Identification and Functional Characterization of Plant MiRNA Under Salt Stress Shed Light on Salinity Resistance Improvement Through MiRNA Manipulation in Crops

Tao Xu*†, Long Zhang††, Zhengmei Yang†‡, Yiliang Wei† and Tingting Dong*†

1 Jiangsu Key Laboratory of Phylogenomics and Comparative Genomics, School of Life Sciences, Jiangsu Normal University, Xuzhou, China, 2 Department of Applied Biology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, South Korea

Salinity, as a major environmental stressor, limits plant growth, development, and crop yield remarkably. However, plants evolve their own defense systems in response to salt stress. Recently, microRNA (miRNA) has been broadly studied and considered to be an important regulator of the plant salt-stress response at the post-transcription level. In this review, we have summarized the recent research progress on the identification, functional characterization, and regulatory mechanism of miRNA involved in salt stress, have discussed the emerging manipulation of miRNA to improve crop salt resistance, and have provided future direction for plant miRNA study under salt stress, suggesting that the salinity resistance of crops could be improved by the manipulation of microRNA.

Keywords: miRNA, plant, salt stress, tolerance, salinity resistance, crop

INTRODUCTION

Salinity, as a major environmental stress factor, restricts crop growth and yield globally. It is reported that salinity affected a land area as large as 800 million hectares across the globe, accounting for 6% of the land (Abdel Latef et al., 2020; Attia et al., 2021). Approximately 20% of the irrigated soils are affected by salinity stress (Zhao et al., 2013), and 50% of arable land will be affected by 2050 (Butcher et al., 2016). Salt stress leads to changes in metabolic activity, cell wall damage, and cytoplasmic dissolution; it reduces the photosynthetic efficiency, accelerates aging, increases respiratory consumption and toxin accumulation, and eventually results in plant death (Osman et al., 2020; Abdel Latef et al., 2021). It is estimated that salinity can result in $27.3 billion in agricultural damage every year (Qadir et al., 2014). On the other hand, regional food scarcity will persist continually, particularly in South Asia, sub-Saharan Africa, the Middle East, and where population increase is rapid but agricultural outputs are low (FAO, 2017). Therefore, breeding and growing salt-tolerant crops to utilize the marginal and high-salinity soils are one of the most
important strategies to meet the increase in food demand required by the estimated population in 2050 of 10 billion people (Mekonnen and Hoekstra, 2016; FAO, 2017; Morton et al., 2019).

MicroRNA (miRNA) is a non-coding single-stranded small RNA with a length of 21–24 nucleotides, and it acts as a gene regulator to control the transcript abundance of its target gene. In the wild, miRNA exists in diverse organisms, including plants, animals, and microorganisms, and it regulates growth, development, signal transduction, response to adversity, and other biological processes. It was firstly discovered in *Caenorhabditis* (Lee and Ambros, 2001) and was then detected in various organisms, including plants, animals, and microorganisms, and it regulates growth, development, signal transduction, response to adversity, and other biological processes. It was firstly discovered in *Caenorhabditis* (Lee and Ambros, 2001) and was then detected in four laboratories at approximately the same time in Llave et al. (2002), Mette et al. (2002), Park et al. (2002), Reinhart et al. (2002). After that, more and more plant miRNAs have been identified and functionally characterized in various plant species. MiRNA family names are listed in the order of publication, and miRNAs with similar sequences (usually fewer than 3 nt in difference) and common functions are classified as members of the same miRNA family (Wang Q. et al., 2014). Both the intraspecific conservation and interspecific differences of miRNAs are environmentally adaptive and evolve with the change in environment (Zhang et al., 2018). However, the evolution of miRNAs is conservative because some key target genes of miRNAs are conservative (Gramzow and Theilen, 2019).

Various enzymes and functional proteins are involved in the plant’s miRNA biosynthesis and functions. The primary miRNA transcripts for plants are produced by RNA polymerase II from miRNA genes, and these then pair with complementary bases to form special hairpin structures (Budak and Akpinar, 2015). Then, the stem ring secondary structure is generated by the DICER-LIKE1 (Bielewicz et al., 2013). After the methylation catalyzed by HUA Enhance 1 at the 3’ end, the double strand was transferred to the cytoplasm with the help of the transport protein HST. In the cytoplasm, this double-stranded miRNA is decomposed into mature single-stranded miRNA and integrated into RNA-induced silencing complex (RISC) cells, where miRNA interacts with the complementary target mRNA and activates the catalytic RISC with the assistance of Argonaute 1 (AGO1) (Koroban et al., 2016). There are two modes for miRNA to regulate gene expression: RNA cleavage and translation inhibition. The first mode is that miRNAs guide the Argonaute component of RISC to cleave a single phosphodiester bond opposite to the 10th and 11th nucleotides of the miRNA within complementary RNA. Then, the RISC will be free by releasing the fragments, and it then subsequently recognizes and cleaves another transcript (Jones-Rhoades et al., 2006). Afterward, the cleavage fragments are released to make the RISC competent for other RNA recognition and cleavage (Jones-Rhoades et al., 2006). MiRNA-mediated translational repression requires the participation of P-body components, a microtubule-severing enzyme, AGO1, and AGO10 (Brodersen et al., 2008). In addition, miRNA possibly prevents translation by triggering the sequestration of miRNA target in P-bodies (Chen, 2009). In addition, each miRNA can control multiple target genes (Haas et al., 2012). For instance, miR156 promotes floral meristem identity transformation by targeting SPL3, SPL4, and SPL5 in *Arabidopsis thaliana* (Xu et al., 2016). A gene can also be regulated by multiple miRNAs. For example, miR31 and miR143 affect steroid hormone synthesis by targeting the FSHR receptor (Zhang et al., 2019).

MiRNAs can regulate plant growth, development, pathogens, and abiotic stress responses. MiR160, miR169, peu-miRn68, and 477b are involved in the hormone signaling crosstalk model of root growth and development in apple rootstock, *A. thaliana* and *Populus* (Sorin et al., 2014; Lian et al., 2018; Meng et al., 2020). Cs-miR414 and cs-miR828 are involved in tea bud dormancy (Jeyaraj et al., 2014). For pathogen stress regulations, miR397 plays a negative regulatory role in apple resistance to hepatitis B virus (Yu et al., 2020), miR396 affects the susceptibility to rice blast (Chandran et al., 2019), and miR528 increases the viral defense ability of *Oryza sativa* (Wu et al., 2017). In the aspect of abiotic stress regulations, miR399 and miR827 are important for the resistance to phosphorus deficiency (Hackenberg et al., 2013; Du et al., 2018). The lack of sulfur induces the expression of miR395 for the regulation of genes in the sulfur assimilation pathway (Kawashima et al., 2009). The expression of miR319 is crucial for the cold tolerance of rice (Yang et al., 2013). MiR399 regulates *Arabidopsis* flowering at different temperatures (Kim et al., 2011). Recently, the comparative antagonistic expression profile of miR169 indicates that the miR169 family is a general regulator of various abiotic stresses (Rao et al., 2020). In addition, the over-expression of miR156 changes the expression level of other miRNAs, thus increasing the contents of anthocyanins, flavonoids, and flavonols and decreasing the total lignin content, suggesting the essential role of miRNAs in nutritional processes (Wang et al., 2020).

Noticeably, it is demonstrated that miRNA plays important roles in plant salinity responses and adaptation through various miRNA-mediated biological processes, including signal transduction, membrane transport, protein biosynthesis and degradation, photosynthesis, and transcription. In the present review, we mainly discuss the recent research progress on salt-stress-related miRNA in plants and the future research direction about miRNA in the salinity stress research field to come up with a strategy to improve the agronomic traits of stress tolerance through the manipulation of miRNAs.

### IDENTIFICATION AND EXPRESSION OF PLANT MiRNAs UNDER SALT STRESS

In recent years, with the rapid development of biotechnology, such as microarray and high-throughput deep sequencing, thousands of plant miRNAs were identified under salt stress. As shown in Table 1, different concentrations (80–600 mM) of NaCl and treatment time (3 h to 15 days) were applied for salt stress treatments for identifying salt-responsive miRNA (Table 1). MiRNAs were detected in leaf, root, stem, and flower separately or in the whole seedling (Table 1). Fu et al. identified 1,077 miRNAs in *Zea mays*, comprising the highest number of identified miRNAs in various crops among the reports (Fu et al., 2017). Moreover, 882, 876, 693, and 650 miRNAs were identified in *Mesembryanthemum*...
# The identification of plant miRNAs under salt stress by deep-sequencing.

| Latin name of sample | Sampling location | Salt stress treatment concentration/time | Number of miRNAs | References |
|----------------------|-------------------|------------------------------------------|------------------|------------|
| Arabidopsis thaliana | Root, bud         | 150 mM NaCl/7 d                          | 118              | Pegler et al., 2019 |
| Brassica juncea      | Seedling          | 150 mM NaCl, 200 mM NaCl/3 h, 6 h, 12 h, 24 h | 51               | Bharati et al., 2014 |
| Brassica oleracea    | Flower            | 80 mM NaCl/15 d                          | 81               | Tian et al., 2014 |
| Cicer arietinum      | Root              | 150 mM NaCl/12 h                         | 181              | Kohli et al., 2014 |
| Cicer arietinum      | Root              | 250 mM NaCl/2 h                          | 284              | Khandal et al., 2017 |
| Eutrema salsugineum | Seedlings         | 300 mM NaCl/0 h, 5 h, 12 h               | 99               | Wu et al., 2016 |
| Glycine max          | Mature nodules    | 125 mM NaCl/6 h                          | 238              | Dong et al., 2013 |
| Halostachys caspica | Root              | 600 mM NaCl/48 h                         | 272              | Yang et al., 2015 |
| Hordeum bulbosum     | Seedling          | 250 mM NaCl/2 w                          | 54               | Liu and Sun, 2017 |
| Hordeum vulgare      | The plant body    | 100 mM NaCl/3 h, 8 h, 27 h               | 152              | Deng et al., 2015 |
| Hordeum vulgare      | Seedling, leaves, roots | 2% NaCl/-                              | 259             | Lv et al., 2012 |
| Ipomoea batatas      | Leaves, roots     | 150 mM NaCl/-                           | 650              | Yang et al., 2020 |
| Lagenaria siceraria   | Root              | 100 mM NaCl/4 h                          | 91               | Xie J. et al., 2015 |
| Leymus chiniensis    | Seedling          | 100 mM NaCl and 200 mM NaHCO3/24 h      | 148              | Zhai et al., 2014 |
| Linum usitatissimum  | -                 | 50 mM NaCl/18 h                          | 332              | Yu et al., 2016 |
| Malvaceae Gossypium  | Leaves            | 150 mM NaCl/2 h, 4 h, 8 h               | 225              | Yin et al., 2017 |
| Medicago sativa      | Root              | 300 mM NaCl/8 h                          | 453              | Long et al., 2015 |
| Medicago truncatula  | Seedling          | 20 mM NaCl + Na2SO4 5 mM Na2CO3 + NaHCO3/72 h | 876          | Cao et al., 2018 |
| Mesembryanthemum crystallinum | Seed | 200 mM NaCl/80 h                        | 967              | Jian et al., 2016 |
| Mesembryanthemum crystallinum | Seedling, root | 200 mM NaCl/8 h                        | 135             | Chiang et al., 2016 |
| Musa nana            | Root              | 0mm (CTR), 100mm (TR100), and 300mm (TR300) NaCl/48 h | 181              | Lee et al., 2015 |
| Onzya glaberrima     | Leaves            | 200 mM NaCl/48 h                         | 498              | Mondal et al., 2018 |
| Onzya coarctata      | Root              | 450 mM NaCl/24 h                         | 433              | Mondal et al., 2015 |
| Onzya sativa         | Leaves            | 200 mM NaCl/15 d                         | 357              | Tripathi et al., 2018 |
| Onzya sativa         | Root, stem        | 256 mM NaCl/9 h                          | 275              | Parmar et al., 2020 |
| Panicum virgatum     | Seedling          | 0.5% NaCl/10 d                          | 273              | Xie et al., 2014 |
| Paulownia            | Seeding           | 0.2%, 0.4% and 0.6% NaCl/20 d           | 187              | Fan et al., 2016 |
| Phoenix dactylfera   | Seedling, leaves and roots | 300 mM NaCl/72 h                   | 422              | Yaish et al., 2015 |
| Populus euphratica   | Leaves, roots     | 300 mM NaCl/3w                          | 428              | Si et al., 2014 |
| Populus tomentosa    | Seeding           | 200 mM NaCl/10 h                         | 187              | Ren et al., 2013 |
| Raphanus sativus     | Root              | 200 mM NaCl/3 h, 6 h, 12 h, 24 h, 48 h, 96 h | 204              | Sun et al., 2015 |
| Reaumuria soongorica | Seed              | 43, 273 mM NaCl/-                        | 101              | Zhang H. et al., 2020 |
| Rhizophora mangle    | Leaves            | 340 mM NaCl/96 h                         | 147              | Gharat and Shaw, 2015 |
| Saccharum officinarum| Shoot, root       | 170 mM NaCl/-                           | 131              | Bottino et al., 2013 |
| Saltmaria europeea   | Root, stem        | 200 mM NaCl/0 h, 12 h, 7 d              | 241              | Feng et al., 2015 |
| Sesamum indicum      | Seeding           | --/12 h, 24 h                           | 442              | Zhang Y. et al., 2020 |
| Solanum melongena    | Root              | 150 mM NaCl/24 h                         | 98               | Zhuang et al., 2014 |
| Spartina alterniflora| Leaf and root     | 500 mM sea salt/6, 12, 24, 72 h         | 902              | Zandkarimi et al., 2015 |
| Suaeda maritima      | Aerial portions   | 255 mM NaCl/9 h                         | 147              | Gharat and Shaw, 2015 |
| Thellungiella salsuginea | Leaves, roots | 200 mM NaCl/24 h                        | 246              | Zhang et al., 2013 |
| Triticum aestivum    | Seeding           | 200 mM NaCl/7 d                         | 317              | Han et al., 2018 |
| Triticum monococcum subsp. monococcum | Leaves, roots | 100 mM NaCl/0, 3 h, 6 h, 12 h, 24 h | 167 | Ünlü et al., 2018 |
| Triticum turgidum ssp. dicoccoides | The plant body | 150 mM NaCl/0 h, 3 h, 6 h, 12 h, 24 h | 212 | Feng et al., 2017 |
| Vicia faba           | Seeding           | 150 mM NaCl/2 w                         | 693              | Alzahrani et al., 2019 |
| Zea mays             | Leaves and roots  | 250 mM NaCl/12 h                        | 1077             | Fu et al., 2017 |
| Zea mays             | Maize ears        | --/--                                    | 102              | Liu et al., 2014 |

* indicates the plant name of halophyte; - indicates no related information.
crystallinum, Medicago truncatula, Vicia faba, and Ipomoea batatas, respectively (Jian et al., 2016; Cao et al., 2018; Alzahrani et al., 2019; Yang et al., 2020). The numbers of identified miRNA vary from dozens to hundreds, which may be due to the plant species, tissue specificity, development stage, and salt stress treatment methods. However, the large-scale identification of miRNAs under salt stress is very necessary and essential, and it lays a solid foundation for the further illumination of the miRNA network.

The expression levels of miRNA are up- or down-regulated by salinity stress. For instance, the expression of miR167 in panicle is negatively correlated with the increase of salt concentration (Jodder et al., 2018). In cotton, miR156, miR157, and miR172 are up-regulated at 0.25% NaCl, but their expression decreases with increasing salt concentration (Wang et al., 2013). The expression of miR164 also decreases with the increase of salt stress in maize (Shan et al., 2020). Macovei et al. found that the expression levels of Osa-miR414, -miR164e, and -miR408 significantly decrease with increased salt stress and further regulate the occurrence of genes to resist external salt stress by increasing the content of helicases (Macovei and Tutu, 2012). In addition, some miRNAs are expressed differently in the early and late stages of salt stress treatment. For example, zma-miR169 displays initial up-regulation and subsequent down-regulation under salt stress (Luan et al., 2015). MiRNAs and their targets, such as cotton miR156-SPL2, miR159-TCP3, miR162-DCL1, miR395-APS1, and miR396-GRF1, exhibit negative correlation on expression levels (Wang et al., 2013).

Table 2 shows the expression levels of some representative miRNAs in plants under salt stress. MiR156, miR319, and miR528 are induced by salinity stress (Wang et al., 2013; Stief et al., 2014; Zhou and Luo, 2014; Xie F. et al., 2015; Yuan et al., 2015), while miR164 and miR397 are repressed (Macovei and Tutu, 2012; Wang et al., 2013; Gupta et al., 2014; Qin et al., 2015; Xie F. et al., 2015; Lu et al., 2017), which were confirmed at least in two plant species (Table 2). Interestingly, the expression levels of nine miRNAs (e.g., miR159, miR168, miR169, miR172, miR393, miR395, miR396, miR399, and miR408) were promoted in some plant species but were inhibited in the other plant species. For instance, salinity stress increases the expression of miR393 in Arabidopsis thaliana, Triticum aestivum, and Agrostis stolonifera, but decreases the expression of miR393 in Oryza sativa, Gossypium sp., and Spartina alterniflora (Xia et al., 2012; Gupta et al., 2014; Iglesias et al., 2014; Qin et al., 2015; Xie F. et al., 2015; Zhao et al., 2019). Similarly, the expression of miR396 is increased by salinity in Solanum lycopersicum, Nicotiana tabacum, and Agrostis stolonifera but decreased in Arabidopsis thaliana, Oryza sativa, and Spartina alterniflora (Gao et al., 2010; Chen L. et al., 2015; Qin et al., 2015; Cao et al., 2016; Yuan et al., 2019). Up- or down-regulated gene expression usually suggests potential positive or negative functional role. However, the same miRNA has an opposite expression pattern in different plant species under salinity stress conditions, suggesting the same miRNA may play a diverse role in different plant species under salt stress. Moreover, the expression levels of some miRNAs, including miR167, miR390, miR394, miR402, and miR414 were only investigated in very few plant species under salinity stress (Table 2). Considering some miRNAs displayed totally different expressions in different species, their expression patterns need to be investigated in more plant species under salinity stress conditions.

**MiRNA STUDIES IN HALOPHYTE PLANTS**

Glycophyte plants, such as Arabidopsis and rice, can only survive at salinity levels 0–100 mM NaCl without any capability to adapt to high salt stress (Horie et al., 2012), whereas some remarkable halophytes can tolerate salinity levels as high as >1000 mM NaCl (Flowers and Colmer, 2008; Munns and Tester, 2008). To an extent, the salt-sensitive glycophytes may not provide enough insights into salt tolerance mechanisms, and the halophytes may have more value for expanding our knowledge about salt resistance mechanisms. Therefore, the exploration of the role of halophyte miRNAs in salinity adaptation can offer compelling contributions for devising strategies of resistance improvement in crops through genetic engineering and plant selection programs. However, there are not many reports on the discovery of salt-responsive miRNAs in halophytes (Table 1).

The halophyte plant *Suaeda maritima* grows naturally along the seashore. The expression of *S. maritima* sma-miR2 and sma-miR5 increases under the influence of seawater, suggesting their metabolic regulatory roles specific to saline environments (Gharat and Shaw, 2015). *Eutrema salsugineum*, a close relative of *A. thaliana*, can thrive in high salt conditions ranging from 100 to 500 mM (Amtmann, 2009). *E. salsugineum* has been developed as a valuable model plant for salt stress-tolerance study because its salinity tolerance is extreme, its lifetime is short, its seed production is copious, and its transformation is easy (Zhu, 2000; Amtmann et al., 2005). Zhang et al. (2013) identified 246 miRNAs candidates in *E. salsugineum*. In addition, 26 conserved miRNAs and 4 novel miRNAs were found to display a significant response to salt stress in *E. salsugineum* (Zhang et al., 2013; Wu et al., 2016). Recently, 88 conserved miRNAs and 13 novel miRNAs were identified from *Reaumuria soongorica* seeds treated with various NaCl concentrations, providing a useful reference for salt resistance improvement of seed germination (Zhang et al., 2020). A total of 135 conserved miRNAs and the hairpin precursor of 12 novel mcr-miRNAs were found from *M. crystallinum* seedlings treated with 200 mM NaCl (Chiang et al., 2016). *Oryza coarctata* is a wild relative of rice and grown in saline water. Mondal et al. found 338 known and 95 novel miRNAs in salt-treated *O. coarctata* leaves, providing a miRNA-target networking that is involved in salt stress adaption (Mondal et al., 2015). *Halostachys caspica* (Bieb.), a salt-tolerant short shrub, can be naturally grown on the field with a salt concentration as high as 100 g/kg dry soil (Song et al., 2006). (Yang et al., 2015) found that 31 conserved miRNAs and 12 novel miRNAs were significantly up-regulated, and 48 conserved miRNAs and 13 novel miRNAs were significantly down-regulated by salinity stress in *H. caspica*. A set of miRNAs were also identified in a salt marsh monocot halophyte smooth cordgrass (*Spartina alterniflora* Loisel) and another plant named salt cord
TABLE 2 | The expression of representative plant miRNAs under salt stress.

| MiRNA      | Expression level                                      | Arabidopsis thaliana | Oryza sativa | Solanum lycopersicum | Gossypium hirsutum | Zea mays | Triticum aestivum | Nicotiana tabacum | Agrostis stolonifera | Spartina alterniflora |
|------------|-------------------------------------------------------|-----------------------|--------------|----------------------|--------------------|----------|-------------------|-------------------|----------------------|----------------------|
| MIR156     | ∨ Stief et al., 2014                                  |                       |              |                      |                    |          | Kang et al., 2020 | Kang et al., 2020  |                      |                      |
| MIR159     | ∨ Xie F. et al., 2015; Wang et al., 2013              |                       |              |                      |                    |          | Wang B. et al., 2014 |                   |                      |                      |
| MIR164     | ∨ Lu et al., 2017                                    |                       |              |                      |                    |          | Fu et al., 2017    | Gupta et al., 2014 |                      |                      |
| MIR167     | ∨ Jodder et al., 2018                                 |                       |              |                      |                    |          |                   | Qin et al., 2015   |                      |                      |
| MIR168     | ∨ Ding et al., 2009                                   |                       |              |                      |                    |          |                   | Gupta et al., 2014 |                      |                      |
| MIR169     | ∨ Zhao et al., 2009                                   |                       |              |                      |                    |          | (1-48h), ∨ (15d) Luan et al., 2014 |                   |                      |                      |
| MIR172     | ∨ Jodder et al., 2018                                 |                       |              |                      |                    |          |                   |                       |                      |                      |
| MIR319     | ∨ Xie F. et al., 2015                                 |                       |              |                      |                    |          |                   | Zhou and Luo, 2014 |                      |                      |
| MIR390     | ∨ Xie F. et al., 2015                                 |                       |              |                      |                    |          |                   |                   |                      |                      |
| MIR393     | ∨ Iglesias et al., 2014                              |                       |              |                      |                    |          | Fu et al., 2017    | Gupta et al., 2014 |                      |                      |
| MIR394a    | ∨ Song et al., 2013                                   |                       |              |                      |                    |          |                   | Zhao et al., 2019  |                      |                      |
| MIR395     | ∨ Song et al., 2013                                   |                       |              |                      |                    |          |                   |                   |                      |                      |
| MIR396     | ∨ Gao et al., 2010                                    |                       |              |                      |                    |          | Wang et al., 2013  |                   |                      |                      |
| MIR397     | ∨ Gao et al., 2010                                    |                       |              |                      |                    |          |                   | Chen L. et al., 2015 |                      |                      |
| MIR398     | ∨ Jagadeeswaran et al., 2009                          |                       |              |                      |                    |          | Wang et al., 2013  |                   |                      |                      |
| MIR399     | ∨ Guddeti et al., 2005                                |                       |              |                      |                    |          |                   |                      |                      |                      |
| MIR402     | ∨ Kim et al., 2010a                                   |                       |              |                      |                    |          |                   |                      |                      |                      |
| MIR408     | ∨ Guo et al., 2018                                    |                       |              |                      |                    |          |                   |                      |                      |                      |
| MIR414     | ∨ Guo et al., 2018                                    |                       |              |                      |                    |          |                   |                      |                      |                      |
| MIR528     | ∨ Yuan et al., 2015                                   |                       |              |                      |                    |          |                   |                      |                      |                      |

↑ and ↓ indicate the expressions of miRNAs are increased and decreased, respectively. % indicates the salt concentration.
### TABLE 3 | The functions of miRNA under salt stress.

| Species | Common Name | MiRNA name | Target gene | Salt tolerance phenotype | Method/Technology | References |
|---------|-------------|------------|-------------|--------------------------|------------------|------------|
| Malus domestica | Apple | MiR156a | MdSPL13 | Overexpressing MiR156a weakened salt resistance in apple, whereas MdSPL13 strengthened | MiR156a and SPL13 overexpression | Ma et al., 2020 |
| Populus euphratica | Creeping bentgrass | Peu-miR164 | AsPCF5, AsPCF6, AsPCF8, AsTOP14 | Enhanced drought, salt tolerance, increased leaf wax content and water retention, but reduced sodium uptake | Overexpressing Osa-miR19a in creeping bentgrass | Zhou and Luo, 2014; Zhou et al., 2013 |
| Glycine max | Soybean | MR169 | GmNFYA3 | Reduced leaf water loss, enhanced drought tolerance and increased sensitivity to high salinity and exogenous ABA in GmNFYA3 overexpression plants | Overexpress GmNFYA3 in Arabidopsis | Ni et al., 2013 |
| Glycine max | Soybean | Gma-miR172c | Glyma01g39520 | Soybean miR172c confers tolerance to water deficit and salt stress, but increases ABA sensitivity in transgenic Arabidopsis thaliana | Overexpress of soybean miR172c | Li et al., 2016 |
| Glycine max | Soybean | MiR172c | NNC1 | Overexpression and knockdown of miR172c activity resulted in substantially increased and reduced root sensitivity to salt stress, respectively | Overexpress miR172c and knockdown miR172c | Sahito et al., 2017 |
| Agrostis stolonifera | Creeping bentgrass | Osa-miR319a | AsPCF5, AsPCF6, AsPCF8, AsTOP14 | Overexpression of Osa-miR319b in Swichgrass | Overexpress Osa-miR319b in Arabidopsis | Liu et al., 2019 |
| Panicum virgatum | Switchgrass | Osa-miR319b | PvPCF5 | Osa-miR319b positively regulated ET synthesis and salt tolerance | Overexpression of Osa-miR319b, target mimic miR319 in swichgrass | Li et al., 2019 |
| Populus spp. | Poplar | MiR390 | ARF3.1, ARF3.2, ARF4 | Stimulated LR development and increased salt tolerance | Overexpress and knockdown (STTM) miR390 in poplar | He et al., 2018 |
| Helianthus tuberosus | Jerusalem artichoke | MiR390 | TAS3, ARF3/4 | May play an active role in salt tolerance | Bioinformatics, gene cloning and RT-qPCR analyses | Wen et al., 2020 |
| Arabidopsis thaliana | Arabidopsis | MiR393 | TIR1, AFB2 | MR393ab mutant shows reduced inhibition of LR number and length, increased levels of ROS in LRs, and reduced APX enzymatic activity | Overexpress miR393ab double mutant was obtained from the cross of miR393a-1 and miR393b-1 | Iglesias et al., 2014 |
| Arabidopsis thaliana | Arabidopsis | MiR393 | TIR1 | Enhanced salt tolerance in mTIR1 transgenic plant | Overexpressing miR393-resistant form mTIR1 in Arabidopsis | Chen Z. et al., 2015 |
| Oryza sativa | Rice | Os-miR393 | OsTIR1, OsAFB2 | Reduced tolerance to salt and drought, increased tillers and early flowering | Overexpressing Os-miR393 in rice | Xia et al., 2012 |
| Oryza sativa | Rice | Osa-miR393 | LOC_Os02g06260, LOC_Os05g41010, LOC_Os05g05800 | Transgenic plants were more sensitive to salt and alkal treatment | Overexpressing Os-miR393 in rice and Arabidopsis | Gao et al., 2011 |
| Agrostis stolonifera | Creeping bentgrass | Osa-miR393a | AsTIR1, AsAFB2 | Improved salt stress tolerance associated with increased uptake of potassium | Overexpressing Osa-miR393a in creeping bentgrass | Zhao et al., 2019 |
| Arabidopsis thaliana | Arabidopsis | MiR394a/b | LCR | MR394a/b over-expression and lcr (LCR loss of function) mutant plants are hypersensitive to salt stress, but LCR over-expressing plants display the salt-tolerant phenotype | Overexpressing miR394a/b and LCR in Arabidopsis | Song et al., 2013 |
| Arabidopsis thaliana | Arabidopsis | MiR395c, MiR395e | APS1, APS3, APS4, SULTR2;1 | Overexpression of miR395c or miR395e retarded and accelerated, respectively, the seed germination of Arabidopsis under high salt or dehydration stress conditions | Overexpression of miR395c or miR395e in Arabidopsis | Kim et al., 2010b |
| Oryza sativa | Rice | Osa-miR396c | LOC_Os01g32750, LOC_Os02g45570, LOC_Os04g5119 | Reduced salt and alkal stress tolerance | Overexpressing osa-miR396c in rice and Arabidopsis | Gao et al., 2010 |

(Continued)
TABLE 3 | Continued

| Species       | Common Name       | MiRNA name        | Target gene | Salt tolerance phenotype                                                                 | Method/Technology | References                 |
|---------------|-------------------|-------------------|-------------|----------------------------------------------------------------------------------------|-------------------|----------------------------|
| Agrostis stolonifera | Creeping bentgrass | Osa-miR396c       | GRF         | Enhanced salt tolerance associated with improved water retention, increased chlorophyll content, cell membrane integrity, and Na+ exclusion during high salinity exposure | Overexpressing Osa-miR396c in creeping bentgrass | Yuan et al., 2019         |
| Solarum pimpinellifolium | Tomato            | Sp-miR396a-5p     | GRF1,GRF3, GRF7,GRF8 | Enhanced its tolerance to salt, drought and cold stresses                              | Overexpressing Sp-miR396a-5p in tobacco | Chen L. et al., 2015       |
| Arabidopsis thaliana | Arabidopsis      | MiR399f           | ABF3, CSP41b | Plants overexpressing miR399f exhibited enhanced tolerance to salt stress, but hypersensitivity to drought | Overexpressing miR399f in Arabidopsis | Baek et al., 2016          |
| Arabidopsis thaliana | Arabidopsis      | MiR402            | DEMETER-LIKE protein3 | Accelerated the seed germination and seedling growth of Arabidopsis under salt stress conditions | Overexpression of miR402 in Arabidopsis | Kim et al., 2010a          |
| Arabidopsis thaliana | Arabidopsis      | MiR408            | Plantacycin, Cupredoxin, Uclacycin, LAC3 | Improved tolerance to salinity, cold and oxidative stress, but enhanced sensitivity to drought and osmotic stress | Overexpressing miR408 in Arabidopsis | Ma et al., 2015            |
| Triticum aestivum | Wheat             | Tae-miR408        | TaCLP1      | Significantly increased cell growth under high salinity and Cu2+ stresses               | Overexpressing TaCLP1 in yeast | Feng et al., 2013          |
| Triticum aestivum | Wheat             | TaemiR408         | TaCP, TaMP, TaBCP, TaFP, TaKRP, TaABP | Enhanced stress tolerance, improved phenotype, biomass, and photosynthesis behavior under salt treatments | Overexpressing TaemiR408 in tobacco | Bai et al., 2018           |
| Salvia miltiorrhiza | -                 | Sm-miR408         | Copper-binding proteins, Laccase | Promoted seed germination and reduced the accumulation of ROS under salt stress, positive responses to salt tolerance | Overexpressing Sm-miR408 in tobacco | Guo et al., 2018           |
| Gossypium spp.    | Cotton            | MiR414c           | GhFSD1      | Overexpressing miR414c increased sensitivity to salinity stress, yielding a phenotype similar to that of GhFSD1-silenced cotton | Silence GhFSD1 in cotton, overexpressing ghr- miR414c and GhFSD1 in Arabidopsis | Wang et al., 2019          |
| Arabidopsis thaliana | Arabidopsis      | MiR417            | At1g04150, At1g17730, At5g66460, At5g49680, At4g11130, At1g48310, At3g06400, At1g19850 | Seed germination of the transgenic plants was retarded under high salt condition | Overexpression of miRNVL417 in Arabidopsis | Jung and Kang, 2007        |
| Agrostis stolonifera | Creeping bentgrass | Osa-miR528        | AsAAO, AsCBP1 | Shortened internodes, increased tiller number, and upright growth, enhances tolerance to salinity stress and nitrogen starvation | Overexpressing Osa-miR528 in creeping bentgrass | Yuan et al., 2015          |
| Gossypium hirsutum | Cotton            | MiRNVL5           | GhCHR       | Arabidopsis constitutively expressing miRNVL5 showed hypersensitivity to salt stress    | Ectopic expression of miRNVL5 and GhCHR in Arabidopsis | Gao et al., 2016           |

(Thellungiella salsuginea) (Zhang et al., 2013; Zandkarimi et al., 2015). These identified miRNAs in halophytes can be further projected as potential miRNAs for developing salt tolerance in glycophyte crops.

FUNCTIONS OF MiRNA UNDER SALT STRESS

Numerous plant miRNAs have been identified under salt stress, but not many miRNAs have been functionally characterized in detail. Table 3 shows us the miRNAs responsive to salt stress, and these which were functionally studied by transgenic approaches, such as overexpression and knocked down/out of the miRNA itself or its targets (Table 3). For instance, miR394a/b over-expression and lcr (functional loss of miR394 target LCR) mutant plants are hypersensitive to salt stress, but LCR over-expressing plants display the salt-tolerant phenotype (Song et al., 2013). MiR393 is a comparative well-studied plant miRNA in different plant species, including Arabidopsis, rice, and creeping bentgrass. MiR393ab mutant shows reduced inhibition of LR (lateral root) number and length, increased levels of ROS in LRs, and reduced APX enzymatic activity (Iglesias et al., 2014). Over-expressing Osa-miR393 in rice and Arabidopsis reduces tolerance to salt and drought and increases tillers and early flowering (Gao et al., 2011; Xia et al., 2012), while
over-expressing miR393-resistant form mTIR1 in Arabidopsis enhances salt tolerance in mTIR1 transgenic plant (Chen Z. et al., 2015). However, over-expressing Osa-miR393a in creeping bentgrass improves salt stress tolerance associated with the increased uptake of potassium (Zhao et al., 2019), suggesting that the same miRNA or different miRNA from the same miRNA family may have different promotion and inhibition effects on salt tolerance in different plants. A similar situation was found for miRNA396, that is, over-expressing Osa-miR396c reduced salt and alkalii stress tolerance in rice and Arabidopsis (Gao et al., 2010), but enhanced salt tolerance associated with improved water retention, increased chlorophyll content, cell membrane integrity, and Na+ exclusion during high salinity exposure in creeping bentgrass (Yuan et al., 2019). Additionally, over-expressing 5p-miR396a-5p in tobacco enhanced its tolerance to salt, drought, and cold stresses (Chen L. et al., 2015). The overexpression of miR395c or miR395e retarded and accelerated, respectively, the seed germination of Arabidopsis under high salt or dehydration stress conditions (Kim et al., 2010b).

Over-expressing miR156a weakens salt resistance in apples, whereas its target gene MdSPL13 strengthens salt resistance (Ma et al., 2020). Transgenic Arabidopsis plants over-expressing the target gene PeNAC070 of miR164 exhibits promoted LR development, delayed stem elongation, and increased sensitivity to salt stress (Lu et al., 2017). Over-expressing the target gene GmNFYA3 of miR169 reduces leaf water loss, enhances drought tolerance, and increases sensitivity to high salinity and exogenous ABA (Ni et al., 2013). Over-expression of miR172c substantially increased the sensitivity of plant roots to salt stress, and the removal of miR172c would decrease the sensitivity of plant roots to salt stress, respectively (Li et al., 2016; Sahito et al., 2017). Osa-miR319a and miR319b positively regulate salt tolerance in creeping bentgrass and switchgrass, respectively (Zhou et al., 2013; Zhou and Luo, 2014; Liu et al., 2019). MiR390 increases LR growth under salt stress via the auxin pathway (He et al., 2018). Additionally, over-expressing miR399f, miR402, and miR408 in Arabidopsis, Tae-miR408 and Sm-MIR408 in tobacco, and Osa-miR528 in creeping bentgrass increases salinity tolerance (Kim et al., 2010a; Feng et al., 2013; Ma et al., 2015; Yuan et al., 2015; Back et al., 2016; Bai et al., 2018; Guo et al., 2018), indicating that these miRNAs enhance plant salt stress adaptation. By contrast, over-expressing miR414c, miR417, and miRNVL5 increases sensitivity to salinity stress (Jung and Kang, 2007; Gao et al., 2016; Wang et al., 2019). Collectively, these results suggest that the agronomic trait of salinity stress tolerance could be enhanced by the manipulation of miRNA or its target.

DISCUSSION AND FUTURE PROSPECTS

In the face of soil salinization, the cultivation of saline-tolerant plants is one of the most economical and effective technologies for biological improvement. Understanding the molecular mechanisms of miRNAs in abiotic stress provides an effective tool for plant breeding, especially in the context of climate and human-induced environmental changes. The essential regulating role of miRNAs in plant salt stress response reveals that miRNA could be applied for salt resistance improvement in crops. The salinity resistance of transgenic plants can be remarkably increased by over-expressing miRNA or knocking down/out the target gene of miRNA. Alternatively, the salinity resistance can be promoted by knocking down/out miRNA, which has a negative effect on salinity response, or over-expressing the target gene of the miRNA. Considering that one miRNA may have more than one targets that would cause totally different effects on plants, we should carefully consider the miRNA effects on crop growth, development, and the sensitivity to other abiotic stresses when optimizing the salinity resistance by miRNA manipulation.

The homologous tetraploid was more tolerant to salt stress than the diploid. Moreover, novel miRNAs induced by genome replication were identified, suggesting salt-responsive miRNAs could be screened by comparative analysis on the plant materials with different ploidy and salinity stress tolerance to explain the key roles of miRNA in achieving better salt stress tolerance. Generally, miRNAs are evolutionarily conserved in their functions in response to salt stress. However, the same miRNAs or different miRNAs from the same miRNA family may have different promotion and inhibition effects on salt tolerance in different plants. Therefore, the function of some miRNAs should be widely studied in different species, especially in crops.

Moreover, considering the significant number of salt-stress-responsive miRNAs identified by using powerful technology (such as high throughput sequencing), only a few miRNAs have been functionally characterized. Therefore, after the identification of plant miRNAs under salinity stress, further studies should be focused on the exploration of function, which will be very crucial for the salt tolerance improvement through miRNA manipulation in crops. Additionally, miRNAs may affect the plant stress tolerance through their interaction with ABA biosynthesis and the regulation of auxin response factors, The investigation of the crosstalk between miRNA and plant hormone will thus expand our knowledge and understanding of the role of plant miRNAs under stress conditions. Finally, the construction of the plant miRNA network in salt stress response will shed light on the salinity resistance improvement through miRNA manipulation in crops.

AUTHOR CONTRIBUTIONS

TX conceived and designed this manuscript. TX, LZ, and ZY wrote the manuscript. YW and TD helped to revise the manuscript. All authors read and approved the manuscript.

FUNDING

This work was supported jointly by the projects of the National Natural Science Foundation of China (32072117 and 31701481), the Natural Science Foundation of Jiangsu Higher Education Institutions of China (19KJA510010), and the Key R&D Program of Xuzhou-Modern Agriculture (KC20039).
REFERENCES

Abdel Latef, A. A., Abu Alhmad, M. F., Kordrostami, M. F., Abo-Baker, A. E., and Zakir, A. (2020). Inoculation with Azospirillum lipoforum or Azotobacter chroococcum reinforces maize growth by improving physiological activities under saline conditions. J. Plant Growth Regul. 39, 1293–1306. doi: 10.1007/s00344-020-10665-9

Abdel Latef, A. A., Omer, A. M., Badawy, A. A., Osman, M. S., and Ragaey, M. M. (2021). Strategy of salt tolerance and interactive impact of Azotobacter chroococcum and/or Alcaligenes faexalis inoculation on canola (Brassica napus L.), plants grown in saline soil. Plants 10:110. doi: 10.3390/plants10010110

Alzahrani, S. M., Alaraidh, I. A., Khan, M. A., Migdadi, H. M., Alghamdi, S. S., and Alshahr, A. A. (2019). Identification and characterization of salt-responsive microRNAs in Vicia faba by high-throughput sequencing. Genes 10:303. doi: 10.3390genes10040303

Amtmann, A. (2009). Learning from evolution: thellungiella generates new knowledge on essential and critical components of abiotic stress tolerance in plants. Mol. Plant 2, 3–12. doi: 10.1093/mp/snn094

Amtmann, A., Bohnert, H. J., and Bressan, R. A. (2005). Abiotic stress and plant genome evolution. Search for new models. Plant Physiol. 138, 127–130. doi: 10.1104/pp.105.059972

Attia, M. S., Osman, M. S., Mohamed, A. S., Mahgoub, H. A., Garada, M. O., Abdelmouty, E. S., et al. (2021). Impact of folic application of chitosan dissolved in different organic acids on isozymes, protein patterns and physiochemical characteristics of tomato grown under salinity stress. Plants 10:388. doi: 10.3390/plants10020388

Baek, D., Chun, H. J., Kang, S., Shin, G., Park, S. J., Hong, H., et al. (2016). A miRNA regulatory module balances growth and rice blast disease-resistance. Front. Plant Sci. 7:1143. doi: 10.3389/fpls.2016.01143

Deng, P., Wang, L., Cui, L., Feng, K., Liu, F., Du, X., et al. (2015). Global identification of microRNAs and their targets in barley under salinity stress. PLoS One 10:e0137990. doi: 10.1371/journal.pone.0137990

Chiang, C. P., Yim, W. C., Sun, Y. H., Ohnishi, M., Mimura, T., Cushman, J. C., et al. (2016). Identification of ice plant (Mesembryanthemum crystallinum L.) microRNAs using RNA-seq and their putative roles in high salinity responses in seedlings. Front. Plant Sci. 7:1143. doi: 10.3389/fpls.2016.01143

Ding, D., Zhang, L., Wang, H., Liu, Z., Zhang, Z., and Zheng, Y. (2009). Differential expression of miRNAs in response to salt stress in maize roots. Ann. Bot. 103, 29–38. doi: 10.1093/aob/mcn205

Dong, Z., Shi, L., Wang, Y., Chen, L., Cai, Z., Wang, Y., et al. (2013). Identification and dynamic regulation of microRNAs involved in salt stress responses in functional soybean nodules by high-throughput sequencing. Int. J. Mol. Sci. 14, 2717–2738. doi: 10.3390/ijms14022717

Du, Q. G., Wang, K., Zou, C., Xu, C., and Li, W. X. (2018). The PILNCR1-miR399 regulatory module is important for low phosphate tolerance in maize. Plant Physiol. 177, 1743–1753. doi: 10.1007/pp.18.00034

Fan, G., Li, X., Deng, M., Zhao, Z., and Yang, L. (2016). Comparative analysis and identification of miRNAs and their target genes responsive to salt stress in diploid and tetraploid Pennisetum glaucum var. fortunei seedlings. PLoS One 11:e0149617. doi: 10.1371/journal.pone.0149617

FAO (2017). The Future of Food and Agriculture - Trends and Challenges. Rome: Food and Agriculture Organization of the United Nations.

Feng, H., Zhang, Q., Wang, Q., Wang, X., Liu, J., Li, M., et al. (2013). Target of tae-miR408, a chenopodin-like protein gene (TaCLP1), plays positive roles in wheat response to high-salinity, heavy cupric stress and stripe rust. Plant Physiol. 163, 433–443. doi: 10.1104/PP.113.01013-01019

Feng, J., Wang, J., Fan, P., Jia, W., Nie, L., Jiang, P., et al. (2015). High-throughput deep sequencing reveals that microRNAs play important roles in salt tolerance of eulahlyphete Salicornia europaea. BMC Plant Biol. 15:63. doi: 10.1186/s12870-015-0451-3

Feng, K., Nie, X., Cui, L., Deng, P., Wang, M., and Song, W. (2017). Genome-Wide identification and characterization of salinity stress-responsive miRNAs in wild emmer wheat (Triticum turgidum ssp. dicoccoides). Genes 8:156. doi: 10.3390/genes8060156

Flowers, T. J., and Colmer, T. D. (2008). Salinity tolerance in halophytes. New Phytol. 179, 945–963. doi: 10.1111/j.1469-8137.2008.02531.x

Frazier, T. P., Sun, G., Burklew, E., and Zhang, B. (2011). Salt and drought stresses induce the aberrant expression of microRNA genes in tobacco. Mol. Biotechnol. 49, 159–165. doi: 10.1007/s12033-011-9387-5

Fu, R., Zhang, M., Zhao, Y., He, X., Ding, C., Wang, S., et al. (2017). Identification of salt tolerance-related microRNAs and their targets in maize (Zea mays L.) using high-throughput sequencing and degradome analysis. Front. Plant Sci. 8:884. doi: 10.3389/fpls.2017.00884

Gao, P., Bai, X., Yang, L., Lv, D., Li, Y., Cai, H., et al. (2010). Over-expression of osa-MIR396c decreases salt and alkaline stress tolerance. Plant 231, 991–1001. doi: 10.1007/PP.1130-10104-2

Gao, P., Bai, X., Yang, L., Lv, D., Fan, X., Li, Y., et al. (2011). Osa-MIR393a: a salinity- and alkaline stress-related microRNA gene. Mol. Biol. Rep. 38, 237–242. doi: 10.1007/s11033-010-0100-8

Gao, S., Yang, L., Zeng, H. Q., Zhou, Z. S., Yang, Z. M., Li, H., et al. (2016). A cotton miRNA is involved in regulation of plant response to salt stress. Sci. Rep. 6:19736. doi: 10.1038/srep19736

Gharat, S. A., and Shaw, B. P. (2015). Novel and conserved miRNAs in the halophyte Suaeda maritima identified by deep sequencing and computational predictions of the ESTs of two mangrove plants. BMC Plant Biol. 15:301. doi: 10.1186/s12870-015-0682-3

Gramzow, L., and Theiflen, G. (2019). Plant miRNA conservation and evolution. Mol. Cells 38, 1–12. doi: 10.1007/s10059-018-0478-1

Xu et al.

IRNeas in Plant Salt Tolerance
Kawashima, C. G., Yoshimoto, N., Maruyama-Nakashita, A., Tsuchiya, Y. N., Saito, K., Kang, T., Yu, C. Y., Liu, Y., Song, W. M., Bao, Y., Guo, X. T., et al. (2020). Subtly

Jian, H., Wang, J., Wang, T., Wei, L., Li, J., and Liu, L. (2016). Identification of

Kim, J. Y., Lee, H. J., Jung, H. J., Maruyama, K., Suzuki, N., and Kang, H. (2010b). Overexpression of microRNA395c or 395e affects differentially the seed germination of Arabidopsis thaliana under stress conditions. Planta 232, 1447–1454. doi:10.1007/s00425-010-1267-x

Kim, W., Ahn, H. J., Chioi, T. J., and Ahn, J. H. (2011). The role of the miR399- PHO2 module in the regulation of flowering time in response to different ambient temperatures in Arabidopsis thaliana. Mol. Cells 32, 83–88. doi: 10.1007/s10059-011-1043-3

Kohli, D., Joshi, G., Deokar, A. A., Bhardwaj, A. R., Agarwal, M., Katiyar-Agarwal, S., et al. (2014). Identification and characterization of WTI and salt stress-responsive microRNAs in chickpea through high-throughput sequencing. PLoS One 9:e108851. doi: 10.1371/journal.pone

Koroban, N. V., Kudryavtseva, A. V., Krasenkov, G. S., Sadridinova, A. F., Fedorova, M. S., Sneszhkina, A. V., et al. (2016). The role of microRNA in abiotic stress response in plants. Mol. Biol. 50, 387–394. doi: 10.7868/s0026898416010105

Lee, R. C., and Ambros, V. (2001). An extensive class of small RNAs in Caenorhabditis elegans. Science 294, 862–864. doi: 10.1126/science.1065329

Lee, W. S., Guidemilla, R., Wong, G. R., Tamimi, M. T., Khalid, N., and Harikrishna, J. A. (2015). Transcripts and microRNAs responding to salt stress in musa acuminate colla (AAA Group) cv. berangan roots. PLoS One 10:e0127526. doi: 10.1371/journal.pone.0127526

Leng, X., Wang, P., Zhu, X., Li, X., Zheng, T., Shangguan, L., et al. (2017). Ectopic expression of CSD1 and CSD2 targeting genes of miR398 in grapevine is associated with oxidative stress tolerance. Funct. Integr. Genomics 17, 697–710. doi: 10.1007/s10248-017-0565-9

Li, W., Wang, T., Zhang, Y., and Li, Y. (2016). Overexpression of soybean miR172c confers tolerance to water deficit and salt stress, but increases ABA sensitivity in transgenic Arabidopsis thaliana. J. Exp. Bot. 67, 175–194. doi: 10.1093/jxb/erw104

Lian, C. L., Yao, K., Duan, H., Li, Q., Liu, C., Yin, W. L., et al. (2018). Exploration of ABA responsive miRNAs reveals a new hormone signaling crosstalk pathway regulating root growth of populus euphratica. Int. J. Mol. Sci. 19:1481. doi: 10.3390/ijms19051481

Liu, B., and Sun, G. (2017). MicroRNAs contribute to enhanced salt adaptation of the autopolyploid Hordeum bulbosum compared with its diploid ancestor. Plant J. 91(1):57–69. doi: 10.1111/tpj.13546

Liu, H., Qin, C., Chen, Z., Zuo, T., Yang, X., Zhou, H., et al. (2014). Identification of miRNAs and their target genes in developing maize ears by combined small RNA and degradome sequencing. BMC Genomics 15:25. doi: 10.1186/1471-2164-15-25

Liu, Y., Li, D., Yan, J., Wang, K., Luo, H., and Zhang, W. (2019). MiR319 mediated salt tolerance by ethylene. Plant Biotechnol. J. 17, 2370–2383. doi: 10.1111/pbi.13154

Llave, C., Kasschau, K. D., Rector, M. A., and Carrington, J. C. (2002). Endogenous and silencing-associated small RNAs in plants. Plant Cell 14, 1605–1619. doi: 10.1105/tpc.003210

Long, R. C., Li, M. N., Kang, J. M., Zhang, T. J., Sun, Y., and Yang, Q. C. (2015). Small RNA deep sequencing identifies novel and salt-stress-regulated microRNAs from roots of Medicago sativa and Medicago truncatula. Physiol. Plant 154, 13–27. doi: 10.1111/plp.12266

Lu, X., Dun, H., Lian, C., Zhang, X., Yin, W., and Xia, X. (2017). The role of pea-miR164 and its target PeNAC genes in response to abiotic stress in Populus euphratica. Plant Physiol. Biochem. 115, 418–438. doi: 10.1016/j.plaphy.2017.04.009

Luan, M., Xu, M., Lu, Y., Zhang, L., Fan, Y., and Wang, L. (2015). Expression of zma-miR169 miRNAs and their target ZmNF-YA genes in response to abiotic stress in maize leaves. Gene 55, 178–185. doi: 10.1016/j.gene.2014.11.001

Luan, M., Xu, M., Lu, Y., Zhang, Q., Zhang, L., Zhang, C., et al. (2014). Family-wide survey of miR169s and NF-Y As and their expression profiles response to abiotic stress in maize roots. PLoS One 9:e91369. doi: 10.1371/journal.pone.0091369

Lv, S., Nie, X., Wang, L., Du, X., Biradara, S. S., Jia, X., et al. (2012). Identification and characterization of microRNAs from barley (Hordeum vulgare L.) by high-throughput sequencing. Int. J. Mol. Sci. 13, 2973–2984. doi: 10.3390/ijms13032973

Ma, C., Burd, S., and Lers, A. (2015). MiR408 is involved in abiotic stress responses in Arabidopsis. Plant J. 84, 169–187. doi: 10.1111/tpj.12999

Ma, Y., Xue, H., Zhang, F., Jiang, Q., Yang, S., Yue, P., et al. (2020). The miR156/SPL module regulates apple salt stress tolerance by activating MdWRKY100 expression. Plant Biotechnol. J. 9, 311–323. doi: 10.1111/pbi.13464
Macovei, A., and Tuteja, N. (2012). MicroRNAs targeting DEAD-box helicases are involved in salinity stress response in rice (Oryza sativa L.). BMC Plant Biol. 12:1833. doi: 10.1186/1471-2229-12-183

Mekonnen, M. M., and Hoekstra, A. Y. (2016). Four billion people facing severe water scarcity. Sci. Adv. 2,e1500323. doi: 10.1126/sciadv.1500323

Meng, Y., Mao, J. P., Tahir, M. M., Wang, H., Wei, Y. H., Zhao, C. D., et al. (2020). MdmiR160 participates in auxin-induced adventitious root formation of apple rootstock. Sci. Horticulturae 270:109442.

Mette, M. F., van der Winden, J., Matzke, M., and Matzke, A. J. (2002). Short RNAs of conserved structure identified in Arabidopsis thaliana. Plant Physiol. 130, 6–9. doi: 10.1104/pp.007047

Mondal, T. K., Ganis, S. A., and Debnath, A. B. (2015). Identification of novel and conserved miRNAs from extreme halophyte, Oryza coarctata, a wild relative of rice. PLoS One 10:e0140675. doi: 10.1371/journal.pone.0140675

Mondal, T. K., Panda, A. K., Rawal, H. C., and Sharma, T. R. (2018). Discovery of microRNA-target modules of African rice (Oryza glaberrima) under salinity stress. Sci. Rep. 8:570. doi: 10.1038/s41598-017-18206-2

Morton, M. J. L., Awlia, M., Al-Tamimi, N., Saade, S., Pailles, Y., Negrão, S., et al. (2019). Profiling the mechanism of maize (Zea mays L.) microRNAs by high-throughput sequencing. New Phytol. 206, 789–804. doi: 10.1111/nph.15744

Xu et al. MiRNAs in Plant Salt Tolerance

Xia, K., Wang, R., Ou, X., Fang, Z., Tian, C., Duan, J., et al. (2012). OsTIR1 and OsTIR1-like proteins are positive regulators of rice flowering. J. Biol. Chem. 287, 4763–4770. doi: 10.1074/jbc.M111.319354

Ren, Y., Chen, L., Zhang, Y., Kang, X., Zhang, Z., and Wang, Y. (2013). Identification and characterization of salt-responsive miRNAs in Populus tomentosa by high-throughput sequencing. Biochimie 95, 743–750. doi: 10.1016/j.biochi.2013.08.009

Qadir, M., Quillere, E., Nanja, V., Murtaza, G., Sopory, S. K., and Sanan-Mishra, N. (2018). Mapping the microRNA expression profiles in glyoxalase over-expressing salinity tolerant rice. Curr. Genomics 19, 21–35. doi: 10.2174/13892027170666170228134530

Ünlüz, E. S., Bataw, S., Aslan, S. D., Sahin, Y., and Zencirci, N. (2018). Identification of conserved miRNA molecules in einkorn wheat (Triticum monococcum subsp. monococcum) by using small RNA sequencing analysis. Turk. J. Biol. 42, 527–536. doi: 10.3906/biy-1802-3

Wang, B., Sun, Y. F., Song, N., Wei, J. P., Wang, X. J., Feng, H., et al. (2014). MicroRNAs involving in cold, wounding and salt stresses in Triticum aestivum L. Plant Physiol. Biochem. 80, 90–96. doi: 10.1016/j.plaphy.2014.03.020

Wang, M., Wang, Q., and Zhang, B. (2013). Response of miRNAs and their targets to salt and drought stresses in cotton (Gossypium hirsutum L.). Gene 530, 26–32. doi: 10.1016/j.plant.2013.08.009

Wang, Q., Wei, L., Guan, X., Wu, Y., Zou, Q., and Ji, Z. (2014). Briefing in family characteristics of microRNAs and their applications in cancer research. Biochim. Biophys. Acta 1844(1 Pt B), 191–197. doi: 10.1016/j.bbapap.2013.08.002

Wang, W., Liu, D., Chen, D., Cheng, Y., Zhang, X., Song, L., et al. (2019). MicroRNA414c affects salt tolerance of cotton by regulating reactive oxygen species metabolism under salinity stress. RNA Biol. 16, 362–375. doi: 10.8002/1076-5517.2018.1574163

Wang, Y., Liu, W. W., Wang, X. W., Yang, R. J., Wu, Z. Y., Wang, H., et al. (2013). MiR3156 regulates anthocyanin biosynthesis through SPL2 and other microRNAs in poplar. Hortic. Res. 7:118. doi: 10.1038/s41438-020-00341-w

Wen, F. L., Yue, Y., He, T. F., Gao, X. M., Zhou, Z. S., and Long, X. H. (2020). Identification of miR390-TAS3-ARF pathway in response to salt stress in Helianthus tuberosus L. Gene 738:144460. doi: 10.1016/j.gene.2020.144460

Wu, L. J., Yang, R. X., Yang, Z. R., Yao, S., Zhao, S. S., Wang, Y., et al. (2017). ROS accumulation and antiviral defence control by microRNA528 in rice. Nat. Plants 3:16203. doi: 10.1038/nplants.2016.203

Wu, Y., Guo, J., Cai, Y., Gong, X., Xiong, X., Qi, W., et al. (2016). Genome-wide identification and characterization of Esteura salusugineum microRNAs for salt tolerance. Physiol. Plant 157, 453–468. doi: 10.1111/pls.12419

Xia, K., Wang, R., Ou, X., Fang, Z., Tian, C., Duan, J., et al. (2012). OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, adventitious root formation of apple rootstock. BMC Plant Biol. 12, 1484–1495. doi: 10.1007/s41598-017-18206-2

Xie, F., Wang, Q., Sun, R., and Zhang, B. (2015). Deep sequencing reveals important roles of microRNAs in response to drought and salinity stress in cotton. J. Exp. Bot. 66, 789–804. doi: 10.1093/jxb/eru437

Wu, L. J., Yang, R. X., Yang, Z. R., Yao, S., Zhao, S. S., Wang, Y., et al. (2017). ROS accumulation and antiviral defence control by microRNA528 in rice. Nat. Plants 3:16203. doi: 10.1038/nplants.2016.203

Xie, F., Stewart, C. N. Jr., Taki, F. A., He, Q., Liu, H., and Zhang, B. (2014). High-throughput deep sequencing shows that microRNAs play important roles in switchgrass responses to drought and salinity stress. Plant Biotechnol. J. 12, 354–366. doi: 10.1111/plb.12142

Xie, F., Wang, Q., Sun, R., and Zhang, B. (2015). Deep sequencing reveals important roles of microRNAs in response to drought and salinity stress in cotton. J. Exp. Bot. 66, 789–804. doi: 10.1093/jxb/eru437
Xie, J., Lei, B., Niu, M., Huang, Y., Kong, Q., and Bie, Z. (2015). High throughput sequencing of small RNAs in the two cucurbita germplasm with different sodium accumulation patterns identifies novel microRNAs involved in salt stress response. *PloS One* 10:e0127412. doi: 10.1371/journal.pone.0127412

Xu, M. L., Hu, T. Q., Zhao, J. F., Park, M. Y., Earley, K. W., Wu, G., et al. (2016). Developmental functions of miR156-regulated SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes in *Arabidopsis thaliana*. *PLoS Genet.* 12:e1006263. doi: 10.1371/journal.pgen.1006263

Yash, M. W., Sunkar, R., Zheng, Y., Ji, B., Al-Yahyai, R., and Farooq, S. A. (2015). A genome-wide identification of the miRNAome in response to salinity stress in date palm (*Phoenix dactylifera* L.). *Front. Plant Sci.* 6:946. doi: 10.3389/fpls.2015.00946

Yang, C. H., Li, D. Y., Mao, D. H., Liu, X., Ji, C. J., Li, X. B., et al. (2013). Overexpression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*Oryza sativa* L.). *Plant Cell Environ.* 36, 2207–2218. doi: 10.1111/pce.12130

Yang, R., Zeng, Y., Yi, X., Zhao, L., and Zhang, Y. (2015). Small RNA deep sequencing reveals the important role of microRNAs in the halophyte *Halostachys caspica*. *Plant Biotechnol. J.* 13, 395–408. doi: 10.1111/pbi.12337

Zhang, Y., Zhu, P., Kang, H., Liu, L., Cao, Q., Sun, J., et al. (2020). High-throughput deep sequencing reveals the important role that microRNAs play in the salt response in sweet potato (*Ipomoea batatas* L.). *BMC Genomics* 21:164. doi: 10.1186/s12864-020-6567-3

Yin, Z., Han, X., Li, Y., Wang, J., Wang, D., Wang, S., et al. (2017). Comparative analysis of cotton small RNAs and their target genes in response to salt stress. *Genes* 8:369. doi: 10.3390/genes8120369

Zhuang, Y., Zeng, P., Zhao, L., Zhou, X. H., and Liu, J. (2014). Conserved miRNAs and their response to salt stress in wild eggplant *Solanum linnaeanum* roots. *BMC Plant Biol.* 14:220. doi: 10.1186/s12870-016-0808-2

Zhu, J. K. (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Signal Behav.* 5, 1341–1350. doi: 10.1186/s11033-015-3880-z

Zhai, J., Dong, Y., Sun, Y., Wang, Q., Wang, N., Wang, F., et al. (2014). Discovery and analysis of microRNAs in *Leucaena chinensis* under saline-alkali and drought stress using high-throughput sequencing. *PloS One* 9:e105417. doi: 10.1371/journal.pone.0105417

Zhang, H., Liu, X., Yang, X., Wu, H., Zhu, J., and Zhang, H. (2020). MiRNA-mRNA integrated analysis reveals roles for miRNAs in a typical halophyte, *Rauamuria soongorica*, during seed germination under salt stress. *Plants* 9:351. doi: 10.3390/plants9030351

Zhang, Q., Zhao, C., Li, M., Sun, W., Liu, Y., Xia, H., et al. (2013). Genome-wide identification of *Thellungiella salassinae* microRNAs with putative roles in the salt stress response. *BMC Plant Biol.* 13:180. doi: 10.1186/1471-2229-13-180

Zhang, Y., Gong, H., Li, D., Zhou, R., Zhao, F., Zhang, X., et al. (2020). Integrated small RNA and degradome sequencing provide insights into salt tolerance in sesame (*Sesamum indicum* L.). *BMC Genomics* 21:494. doi: 10.1186/s12864-020-06913-3

Zhang, Y., Yun, Z., Gong, L., Qu, H. X., Duan, X. W., Jiang, Y. M., et al. (2018). Comparison of miRNA evolution and function in plants and animals. *Microrna* 7, 4–10. doi: 10.2174/2211356660766180126163031

Zhang, Z., Chen, C. Z., Xu, M. Q., Zhang, L. Q., Liu, J. B., Gao, Y., et al. (2019). MiR-31 and miR-143 affect steroid hormone synthesis and inhibit cell apoptosis in bovine granulosa cells through FSHR. *Theriogenology* 123:45–53. doi: 10.1016/j.theriogenology.2018.09.020

Zhao, B., Ge, L., Liang, R., Li, W., Ruan, K., Lin, H., et al. (2009). Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. *BMC Mol. Biol.* 10:29. doi: 10.1186/1471-2199-10-29

Zhu, J., Yuan, S., Zhou, M., Yuan, N., Li, Z., Hu, Q., et al. (2019). Transgenic creeping bentgrass overexpressing Osa-miR393a exhibits altered plant development and improved multiple stress tolerance. *Plant Biotechnol. J.* 17, 233–251. doi: 10.1111/pbi.12960

Zhuo, Q., Zhang, H., Wang, T., Chen, S. X., and Dai, S. J. (2013). Proteomics-based investigation of salt-responsive mechanisms in plant roots. *J. Proteome Res.* 82, 230–253. doi: 10.1021/jpr30103.126163031

Zhou, M., Li, D., Li, Z., Hu, Q., Yang, C., Zhu, L., et al. (2013). Constitutive expression of a miR319 gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. *Plant Physiol.* 161, 1375–1391. doi: 10.1104/pp.112.208702

Zhou, M., and Luo, H. (2014). Role of microRNA319 in creeping bentgrass salinity and drought stress response. *Plant Signal Behav.* 9:e28700. doi: 10.4161/pdb.28700

Zhu, J. K. (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiol.* 124, 941–948. doi: 10.1104/pp.124.3.941

Zhuang, Y., Zhou, X. H., and Liu, J. (2014). Conserved microRNAs and their response to salt stress in wild eggplant *Solanum linnaeanum* roots. *Int. J. Mol. Sci.* 15, 839–849. doi: 10.3390/ijms15010839

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Xu, Zhang, Yang, Wei and Dong. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.