

Impact of Nanomaterials on the Regulation of Gene Expression and Metabolomics of Plants under Salt Stress

Zainul Abideen ¹, Maria Hanif ², Neelma Munir ^{2,*} and Brent L. Nielsen ^{3,*}

¹ Dr. Muhammad Ajmal Khan Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi 75270, Pakistan; zuabideen@uok.edu.pk

² Department of Biotechnology, Lahore College for Women University, Lahore 54000, Pakistan; mariahanif81@yahoo.com

³ Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84602, USA

* Correspondence: neelma.munir@yahoo.com (N.M.); brentnielsen@byu.edu (B.L.N.)

Abstract: Plant salinity resistance results from a combination of responses at the physiological, molecular, cellular, and metabolic levels. This article focuses on plant stress tolerance mechanisms for controlling ion homeostasis, stress signaling, hormone metabolism, anti-oxidative enzymes, and osmotic balance after nanoparticle applications. Nanoparticles are used as an emerging tool to stimulate specific biochemical reactions related to plant ecophysiological output because of their small size, increased surface area and absorption rate, efficient catalysis of reactions, and adequate reactive sites. Regulated ecophysiological control in saline environments could play a crucial role in plant growth promotion and survival of plants under suboptimal conditions. Plant biologists are seeking to develop a broad profile of genes and proteins that contribute to plant salt resistance. These plant metabolic profiles can be developed due to advancements in genomic, proteomic, metabolomic, and transcriptomic techniques. In order to quantify plant stress responses, transmembrane ion transport, sensors and receptors in signaling transduction, and metabolites involved in the energy supply require thorough study. In addition, more research is needed on the plant salinity stress response based on molecular interactions in response to nanoparticle treatment. The application of nanoparticles as an aspect of genetic engineering for the generation of salt-tolerant plants is a promising area of research. This review article addresses the use of nanoparticles in plant breeding and genetic engineering techniques to develop salt-tolerant crops.

Keywords: salinity; ecophysiology; environment; salt tolerance; photosynthesis



Citation: Abideen, Z.; Hanif, M.; Munir, N.; Nielsen, B.L. Impact of Nanomaterials on the Regulation of Gene Expression and Metabolomics of Plants under Salt Stress. *Plants* **2022**, *11*, 691. <https://doi.org/10.3390/plants11050691>

Academic Editors: Milan S. Stankovic, Paula Baptista and Petronia Carillo

Received: 27 January 2022

Accepted: 28 February 2022

Published: 3 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Soil salinization of land poses a serious threat and harms the environment, agriculture, and the economy. Salinity stress in plants may cause changes at the molecular as well as the physiological level [1]. Some plants contain salt tolerance genes while many have a salt-sensitive genetic makeup. Various complex mechanisms may alter the genetic responses in plants under abiotic conditions. Modifications in the expression of salt-responsive genes make the plants more resistant to salinity stress. Ecophysiological traits of plants and their importance for biomass production in response to variable climate change are critical for sustainable agricultural productivity [2–4]. Plants can change their ecophysiological mechanism in five known constraints including growth, water dynamics, mineral nutrition, photosynthesis rate, and oxidative stability [5,6].

The adaptation of a plant to a stressful environment is a complex and sensitive phenomenon [7,8]. This acclimation is governed by multiple genes and regulatory pathways [9]. Once the plant detects a stress, it first senses and then transduces a stress signal. Plants utilize various components for signal transduction including transcription factors, ion transporters, kinases, calcium, and hormones [10]. During abiotic stress, many physical modifications occur such as alteration in protein and other metabolites along with changes

in the cellular matrix and segregation of nucleic acid strands [11]. All these alterations may result in altered regulation of abiotic stress-responsive genes. It was observed by Tang [12] that superoxide dismutase is responsible for oxidative stress tolerance. Enhanced salt resistance in plants is due in part to the overexpression of chloroplast protein-increasing stress tolerance (CEST) [13]. The assimilation of methylglyoxal in a saline stressed potato plant was inhibited by glyoxalase activity [14]. Hasanuzzaman et al. [15] reported that selenium protects plants from damaging free radicals, improves the antioxidant defense system, and methylglyoxal detoxification. It was observed that the use of selenium nanoparticles with bitter melon induced alterations in the methylation of cytosine in DNA resulting in epigenetic modifications. The up-regulation of the *WRKY1* transcription factor was induced by a high dose of selenium nanoparticles. The transcription of phenylalanine ammonia-lyase (*PAL*) and 4-CoA-ligase (*4CL*) genes have also been affected by selenium nanoparticles [10].

The application of nanoparticles to plants helps to mitigate salinity stress. Nanoparticles can be used to alter plant genetic makeup to become resistant to salt stress. Nanoparticles are identified as particles that have a size of less than 100 nm in diameter [16]. They are found naturally in various resources such as minerals or as a product of bacteria and clays. Nanoparticles have been used historically for coloring metals and other purposes, with new applications over the past several years [17]. Nanoparticles that are engineered have some significant specific properties. These nanoparticles have different sizes and shapes and their composition also varies, and they differ widely from naturally occurring nanoparticles [18]. Metal and metal oxide nanoparticles reveal various physiochemical properties such as high density and possess microscopic edges on their surface. The sizes of nanoparticles vary due to differences in composition, such as Cu^{+2}O , $\text{Zn}^{+2}\text{O}^{-2}$, $\text{Sn}^{+4}\text{O}^{-2}$, $\text{Al}^{+3}_2\text{O}^{-2}$, $\text{Mg}^{+2}\text{O}^{-2}$, $\text{Ti}^{+4}\text{O}^{-2}$, and $\text{Ce}^{+4}\text{O}^{-2}$. Due to the changes in nanoparticles size, many properties including magnetic, electronic, and chemical properties are altered. Magnetic nanoparticles have achieved significant importance due to their variations in size and shape [19]. Surface, optical, thermal, and electrical properties can also be incorporated into these nanoparticles. The process of metal/metal oxide nanoparticle synthesis includes the reduction as well as oxidation of respective metal salts [20]. There are many different factors that contribute to nanoparticle reactivity with desired biomaterials. These factors are the size, dimension, and stability of the nanoparticles [21]. In the past few decades, synthesized nanoparticles have been used for various industrial and household purposes. There is continuing effort to synthesize new nanomaterials to enhance quality products. However, the environment can be contaminated due to the excess use of nanoparticles due to improper disposal of industrial wastes and other by-products [22].

Nanoparticles can be adapted for environmental conditions and their aggregation and oxidation state can be engineered [23]. The stability and behavior of nanoparticles can be affected by chemicals in the environment and by physical parameters. The properties of nanoparticles depend on their composition. The composition of nanoparticles also affects their rate of reaction, penetration ability, and translocation inside the plant. Hence, the same nanoparticles may show different responses in plants under different conditions. For instance, it was observed by Barrios et al. [24] that plant responses were influenced by citric acid-coated nanoparticles compared to bare nanoparticles. Plants constantly interact with the surrounding medium, such as water, air, and soil. The engineered nanoparticles can cause different effects caused by quantum dots, carbon-based and metal-based effects on plant growth variations, physiological and biochemical traits, food production, and quality of food. Thorough interaction studies between engineered nanoparticles and plants are needed to analyze the toxicity levels and the remediation scheme to build a sustainable environment for agriculture [25]. Plants play a significant part in the ecosystem and in the food chain. However, the effects of nanoparticles on plants are not well known. The study of nanoparticles is difficult due to a lack of detection methods in plants [26]. The most suitable technique for the identification of nanoparticles in plants is inductively coupled plasma mass spectroscopy (ICP-MS). Due to the size, shape, composition, and stability

of nanoparticles, the plant may show positive or negative impacts due to nanoparticle application. Several reported studies showed that some nanoparticles have a negative impact on plants such as declines in plant growth, production rate, and pigments [27]. Conversely, some nanoparticles may be beneficial for plants. In order to maintain their stability in agricultural crop production, synthetic nanoparticles are mostly used. These nanoparticles are used as biofertilizers, growth stimulators, soil-improving agents, and are also used as sensors [28].

2. Engineered Nanoparticles and their Effect on Plant Salt Tolerance Genes: Enzymatic Expression

Engineered nanoparticles can interact chemically and mechanically with plants. These interactions are based on their properties such as size, surface area, and catalytic interactions. Few studies have been reported regarding the effect of nanoparticles at the molecular level [29–31]. Various plant species are highly affected by ZnO nanoparticles. Nanoparticles penetrate the plant leaf and accumulate in the edible parts while some assimilate into the soil in the surrounding area of the plant. Some metal and metal oxide nanoparticles are toxic to the environment, such as Ag^+ , Fe^{+3} , Zn^{+2} , Al^{+3} , and Ti^{+4} [32]. It was observed that when *Brassica juncea* was treated with silver nanoparticles it resulted in increased levels of antioxidant enzymes, for instance, guaiacol peroxidase, catalase, and ascorbate peroxidase, which resulted in decreased levels of reactive oxygen species (ROS) activity [33]. The activity of enzymes such as super oxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase increased after the treatment of *Brassica juncea* with gold nanoparticles [34]. It was found that H_2O_2 and proline content increases in gold nanoparticle-treated plants. The activity of ascorbate peroxidase, glutathione reductase, and guaiacol peroxidase is stimulated in the presence of up to 400 ppm of gold nanoparticles, while on the other hand, the activity of guaiacol peroxidase increases with 200 ppm gold nanoparticles. Plant molecular responses to silver nanoparticle treatment were analyzed in *Arabidopsis* by reverse transcription-polymerase chain reaction [35]. A whole-genome cDNA expression microarray was also used for the transcriptional response analysis of *Arabidopsis* plants subjected to silver nanoparticles. This resulted in the identification of 286 upregulated genes, including those involved with metal and oxidative stress responses such as the vacuolar proton exchanger, SOD, cytochrome P450-dependent oxidase, and peroxidase. It also identified about 81 downregulated genes along with genes that help in the plant defense system. These included auxin-regulated genes, ethylene signaling pathway, and SAR against pathogens.

A proteomic analysis of rice treated with silver nanoparticles was carried out. It was found that silver nanoparticle-responsive proteins were associated with various metabolic functions such as transcription and protein degradation, the oxidative stress response pathway, and the calcium signaling pathway [36]. Treatment with zinc oxide nanoparticles in *Arabidopsis thaliana* identified 660 up- and 826 down-regulated genes. Seedling growth and seed germination of tomato was enhanced by the up-regulation of stress-related gene expression employing multi-walled carbon nanotube-based treatment [37]. The effect of engineered nanoparticles on plant traits is shown in Figure 1.

Iron (Fe) is considered to be essential for plant growth and development as it plays a significant role in enzymatic reactions, helps in photosynthesis, and aids to improve the performance of photosystems. In plants, Fe is present in the insoluble form, i.e., Fe^{3+} . The increase in pH and aerobic conditions leads to a decreased concentration of Fe in the soil. The use of iron nanoparticles helps to improve plant resistance to different environmental abiotic stresses. The application of iron nanoparticles reacts at the molecular level of plants, which helps to enhance the nutrient uptake ability [38]. Toxicity in plants may be caused by an excess concentration of iron nanoparticles. A higher amount of free Fe ions such as Fe^{2+} and Fe^{3+} leads to the production of ROS in plants. It was reported by Rodríguez et al. [39] that in some plants, down-regulation of detoxifying proteins such as CAT2 (CATALASE 2; AT4G35090) protein and AP2 (PEROXIDASE 2; AT5G06720) protein has been observed.

A deficiency of Fe in the roots of *M. truncatula*, *P. dulcis*, and *P. persica* was correlated with superoxide dismutase expression, i.e., ATMSD1 (ARABIDOPSIS-SIS-MANGANESE SUPEROXIDE DISMUTASE 1; AT3G10920) [39]. Fe deficiency may cause the production of non-enzymatic ROS. Under Fe deficiency in *A. thaliana*, two enzymes have been reported to be expressed: GST1 (ARABIDOPSIS GLUTATHIONE S-TRANSFERASE 1; AT1G02930) and MDAR1 (MONODEHYDROASCORBATE REDUCTASE 1; AT3G52880) [40]. The ROS-eliminating enzyme aids in the stimulation of the ascorbate-glutathione cycle from GPX3 (GLUTATHIONE PEROXIDASE 3; AT2G43350) [40]. Due to the magnetic properties of superparamagnetic iron oxides, Fe₂O₃ (maghemite) and Fe₃O₄ (magnetite) nanoparticles are widely used in various applications including the mitigation of salinity effects of plants. High Fe₃O₄ nanoparticle concentration has a high impact on seed germination and root elongation of cucumber [41]. In cucurbits, the Fe₃O₄ nanoparticle aggregation occurred in the stem and roots [42]. The toxicity of superparamagnetic iron oxide nanoparticles has been tested in *Lemna gibba* [43]. It has been observed that plant chlorophyll content decreased while the photosynthetic activity and growth were also highly affected. The size and stability of nanoparticles are responsible for their toxicity level. The effect of Fe₃O₄ nanoparticles has been investigated in *Cucumis sativus*, and it was observed that seed germination and root elongation were highly affected [41]. It has been shown that Fe₃O₄ nanoparticles are translocated towards the foliage, stem, and below-ground root. Aggregation of Fe₃O₄ nanoparticles in plants may decrease the root hydraulic movement and water transport. The growth parameters of *S. lycopersicum* were studied by the application of Fe₂O₃ nanoparticles. It has been observed that these nanoparticles were clogged in root hairs, root tips, and the nodal portion of plants. Increases in Fe₂O₃ nanoparticle concentration improved iron content in plants [44]. In *Arachis hypogaea*, root length and plant height increased due to the use of Fe₂O₃ nanoparticles in saline conditions [45].

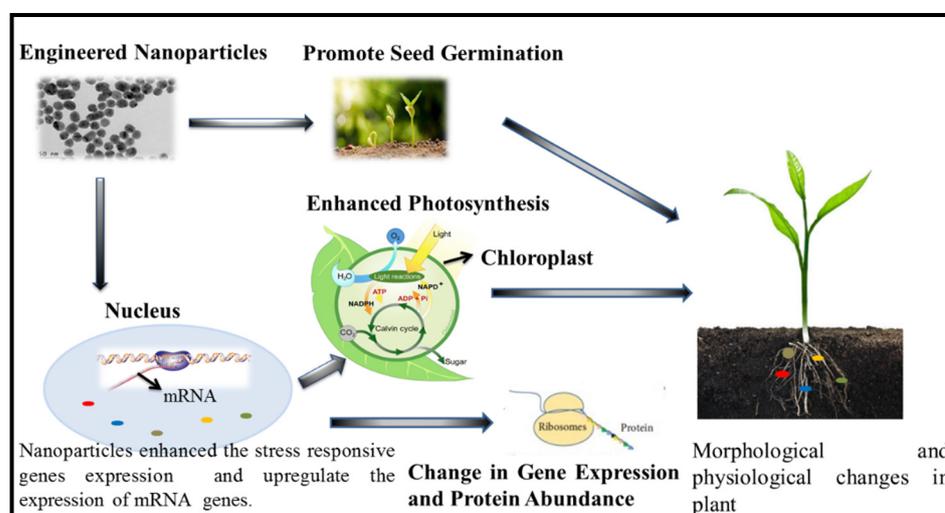


Figure 1. Effects of engineered nanoparticles on plant ecophysiological mechanisms in response to salinity.

While a number of genes with the potential for the engineering of salt tolerance have been identified and tested, additional genes and regulatory pathways need to be identified. Work in many labs is ongoing to develop genomic, transcriptomic, proteomic, and metabolomic resources.

3. Plant Metabolomics and the Linkage of Molecular Functions to Nanomaterial Application

The by-products of cellular regulatory mechanisms are metabolites. These metabolites are secreted in response to the external stimuli faced by the organism. More than 200,000 metabolites are secreted by plants and these metabolites are divided into two

classes; these are primary and secondary metabolites for plant growth and development [46]. Primary metabolites are essential and include carbohydrates, fatty acids, vitamins, amino acids, and organic acids [47]. Polyketides, alkaloids, terpenoids, glucosinolates, and phenylpropanoids are secondary metabolites synthesized from primary metabolites and are required by plants for adaptation and defense responses [48]. Throughout the plant kingdom, primary metabolites are common in all plants and conserved in their structure, while on the other hand, plant secondary compounds may vary in their chemical composition and are species-specific. Figure 2 shows the metabolomics analysis of plants exposed to engineered nanomaterials. In xenobiotic plants, the modifications in plant physiology induced by engineered nanoparticles are monitored by molecular events. These molecular events also reflect the metabolites that participate in biological pathways, for instance, the citric acid cycle, glycolysis, gluconeogenesis, and amino acid and secondary metabolite biosynthesis, nitrogen, and fatty acid metabolism. In order to defend against or adapt to various abiotic stresses, plant roots excrete metabolites as signaling molecules. Plants also alter soil chemistry and biochemical pathways to enhance nutrient bioavailability [49]. A list of halophytic species and their secondary metabolites is shown in Table 1.

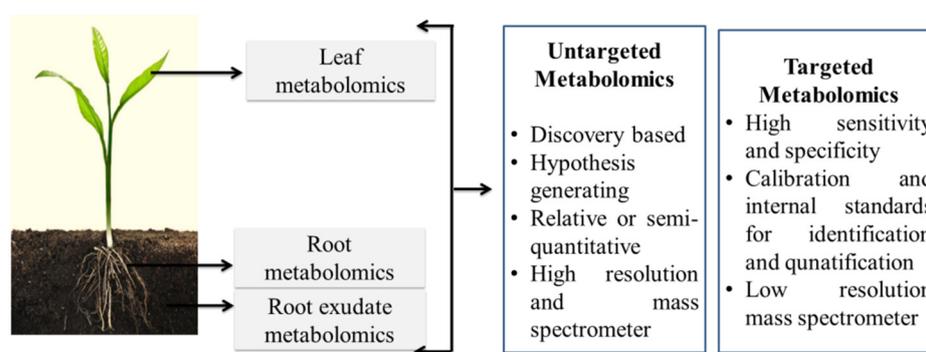


Figure 2. Metabolomics analysis in plants exposed to engineered nanomaterials.

Table 1. List of plant species and their secondary metabolites [50].

Fodder Crops	Secondary Metabolites
<i>Atriplex nummularia</i>	Saponin, Alkaloids, Tannins, Nitrate
<i>Atriplex leucoclada</i>	Saponin, Alkaloids, Tannins
<i>Atriplex halimus</i>	Saponin, Flavonoids, Alkaloids, Tannins, Nitrate
<i>Diplache fusca</i>	Flavonoids, Alkaloids
<i>Halocnemum strobilecum</i>	Saponin, Flavonoids, Alkaloids, Tannins, Nitrate
<i>Haloxyton salicornicum</i>	Saponin, Flavonoids, Alkaloids, Tannins
<i>Kochia eriophora</i>	Alkaloids, Tannins
<i>Juncus acutus</i>	Flavonoids, Alkaloids, Tannins, Nitrate
<i>Juncus arabicus</i>	Alkaloids, Tannins
<i>Juncus subulatus</i>	Alkaloids, Tannins, Flavonoids
<i>Limonium pruinosum</i>	Saponin, Alkaloids, Tannins
<i>Nitraria retusa</i>	Saponin, Tannins
<i>Salsola glauca</i>	Saponin, Flavonoids, Alkaloids
<i>Suaeda fruticosa</i>	Alkaloids, Tannins, Nitrate
<i>Tamarix aphylla</i>	Saponin, Tannins
<i>Salsola tetrandra</i>	Nitrate
<i>Tamarix mannifera</i>	Saponin, Tannins
<i>Zygophyllum album</i>	Saponin, Flavonoids, Alkaloids, Tannins, Nitrate
<i>Sesbania sesban</i>	Saponin, Alkaloids

4. Plant Genetic Responses to Salinity Stress

Under salt stress, genetic responses in plants occur by a complex mechanism. The synthesis of specific gene products (RNA or protein) is up-regulated while others are down-regulated. It was observed that these mechanisms may occur at different phases of the cen-

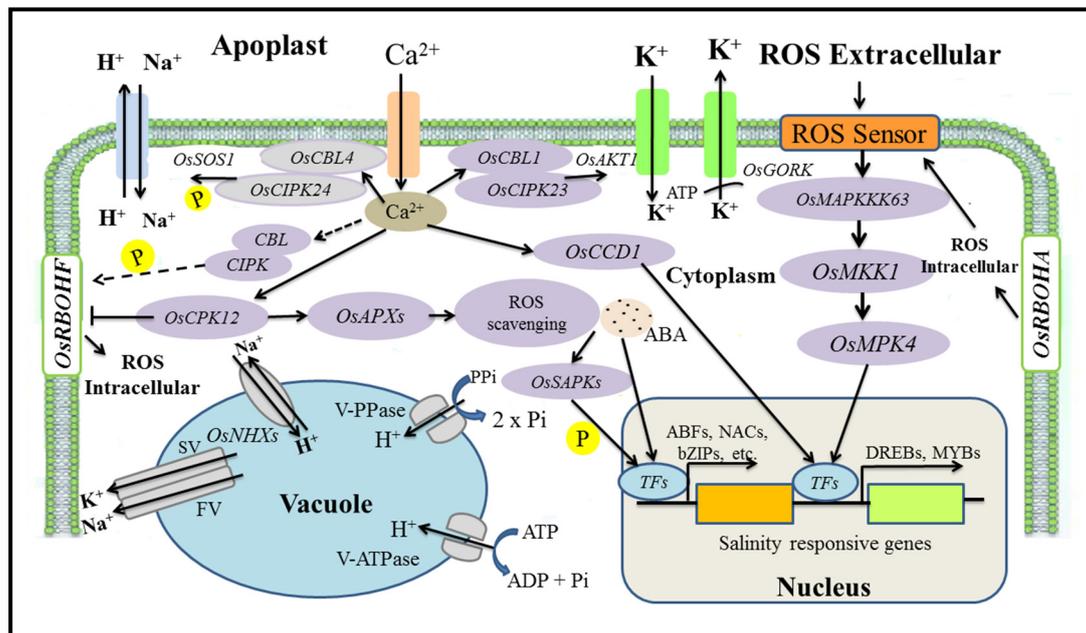
tral dogma process, such as transcription initiation, RNA processing, post-transcriptional processing, translation, and modification [51]. In order to identify the genes responsible for the stress response, transcriptional profiling is most often used, leading to the creation of extensive databases. To date, considerable information is available on the transcription factors that are included in the up- and down-regulation of plant genes and salt-responsive genes [52]. These genomic methodologies play a vital function in the encoding, cloning, and characterization of salt-responsive genes. These factors are considered important for the up-regulation and down-regulation of gene expression. *bZIP*, *MYB*, *WRKY*, *AP2*, *C2H2* zinc finger gene, *NAC*, and *DREB* family proteins are stress-responsive gene family members. The cis-acting binding of a transcription factor at a promoter region can alter the expression of individual genes [53].

Under salinity stress, up-regulation in *bZIP* gene expression was observed in a salt-sensitive variety of wheat while in the salt-tolerant cultivar, down-regulation of the gene occurred [54]. Rice contains transcriptional regulators that play a vital function under stress responses. These regulators are *DREB1/CBF*, *DREB2*, and *AREB/ABF* [55]. Under salt stress, up-regulation of transcription factors (*OsNAC5* and *ZFP179*) occurs. The up-regulation of these factors may affect the synthesis of proline, LEA proteins, and sugar, which in turn plays a significant role in abiotic stress tolerance [56]. Zhang et al. [57] determined that one member of one gene family, i.e., the *MYB* gene, responds to abiotic stress. Sixty full-length cDNA sequences were isolated that encode wheat *MYB* proteins. The phylogenetic tree that includes wheat, rice, and *Arabidopsis* *MYB* proteins was used to analyze their function and evolutionary relationship. Up-regulation of *AtWRKY8* gene expression occurs in *Arabidopsis* plants under salinity stress [58]. In diverse species of plants, many genes and transcription factors are up-regulated under salinity stress as presented in Table 2 [59], which shows associated genes, molecular chaperones, and dehydration-related transcription factors. For ion homeostasis, the *SOS* gene family plays a vital function in salt tolerance [60]. It was observed by Schmidt et al. [61] that more than 10 genes involved in the osmotic regulation process are up-regulated in *Spartia alterniflora* under salinity stress.

The salt-responsive transcription factor *ERF1* (*SERF1*) gene was characterized by Schmidt et al. [61] in *Oryza sativa*. It was observed that salt treatment and H_2O_2 may induce the expression of this gene in roots. Plants lacking the transcription factor *SERF1* are less resistant to salinity stress than wild-type plants. However, the overexpression of *SERF1* may improve salt resistance in plants. Kinases play a vital role in the adaptation of plants to salinity stress and they also regulate the activity of transcription factors. In rice, *OsRMC* acts as a negative regulator during salinity stress and it also encodes a receptor-like kinase [62]. Negative gene expression was also shown by two transcription factors, *OsEREBP1* and *OsEREB*, which belong to the *AP2/ERF* family. As with the *OsRMC* promoter, the above-mentioned transcription factors bind to the same GCC-like DNA motif. One of the major transcription factors, the basic region/leucine zipper (*bZIP*), contains a specific region that binds to the DNA and a leucine zipper dimerization motif. One of the major *ABRE*-binding transcription factors, *OSBZ8*, was also recognized in rice and is identified as the most expressed gene in salt-tolerant cultivars [63]. To respond to environmental stresses such as abiotic stress, the alternative splicing of RNA/mRNA plays a significant role by switching on/off transcriptional activities. The spliceosomal proteins play a significant function in plant development, response to harsh environmental factors, the plant immune system, and tolerance efficiency [64]. Figure 3 summarizes the signaling pathway in rice under salt stress.

Table 2. Salt responsive genes in various plant species along with their respective gene functions.

Gene Name	Plants	Gene Functions	References
<i>SOS1, SOS2, AtNHX1</i>	<i>Brassica campestris Brassica juncea</i>	Na ⁺ /K ⁺ plasma membrane antiporter, calcium-binding protein, and protein kinase	[65]
<i>AtSKIP</i>	<i>Arabidopsis thaliana</i>	Transcription factor, splicing, and polyadenylation	[66]
<i>OsHsp17.0</i> <i>OsHsp23.7</i>	<i>Oryza sativa</i> L.	Transporting proteins and heat-shock proteins	[67]
<i>DcHsp17.7</i>	<i>Daucus carota</i>	Cell viability and membrane stability under heat stress	[68]
<i>JcDREB</i>	<i>Arabidopsis thaliana</i>	Transcription factor	[69]
<i>AtNHX1</i>	<i>Arabidopsis thaliana</i>	Calcium-binding protein, vacuolar Na ⁺ /K ⁺ antiporter	[70]
<i>OsRab7</i>	<i>Oryza sativa</i> L.	Vesicle trafficking gene enhanced growth and proline	[71]
<i>PeXTH</i>	<i>Populus euphratica</i>	Higher cell viability, water holding capacity, and membrane integrity	[72]
<i>CYP94</i>	<i>Oryza sativa</i>	Enhanced <i>CYP94C2b</i> expression	[73]
<i>SOS1</i>	<i>Nicotina tabacum</i>	Plasma membrane, Na ⁺ /K ⁺ and vacuolar Na ⁺ /K ⁺ antiporter	[74]
<i>mtlD</i>	<i>Escherichia coli</i>	Higher mannitol 1 phosphate dehydrogenase levels	[75]
<i>TaSTRG</i>	<i>Triticum aestivum</i>	Increase salinity and water deficit resistance	[76]
<i>AtSTO1</i>	<i>Arabidopsis thaliana</i>	Higher root, pith size, and photosynthesis	[77]

**Figure 3.** Signaling pathways in rice under salt stress.

MicroRNAs and small interfering RNA (siRNAs) could play a significant role in the abiotic stress responses of plants. However, post-transcriptional gene regulation has a significant impact on plant salinity resistance [78]. It has been observed that miRNAs are sensitive to biotic as well as abiotic stress. Plants face various environmental abiotic stresses such as drought, salinity, and cold. The overexpression of miR402 is induced by these environmental stresses. Figure 4 shows the post-transcriptional regulation of plant salt stress-responsive genes mediated by miRNA as studied in *Arabidopsis thaliana* and *Oryza sativa* to determine the significant role of miRNA in salt stress [79].

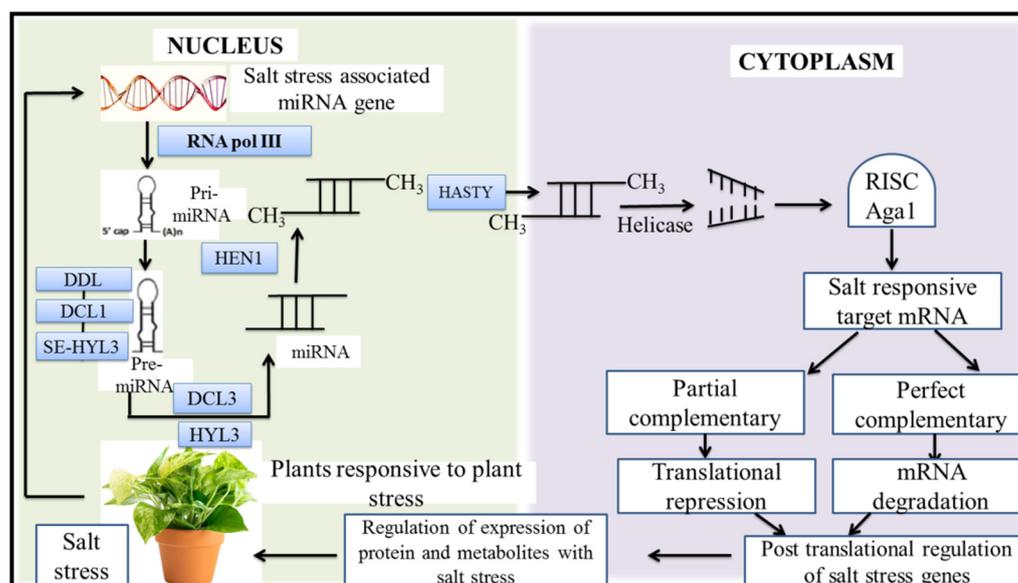


Figure 4. Pathway showing post-transcriptional regulation of salt stress-responsive plant genes mediated by miRNA.

5. Mechanisms for the Regulation of Salt Tolerance Genes

Plant transcription factors act to link salt-sensitive pathways to several tolerance responses. Certain transcription factor family genes are expressed specifically in response to external salinity stress. These transcription factor gene families include *bZIP*, *WRKY*, *APETALA2/Ethylene Response Factor (AP2/ERF)*, *MYB*, basic helix-loop-helix (*bHLH*), and *NAC*. These transcription factors play a vital function in regulating the expression level of genes that may enhance the plant salt tolerance level [80]. Salt stress-induced *bZIP* transcription factor *bZIP24* is a primary example involved in adaptation to salt resistance in plants. In *Arabidopsis*, this transcription factor induces the expression of many stress-responsive genes [81]. Salt tolerance is increased in *Arabidopsis* due to the expression of *bZIP24*. It was observed that *bZIP24* down-regulated *AtHKT1;1* directly or indirectly [82]. *bZIP24* was identified by comparison of transcript regulation patterns in *Arabidopsis* and in the halotolerant *Lobularia maritima*. This halophilic model species can be utilized for the identification of novel salt tolerance mechanisms [83].

5.1. Salinity Tolerance Mechanisms

AtHKT1;1 regulates the sodium level in leaves and salt tolerance in plants. In order to modify a Na^+ -resistant crop, the molecular mechanism regulating *AtHKT1;1* expression should be determined [84]. The plant salinity tolerance mechanism was analyzed by the study of the model plant *Arabidopsis* [85]. *Rice transcription factor SALT-RESPONSE ERF1 (SERF1)* functions as an enhancer of ROS-activated MAP kinase under salinity stress. The high salinity rate may induce the expression of *SERF1*. It was observed that rice plants deficient in *SERF1* exhibit a drop in salt stress tolerance genes. *serf1* mutants grown hydroponically for 3–4 weeks were observed to be salt-sensitive while *SERF1*-overexpression lines showed increased salt tolerance. The Na^+ over K^+ ratio in the foliage of the *serf1* mutant was higher than the wild-type [86].

5.2. Traditional Plant Breeding

Many approaches including conventional plant breeding have been used to enhance the salinity resistance of plants that are economically important. These traditional breeding programs have been successful in improving salt stress tolerance [87]. However, multiple salt-responsive genes are involved in tolerance to salinity in plants. These genes also function with other parts of stress signal transduction pathways. The results obtained by

traditional plant breeding may not identify genomic regions such as in perennial quantitative trait loci (QTL) that control salt resistance. By the improvement of high-throughput genotyping methods, molecular marker technology was developed to identify QTL, which is considered to be accountable for salt tolerance. In breeding programs, the selection efficiency is based on the identification of QTL regions [88]. The results of gene expression analysis and the correlation of QTLs may be easily detected at the seedling stage as compared to the developmental stages, while at the reproductive stages they are fully recognizable [89]. In recent studies, the antioxidant response in tomato plants under salinity stress was identified. The QTLs related to antioxidant content were also analyzed. The development of tomato cultivars having higher antioxidant levels may be accomplished using QTLs [90].

By using traditional breeding methods, multiple traits have been introduced into crop plants to manipulate salt tolerance. In addition, the overexpression of single genes can be used for improving plant salt tolerance. High salt tolerance has also been observed in genetically modified plants by the overexpression of genes that code for the synthesis and assimilation of osmoprotectants (proline and glycine betaine are used for osmotic adjustment). In addition, some enzymes such as glutathione S-transferase, peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase are also used to modify plant salt resistance and oxidative protection [91]. Plant genetic engineering holds great promise for producing salt-tolerant crops. Salinity tolerance is multi-genic in nature, but most genetically engineered plants possess a single transgene. Various genetic tools are developed that can be utilized in gene function analysis. In order to develop salt-tolerant cultivars, the use of nanoparticle carriers to facilitate genetic engineering will be helpful to understand the mechanisms and regulation of gene expression, candidate gene usage, and tissue-specific and inducible promoters.

6. Salt Responsive Genes Present in Halophytes

Limited literature is available on the molecular defense mechanism of halophytes against salinity stress. In various studies, the ecological, physiological, anatomical, and biochemical responses of halophytes towards salinity were studied [92,93]. For the investigation of salinity stress defense molecular mechanisms, *Arabidopsis thaliana* was used as a model plant. This species was also utilized for the analysis of salt stress-responsive genes to enhance salinity tolerance in genetically modified plants [94]. It is generally understood that halophytes are salt-tolerant and glycophytes are salt sensitive. However, there are some halophyte species that are sensitive to abiotic stresses and some glycophytes are tolerant to salt. Halophytes are considered to be suitable model plants for the analysis of salt tolerance mechanisms [95]. It was observed that almost all plants likely possess similar salt tolerance regulatory mechanisms [96].

Many genomic methods have been used for the isolation and identification of salinity-responsive genes from halophytes. The overexpression of these halophytic genes under the control of the constitutive, non-specific 35SCaMV promoter in glycophytic recipients may increase abiotic stress tolerance. Many of these genes code for Na^+/H^+ antiporters, vacuolar pyrophosphatase, potassium transporters, ion channels, antioxidants, and ROS scavengers. These genes also code for proteins that are included in signal transduction and various protective functions. Many other salt-responsive genes have been identified from halophytes such as *Salicornia brachiata* [97]. Many model plants including *Arabidopsis thaliana* and salt-tolerant *Thellungiella salsuginea* were used for the study of abiotic stress tolerance. *Thellungiella* exhibits high tolerance to salt and drought stress [98]. The genetic basis of the abiotic stress defense mechanism was obtained from the analysis of the genomic sequence of *Thellungiella salsuginea*. This species was identified as the gene resource for cation transporters, abscisic acid signaling genes, and many other genes that respond to abiotic stresses [99]. The results obtained from microarray analysis revealed that only a few genes were affected under salt stress in *Thellungiella salsuginea* compared to *Arabidopsis* [100]. In another study, it was observed that in *Thellungiella salsuginea*, about 154 genes were

identified as compared to *Arabidopsis* under various stresses [101]. A diagram illustrating salt stress tolerance mechanisms in halophytes is shown in Figure 5.

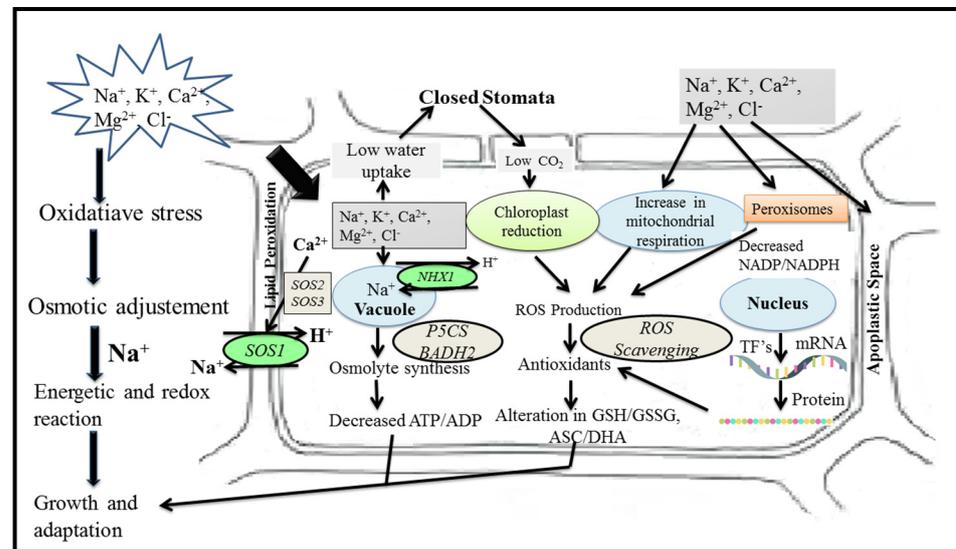


Figure 5. Salt stress tolerance mechanisms of halophytes in a saline environment.

Arabidopsis and *Lepidium crassifolium* have been studied as model plants to contrast a glycophyte with a halophyte, respectively, and to identify genes involved in oxidative and osmotic stress tolerance. Independent transgenic lines expressing random genes from *L. crassifolium* in *Arabidopsis thaliana* enhanced plant salinity tolerance [102]. Other studies were conducted with *Salicornia brachiata*, which grows in saline marshes under extreme abiotic stresses. This plant is considered to be an efficient source of stress response genes and promoters as it has the potential to grow under adverse environmental conditions [97]. Salinity-resistant transgenic plants including *Jatropha*, Cumin, and Castor were developed using salt-responsive genes isolated from *Salicornia brachiata* [103]. *Salicornia* species are considered functional foods as they contain metabolites and sulfur-rich seed storage proteins [104]. *Porteresia coarctata* is a wild halophyte that has the ability to grow in extreme saline soils. Around 152,367 unique transcript sequences were identified; 15,158 of these genes are related to salinity and submerged tolerance and the analysis of these genes will help unravel the key metabolic pathways involved in tolerance. These genes can also be utilized to introduce salinity and submerged tolerance traits in rice [105]. Table 3 shows the salt-responsive genes present in halophytes and recipient plants that express the genes.

Table 3. Salt-responsive genes present in halophytes and recipient plants.

Halophytes	Genes	Description	Recipient Plants	References
<i>Aeluropus litoralis</i>	<i>AtNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Nicotiana tabacum</i>	[106]
<i>Atriplex hortensis</i>	<i>AhBADH</i>	Glycine betaine synthesis	<i>Solanum lycopersicum</i>	[107]
<i>Avicennia marina</i>	<i>AmMDHAR</i>	ROS scavenging	<i>Nicotiana tabacum</i>	[108]
<i>Salicornia brachiata</i>	<i>SbASR1</i>	Ascorbate regeneration and ROS scavenging	<i>Nicotiana tabacum</i>	[108]
<i>Salicornia brachiata</i>	<i>SbpAPX</i>	Peroxisomal ascorbate peroxidase	<i>Nicotiana tabacum</i>	[109]
<i>Salicornia brachiata</i>	<i>SbpAPX</i>	Peroxisomal ascorbate peroxidase	<i>Arachis hypogea</i>	[110]
<i>Salsola soda</i>	<i>SsNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Alfalfa</i>	[111]
<i>Suaeda liaotungensis</i>	<i>SIBADH</i>	Glycine betaine synthesis	<i>Zea mays</i>	[112]
<i>Suaeda salsa</i>	<i>SsCAX1</i>	Vacuolar H ⁺ /Ca ²⁺ transporter	<i>Arabidopsis</i>	[113]

Table 3. Cont.

Halophytes	Genes	Description	Recipient Plants	References
<i>Suaeda salsa</i>	<i>SsGST</i>	Glutathione S-transferase	<i>Oryza sativa</i>	[114]
<i>Suaeda salsa</i>	<i>SsVP</i>	Vacuolar-H ⁺ -pyrophosphatase	<i>Arabidopsis</i>	[115]
<i>Thellungiella halophila</i>	<i>ThSOS1</i>	Salt overly sensitive gene	<i>Arabidopsis</i>	[116]
<i>Thellungiella salsuginea</i>	<i>TsTIP1</i>	Tonoplast AQP gene	<i>Arabidopsis</i>	[117]
<i>Tamarix androssowii</i>	<i>TaMnSOD</i>	Manganese superoxide dismutase	<i>Populus</i>	[118]
<i>Spartina alterniflora</i>	<i>SaVHAc1</i>	Vacuolar H ⁺ -ATPase subunit Cl	<i>Oryza sativa</i>	[119]
<i>Salicornia europaea</i>	<i>SeCMO</i>	Enhanced glycine betaine synthesis	<i>Nicotiana tabacum</i>	[120]
<i>Kalidium foliatum</i>	<i>V-ATPase</i>	Vacuolar-H ⁺ -pyrophosphatase	<i>Arabidopsis</i>	[121]
<i>Atriplex gmelini</i>	<i>AgNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Oryza sativa</i>	[122]

7. Promoters for Salt-Responsive Halophytic Genes

A strong promoter is needed for the genetic engineering of plant crops to attain the desired level of transgene expression. In *T. halophila*, stress-related genes are expressed constitutively as compared to *A. thaliana* where they are not [123]. This study revealed that in halophytes, a transcriptional regulatory network for stress-responsive genes is fully functional. Another study was carried out in halophytes that led to the identification of *cis*-regulatory elements of stress-responsive genes and stress-inducible motifs [124]. The promoter of the *AcBADH* gene from *Atriplex centralasiatica* contains two salt-responsive enhancer regions and one silencer region. The enhancer regions are located from −1115 to −890 and −462 to −230, while the silencer region is from −890 to −462. The *AcBADH* promoter is strongly induced by salinity stress [125].

Another strong and salt-inducible promoter is *SIBADH*; the promoter fragment (−300 bp) was identified in *Suaeda liaotungensis*. This promoter supported a 6.3-fold higher expression under salinity in contrast to non-stressed conditions [106]. To measure expression levels, GUS is widely used as a reporter gene in transformation in microorganisms and animals. It is found in various bacterial species such as *Shigella*, *Bacteroides* and *Clostridium*, *S. liaotungensis*, and *Salicornia europaea* that contain CMO genes [120]. GUS showed increased expression in transgenic *Arabidopsis*, while a halophyte *T. halophila* contains a *TsVP1* gene promoter having a 130 bp specific *cis*-acting element responsive to salt stress. The *SIPEAMT* gene of *S. liaotungensis* with an 897 bp promoter region also showed an 18.6-fold increase in the beta-glucuronidase (GUS) activity under 200 mM NaCl stress [126]. They determined that even a small portion of the promoter contains a *cis*-acting element that allows regulation of gene expression under salinity stress. A choline monooxygenase (CMO) gene was found in Chenopodiaceae and Amaranthaceae. In plants, usually, the activity of CMO is low and unstable but it can be a critical rate-limiting step in the biosynthesis of glycine betaine [127]. Promoters of these genes are inducible under salinity stress. Crassulacean acid metabolism (CAM) genes were studied in *M. crystallinum*, and the transcriptional activation of salt-responsive genes occurs due to the enhancer and silencer regions of the gene promoter [128]. CAM genes were found in various plant species. In model plant species such as *Arabidopsis* and rice, it was revealed that CAM proteins are encoded by gene families. These genes play a significant role in the regulation of growth, development, and abiotic stress resistance in plants [129].

A tissue-specific promoter *AISAP* was examined in *Aeluropus littoralis* [130]. It was found that the expression level of a *gusA* fusion with this promoter was the same in transgenic rice under the control of the *AISAP* gene as in *A. littoralis* [131]. *AISAP* and *OsSAP9* are two orthologs of the regulatory region of the promoter and provide the basis for variation in regulation specificity and stress induction in rice. The *TsVP1* gene from the halophyte *T. halophila* contains a 130 bp *cis*-acting element in the promoter region of vacuolar H⁺-pyrophosphatase. It helps to enhance GUS fusion expression under

salinity stress in transgenic *Arabidopsis* [132]. Under the conditions of biotic and abiotic stresses, the expression of the reporter gene can be controlled by the *CBL1* gene promoter obtained from *Ammopiptanthus mongolicus* [133]. The enhancer and repressor binding sites in the *cis*-regulatory region were also found in the *SbpAPX* gene. This gene was found in *S. brachiata* [134]. The salt stress-responsive *cis*-regulatory motifs were present in the *SbGSTU* promoter. In *S. brachiata*, these motifs regulate the expression of the *GSTU* gene [124]. Thus, halophytes can be utilized as a source of genes for engineering abiotic stress tolerance in crops. Table 4 shows the details of promoters used to stimulate salinity resistance traits in plants.

Table 4. Promoters used to improve salt tolerance traits in crop plants [58].

Transgene	Gene Isolated	Promoters	Transgenic Crop
Ion exclusion Na ⁺ /H ⁺ antiporter (<i>SOS1</i>)	<i>Arabidopsis</i>	Constitutive	<i>Nicotiana tabacum</i>
Na ⁺ /H ⁺ antiporter (<i>SOD2</i>)	<i>Salicornia brachiata</i>	Stress inducible	<i>Oryza sativa</i>
Tissue tolerance Na ⁺ /H ⁺ antiporter (<i>NHX</i>)	<i>Arabidopsis</i>	Constitutive	<i>Fagopyrum esculentum</i>
Tissue tolerance Trehalose-6-phosphate synthase (<i>TPS</i>)	Yeast	Constitutive	<i>Medicago sativa</i>
Tissue tolerance Trehalose-6-phosphate phosphatase (<i>TPP</i>)	Rice	Stress inducible	<i>Solanum lycopersicum</i>
Mannitol-1-phosphate dehydrogenase (<i>mt1D</i>)	<i>E.coli</i>	Shoot expression	<i>Oryza sativa</i>
Myoinositol O-methyltransferase	<i>M. crystallinum</i>	Constitutive	<i>Triticum aestivum</i>
Tissue tolerance Ascorbate (<i>APX</i>)	<i>Arabidopsis</i>	Constitutive	<i>Nicotiana tabacum</i>
Glutathione S-transferase (<i>GST</i>)	Tomato	Protein targeted to chloroplast/cytosol	<i>Oryza sativa</i>
Mitogen activated protein kinase (<i>MAPK</i>)	Chickpea	Constitutive	<i>Nicotiana tabacum</i>
Sucrose protein kinase	Rice	Inducible	<i>Oryza sativa</i>
Transcription factors <i>DREB</i>	<i>Pennisetum glaucum</i>	Constitutive & inducible	<i>Nicotiana tabacum</i>

8. Transgenic Approach for Engineered Plants Having Enhanced Salt Tolerance

It was reported by Rao et al. [53] that for salt tolerance in plants, the breeding strategy is not particularly recommended due to reproductive restrictions and there is a high probability for the transfer of undesirable traits. Genetic engineering is considered to be suitable for single gene transfer [58]. Plants have been developed from a single plant cell by the direct transfer of the gene of interest into the genome to create transgenic plants. The use of genetic engineering techniques involves traits such as resistance to pesticides, pests, better nutritional value, and improved product shelf life, which can contribute to improvements in sustainable agriculture [135]. Figure 6 shows the factors involved in enhanced salt tolerance in plants. The transgenic approach is also utilized to enhance the resistance to abiotic stress in plants [136]. Table 5 summarizes reports on gene transfer into target plants for enhanced salt tolerance.

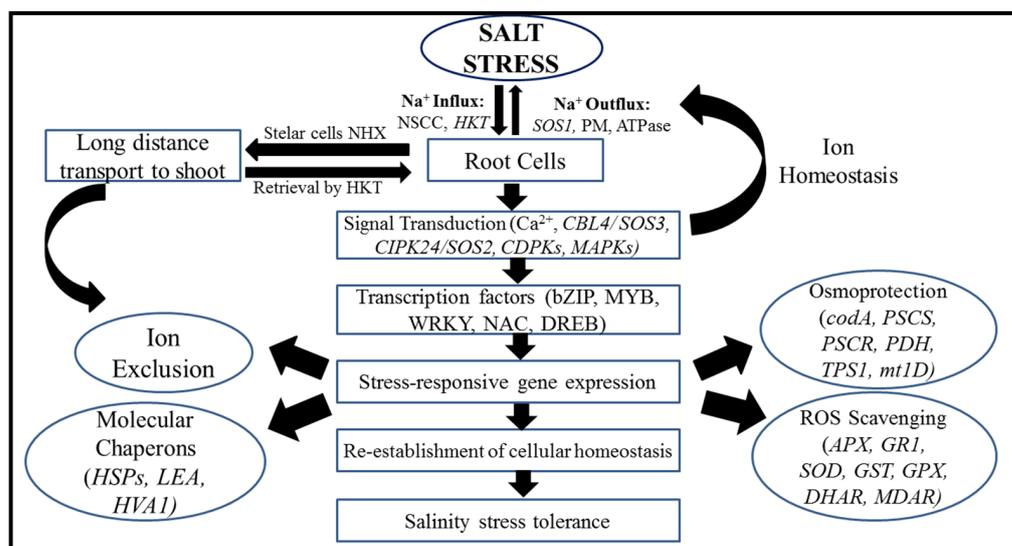


Figure 6. Factors involved in enhanced plant salt tolerance.

Table 5. Gene transfer into target plants for enhanced salt tolerance.

Desired Gene	Donor Plant	Target Plant	References
<i>codA</i>	<i>Arthrobacter globiformis</i>	<i>Solanum lycopersicum</i>	[137]
<i>Cox</i>	<i>Arthrobacter pascens</i>	<i>Oryza sativa</i>	[138]
<i>TPS1</i>	Yeast	<i>Solanum lycopersicum</i>	[139]
<i>AtTPS1</i>	<i>Arabidopsis</i>	<i>Nicotiana tabacum</i>	[140]
<i>mtID</i>	<i>Triticum aestivum</i>	<i>Escherichia coli</i>	[141]
<i>S6PDH</i>	<i>Malus domestica</i>	<i>Japanese Persimmon</i>	[142]
<i>P5CS</i>	<i>Vigna acontifolia</i>	<i>Nicotiana tabacum</i>	[143]
<i>nhaA</i>	<i>E.coli</i>	<i>Arabidopsis</i>	[141]
<i>AtNHX1</i>	<i>Arabidopsis</i>	<i>Solanum lycopersicum</i>	[144]
<i>BnNHX1</i>	Brassica	<i>Nicotiana tabacum</i>	[145]
<i>GlyII</i>	<i>Oryza sativa</i>	<i>Nicotiana tabacum</i>	[146]
<i>GmbZIP1</i>	Soybean	<i>Arabidopsis, Nicotiana tabacum</i>	[147]
<i>BrERF4</i>	Brassica	<i>Arabidopsis</i>	[148]
<i>T30hsp70</i>	<i>Trichoderma harzianum</i>	<i>Arabidopsis</i>	[149]
<i>GhMPK2</i>	Cotton	<i>Nicotiana tabacum</i>	[150]

9. Development of Salt Tolerant Glycophytes using Halophytic Salt Tolerance Genes

Plants are classified into two groups: salt-tolerant halophytes and salt-sensitive glycophytes depending on their growth ability in saline environments. Halophytes have the ability to grow in a saline environment including coastal marshes and inland deserts. Monocot halophytes have the potential to achieve optimum growth at less than 50 mM NaCl while in the case of dicot halophytes they can grow at approximately 100–200 mM salt [151]. Glycophytes are highly affected by saline habitats and do not grow well at 100–200 mM NaCl [92]. Various studies were conducted to identify salt tolerance genes in halophytes [96]. Over the last several years, genetic engineering has been commonly used to introduce salt tolerance in glycophytes by the transfer of salt tolerance responsive genes from halophytes [152]. For this purpose, different plants are used as model plants for the introduction of salinity tolerance genes. *Arabidopsis*, tobacco, and many other crop plants have been used to enhance ion homeostasis and salt tolerance [73].

The most commonly monitored phenomena related to salinity are the dispersion of sodium ions in vacuoles, sodium ion efflux, and the prevention of sodium ion influx by the antiporter [153]. Many antiporters have been characterized functionally after their isolation from glycophytes and halophytes. It was observed that some of the glycophytic transporters that are encoded by the *NHX*, *ATPase*, *SOS*, and *HKT* genes led to salt tolerance

in the range of 150–250 mM NaCl when expressed under the control of the constitutive CaMV35S promoter in transgenic plants [154]. For evolving salt tolerance in various crops such as tomato, maize, brassica, and wheat, constitutive expression of the glycophytic *NHX* gene obtained from *Arabidopsis thaliana* was used [155]. However, other genes such as *BnNHX1* (*Brassica napus*), *HbNHX1* (*Hordeum brevisubulatum*), and *GhNHX1* (*Gossypium hirsutum*) were used to develop salt tolerance in tobacco. It was reported that salt tolerance was conferred by the *NHX1* gene obtained from both halophyte and glycophytes, although they differ in the level of salt tolerance. A 75% amino acid sequence similarity was observed for the antiporter *AgNHX1* from *Atriplex 15melina* and *AtNHX1* from *Arabidopsis thaliana*. As compared to glycophytes, transgenic plants overexpressing *AgNHX1*, *SaNHX1*, or the *SsNHX1* gene showed up to 300–400 mM NaCl tolerance [156]. In genetically modified tobacco plants, upregulation of the *SbNHX1* gene showed salt tolerance up to 200 mM NaCl while in transgenic jatropha and castor plants, salt tolerance was observed up to only 100 mM NaCl [157]. However, in transgenic plants, the upregulation of other halophytic genes such as *SbpAPX*, *SbUSP*, and *SbGSTU* also showed salt tolerance up to 200–300 mM NaCl [158]. The *TIP1* gene obtained from the halophyte *T. salsuginea* revealed enhanced salt tolerance in transgenic *Arabidopsis* plants as compared to the glycophyte *Panax ginseng* [159]. Genes from rice such as *APX* and *GST* showed tolerance of up to 150–200 mM NaCl when compared to similar genes obtained from the halophyte *S. brachiata* in transgenic plants [160]. Different levels of abiotic stress tolerance in rice, tobacco, and wheat were observed by up-regulation of the stress-associated protein *AISAP* from *Allocasuarina littoralis* [131]. It was reported that many abiotic stresses induced *AISAP* transcripts but the rice gene *OsSAP9* is also influenced by cold and heat treatments. The negative regulators of *AtHKT1;1* expression are shown in Figure 7.

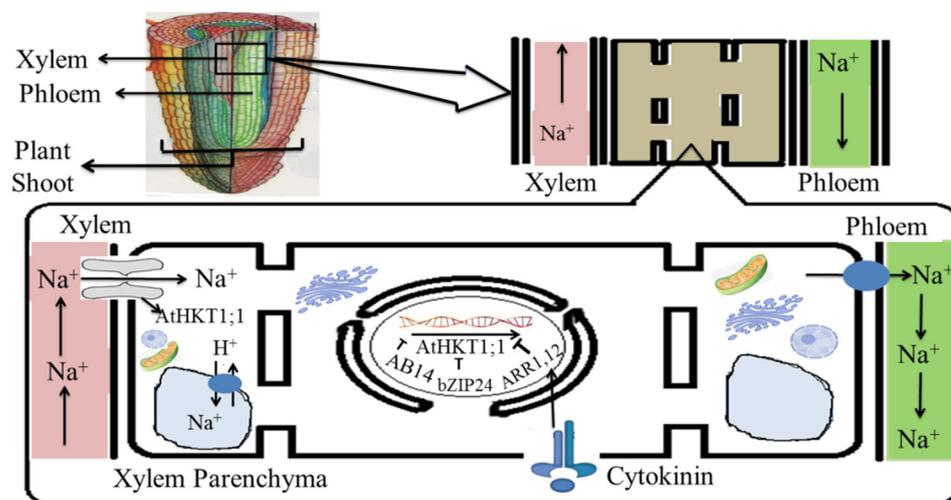


Figure 7. Model showing *AtHKT1;1* and the negative regulators of *AtHKT1;1* expression.

The antiporter *SOS1* gene and its over-expression were studied in *Thellungiella* and contrasted to expression in *Arabidopsis* [161]. In *Thellungiella*, *SOS2*, *NHX1*, and *HKT1*, which are involved in sodium exclusion and compartmentation, have been expressed at higher levels [123]. Halophytes can serve as model plants to discover different stress-responsive genes for enhancing the salt resistance of glycophytes to allow cultivation in saline and arid areas for sustainable agriculture.

10. MicroRNAs (miRNA), a New Target for Improving Plant Tolerance to Salt Stress

Microarray and high throughput deep sequencing methods are used to identify plant miRNAs induced under salinity, as presented in Table 6. It was observed that plant miRNAs are present in all of the main plant parts such as the leaf, root, stem, and flower (Table 5). It was reported by Fu et al. [162] that among all the crops, the highest numbers of miRNAs have been identified in *Zea mays* (1077 miRNAs). *Mesembryanthemum crystallinum*, *Medicago*

truncatula, *Vicia faba*, and *Ipomoea batatas* contain 882, 876, 693, and 650 miRNAs, respectively, under saline conditions [163]. The numbers of miRNAs in plants may vary and depend on plant species, tissue specificity, development stages, and intensity of salinity stress. The degree of salt stress may up- and down-regulate the expression level of miRNAs in plants. Jodder [164] observed that the expression of miR167 in oat panicles is negatively associated with an increase in the degree of salt stress. With a 0.25% increase in NaCl, miR156, miR157, and miR172 are up-regulated in cotton, and the expression decreases with a further increase in salt concentration. Nanoparticles have the ability to affect the expression level of plant miRNAs [165].

Table 6. Numbers of salt-responsive miRNAs identified under salt stress at varying concentrations of NaCl.

Plants	NaCl Concentration	miRNA Number	References
<i>Arabidopsis thaliana</i>	150 mM	118	[166]
<i>Glycine max</i>	125 mM	238	[167]
<i>Leymus chinensis</i>	100 mM	148	[168]
<i>Medicago truncatula</i>	20 mM	876	[169]
<i>Musa nana</i>	300 mM	181	[170]
<i>Oryza sativa</i>	200 mM	498	[171]
<i>Panicum virgatum</i>	0.5 %	273	[172]
<i>Suaeda maritima</i>	255 mM	147	[173]
<i>Zea mays</i>	250 mM	1077	[174]
<i>Vicia faba</i>	150 mM	693	[175]
<i>Thellungiella salsuginea</i>	200 mM	246	[176]
<i>Raphanus sativus</i>	200 mM	204	[177]

The expression levels of miRNAs highly depend on plant developmental stages, as it was reported that few miRNAs are expressed in early growth under saline conditions while others appear in the late stages. It was observed by Luan et al. [178] that zma-miR169 shows initial up-regulation and then down-regulation under salinity stress. In cotton, miRNAs and their targets such as miR156-SPL2, miR159-TCP3, miR162-DCL1, miR395-APS1, and miR396-GRF1 show a negative correlation of expression levels [165]. Some of the miRNAs are induced under salt stress such as miR156, miR319, and miR528 while miR164 and miR397 are repressed [179]. The degree of salinity stress may increase or decrease the expression level of some miRNAs in plants. For instance, in *Arabidopsis thaliana*, *Triticum aestivum*, and *Agrostis stolonifera*, the expression level of miR393 increases under salt stress. However, in contrast, the expression level of miR393 decreases in *Oryza sativa*, *Gossypium* sp., and *Spartina alterniflora* under similar conditions [180]. The expression level of some miRNAs such as miR167, miR390, miR402, and miR414 have been observed only in a few plant species under salinity stress.

Glycophytes cannot tolerate high salinity levels while halophytes can tolerate and survive at up to 1000 mM NaCl. Glycophyte plants may have the potential to adapt a salt tolerance mechanism by following various strategies of gene regulation used by halophytes. The role of halophyte miRNAs may follow various strategies of salinity resistance improvements in crops by incorporating them in genetic engineering and plant selection programs. For instance, Gharat [173] observed that the expression of *Suaeda maritima* sma-miR2 and sma-miR5 increases in seawater, suggesting that their metabolic regulatory roles are restricted to saline environments. About 246 miRNAs have been identified in *E. salsugineum*. A significant response to salt stress in *E. salsugineum* was observed by the expression of 26 conserved miRNAs and four novel miRNAs [181]. Seedlings of *M. crystallinum* were treated with 200mM NaCl and it was observed that 135 conserved miRNAs and the hairpin precursor of 12 novel mcr-miRNAs were expressed [182]. In another example, *Halostachys capsica*, a salt tolerant shrub, was treated with salt and it was observed that 31 conserved miRNAs and 12 novel miRNAs were up-regulated while 48 conserved miRNAs and 13 novel miRNAs were down-regulated by salinity stress in *H. caspica* [183].

11. Conclusions and Future Perspectives

Plants integrate cellular, physiological, and molecular responses for salt stress tolerance. Various studies have been carried out on the plant salt resistance mechanisms that control ion homeostasis, osmoregulation, ROS detoxification, hormone metabolism, and stress signaling, but there is still a lack of information from genomic, transcriptomic, and proteomic studies. Genetic engineering of salt tolerance in plants has great potential. However, the continuous release of nanoparticles into the surrounding soil may affect plant growth and development. Nanoparticles may alter seed germination as well as various stages of crop production. Various kinds of nanoparticles have been found in the environment such as ZnO, CuO, TiO₂, and Fe₃O₄. All of these nanoparticles showed some positive and negative results against seed germination, root and shoot growth, biomass production, and physiological as well as biochemical activities. These nanoparticles become adsorbed onto the plant surface and are transported to different plant tissues. However, the low concentration of nanoparticles does not show any negative effect on plants and appears to be beneficial for their growth and developmental process. Higher concentrations of nanoparticles cause toxicity by ROS which leads to the disruption of the cellular membrane. It has been considered that some nanoparticles could replace the use of toxic chemicals and fertilizers in the near future. Still, further research needs to be carried out to analyze the effect of nanoparticles on plants and the surrounding environment.

Progress has been made in developing salt-tolerant cultivars, but there are still many questions related to salt stress tolerance in plants that need to be addressed with the help of molecular marker development for gene mapping, EST library development, and integration of complete genome sequences for Arabidopsis, rice, and maize. High throughput sequencing is the most powerful technology for the identification of salt stress-responsive miRNAs. After the identification of miRNAs in plants, there is still an empty space that needs to be filled for the analysis of function carried out for salt tolerance improvement through miRNA manipulation in crops. Applications of nanoparticles will play a significant role in the modification of salt-tolerance genes in plants. Many advanced strategies have been used to date to modulate genes in plants under salinity stress, including nanoparticle transport across the plant cell and chloroplast membranes to target their genetic makeup. In addition, miRNAs can be introduced to develop salt resistance in crops. The over-expression and knocking down of miRNAs may promote the development of salinity resistance in transgenic plants.

The various modifications in small RNA sequencing technologies and analysis of miRNAs will be important for the development and growth of salt-tolerant cultivars. Many advances in genomics and metabolomics analyses of crop plants may improve the resolution of complex networks and unravel the mechanism(s) of abiotic stress tolerance. It will be essential to identify candidate gene(s) that have the ability to confer stress tolerance in plants without affecting growth and yield. It has been observed that conventional breeding methods did not significantly improve salt stress tolerance in plants and in addition, the crossing method reduced crop yield. In order to develop salt-tolerant plants, some points need to be addressed such as (i) how under the unstable natural environment genetically modified plants respond to soil salinity conditions, (ii) how soil salinity affects the transgenic plants at different growth stages including seed germination and reproductive stage, (iii) the effects of transgene expression on plant growth and development as well as nitrogen use efficiency (NUE) under saline conditions, (iv) yield of transgenic plants, and (v) disease resistance. Additional research still needs to be performed to understand stress perception, signaling, transcription factors, and genes associated with the salinity stress response.

Author Contributions: Conceptualization Z.A. and M.H.; formal analysis, Z.A. and M.H.; investigation, Z.A. and M.H.; writing—original draft preparation, Z.A. and M.H.; writing—review and editing, N.M. and B.L.N.; visualization, N.M. and B.L.N.; supervision, N.M. and B.L.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abideen, Z.; Qasim, M.; Hussain, T.; Rasheed, A.; Gul, B.; Koyro, H.W.; Ansari, R.; Khan, M.A. Salinity improves growth, photosynthesis and bioenergy characteristics of *Phragmites karka*. *Crop Pasture Sci.* **2018**, *69*, 944–953. [\[CrossRef\]](#)
2. Ehsen, S.; Abideen, Z.; Rizvi, R.F.; Gulzar, S.; Aziz, I.; Gul, B.; Khan, M.A.; Ansari, R. Ecophysiological adaptations and anti-nutritive status of sustainable cattle feed *Haloxylon stocksii* under saline conditions. *Flora* **2019**, *257*, 151425. [\[CrossRef\]](#)
3. Shoukat, E.; Ahmed, M.Z.; Abideen, Z.; Azeem, M.; Ibrahim, M.; Gul, B.; Khan, M.A. Short and long term salinity induced differences in growth and tissue specific ion regulation of *Phragmites karka*. *Flora* **2020**, *263*, 151550. [\[CrossRef\]](#)
4. Hussain, M.I.; Abideen, Z.; Qureshi, A.S. Soil degradation, resilience, restoration and sustainable use. In *Sustainable Agriculture Reviews*; Springer: Cham, Switzerland, 2021; Volume 52, pp. 335–365. [\[CrossRef\]](#)
5. Abideen, Z.; Koyro, H.W.; Huchzermeyer, B.; Ahmed, M.Z.; Gul, B.; Khan, M.A. Moderate salinity stimulates growth and photosynthesis of *Phragmites karka* by water relations and tissue specific ion regulation. *Environ. Exp. Bot.* **2014**, *105*, 70–76. [\[CrossRef\]](#)
6. Shoukat, E.; Abideen, Z.; Ahmed, M.Z.; Gulzar, S.; Nielsen, B.L. Changes in growth and photosynthesis linked with intensity and duration of salinity in *Phragmites karka*. *Environ. Exp. Bot.* **2019**, *162*, 504–514. [\[CrossRef\]](#)
7. Munir, N.; Hasnain, M.; Roessner, U.; Abideen, Z. Strategies in improving plant salinity resistance and use of salinity resistant plants for economic sustainability. *Crit. Rev. Environ. Sci. Technol.* **2021**, 1–47. [\[CrossRef\]](#)
8. Abideen, Z.; Koyro, H.W.; Huchzermeyer, B.; Ahmed, M.; Zulfiqar, F.; Egan, T.; Khan, M.A. *Phragmites karka* plants adopt different strategies to regulate photosynthesis and ion flux in saline and water deficit conditions. *Plant Biosyst.-An Int. J. Deal. All Asp. Plant Biol.* **2021**, *155*, 524–534. [\[CrossRef\]](#)
9. Lohani, N.; Jain, D.; Singh, M.B.; Bhalla, P.L. Engineering multiple abiotic stress tolerance in Canola, *Brassica napus*. *Front. Plant Sci.* **2020**, *11*, 3–11. [\[CrossRef\]](#)
10. Rajaei, B.S.; Iranbakhsh, A.; Ebadi, M.; Majd, A.; Ardebili, Z.O. Red elemental selenium nanoparticles mediated substantial variations in growth, tissue differentiation, metabolism, gene transcription, epigenetic cytosine DNA methylation, and callogenesis in Bittermelon (*Momordica charantia*); an In vitro experiment. *PLoS ONE* **2020**, *15*, e0235556.
11. Derbali, W.; Manaa, A.; Spengler, B.; Goussi, R.; Abideen, Z.; Ghezellou, P.; Abdely, C.; Forreiter, C.; Koyro, H.W. Comparative proteomic approach to study the salinity effect on the growth of two contrasting quinoa genotypes. *Plant Physiol. Biochem.* **2021**, *163*, 215–229. [\[CrossRef\]](#)
12. Tang, W. Heterologous expression of transcription factor *ATWRKY57* alleviates salt stress-induced oxidative damage. *Open Biotechnol. J.* **2018**, *12*, 204–218. [\[CrossRef\]](#)
13. Yokotani, N.; Higuchi, M.; Kondou, Y.; Ichikawa, T.; Iwabuchi, M.; Hirochika, H.; Matsui, M.; Oda, K. A novel chloroplast protein, CEST induces tolerance to multiple environmental stresses and reduces photooxidative damage in transgenic *Arabidopsis*. *J. Exp. Bot.* **2011**, *62*, 557–569. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Upadhyaya, C.P.; Venkatesh, J.; Gururani, M.A.; Asnin, L.; Sharma, K.; Ajappala, H.; Park, S.W. Transgenic potato overproducing l-ascorbic acid resisted an increase in methylglyoxal under salinity stress via maintaining higher reduced glutathione level and glyoxalase enzyme activity. *Biotechnol. Lett.* **2011**, *33*, 2297–2307. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Hasanuzzaman, M.; Hossain, M.A.; Fujita, M. Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in Rapeseed seedlings. *Biol. Trace Elem. Res.* **2011**, *143*, 1704–1721. [\[CrossRef\]](#)
16. Auffan, M.; Rose, J.; Bottero, J.Y.; Lowry, G.V.; Jolivet, J.P.; Wiesner, M.R. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat. Nanotechnol.* **2009**, *4*, 634–641. [\[CrossRef\]](#)
17. Maurer-Jones, M.A.; Gunsolus, I.L.; Murphy, C.J.; Haynes, C.L. Toxicity of engineered nanoparticles in the environment. *Anal. Chem.* **2013**, *85*, 3036–3049. [\[CrossRef\]](#)
18. Radad, K.; Al-Shraim, M.; Moldzio, R.; Rausch, W.D. Recent advances in benefits and hazards of engineered nanoparticles. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 661–672. [\[CrossRef\]](#)
19. Chavali, M.S.; Nikolova, M.P. Metal oxide nanoparticles and their applications in nanotechnology. *SN Appl. Sci.* **2019**, *1*, 3199. [\[CrossRef\]](#)
20. Sanchez-Dominguez, M.; Boutonnet, M.; Solans, C. A novel approach to metal and metal oxide nanoparticle synthesis: The oil-in-water microemulsion reaction method. *J. Nanopart. Res.* **2009**, *11*, 1823–1829. [\[CrossRef\]](#)
21. Rana, A.; Yadav, K.; Jagadevan, S. A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity. *J. Clean. Prod.* **2020**, *272*, 122880. [\[CrossRef\]](#)
22. Dumont, E.; Johnson, A.C.; Keller, V.D.; Williams, R.J. Nano silver and nano zinc-oxide in surface waters—exposure estimation for Europe at high spatial and temporal resolution. *Environ. Pollut.* **2015**, *196*, 341–349. [\[CrossRef\]](#)

23. Levard, C.; Hotze, E.M.; Lowry, G.V.; Brown, G.E., Jr. Environmental transformations of silver nanoparticles: Impact on stability and toxicity. *Environ. Sci. Technol.* **2012**, *46*, 6900–6914. [[CrossRef](#)]
24. Barrios, A.C.; Rico, C.M.; Trujillo-Reyes, J.; Medina-Velo, I.A.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on Tomato plants. *Sci. Total Environ.* **2016**, *563*, 956–964. [[CrossRef](#)] [[PubMed](#)]
25. Gardea-Torresdey, J.L.; Rico, C.M.; White, J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* **2014**, *48*, 2526–2540. [[CrossRef](#)] [[PubMed](#)]
26. Mahdi, K.N.; Peters, R.J.; Klumpp, E.; Bohme, S.; Van der Ploeg, M.; Ritsema, C.; Geissen, V. Silver nanoparticles in soil: Aqueous extraction combined with single-particle ICP-MS for detection and characterization. *Environ. Nanotechnol. Monit. Manag.* **2017**, *7*, 24–33. [[CrossRef](#)]
27. Tripathi, D.K.; Singh, S.; Singh, S.; Srivastava, P.K.; Singh, V.P.; Singh, S.; Prasad, S.M.; Singh, P.K.; Dubey, N.K.; Pandey, A.C. Nitric oxide alleviates silver nanoparticles (Ag-NPs)-induced phytotoxicity in *Pisum sativum* seedlings. *Plant Physiol. Biochem.* **2017**, *110*, 167–177. [[CrossRef](#)] [[PubMed](#)]
28. Wang, P.; Lombi, E.; Zhao, F.J.; Kopittke, P.M. Nanotechnology: A new opportunity in plant sciences. *Trends Plant Sci.* **2016**, *21*, 699–712. [[CrossRef](#)] [[PubMed](#)]
29. Rao, S.; Shekhawat, G.S. Phytotoxicity and oxidative stress perspective of two selected nanoparticles in *Brassica juncea*. *3 Biotech* **2016**, *6*, 244. [[CrossRef](#)]
30. Taran, N.; Batsmanova, L.; Kovalenko, M.; Okaneneko, A. Impact of metal nanoform colloidal solution on the adaptive potential of plants. *Nanoscale Res. Lett.* **2016**, *1*, 11–89. [[CrossRef](#)]
31. Zohra, E.; Ikram, M.; Omar, A.A.; Hussain, M.; Satti, S.H.; Raja, N.I.; Ehsan, M. Potential applications of biogenic selenium nanoparticles in alleviating biotic and abiotic stresses in plants: A comprehensive insight on the mechanistic approach and future perspectives. *Green Process. Synth.* **2021**, *10*, 456–475. [[CrossRef](#)]
32. Munir, N.; Hanif, M.; Dias, D.A.; Abideen, A. The role of halophytic nanoparticles towards the remediation of degraded and saline agricultural lands. *Environ. Sci. Pollut. Res.* **2021**, *28*, 60383–60405. [[CrossRef](#)]
33. Sharma, P.; Bhatt, D.; Zaidi, M.; Saradhi, P.P.; Khanna, P.; Arora, S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl. Biochem. Biotechnol.* **2012**, *167*, 2225–2233. [[CrossRef](#)]
34. Gunjan, B.; Zaidi, M. Impact of gold nanoparticles on physiological and biochemical characteristics of *Brassica juncea*. *J. Plant Biochem. Physiol.* **2014**, *2*, 67–73. [[CrossRef](#)]
35. Mazumdar, H.; Ahmed, G. Phytotoxicity effect of silver nanoparticles on *Oryza sativa*. *Int. J. ChemTech Res.* **2011**, *3*, 1494–1500.
36. Mirzajani, F.; Askari, H.; Hamzelou, S.; Schober, Y.; Römpf, A.; Ghassempour, A.; Spengler, B. Proteomics study of silver nanoparticles toxicity on *Oryza sativa* L. *Ecotoxicol. Environ. Saf.* **2014**, *108*, 335–339. [[CrossRef](#)]
37. Khodakovskaya, M.; Dervishi, E.; Mahmood, M.; Xu, Y.; Li, Z.; Watanabe, F.; Biris, A.S. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano* **2009**, *3*, 3221–3227. [[CrossRef](#)]
38. Tawfik, M.; Mohamed, M.H.; Sadak, M.S.; Thalooh, A.T. Iron oxide nanoparticles effect on growth, physiological traits and nutritional contents of *Moringa oleifera* grown in saline environment. *Bull. Natl. Res. Cent.* **2021**, *45*, 177. [[CrossRef](#)]
39. Rodríguez-Celma, J.; Lattanzio, G.; Grusak, M.A.; Abadía, A.; Abadía, J.; López-Millán, A.F. Root responses of *Medicago truncatula* plants grown in two different iron deficiency conditions: Changes in root protein profile and riboflavin biosynthesis. *J. Proteome Res.* **2011**, *10*, 2590–2601. [[CrossRef](#)] [[PubMed](#)]
40. Mai, H.J.; Lindermayr, C.; Toerne, C.; Fink-Straube, C.; Durner, J.; Bauer, P. Iron and fer-like iron deficiency-induced transcription factor-dependent regulation of proteins and genes in *Arabidopsis thaliana* roots. *Proteomics* **2015**, *15*, 3030–3047. [[CrossRef](#)] [[PubMed](#)]
41. Mushtaq, Y.K. Effect of nanoscale Fe₃O₄, TiO₂ and carbon particles on cucumber seed germination. *J. Environ. Sci. Health* **2011**, *46*, 1732–1735. [[CrossRef](#)]
42. Zhu, H.; Han, J.; Xiao, J.Q.; Jin, Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by Pumpkin plants. *J. Environ. Monit.* **2008**, *10*, 713–717. [[CrossRef](#)] [[PubMed](#)]
43. Barhoumi, L.; Oukarroum, A.; Taher, L.B.; Smiri, L.S.; Abdelmelek, H.; Dewez, D. Effects of superparamagnetic iron oxide nanoparticles on photosynthesis and growth of the aquatic plant *Lemna gibba*. *Arch. Environ. Contam. Toxicol.* **2015**, *68*, 510–520. [[CrossRef](#)] [[PubMed](#)]
44. Shankamma, K.; Yallappa, S.; Shivanna, M.B.; Manjanna, J. Fe₂O₃ magnetic nanoparticles to enhance *S. lycopersicum* (tomato) plant growth and their biomineralization. *Appl. Nanosci.* **2016**, *6*, 983–990. [[CrossRef](#)]
45. Rui, M.; Ma, C.; Hao, Y.; Guo, J.; Rui, Y.; Tang, X.; Zhao, Q.; Fan, X.; Zhang, Z.; Hou, T.; et al. Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Fron. Plant Sci.* **2016**, *7*, 815. [[CrossRef](#)]
46. Dixon, R.A. Natural products and plant disease resistance. *Nature* **2001**, *411*, 843–847. [[CrossRef](#)]
47. Fiehn, O. Metabolomics—The link between genotypes and phenotypes. *Funct. Gen.* **2002**, *48*, 155–171. [[CrossRef](#)]
48. Hounsome, N.; Hounsome, B.; Tomos, D.; Edwards, J.G. Plant metabolites and nutritional quality of vegetables. *J. Food Sci.* **2008**, *73*, R48–R65. [[CrossRef](#)]
49. Mhlongo, M.I.; Piater, L.A.; Madala, N.E.; Labuschagne, N.; Dubery, I.A. The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. *Front. Plant Sci.* **2018**, *9*, 112–121. [[CrossRef](#)]

50. Attia-Ismail, S.A. Plant secondary metabolites of halophytes and salt tolerant plants. In *Halophytic and Salt-Tolerant Feedstuffs*; CRC Press: Boca Raton, FL, USA, 2015; Volume 1, pp. 127–142. [[CrossRef](#)]
51. Guo, J.; Li, Y.; Han, G.; Song, J.; Wang, B. NaCl markedly improved the reproductive capacity of the euhalophyte *Suaeda salsa*. *Funct. Plant Biol.* **2018**, *45*, 350–361. [[CrossRef](#)]
52. Yao, W.; Zhao, K.; Cheng, Z.; Li, X.; Zhou, B.; Jiang, T. Transcriptome analysis of Poplar under salt stress and over-expression of transcription factor *NAC57* gene confers salt tolerance in transgenic *Arabidopsis*. *Front. Plant Sci.* **2018**, *9*, 11–21. [[CrossRef](#)]
53. Rao, A.Q.; Ud Din, S.; Akhtar, S.; Sarwar, M.B.; Ahmed, M.; Rashid, B.; Khan, M.A.U.; Qaisar, U.; Shahid, A.A.; Nasir, I.A. Genomics of salinity tolerance in plants. In *Plant Genomic*; InTechOpen: London, UK, 2016; pp. 273–299.
54. Johnson, R.R.; Wagner, R.L.; Verhey, S.D.; Walker-Simmons, M.K. The abscisic acid-responsive kinase *PKABA1* interacts with a seed-specific abscisic acid response element-binding factor, TaABF, and phosphorylates TaABF peptide sequences. *Plant Physiol.* **2002**, *130*, 837–846. [[CrossRef](#)] [[PubMed](#)]
55. Fujita, Y.; Yoshida, T.; Yamaguchi-Shinozaki, K. Pivotal role of the *AREB/ABF-SnRK2* pathway in *ABRE*-mediated transcription in response to osmotic stress in plants. *Physiol. Plant* **2013**, *147*, 15–27. [[CrossRef](#)] [[PubMed](#)]
56. Su, H.; Zhang, S.; Yuan, X.; Chen, C.; Wang, X.F.; Hao, Y.J. Genome-wide analysis and identification of stress-responsive genes of the *NAM-ATAF1, 2-CUC2* transcription factor family in Apple. *Plant Physiol. Biochem.* **2013**, *71*, 11–21. [[CrossRef](#)] [[PubMed](#)]
57. Zhang, L.; Zhao, G.; Jia, J.; Liu, X.; Kong, X. Molecular characterization of 60 isolated wheat *MYB* genes and analysis of their expression during abiotic stress. *J. Exp. Bot.* **2012**, *63*, 203–214. [[CrossRef](#)]
58. Roy, S.; Negra, S.; Tester, M. Salt resistant crop plants. *Curr. Opin. Biotechnol.* **2014**, *26*, 115–124. [[CrossRef](#)]
59. Hu, Y.; Chen, L.; Wang, H.; Zhang, L.; Wang, F.; Yu, D. Arabidopsis transcription factor *WRKY8* functions antagonistically with its interacting partner *VQ9* to modulate salinity stress tolerance. *Plant J.* **2013**, *74*, 730–745. [[CrossRef](#)]
60. Ji, H.; Pardo, J.M.; Batelli, G.; Van Oosten, M.J.; Bressan, R.A.; Li, X. The salt overly sensitive (SOS) pathway: Established and emerging roles. *Mol. Plant* **2013**, *6*, 275–286. [[CrossRef](#)]
61. Schmidt, R.; Mieulet, D.; Hubberten, H.M.; Obata, T.; Hoefgen, R.; Fernie, A.R.; Fisahn, J.; San Segundo, B.; Guiderdoni, E.; Schippers, J.H. Salt-responsive *ERF1* regulates reactive oxygen species-dependent signaling during the initial response to salt stress in Rice. *Plant Cell* **2013**, *25*, 2115–2131. [[CrossRef](#)]
62. Steinfeld, B.; Scott, J.; Vilander, G.; Marx, L.; Quirk, M.; Lindberg, J.; Koerner, K. The role of lean process improvement in implementation of evidence-based practices in behavioral health care. *J. Behav. Health Serv. Res.* **2015**, *42*, 504–518. [[CrossRef](#)]
63. Kakali, M.; Roy, C.; Bhaskar, G.; Sudhiranjan, G.; Sengupta, D. An *ABRE*-binding factor, *OSBZ8*, is highly expressed in salt tolerant cultivars than in salt sensitive cultivars of *Indica Rice*. *BMC Plant Biol.* **2006**, *6*, 18. [[CrossRef](#)]
64. Staiger, D.; Brown, J.W. Alternative splicing at the intersection of biological timing, development, and stress responses. *Plant Cell* **2013**, *25*, 3640–3656. [[CrossRef](#)] [[PubMed](#)]
65. Chakraborty, K.; Sairam, R.K.; Bhattacharya, R. Differential expression of salt overly sensitive pathway genes determines salinity stress tolerance in *Brassica* genotypes. *Plant Physiol. Biochem.* **2012**, *51*, 90–101. [[CrossRef](#)] [[PubMed](#)]
66. Lim, G.H.; Zhang, X.; Chung, M.S.; Lee, D.J.; Woo, Y.M.; Cheong, H.S.; Kim, C.S. A putative novel transcription factor, *AtSKIP*, is involved in abscisic acid signalling and confers salt and osmotic tolerance in *Arabidopsis*. *New Phytol.* **2010**, *185*, 103–113. [[CrossRef](#)] [[PubMed](#)]
67. Zou, J.; Liu, C.; Liu, A.; Zou, D.; Chen, X. Overexpression of *Oshsp17.0* and *OSHSP23.7* enhances drought and salt tolerance in Rice. *J. Plant Physiol.* **2012**, *169*, 628–635. [[CrossRef](#)] [[PubMed](#)]
68. Song, N.H.; Ahn, Y.J. *DcHsp17.7*, a small heat shock protein in Carrot, is tissue-specifically expressed under salt stress and confers tolerance to salinity. *New Biotechnol.* **2011**, *28*, 698–704. [[CrossRef](#)] [[PubMed](#)]
69. Tang, M.; Liu, X.; Deng, H.; Shen, S. Over-expression of *JcDREB*, a putative AP2/EREBP domain-containing transcription factor gene in woody biodiesel plant *Jatropha curcas*, enhances salt and freezing tolerance in transgenic *Arabidopsis thaliana*. *Plant Sci.* **2011**, *181*, 623–631. [[CrossRef](#)] [[PubMed](#)]
70. Sottosanto, J.B.; Saranga, Y.; Blumwald, E. Impact of *AtNHX1*, a vacuolar Na^+/H^+ antiporter, upon gene expression during short-and long-term salt stress in *Arabidopsis thaliana*. *BMC Plant Biol.* **2007**, *7*, 18. [[CrossRef](#)] [[PubMed](#)]
71. Peng, X.; Ding, X.; Chang, T.; Wang, Z.; Liu, R.; Zeng, X.; Cai, Y.; Zhu, Y. Overexpression of a vesicle trafficking gene, *OsRab7*, enhances salt tolerance in Rice. *Sci. World J.* **2014**, *2014*, 483526. [[CrossRef](#)]
72. Han, Y.; Wang, W.; Sun, J.; Ding, M.; Zhao, R.; Deng, S.; Wang, F.; Hu, Y.; Wang, Y.; Lu, Y. *Populus euphratica* XTH overexpression enhances salinity tolerance by the development of leaf succulence in transgenic Tobacco plants. *J. Exp. Bot.* **2013**, *64*, 4225–4238. [[CrossRef](#)]
73. Kurotani, K.I.; Hayashi, K.; Hatanaka, S.; Toda, Y.; Ogawa, D.; Ichikawa, H.; Ishimaru, Y.; Tashita, R.; Suzuki, T.; Ueda, M. Elevated levels of *CYP94* family gene expression alleviate the Jasmonate response and enhance salt tolerance in Rice. *Plant Cell Physiol.* **2015**, *56*, 779–789. [[CrossRef](#)]
74. Yue, Y.; Zhang, M.; Zhang, J.; Duan, L.; Li, Z. *SOS1* gene overexpression increased salt tolerance in transgenic Tobacco by maintaining a higher K^+/Na^+ ratio. *J. Plant Physiol.* **2012**, *169*, 255–261. [[CrossRef](#)] [[PubMed](#)]
75. Bhauso, T.D.; Thankappan, R.; Kumar, A.; Mishra, G.P.; Dobarra, J.R.; Rajam, M.V. Over-expression of bacterial *MTLD* gene confers enhanced tolerance to salt-stress and water-deficit stress in transgenic Peanut (*Arachis hypogaea*) through accumulation of mannitol. *Aust. J. Crop Sci.* **2014**, *8*, 413–424.

76. Zhou, W.; Li, Y.; Zhao, B.C.; Ge, R.C.; Shen, Y.Z.; Wang, G.; Huang, Z.J. Overexpression of *TaSTRG* gene improves salt and drought tolerance in Rice. *J. Plant Physiol.* **2009**, *166*, 1660–1671. [[CrossRef](#)] [[PubMed](#)]
77. Lawson, S.S.; Michler, C.H. Overexpression of *AtSTO1* leads to improved salt tolerance in *Populus tremula* × *P. alba*. *Transgenic Res.* **2014**, *23*, 817–826. [[CrossRef](#)] [[PubMed](#)]
78. Mangrauthia, S.K.; Agarwal, S.; Sailaja, B.; Madhav, M.S.; Voleti, S. MicroRNAs and their role in salt stress response in plants. In *Salt Stress in Plants*, 3rd ed.; Springer: Cham, Switzerland, 2013; pp. 15–46.
79. Lu, S.; Sun, Y.H.; Chiang, V.L. Stress-responsive microRNAs in *Populus*. *Plant J.* **2008**, *55*, 131–151. [[CrossRef](#)]
80. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J.I. Plant salt-tolerance mechanisms. *Trends Plant Sci.* **2014**, *19*, 371–379. [[CrossRef](#)] [[PubMed](#)]
81. Gollmack, D.; Lüking, I.; Yang, O. Plant tolerance to drought and salinity: Stress regulating transcription factors and their functional significance in the cellular transcriptional network. *Plant Cell Rep.* **2011**, *30*, 1383–1391. [[CrossRef](#)]
82. Yang, O.; Popova, O.V.; Süthoff, U.; Lüking, I.; Dietz, K.J.; Gollmack, D. The *Arabidopsis* basic leucine zipper transcription factor AtbZIP24 regulates complex transcriptional networks involved in abiotic stress resistance. *Gene* **2009**, *436*, 45–55. [[CrossRef](#)]
83. Popova, O.V.; Yang, O.; Dietz, K.J.; Gollmack, D. Differential transcript regulation in *Arabidopsis thaliana* and the halotolerant *lobularia maritima* indicates genes with potential function in plant salt adaptation. *Gene* **2008**, *423*, 142–148. [[CrossRef](#)]
84. Munns, R.; James, R.A.; Xu, B.; Athman, A.; Conn, S.J.; Jordans, C.; Byrt, C.S.; Hare, R.A.; Tyerman, S.D.; Tester, M. Wheat grain yield on saline soils is improved by an ancestral Na⁺ transporter gene. *Nat. Biotechnol.* **2012**, *30*, 360–364. [[CrossRef](#)]
85. Yamaguchi, T.; Blumwald, E. Developing salt-tolerant crop plants: Challenges and opportunities. *Trends Plant Sci.* **2005**, *10*, 615–620. [[CrossRef](#)]
86. Reddy, I.N.B.L.; Kim, B.K.; Yoon, I.S.; Kim, K.H.; Kwon, T.R. Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Sci.* **2017**, *24*, 123–144. [[CrossRef](#)]
87. Silva, P.; Gerós, H. Regulation by salt of vacuolar H⁺-ATPase and H⁺-pyrophosphatase activities and Na⁺/H⁺ exchange. *Plant Signal. Behav.* **2009**, *4*, 718–726. [[CrossRef](#)] [[PubMed](#)]
88. Flowers, T.; Koyama, M.; Flowers, S.; Sudhakar, C.; Singh, K.; Yeo, A. QTL: Their place in engineering tolerance of Rice to salinity. *J. Exp. Bot.* **2000**, *51*, 99–106. [[CrossRef](#)] [[PubMed](#)]
89. Alam, R.; Sazzad-ur-Rahman, M.; Seraj, Z.I.; Thomson, M.J.; Ismail, A.M.; Tumimbang-Raiz, E.; Gregorio, G.B. Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* l. Pokkali. *Plant Breed.* **2011**, *130*, 430–437. [[CrossRef](#)]
90. Frary, A.; Göll, D.; Keleş, D.; Ökmen, B.; Pinar, H.; Şığva, H.Ö.; Yemencioğlu, A.; Doğanlar, S. Salt tolerance in *Solanum pennellii*: Antioxidant response and related QTL. *BMC Plant Biol.* **2010**, *10*, 58. [[CrossRef](#)] [[PubMed](#)]
91. Yang, Q.; Chen, Z.Z.; Zhou, X.F.; Yin, H.B.; Li, X.; Xin, X.F.; Hong, X.H.; Zhu, J.K.; Gong, Z. Overexpression of SOS (salt overly sensitive) genes increases salt tolerance in transgenic *Arabidopsis*. *Mol. Plant* **2009**, *2*, 22–31. [[CrossRef](#)] [[PubMed](#)]
92. Shabala, S. Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.* **2013**, *112*, 1209–1221. [[CrossRef](#)]
93. Ventura, Y.; Eshel, A.; Pasternak, D.; Sagi, M. The development of halophyte-based agriculture: Past and present. *Ann. Bot.* **2015**, *115*, 529–540. [[CrossRef](#)]
94. Zhu, J.K. Plant salt tolerance. *Trends Plant Sci.* **2001**, *6*, 66–71. [[CrossRef](#)]
95. Himabindu, Y.; Chakradhar, T.; Reddy, M.C.; Kanygin, A.; Redding, K.E.; Chandrasekhar, T. Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. *Environ. Exp. Bot.* **2016**, *124*, 39–63. [[CrossRef](#)]
96. Muchate, N.S.; Nikalje, G.C.; Rajurkar, N.S.; Suprasanna, P.; Nikam, T.D. Plant salt stress: Adaptive responses, tolerance mechanism and bioengineering for salt tolerance. *Bot. Rev.* **2016**, *82*, 371–406. [[CrossRef](#)]
97. Udawat, P.; Mishra, A.; Jha, B. Heterologous expression of an uncharacterized universal stress protein gene (SbUSP) from the extreme halophyte, *Salicornia brachiata*, which confers salt and osmotic tolerance to *E. coli*. *Gene* **2014**, *536*, 163–170. [[CrossRef](#)] [[PubMed](#)]
98. Bartels, D.; Dinakar, C. Balancing salinity stress responses in halophytes and non-halophytes: A comparison between *Thellungiella* and *Arabidopsis thaliana*. *Funct. Plant Biol.* **2013**, *40*, 819–831. [[CrossRef](#)]
99. Wu, H.J.; Zhang, Z.; Wang, J.Y.; Oh, D.H.; Dassanayake, M.; Liu, B.; Huang, Q.; Sun, H.X.; Xia, R.; Wu, Y. Insights into salt tolerance from the genome of *Thellungiella salsuginea*. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 12219–12224. [[CrossRef](#)]
100. Taji, T.; Seki, M.; Satou, M.; Sakurai, T.; Kobayashi, M.; Ishiyama, K.; Narusaka, Y.; Narusaka, M.; Zhu, J.K.; Shinozaki, K. Comparative genomics in salt tolerance between *Arabidopsis* and arabidopsis-related halophyte salt stress using *Arabidopsis* microarray. *Plant Physiol.* **2004**, *135*, 1697–1709. [[CrossRef](#)]
101. Wong, C.E.; Li, Y.; Labbe, A.; Guevara, D.; Nuin, P.; Whitty, B.; Diaz, C.; Golding, G.B.; Gray, G.R.; Weretilnyk, E.A. Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in *Thellungiella*, a close relative of *Arabidopsis*. *Plant Physiol.* **2006**, *140*, 1437–1450. [[CrossRef](#)]
102. Rigó, G.; Valkai, I.; Faragó, D.; Kiss, E.; Van Houdt, S.; Van de Steene, N.; Hannah, M.A.; Szabados, L. Gene mining in halophytes: Functional identification of stress tolerance genes in *Lepidium crassifolium*. *Plant, Cell Environ.* **2016**, *39*, 167–181. [[CrossRef](#)]
103. Pandey, S.; Patel, M.K.; Mishra, A.; Jha, B. In planta transformed Cumin (*Cuminum cyminum* l.) plants, overexpressing the SbNHX1 gene showed enhanced salt endurance. *PLoS ONE* **2016**, *11*, e0159349.

104. Jha, B.; Singh, N.P.; Mishra, A. Proteome profiling of seed storage proteins reveals the nutritional potential of *Salicornia brachiata* Roxb., an extreme halophyte. *J. Agric. Food Chem.* **2012**, *60*, 4320–4326. [[CrossRef](#)]
105. Garg, R.; Verma, M.; Agrawal, S.; Shankar, R.; Majee, M.; Jain, M. Deep transcriptome sequencing of wild halophyte Rice, *Porteresia coarctata*, provides novel insights into the salinity and submergence tolerance factors. *DNA Res.* **2014**, *21*, 69–84. [[CrossRef](#)] [[PubMed](#)]
106. Zhang, G.H.; Su, Q.; An, L.J.; Wu, S. Characterization and expression of a vacuolar Na⁺/H⁺ antiporter gene from the monocot halophyte *Aeluropus litoralis*. *Plant Physiol. Biochem.* **2008**, *46*, 117–126. [[CrossRef](#)] [[PubMed](#)]
107. Jia, G.X.; Zhu, Z.Q.; Chang, F.Q.; Li, Y.X. Transformation of Tomato with the *BADH* gene from *Atriplex* improves salt tolerance. *Plant Cell Rep.* **2002**, *21*, 141–146. [[CrossRef](#)]
108. Harris, P.A.; Taylor, R.; Minor, B.L.; Elliott, V.; Fernandez, M.; O'Neal, L.; McLeod, L.; Delacqua, G.; Delacqua, F.; Kirby, J. The REDCap consortium: Building an international community of software platform partners. *J. Biomed. Inform.* **2019**, *95*, 15–32. [[CrossRef](#)]
109. Singh, N.; Mishra, A.; Jha, B. Over-expression of the peroxisomal ascorbate peroxidase (SbpAPX) gene cloned from halophyte *Salicornia brachiata* confers salt and drought tolerance in transgenic tobacco. *Mar. Biotechnol.* **2014**, *16*, 321–332. [[CrossRef](#)]
110. Singh, N.; Mishra, A.; Jha, B. Ectopic over-expression of peroxisomal ascorbate peroxidase (SbpAPX) gene confers salt stress tolerance in transgenic Peanut (*Arachis hypogaea*). *Gene* **2014**, *547*, 119–125. [[CrossRef](#)]
111. Li, W.; Wang, D.; Jin, T.; Chang, Q.; Yin, D.; Xu, S.; Liu, B.; Liu, L. The vacuolar Na⁺/H⁺ antiporter gene *SSNHX1* from the halophyte *Salsola soda* confers salt tolerance in transgenic Alfalfa (*Medicago sativa* L.). *Plant Mol. Biol. Rep.* **2011**, *29*, 278–290. [[CrossRef](#)]
112. Wu, W.; Su, Q.; Xia, X.; Wang, Y.; Luan, Y.; An, L. The *Suaeda liaotungensis* KITAG betaine aldehyde dehydrogenase gene improves salt tolerance of transgenic maize mediated with minimum linear length of DNA fragment. *Euphytica* **2008**, *159*, 17–25. [[CrossRef](#)]
113. Qiu, W.; Liu, M.; Qiao, G.; Jiang, J.; Xie, L.; Zhuo, R. An isopentyl transferase gene driven by the stress-inducible RD29A promoter improves salinity stress tolerance in transgenic Tobacco. *Plant Mol. Biol. Rep.* **2012**, *30*, 519–528. [[CrossRef](#)]
114. Zhao, F.; Zhang, H. Salt and paraquat stress tolerance results from co-expression of the *Suaeda salsa* glutathione s-transferase and catalase in transgenic Rice. *Plant Cell Tissue Organ Cult.* **2006**, *86*, 349–358. [[CrossRef](#)]
115. Guo, S.; Yin, H.; Zhang, X.; Zhao, F.; Li, P.; Chen, S.; Zhao, Y.; Zhang, H. Molecular cloning and characterization of a vacuolar H⁺-pyrophosphatase gene, *SsVp*, from the halophyte *Suaeda salsa* and its overexpression increases salt and drought tolerance of *Arabidopsis*. *Plant Mol. Biol.* **2006**, *60*, 41–50. [[CrossRef](#)] [[PubMed](#)]
116. Oh, D.H.; Leidi, E.; Zhang, Q.; Hwang, S.M.; Li, Y.; Quintero, F.J.; Jiang, X.; D'Urzo, M.P.; Lee, S.Y.; Zhao, Y. Loss of halophytism by interference with *SOS1* expression. *Plant Physiol.* **2009**, *151*, 210–222. [[CrossRef](#)] [[PubMed](#)]
117. Wang, L.L.; Chen, A.P.; Zhong, N.Q.; Liu, N.; Wu, X.M.; Wang, F.; Yang, C.L.; Romero, M.F.; Xia, G.X. The *Thellungiella salsuginea* tonoplast aquaporin *TsTIP1; 2* functions in protection against multiple abiotic stresses. *Plant Cell Physiol.* **2014**, *55*, 148–161. [[CrossRef](#)] [[PubMed](#)]
118. Lin, F.; Xu, J.; Shi, J.; Li, H.; Li, B. Molecular cloning and characterization of a novel glyoxalase I gene *TaGly I* in Wheat (*Triticum aestivum* L.). *Mol. Biol. Rep.* **2010**, *37*, 729–737. [[CrossRef](#)]
119. Baisakh, N.; RamanaRao, M.V.; Rajasekaran, K.; Subudhi, P.; Janda, J.; Galbraith, D.; Vanier, C.; Pereira, A. Enhanced salt stress tolerance of Rice plants expressing a vacuolar H⁺-ATPase subunit *c1* (*SaVHAc1*) gene from the halophyte grass *Spartina alterniflora* Loisel. *Plant Biotechnol. J.* **2012**, *10*, 453–464. [[CrossRef](#)]
120. Wu, S.; Su, Q.; An, L. Isolation of choline monoxygenase (CMO) gene from *Salicornia europaea* and enhanced salt tolerance of transgenic Tobacco with CMO genes. *Ind. J. Biochem. Biophys.* **2010**, *47*, 298–305.
121. Yin, X.Y.; Yang, A.F.; Zhang, K.W.; Zhang, J.R. Production and analysis of transgenic maize with improved salt tolerance by the introduction of *AtNHX1* gene. *Acta Bot. Sin.-Engl. Ed.* **2004**, *46*, 854–861.
122. Ohta, M.; Hayashi, Y.; Nakashima, A.; Tsunetomi, N.; Hamada, A.; Tanaka, A.; Nakamura, T.; Hayakawa, T. Salt tolerance of Rice is conferred by introduction of a Na⁺/H⁺ antiporter gene from *Atriplex gmelini*. *Plant Cell Physiol.* **2002**, *532*, 279–282. [[CrossRef](#)]
123. Taji, T.; Komatsu, K.; Katori, T.; Kawasaki, Y.; Sakata, Y.; Tanaka, S.; Kobayashi, M.; Toyoda, A.; Seki, M.; Shinozaki, K. Comparative genomic analysis of 1047 completely sequenced cDNA's from an arabidopsis-related model halophyte, *Thellungiella halophila*. *BMC Plant Biol.* **2010**, *10*, 261. [[CrossRef](#)]
124. Tiwari, V.; Patel, M.K.; Chaturvedi, A.K.; Mishra, A.; Jha, B. Functional characterization of the Tau class glutathione-s-transferases gene (*SbGSTU*) promoter of *Salicornia brachiata* under salinity and osmotic stress. *PLoS ONE* **2016**, *11*, e0148494. [[CrossRef](#)]
125. Yin, X.; Zhao, Y.; Luo, D.; Zhang, H. Isolating the promoter of a stress-induced gene encoding betaine aldehyde dehydrogenase from the halophyte *Atriplex centralasiatica* Iljin. *Biochim. Biophys. Acta-Gene Struct. Express* **2002**, *1577*, 452–456. [[CrossRef](#)]
126. Li, Q.L.; Xie, J.H.; Ma, X.Q.; Li, D. Molecular cloning of phosphoethanolamine n-methyltransferase (PEAMT) gene and its promoter from the halophyte *Suaeda liaotungensis* and their response to salt stress. *Acta Physiol. Plant* **2016**, *38*, 39–45. [[CrossRef](#)]
127. Yang, X.; Lu, M.; Wang, Y.; Wang, Y.; Liu, Z.; Chen, S. Response mechanism of plants to drought stress. *Horticulturae* **2021**, *7*, 50. [[CrossRef](#)]
128. Schaeffer, H.J.; Forsthoefel, N.R.; Cushman, J.C. Identification of enhancer and silencer regions involved in salt-responsive expression of crassulacean acid metabolism (CAM) genes in the facultative halophyte *Mesembryanthemum crystallinum*. *Plant Mol. Biol.* **1995**, *28*, 205–218. [[CrossRef](#)]

129. Zhao, Y.; Liu, W.; Xu, Y.P.; Cao, J.Y.; Braam, J.; Ca, X.Z. Genome-wide identification and functional analyses of Calmodulin genes in *Solanaceous* species. *BMC Plant Biol.* **2013**, *13*, 70. [[CrossRef](#)] [[PubMed](#)]
130. Saad, R.B.; Romdhan, W.B.; Zouari, N.; Azaza, J.; Mieulet, D.; Verdeil, J.L.; Guiderdoni, E.; Hassairi, A. Promoter of the *ALSAP* gene from the halophyte grass *Aeluropus litoralis* directs developmental-regulated, stress-inducible, and organ-specific gene expression in transgenic Tobacco. *Transgenic Res.* **2011**, *20*, 1003–1018. [[CrossRef](#)]
131. Ben-Saad, R.; Meynard, D.; Ben-Romdhane, W.; Mieulet, D.; Verdeil, J.L.; Al-Doss, A.; Guiderdoni, E.; Hassairi, A. The promoter of the *ALSAP* gene from the halophyte grass *Aeluropus litoralis* directs a stress-inducible expression pattern in transgenic Rice plants. *Plant Cell Rep.* **2015**, *34*, 1791–1806. [[CrossRef](#)]
132. Sun, Q.; Gao, F.; Zhao, L.; Li, K.; Zhang, J. Identification of a new 130 bp cis-acting element in the TSVP1 promoter involved in the salt stress response from *Thellungiella halophila*. *BMC Plant Biol.* **2010**, *10*, 90. [[CrossRef](#)]
133. Guo, L.; Yu, Y.; Xia, X.; Yin, W. Identification and functional characterisation of the promoter of the calcium sensor gene *CBL1* from the xerophyte *Anemoptanthus mongolicus*. *BMC Plant Biol.* **2010**, *10*, 18. [[CrossRef](#)]
134. Tiwari, V.; Chaturvedi, A.K.; Mishra, A.; Jha, B. The transcriptional regulatory mechanism of the peroxisomal ascorbate peroxidase (*pAPX*) gene cloned from an extreme halophyte, *Salicornia brachiata*. *Plant Cell Physiol.* **2014**, *55*, 201–217. [[CrossRef](#)]
135. James, C. *Global Status of Commercialized Biotech/GM Crops, 2nd ed*; International Service for the Acquisition of Agri-Biotech Applications (ISAAA): Ithaca, NY, USA, 2007; pp. 22–45.
136. Ashraf, M.; Athar, H.; Harris, P.; Kwon, T. Some prospective strategies for improving crop salt tolerance. *Adv. Agron.* **2008**, *97*, 45–110. [[CrossRef](#)]
137. Goel, D.; Singh, A.K.; Yadav, V.; Babbar, S.B.; Murata, N.; Bansal, K.C. Transformation of Tomato with a bacterial *CODA* gene enhances tolerance to salt and water stresses. *J. Plant Physiol.* **2011**, *168*, 1286–1294. [[CrossRef](#)] [[PubMed](#)]
138. Su, J.; Hirji, R.; Zhang, L.; He, C.; Selvaraj, G.; Wu, R. Evaluation of the stress-inducible production of choline oxidase in transgenic Rice as a strategy for producing the stress-protectant glycine betaine. *J. Exp. Bot.* **2006**, *57*, 1129–1135. [[CrossRef](#)] [[PubMed](#)]
139. Cortina, C.; Culiáñez-Macià, F.A. Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. *Plant Sci.* **2005**, *169*, 75–82. [[CrossRef](#)]
140. Almeida, A.M.; Villalobos, E.; Araújo, S.S.; Leyman, B.; Van Dijck, P.; Alfaro-Cardoso, L.; Fevereiro, P.S.; Torné, J.M.; Santos, D.M. Transformation of tobacco with an *Arabidopsis thaliana* gene involved in trehalose biosynthesis increases tolerance to several abiotic stresses. *Euphytica* **2005**, *146*, 165–176. [[CrossRef](#)]
141. Abebe, T.; Guenzi, A.C.; Martin, B.; Cushman, J.C. Tolerance of mannitol-accumulating transgenic Wheat to water stress and salinity. *Plant Physiol.* **2003**, *131*, 1748–1755. [[CrossRef](#)]
142. Gao, M.; Tao, R.; Miura, K.; Dandekar, A.M.; Sugiura, A. Transformation of Japanese Persimmon (*Diospyros kaki thunb.*) with Apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase. *Plant Sci.* **2001**, *160*, 837–845. [[CrossRef](#)]
143. Hmida-Sayari, A.; Gargouri-Bouزيد, R.; Bidani, A.; Jaoua, L.; Savouré, A.; Jaoua, S. Overexpression of δ 1-pyrroline-5-carboxylate synthetase increases proline production and confers salt tolerance in transgenic potato plants. *Plant Sci.* **2005**, *169*, 746–752. [[CrossRef](#)]
144. Zhang, H.X.; Hodson, J.N.; Williams, J.P.; Blumwald, E. Engineering salt-tolerant Brassica plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12832–12836. [[CrossRef](#)]
145. Wang, J.; Zuo, K.; Wu, W.; Song, J.; Sun, X.; Lin, J.; Li, X.; Tang, K. Expression of a novel antiporter gene from *Brassica napus* resulted in enhanced salt tolerance in transgenic Tobacco plants. *Biol. Plant* **2004**, *48*, 509–515. [[CrossRef](#)]
146. Singla-Pareek, S.; Reddy, M.; Sopory, S. Genetic engineering of the glyoxalase pathway in Tobacco leads to enhanced salinity tolerance. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14672–14677. [[CrossRef](#)] [[PubMed](#)]
147. Gao, S.Q.; Chen, M.; Xu, Z.S.; Zhao, C.P.; Li, L.; Xu, H.J.; Tang, Y.M.; Zhao, X.; Ma, Y.Z. The Soybean *GmbZIP1* transcription factor enhances multiple abiotic stress tolerances in transgenic plants. *Plant Mol. Biol.* **2011**, *75*, 537–553. [[CrossRef](#)] [[PubMed](#)]
148. Seo, Y.J.; Park, J.B.; Cho, Y.J.; Jung, C.; Seo, H.S.; Park, S.K.; Nahm, B.H.; Song, J.T. Overexpression of the ethylene-responsive factor gene *BrERF4* from *Brassica rapa* increases tolerance to salt and drought in *Arabidopsis* plants. *Mol. Cells* **2010**, *30*, 271–277. [[CrossRef](#)] [[PubMed](#)]
149. Montero-Barrientos, M.; Hermosa, R.; Cardoza, R.E.; Gutierrez, S.; Nicolas, C.; Monte, E. Transgenic expression of the *Trichoderma harzianum* *HSP70* gene increases *Arabidopsis* resistance to heat and other abiotic stresses. *J. Plant Physiol.* **2010**, *167*, 659–665. [[CrossRef](#)] [[PubMed](#)]
150. Zhang, L.; Xi, D.; Li, S.; Gao, Z.; Zhao, S.; Shi, J.; Wu, C.; Guo, X. A Cotton group c map kinase gene, *GhMPK2*, positively regulates salt and drought tolerance in Tobacco. *Plant Mol. Biol.* **2011**, *77*, 17–31. [[CrossRef](#)]
151. Flowers, T.J.; Colmer, T.D. Salinity tolerance in halophytes. *New Phytol.* **2008**, *179*, 945–963. [[CrossRef](#)]
152. Bose, J.; Rodrigo-Moreno, A.; Shabala, S. Ros homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* **2014**, *65*, 1241–1257. [[CrossRef](#)]
153. Kronzucker, H.J.; Britto, D.T. Sodium transport in plants: A critical review. *New Phytol.* **2011**, *189*, 54–81. [[CrossRef](#)]
154. Volkov, V. Salinity tolerance in plants. Quantitative approach to ion transport starting from halophytes and stepping to genetic and protein engineering for manipulating ion fluxes. *Front. Plant Sci.* **2015**, *6*, 873–884. [[CrossRef](#)]
155. Mishra, A.; Tanna, B. Halophytes: Potential resources for salt stress tolerance genes and promoters. *Front. Plant Sci.* **2017**, *8*, 829. [[CrossRef](#)]

156. Lan, T.; Duan, Y.; Wang, B.; Zhou, Y.; Wu, W. Molecular cloning and functional characterization of a Na⁺/H⁺ antiporter gene from halophyte *Spartina anglica*. *Turk. J. Agric. For.* **2011**, *35*, 535–543.
157. Patel, M.K.; Joshi, M.; Mishra, A.; Jha, B. Ectopic expression of *SbNHX1* gene in transgenic Castor (*Ricinus communis* L.) enhances salt stress by modulating physiological process. *Plant Cell Tissue Organ Cult.* **2015**, *122*, 477–490. [[CrossRef](#)]
158. Udawat, P.; Jha, R.K.; Sinha, D.; Mishra, A.; Jha, B. Overexpression of a cytosolic abiotic stress responsive universal stress protein (*SbUSP*) mitigates salt and osmotic stress in transgenic Tobacco plants. *Front. Plant Sci.* **2016**, *7*, 518–529. [[CrossRef](#)] [[PubMed](#)]
159. Peng, Y.; Lin, W.; Cai, W.; Arora, R. Overexpression of a *Panax ginseng* tonoplast aquaporin alters salt tolerance, drought tolerance and cold acclimation ability in transgenic Arabidopsis plants. *Planta* **2007**, *226*, 729–740. [[CrossRef](#)] [[PubMed](#)]
160. Sharma, R.; Sahoo, A.; Devendran, R.; Jain, M. Over-expression of a Rice Tau class glutathione s-transferase gene improves tolerance to salinity and oxidative stresses in Arabidopsis. *PLoS ONE* **2014**, *9*, e92900.
161. Oh, D.H.; Dassanayake, M.; Haas, J.S.; Kropornika, A.; Wright, C.; d'Urzo, M.P.; Hong, H.; Ali, S.; Hernandez, A.; Lambert, G.M. Genome structures and halophyte-specific gene expression of the extremophile *Thellungiella parvula* in comparison with *Thellungiella salsuginea* (*Thellungiella halophila*) and Arabidopsis. *Plant Physiol.* **2010**, *154*, 1040–1052. [[CrossRef](#)]
162. Fu, R.; Zhang, M.; Zhao, Y.; He, X.; Ding, C.; Wang, S. Identification of salt tolerance-related microRNAs and their targets in maize (*Zea mays* L.) using high-throughput sequencing and degradome analysis. *Front. Plant Sci.* **2017**, *8*, 864. [[CrossRef](#)]
163. Yang, Z.; Zhu, P.; Kang, H.; Liu, L.; Cao, Q.; Sun, J. High-throughput deep sequencing reveals the important role that microRNAs play in the salt response in sweet potato (*Ipomoea batatas* L.). *BMC Genom.* **2020**, *21*, 164. [[CrossRef](#)]
164. Jodder, J.; Das, R.; Sarkar, D.; Bhattacharjee, P.; Kundu, P. Distinct transcriptional and processing regulations control miR167a level in tomato during stress. *RNA Biol.* **2018**, *15*, 130–143. [[CrossRef](#)]
165. Wang, M.; Wang, Q.; Zhang, B. Response of miRNAs and their targets to salt and drought stresses in cotton (*Gossypium hirsutum* L.). *Gene* **2013**, *530*, 26–32. [[CrossRef](#)]
166. Pegler, J.L.; Oultram, J.M.J.; Grof, C.P.L.; Eamens, A.L. Profiling the abiotic stress responsive microRNA landscape of *Arabidopsis thaliana*. *Plants* **2019**, *8*, 58. [[CrossRef](#)] [[PubMed](#)]
167. Dong, Z.; Shi, L.; Wang, Y.; Chen, L.; Cai, Z.; Wang, Y. Identification and dynamic regulation of microRNAs involved in salt stress responses in functional soybean nodules by high-throughput sequencing. *Int. J. Mol. Sci.* **2013**, *14*, 2717–2738. [[CrossRef](#)] [[PubMed](#)]
168. Zhou, M.; Luo, H. Role of microRNA319 in creeping bentgrass salinity and drought stress response. *Plant Signal Behav.* **2014**, *9*, e28700. [[CrossRef](#)] [[PubMed](#)]
169. Cao, C.; Long, R.; Zhang, T.; Kang, J.; Wang, Z.; Wang, P. Genome-wide identification of microRNAs in response to salt/alkali stress in *Medicago truncatula* through high-throughput sequencing. *Int. J. Mol. Sci.* **2018**, *19*, 4076. [[CrossRef](#)] [[PubMed](#)]
170. Lee, W.S.; Gudimella, R.; Wong, G.R.; Tammi, M.T.; Khalid, N.; Harikrishna, J.A. Transcripts and microRNAs responding to salt stress in *Musa acuminata colla* (AAA Group) cv. berangan roots. *PLoS ONE* **2015**, *10*, e0127526. [[CrossRef](#)]
171. Tripathi, A.; Chacon, O.; Singla-Pareek, S.L.; Sopory, S.K.; Sanan-Mishra, N. Mapping the microRNA expression profiles in glyoxalase over-expressing salinity tolerant rice. *Curr. Genom.* **2018**, *19*, 21–35. [[CrossRef](#)]
172. Xie, F.; Stewart, C.N., Jr.; Taki, F.A.; He, Q.; Liu, H.; Zhang, B. High-throughput deep sequencing shows that microRNAs play important roles in switchgrass responses to drought and salinity stress. *Plant Biotechnol. J.* **2014**, *12*, 354–366. [[CrossRef](#)]
173. Gharat, S.A.; Shaw, B.P. Novel and conserved miRNAs in the halophyte *Suaeda maritima* identified by deep sequencing and computational predictions using the ESTs of two mangrove plants. *BMC Plant Biol.* **2015**, *15*, 301. [[CrossRef](#)]
174. Xu, T.; Zhang, L.; Yang, Z.; Wei, Y.; Dong, T. Identification and functional characterization of Plant miRNA under salt stress shed light on salinity resistance improvement through miRNA manipulation in crops. *Front. Plant Sci.* **2021**, *12*, 665439. [[CrossRef](#)]
175. Alzahrani, S.M.; Alaraidh, I.A.; Khan, M.A.; Migdadi, H.M.; Alghamdi, S.S.; Alsahli, A.A. Identification and characterization of salt-responsive microRNAs in *Vicia faba* by high-throughput sequencing. *Genes* **2019**, *10*, 303. [[CrossRef](#)]
176. Zhang, Q.; Zhao, C.; Li, M.; Sun, W.; Liu, Y.; Xia, H. Genome-wide identification of *Thellungiella salsuginea* microRNAs with putative roles in the salt stress response. *BMC Plant Biol.* **2013**, *13*, 180. [[CrossRef](#)] [[PubMed](#)]
177. Sun, X.; Xu, L.; Wang, Y.; Yu, R.; Zhu, X.; Luo, X. Identification of novel and salt-responsive miRNAs to explore miRNA-mediated regulatory network of salt stress response in radish (*Raphanus sativus* L.). *BMC Genom.* **2015**, *16*, 197. [[CrossRef](#)] [[PubMed](#)]
178. Luan, M.; Xu, M.; Lu, Y.; Zhang, L.; Fan, Y.; Wang, L. Expression of zma-miR169 miRNAs and their target ZmNF-YA genes in response to abiotic stress in maize leaves. *Gene* **2015**, *55*, 178–185. [[CrossRef](#)]
179. Yuan, S.; Li, Z.; Li, D.; Yuan, N.; Hu, Q.; Luo, H. Constitutive expression of rice microRNA528 alters plant development and enhances tolerance to salinity stress and nitrogen starvation in creeping bentgrass. *Plant Physiol.* **2015**, *169*, 576–593. [[CrossRef](#)]
180. Yuan, S.; Zhao, J.; Li, Z.; Hu, Q.; Yuan, N.; Zhou, M. MicroRNA396-mediated alteration in plant development and salinity stress response in creeping bentgrass. *Hortic. Res.* **2019**, *6*, 48. [[CrossRef](#)] [[PubMed](#)]
181. Wu, Y.; Guo, J.; Cai, Y.; Gong, X.; Xiong, X.; Qi, W. Genome-wide identification and characterization of *Eutrema salsugineum* microRNAs for salt tolerance. *Physiol. Plant* **2016**, *157*, 453–468. [[CrossRef](#)]
182. Chiang, C.P.; Yim, W.C.; Sun, Y.H.; Ohnishi, M.; Mimura, T.; Cushman, J.C. Identification of ice plant (*Mesembryanthemum crystallinum* L.) microRNAs using RNA-seq and their putative roles in high salinity responses in seedlings. *Front. Plant Sci.* **2016**, *7*, 1143. [[CrossRef](#)]
183. Yang, R.; Zeng, Y.; Yi, X.; Zhao, L.; Zhang, Y. Small RNA deep sequencing reveals the important role of microRNAs in the halophyte *Halostachys caspica*. *Plant Biotechnol. J.* **2015**, *13*, 395–408. [[CrossRef](#)]