF transcription factors in cold acclimation in Arabidopsis. Plant Physiology. 2016; 171:2744-2759
- [102] Lou D., H. Wang, G. Liang, and Y. Diqiu. OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. Frontiers in Plant Science, 2017; 8:993
- [103] Guo M., M. A. Rupe, J. Wei et al. Maize ARGOS1 (ZAR1) transgenic alleles increase hybrid maize yield. Journal of Experimental Botany. 2014; 65(1):249–260.
- [104] Shi J., J. E. Habben, R. L. Archibald et al. Over expression of ARGOS genes modifies plant sensitivity to ethylene, leading to improved drought tolerance in both Arabidopsis and maize. Plant Physiology. 2015; 169(1):266-282.
- [105] Zhao Y, Zhang C, Liu W, Gao W, Liu C, Song G ET AL. An alternative strategy for targeted gene replacement in plants using a dual-sgRNA/Cas9 design. Sci Rep. 2016; 6, 23890
- [106] Roca Paixao, Gillet JF, Ribeiro FX, Bournaud TP, Lourenco-Tessutti C, Noriega DD et al. Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a Histone Acetyl Transferase. Sci. Rep. 2019, 9, 8080.
- [107] Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M et al. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol. J. 2017; 15:207–216.
- [108] Char SN, Neelakandan AK, Nahampun H, Frame B, Main M, Spalding M.H et al. An Agrobacteriumdelivered CRISPR/Cas9 system for high frequency targeted mutagenesis in maize. Plant Biotechnol. J. 2017; 15:257–268.
- [109] Chen R, Xu Q, Liu Y, Zhang J, Ren D, Wang G, Liu, Y. Generation of Transgene-Free Maize Male Sterile Lines Using the CRISPR/Cas9 System. Front. Plant Sci. 2018: 9, 1180.
- [110] Feng C, Su H, Bai H, Wang R, Liu Y, Guo X, Liu C et al. High-efficiency genome editing using a dmc1 promoter-controlled CRISPR/Cas9 system in maize. Plant Biotechnol. J. 2018; 16:1848–1857.

- [111] Feng C, Yuan J, Wang R, Liu Y, Birchler JA, Han F. Efficient Targeted Genome Modification in Maize Using CRISPR/Cas9 System. J. Genet. Genom. 2016; 43:37–43.
- [112] Kim D, Alptekin B, Budak H. CRISPR/Cas9 genome editing in wheat. Funct. Integr. Genom. 2018; 18:31–41
- [113] Bhowmik P, Ellison E, Polley B, Bollina V, Kulkarni M, Ghanbarnia K et al. Targeted mutagenesis in wheat microspores using CRISPR/Cas9. Sci. Rep. 2018;8, 6502
- [114] Sanchez-Leon S, Gil-Humanes J, Ozuna CV, Gimenez MJ, Sousa C, Voytas D.F et al. Low-gluten, non transgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J. 2018; 16:902–910.