Review

The Role of MicroRNA in Plant Response to Abiotic Stress

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The Role of MicroRNA in Plant Response to Abiotic Stress

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Abstract: MicroRNAs (MiRNAs) are a class of non-coding single-stranded RNA molecules of approximately 20-24 nucleotides in plants that play an important regulatory role in a variety of biological processes such as plant growth and development and response to various abiotic stresses. For example Drought, Salt, Cold, High temperature, Heavy metals and Nutrition. MiRNAs affect gene expression by manipulating the cleavage, translational expression or DNA methylation of target mRNAs. This review describes the current progress made on the way miRNAs are produced and regulated and the way miRNA/target gene is used in plant responses to abiotic stresses. Studying the molecular mechanism of action of miRNAs downstream target genes can optimize the genetic manipulation of crop growth and development conditions that provide a more theoretical basis for improving crop production.

Keywords: microRNA; target gene; plant growth and development; abiotic stress response

1. Introduction

MicroRNAs, a class of plant non-coding single-stranded RNA molecules of approximately 20-24 nucleotides in length encoded by endogenous genes, have a variety of important regulatory roles in cells, participating in the regulation of plant growth and development, stress responses and hormone signalling through the negative regulation of plant gene expression, and post-transcriptional regulation of gene expression in plants [1]. microRNAs can complementarily bind to the 3'UTR region of the target mRNA, thus achieving negative regulation of gene expression. Several miRNAs can also regulate the same gene and be regulated by a combination of several miRNAs. It has been shown that miRNAs are not only conserved in gene location, but also exhibit a high degree of sequence homology, that most mammalian miRNAs are located in transcriptionunits (TUs), and most of them are located in intronic regions [2-4]. The high degree of conservation is strongly related to its functional importance, potentially demonstrating that homologous miRNAs serve similar functions across different species. The purpose of this review is to provide a deeper understanding of molecular mechanisms of miRNA involvement in response to abiotic stresses, For example Drought, Salt, Cold, High temperature, Heavy metals and Nutrition. Since the first discovery of microRNAs in animals in 1993, more and more researchers have become interested in this non-coding RNA [3]. Furthermore, the aim of this paper is to provide a functional perspective on the role of miRNAs in plant adaptation to agronomic abiotic stress conditions, as well as to provide a theoretical basis for miRNA improving plant resistance in abiotic stress with a view to increasing crop yields.

2. Production and Mechanism of miRNAs

2.1. Production of miRNAs

Gene transcription processing: RNA polymerase II in most cases and RNA polymerase III in a few cases transcribe from genomic sequences to form the precursor pri-miRNA. pri-miRNA is very

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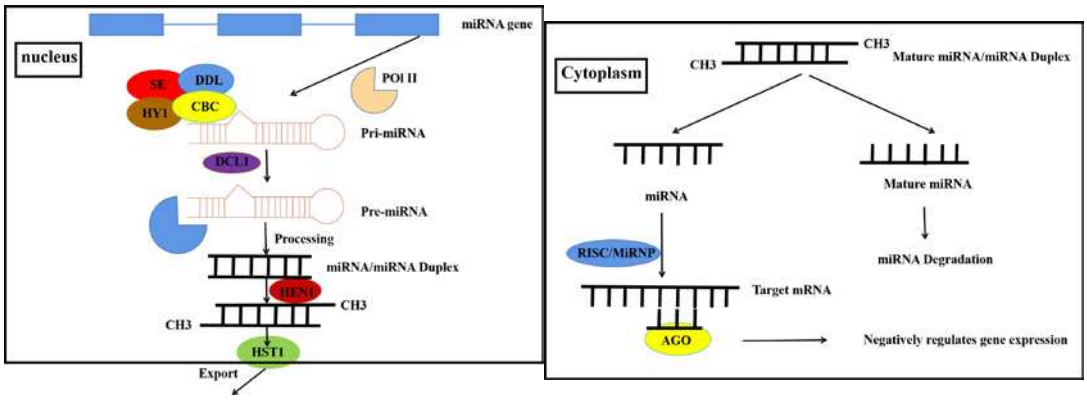
long, ranging from a few hundred to several thousand nucleotides. pri-miRNA maintains its non-activated state by cap structure and polyadenylation and coiling of the spatial structure. Intranuclear processing: pri-miRNA is edited by the adenylate deaminase of RNA and forms pre-miRNA hairpin sequences after being sheared by the microprocessor formed by the enzyme.

Nuclear export processing: The nucleoplasmic transporter protein recognizes the two nucleotides protruding from the 3′ ends of the pre-miRNA hairpin sequence and transports the pre-miRNA from the nucleus to the cytoplasm. Nucleus processing: The nuclease Dicer begins to shear the stem-loop structure in the hairpin sequence, after which it is able to produce miRNA double strands. Pre-miRNA precursors are processed into miRNA/miRNA double-stranded bodies via the DICER-LIKE 1 (DCL1) protein, nucleocapsid binding complex (CBC), mushroom (DDL) and sawtooth (SE), then the double-stranded body is translocated into the cytoplasm.The pre-miRNA is then processed into a mature double-stranded miRNA/miRNA complex with the help of the HUA ENHANCER 1 (HEN1) protein helper complex. When the mature miRNA is embedded in an RNA-induced silencing complex, the antisense miRNA is degraded in the cytoplasm. The RNA-induced silencing complex (RISC), known as the miRNA-ribonucleoprotein complex (miRNP), contains mature miRNAs and proteins. The ribonucleoprotein complex miRNP interacts with target mRNAs through its cognate mature miRNAs, negatively regulates the expression of target genes at the post-transcriptional level, leading to mRNA degradation or translational repression under specific mechanisms of action [5-7](figure 1).



(**a**) (**b**)

Figure 1. The origin, biogenesis of miRNAs in the plant: (**a**) RNA polymerase II in most cases and RNA polymerase III in a few cases transcribe from genomic sequences to form the precursor pri-miRNA. In the nuclear pri-miRNA is edited by the adenylate deaminase of RNA and forms pre-miRNA hairpin sequences after being sheared by the microprocessor formed by the enzyme. (b) The nucleoplasmic transporter protein recognises the two nucleotides protruding from the 3' end of the pre-miRNA hairpin sequence and transports the pre-miRNA from the nucleus to the cytoplasm. The nuclease begins to shear the stem-loop structure in the hairpin sequence, after which it is able to produce the miRNA duplex. The pre-miRNA precursor is processed into a mature double-stranded miRNA/miRNA complex by processing the miRNA/miRNA double-stranded body and then the double-stranded body translocates with the help of a protein helper complex. When the mature miRNA is embedded in the RNA-induced silencing complex, the antisense miRNA is degraded in the cytoplasm. The ribonucleoprotein complex miRNP negatively regulates the expression of target genes at the post-transcriptional level through the interaction of its cognate mature miRNA with the target mRNA.

2.2. Mechanism of miRNAs

Since most plant miRNAs are derived from the reverse copy of the target gene, the bases of the miRNA are complementary to those of the target mRNA. after the plant miRNA recognizes and binds to the target mRNA, the AGO will shear the target mRNA at the 10th and 11th nucleotides of the miRNA binding site. the AGO is the protein for the miRNA to carry out its function, miRNAs must

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bind to AGO to function. Since most plant miRNAs are derived from the reverse replication of target genes, the bases of miRNAs are complementary to those of target mRNAs, and when plant miRNAs bind to target mRNAs, the AGO will shear the target mRNA of the miRNA binding site [8-9]. Ten AGOs have been identified in Arabidopsis, which AGO1 has four structural domains, PAZ, Mid, PIWI and the N-terminal domain, respectively. The PIWI domain has nucleic acid endonuclease activity and functions as a shear for its target mRNA. Complete base complementarity between bases near the miRNA shear site and the target mRNA is important for AGO shear [8] (figure 2A).

Another mechanism of plant miRNAs is through translational repression. The miRNA does not bind to the 3′ UTR region of the target mRNA in its entirety and the RNA-induced silencing complex inhibits the initiation of translation or the specific degradation of the synthetic ribosome to achieve translation inhibition. Aukerman found that overexpression of *miR172* did not reduce the expression abundance of target mRNAs, but the corresponding levels of proteins encoded by target mRNAs were significantly reduced [10]. they therefore proposed for the first time that plant miRNAs could also repress translation of target genes. Subsequently, several studies have shown that translational repression of target genes by plant miRNAs is also a common phenomenon, and even the same miRNA may regulate target genes in both a shearing and translational repression manner. This is one of the reasons why plant miRNAs are not fully complementary to the expression of their target genes [11-12] (figure 2B). In plants, miRNAs are an important modality for mRNA regulation through translational repression.

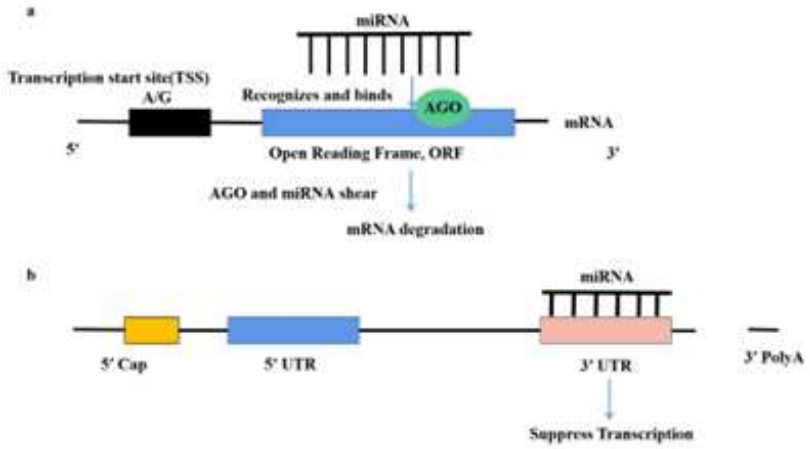

Figure 2. The mechanism of miRNAs in the plant: (**a**) After the plant miRNA recognizes and binds to the target mRNA, the AGO will shear the target mRNA at the 10th and 11th nucleotides of the miRNA binding site. When plant miRNAs bind to target mRNAs, the AGO will shear the target mRNA of the miRNA binding site. (**b**) Another mechanism of plant miRNAs is through translational repression. The miRNA does not bind to the 3′ UTR region of the target mRNA in its entirety and the RNA-induced silencing complex inhibits the initiation of translation or the specific degradation of the synthetic ribosome to achieve translation inhibition.

3. For miRNA and Drought Stress

Water is a vital resource for the survival of all life and has played an important role in the evolution of life. The most abundant substance in plant cells is water, which is an essential component of the plant body. With sufficient water, the stalks and branches of plants can stand up and stretch in the air, and the flowers can bloom better and facilitate the completion of pollination. Water is also one of the raw materials for photosynthesis in green plants and if there is a lack of water, the plant's photosynthesis will be weakened. Leaves will wilt and in severe cases can lead to the death of the plant [13-15]. MiRNA156 was one of the first miRNAs identified in plants, and numerous studies have linked miRNA156 to drought stress. anthocyanins act as a secondary metabolite by scavenging ROS to protect plants from stress. In Arabidopsis, rice, alfalfa and poplar, miR156/SPL is present in Arabidopsis, rice, alfalfa and poplar by regulating anthocyanin accumulation levels in response to plant drought stress. mechanism in response to plant drought stress by regulating the level of

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anthocyanin accumulation [16-17]. López-Galiano et al showed that drought conditions lead to down-regulation of miRNA159 and up-regulation of its target gene transcription factor *MYB33* in tomato [18]. Reyeset al showed that during Arabidopsis seed germination, ABA induces the accumulation of microRNA 159 (miR159) in an ABI3-dependent manner, and miRNA159 mediates the cleavage of MYB101 and MYB33 transcripts in vitro and in vivo [19]. Zhang et al. showed that fine localization and functional analysis identified the candidate gene *ZmLRT* of *qLRT5-1* as expressing the major transcript of microRNA miR166a, and that knockdown of ZmLRT enhanced drought tolerance in maize seedlings [20].

Stomata play a central role in the exchange of gases between plants and their environment, and their opening and closing is influenced by environmental signals, as well as being regulated by endogenous hormones, which in turn affect the plant's response and tolerance to drought stress. ABA is the most critical hormone in drought stress, it regulating water loss, stomatal opening and closing [21-24]. MiR393 positively regulated stomatal density and negatively regulated guard cell length, while overexpressing plants had the opposite phenotype to the deletion mutant, possibly due to miR393 regulating the expression of growth hormone response factor 5 (*ARF5*) and three stomatal development-related genes, *epidermal pattern factor 1* (*EPF1*), *SPEECHLESS (SPCH)* and *MUTE*. The *miR393* overexpression strain was more sensitive to drought treatment, accumulating more malondialdehyde (MDA) and hydrogen peroxide (H2O2) compared to the wild type, and also inhibiting the accumulation of ABA in leaves. These results also demonstrate that miR393 responds to plant drought stress by interacting with ABA and regulating stomatal density [25]. Zhao et al. showed that plants overexpressing *miR393a* exhibited enhanced drought stress tolerance associated with stomatal density and epidermal densification. MiR393 regulates the expression of *Auxin signalling F-BOX 2 (AsAFB2)* and *TRANSPORTINHIBITOR RESPONSE 1 (AsTIR1)* [26]. To adapt to drought stress plants require a hormone monocrotaline lactone, and exogenous monocrotaline lactone applied to tomato induces the accumulation of miR156. *miR156-OE* and monocrotaline lactone treatments both result in reduced stomatal conductance and increased ABA sensitivity in plants [27]. MiR398c was able to negatively regulate drought resistance in soybean. Overexpression of *miR398c* in Arabidopsis reduced the expression of *GmCSD1a/b*, *GmCSD2a/b/c* and *GmCCS*, impaired the ability to scavenge active oxygen, and increased relative electrolyte leakage and stomatal opening. This resulted in reduced germination, increased water loss from leaves, reduced survival under water deficit and demonstrated sensitivity to drought during seed germination and seedling growth [28].

Plants can also improve their drought stress tolerance by altering root conformation and adjusting leaf size and curl to reduce water evaporation. Hang et al. showed that the increased drought tolerance in *OsmiR408* transgenic plants may be due to changes in leaf morphology that facilitate the maintenance of water status, as well as increased antioxidant capacity to protect against damage from reactive oxygen specie（ROS）under stress [29]. Wang et al. showed that *miR9674a* showed progressive up-regulation in response to drought stress treatment. Overexpressing *miR9674a* lines exhibited different growth characteristics under drought and salt treatment in tobacco, with significant improvements in plant biomass, leaf area and root length, while its knockout expression line showed significant alleviation in the above growth traits compared to the wild type [30].

4. For miRNA and Salt Stress

Soil salinity affects around 6% of the world's land and 23% of arable land, causing considerable economic losses through crop stress and reduced yields. Because salinity plays a vital role in plant growth, above a certain limit, excess soluble salts will have a toxic effect on plants, affecting the levels of a wide range of endogenous plant signalling molecules such as ABA, ethylene, gibberellin (GA), ROS, and Nitric oxide (NO), These hormones can greatly affect the growth and development of plants, ultimately resulting in reduced yields [31-32]. In recent years, a number of miRNAs have been identified through miRNA studies in response to salt stress. The increased abundance of miR399 under salt stress, and therefore the altered expression of *PHO2* target genes, resulted in significant changes in the expression levels of two PO4 transporter genes, *PHOSPHATE TRANSPORTER1;4*

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(PHT1;4) and *PHT1;9*. In salt-stressed Arabidopsis would enhance PO4 transport from roots to shoot tissues, and these aerial tissues could use these resources to maintain essential biological processes or to generate adaptive responses to salt stress[33]. The mRNA for PpDCL1a encodes an essential Dicer protein for microRNA (miRNA) biogenesis and contains an intron miRNA (miR1047). Precise deletion of the intron containing MIR1047 to abrogate PpDCL1a autoregulatory feedback control revealed a hypersensitive response to salt stress and an insensitive response to the phytohormone ABA, and the physiological importance of feedback control of miR1047 on the abundance of PpDCL1a transcripts, which controls miRNA expression and its homologous target RNAs during salt stress adaptation[34]. Overexpression of *sly-miR398b* inhibited plant growth under salinity conditions in tomato, including less above-ground and root biomass and shorter plant height. Further analysis showed that overexpression of *sly-miR398b* down-regulated the expression of Cu/Zn superoxide dismutase (CSD) [35]. Liu et al. identified that two contrasting F. velutina cuttings clones, salt-tolerant (R7) and salt-sensitive (S4), and found to exhibit higher salt tolerance in R7 than in S4. In R7 leaves, miR164d, miR171b/c, miR396a and miR160g targeting *NAC1*, *SCL22*, *GRF1* and *ARF18*, respectively, were involved in salt tolerance. In R7 roots, miR396a, miR156a/b, miR8175, miR319a/d and miR393a targeting *TGA2.3*, *SBP14*, *GR-RBP*, *TCP2/4* and *TIR1*, respectively. That were involved in salt stress response [36]. Yuan et al found that *Osa-miR396c* transgenic plants exhibited reduced biomass, shorter internodes, reduced leaf area and reduced leaf size compared with wild-type, while The transgenic plants showed increased water retention under high salt stress [37].

5. For miRNA and Temperature Stress

5.1. For miRNA and Cold Stress

Temperature is the main environmental factor affecting plant growth and development and the quality of life of the fruit after harvest. Low temperature can inhibit plant growth and is a very important abiotic stressor. A variety of miRNAs can be involved in the low temperature stress response of plants by affecting the IAA or ABA signalling pathway [38]. Wang et al found that miR319 has been shown to target the *TEOSINTE BRANCHED/CYCLOIDEA/PCF (TCP)* transcription factors, which are involved in regulating multiple processes in plant growth and development by controlling cell proliferation. *miR319* expression is down-regulated by low temperature induction, while its target genes, *OsPCF6* and *OsTCP21* are reversed. *Osa-miRNA319* overexpression enhanced tolerance to low temperature stress [39]. Overexpression of *miRNA156* resulted in increased cell viability and growth rate under cold stress in Arabidopsis, pine and rice. OsmiR156 increased plant cold tolerance by targeting *OsSPL3*, which positively regulates the expression of *OsWRKY71*, a negative regulator of the transcription factors *OsMYB2* and *OsMYB3R-2* [40]. Dong et al found that SlNAM3 enhances cold tolerance and Sl-miR164a/b-5p plays a negative role in cold tolerance by repressing the expression upstream of *SlNAM3*. miR164a-NAM3 module induces ethylene synthesis by directly regulating the expression of *SlACS1A*, *SlACS1B*, *SlACO1* and *SlACO4*, thereby conferring cold tolerance in tomato [41]. The APETALA2/ethylene response factor (ERF) transcription factor OsERF096 was identified as a target of *miR1320*, which negatively regulates cold stress tolerance. Overexpression *miR1320* leads to increased cold tolerance, while *miR1320* knockdown lines decreases cold tolerance. The MiR1320-OsERF096 module regulates cold tolerance by inhibiting the JA-mediated cold signalling pathway [42].

5.2. For miRNA and High Temperature Stress

The response of plants to temperature stress is a complex process involving a variety of metabolic and biochemical processes. Not only low temperatures affect plant growth and development, but also high temperatures negatively affect processes such as growth, development and reproduction [43-48]. Wang et al found that SRL10, a double-stranded RNA-binding protein, regulates leaf morphology and heat tolerance in rice by altering microRNA biogenesis. The *srl10* mutant has a semi-curled leaf phenotype and increased heat sensitivity. SRL10 interacts directly with catalase isozyme B (CATB) to enhance hydrogen peroxide (H2O2) scavenging, thereby promoting

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heat tolerance [49]. Li et al found that Overexpression of cucumber *miR9748* in Arabidopsis thaliana increased high temperature tolerance. Transcriptome analysis suggests that miR9748 may mediate high temperature tolerance through the phytohormone signalling pathway. The target gene of miR9748 is *CsNPF4.4*, which negatively regulates high temperature stress tolerance by repressing the JA signalling pathway [50]. Ahmed et al found that Novel and conserved heat responsive miRNAs were identified in Chinese cabbage using a high-throughput sequencing approach using heat stress treatment at 38°C . This analysis identified 41 conserved miRNAs from 19 families, with *miRNA156*, *miRNA159*, *miRNA168*, *miRNA171* and *miRNA1885* having the most abundant molecules [51].

6. For miRNA and Heavy Metals Stress

The excessive accumulation of heavy metals in plants can poison plants, affect their growth and productivity, affect humans and animals through enrichment in the food chain, cause disease and induce cell damage. Metal elements include essential and non-essential elements. Essential metals are required for many physiological processes in living organisms, such as zinc, manganese and copper, non-essential metals are cadmium, lead or mercury [52-62]. Zhang et al found that overexpressing *miR156* accumulated significantly less Cd in their branches and showed enhanced tolerance to Cd stress in plants. The reason for this is that miR156 positively regulates Cd stress tolerance by regulating ROS levels and Cd uptake/transport gene expression [63]. Plants overexpressing *miR408* showed severe susceptibility to low sulphur (LS), arsenite As(III) and LS+As(III) stresses due to altered and *miR408* knockout mutants showed tolerance that regulated expression of genes involved in the sulphur reduction pathway and affecting the accumulation of sulphate and glutathione [64]. Nie et al found *miRNA167a*, *novel_miRNA15*, *novel_miRNA22* and their targets may be involved in Cr transport and chelation. In addition, *miRNA156a*, *miRNA164*, *miRNA396d* and *novel_miRNA155* were identified as being involved in the detoxification of plant Cr [65]. Zhou et al by comparing miRNAs and transcriptome analysis, a total of three known and 19 new differentially expressed *microRNAs* (DEMs) and 1561 differentially expressed genes (DEGs) were identified following Cd treatment, mainly because miRNAs play an important role in Cd-stressed wheat by regulating targets such as *TaHMA2;1* [66]. Overexpression of *miR393* abolished the inhibition of root elongation by aluminium ions. In addition, overexpression *miR393* attenuated the effect of exogenous growth hormone on aluminium-induced root growth inhibition and down-regulated the expression of growth hormone-responsive genes under aluminium stress [67].

7. For miRNA and Nutrient Stress

The nutrients of plants include nitrogen, phosphorus and potassium, which play a very important role in the growth and development of plants [68-71]. Nitrogen is a major component of many important compounds in plants, participating in a range of biochemical reactions and playing an important role in crop biomass accumulation and yield enhancement [72]. Phosphorus is involved in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and a number of other processes in the plant [73]. Potassium is involved in osmoregulation, material transport and other processes, and can improve plant stress tolerance [74]. It has been found in studies that nutrient deficiencies in plants cause plants to exhibit reduced dry weight of tissues in the above and below ground parts, reduced root length, root surface area, root volume, root vigour and reduced root respiration. Therefore, a deficiency of elements will greatly affect plant growth and in severe cases cause plant death [75].

Nitrogen stress：In past studies miRNA functions were identified in response to nitrate and N deficiency. MiR167 is able to limit root growth, mainly because it controls the response of adventitious plants to N and even controls N metabolizing enzymes produced downstream of nitrification and uptake, thereby affecting plant growth through N [76]. miR393 is activated by N signalling transmitted during nitrification and uptake. Nitrate had no effect on primary root development in overexpressing *miR393* plants or afb3-1 mutants, but it controlled horizontal root development in response to nitrate treatment [77-78].

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Phosphorus stress：In past studies miRNA functions were identified in response to phosphorus. MiR399 is an important component of the phosphorus starvation signalling pathway. The function of miR399 in phosphorus starvation signalling was first elucidated in Arabidopsis. *miR399* expression was increased under phosphorus starvation conditions, increasing the uptake and translocation of inorganic phosphorus by plants in response to phosphorus deficiency [79]. Hu et al. showed that up-regulation of genes in response to phosphorus starvation, many genes involved in iron, potassium, sodium and calcium uptake were also significantly up-regulated in overexpression miR399 strains, with increased concentrations of iron, potassium, sodium and calcium. In addition , function of *Ospho2* mutant also resulted in increased concentrations of these nutrients as well as upregulation of related genes. This demonstrates that miRNA399 influences plant responses to nutrient stress by regulating *PHO2* expression [80].

Potassium stress：In past studies miRNA functions were identified in response to potassium. Researchers have demonstrated that miRNA expression in cotton and wheat is altered by low dietary potassium utilisation. K deficiency treatment resulted in altered expression of 16 of the 20 miRNAs. As a response to K deficiency, wheat increases root growth and nutrient uptake through molecular mechanisms. In peanut plants, root development is influenced by miRNAs, which play a key role in K deficiency conditions. *miR156* and *miR390*, together with *miR160*, *miR164* and *miR393*, are proposed to be up-regulated in response to potassium deficiency [81-82]. Under low K stress in barley, many miRNAs appear to be differentially expressed including *Hvu-miR160a*, *Hvu-miR169h* and *Hvu-miR396c*. Due to the induction of Hvu-miR319 under low K, it is able to repress the expression of growth response factor (HvGRF) and thus promote Hvu-miR396 transcription in barley [83]. The dormancy-associated MADS-box (*OsMADS23*) target gene is significantly up-regulated in response to potassium deficiency, while Osa-miR444a clearly regulates N and P accumulation [84].

Except nitrogen, phosphorus and potassium, there are other elements in plants that play a very important role in plant growth, such as magnesium (Mg), Iron (Fe), sulfate (S), manganese (Mn), copperand (Cu), and boron (B). Mg is one of the main components of chlorophyll and promotes the activation of phosphatase and glucose convertase, facilitating the conversion of monosaccharides. Fe is an essential element for chlorophyll formation and is directly or indirectly involved in the formation of chloroplast proteins. S is protein, amino acid, vitamin and enzyme component. Promotes redox, growth regulation and is involved in chlorophyll formation and sugar metabolism. Cu is a core element in the activation groups of various oxidative enzymes in the crop and plays an important role in catalysing redox reactions in the crop. Mn is an activator of enzymes and a component of chloroplasts. B is involved in water, sugar and nitrogen metabolism and cell membrane pectin formation, it is involved in promoting the differentiation of meristematic tissues, the development of flowering organs and seed formation [85-89]. During sulfate limitation, *miR395* expression is significantly upregulated. miR395 targets two genes capable of participating in the sulfate metabolism pathway, ATP sulfatase (encoded by the APS gene) and sulfate transporter protein 2;1 (SULTR2;1, also known as AST68) [90]. Valdés et al. found Discovery of novel common bean stress response miRNAs for manganese toxicity [91]. Kayihan et al. found expression levels of miRNAs for transcription factors related to JA and ethylene metabolism were significantly induced in moderate B toxicity but not in severe B toxicity, with the most significant regulation obtained in Arabidopsis by *miR172* and *miR319* [92]. Ozhuner et al. found a total of 31 known miRNAs and 3 new miRNAs were identified in barley, 25 of these were found to be responsive to boron treatment [93]. Thus, miRNAs may therefore plant regulate the expression of downstream genes to help plants to resist the stress.

8. Conclusion and Prospects

Environmental stresses, such as drought, salt, temperature, heavy metals and nutrient stress, affect the metabolic processes of plants, which in turn regulate the expression of secondary metabolites, the synthesis of which reduces the toxic effects of reactive oxygen groups through signal transduction, redox and other mechanisms to ensure the continued survival of the plant (Table 1). Much research have shown that differential expression of miRNAs is induced in plants in response

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to different environmental stresses. MiRNAs are important regulators in the gene regulatory network and have various functions in regulating the growth, development, programmed cell death and metabolism of organisms [120-121]. MiRNAs can cause changes in the expression of various genes in plants, and therefore their study can help improve the resistance of plants to abiotic stresses. Although miRNAs have been studied for a long time, there is little data available on the link between secondary metabolites and abiotic stresses, and there are still many plant miRNA functions that have not yet been verified. Most articles focus on the role of miRNAs and their target genes in biological processes, while the molecular mechanisms of how miRNAs receive upstream signals and influence various downstream regulatory pathways through cascade responses are still unclear.

With in-depth research on the formation, function and mechanism of action of plant miRNAs and continuous improvement and innovation in miRNA research method, more and more miRNAs will be validated to play a critical role in plant resistance to abiotic stresses, laying the foundation for a more systematic miRNA regulatory network. In summary, miRNAs are essential for plants to regulate mRNA translation in plants, and research to explore the mechanisms of miRNA downstream target gene action can provide a more theoretical basis for improving food production. In the future researchers focus on miRNAs, where genomic information is scarce, will be of great significance in broadening the scope of species and research areas.

Table 1. Abiotic stress responsive miRNAs: their regulations and target genes in plants.

Abiotic Stress Type	miRNA	Expression	Species	Target Genes	Reference
Drought	MicroRNA-157	Upregulated	Arabidopsis thaliana	SPB Transcription factor	[94]
Drought	MicroRNA-159	Upregulated	Arabidopsis thaliana	MYB and TCP Transcription factors	[95]
Drought	MicroRNA-160	Downregulated	Arabidopsis thaliana	ARF10, ARF16, ARF17	[96]
Drought	MicroRNA-166	Upregulated	Medicago truncatula	HD-ZIPIII Transcription factors	[97-98]
Drought	MicroRNA-167	Upregulated	Arabidopsis thaliana	ARF6,ARF8	[94]
Drought	MicroRNA-168	Upregulated	Arabidopsis thaliana	ARGONAUTE, MAPK	[94]
Drought	MicroRNA-169	Downregulated	Arabidopsis thaliana	NF-YA transcription factor, SIMRP1	[99]
Drought	MicroRNA-171	Upregulated	Arabidopsis thaliana	GRAS transcription factor	[94]
Drought	MicroRNA-319	Upregulated	Arabidopsis thaliana	TCP Family	[100]
Drought	MicroRNA-390	Upregulated	Vigna unguiculata	ARF Family	[101]
Drought	MicroRNA-393	Upregulated	Arabidopsis thaliana	(TIR1, AFB2, AFB3) (ARF5, EPF1, SPCH)	[102-103]
Drought	MicroRNA-396	Upregulated	Arabidopsis thaliana	GRL transcription factor	[94]
Drought	MicroRNA-397	Downregulated	Oryza sativa	Laccase genes	[104]
Drought	MicroRNA-398	Upregulated	Medicago truncatula	Superoxide dismutase	[98]
Drought	MicroRNA-398c	Downregulated	Soybean	GmCSD1a/b,GmCSD2a/b/c, GmCCS	[28]

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Drought	MicroRNA-408	Upregulated	Arabidopsis thaliana	Chemocyanin precursor, kinases	[94]
Drought	MicroRNA-474	Upregulated	Zea mays	PDH, PPR	[105]
Drought	MicroRNA-528	Downregulated	Zea mays	POD	[105]
Drought	MicroRNA-811	Downregulated	Catharanthus roseus	MYB transcription factor	[106]
Drought	MicroRNA-814	Downregulated	Phaseolus vulgaris	Hydroxyproline-rich glycoprotein	[106]
Drought	MicroRNA-835	Downregulated	Ricinus communis	Aquaporin	[106]
Drought	MicroRNA-4398	Downregulated	Solanum tuberosum	WRKY transcription factor	[106]
Salt	MicroRNA-319b	Upregulated	Switchgrass	PvPCF5	[107]
Salt	MicroRNA-390	Downregulated	Poplar	ARF3.1, ARF3.2,ARF4	[108]
Salt	MicroRNA-390a	Downregulated	Creeping bentgrass	AsTIR1, AsAFB2	[26]
Salt	MicroRNA-396c	Upregulated	Creeping bentgrass	GRF	[37]
Salt	MicroRNA-408	Upregulated	Wheat	TaCP,TaMP,TaBCP, TaFP,TaKRP,TaABP	[109]
Salt	MicroRNA-408	Upregulated	Salvia miltiorrhiza	NbSOD, NbPOD, NbCAT	[110]
Salt	MicroRNA-414c	Downregulated	Cotton	GhFSD1	[111]
Cold	MicroRNA-160	Downregulated	Maize		[112]
Cold	MicroRNA-319	Downregulated	Rice	PCF6/TCP21	[113]
Cold	MicroRNA-319	Downregulated	Maize		[112]
Cold	MicroRNA-408a	Upregulated	Maize		[112]
Cold	MicroRNA-528	Upregulated	Maize		[112]
Cold	MicroRNA-5125	Upregulated	Potato	ABF8011	[114]
Cold	MicroRNA-10881	Upregulated	Potato	GA3ox123158	[114]
High temperature	MicroRNA-156	Downregulated	Arabidopsis thaliana	SPL transcription factor	[115]
High temperature	MicroRNA-159	Downregulated	Maize	MYB transcription factor	[116]
High temperature	MicroRNA-164	Downregulated	Maize	NAC transcription factor	[116]
High temperature	MicroRNA-166	Downregulated	Maize	HD zip	[116]
High temperature	MicroRNA-169	Downregulated	Maize	SBP	[116]
High temperature	MicroRNA-172	Downregulated	Maize	AP2/ERF	[116]
High temperature	MicroRNA-396	Downregulated	Maize	GRF,	[116]
High temperature	MicroRNA-5381	Downregulated	Maize	SAC2	[116]
Heavy metals-Cd	MicroRNA-167		Zea mays		[117]
Heavy metals-Cd	MicroRNA-393		Zea mays		[117]

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Heavy metals-Cu	MicroRNA-398		Grape	VvCSD1 and VvCSD2	[63]
Heavy metals-Al	MicroRNA-160		Sugarcance		[117]
Heavy metals-Al	MicroRNA-162		Sugarcance		[117]
Heavy metals-Al	MicroRNA-164		Sugarcance		[117]
Heavy metals-Al	MicroRNA-166		Sugarcance		[117]
Heavy metals-Al	MicroRNA-167		Sugarcance		[117]
Nutrients-Zn	MicroRNA-158	Upregulated	Brassica juncea	FUT1	[118]
Nutrients-K	MicroRNA-169		Triticum aestivum	Pentose pathway	[119]
Nutrients-N	MicroRNA-169	Downregulated	Arabidopsis thaliana	HAP2	[81]
Nutrients-B	MicroRNA-319	Upregulated	Riticum aestivum	MYB transcription factor	[92]
Nutrients-K	MicroRNA-319	Downregulated	Hordeum vulgare	TCP	[92]
Nutrients-K	MicroRNA-396	Downregulated	Hordeum vulgare	GRF	[83]
Nutrients-P	MicroRNA-399	Downregulated	Arabidopsis thaliana	Ubiquitin conjugase E2	[117]
Nutrients-Mn	MicroRNA-781	Upregulated	Arabidopsis thaliana	MCM2	[117]
Nutrients-Mn	MicroRNA-826	Upregulated	Arabidopsis thaliana	Alkenyl hydroxalkyl producing 2	[117]

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