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Chapter

Molecular Mechanisms and Strategies Contributing toward Abiotic Stress Tolerance in Plants

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Abstract

Plants respond to climate change *via* sensing the extreme environmental conditions at cell level, which initiated significant changes in their physiology, metabolism, and gene expression. At the cell membrane, plants activate certain genes (like GRP, PRP, AGP) to provide strengthening to cell wall. Drought and salinity stress tolerance attained by osmotic adjustments, activation of transcriptional factors (like AREB, ABF, DREB2), and regulation of Na⁺ homeostasis *via* transporters (like NSCC, NHX1, SOS1, HKT1, LTC1). For adaptations to chilling and frost stress, plants use hydrophobic barriers (waxes/cuticles), antinucleator (cryoprotective glycoprotein), and antifreeze proteins. Higher expression of HSPs (heatshock proteins such as HSP70, HSP100, HSP90, HSP60) is important for thermal tolerance. Tolerance to heavy metal (HM) stress can be achieved *via* vacuolar sequestration and production of phytochelatin, organic acids and metallothionein. ROS generated due to abiotic stresses can be alleviated through enzymatic (APX, CAT, POD, SOD, GR, GST) and nonenzymatic (ascorbate, glutathione, carotenoids, flavonoids) antioxidants. Genetic manipulation of these genes in transgenic plants resulted in better tolerance to various abiotic stresses. Genetic engineering of plants through various genome editing tools, such as CRISPR/Cas9, improve the abiotic stress tolerance as well as enhance the crops' quality, texture, and shelf life.

Keywords: climate change, molecular mechanisms, abiotic stress, adaptations, genome editing

1. Introduction

Climatic conditions are important for the well-functioning of any ecosystem. Altered climatic conditions have direct effects on plant health, productivity, and yield. Earth's climate is changing very rapidly mainly due to human activities, having negative effects on human and the ecosystem (particularly on the agriculture sector) [1]. Altered climatic conditions contribute negatively due to increased levels of greenhouse gases, global warming, higher emission of CO₂, deforestation, and excessive use of fossil fuels. Plants are the primary producers and a very important component of the ecosystem. Temperature fluctuations, particularly due to altered climate conditions, may trigger other factors, such as drought, flood, soil erosion, waterlogging, and salinity, which ultimately led to lower crop productivity and yield.

As the world population is increasing rapidly, demand for the food is also increasing. We need good quality and quantity of crops to fulfill the feeding requirements of the world population. Natural stress factors (light intensity, temperature, water stress, and nutrient availability) and anthropogenic stress factors (mainly HM pollution, excessive use of herbicides, acid rain, and enhanced UV-B radiations) contribute strongly to deteriorate crop health and productivity. Continuously changing climatic conditions induce higher stress on crops due to irregular patterns of moisture contents, more pest and disease infection, more waterlogging conditions, increased soil erosion, and global warming [2]. Climate change and food shortage are the most challenging factors of this century, which need our serious efforts and attention. It is very important to develop crops that are better able to tolerate abrupt climate changes and associated abiotic stresses to keep a balance between environment and agricultural crop production [3]. Among other techniques, the plant genetic engineering approach could also be used for abiotic stress management in crops. Transgenic plants have better tolerance level to various kinds of abiotic stress. They also have improved fruit quality, shelf life, and plant architecture. Genetic engineering of plants also results in reduced postharvest losses, which improve productivity and yield [4]. Induction of the expression of stress-related TFs (MYC, bzip, DREB1A, DREB1B, DREB1C, CBF1, CBF2), stress-responsive genes, signaling pathway kinases (MAPK, CDPK, S6K, PIP5K) hormonal biosynthesis (ABA, ethylene), antioxidant and ROS scavenging mechanism (APX, GSH, GR, GST, SOD, flavonoids, carotenoids), regulatory proteins (HSPs, LEA, dehydrins, aquaporins, metallothioneins, phytochelatins) osmolytes, and compatible solutes (proline, sorbitol, mannitol, polyamines, amino acids, glycine betaine), transporters (NHX, HKT, HMAs) improve the crop performance under altered environmental conditions. Through genetic engineering and genome editing tools, transgenic plants have developed, which are better able to adapt to climate changes without affecting their productivity and yield. Genotyping, sequencing, transcriptomics, proteomics, metabolomics, and functional genomics can be integrated collectively for the identification of stress-responsive genes/gene products and their expression in targeted plants to develop abiotic stress-tolerant cultivars [3]. Different genome editing tools are being used like CRIPR/Cas9, which is of prime importance due to its rapid and effective outcomes. It is an environment-friendly technique to produce transgenic plants, which are better adapted to stress conditions that emerges due to climate change. The CRISPR system is based on candidate gene knockout/insertion or gene replacement, which results in either loss of function, downregulated, or over-expression of gene for abiotic stress tolerance [5].

2. Drought stress

Decreased water availability in soil or excessive loss of water from plants due to high transpiration rate causes drought stress. At the vegetative stage, it affects growth, development, turgidity, and stomatal conductance. At the cellular level, drought stress causes damage to cell division, expansion, nutrient uptake, chlorophyll content, and CO₂ assimilation [6].

2.1 Molecular responses of genetically engineered plants

Genetic engineering basically focuses on the identification of key genes involved in drought stress resistance mechanisms and their potential transfer to different crops

through recombinant DNA technology. These candidate genes are involved in osmotic adjustments, induction of dehydrins, synthesis of abscisic acid (ABA), indole-3-acetic acid (IAA), polyamines for maintaining turgidity, tissue water potential, net photosynthesis, stomatal conductance, growth of plants, and development of deeper and prolific root system [7].

2.1.1 Induction of trehalose biosynthesis

Trehalose is a compatible osmoprotectant that plays its role in improving root architecture, maintaining the integrity of thylakoid membrane and sugar signaling pathway, and increasing photosynthesis, stomatal conductance, and drought resistance [8]. The bacterial OtsB gene is introduced in tobacco for better growth and development under drought stress by promoting a deeper and more prolific root system. Such transgenic tobacco plants show more photosynthetic and water-retaining capacity [9]. The yeast TPS1 gene is involved in developing drought tolerance in transgenic plants by regulating carbohydrate levels through SnRK1 and ABA signaling pathways to regulate stomatal conductance [10].

2.1.2 Induction of LEA protein

LEA (late embryogenic abundant protein) plays a role in the sequestration and compartmentalization of ions. They also protect other proteins from degradation during cellular dehydration. HVA1 gene isolated from barley is introduced in rice for higher growth rates, hydraulic conductivity, and water permeability under drought stress [11]. Transgenic wheat containing barley HVA1 gene showed overexpression of aquaporins (PIP1, PIP2, NIP, TIP3, XIP) for cell proliferation, ions transport, germination, and morphogenesis under drought stress [9].

2.1.3 Induction of proline and polyamines biosynthetic pathways

Proline and polyamines are compatible solutes, whose expression is regulated under drought stress. They are of low molecular weight and are highly soluble in the cytosol. P5CS gene isolated from moth bean is inserted in tobacco plants for detoxification of ROS and stabilizing structures of membranes, enzymes, and proteins [10]. Transgenic soybean plants modified with P5CR gene showed increased proline accumulation for plant growth and development under drought stress. It also protects the lipid bilayer from damage during cellular dehydration [11]. Genetic engineering of the induction of polyamine biosynthetic pathway mainly focuses on two species rice and tobacco. Transgenic rice modified with oats ADC (arginine decarboxylase) shows increased biomass due to regulated plant growth, antioxidant defense, and metabolism [12].

2.1.4 Induction of transcriptional factors (TFs)

Transcription factors are proteins that bind to the promoter of the respective gene to regulate its expression. Under drought stress, different transcription factors are activated, such as DREB (DREB1A, DREB1B, DREB1C) and CBF (CBF1, CBF2, CBF3). Transgenic wheat modified with DREB1A gene from *Arabidopsis thaliana* showed increased drought resistance by overexpressing drought-tolerant genes [13].

Gene	Source	Transgenic plant	Adaptations	References
P5CS1	<i>Vigna aconitifolia</i>	<i>Nicotiana tabacum</i>	Delayed wilting of transgenic plants under drought stress.	[15]
Delta OAT1	<i>Arabidopsis thaliana</i>	<i>Oryza sativa</i>	Transgenic plants showed higher growth rate under drought stress.	[16]
DREB1A CBF3	<i>A. thaliana</i>	<i>Festuca arundinacea</i>	ABA dependent signaling pathway that activates drought-responsive genes.	[17]
betA	<i>E.coli</i>	<i>Gossypium hirsutum</i>	Higher RWC and photosynthesis rate, reduced ion leakage, and lipid membrane peroxidation.	[18]
TPS1	<i>Pichia angusta</i>	<i>Solanum tuberosum</i>	Increased yield and metabolite production, such as sugar, proteins, osmolytes, and hormones.	[10]
OtsA OtsB	<i>E.coli</i>	<i>O. sativa</i>	Less photo-oxidative damage and improved mineral balance under drought stress.	[19]
codA	<i>E.coli</i>	<i>S. tuberosum</i>	Drought stress-inducible glycine-betaine (GB) production for protecting proteins, enzymes, and biological membranes.	[18]

Table 1.
Molecular responses of transgenic plants to drought stress.

2.1.5 Induction of MAPK pathway

MAPK is involved in phosphorylating other protein molecules that initiate a downstream oxidative signaling cascade. NPK1 gene isolated from tobacco is inserted in maize to increase drought tolerance of transgenic maize by protecting photosynthetic machinery under drought stress [14].

2.1.6 Induction of ABA signaling pathway

Under drought stress, the level of ABA increases to cause the closure of stomata and to prevent water loss through transpiration. Genetically engineered plants modified with ERA1 gene isolated from *A. thaliana* showed better growth, development, and net photosynthesis under drought stress as compared to control. Identification and transference of candidate genes from donor to transgenic plant and their molecular responses have been summarized in **Table 1**.

3. Temperature stress (chilling/freezing and heat stress)

Temperature variations across the world have a direct effect on plant productivity. There is a prominent change in the growth and survival patterns of plants under temperature stress. Temperature stress is divided into two categories, that is, low-temperature stress (chilling injury) and high-temperature stress (heat stress).

3.1 Chilling injury

Morphologically, chilling injured leaves become purple or red in color, and wilting of leaves is also observed. Growth is retarded and foliage of leaves appears soggy. At the cellular level, changes in membrane structure and composition due to decreased fluidity and permeability of plasma membrane were observed. Decreased photosynthesis activity under low temperature is mainly due to distorted and swollen thylakoids, reduction in size and number of starch granules, unstacking of grana, and disappearance of the chloroplast envelope. Condensation of chromatin, alternation in the appearance of nucleolus, Golgi apparatus, and endoplasmic reticulum has also been reported under low temperatures.

3.2 Molecular responses of genetically engineered plants

3.2.1 Induction of dehydration-responsive element (DRE)

DREB1A is a transcription factor that interacts with DRE to induce the expression of cold-responsive genes (COR). At low-temperature, ICE transcription factor turn on the expression of CBF/DREB genes, which induces freezing tolerance [20]. Transgenic *A. thaliana* over-expressing ICE-1 showed increased tolerance to chilling stress by regulating CBF and other cold-responsive regulons [21]. In wheat, ICE-1 homologs TaICE141 and TaICE187 are overexpressed to activate the wheat CBF family. Transgenic *A. thaliana* modified with these homologs showed increased freezing tolerance by transcriptional and post-transcriptional changes [22].

3.2.2 Induction of cold-responsive LEA proteins

LEA proteins act as antifreeze proteins that prevent ice nucleation and the formation of ice crystals. They slowed the growth and recrystallization of ice. PmLEAS is a cold-responsive gene, which is expressed in *Prunus mume* under chilling stress. Transgenic tobacco modified with PmLEAS showed increased freezing tolerance by modifying the composition of the lipid bilayer to increase the proportion of unsaturated fatty acid. It also increases the activity of the desaturase enzyme [23].

3.2.3 Expression of LcFIN1 gene in transgenic *A. thaliana*

LcFIN1 gene is overexpressed in sheep grass to provide adaptation to cold stress. Transgenic *A. thaliana* modified with LcFIN1 gene showed high germination rates and long survival time period due to the accumulation of compatible solutes, membrane stabilization, reduced ROS generation, and expression of COR genes [24].

3.2.4 Induction of dehydrins

Dehydrins are thermostable, hydrophilic, and cryoprotective protein molecules. They are molecular chaperone. Chilling stress results in the formation of secondary structures of RNA. Dehydrins prevent the formation of secondary structures by acting as molecular chaperons. They also protect other proteins and enzymes from denaturation. ABA treatment induces the expression of dehydrins. PmLEA

Gene	Source	Transgenic plant	Adaptations	References
CodA	<i>Arthrobacter globiformis</i>	<i>Brassica campestris</i>	Transgenic plants showed an increased net photosynthetic rate.	[29]
CodA	<i>A. globiformis</i>	<i>Lycopersicon esculentum</i>	Chloroplastic synthesis of glycine betaine showed increased tolerance against chilling stress.	[30]
GmTCF1a	<i>Glycine max</i>	<i>Arabidopsis thaliana</i>	Improved survival rate and decreased electrolyte leakage.	[31]
BoCRP1	<i>Brassica oleracea</i>	<i>L. esculentum</i>	Increased accumulation of osmoprotectants and increased activity of ROS-scavenging enzymes.	[32]
DaCB4/ DaCBF7	<i>Deschampsia antactica</i>	<i>Oryza sativa</i>	Increased expression of cold-responsive genes and Ca signaling pathway.	[33]

Table 2.
Molecular responses of transgenic plants to chilling stress.

is a cold-responsive gene of *P. mume*, which is overexpressed under chilling stress. Transgenic tobacco modified with PmLEA showed increased chilling tolerance due to reduced lipid peroxidation and electrolyte leakage [25]. Similarly, the maize ZmDHN2B gene inserted in tobacco provides cold adaptations by preventing the destabilization of membranes. It also increases the unsaturated to saturated fatty acid ratio to prevent ice crystals formation [26].

3.2.5 Induction of compatible solutes

Compatible solutes, such as amino acids, proline, polyamines, and sugars, provide molecular adaptations under chilling stress. Glycine betaine is very important for osmotic adjustments and subcellular functions. CodA (choline oxidase) isolated from *Arthrobacter globiformis* is inserted in transgenic *A. thaliana* provides cold acclimation [27]. Zinc finger protein gene OSISAP1 from rice is inserted into tobacco plants that showed increased growth and survival rates under chilling stress. OSISAP1 encodes for proline biosynthesis enzyme [28]. A short review of molecular responses of some transgenic plants has been summarized in **Table 2**.

4. Heat stress

Heat stress means temperature above a threshold level, which causes irreversible damage to plant growth and development. Scorching of leaves, leaf senescence, abscission, fruit discoloration, reflective leaf hair, leaf curling, and vertical leaf orientation are the main morphological effects of higher temperature. Heat stress induces the production of NH₃ within plant tissues. It leads to ammonia toxicity. CAM pathway is responsible for the high production of organic acids, such as pyruvate, citrate, malate, PEP, and oxaloacetic acid. These organic acids prevent ammonia toxicity within the cytoplasm under heat stress.

4.1 Molecular responses of genetically engineered plants

4.1.1 Induction of heat-shock proteins (HSPs)

Expression of genes for the synthesis of various hormones, such as ABA, ethylene, salicylic acid, and brassinosteroids, are very important for thermotolerance. These hormones stabilize the heat-shock transcription factors and help them to bind with heat shock-related genes. DcHSP17.7 is a heat shock-related gene of carrots. It was inserted into the potato under the control of 35S promoter. Transgenic tomatoes showed increased tolerance to heat stress by stabilization of the tertiary structure of proteins and enzymes [34]. Fad8 is a cytosolic protein of *Brassica napus*, which is over-expressed in the tobacco plant. Transgenic tobacco showed much more heat sensitivity, which shows that silencing of fad8 is important for heat stress tolerance because fad8 encodes for desaturase enzyme [35]. OsHSA2e was isolated from *oryza sativa* and was introduced in *A. thaliana*. Resultant transgenic *A. thaliana* showed increased thermotolerance by upregulation of HSF-related genes [35]. Slhsp gene isolated from *Solanum lycopersicon* was introduced in *N. tabacum*. hsp101 gene was isolated from *A. thaliana* and was introduced in *O. sativa*. Both of the transgenic plants showed increased thermotolerance due to prevented protein aggregation [36]. *A. thaliana* AtPLC9 gene is responsible for heat tolerance as it induces the expression of HSPs and HSFAs. AtPLC9 gene was inserted into *O. sativa*. Transformed rice plants showed increased heat stress tolerance due to the over-expression of OsHSFAs, calcium ions, and calmodulin-related genes [37].

4.1.2 Induction of membrane associated lipid metabolism

Heat stress increases membrane fluidity, which causes disruption of cellular functions and membrane permeability. Plants achieve adaptation to heat stress by

Gene	Source	Transgenic plant	Adaptations	Reference
hsp21	<i>A. thaliana</i>	<i>S. lycopersicon</i>	Protection of PSII from heat-induced oxidative stress and detoxification of photo-induced H ₂ O ₂ .	[39]
OsHsfA2e	<i>O. sativa</i>	<i>A. thaliana</i>	Transformed <i>A. thaliana</i> showed increased thermotolerance.	[40]
TaHsfA6f	<i>Triticum aestivum</i>	<i>A. thaliana</i>	Increased thermotolerance by inducing the expression of ABA metabolism and other heat stress-responsive genes (<i>APX</i> , <i>LEA3</i> , <i>LTP3</i>).	[40]
Badh	<i>Spinacia oleracea</i>	<i>N. tabacum</i>	Overexpression of chloroplastic glycine betaine providing thermotolerance during vegetative stage.	[40]
TaHSP23.9	<i>T. aestivum</i>	<i>A. thaliana</i>	High thermotolerance by preventing irreversible protein unfolding and aggregation.	[41]

Table 3.
 Molecular responses of transgenic plants to heat stress.

increasing saturated fatty acids in membrane composition. Fad7 gene isolated from *A. thaliana* was introduced in *N. tabacum*. This gene encodes for the desaturase enzyme. Transformants that showed silencing of fad7 gene were able to adapt to heat stress more effectively. Similarly, fad8 isolated from *B. napus* was introduced in *N. tabacum*. Transformants with silenced fad8 gene showed better growth, chlorophyll content, and photochemical efficiency [38]. A short review of molecular responses of transgenic plants to heat stress has been summarized (Table 3).

5. Heavy metal stress

Plants required a small amount of HM for the proper functioning of their physiological processes. When the concentration exceeds the threshold value, these HM become toxic for plants. Excess of arsenic (As) causes photosynthesis inhibition, and decreases biomass and yield; cadmium (Cd) toxicity causes chlorosis, reduced water, and nutrient uptake, browning of root tips, and ultimate death; chromium (Cr) and lead (Pb) stress cause reduced nutrient uptake and disturbance in metabolic pathways, respectively. Mercury (Hg) and zinc (Zn) toxicity cause reduced photosynthesis due to the inhibition of photosystems I & II. Excess of nickel (Ni) causes retarded seed germination, reduced plant height, reduced root length, and reduced chlorophyll content [42].

5.1 Molecular responses of genetically engineered plants

5.1.1 Induction of the organic acid biosynthetic pathway

TaALMT1 gene isolated from *Triticum aestivum* was inserted into tobacco and barley crops. Transgenic tobacco and barley showed increased tolerance to HM stress because TaALMT1 induces the expression of the malate biosynthetic pathway. Malate acts as a metal chelator and causes metal efflux. SbMATE gene isolated from sorghum was inserted into *A. thaliana*. Transgenic *A. thaliana* showed increased HM-stress tolerance. SbMATE induces the expression of citrate transporter for metal efflux [43].

5.1.2 Genetic engineering for Cd toxicity tolerance

Cd is a highly toxic metal due to its fast mobility and persistency. A very small concentration of Cd is lethal to plants. Different genetic engineering approaches have been implied to develop transgenic plants that can withstand Cd toxicity. *gsh1* isolated from *E. coli* was inserted into *Brassica juncea*, which showed increased Cd tolerance. *gsh1* gene encodes for γ -glutamylcysteine synthetase for the synthesis of glutathione (GSH) and phytochelatin (PCs). GSH plays role in HM-induced ROS scavenging by initiating the ascorbate-glutathione cycle. PCs form a complex with HM (HM-PC), which is transported to the vacuole for detoxification. *N. tabacum* modified with RCS1 gene of *O. sativa* showed higher cysteine synthase activity [44]. CDna-LTC1 a nonspecific transporter of Cd was introduced in tobacco that showed increased Cd tolerance due to less storage of Cd in roots [45].

5.1.3 Induction of the expression of metallothioneins (MT)

MTs act as chelator that binds with free metals and releases them slowly. MT1 gene isolated from chickpea was inserted into *A. thaliana*, which showed increased HM-stress

Gene	Source	Transgenic plant	Adaptations	References
NAS1	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i>	Increased Ni tolerance and accumulation.	[51]
MTA1	<i>Pisum sativum</i>	<i>Populus alba</i>	Increased Cu and Zn tolerance.	[52]
HMA4/ MT2	<i>A. thaliana</i>	<i>N. tabacum</i>	Increased Cd tolerance and Cd/Zn translocation efficiency.	[51]
MRP7	<i>A. thaliana</i>	<i>N. tabacum</i>	Increased tolerance to Cd and increased metal accumulation in roots.	[51]
merA	<i>E.coli</i>	<i>N. tabacum</i>	More efficient Hg volatilization and tolerance.	[53]
ECS/ arsC	<i>E.coli</i>	<i>A. thaliana</i>	Enhanced As tolerance and hyperaccumulation.	[53]

Table 4.
 Molecular responses of transgenic plants to HM stress.

tolerance by upregulation of antioxidative enzymes (APX, GPX, GSH, GR) and reduced electrolyte leakage [46]. OsMT1e-P, a MT gene of *Oryza sativa* was inserted into tobacco that showed improved HM-stress tolerance (Cu and Zn) by metal ions compartmentalization and vacuolar sequestration [47]. Human MT2 was inserted into tobacco and oil seed crops. Transference of various MT genes (human MTIA, human MTII, yeast CUPI, pea PsMTA, and TaMT) into *A. thaliana*, *Brassica campestris*, and *N. tabacum* showed increased HM-stress tolerance due to overexpression of GSH-S-transferase activity. BcMT1 and BcMT2 genes from *Brassica campestris* were inserted into *A. thaliana* and showed improved tolerance to HM stress by upregulation of the activity of anti-oxidative enzymes [48].

5.1.4 Induction of the expression of metal transporter genes

Metal transporters are important for the transportation and compartmentalization of free metal ions. Genetic engineering mainly focuses on the expression of metal transporter genes in plants. Induction of AtPHT1/AtPHT7 genes isolated from *A. thaliana* along with YCF1 gene of *Saccharomyces cerevisiae* in tobacco showed much more As tolerance and accumulation. TgMTP1 gene from *N. Goesingense* was genetically engineered into *A. thaliana* that showed improved Zn tolerance [49]. *Znta* gene isolated from *E.coli* was inserted into *A. thaliana* that showed increased resistance to Pb and Cd. *Znta* gene encodes for V-type ATPase metal transporter that transports free metal ions from cytoplasm to vacuole for sequestration [50]. PvACR3 transporter gene isolated from *Pteris vittata* was introduced in *A. thaliana*, which showed increased tolerance to As [49]. A brief review of the molecular responses of transgenic plants to HM stress has been summarized in **Table 4**.

6. Salinity stress

Increased concentration of soluble salts in the soil causes salinity stress. Salinity stress causes high-level accumulation of Na⁺ and Cl⁻ ions within the cytoplasm, which disturb enzyme activities and photosynthetic processes. It also

causes ROS-induced oxidative damage to lipids, proteins, and nucleic acids. Other adverse effects include decreased nutritional value of plants, salinity-induced osmotic stress, decreased rate of seed germination, and decreased plant growth and productivity.

6.1 Molecular responses of genetically engineered plants

6.1.1 Induction of the expression of Na⁺/H⁺ antiporter

Genetic engineering approaches focus on the identification of various genes that encode ion transporters, antiporters, cationic channels, compatible solutes, osmoprotectants, etc. Ion transporters play important role in the selective transport of ions and maintain the optimal level of these ions. Vacuolar Na⁺/H⁺ antiporter catalyzes the exchange of Na⁺ from the cytoplasm to vacuole for sequestration. It helps in maintaining cellular homeostasis, pH, and cell turgidity. *B. napus* modified with AtNHX gene from *A. thaliana*. AtNHX gene encodes Na⁺/H⁺ antiporter. The transgenic plant showed increased salt tolerance, growth, and photosynthetic rate. Similar results were observed when *Brassica juncea* was transformed with pgNHX1 gene [54]. *T. aestivum* was modified by vacuolar Na⁺/H⁺ antiport gene AtNHX1 from *A. thaliana*. The transgenic wheat plant showed a lower accumulated level of Na⁺ in leaves. Transformed *A. thaliana* with a high expression level of AtNHX1 gene showed high salt tolerance. AtNHX1 gene is responsible for the compartmentalization and sequestration of Na⁺ into the vacuole [55]. *O. sativa* was modified by Na⁺/H⁺ antiport gene nhaA from *E.coli*. Transgenic rice showed better salt tolerance, seed germination rates, growth, and productivity [56].

6.1.2 Induction of the expression of SOS gene

The high salt level is detected by receptors, which increases the cytosolic level of calcium. SOS3 binds to free Ca and activates the expression of SOS3 protein kinase. SOS3-SOS2 complex induces the expression of SOS1 gene, which encodes Na⁺/H⁺ antiporter. *ThSOS1-ThSOS5* genes were isolated from *T. hispidia* and inserted into *A. thaliana*. The transformed plant showed increased salt tolerance due to increased ROS scavenging activity, and lower MDA and H2O2 levels [57]. *SOS1* and *AHA* genes were isolated from *Sesuvium portulacastrum* and coexpressed in *A. thaliana*. Transgenic *A. thaliana* showed increased salt tolerance due to rapid Na⁺ extrusion and regulated cellular homeostasis [58].

6.1.3 Induction of the expression of HKT1-type transporters

HKT1 transporters are responsible for regulating Na homeostasis by keeping a balance between Na and K in the cytoplasm. PpHKT1 gene isolated from almond rootstock was inserted into *A. thaliana*. The transgenic plant showed reduced electrolyte leakage, longer lateral roots, and increased salt tolerance [59]. McHKT2 gene isolated from *Mesembryanthemum crystallinum* was inserted into *A. thaliana*. Transgenic *A. thaliana* showed increased salt tolerance due to lower root Na uptake and lower Na concentration in xylem sap. A short review of molecular responses of transgenic plants to salt stress has been summarized in **Table 5**.

Gene	Source	Transgenic plant	Adaptations	References
Vacuolar Na ⁺ /H ⁺ antiporter MsNHX1	Alfalfa (<i>Medicago sativa</i>)	<i>A. thaliana</i>	Increased osmotic balance. Reduced aggregation of Na ⁺ and increased accumulation of K ⁺ in leaves.	[60]
Vacuolar Na ⁺ /H ⁺ antiporter AtNHX1	<i>Aeluropus litoralis</i>	<i>Nicotiana tabacum</i>	Compartmentalization of Na ⁺ in roots. Maintenance of K ⁺ /Na ⁺ ratio in the leaf.	[60]
GhNHX1	<i>Gossypium hirsutum</i>	<i>N. tabacum</i>	Na ⁺ extrusion and vacuolar sequestration.	[54]
AgNHX1	<i>Atriplex gmelini</i>	<i>Oryza sativa</i>	Na ⁺ vacuolar sequestration.	[54]
ProDH	<i>A. thaliana</i>	<i>A. thaliana</i>	Proline biosynthesis for osmotic homeostasis.	[54]
Avp1	<i>A. thaliana</i>	<i>O. sativa</i>	Vacuolar H ⁺ pyrophosphatase synthesis. Vacuolar acidification.	[54]

Table 5.
 Molecular responses of transgenic plants to salt stress.

7. Genome editing strategy (CRISPR/Cas9) for transgenic plants

Different genome editing strategies are being used to develop transgenic-resistant crops to deal with food insecurity issues. CRISPR/Cas9 is the most powerful system to develop a genetically engineered crop system that is able to adapt to different climate change-induced stresses more effectively. It also helps in producing high-yielding and stress-resistant crops. It is environment friendly, fast, rapid, accurate and economical. SgRNA/Cas9 construct is established to target the specific genome sequence of a plant. This construct is introduced in the targeted crop by means of various transformation methods, such as Agrobacterium-mediated transformation and protoplast transfection, to produce abiotic stress-resistant variety [61]. *B. napus* was modified with CRISPR technology to downregulate the expression of CLVTA3. Transgenic *B. napus* showed more seed production. TaGW2 gene's expression was knocked down using the CRISPR system in wheat. This modification helped in increasing the seed size of wheat. Overexpression of SIMAPK3 gene by CRISPR/Cas9 in *S. lycopersicon* showed improved drought tolerance. Knocking down the expression of Osann3 by CRISPR/Cas9 in rice produced more cold stress tolerance [62]. CRISPR/Cas9-mediated genome editing technology is very efficient to develop transgenic crops, which are environment-friendly and able to adapt to climate changes efficiently. A short review of the application of CRISPR/Cas9 in abiotic stress tolerance has been summarized in **Table 6**.

8. Conclusion

In this chapter, molecular responses of transgenic plants to different types of abiotic stresses have been discussed. This review has thrown light on the effects of

Specie	Targeted gene/s	Genome editing strategy	Improved trait	References
<i>Oryza sativa</i>	OsHLH024	Knockdown expression by CRISPR/Cas9.	Salt tolerance	[5]
<i>O. sativa</i>	OsHKT1;3, SOS1, OsHAK7	Enhanced expression of ion transporter genes by CRISPR/Cas9.	Salt tolerance	[5]
<i>Zea mays</i>	ARGOS8	Replacing ARGOS8 with GOS2 by CRISPR/Cas9 to improve yield.	Drought tolerance	[5]
<i>S. lycopersicon</i>	SILBD40	CRISPR/Cas9-mediated gene mutation.	Drought tolerance	[5]
<i>Z. mays</i>	ZmWRKY106	Enhanced expression by CRISPR/Cas9.	Heat stress tolerance	[63]
<i>O. sativa</i>	OsMYB30	CRISPR/Cas9-mediated gene knockout.	Cold tolerance	[63]
<i>O. sativa</i>	OsNramp, OsCd1, and OsNramp5	Overexpression of metal transporter genes by CRISPR/Cas9.	HM-stress tolerance.	[63]
<i>A. thaliana</i>	AtPDF2.6	Overexpression by CRISPR/Cas9.	HM-stress tolerance by chelation of cytoplasmic Cd.	[64]

Table 6.
Genetic engineering of plants through CRISPR/Cas9 to adapt abiotic stress.

climate change-induced stress factors for plants and how genetic engineering can help to develop transgenic plants that are able to respond to these stresses at the molecular level. Climate change is a major threat factor for the agricultural sector as it causes lower yield and productivity. It causes increased food demand leading to hunger and starvation. Drought stress, salinity stress, temperature stress, and heavy metal stress are major types of abiotic stresses that cause retarded and stunted growth, lower yield, and productivity. Molecular adaptations through genetic engineering can be achieved by expressing stress-related genes, accumulation of compatible solutes, activation of signaling pathways, activation of transcriptional factors, action of various transporters, synthesis of heat-shock proteins and secondary metabolites, and enzymatic and nonenzymatic defense mechanisms, etc. through various genome editing tools, such as CRISPR/Cas9. This review has covered a detailed analysis of each type of abiotic stress and responses of transgenic plants at the molecular level.

Most of the studies of transgenic plants are based on Arabidopsis, rice, and tobacco. Further studies are required by using model plants on different staple crops, such as wheat, cereals, and legumes. to meet food requirements. Climate is changing very rapidly and it has adverse effects on plant growth, productivity, and yield. Climate change-induced stress factors can cause famine and starvation. Different technologies and genome editing tools are being used worldwide to produce resistant transgenic crops, such as CRISPR/Cas9, OMICS, TALEN, QTL, nanobiotechnology, and miRNAs/siRNAs. These technologies focus on genome editing of transcription factors and stress-responsive genes to introduce novel modifications in the plant genome. The development of transgenic crops through genetic engineering is a need of time and demands serious efforts.

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