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# Genomics of Salinity Tolerance in Plants

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Additional information is available at the end of the chapter

## Abstract

Plants are frequently exposed to wide range of harsh environmental factors, such as drought, salinity, cold, heat, and insect attack. Being sessile in nature, plants have developed different strategies to adapt and grow under rapidly changing environments. These strategies involve rearrangements at the molecular level starting from transcription, regulation of mRNA processing, translation, and protein modification or its turnover. Plants show stress-specific regulation of transcription that affects their transcriptome under stress conditions. The transcriptionally regulated genes have different roles under stress response. Generally, seedling and reproductive stages are more susceptible to stress. Thus, stress response studies during these growth stages reveal novel differentially regulated genes or proteins with important functions in plant stress adaptation. Exploiting the functional genomics and bioinformatics studies paved the way in understanding the relationship between genotype and phenotype of an organism suffering from environmental stress. Future research programs can be focused on the development of transgenic plants with enhanced stress tolerance in field conditions based upon the outcome of genomic approaches and knowing the mystery of nucleotides sequences hidden in cells.

**Keywords:** Salt tolerant genes, Salt Tolerance, Transgenic Plants, MicroRNA, Quantative Trait loci

## 1. Introduction

Nature's rage influences plants in the form of various biotic and abiotic stresses. Extreme abiotic stress conditions, such as salinity, flooding, heat, drought, and cold, as well as heavy metal toxicity and oxidative stress affect plants in many different ways. Human activities exacerbate these stress conditions to a greater extent. All the abiotic and biotic stresses, including

various pathogens, cause havoc to plants eventually limiting their growth and yield potentials. About 50% of crop yields are reduced due to abiotic stresses, making them the major cause of crop failure worldwide [1]. Abiotic stresses are a serious threat to the sustainability of agricultural industry. Naturally, a number of stresses combine with each other and act together; therefore, the negative effects are aggravated to a greater extent when compared to a single stress factor. To combat these stresses, combinations of diverse pathways are triggered [2].

In physical terms, stress is defined as a mechanical force per unit area applied to an object. It is difficult to measure the exact force applied by the stresses because the plants are immotile. This makes it harder to define stress in biological terms. A condition, which may act as a stress for one plant, may be ideal for another plant. Hence, a biological stress can most suitably be defined as a harsh condition or force that impedes the normal functioning of a biological system such as plants [3].

The plasma membrane serves as a barrier that separates a cell from its surrounding environment. Some of the small lipid molecules like steroid hormones are able to pass through this membrane and diffuse into the cytoplasm, whereas the membrane does not allow the water soluble molecules, such as ions, proteins, and other large molecules, to pass through it. Cells start responding when extracellular molecules come in contact with the plasma membrane. This foreign molecule is called an elicitor, and the protein that is present on the cell membrane and interacts with the elicitor is called a receptor. A number of biotic and abiotic stress signals serve as elicitors for the plant cells [4].

## 2. Salinity stress and its causes

Total amount of dissolved mineral salts in water and soil is termed as salinity [5]. These salts comprise electrolytes of anions (majorly $CO_3^{2-}$, $SO_4^{2-}$, $Cl^-$, $NO_3^-$, $HCO_3^-$) and cations (majorly $Ca_2^+$, $K^+$, $Mg_2^+$, $Na^+$). Salts that are soluble in water get deposited in the upper layer of soil to a greater extent that hinders the agricultural productivity of that land area [6]. Although fewer salts are present in the rainwater, these salts can be accumulated in the soil over a certain period of time. Salts can also be deposited by soil transported by wind from far off places. Impure irrigation water also contributes to the level of deposited salts in the agricultural lands [7].

Salinity stress is one of the main abiotic stresses and is considered as a restraint to crop yield. Increased salinization of cultivable land has disastrous effects worldwide [8]. Hyperosmotic and hyperionic stresses are caused by increased salinity, which can lead to plant death [9]. A number of factors are responsible for causing salinity in a given area such as the extent of precipitation or evaporation and weathering of rocks. Deserts have high salinity due to the fact that the rate of evaporation is greater than the rate of precipitation.

All the key processes within a plant are significantly influenced when the plant is exposed to salt stress [10]. The water stress resulting under salt stress affects the leaf growth and development. Cell division and expansion as well as stomatal opening and closing are negatively influenced by the salinity stress [11]. If the stress condition prevails, then the ionic stress strikes, and eventually, a major decline in photosynthetic rate occurs, and the leaves start to die [12].

Deforestation is a leading cause of salt stress. Heavy salt–rich irrigation is the major cause of salinity in agricultural lands. The process of evapotranspiration is responsible for the retention of excessive amounts of salt in the soil every year. This is due to abundant loss of water as a result of both evaporation and transpiration. Almost all of the main agricultural crops are sensitive to salt stress that results in serious damage to the yields of the crops [13]. Soil contents altered by the deposition of large amounts of salt in the soil, and as a result, soil becomes less porous reducing soil aeration and water transport [14]. Salinity stress and drought stress are quite similar in terms of physiology [15].

## 3. Stress signaling pathways

The receptors present on the plant cell surface receive the stress signals and transfer them downstream, resulting in the production of secondary messengers, e.g. reactive oxidative stress (ROS), calcium, and inositol phosphates [16]. Calcium level is further controlled by these messengers within the cell. As a result of this disturbance in the intracellular $Ca^{2+}$ level, the $Ca^{2+}$ sensors are triggered, which change their conformation in a calcium-dependent manner [14]. These sensors initiate a phosphorylation cascade by interacting with their respective partners and activate the stress responsive genes or the transcription factors that regulate stress response genes. The products of stress response genes help in plant survival and mitigate the stress conditions. Production of hormones (such as ethylene, salicylic acid, and abscisic acid (ABA) takes place because of changes in gene expression under the stress. Initial signal is amplified by messenger 'Sensor' stress response molecules, and a secondary signaling pathway may be induced. Such molecules which do not take part directly in signaling but play a role in alteration of signaling components are called accessory molecules [17].

The stress responsive genes can be divided into two major categories: early- and late-induced genes. Early-induced genes are prompted immediately after stress signals are received, and most of the times, they express in shorter period. In this category, a number of transcription factors are included because they do not require synthesis of new proteins for their stimulation. In contrast, late-induced genes are expressed slowly under the stress condition, i.e., express after hours of receiving stress signals, and their expression is persistent [18]. In this gene category, major stress responsive genes, such as (COR cold responsive), KIN (cold induced), or RD (responsive to dehydration), and membrane stabilizing proteins, osmolytes, antioxidants, and LEA (late embryogenesis abundant)-like proteins are included [19].

## 4. Salt tolerance

The percentage of biomass production in saline conditions in comparison with normal growing environment during an extended period is known as salt tolerance. In this regard, vivid variations are found among different plants due to the fact that decline in growth is dependent on the length of time over which plants are growing in the salinity-affected soils. For example,

a salt-tolerant plant like sugar beet may undergo 20% decrease in dry weight when grown in 200 mM NaCl [20]. In contrast, a moderately tolerant plant like cotton may undergo 60% decrease in the dry weight, whereas a sensitive plant like soybean may become dead [21] and a halophyte like *Suaeda maritima* may grow to its full potential in the 200 mM NaCl condition [22]. Evaluation of salt tolerance for perennial plant species can also be done based on survival rate. A marked decline in growth rate is observed in both salt-tolerant as well as nontolerant species during a short time in salt stress. This has been seen in the case of durum wheat and bread wheat, where durum wheat is more salt sensitive [23] and the same was also observed in the case of barley and triticale [15]. This led to realizing the importance of time frame and the mechanisms that different plants use at different growth stages when exposed to salinity.

## 5. Mechanisms of salt tolerance

The initial discovery of biochemists that enzymes of halophytes and nonhalophytes are equally tolerant to increased levels of NaCl is found to be true [24]. This was explained by the example of enzymes obtained from a halophyte *Atriplex spongeosa* and those obtained from peas or beans that were equally sensitive to NaCl [21, 25]. This is because most enzymes get inhibited at $Na^+$ concentration more than 100 mM, and some are observed in the case of $Cl^-$. Even $K^+$ can also inhibit enzymes when present in 100–200 mM concentrations [26]. Hence, the salt-tolerant mechanisms can be divided into two main categories: (1) preventing or reducing the amount of salt being uptake by plant tissue and (2) reducing the concentration of salt present in the cytoplasm. These both types of mechanisms are found in halophytes, which not only exclude salt very effectively but also quite effectively compartmentalize the excess salt in cell vacuoles. Due to this reason, halophytes are able to grow in saline soils far better and for longer time spans than other plants.

## 6. Conventional ways to manage salinity

Accumulation of large amounts of salts in the water around the root area is referred to as soil salinity [6]. Plants can tolerate soil salinity by two processes: salt exclusion and salt inclusion [27]. Plants, which are able to eliminate salts from the whole plant or specific plant tissues, are known as salt excluders. Such plants possess low $Na^+$ and $Cl^-$ content as the membrane permeability prefers $K^+$ over $Na^+$ uptake in these plants. On the other hand, salt accumulators can withstand high salt concentrations by two approaches. The first approach is the enduring increased amounts of intercellular salts. The second approach is through the elimination of surplus amounts of salt from the plant because the roots of these plants can absorb salt ions but prevent their harmful effects [28].

To recover the agricultural lands from salt stress and for increased yields, it is necessary to remove excess amounts of salts from the root region. The common strategies used for this purpose are leaching, scraping, and flushing. As these methods were quite costly, new

approaches were introduced for contending salt stress. One of them is the use of halophytes in salinity-affected lands. Halophytes are the plants that can exclude the deposited salts from the soil surface in addition to withstanding high levels of accumulated salts [29]. For this purpose, some halophytes possess salt glands, which are specialized leaf cells having the ability to expel salt. Some others use salt hairs present on stems for this purpose while some have stomatal guard cells, which regulate the rate of transpiration according to the surrounding salt concentration. Another strategy used to protect the plants from the injurious effects of salinity is foliar feeding of nutrients. This enhances plant salt tolerance by relieving plants from $Na^+$ and $Cl^-$ injury [30].

Soil salinity can also be controlled using better farm management practices. In this regard, improved irrigation methods, such as drip irrigation, can be used to apply controlled amount of water to the land. In rain-fed areas, crop rotation of annual crops with perennial crops (having deep roots) should be practiced to re-establish the equilibrium between rainfall and used water. This will avert the water tables from rising and delivering salts to the surface [31].

## 7. Genetic responses to salinity

Genetic response in case of salinity stress takes in a complex mechanism that is used by plants to up-regulate or down-regulate (increase or decrease) the production of specific gene products (protein or RNA). These mechanisms have been recognized at different stages of central dogma process like from transcriptional initiation to RNA processing, post-transcriptional modification, and translation, and to the post-translational modification of a protein [32]. Understanding about the transcriptional behavior of plants provides a detailed knowledge about the gene expression at mRNA level. Transcriptional profiling is widely used to screen out candidate genes involved in stress responses. Till now, massive information about the salt responsive genes, transcription factors which either up-regulated or down-regulated, has been identified using transcriptome profiling methodology. Further genomic approaches contribute significant role in encoding, cloning, and characterization of these genes. Gene expression under the certain conditions altered by transcription factors. These factors are considered the most important switches that up-regulate or down-regulate the gene expression. Among them, *bZIP*, *WRKY*, *AP2*, *NAC*, *C2H2* zinc finger gene, MYB and DREB family proteins comprise a large number of stress-responsive members. These transcription factors have the capacity to alter the gene expression by cis-acting specific binding in the promoter region of broad range of genes.

Up-regulation in the expression of bZIP genes were observed in sensitive wheat cultivar under persistent salinity stress and down-regulation in salt-tolerant variety [33]. It predicts the role of NAC transcription factor in salinity tolerance in both rice and wheat cultivars. In rice, transcriptional regulators, such as DREB1/CBF, DREB2, and AREB/ABF, have been demonstrated to play a significant role in abiotic stress responses [34, 35]. Transcription factors, such as OsNAC5 and ZFP179, show an up-regulation under salinity stress, which may regulate the synthesis and accumulation of proline, sugar, and LEA proteins that in turn play an integral role in stress tolerance [36].

Full-length cDNA is a vital resource for studying the full functional genes in wheat. A group of gene "MYB gene" family analyzed by Zhang et al. [37] that respond to one or more abiotic stress treatments. They isolated 60 full-length cDNA sequences encoding wheat MYB proteins and also construct phylogenetic tree with other wheat, rice, and *Arabidopsis* MYB proteins to understand their evolutionary relationships and the putative functions of wheat MYB proteins based on *Arabidopsis* MYB proteins with known functions. Tissue-specific analysis and abiotic stress response expression profiles were also carried out to find potential genes that participate in the stress signal transduction pathway, including the analysis of transgenic *Arabidopsis* plants expressing the MYB gene, TaMYB32 [38]. In *Arabidopsis*, salt stress results in up-regulation of AtWRKY8 gene expression, which directly binds with the promoter of RD29A [39]. A large number of genes and transcription factors are up-regulated in response to salinity in different plant species, which serve diverse functions [40]. Some of the examples of salt-responsive genes are listed in the Table 1, and these genes are mainly classified into the following functional categories: ion transport or homeostasis (e.g., SOS genes, AtNHX1, and $H^{+}$-ATPase), senescence-associated genes (e.g., SAG), molecular chaperones (e.g., HSP genes), and dehydration-related transcription factors (e.g., DREB). Among stress-responsive genes, the SOS transcription gene family is considered to play a very stimulating role in ion homeostasis, thereby conferring salt tolerance [32, 41]. Most of the salinity responsive genes, such as ROS-scavenging and osmotic-regulating genes, are also up-regulated by salinity in salinity tolerant species. Schmidt et al. [42] observed more than 10 extensively up-regulated genes in the halophyte plant species *Spartina alterniflora* under salt stress. Most of these genes were related to osmotic regulation process.

| Gene Name | Species | NaCl Concentration | Gene functions | References |
|---|---|---|---|---|
| SOS1 | | | (1) Plasma membrane $Na^{+}/K^{+}$ antiporter | |
| SOS2 | *Brassica juncea* | 25 and 50 mM | (2) Protein kinase | [40] |
| SOS3 | *Brassica campestris* | | (3) Calcium-binding protein | |
| AtNHX1 | | | (4) Vacuolar $Na^{+}/K^{+}$ antiporter | |
| PRP | | | (1) Proline-rich proteins and cell wall protection | |
| SAG | *Oryza sativa* | 50 mM | (2) Senescence-associated genes, regulatory processes, and cellular signal transduction | [43] |
| HSPC025 | | | (3) Heat-shock proteins, protein stabilizing | |
| OsHSP23.7 | | | Heat-shock proteins, molecular | |
| OsHSP71.1, | *Oryza sativa* | 100 mM | chaperones, folding, assembling and | [44] |
| OsHSP80.2 | | | transporting proteins | |
| AtSKIP | *Arabidopsis thaliana* | 150 mM | Transcription factor, transcriptional preinitiation, splicing, and polyadenylation | [45] |
| OsHsp17.0, | | | Heat-shock proteins, molecular | |
| OsHsp23.7 | *Oryza sativa* | 200 mM | chaperones, folding, assembling and transporting Proteins | [46] |

| Gene Name | Species | NaCl Concentration | Gene functions | References |
|-----------|---------|--------------------|----------------|------------|
| *DcHsp17.7* | *Carrot* | 300 mM | Cell viability and membrane stability under heat stress | [47] |
| *JcDREB* | *Arabidopsis thaliana* | 300 mM | Transcription factor | [48] |
| *katE gene* | *Escherichia coli* | 150 mM | Membrane stability | [49] |
| *Salt overly sensitive (SOS) genes* | *Ipomoea batatas* | 120 mmol L$^{-1}$ | Ion homeostasis Improve biochemical indicators | [50] |
| *AtNHX1* | *Arabidopsis thaliana* | 220 mM | Calcium-binding protein, vacuolar Na$^+$/K$^+$ antiporter | [51] |
| *SNAC1* | *Oryza sativa* | 200mM | Enhancing root development and reducing transpiration rate Biochemical adjustment | [52] |
| *OsRab7* | *Oryza sativa* | 250 mM | Vesicle trafficking gene enhanced seedling growth and increased proline content | [53] |
| *PtSOS2* | *Populus tremula* | 85 mM | Protein kinases enhanced photosynthetic pigments and physiological parameters | [54] |
| *TaSC* | *Triticum aestivum* | 150mM | Enhanced membrane stability | [55] |
| *PeXTH* | *Populus euphratica* | 200 mM | Cell viability and membrane stability enhanced water holding capacity | [56] |
| *StP5CS* | *Solanum tuberosum* | 150 mM | Osmolyte accumulation | [57] |
| *CYP94 (cytochrome P450)* | *Oryza sativa* | 200 mM | Enhanced CYP94C2b expression | [58] |
| *TaSC* | *Triticum aestivum* | 120 mM | Regulate the gene expression program | [55] |
| *H3K4me3* | *Arabidopsis thaliana* | 150 mM | Gene priming, regulate the gene expression program | [56] |
| *WsSGTL1* | *Withania somnifera* | 100 mM | Stabilized the phenotypic and physiological parameters | [59] |
| *GmPIP1;6* | *Glycien max* | 100 mM | Multifunctional aquaporin involved in root water transport, photosynthesis, and seed loading | [60] |
| *AtSTO1* | *Arabidopsis thaliana* | 150 mM | Enhanced the salt tolerance increased concentrations of 9-cis-epoxycarotenoid dioxygenase3 | [61] |
| *ONAC045* | *Oryza sativa* | 200 mM | Functioned as a transcriptional activator | [62] |
| *SOS1* | *Nicotina tabacum* | 150 mM | (1) Plasma membrane Na$^+$/K$^+$ antiporter (2) Protein kinase | [63] |

| Gene Name | Species | NaCl Concentration | Gene functions | References |
|-----------|---------|--------------------|----------------|------------|
| | | | (3) Calcium-binding protein | |
| | | | (4) Vacuolar $Na^+/K^+$ antiporter | |
| mtlD | Escherichia coli | 200 mM | Enhanced the production of mannitol 1-phosphate dehydrogenase | [64] |
| glyoxalase II | Oryza sativa | 200 mM | Detoxification of cytotoxic 2-oxo-aldehydes | [65] |
| HAL5 | | 100 mM | Regulate $Na^{(+)}/K^{(+)}$ homeostasis, lower leaf $Na^{(+)}$ accumulation, reducing $Na^{(+)}$ transport from root to shoot, maintaining $Na^{(+)}/K^{(+)}$ homeostasis | [66] |
| AtSTO1 | Arabidopsis thaliana | 200 mM | Increased concentrations of 9-cis-epoxycarotenoid dioxygenase3, greater overall biomass, greater root biomass, improved photosynthesis, and greater pith size | [61] |
| TaSTRG | Triticum aestivum | 200 mM | Higher salt and drought tolerance, lower intracellular $Na^{(+)}/K^{(+)}$ ratio, higher survival rate, fresh weight and chlorophyll content, accumulated higher proline and soluble sugar contents and had significantly higher expression levels of putative proline synthetase and transporter genes | [67] |

**Table 1.** Salt responsive genes with their origin and possible functions

Recently, Schmidt et al. [42] characterized root-specific salt-responsive *ERF1* (*SERF1*) transcription factor gene in *Oryza sativa* that showed a root-specific induction upon salt and $H_2O_2$ treatment. Plants deficient for *SERF1* are more sensitive to salt stress compared with the wild type, although constitutive overexpression of *SERF1* improves salinity tolerance. Different types of kinases also regulate the activity of transcription factors and have been found to be significant players of plant adaptation to salinity stress. Serra et al. [68] studied the *OsRMC* encodes a receptor-like kinase and described as a negative regulator of salt stress responses in rice. Two transcription factors, *OsEREBP1* and *OsEREBP2*, belonging to the AP2/ERF family were shown to bind to the same GCC-like DNA motif in *OsRMC* promoter and to negatively regulate its gene expression. Basic region/leucine zipper (*bZIP*) TFs (Transcription factors are proteins involved in the process of converting, or transcribing, DNA into RNA. Transcription factors include a wide number of proteins, excluding RNA polymerase, that initiate and regulate the transcription of genes) possesses a basic region that binds DNA and a leucine zipper dimerization motif. A *bZIP* class of ABRE-binding transcription factor, known as *OSBZ8*, has also been identified from rice and has been shown to be highly expressed in salt-tolerant cultivars than

in salt-sensitive one [69]. Moreover, *OSBZ8* has been shown to be activated/phosphorylated by a SNF-1 group of serine/threonine kinase in the presence of Spd during salinity stress [69].

Sairam et al. [70] isolated and analyzed the expression response of wheat *lip19* (encoding *bZIP*-type transcription factors) against cold stress. Further analysis confirmed the upregulation of *Wlip19* gene in a freezing-tolerant wheat cultivar than in a freezing-sensitive cultivar, while under drought and exogenous ABA application, higher activity of *Wlip19* also observed. Heterologous expression of Wlip19 in tobacco has showed a significant increase in abiotic stress tolerance. Alternative splicing of RNA/mRNA played a critical role to cope stress condition especially abiotic stress by switching on/off the transcriptional activities. These splicing factors and spliceosomal proteins mainly involved in plant growth, development process, responses to external environmental factor by affecting the cellular process, cell fate, plant immune/defense system, and tolerance efficiency. All these processes point to critical role of the splicing/alternative splicing under abiotic stress environment [71].

In addition to protein coding genes, recently discovered microRNAs (miRNAs) and endogenous small interfering RNAs (siRNAs) have emerged as important players in plant stress responses. Therefore, post-transcriptional gene regulation plays a crucial role in the plant salt response (Figure 1) [72]. Initial clues suggesting that small RNAs are involved in plant stress responses stem from studies showing stress regulation of miRNAs and endogenous siRNAs, as well as from target predictions for some miRNAs [73]. There has been strong evidence leading to the proposal that miRNAs are hypersensitive to abiotic or biotic stress as well as to diverse physiological processes [74, 75]. Drought, cold, and salinity are major abiotic stresses for plants; all of these conditions strongly induced miR402 overexpression. Numerous studies on plants, such as *Arabidopsis thaliana* and *Oryza sativa*, have been studied with respect to miRNA expression analysis and have revealed an important role for miRNAs in response to abiotic stress.

Various studies with respect to miRNAs profiling under abiotic stress point out the several differentially expressed miRNAs. In response to salt stress, miRNAs, such as miR396, miR394, miR393, miR319, miR171 miR169, miR168, and miR167, were up-regulated, whereas the miR398 was down-regulated in *Arabidopsis*, thus indicate a role for miRNAs in the response to salt stress [76].

Up-regulation of miRS1 and miR159.2 in response to salt stress was observed in *Phaseolous vulgaris* [77]. The expression of miR530a, miR1445, miR1446a-e, miR1447, and miR171l-n was increased, whereas the expression of miR482.2 and miR1450 was decreased during salt stress in *Populus trichocarpa* [76]. Furthermore, two members of miR169 family namely miR169g and miR169n showed enhanced expression during salinity. With the development of genomics tools and computational algorithms to predict and identify the miRNAs in various plant species, the number of miRNAs associated with salt stress response is increasing. A comprehensive understanding of miRNA-based gene regulation under salt stress will definitely help in elucidating the complex network of regulatory factors, proteins, and metabolites.
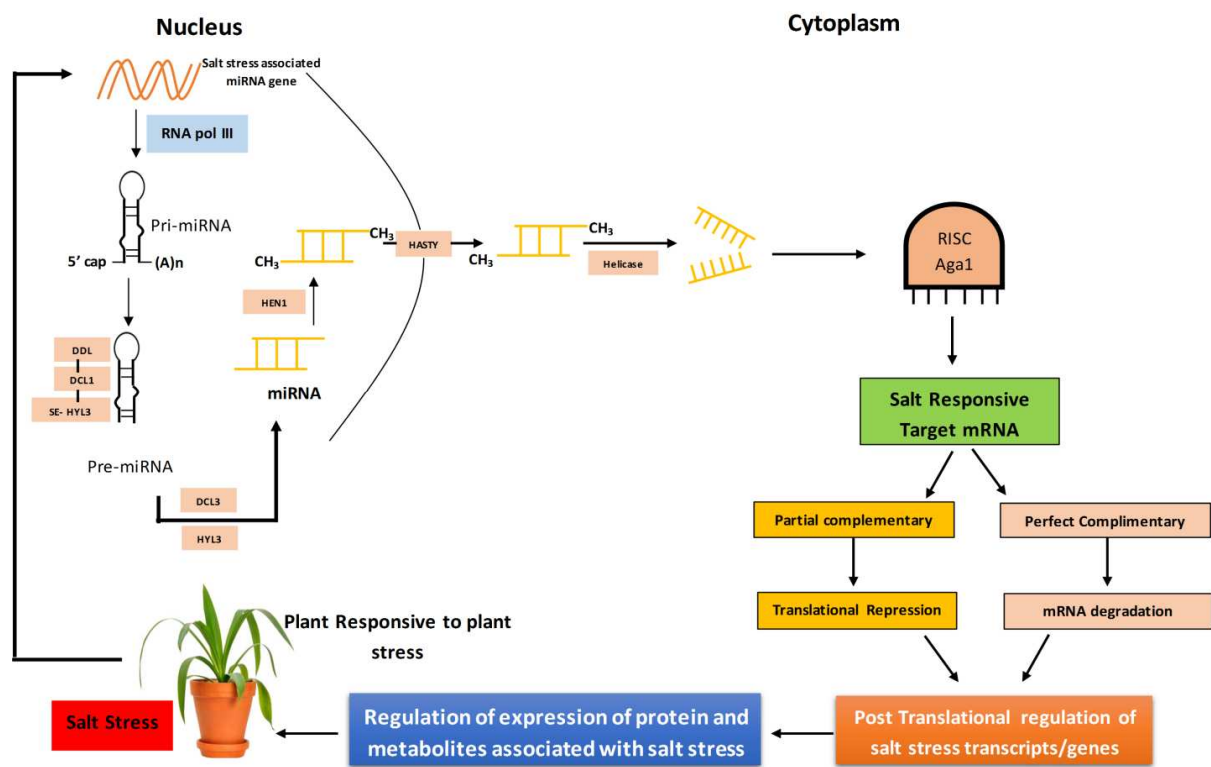
**Figure 1.** A pathway showing miRNA-mediated post-transcriptional regulation of salt stress–responsive plant genes

## 8. QTL mapping in relation to plant salinity tolerance

A quantitative trait locus (QTL) is a section of DNA (the locus) that correlates with variation in a phenotype (the quantitative trait). The QTL typically is linked to, or contains, the genes that control that phenotype. Genetic marker is an identifiable fragment of DNA that is linked with a specific point and indicates genetic differences within the genome. Molecular markers act as a 'signs' or 'flags' should not be considered as normal genes. Genetic markers that are tightly linked are referred to as "gene tags" [78]. The DNA markers can be grouped in various categories based on their technical requirements, the number of genetic markers that can be detected throughout the genome, and the amount of genetic variation found at each marker [79]. Restriction fragment length polymorphisms (RFLPs) are one of the earliest types of DNA-based marker system, which detect the variation in restriction fragment length by Southern hybridization, which cause single base changes that led to the creation or removal of a restriction endonuclease recognition site to detect shift in fragment size. Although this technique is an important tool in breeding programs, it has been superseded by microsatellite or simple sequence repeat (SSR) markers and is now rarely used. SSR markers detect variation in the number of short repeat sequences, usually two or three base repeats that allow the detection of multiple alleles. The expressed sequence tag (EST) databases have now opened the opportunity for the identification of single nucleotide polymorphisms (SNPs) that occur at varying frequencies depending on the species and genome region being considered [80].

These DNA marker types could be associated with quantitative traits, which are known as quantitative trait loci (QTLs). Mapping of QTLs for salt tolerance have been a slow process due to the complexity of this trait and poor understanding about it. Ren et al. [81] discovered a gene locus named as QTL SKC1, which codes for a transporter that removes $Na^+$ from the xylem [82]. Several QTLs have been identified in different crop plants. QTLs for yield and physiological characteristics were identified at a late stage of growth of barley under salinity stress [83]. A total of 10 traits were considered for which 30 QTLs were identified under salt stress and nonstress conditions. Of these 30, 13 QTLs were discovered under salt stress [83]. In white clover, QTLs for salt tolerance were identified at the vegetative stage of plants and the results showed that, in white clover, multiple QTLs are responsible for controlling the salt tolerance [84]. However, QTLs for salt tolerance in tomato were detected at the seedling stage of *Solanum pennellii* and *Solanum lycopersicoides* plants. In *S. pennellii*, four major QTLs were detected, for salt tolerance, on chromosomes 6, 7, and 11, whereas in *Solanum lycopersicoides* six major QTLs were identified under salt stress on chromosomes 4, 6, 9, and 12 [85]. QTLs for salt tolerance in soybean were identified on chromosome 3 [86]. QTLs identified by SSR markers in various plants are given in Table 2.

| Crop plants | Locus | Traits | Reference |
|---|---|---|---|
| Wheat (*Triticum aestivum* L.) | *Kna1* | Controls the selectivity of Na+ and K+ transport from root to shoot and maintains high $K^+/Na^+$ ratio | [88, 89] |
| | *Nax1* | Both are involved in decreasing Na+ uptake and enhancing $K^+$ loading into the xylem | [90, 91] |
| Rice (*Oryza sativa* L.) | *qRL-7, qDWRO-9a* and *qDWRO-9b qBI-1a* and *qBI-1b* | Play important roles in root length and root dry weight at seedling stage under saline conditions | [92] |
| | *QNa, QNa:K, SKC1/ OsHKT8* | Regulate $K^+/Na^+$ homoeostasis | [81] |
| | *qDM-3* and *qDM-8, qSTR-6* | Improve Na+/K+ ratio under saline conditions | [93] |
| | *qNAK-2* and *qNAK-6* | Improve $Na^+/K^+$ ratio | [94, 95, 96, 97] |
| | *Saltol* | Controls shoot $Na^+/K^+$ homoeostasis | |
| | *Saltol* and *nonSaltol* | Control shoot $Na^+/K^+$ homoeostasis | |
| | *QKr1.2* | Controls $K^+$ content in root | |
| Barley (*Hordeum vulgare*) | Five QTL for ST were identified on chromosomes 1H, 2H, 5H, 6H, and 7H, which accounted for more than 50% of the phenotypic variation | Enhance vegetative growth under saline stress | [98] |
| | A locus *HvNax3* on the short arm of | Reduces shoot $Na^+$ content by 10–25% in plants grown under salt stress (150 mM NaCl) | [99] |

| Crop plants | Locus | Traits | Reference |
|---|---|---|---|
| | chromosome 7H in wild barley (*Hordeum vulgare* ssp. *spontaneum*) accession CPI-71284-48 | | |
| White clover (*Trifolium repens* L.) | Several QTLs for ST, some at common locations, but each of low scale | Affect ST during vegetative stage | [84] |
| Soybean (*Glycine max* (L.) Merr.) | A major QTL for ST was identified near the Sat091 SSR marker on linkage group (LG) N Eight QTLs for ST were detected A major QTL for ST was detected | Maintains growth under salt stress Maintains growth under salt stress Maintains healthy growth under salt stress | [100] [101] [102] |

**Table 2.** QTLs for 'Salt Tolerance' (ST) in various plants identified by SSR markers [87]

## 9. Engineering plants for enhanced salt tolerance: Transgenic approach

Plant breeding strategy for salt tolerance is not much successful due to the reproductive barrier and also as it involves the risk of other undesirable traits transfer. Reproductive barriers and uncontrolled transfer of the traits make the conventional approach of plant breeding and genetics less desirable technique for abiotic stress tolerance in varieties' development. Other advanced techniques like genetic engineering for single gene transfer are considered more powerful to deal with this problem [38]. Transgenic plants are those plants, which have desired gene of interest directly integrated into the plant genome and developed from only a single plant cell. Transgenic plants with improved traits, including resistance to pests, pesticides, diseases or adverse environmental conditions, improved nutritional value, and enhanced product shelf life, have been developed through different genetic engineering techniques. Despite a number of social, political, and legal concerns, many countries are now allowing transgenic crop production in conjunction with their conventional crop production [103]. Transgenic approaches are being successfully pursued by researchers in some crops not only to improve the quality but also to increase the tolerance to abiotic stress, but tolerance trait is a quantitative complex trait and involves a number of genes. Thus, improving crop salt tolerance by genetic engineering is not so easy. Genes that encode ion transport proteins, compatible organic solutes, antioxidants, heat-shock and late embryogenesis abundant proteins, and transcription factors for gene regulation have focused by the biologist for improving the salt tolerance trait in various trait through genetic engineering techniques [104].

| Gene | Type of product | Source | Target plant | Reference |
|------|----------------|--------|--------------|-----------|
| *coda* | Glycine betaine | *Arthrobacter globiformis* | Tomato | [106] |
| *coda* | Glycine betaine | *Arthrobacter globiformis* | *Brassica juncea* | [107] |
| *Cox* | Glycine betaine | *Arthrobacter pascens* | Rice | [108] |
| *OsTPS1* | Trehalose-6-phosphate synthase | Rice | Rice | [109] |
| *TPS1* | Trehalose-6-phosphate synthase | Yeast | Tomato | [110] |
| *OstA, ostB* | Trehalose | *Escherichia coli* | Rice | [111] |
| *AtTPS1* | Trehalose | *Arabidopsis* | Tobacco | [112] |
| *mtlD* | Mannitol | Tobacco | Tobacco | [113] |
| *mtlD* | Mannitol | Wheat | *Escherichia coli* | [114] |
| *M6PR* | Mannitol | Celery | *Arabidopsis* | [115] |
| *S6PDH* | Sorbitol | Apple | *Japanese Persimmon* | [116] |
| *P5CS* | Proline | Rice | Mouth-bean | [117] |
| *P5CS* | Proline | *Vigna acontifolia* | *Nicotiana tabacum* | [118] |
| *SOD2* | Na+/H+ antiporter | *Schizosaccharomyces pombe* | *Arabidopsis* | [119] |
| *nhaA* | Na+/H+ antiporter | *E. coli* | *Arabidopsis* | [120] |
| *AVP1* | Vacuolar H+-pyrophosphates | *Arabidopsis* | Cotton | [121] |
| *AtNHX1* | Vacuolar Na+/H+ antiporter | *Arabidopsis* | Tomato | [122] |
| *AgNHX1* | Vacuolar Na+/H+ antiporter | *Atriplex gmelini* | Rice | [123] |
| *BnNHX1* | Vacuolar Na+/H+ antiporter | Brassica | Tobacco | [124] |
| *GhNHX1* | Vacuolar Na+/H+ antiporter | Cotton | Tobacco | [125] |
| *GlyII* | GlyoxylaseII | Rice | Tobacco | [126] |
| *OsNAC5* | NAC1 Transcription factor | Rice | Rice, *Arabidopsis* | [36] |
| *GmbZIP1* | bZIP Transcription factor | Soybean | *Arabidopsis*, tobacco | [127] |
| *TaMYB2A* | MYB2A transcription factor | Wheat | *Arabidopsis* | [128] |
| *BrERF4* | Ethylene responsive element 4 | Brassica | *Arabidopsis* | [129] |
| *MCM6* | DNA helicase | Pea | Tobacco | [130] |
| *T30hsp70* | Heat-shock protein | *Trichoderma harzianum* | *Arabidopsis* | [130] |
| *HVA1* | LEA protein | *Hordeum vulgare L* | Rice | [131] |
| *GhMPK2* | MAP kinase | Cotton | Tobacco | [132] |

**Table 3.** List of various genes responsible for salinity tolerance in plants with their role, source, and target plants (transgenic plants).

Plants try to survive with salinity by bringing various metabolic changes, such as a production of osmolytes, antioxidative enzymes, and up-regulating various genes involved in stress response like ion transporters, ion channels, transcriptional factors, and various signaling pathway components. The scientist studied various pathway responses that altered due to the salinity as mentioned above to generate the transgenic plants by transferring the salt-responsive genes into the salt susceptible plants from different genetic background (relatively salt-tolerant plants) or altering the expression of existing genes [105]. There are a number of gene(s) known which are responsible for salinity tolerance when transferred in plants through genetic engineering (Table 3).

Discovery of salt-tolerant genes is essential to induce salt tolerance in crop plants to enable them to grow on saline soils. Successful examples of identification and expression of salt tolerance genes include: over expression of AtNHX1 in *Arabidopsis* [133], tomato [134], *Brassica napus* [122], and cotton [135]. Likewise, overexpression of SOS1 gene in *Arabidopsis* also induces salt tolerance [136]. YCF1 is a yeast protein, which belongs to the ATP-binding cassette transporter family. Expression of this protein in *Arabidopsis* enhanced the salt tolerance in the transgenic plants to a significant level [137]. Since last several years' identification and transformation of salt tolerance genes in crop plants have been done [30]. When *AtNHX1* (vacuolar $Na^+/H^+$ antiporter from *A. thaliana*) was over expressed in tomato, Brassica [122, 134], and *Arabidopsis* [133], the transformed plants showed enhanced salt tolerance and were able to grow at 200 mM NaCl concentration. On the basis of growth responses of these transgenic plants, it has been concluded that they can grow on saline soils very well. Over expression of another $Na^+/H^+$ antiporter from *Atriplexgmelini* (*AgNHX1*) in rice enabled the transformed plants to grow at 300 mM concentration of NaCl for 3 days. Similar findings were observed when the same gene was transformed in wheat [138] and maize [139].

Overexpression of $Na^+/H^+$ antiporter from rice in the same species showed enhanced yield under salt stress [140]. Overexpression of HKT1-1 transporter in root cells surrounding the xylem of *Arabidopsis thaliana*, resulted in more removal of $Na^+$ ions from the xylem and into specialized compartments in the root tissues preventing the premature injury of shoots and leaves that could occur due to $Na^+$ accumulation [141]. The reason behind low number of successful transformations of salt-tolerant genes in crops is that these efforts have mostly been restricted to model plants like *Arabidopsis*, rice, and tobacco. Furthermore, there are some problems in applying this technology to other crop plants, such as monocots, due to the difficulty of obtaining series of independent T2 lines because the process is labor intensive and expensive [142].

## 10. Conclusion

Agriculture is immensely affected by salinity worldwide and is predicted to be a larger problem in near future. The damaging effects of high salinity can be seen in plants at organismic level, leading to immature death or decreased productivity. Some plant species are more tolerant to these detrimental effects than others. Salt stress leads to high yield losses worldwide.

Therefore, the changes aimed at overcoming these issues need to be fully implemented as soon as possible. Information related to the biochemical indicators at the cellular level may act as selection criteria for salt tolerance in different crops. There are many transgenic plants with high stress tolerance towards abiotic stress, yet stress tolerance has complex mechanism that includes multiple physiological and biochemical changes and multiple genes. Transgenic plants, which are commercially valuable, should have relatively high productivity and other traits important for their yield. Genetic modification, moreover, should be combined with marker-assisted breeding along with stress-related genes and QTLs. These strategies must be integrated, and such approaches should be combined to effectively increase plant stress tolerance

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