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# Salt Stress and Plant Molecular Responses

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and Ali Fuat Gökçe*

## Abstract

Plants being sessile suffer from several abiotic stresses during growth. These include drought, salinity, heavy metal, temperature, and nutrient stress. Salt stress is one of the devastating abiotic stresses that plant suffers under natural growth conditions. It is more common in arid regions due to excessive evaporation, which causes the accumulation of inorganic salts disrupting the plant metabolism. It also triggers the influence of drought stress, as plants are unable to absorb water. Additionally, it also causes oxidative stress in plant tissues. Thereby, plant adaptation to salt stress, rely on signals and pathways that help plant in establishing cellular ionic and osmotic homeostasis. Stress-responsive transcription factors play crucial roles in the regulation of gene expression in responses to salt stress. Moreover, genome editing has gained much attention for the engineering of traits for the better adaptation of plants to salt stress. This chapter elucidated the plant's physio-biochemical responses and molecular mechanisms to salt stress.

**Keywords:** genome editing, oxidative stress, sensing, salt stress, transcription factors

## 1. Introduction

Abiotic stress is an external environmental factor that influences plant growth stages. It includes a fluctuation in temperature ranging from chilling to heat stress, deposition of heavy metals in soil due to anthropogenic activities also results in heavy metal stress/toxicity. Limited availability of water for a shorter or longer period causes drought stress [1]. Most importantly and thoroughly discussed is the salt stress that arises due to deposition of higher salt contents in the soil layers due to poor application of irrigation water, higher evaporation that also deposits salt in the upper surface layer of the soil [2]. Currently, climate change is also stimulating several abiotic stresses on a yearly basis and the salinity problem is one of them. Globally, the number of salt-affected soils is increasing and approximately 20% of the agricultural land has been affected [3].

Salt stress also causes secondary stresses such as osmotic, water, ionic, and oxidative stresses. Osmotic stress is formed as soil salinity decreases the soil and leaf water potential, that disrupts the water relation of plants and decreases the turgor pressure of plants [4]. Plant roots serve as a medium of nutrient uptake, but the excessive salt solution in the vicinity of the roots forces its uptake that causes ionic toxicity in plants. Additionally, plant roots are unable to extract/absorb water that also further aggravates plants growth by acclimatizing its water stress.

Plants start their journey from seed germination to seedling establishment, plant growth, and fruiting, however higher salt contents in the soil cause poor germination and weakened morphological growth leading to declined yield [5].

Salt stress also disrupts the physiological functioning of the plants, such as the closure of stomata, reduction in gaseous exchange, damage to photosynthetic machinery, decrease in chlorophyll contents, and distortion of PSII system [6, 7]. Salt stress also disrupts the biochemical functioning of the plants with the excessive production of reactive oxygen species (ROS) resulting in oxidative stress [8]. The ROS is favorable at a lower concentration that is crucial for cellular signaling processes for the induction of positive response in antioxidant defense likewise biological processes to help improve stress adaptation mechanisms [9]. However, higher ROS causes oxidative stress, and plants have the inherent ability to alleviate ROS species with the upregulation of genes that synthesize antioxidant enzymes [10]. Plants can tolerate salt stresses by maintaining antioxidant enzyme activity or increase their levels to cope with oxidative stress caused due to environmental adversities in various plant species [11]. Abiotic stress causes gene expression changes in plants and a comparison of these changes may provide information on adaptation and stress tolerance/sensitivity [12].

Transcription factors are involved in several biological processes in plants to regulate stress tolerance. Transgenic approaches have extensively used transcription factors that function in salt stress signaling pathways for the genetic engineering crops to confer stress tolerance. Most used transcription factors include ABA-binding factor (ABF), basic leucine zipper (bZIP), dehydration responsive element binding (DREB), myeloblastosis (MYB), NAC, and WRKY due to their significance in the signaling against salt stress [13]. Their specificity depends upon homodimerization, heterodimerization, and modifications at post transcription, post translation by small RNA (miRNA). Additionally, modification in histone modification and DNA methylation are involved to perform the main role for regulating gene expression in response to salt stress. The transcription factors bind to cis-acting elements in promoter regions to regulate gene expression. Therefore, they exhibited potential for controlling the expression of target genes, as some transcription factors are early stress-responsive genes [13, 14].

Currently with the introduction of novel genome editing techniques also assisted in developing stress-resilient genotypes. Generally, nowadays clustered regularly interspaced short palindromic repeats (CRISPR) is the most used genome editing technique due to simplicity and ease of availability. It is believed that unraveling of molecular mechanisms and genome editing approaches are the best options to pave the path to understand and develop stress tolerant genotypes to cope with future climate and ensure food security [15].

## **2. Salt stress and plant growth**

Roots provide anchorage, access to soil water, and essential nutrients required throughout the growth stages. Its morphological characteristics play an important role in the growth and production of plants [16]. During stress, the plant roots are the first organ that senses adverse conditions such as excessive salt in the soil. The study of the root system is of great interest as the structure and distribution of roots assist in higher water uptake. It also maintains the productivity of plants under salt stress conditions [17]. Salt stress interrupts the normal growth of the plants as excessive salt accumulation in the vicinity of roots and its uptake cause a toxic environment for plants. The typical symptoms of morphological growth of plant include retardation of vegetative growth, increase in plant development, senescence

sometimes resulting in death [8]. The inhibited growth is the first noticeable symptom of salt stress that might results by alteration of cell division due to salinity shock. Other obvious symptoms of sodium accumulation in plants are necrosis of older leaves, it starts from the tip leading towards the leaf base. Therefore, it also causes early senescence of leaves resulting in poor plant biomass and yield. We also observed that higher salt contents resulted in a decreased number of leaves, plant height, and reduction in bulb yield of onion [6, 18]. Plant growth reduction with the acclimatization to salt stress is due to disruption of normal physio-biochemical in plants with the development of secondary stresses that are discussed below.

### **3. Salt stress and physiological processes**

Salt stress disrupts the physiological functioning of the plant, as the first response of the plant is to limit gaseous exchange with the closure of stomata. It also helps in reducing the water loss of the plant. Higher salt accumulation along with stomatal closure limits CO<sub>2</sub> intake that disturbs the photosynthesis of the plants. Likewise, photosynthesis of the plant decreases that ultimately affects vegetative growth and harvest yield [19]. It also aggravates ionic toxicity and osmotic stress to the plants. It is reported that abscisic acid (ABA) results in stomatal closure. The ABA is the main chemical compound that is produced in the roots in response to stress. It sends signals for the closure of stomata. It is directly linked to the rate of photosynthesis, therefore, damage to photosynthetic machinery and pigments [20]. Additionally, the plant also accumulates sucrose that changes electron transport. Increased salt contents in photosynthetic tissues also cause shrinkage of the thylakoid membrane. There is another perspective that decreased stomatal conductance is beneficial for the plant as it helps in limiting the entry of toxic ions via roots. It was corroborated by a couple of studies that decreased stomatal conductance resulted in a reduction of the uptake of ions to the transpiration stream [21]. Plants uptake higher Na<sup>+</sup> contents from the soil under salt stress that decreases the uptake of other essential nutrients required by plants for their functioning. It causes an ionic imbalance in a plant that replaces K<sup>+</sup> loading by Na<sup>+</sup> to the chloroplast resulting in damaged photosystem II. It is the protein complex that functions in the catalyzation of light-induced water oxidation to the evolution of oxygen. Salt stress decreases the activity of PSII that decreases the quantum yield of PSII. It has been reported that NaCl inhibited the photochemical efficiency of PSII. It is sensitive to Na<sup>+</sup> toxicity and directly reduces the photosynthesis process of the plant [7]. Osmotic stress is regarded as the deficiency of water in plant tissues due to excessive accumulation of salts. It is caused due to the inability of plant roots to absorb water. Higher Na<sup>+</sup> also decreases the turgor potential of the plant [22]. Resultantly the supply of water to the leaf decreases that causes lower leaf development. Decreased number of leaves or senescence directly reduces the photosynthetic rate and damage to chlorophyll contents. Salt-sensitive genotypes that are unable to cope with stress suffer from dehydration caused by a disturbance in turgor pressure resulting in the death of plant tissues [23].

### **4. Salt induced oxidative stress**

Salt stress directly causes ionic imbalances and with the severity of the stress to ionic toxicity. Moreover, it also triggers oxidative stress with the higher production ROS. The toxic influence of higher ROS accumulation causes increased lipid peroxidation, membrane damage, in addition DNA and protein damage [24]. The ROS are

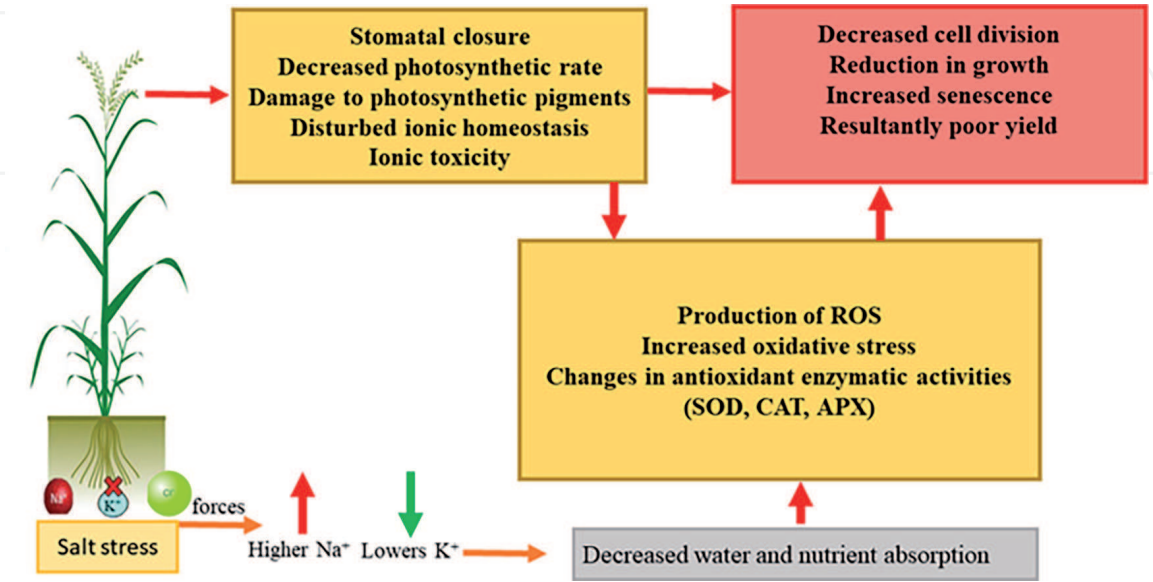


mostly produced in chloroplast and mitochondria that include singlet oxygen ( $^1\text{O}_2$ ), superoxide radical ( $\text{O}^{2-}$ ), hydroxyl radical ( $\cdot\text{OH}$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). It includes superoxide dismutase that works as a first in the antioxidant system by catalyzing the dismutation of superoxide radical ( $\text{O}^{2-}$ ) to  $\text{H}_2\text{O}_2$  and  $\text{O}_2$  in the cytosol. Ascorbate peroxidase is the main player in detoxifying hydrogen peroxide in plant chloroplast, mitochondria, cytosol, and peroxisomes during stress conditions. It utilizes ascorbic acid as an electron donor for the reduction of  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$ . Catalase also alleviates oxidative damages by converting  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$  [25].

Secondary metabolites such as tocopherol play role in the stabilization of the membrane integrity. It also works as a signaling molecule in response to stress between cells [26]. Ascorbic acid is an important antioxidant that is found abundantly in the apoplast and cellular organelles. It scavenges  $^1\text{O}_2$ ,  $\text{O}^{2-}$ , and  $\cdot\text{OH}$  for plant adaptation against salinity. Carotenoids are localized in the chloroplast that is reported to help plants in light reception to continue the process of photosynthesis, furthermore, they are also known as a protective compound for scavenging ROS species [27]. Putrescine supplementation is beneficial to restrain the production of ROS, as it speeds up the antioxygenic enzymes that ultimately help in protecting the chloroplast membrane [28]. In one of the study, putrescines are reported to enhance the level of carotenoids and glutathione in response to salt stress [29].

Several studies have reported the higher accumulation of ROS is a response to stress leading to cellular damages [7]. Additionally, it was also observed that higher production ROS due to salt stress caused decreased crop production. Therefore, it is essential to control the regulation of ROS to protect plant tissues from cellular damage caused by oxidative burst. Moreover, C4 plants have been reported to show higher resistance to salinity compared with C3 plants. As they have a better capability of preserving photosynthetic mechanisms against oxidative stress [30]. Salt stress influence on plant oxidative stress is shown in **Figure 1**.

Several genes have been identified that encode antioxidant enzymes SOD, CAT, APX, and glutathione reductases (GRs), that are involved to scavenge ROS in plants. These genes are described as T genes as they contribute to stress tolerance [9]. Contrarily S genes have been reported that result in excessive ROS production. They are responsible for higher oxidative stress, increased programmed cell death,



**Figure 1.** Plant responses to salinity stress. Salinity favors the higher uptake of  $\text{Na}^+$  and lower uptake of  $\text{K}^+$  that causes ionic homeostasis leading oxidative burst and reduced growth and yield.

| Crops   | Gene               | Function  | Reference |
|---------|--------------------|---|-----------|
| Tobacco | CuZnSOD, APX       | Reduced oxidative stress                            | [31]      |
| Wheat   | P5CS               | Higher proline accumulation                         | [32]      |
| Onion   | Cat                | Improved catalase activity                          | [7]       |
| Rice    | OsGR2              | Alleviated oxidative stress                         | [33]      |
| Tomato  | SOD                | Decreased ROS production                            | [34]      |
| Maize   | APX, SOD, CAT, POD | Higher antioxidant enzymatic activities to cope ROS | [35]      |

**Table 1.**  
*Genes responsible for the alleviation of oxidative stress in crops.*

decreased antioxidant activity. Other gene group that causes disturbances in hormonal concentration, or ionic distribution have also been reported. Plant molecular breeders have utilized these identified T genes to incorporate into plants to confer salt stress tolerance. The list of some antioxidant genes is given in **Table 1**.

5. Salt stress inducible genes

Plant response to salinity stress is a complicated process that includes a various numbers of genes to finally activate physiological and biochemical mechanisms. The study of these genes provided a new horizon to identify their functions to cope with stress. These genes initiate a transcriptional response in plants with a set of several genes and their transcription also varies with the severity and duration of the stress. As they are categorized as early responsive genes that protect the plant by stimulating genes for free radical scavengers, detoxification enzymes, osmoprotectants, etc. The second ones are transcription factors, protein kinases, whereas the third group includes ion transporters and aquaporins [36].

6. Salt stress signaling

Plants being sessile must suffer from salt stress and suffer from several secondary stresses caused by Na<sup>+</sup>. They undergo several morpho-physiological and biochemical changes to adapt/cope with stress that has been thoroughly studied. However, until now, the identification of Na<sup>+</sup> sensors and perception by plants is still ambiguous. Under salt stress conditions, cellular organelles, and compartments such as the nucleus, chloroplast, peroxisome, plasma membrane, apoplast, and cell wall play crucial roles. Regardless of all the recent progress in unraveling complex stress mechanisms, there is still needed to find out how stress signals are generated, integrated, and coordinated in/from different organelles. Furthermore, scientists have identified specific genes, genomic regions, proteins, and metabolites against salt stress. The high-throughput transcriptomics and proteomics are potent functional genomic that can elucidate cellular networks for the perception of salt stress. Higher Na<sup>+</sup> causes a higher accumulation of ROS in plant tissues that disturbs the K<sup>+</sup> and Ca<sup>2+</sup> homeostasis [37, 38]. These stress constraints theoretically may be sensed either by cytosolic or membrane-bound sensors that later translated into physio-biochemical and genetic modifications for the optimization of plant growth. There is also a high probability that a couple of sensory mechanisms might operate in the same cell, and at same time translating information related to the severity of

stress. Therefore, it remained a great challenge to understand the mechanisms of signaling. Higher salt concentration disturbs the ionic homeostasis causing ionic stress. It is a general perspective that it triggers the elevated  $\text{Ca}^{2+}$  levels in the cytosol within seconds after exposure to salinity. It is further aided due to the activation of slow-activating vacuolar/two-pore channel 1 that results in the release of vacuolar  $\text{Ca}^{2+}$ . Therefore, the identification of proteins involved in the quick influx of  $\text{Ca}^{2+}$  is efficient for the discovery of salinity sensors. *Arabidopsis thaliana* a model plant has 20 different glutamate receptor-like proteins that are suggested for their function as cation channels [39].

Additionally, non-selective cation channels include a diverse group of ionic channels having the capability to discriminate several essential nutrient and toxic ions to plant under salinity conditions [40]. Increased cytosolic  $\text{Ca}^{2+}$  is largely accumulated in the cortex and endodermis of the root. Its higher influx also stimulates  $\text{Ca}^{2+}$ /calmodulin (CaM)-dependent kinases, that trigger plasma-membrane  $\text{H}^+$  ATPase [41]. Higher ROS burst is also due to elevated levels of cytosolic  $\text{Ca}^{2+}$ . Therefore, both higher ROS and  $\text{Ca}^{2+}$  regulate the release of abscisic acid resulting in the initiation of transcriptional responses [42].

## 7. Salt stress and signaling pathways

Stress stimulus perception needs cell-surface harboring sensors or receptors to understand different stresses for the transduction of signal via signaling pathways. The main signaling pathways include receptor-like protein kinases (RLK), mitogen-activated protein kinase (MAPK) that are known as molecular switches for upstream signaling for the determination of the fate of plants in response to environmental stresses [43]. Thus, to understand how plants recognize high concentrations of salt and ultimately adapt themselves to salt stress is essential. The MAPK signaling cascade is the main pathway. It connects the external environment to the endogenic cellular activities of the plant. It is conserved in *Arabidopsis* which exhibited 20 MAPKs [44]. Their activation targets specific substrates i.e., transcription factors and other kinases. It confers salt stress tolerance by regulating salt-related genes for the homeostasis of oxidative burst and likewise osmotic stress [45]. With the exposure to salt stress, activated MAPK cascades initiated the varied transcriptional response of salt-related genes [46]. It suggested the link between salt sensors and target genes. Transcriptome analysis of *Arabidopsis* revealed that 152 genes changed their expression with the over-expression of MAPK. Modified genes can be clustered into different types. Some genes are regarded as stress marker genes, and others as ethylene, jasmonic acid, and auxin signaling [47, 48].

## 8. Salt stress and transcription factors

Transcription factor is a protein that attaches to the DNA-regulatory sequences usually found in the 5'-upstream region of target genes to initiate the rate of transcription of the gene. They help turn "on" or "off" the specific genes with the binding to the neighboring DNA. This results in increasing or decreasing the transcription of the gene, synthesis of protein, and ultimately modifies cellular functioning against salt stress. Additionally, they also perform a function to control cellular processes such as cell cycle mechanisms, intercellular regulation mechanisms, reproduction, growth [49]. Their most important salt stress responses make valuable for the characterization of their functions [50, 51]. Some transcription factors are highly conserved in plants that modify gene expression to confer

stress tolerance against salt [52]. The main transcription factors that respond to abiotic stresses are bZIP, NAC, WRKY, AP2, MYB, DREB, and bHLH. They follow ABA-dependent and ABA-independent signal pathways. These pathways are highly involved in controlling gene expression [53]. They are essential for the association of signaling pathways for their involvement in gene expression, thereby it signifies the importance of these factors in the plant genome. As arabidopsis genome sequence revealed 1500 transcription factors [54]. The transcription factors play a crucial role in plant adaptation against different environments [55]. One transcription factor is responsible for controlling the expression of numerous genes. In this way, the engineering of these factors further highlighted their importance to upregulate or downregulate the gene expression for plant adaptation to salt stress. The union of these factors developed a transcription unit called regulon (a group of numerous genes that turns on/off as a unit). Very few regulons have been reported in plants in response to abiotic stresses. One of them is DREB regulon and its overexpression exhibited tolerance against major abiotic stresses such as cold, drought, and salt stress [56]. The wheat cultivars exposed to long-term salinity and bZIP gene expression was noticed significantly upregulated that conferred salinity tolerance in sensitive cultivars, contrarily its expression was lower in tolerant cultivars [57]. The other transcription factor NAC genes were overexpressed. Its higher expression alleviated the harmful effects and rendered salt stress tolerance [58]. Another study also reported that NAC5 gene showed upregulated gene expression in response to salt stress. Moreover, it might also regulate the production and aggregation of proline, sugar, LEA proteins to mitigate oxidative stress in plants [59]. Rice is a monocot model plant therefore it has been explored to unravel information about transcription factors. In rice SALT-RESPONSIVE ERF1 (SERF1), the gene showed root-specific stimulation after being acclimatized to salinity. The SERF1 gene is responsive against  $H_2O_2$ , which means that it has the potential to minimize ROS burden in plants [60].

## 9. Salt stress and ion transporters

Salt stress disrupts the ionic homeostasis in plant cells and causes ionic stress. The increased concentration of  $Na^+$  and  $Cl^-$  ions disturbs the  $K^+/Na^+$  ratio with the formation of a toxic environment for plant cells. It is mainly caused due to favoring higher uptake of  $Na^+$  through the same pathway that uptakes  $K^+$ . It is also reported that it is difficult for transporter proteins. Another assumption is that it is hard for plant transporter protein to differentiate between  $Na^+$  and  $K^+$  due to similarity. Additionally, both ions are competitors of each other to occupy binding sites. Therefore, the plant uptakes higher  $Na^+$  ions and results in  $Na^+$  toxicity. The  $Na^+$  influx in cytosol increases making plants difficult to thrive due to salt stress. Higher  $Na^+$  efflux into the cytoplasm results in depolarization of the membrane potential, which also activates outward rectifier channels ( $K^+$ ) i.e. guard cell outward rectifying  $K^+$  channel (GORK) for the extrusion of  $K^+$  [61].

Conversely, plants have adopted a mechanism to minimize the influx of  $Na^+$  ions to avoid depolarization of membrane by retaining a negative potential to enhance retention of intracellular  $K^+$  [62]. The antiporters  $Na^+/H^+$  helps in vacuolar  $Na^+$  compartmentation and extrusion from the cell. It is considered as a vital process for the detoxification of  $Na^+$  from the cytosol and helps in osmotic homeostasis to confer salt stress tolerance. In salt stress, several selective pumps activate to favor  $K^+$  uptake instead of  $Na^+$ . They use high and low-affinity potassium transporters for the uptake of  $K^+$ . The transporters include  $Na^+/H^+$  exchangers (NHX), salt overly sensitive 1 (SOS1) antiporter are crucial to decrease salt stress [62, 63].



High-affinity potassium transporter (HKT) protein was reported to be the first selective transporter that performs the selective role for  $K^+$  uptake instead of  $Na^+$  [64]. It plays an important role in the exclusion of  $Na^+$  and maintains  $K^+$  homeostasis. The transporter HKT2;1 has been reported for conferring tolerance against salt stress. The NHX that are localized in the tonoplast also performs a key role to restrict the entry of  $Na^+$  by promoting the outer movement of  $Na^+$  to the vacuole. The SOS1 functions as an exchanger in the plasma membrane. Its overexpression conferred salt tolerance [65].

## **10. Salt stress and epigenetics**

In the field of functional genomics in plants epigenetics has gained much attention due to its potential for the adaptation of plants against stress. Salt stress significantly reduces the vegetative growth and yield of the crops. In past conventional breeding, approaches helped a lot for the selection and adaptation of plants but they are time-consuming [66]. Currently, new genetic approaches such as epigenetics that modifies plant genome without changing the sequence of DNA such as histone modification, methylation and demethylation of DNA, and gene imprinting are helping hand for the development of stress-resilient genotypes [67]. It also refers to the heritable variation in the regulation of genes leading to DNA modifications and protein. These modifications are reversible that can alter the phenotype of the plant. As methylation of cytosine is a conserved mechanism of epigenetics that is involved in several biological processes e.g., the proliferation of transposons, and regulation of gene expression in response to salt stress. Generally, it inactivates the genes whereas demethylation is responsible for the activation of the gene. It was further corroborated by genome-wide mapping that methylated genes were 8% that showed activation, whereas methylation of the transcribed region exhibited 31% expressed genes. Salt stress influence on cytosine methylation has been reported in many important crops. It is a conserved epigenetic mechanism that plays a crucial role in several biological processes in plants. It includes transposon proliferation, genomic imprinting, and gene expressional changes. Generally, it is related with the inactivation/suppression of genes expression, contrarily demethylation causes activation of genes [68, 69]. A study reported that salinity-induced methylation resulted in down-regulation of negative regulator, whereas it upregulated positive regulated [70]. The H3K9ac and H3K4me3 positively regulate gene expression and H3K9 methylation is reported as a repressive chromatin marker [71].

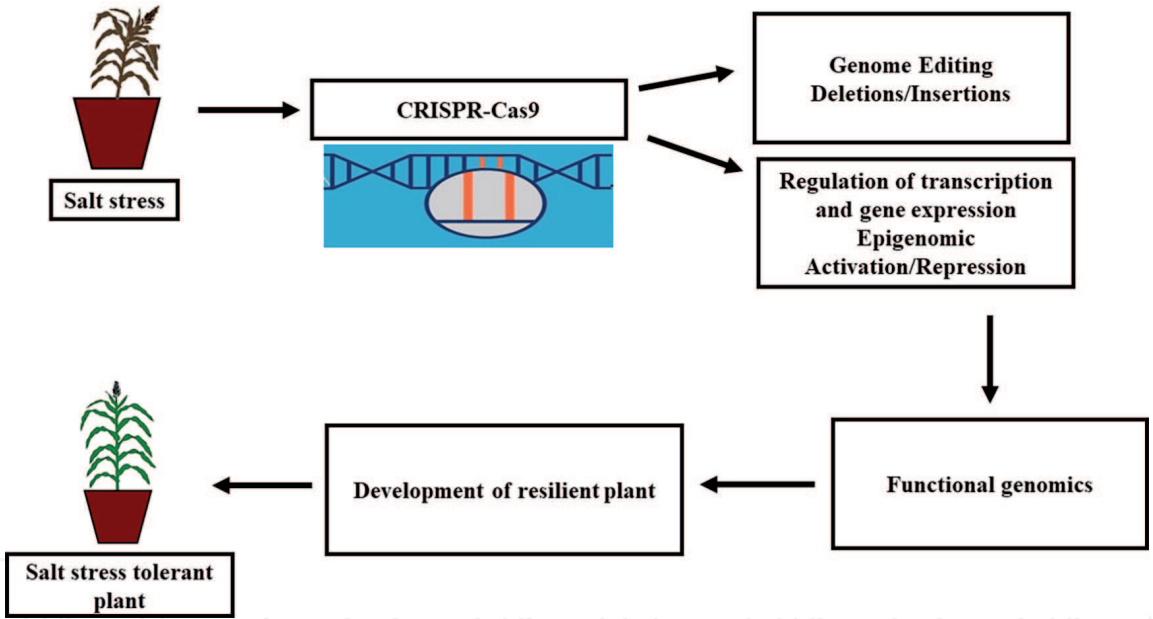
## **11. Salt stress and genome engineering**

Change, any alteration, or target mutagenesis of the genome is known as genome editing. With the advent of this modern technology, it allows the introduction of specific changes with more precision at specific sites. It utilizes engineered nucleases that cleave the sequence of target DNA strand breaks, that are repaired by either homologous recombination or non-homologous end-joining. The customized modifications of DNA that include insertions, deletions, can be obtained via DNA repair mechanisms. Until now four different genome engineering techniques have been used in plants. These include meganucleases, zinc finger nucleases, transcription activator-like effector nucleases, and the most recently introduced is CRISPR/Cas RNA-guided nucleases [72]. CRISPR is a new versatile tool for genome editing derived from a bacterial system. This eminent technology showed promising results

to edit genomes of different plants, resulting in improved plant biotechnology research. Currently is being employed for the development of new varieties with improved traits against salt stress shown in **Figure 2** [73].

This technology has the ability of multiplexing that can co-target multiple loci, therefore it has the great potential to knockout the complete cassette of non-desirable genes that halts plant growth in response to salt stress. It can be used to disrupt the functions of a negative regulators, such as RMC transcription factor negatively regulates in response to salt stress [74]. Several genes have not been explored that are responsible for the negative regulation of salt stress. The RING finger protein 1 is a ubiquitin ligase E3 that is a negative regulator that enhances the production of H<sub>2</sub>O<sub>2</sub> and suppresses antioxidant enzyme activities. However, the knockdown of this negative regulator resulted in disruption of H<sub>2</sub>O<sub>2</sub> synthesis and positively regulated antioxidant enzymatic activities [75]. Disruption of the functions of genes by CRISPR to confer salt stress tolerance is given in **Table 2**.

The CRISPR-based epigenome editing helps in protecting the plants from the harmful effects of salt stress [82]. The CRISPR/Cas9 protein has been used as a dCas9 is attached to the modifier of epigenetics for the alteration of gene expression [83].



**Figure 2.**  
*Salt stress and role of genome editing for the development of salt tolerant plants.*

| Crops  | Gene   | Function   | Reference |
|--------|--------|--|-----------|
| Rice   | OsDST  | Decreased stomatal density and tolerance to osmotic stress | [76]      |
| Tomato | HyPRP1 | Negative regulator of salt stress                          | [73]      |
| Rice   | OTS1   | Regulator for salt stress response                         | [77]      |
| Maize  | HKT1   | Potassium transporter                                      | [78]      |
| Tomato | CLV3   | Regulates shoot development                                | [79]      |
| Tomato | SLARF4 | Tolerance to osmotic stress                                | [80]      |
| Wheat  | TaERF4 | Transcription repressor of ABA                             | [81]      |

**Table 2.**  
*Genes knockdown by CRISPR/Cas for salinity tolerance.*

## 12. Conclusion

Plant tolerance to salt stress involves several complex molecular mechanisms to respond for better morphological growth and maintain physiological functioning. Extensive research has been conducted to elucidate the physio-biochemical responses to control  $\text{Na}^+$  uptake, its transport, and cellular homeostasis by minimizing oxidative stress. In this regard plant sensors and signaling played a crucial role in the adaptation of plants. Additionally, regulation of gene expression of the positive regulators also assists in salt stress tolerance. Conventional breeding techniques have been used for developing new varieties but that is time consuming however with the advent of genome editing as a tool for plant breeder, tolerant plants can be developed within a less period by disrupting the function of negative regulators or improving traits for salinity tolerance. Additionally, in the future to cope with the negative influence of harsh salt stress problems plant epigenetics can be explored for a better understanding of plant salt stress responses. It can also be potentially used for the regulation of gene expression that can confer salt stress tolerance.

## Conflict of interest

The authors declare no conflict of interest.

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