***Review Article***

# INTEGRATIVE APPROACHES FOR ENHANCING ABIOTIC STRESS TOLERANCE IN CROPS THROUGH MOLECULAR AND BIOTECHNOLOGICAL INTERVENTIONS

## Abstract

Currently, there is need to have more stress-tolerant crops for which reason there is need to know the cellular, biochemical and molecular processes to stress. Natural breeding takes longer time and is only restrictive to sexually reproducing species while advances in reproductive sciences in the 20th century new biotechnologies and rDNA technologies offer precise alterations of genes. Stress induced genes and compatible solutes such as proline, glycine betaine and polyamine which are synthesized in plants, help in improving tolerance to abiotic stresses. Another stress protective protein is late embryogenesis abundant (LEA) proteins to protect structures and uphold osmotic stability. Heat stress proteins and transcription factors take central roles in improving stress tolerance in plants. HSPs, conserved between species, function in protein folding, stabilization and protection from stress, which enhances photosynthetic rates and decreases the oxidative injury. NAC, MYB, bZIP, and WRKY are the principal families of transcription factors augmenting stress tolerances through changes in the physiology and biochemistry. Some of the antioxidant defense committed enzymatic system comprising of SOD, CAT, and APX scavenges ROS by minimizing the impact of the oxidative stress response. The breakthroughs in the genome editing method and gene regulation especially by CRISPR/Cas9 permit stress response genes to be fine-tuned opening new possibilities to create improved crop resistance. The progress in biotechnology especially in genetic engineering is seen to have great possibilities of increasing resistant crop plants in unfavorable environments.

**Keywords:** Cellular responses, Compatible solutes, Heat shock proteins, Transcription factors, Genome editing, Crop resilience

## Introduction

To be able to breed more tolerant crops under all form of stresses, there is need to determine the cellular, biochemical and molecular responses to stress conditions. It is basics that are vital in biotechnology focusing on enhancement of stress factors like salt concentration, low temperatures, and water deficit(Chaudhary *et al.*, 2024). Choosing the necessary genes for the improvement of the overall tolerance and their subsequent overexpression can increase plants' genetically modified resistance to the abiotic stress. Furthermore, the specific as to how plants receive and relay stress signals is critical in crop engineering(Desa *et al.*, 2023).A number of molecular and biochemical investigations have helped understand the roles of different signaling cascades in plant's reaction to abiotic stress. Such studies aid in providing a better understanding of pathways that are involved with stress(Bhattacharjee *et al.*, 2024). The conventional breeding techniques have some drawbacks including time consuming due to cross and selection cycling and applicable only to sexually reproducing species(Akhtar *et al.*, 2023). Besides, with classical breeding, there are tendencies in which undesirable characters are inherited along with the desired genes.These limitations are solved using recombinant DNA tech- nology in modern biotechnology that allows the alteration of plant genetic structures using the genes of other organisms(Begna *et al.*, 2020). It enables the transfer or interchanging of genetic material between two different species which are sexually in compatible. Thus, genes for resistance and/ or tolerance include those for viruses, bacteria, nematodes, fungi and herbivores as well as those for abiotic stresses such as drought, salinity, temperature and cold are among the most commonly used genes in production of GMOs(Ijaz *et al.*, 2021).Stress factor genes have been identified in plants and plants use signal transduction networks for the regulation of other stress-responsive genes including kinases, molecular chaperones, osmoprotectants, and transcription factor(Bhat *et al.*, 2024). This network helps one to understand how plants develop what is referred to as stress tolerance. Since these pathways are regulated by many genes, it becomes difficult to obtain the abiotic stress tolerance totally through one gene(Shakespear *et al.*, 2024).Some of the molecules that have been identified to have protectant roles against the impact of abiotic stress includes: Proline perform osmoprotectant role, metallochelating role, as well as antioxidative defense molecule and signaling molecule. Trehalose plays roles in stress as an osmoprotectant and is also participates in ROS elimination(Yang *et al*., 2022). Molecular chaperones involved in protein folding, assembly, translocation, and degradation are called heat shock proteins. Antioxidant and protease, late embryogenesis abundant (LEA)proteins preserve proteins, membranes, and ions (Arranz-Solís *et al*. 2023). While CAL proteins are calcium sensors that are involved in signal transduction, aquaporins are involved in the transport of water and neutral molecules (Tang *et a*l., 2023). NAC, WRKY, MYB, bZIP, DREB/CBF stars, kinases, and phosphatases are implicated in stress receival, signal transduction and differential regulating the stress-responsive genes(Lata *et al*., 2011).Thus, molecular techniques that include identification of genes, isolation, characterization, and transformation of abiotic stress responsive genes, is one of the most efficient methods in understanding of these genes and their products involvement in plant response, behaviour and phenotypic traits(Goyal *et al*., 2023).

## Genetic Engineering of Osmo protectants for Improved Crop Performance

Around the world, abiotic stresses like salinity, drought, and extremely high temperatures caused significant crop losses. These stresses alter a plant's morphology, physiology, metabolism, and molecular makeup, all of which are harmful to the growth and survival of the plant, e.g., salinity causes biphasic responses in plants, first disrupting cellular homeostasis and then ionic homeostasis, which inhibits plant growth (Adem *et al*., 2014; Li and colleagues, 2015).To counter such stresses, plants have developed tolerance mechanisms like ion exclusion, compartmentalization and Osmo protection (Chen and Murata, 2008; ). Osmo protection is an efficient mechanism to combat the stress conditions. Here plants upgrade the level of two types of low-molecular-weight compounds called osmolytes and carbon-based metabolites(Roychoudhury *et al*., 2015). Osmolytes consist of polar, highly soluble, highly hydrophilic compounds that protect macromolecule membrane structures in a dehydrated condition. Well-known osmolytes are free amino acids, such as proline; quaternary amines, such as glycine betaine, polyamines and acidic zwitterionic compounds; and, sugars and sugar alcohols, such as trehalose, fructose, mannitol and sorbitol. Osmolytes play a role in osmoregulation by decreasing the osmotic potential of water and maintaining the tissue water content under a saline condition (Niu *et al*., 1995). They act as molecular chaperones by preventing protein misfolding and help stabilize proteins and membrane structures by hydrogen-bond formation (Kumar, 2009; Slama *et al*., 2015). Moreover, they function as scavengers of hydroxyl radicals, i.e., lowering the oxidative damage brought on by reactive oxygen species (ROS), which are created when abiotic stresses attack plant organs. Reduction in the level of lipid peroxidation is a proof of reduced ROS damage (Ozgur *et al*., 2013). The beneficial protective roles of osmolytes are used to transform plants to make transgenic forms that overexpress the genes responsible for osmolyte synthesis (Table 1) (Wani *et al*., 2017).

One of the most basic compounds involved in plant growth, development, and stress physiology is proline (PL), a derivative of α-amino acids. PL can be synthesized as an osmo protectant in all higher plants under both non-stress and stress conditions, mainly in leaves (Huang *et al*. 2008). It is one of the most highly water soluble compounds: its solubility in water is 1.54 kg/L water (Huang *et al*., 2008). In non-stressful conditions, it serves as a precursor for the synthesis of proteins and enzymes (Nanjo *et al.,* 1999) and control the development of seeds and embryos (Mattioli *et al.,* 2009). When plants show high concentration of proline, it implies that they are subjected to salinity and drought stress, mediating oxidative and osmotic stresses as an osmoprotectant (Suprasanna *et al*., 2014).In order for proline to be a feasible solute, it is essential that appreciable amounts are produced naturally. According to certain studies, overexpressing the genes responsible for proline biosynthesis can raise proline levels and enhance stress tolerance. In plants of transgenic tobacco, Kishor *et al.* (1995) increased the *Vigna aconitifolia* P5CS gene's expression level, which led to a proline accumulation that was 10–18 times higher than that of controls while also resulting in increased root biomass and flower development. The range of advantages of manipulating the proline synthesis process has been broadened by recent research. As an example, Surekha *et al.* (2014) overexpressed *V. aconitifolia* P5CSF129A gene in *Cajanus cajan* which accumulated four times greater amounts of the amino acid during chloride irrigation; hence recorded better growth rates coupled with increased chlorophyll levels. The study conducted by Zhang *et al.* (2014) revealed that the expression of rice OsP5CS1 and OsP5CS2 genes in tobacco led to a significant increase in free-proline accumulation levels, which in turn improved tolerance against various environmental conditions. Furthermore, Chen and colleagues. (2013) enhanced proline accumulation and enhanced salt tolerance in *A. thaliana* by co-expressing the *Phaseolus vulgaris* P5CS genes.

Particularly in response to abiotic stressors like salinity, drought, and high temperatures, plants such as sugar beet, maize, spinach, and barley naturally contain small amounts of glycine betaine (GB) (Wani *et al*., 2013). GB helps in osmotic maintenance of cells, protection of photosynthetic apparatuses, scavenging oxidative species and stabilization of proteins (Allakhverdiev*et al.*, 2003). Genetically engineering has boosted the levels of endogenous GB in crops such as rice, potatoes, soybeans and maize by employing genes from bacteria like *Arthrobacter globiformis* (Ranganayakulu*et al*., 2013). Wei *et al*. (2017) successfully generated transgenic plants with increased GB accumulation that had superior growth and yield under stress by utilizing the codA gene from various Arthrobacter species. As an illustration, transgenic rice overexpressing codA accumulated significantly higher GB levels compared to wild types (Sakamoto and Murata, 1998). Transgenic tomatoes containing the codA gene produced more fruit at low temperatures than controls did (Park *et al*., 2004). Similarly, under salt and cold conditions, transgenic potatoes expressing the codA gene showed normal growth rates and high tuber yields (Ahmad *et al*., 2014). Synonymously, spinage BADH gene upregulation in potatoes and sweet potatoes has caused increased GB accumulation which could reduce the effect of salinity, low temperatures, or oxidative stress (Fan *et al*., 2012). When exposed to salt stress, transgenic tomato plants carrying the codA gene demonstrated increased rates of photosynthetic activity and antioxidant capacities (Wei *et al.* 2017,). Based on these results, GB is a valuable trait for genetic manipulation in agriculture because it helps improve plant growth, development, and tolerance to abiotic stresses.

Animals, plants, and bacteria all naturally contain small molecular weight aliphatic amines called polyamines (PAs) (Hussain *et al*., 2011). Plants contain three main types of polyamines: putrescine (Put), spermidine (Spd), and spermine (Spm) (Tiburcio *et al*., 2014). Plant physiology depends on PAs for growth, development, and the performance of numerous biological tasks like cell division or differentiation or transcriptional regulation among others. Furthermore, they contribute to elongation growth during flower initiation or fruit ripening processes while also being involved apoptosis program during leaf senescence amongst other developmental programs such as floral initiation through control of these process polyamine level is regulated by specific. In order to achieve long-term stress tolerance, genetic engineering has demonstrated great promise for raising endogenous polyamine levels. By introducing the Cucurbita ficifolia spermidine synthase gene into a thaliana, Kasukabe*et al.* (2004) improved stress tolerance and spermidine concentration. Rice's putrescine content was increased by Capell *et al*. (2004) through the use of the *Datura stramonium* ADC gene, which also caused the rice to produce more spermidine and spermine in response to drought stress. After inserting the *D. stramonium* SPDS gene into tobacco plants, Franceschetti *et al.* (2004) observed increased spermidine accumulation and improved growth in saline environments. He *et al.* (2008) reported that during saline stress, transgenic *Pyrus communis* expressing the apple SPDS gene showed reduced ROS levels and elevated activity of antioxidant enzymes.

A number of key sugars and the alcohol derivatives that correspond to them, known as "polyols," are greatly impacted by stresses such as salt, cold, and water shortage. These stresses also have a substantial impact on cell carbon metabolism. It is worth noting that these stresses are often associated with high sugar concentrations like trehalose or fructose and high amounts of sugar alcohols such as mannitol, sorbitol or ononitol. These molecules serve osmotic adjusters in cells; they act as sinkholes for carbon while at the same time they scavenge reactive oxygen species (ROS), maintain membrane integrity, help preserve osmotic balance and function in protein folding among others provided that they are able to acquire this ability through evolution. Trehalose prevents cellular membranes and proteins from getting damaged by drying in the sun and salinity and it has other functions like cell proliferation, differentiation, homeostasis (Ali *et al*., 2012). When nutrients are being restored following exposure to abiotic stress, fructans serve as storage sinks for carbohydrates. Transgenic plants that are resistant to stress have been created using genes associated with fructan biosynthesis. For example, Li *et al.* (2007) produced transgenic tobacco with Lactuca sativa 1-SST gene demonstrating higher content of soluble carbohydrates and fructans, as well as increased photosynthetic rates under frosty conditions. Fructose 6 phosphate is converted into mannitol, a six-carbon sugar alcohol. Twenty years ago, the mtlD gene was inserted into Arabidopsis by Thomas *et al.* (1995). resulting into high mannitol content which was responsible for improving growth when plants experienced stress from NaCl. Similarly, Transgenic potato lines with the mtlD gene also demonstrated reduced growth reduction under salt stress (Rahnama *et al*., 2011). On the other hand, Nguyen *et al*. (2013) did create maize that is transgenic with a construct containing the Hordeum vulgare HVA1 and mtlD genes showing improved survival and biomass under high salinity and drought stress. This has been linked to the above, where transgenic plants having sorbitol biosynthesis genes have displayed increased tolerance towards abiotic stress. Transgenic tobacco plants expressing the apple S6PDH gene were made by Sheveleva*et al*. (1998), and this enhanced salt stress tolerance and sorbitol accumulation were the outcomes. Transgenic Japanese persimmon cultivars carrying the S6PDH gene were created by Gao *et al*. (2001). These cultivars accumulated sorbitol, displayed restored photosynthetic activity, and had higher chlorophyll content when subjected to salt stress. Global crop productivity is hampered by abiotic stress on plant growth. Plant breeders are now interested in compatible solute engineering for creating genetically modified plants that can withstand stressful conditions. Many genes responsible for osmolyte biosynthesis have been identified and introduced into major crops thereby improving their stress tolerances again. However, lack of successful field trials means that most research happens only in laboratories among others. In future trials, should we not develop multigenic traits through combining osmolyte biosynthesis genes with different stress related ones especially those that influence plant growth, survival and yield even when subjected to more than one abiotic stress conditions.

## Role of Late Embryogenesis Abundant (LEA) Proteins in Plant Stress Tolerance

During the late embryogenesis of plant seeds, there is an accumulation of late embryogenesis abundant proteins (LEAs) in their mature embryos and their encoding genes are found in various higher plants (Jin *et al*., 2019). Hydrophilic amino acids like lysine and glycine are present in several LEA proteins. Some characteristics of LEA proteins include thermal stability, hydrophilicity, and highly conserved amino acid sequences. LEA plays two different roles in cellular processes. It serves as an osmoregulation protein that helps to retain the water content inside cells thus maintaining the osmotic pressure. Additionally, it protects other proteins, and membrane structures against dehydration ensuring its stability. This protective role is important for plants undergoing stressful conditions like drought or excessive salinity

(Arroyo *et al.*, 2000; Garay-Arroyo *et al.*, 2000). Some LEA proteins function as chaperones, helping to stabilize membrane structure, proteins, and vesicles in plants during stress response (Allagulova*et al.*, 2003). Motif composition differences coupled with amino acid sequence variations and phylogenetic relationships lead to the classification of at least eight different groups of LEA proteins in the Pfam database; LEA1, LEA2, LEA 3, LEA 4, LEA 5, LEA6, dehydrin, and seed matures protein (SMP) (Finn *et al.*, 2016). Several groups have been proven to be water tolerant, including 2, 3, 5, and dehydrin. A cotton subgroup gene, LEA 2, promotes root growth in Arabidopsis, resulting in drought tolerance (Magwanga*et al.*, 2018). *PhellodendronamurenseRupr.* has also been reported to overexpress the wheat LEA3 subfamily genes. The transgenic plant has faster stomatal closure and increased drought tolerance when subjected to drought stress (Yang *et al*., 2018).

In tobacco, ectopic expression of peanut LEA 5 family genes increased dehydration tolerance, suggesting the family members contribute to stress resistance (Sharma *et al*., 2016). As members of the group II, D 11 family of highly hydrophilic proteins known as LEA, DHN proteins accumulate in the later stages of embryogenesis. Plant stress tolerance and DHNs gene expression have been found to be positively connected. Transgenic Arabidopsis showed enhanced drought tolerance due to Arachis duranensisKrapov's dehydrogenase (Mota *et al.*, 2019). In line with Cao *et al.* (2017) found that eliminating ROS increased stress tolerance in Arabidopsis through transgenic expression of the oak dehydrogenase genes HbDHN1 and HbDHN2. Arabidopsis transgenic plants with overexpression of dehydrin subfamily genes show robust resistance to osmotic stress (Chiappetta *et al.*, 2015). According to Brini*et al.* (2007), the wheat Dhn-5 gene improves transgenic Arabidopsis's resistance to dehydration stress. For instance, overexpressing the barley LEA (HVA1) gene in rice and wheat can protect plant cell membranes from damage and increase the plant's resistance to drought stress (Babu *et al.*, 2004,). Improved early dehydration tolerance was demonstrated by transgenic rice that overexpressed the wheat LEA gene PMA80 (Cheng *et al.*, 2002). The transgenic plants' tolerance to salt and osmotic stress is increased when the soybean LEA protein gene GsPM30 is overexpressed in Arabidopsis, as this gene responds to both conditions (Sun *et al*., 2019). In response to drought stress, many LEA genes are induced to express in upland cotton (Magwanga*et al.*, 2018,). OsLEA1a in rice enhances resistance to a range of abiotic stresses by averting damage to cell membranes and enhancing transgenic rice's ability to scavenge ROS (Wang *et al*., 2021). In response to drought stress, maize expresses ZmLEA5C, and overexpressing this gene can make tobacco more resistant to drought stress (Liu *et al*., 2014). Plants overexpressing this gene showed higher resistance to severe drought than plants of the wild type, and several abiotic stresses induce Arabidopsis AtLEA4-5 (Cuevas-Velazquez *et al*., 2016). Kim et al. (2005) found that tobacco plants with overexpression of CaLEA6 exhibited enhanced resistance to dehydration based on the fresh weights, chlorophyll contents, and general health of the leaves. IbLEA14 may improve lignification in sweet potatoes (*Ipomoea batatas*), which would improve their reaction to drought stress (Park *et al*., 2011). SiLEA14 overexpression in Arabidopsis revealed enhanced resistance to osmotic stress and salt in foxtail millet (*Setaria italica*) compared to the wild type (Wang *et al.*, 2014).

## Role of Heat Shock Proteins in Plant Stress Tolerance

It was originally discovered that heat shock proteins are produced by all classes of organisms, including those in the eukaryotic and prokaryotic kingdoms, in reaction to temperature increases. The subsequent research has established that they are involved with different abiotic stresses. Highly stable proteins known as HSPs are produced in reaction to a range of environmental and physiological stressors. These proteins are mostly found in the cytoplasm, but they are also present in other organelles, such as the endoplasmic reticulum, mitochondria, nucleus, and chloroplasts (Park *et al.*, 2015). HSPs themselves are evolutionarily conserved across the domains of prokaryotes and eukaryotes and also equally present in plants. Their molecular weights are used to divide them into five groups: These families comprise the minor heat shock proteins and the heat shock proteins, such as HSP60, HSP70, HSP90, and HSP100. HSPs are up-regulated by heat in these populations, but they are also up-regulated by salt, cold, and drought. In model species, they are involved in functions such as germination of seeds, embryogenesis, and fruiting(Chauhan *et al*., 2012). For example, the chloroplastic sHSP26 gene from wheat (*Triticum aestivum*) when introduced in transgenic Arabidopsis thaliana enhances heat tolerance in plants and helps the growth of photosynthetic pigments, biomass, and seed yield in plants (Chauhan *et al*., 2012). Similarly, under heat stress, transformants of the rice (*Oryza sativa*) HSP26 gene reduce electrolyte leakage and the amount of TBARS. This gene aims to produce a tiny heat shock protein localised in the chloroplast. In comparison to the control plants, these transgenic plants also show increased PS II (Fv/Fm) photochemical efficiency at 42˚ C (Kim *et al*., 2012). Other information also shows that sHSPs are involved in the removal of ROS. For instance, overexpressing OsHSP18.6 in transgenic plants improves thermotolerance by increasing the removal of ROS with greater efficiency, which reduces MDA buildup and increases the activities of SOD and CAT in response to heat and drought stress (Wang *et al*., 2015). Another important heat shock protein is known as HSP70 which helps in the stabilization of proteins so that they do not get denatured and aggregate. In order to maintain protein homeostasis in the cell, it is also engaged in the transport of specific protein molecules, appropriate folding of freshly synthesised proteins, unfolding of undesirable proteins, and the creation and dissolution of protein complexes (Hoang *et al*., 2015). In order to increase salt tolerance and reduce ROS production and plant damage, transgenic rice plants were created using genes that prevent apoptosis, such as AtBAG4 from *Arabidopsis thaliana*, Hsp70 from the Citrus tristeza virus, and p35 from Baculovirus (Hoang *et al*., 2015). Under saline stress, transgenic sugarcane (Saccharum spp. Hybrid) plants overexpressing the *Erianthusarundinaceus* HSP70 gene showed increased expression of stress-related genes, which resulted in germination and chlorophyll contents (Augustine *et al*., 2015). Being one of the most conserved proteins throughout evolution and present in high concentrations in many organisms, HSP90 takes part in stress-induced signaling, the regulation of protein degradation, the cell cycle, and protein transportation. In Arabidopsis thaliana, growing overexpression of GmHsp90A2, GmHsp90A4, GmHsp90B1, GmHsp90C1. 1, and GmHsp90C2. 1 protects against abiotic stress damage, and increased proline content has a significant impact on proline synthesis via the AtP5CS1 gene (Xu *et al*., 2013). Some of the mechanisms of polyamine homeostasis include transport, degradation, and change of amino group that affects the synthesis of HSP under heat stress. In Arabidopsis thaliana, knocking down genes encoding HSP90 leads to an increase in soluble spermidine, acetylated spermidine, and acetylated spermine. HSP90s and POs are co-responsible for the control of polyamine acetylation, oxidation, and PA/$H_2O_2$ equilibrium (Toumi *et al*., 2019).

## The Role of Transcription Factors in Enhancing Plant Stress Tolerance

Developing genetically modified plants that can tolerate different stresses requires characterizing and identifying stress-responsive genes. Regulatory genes, such transcription factors (TFs), are critical for regulating the expression of several genes associated to stress (Table 2). Important TF families involved in stress response include AP2/EREBP, NAC, WRKY, MYB, and bZIP. According to Wang *et al.* (2016), NAC protein is a major component of several TF families in plants and is involved in both development and stress response. Flower and lateral root development, cell division, phytohormone homeostasis, senescence processes, responses to both abiotic and biotic stresses secondary cell wall formation and so many others are regulated by them or made possible through their actions Numerous investigations have demonstrated a link between NAC transfer into plants and enhanced resistance to abiotic stressors.

In an example, Huang *et al.* (2015) introduced the TaNAC29 gene from wheat (Triticum aestivum) into Arabidopsis thaliana. Consequently, overexpression of TaNAC29 facilitated resistance of these transgenic plants to high salt and dehydration. Stress-induced high activities of superoxide dismutase (SOD) and catalase (CAT), coupled with reduced levels of malondialdehyde (MDA) among other things indicated a robust stress tolerance mechanism that is mediated by ABA-signalling pathway and antioxidant enzymes. In the same way for instance, transgenic wheat with TaNAC67 overexpression had increased expression of many stress-related genes like DREB1A, DREB2A, RD29B, RD22, COR15, Rab18, and RD29A. This led to improved membrane stability in addition to enhanced chlorophyll content leading to better Na+ efflux due to the presence of osmotic adjustment molecules leading to increased drought and salinity tolerance as well as cold hardiness (Mao *et al*., 2014; Shah *et al*., 2014). Similarly, NAC transcription factors MYB also have key roles they play in regulating physiological processes through biochemical pathways. Among these include reactions to biotic and abiotic stressors, primary and secondary metabolism, hormone signalling, cell cycle regulation, and signal transduction (Wang *et al*., 2016).

Previous studies have demonstrated the stress tolerance of transgenic plants that overexpress MYB transcription factors. For instance, transgenic Arabidopsis plants with the GmMYBJ1 gene showed greater resistance to salt and drought than non-transgenic plants. These plants also have higher herbal stature and less water loss in dehydration and cold stress as well as less accumulation of malondialdehyde (MDA) under the stress condition because of MYB overexpression (Su *et al.*, 2014). Also, the TaMYB3R1 (*Triticum aestivum*) transcription factor enhanced drought tolerance in Arabidopsis through stomatal effective closure and a decrease in stomatal aperture during stress (Cai *et al*., 2015). The basic leucine zipper (bZIP) transcription factors, which have a bZIP region for DNA binding and dimerisation, are another class of transcription factors involved in stress responses. For instance, the transgenic Nicotiana tabacum plants with LrbZIP (*Nelumbo nucifera*) gene silencing have shown the higher salt stress tolerance ability that is proved by the low electrolyte leakage and higher chlorophyll content at the stage of salt stress (Cheng *et al*., 2013). Likewise, the CaBZ1 (*Capsicum annuum*) bZIP transcription factor alleviates reduced water loss, and upregulation of ABA and stress-associated genes in Solanum tuberosum under drought stress (Moon *et al.*, 2015). WRKY proteins are composed of at least two conserved WRKY subsequences and a zinc-finger-like sequence; they participation in diverse developmental and physiological actions which include plant senescence, biosynthetic/metzincin pathways, hormones, embryogenesis, and the trichome. These TFs are also vital for the abiotic stress response. For instance, the overexpression of ZmWRKY33 (*Zea mays*) activated stress-induced genes and was significant to stress tolerance acquisition (Li *et al.*, 2013). Therefore, under stressful circumstances, WRKY TFs have also been shown to improve physiological characteristics like as seed germination, root length, and chlorophyll content. Symbol TaWRKY79 (*Triticum aestivum*) transforms Arabidopsis thaliana plants and provides salt tolerance and ion stress through increased root elongation potential under salt stress. Similarly, it was shown that Gossypium hirsutum plants overexpressing GhWRKY34 showed increased salt stress tolerance along with a decreased Na+/K+ ratio in both the leaves and the roots (Zhou *et al*., 2015).AP2/ERF family proteins consists of a conserved region called the AP2/EREB domain of about 40-70 amino acids that has some part in binding to DNA. This family is categorized into four main groups: Apetala2 (AP2), as RAV, DREB, ERF which is similar to Abscisic Acid Intensive 3/Viviparous. These transcription factors also explore parts in regulating development within the plant besides in the tackling of other forms of stresses (Sharoni *et al*., 2011). Out of all the ERF family members some of them are members of the AP2 group and hence play a crucial role in the ability of the plant to survive stress through activating both the jasmonate and abscisic acid signalling pathways. However, the concentrations of α-linolenate, certain derivatives of jasmonate, and abscisic acid were greater in rice where the EREB1 gene (AP2/ERF family) was inserted. This gene family is thought to have a role in the regulation of both biotic and abiotic stressors, making it crucial for improving plants' ability to withstand multiple stress. DREBs and the genes of polyamine biosynthesis have synergistic effects for plants under the situation of salt stress (Peng *et al*., 2013). For instance Arabidopsis plants with the LcERF054 gene from Leymus chinensis had higher relative moisture content soluble sugar and proline content it also shown better salinity stress tolerance among transgenic plants (Sun *et al*., 2014). Moreover, the higher stress scores with respect to HOS genes such as COR15A, LEA4-5, P5CS1 and RD-29A were also noticed in these plants. Like other members of AP2/ERF family, functions of DREB transcription factors in stress tolerance continue to substantiate the idea of genetic engineering for stress resistance (Ravikumar *et al*., 2014).

## Antioxidant Defense Mechanisms and Their Role in Mitigating Abiotic Stress in Plants

Stress influences the entire plant and the stress signals are conveyed through different hormones such as abscisic acid (ABA) and jasmonate for abiotic stress hence the need to control stress in order to keep the plant healthy (Sami *et al*., 2018). There are specific suggestions about how oxidative metabolism of reactive oxygen species, sensory activity, and signal transduction pathways participate in the alleviation of developmental and environmental stresses (Apel and Hirt, 2004). ROS accumulation marked by low electron transport in the Calvin cycle and high leakage at the time of photosynthesis manifests by high respiration and low photosynthesis. factors like salt, heat, and drought increase photorespiration and result in hydrogen peroxide ($H_2O_2$) that coupling with $O^{2-}$ is the second messenger organism (Rhee, 1999). Nevertheless, high ROS levels are toxic because they initiate lipid peroxidation which results in damage of cell membranes, lipids, nucleic acids and proteins leading to cell death. During stress, ROS overproduction negatively impacts crop production. When endogenous and exogenous stimuli are present, enzymes like NADPH oxidases and polyamine oxidases increase the generation of ROS. Therefore, it can be concluded that increasing antioxidant capacity is a viable solution for decreasing ROS damage

(Zulfiqar and Ashraf, 2021). Specific plant lines are resistant and demonstrate better ROS elimination and membrane integrity than susceptible ones: thus, plants should possess functional ROS-removal systems under stress (Gill and Tuteja, 2010). Under basal state, rise in oxidative stress occurs in cells and generates reactive oxygen species (ROS) mildly. However, abiotic stresses increase the ROS levels which in turn triggers the stress signals within the plant cells. The processes of cleaning and maintenance of ROS are expressed via interdependence of several enzymatic and non-enzymatic antioxidant systems with multiple targets in various organelles (Aranda-Rivera *et al.*, 2022). A proteomic investigation by Ford *et al.* (2011) on wheat also observed different antioxidative enzymes under stress and out of all the enzymes, the catalase (CAT) represented the highest proportion. CAT is required for the detoxification of photorespiratory $H_2O_2$ in plants and especially during instances when the plant suffers from water deficit. Mannitol and other sugars can decrease the oxidative damage of chloroplasts through sugar signaling pathways that in response to stress affect specific genes such as SOD, HSP, GST, and other stress related proteins.

Enhanced superoxide dismutase (SOD) activity has been observed in various Phaseolus vulgaris cultivars under water deficiency (He *et al.*, 2020) and in Oryza sativa (Sharma *et al.*, 2005). Trifolium repens leaves also show increased SOD activity with limited water irrigation (Chang-Quan *et al.*, 2008). Saline stress enhances SOD activity in Cicer arietinum (Kukreja *et al.*, 2005) and Solanum lycopersicum (Gapinska*et al.*, 2008). All three SOD isoforms contribute to saline condition tolerance in C. arietinum (Eyidogan*et al.*, 2007). Additionally, Wang *et al.* (2016) reported that transgenic Arabidopsis thaliana that overexpress Mn-SOD show notable improvements in salt tolerance. Supplemental UV-B radiation increases SOD activity in *Munga radiata* and *Triticum aestivum* under field circumstances; different responses are seen in different cultivars of *Glycine max* (Agrawal *et al.*, 2009). The catalase (CAT) gene family is subject to many levels of regulation, which include transcriptional control by transcription factors and post-transcriptional control by mRNA sponging and alternative splicing (Wang *et al.*, 2018). In *Nicotiana tabacum* lines, overexpression of TaMIR1119, a microRNA derived from *T. aestivum*, led to elevated activities of SOD, CAT, and peroxidase (POD) in the presence of water shortage (Shi et al., 2018). Higher CAT activity has been seen in the roots and leaves of C. arietinum under salt stress (Eyidogan*et al.*, 2007) and in sensitive T. aestivum cultivars under water stress (Simova-Stoilova*et al.*, 2010). When compared to heat-sensitive mutants, the O. sativa mutant HTT-121, which has been found to be heat-tolerant, exhibits higher CAT activity (Zafar *et al.*, 2020). According to Roychoudhury *et al*. (2020), there is evidence that under a variety of abiotic and biotic stressors, glutathione peroxidase (GPX) activity increases. Under cadmium stress, both salt-sensitive and salt-tolerant O. sativa cultivars showed increased GPX and ascorbate peroxidase (APX) activity, with the salt-tolerant cultivars showing larger increases. Increased resistance to oxidative stress and drought was provided upon *Salvia miltiorrhiza* by the expression of the GPX gene from Rhodiola crenulata under a constitutive promoter (Roychoudhury *et al.*, 2020). When overexpressed in Arabidopsis, the glutathione reductase (GR) gene (SpGR) from *Stipa purpurea*, which has an open reading frame of 1497 bp and codes for 498 amino acids, improves salt tolerance (Wang *et al.*, 2018). Three GR genes from *Populus trichocarpa* were recently cloned and characterized, with PtGR1.1 and PtGR1.2 localized in the cytoplasm and PtGR2 in the chloroplast (Liu *et al.*, 2020). Research has been done on ascorbate peroxidase (APX) activity in *A. graveolens*. AgAPX1 gene expression increases dramatically under drought stress and exhibits optimum activity at 55˚ C. Transgenic Arabidopsis lines were given drought tolerance by transformation with AgAPX1 (Liu e*t al*., 2019). Cloning and introducing the APX gene from Dioscorea alata cv. MH1 into Arabidopsis resulted in enhanced resistance to flood stress and freezing. H2O2 spraying can increase APX activity and enhance D. alata's resistance to various shocks, since low APX expression is associated with decreased stress tolerance (Barros *et al*., 2019). Analogously, by strengthening the antioxidative defence system, the constitutive promoter-induced transformation of APX (Apx1) from A. thaliana into Brassica juncea increased resistance to salt stress (Saxena *et al*., 2020).

## Genome Editing Applications for Abiotic Stress Tolerance in Plants with an Emphasis on Recent CRISPR Applications

This technique has been used for the development of crop plants for growth in abiotic stress and other biotechnological applications since the work of Jinek *et al.* (2012), where the bacterial defence mechanism against viruses, known as

CRISPR/Cas9 - detection and elimination of invader nucleic acids has been proposed as a tool for site-specific genome editing. As CRISPR/Cas9 may be used for both gene silencing (CRISPRi) and gene activation (CRISPRa), it can be applied to any of the abiotic stress tolerance mechanisms that were previously discussed in this chapter and have been identified as the location for gene transfer applications. According to Zafar et al. (2020), this dual capacity permits the expression of tolerance genes (T genes) and the muting of sensitivity genes (S genes). Hormonal control is thought to be one of the most practical methods for abiotic stress tolerance. The actions of ABA include seed dormancy, stomatal pore regulation, plant growth and development, and responses to various stressors and environmental cues. In rice, the gene 9-cis-epoxycarotenoid dioxygenase (NCED), which influences ABA, was directly on the decrease for a number of abiotic stress tolerances inclusive of salinity, drought, and $H_2O_2$ stress. Rice plants with NCED3 fully silenced, and therefore, contained comparatively low levels of ABA were highly susceptible to these stresses. On the other hand, when OsNCED3 was overexpressed in rice through the application of the CRISPRa system, the ABA level and stress endurance to salinity and drought enhanced (Huang *et al.*, 2018). Using CRISPRa, it is possible to upregulate other target genes of ABA signalling, such as ABA-responsive element binding proteins ABRE binding factors (AREB/ABFs), which improve Arabidopsis's ability to withstand drought (Roca et al., 2019). As was previously noted, transcription factors (TFs) are the gene regulatory systems found in the promoter regions of plants. For this reason, TFs are regarded as key targets for genome editing and genetic engineering aimed at enhancing abiotic stress tolerance. For instance, the CRISPRi system to precisely control the expression of the B-type response regulator transcription factor gene OsRR22 exhibited enhanced salinity tolerance in rice because of cytokinin signal transduction and cytokinin metabolite (Zhang *et al.*, 2019). In maize, the ARGOS genes, which are involved in ethylene synthesis and which are that negatively regulate responses to of this plant hormone, were thereby overexpressed with the use of CRISPRa also improved drought tolerance and grain yield (Shi *et al.*, 2017). Similarly, downregulation of SlARF4 by employing CRISPR/Cas9 rendered salinity as well as osmotic stress tolerance in tomato (Bouzroud*et al.*, 2020). Thus, on the basis of the presented facts and consideration of the ROS implications for the cellular damage, the antioxidant scavenging is stated to play a crucial role in abiotic stress tolerance. Increased antioxidant enzyme potentials by transgenic approaches has been proved to be effective. For instance, Oryza sativa stress response reckon RING Finger Protein 1 (OsSRFP1) was downregulated by RNA interference, which improved salinity and cold tolerance due to increased antioxidant capability and identified OsSRFP1 as a promising CRISPRi target (Fang *et al.*, 2016). Another important factor in abiotic stress tolerance, especially with regard to salinity, is believed to be the maintenance of ion homeostasis. To compare the effect of K+ uptake under salt stress, two members of the Cucurbitaceae family cucumber and pumpkin with salt tolerance and sensitivity, respectively, were employed. In this study, effective silencing of SlNADPH oxidase (SI RBOHD) in cucumber or overexpression of pumpkin RBOHD in Arabidopsis reduced or increased salinity tolerance, respectively (Huang *et al.*, 2019). CRISPR/Cas9 can also be applied to understand the function of certain gene regarding abiotic stress tolerance.For example, the researchers showed that NPR1 is involved in drought stress by utilising CRISPRi to knock down the nonexpressor of pathogenesis-related gene 1 (NPR1) gene in tomatoes (Li *et al.*, 2019). Similarly, it was known that the signalling molecules involved in the CRISPRi of tomato mitogen-activated protein kinases (MAPKs) contribute to drought tolerance by controlling the differentially expressed genes that respond to drought (Wang *et al.*, 2017). As for the second milestone, employing CRISPRi to intervene the Ca2 + -dependent phospholipid-binding proteins like annexins revealed its function in cold stress tolerance in rice (Shen *et al.*, 2017). In tomato using CRISPR interference, receptor known as C-repeat binding factors (CBFs) it was seen that they were involved in chilling stress (Li *et al.*, 2018). However, there are limitations that go beyond the technique of genome editing, especially in relation to legislation on the use of genetically modified plants and the population's perception of such plants. There is debate about whether or not items made using CRISPR/Cas9 should be subject to regulation, even as certain GE products are subject to stricter regulations. As an example, consider the 2018 ruling by the Court of Justice of the European Union (CJEU) on the regulation of genome-edited goods in the same manner as genetically altered products. On the other hand, the USDA and other countries such as Brazil, Argentina, and Australia have stated that regulation is not required in genome-edited mutations because the change can be naturally induced (Ledford *et al.*, 2019). One new concept which can be applied to these issues is DNA-free genome editing. Instead of intoducing RNA-guided nucleases into plants through Agrobacterium tumefaciens or plasmid transfection, Cas9-gRNA ribonucleoproteins

(RNPs) constructs are transfection directly into protoplast cells. Owing to this, it has been possible to modify genes in plants such as Arabidopsis, tobacco, lettuce, and rice using a technique that does not use recombinant DNA, suggesting that this technique may not comply with the present regulations governing genetically modified plants (Woo et al., 2015). This direct delivery method has also been adopted for other plants while the local delivery has be done by; For example grapevine genes related to powdery mildew and genes related in apple.

## Conclusions

There are environmental stresses that prevail include salinity, drought, and extreme temperature threatening food production worldwide require enhancement of plant tolerance. Solute stabilization under osmotic stress, LEA proteins, and HSPs, have been pointed out to be essential in providing plant cells with better tolerance to these stresses and transcription factors and antioxidant defence systems also play an important role. Transgenic plants have been created using Genetic engineering especially through osmoprotectant synthesis genes; this has presented high stress tolerance. HSPs, chaperonins, and LEA proteins protect proteins and cell structures, whereas MYB and NAC transcription factors control the expression of genes that respond to stress. Antioxidant defence systems regulate stress circumstances by assisting in the management of the harmful presence of reactive oxygen species (ROS). Further, the improvement of abiotic stress tolerance in crops is well supported by the modern methods of genome editing such as CRISPR/Cas9. What is more, the application of CRISPR/Cas9 technology allows targeting and modifying the genes by which the stress response pathways are regulated to create crops more resistant to stress and with higher yields. Since this element can be used both in activating and repressing certain genes, it is indeed a very useful tool for plant genetic engineering. Thus, it can be stated that the application of selective breeding and efficient pollination in combination with the methods of genetic engineering and genome editing could be promising for creating the crop varieties with enhanced abiotic stress tolerance. It is only now that exhaustive knowledge of the molecular and biochemical triggers of stress tolerance and managing these by using modern tools of genetics will be able to respond to the problems posed by climate change and guarantee stable crop yield. Further research and development in this area are crucial and require looking into better crop systems' sustainability and consequently, food security.

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**Table 1. List of transgenic plants that overexpressing potential genes for compatible solute biosynthesis for abiotic stress tolerance**

| Sl no. | Transgene | Host | Target crop plants/trees | Remark | Reference |
|---|---|---|---|---|---|
| 1 | *mtlD* | *E. coli* | *Solanum tuberosum* | Increased mannitol content, which results in 65% of plants surviving salt stress. | Rahnama *et al.* (2011) |
| 2 | *HVA1+mtlD* | *Hordeum vulgare* and *E. coli* | *Zea mays* | Increased rate of plant survival and biomass in the shoots and roots under various abiotic stress situations. | Nguyen *et al.* (2013) |
| 3 | *PvP5CS1, PvP5CS2* | *Phaseolus vulgaris* | *Arabidopsis thaliana* | Enhancedproline content by 1.9 times, as well as seed and flower development. | Chen *et al.* (2013) |
| 4 | *P5CSF129A* | *Vigna acontifolia* | *Cajanus cajan* | Higher levels of proline accumulation, chlorophyll content, and reduced levels of lipid peroxidation. | Surekha *et al.* (2014) |
| 5 | *P5CS* | *Vigna acontifolia* | *Sugarcane* | Increased proline content, biomass generation, reduced lipid peroxidation, and defence against oxidative stress. | Guerzoni*et al.* (2014) |
| 6 | *IbP5CR* | *Ipomoea batatas* | *Ipomoea batatas* | Increased tolerance to salt and increased proline content. | Liu *et al.* (2014) |
| 7 | *GSMT* and *DMT A* | *Aphanothecehalophytica* | *Oryza sativa* | Improvedtolerance to salt, cold, and glycine betaine biosynthesis | Niu *et al.* (2014) |
| 8 | *codA* | *Arthrobacter globiformis* | *Solanum tuberosum* | Increased glycine betaine content, salt and cold stress tolerance. | Ahmad *et al.* (2014) |
| 9 | *LrP5CS1, LrP5CS2* | *Lilium regale* | *Arabidopsis thaliana* | Increased proline accumulation and tolerance to osmotic stress, salt, and dryness. | Wei *et al.* (2016) |
| 10 | *AvADC* | *Avena sativa* | *Medicago truncatula* | Increased seed production, polyamine concentration, and tolerance to desiccation stress. | Duque *et al.* (2016) |
| 11 | *codA* | *Arthrobacter globiformis* | *Solanum lycopersicum* | Elevated levels of antioxidant enzymes, salt tolerance, and glycine betaine biosynthesis. | Wei *et al.* (2017) |
| 12 | *LcSAMDC1* | *Leymus chinensis* | *Arabidopsis thaliana* | Increased levels of proline, chlorophyll, and spremine when exposed to cold and salt stress. | Liu *et al.* (2017) |
| 13 | *PgP5CS* | *Pennisetum glaucum* | *Nicotiana tabacum* | Transgenic plants have reduced levels of malondialdehyde (MDA) and increased levels of proline, relative water, and chlorophyll in heat and | Sellamuthu*et al.* (2024) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | drought conditions. | |

**Table 2. List of transgenic plants with overexpressed transcription factor genes that are tolerant to abiotic stress.**

| Sl no. | Gene action | Gene source | Target trait | Target crop plants/trees | Reference |
|---|---|---|---|---|---|
| 1 | *AP2-ERFBP* | *TaPIE1* from *Triticum aestivum* | Freezing | *Triticum aestivum* | Zhu et al. (2024) |
| 2 | *AP2-ERFBP* | *VrDREB2A* from *Vigna radiata* | Drought<br>Salinity | *Arabidopsis thaliana* | Chen et al. (2016) |
| 3 | *AP2-ERF* | *OsEREBP1* from *Oryza sativa* | Drought | *Oryza sativa* | Jisha et al. (2015) |
| 4 | *bZIP* | *OsbZIP71* from *Oryza sativa* | Drought<br>Salinity | *Oryza sativa* | Liu et al. (2014) |
| 5 | *bZIP* | *TabZIP60* from *Triticum aestivum* | Drought<br>Salinity<br>Freezing | *Arabidopsis thaliana* | Zhang et al. (2015) |
| 6 | *bZIP* | *AtTGA4* from *Arabidopsis thaliana* | Drought | *Arabidopsis thaliana* | Zhong et al. (2015) |
| 7 | *MYB* | *GmMYBJ1* from *Glycine max* | Drought<br>Cold | *Arabidopsis thaliana* | Su et al. (2014) |
| 8 | *MYB* | *TaMYB3R1* from *Triticum aestivum* | Drought<br>Salinity | *Arabidopsis thaliana* | Cai et al. (2015) |
| 9 | *NAC* | *OsNAP*from *Oryza sativa* | Cold<br>Salinity<br>Drought | Oryza sativa | Chen et al. (2014) |
| 10 | *NAC* | *MLNAC5* from *Miscanthus lutarioriparius* | Drought<br>Cold | *Arabidopsis thaliana* | Yang et al. (2015) |
| 11 | *NAC* | *TaNAC47* from *Triticum aestivum* | Salt<br>Cold<br>Drought | *Arabidopsis thaliana* | Zhang et al. (2016) |
| 12 | *WRKY* | *GhWRKY34* from *Gossypium hirsutum* | Salinity | *Arabidopsis thaliana* | Zhou et al. (2015) |
| 13 | *WRKY* | *MtWRKY76* from *Medicago truncatula* | Drought<br>Salinity | *Medicago truncatula* | Liu et al. (2016) |
| 14 | *HomeoBox 4* | *HaHB4* from *Helianthus annuus* | Drought | *Glycinemax*<br>*Triticum vulgaris* | Gonzalez et al. (2020) |
| 15 | *WRKY* | *SlWRKY23* from *Solanum lycopersicum* | Salt<br>Osmotic | *Arabidopsis thaliana* | Singh et al. (2023) |
| 16 | *WRKY* | *PtrWRKY51*from *Populus trichocarpa* | Salt stress | *Arabidopsis thaliana* | Zhou et al. (2023) |
| 17 | *AP2/ERF* | *EgAP2.25* from *Elaeis guineensis* | Salinity<br>Cold<br>Drought | *Nicotiana tabacum* | Zhou et al. (2024) |