



## Role of MicroRNAs in Abiotic Stress Response of Plants

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### ABSTRACT

Abiotic stresses, which encompass different environmental stimuli, constantly affect plant sustainability throughout their lifetime. Plants have developed several complex mechanisms to respond against these abiotic stresses. It is reported in recent studies that miRNAs play an important role to bring down stress-responsive genes and help plants to withstand abiotic stresses such as drought, salinity, temperature, heavy metals, etcetera. The gene regulation by miRNAs helps in plant growth and development and also regulates various physiological processes like identification of floral organs, morphogenesis of leaves, development of roots, etc. Micro RNAs are small endogenous non-coding double-stranded RNA, ranging from 20 -24 nucleotide base pairs in size. The miRNA is formed by a Dicer-like1 complex from a hairpin-like precursor. The miRNAs target mRNAs, either cleaving the mRNAs or mediating transitional repression of particular mRNAs according to their size through epigenetic modification. Several modern technologies and approaches including molecular cloning and throughput sequencing (e.g: Next Generation Sequencing) using computational tools have evolved to characterize miRNA expression patterns during abiotic stress. In this context assessing the relationship between the various miRNAs and their coordinated functioning for the control of stress signaling and biomass production. Various targeted genes of miRNAs are transcription factors that further control a set of downstream genes to affect physiological responses. In this review, we have explored the current knowledge about miRNAs i.e., their development, roles, functions, and target genes under abiotic stress conditions.

**Keywords:** Oxidative stress, Abiotic Stress, MiRNA, Stress Adaptation, Physiological stress.

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### INTRODUCTION

MicroRNAs or miRNAs are tiny non-coding RNA molecules, 20 to 24 nucleotides in length. The discovery of microRNA or miRNA by Ambros and Ruvkun in 1993 from the species *C. elegans*, had revolutionized molecular biology<sup>1</sup>. It was established that a single micro-RNA can regulate the expression of hundreds of mRNAs within a given cell type, and on the other hand, a single mRNA expression can be regulated by multiple miRNAs. As per an estimation, over half of the human transcriptome is directly under the regulation of miRNAs<sup>2</sup>. Ten years later, in 2002 miRNA was first discovered in the plant species, *Arabidopsis thaliana*<sup>1</sup>. Since the discovery of hundreds of microRNA in the organisms of *C. elegans* in 1993, their role in regulating the diversity of physiological and pathological parameters including growth, differentiation, immune response, and stress adaptation has been found<sup>3</sup>. Micro RNAs have an ancestor structure with a hair-pin-like secondary structure that is conserved across species<sup>4</sup>. From recent studies, it has been

found that miRNAs play a key role in regulating plant growth and development. In response to alterations in their environment and interactions with other animals, a plant's evolution has been adopted sophisticatedly. A large number of these strategies depend on the expression of various genes, and in this context, miRNAs are essential regulator molecules. They can be activated or inhibited to affect the expression of target genes by post-transcriptional gene silencing or translational inhibition of their target mRNA<sup>5</sup>.

The knowledge of post-transcriptional gene regulation has changed as a result of the discovery that microRNAs (miRNA) in plants and animals<sup>6</sup>. miRNAs are often split into two categories: species-specific miRNAs, which are abundant and expressed at low levels, and preserved miRNAs, which are observed in many families and form a significant percentage of RNAs of the type<sup>7</sup>. microRNAs, or miRNAs, are tiny, non-coding RNA molecules, that are crucial for regulating gene expression in several signaling and developmental pathways. They generally include 20–24 nucleotide base pairs<sup>8</sup>. They are endogenous and have been conserved throughout evolution, suggesting their importance in biological function<sup>9</sup>. Since their initial discovery in the plant species *Arabidopsis thaliana*, it has been demonstrated that miRNA is the primary regulator of gene expression in every aspect of a plant's physiological development by directing a defense response against infection brought on by different biotic stresses, such as viral, fungal, and pathogen infection, or by aiding in the



accumulation against abiotic stress, such as stress from drought, salinity stress, temperature stress, etc <sup>1</sup>. Mature miRNA contains a 3' end with a hydroxyl group and a 5' end with a phosphate group, which is abundant in RNA Inducing Silencing Complex (RISC). Through binding to a particular location in the target mRNA's 3' UTR to obtain substrate specificity, these changes affect post-transcriptional silencing, which modifies gene regulation by mRNA cleavage and/or translational repression <sup>10,11</sup>. miRNA-miRNA pairs regulate an extremely complex signaling network because a single miRNA may target hundreds of mRNAs or a solitary mRNA can be targeted by several distinct miRNAs <sup>3</sup>. In eukaryotes, miRNA negatively controls the expression of genes. According to several studies, proteins interact with one another to form the biosynthetic pathway through which miRNAs are produced and function. Additionally, post-transcriptional modification and the control of gene expression may result in the recombination of protein-protein interactions <sup>11</sup>. In contrast to animal targets, which often have several, weakly complementary sites within the 3' untranslated region, The majority of plant miRNA targets contain a single site that is complementary to a single miRNA family <sup>12</sup>.

Most plant miRNA genes are transcribed as primary miRNA transcripts by RNA Polymerase II (pri-miRNAs), which are next capped and polyadenylated <sup>13</sup>. Pri-miRNA is subsequently degraded in the nucleus by the ribonuclease enzyme Dicer-like 1 (DCL1) to produce a precursor-miRNA (pre-miRNA), which DCL1 then further degrades with the aid of the proteins Hyponastic leaves 1 (HYL1) and serrate (SE) to produce miRNA: miRNA\* duplex complex<sup>14</sup>. The primary feature of plant miRNA processing is 2'-O-methylation of miRNA: miRNA \* duplex by nuclear RNA methyl transferase protein Hua -Enhancer 1 (HEN1) <sup>15</sup>. HASTY, the Arabidopsis equivalent of exoprotein 5, moves the miRNA: miRNA\* duplex from the nucleus to the cytoplasm during the production of miRNAs <sup>16</sup>. The RNA-induced silencing complex is formed in the cytoplasm by the interaction of single-stranded miRNA loaded with the Argonaute1 (AGO1) protein. Target genes are down-regulated by RISC either by cleaving mRNAs or by suppressing translation <sup>4</sup>.

The two main mechanisms by which miRNAs function, are cleavage of the target mRNA and translational repression. The degree of complementarity between the base pair of the miRNA and its binding site with the target mRNA in turn determines the manner of action. The target mRNA which has a high degree of complementarity with the miRNA is cut by the miRNA, while the target mRNA which has relatively less or partial complementarity is repressed by translation <sup>17</sup>. Most plant miRNAs are thought to have high levels of complementary activity by cleaving the target mRNA to control the expression of the gene <sup>4</sup>. Abiotic stimuli cause plant microRNAs to react in one of three ways; i) in a genotype-dependent way, ii) in Stress dependent way, and c) in a miRNA-dependent manner <sup>6</sup>.

Throughout their life cycles, plants must contend with changing environmental obstacles. These abiotic stress or environmental disturbances include drought, excessive soil salinity, severe temperatures, and dangerous heavy metals <sup>9</sup>. It is well known that these abiotic stress factors significantly affect plant endurance, enlargement, and maturity; which degrade the plant quality, biomass production, and yield. One of the most well-known mechanisms for the abiotic stress response is miRNA regulation of gene expression <sup>7</sup>. Stress is marked as the most dynamic environmental difficulty that plants experience during their cycle of life <sup>9</sup>. The two main categories of causes of plant stress are abiotic (non-living) and biotic (living). The most frequent biotic influences are insects, bacteria, and fungi, whereas the most frequent abiotic factors are light, water, salinity, temperature, and heavy metals. It is well recognized that these abiotic stress factors, which plants regularly experience one at a time or in combination with other factors, have an important effect on the plant's capacity to withstand, develop, and mature, which decreases plant quality, biomass production, and yield <sup>18</sup>. A variety of complex mechanisms have been developed by plants, such as changes in gene expression and regulatory networks, to respond to all of these factors. These abiotic stressors are said to reduce agricultural output around the globe <sup>19</sup>. In this article, we will review the role of miRNAs in combating these abiotic stressors for plants.

#### Role of miRNA in drought Response:

Numerous plant species have been studied to investigate how miRNAs function in the drought response. According to some research, miRNAs may be one of the most important stress-related regulators<sup>20</sup>. Research by various scientists has shown that numerous miRNAs are involved in this and they achieve it by overexpression as well as downregulation in response to drought. While miR161, miR169, miR171a, miR168a, miR168b, and miR319a are some of the downregulated miRNAs in Arabidopsis; miR159, miR167, miR168, miR156, miR319, miR171, miR393, miR394a, miR396 miR395c, and miR397 are upregulated <sup>21</sup>. For instance, *A. thaliana* responded to drought, salt, and ABA by upregulating miR393 and miR397b. ABA is one of the main hormones which is responsible for stress in a plant <sup>22</sup>. The nuclear transcription factor YA (NF-YA) is the target of miR169, which when overexpressed in *Arabidopsis thaliana*, makes the plant more vulnerable to drought. On the other hand, miR169c is overexpressed, which regulates a gene involved in the stomatal opening and closing in tomato plants <sup>23</sup>. Upregulated miR157 and miR159 target the SPB family transcription factor as well as transcription factors from the MYB and TCP family proteins and miR160 downregulated by targeting ARF 16, ARF 10, and ARF 17 proteins in drought stress<sup>24,25</sup>.

In barley plants under drought stress, the negative relationship between the targets and the miRNAs such as miR171, miR166, miR156, and miR408 has been identified



<sup>26</sup>. The SBP family of transcription factors are the primary targets of miR156 upregulation, which affect the phase change and blooming period, miR166 targets promoter binding proteins such as transcription factors to lower the expression of HvSCL genes, whereas miR171 targets HvSCL genes, and targets miR408 are yet unknown <sup>27</sup>. The response of these miRNAs to drought stress is demonstrated by the upregulation of miR 398a/b and miR408 in the *Medicago truncatula* plant and the observable downregulation of their respective targeted genes that encode the copper proteins COX5B (Subunit 5 mitochondrial cytochrome c oxidase) and plantacyanin<sup>28</sup>.

Abiotic stress resulted in a decrease in the expression levels of miR1445, miR530a, miR1447, and miR1446a-e in *Populus trichocarpa*. However, the expression pattern of miR1450 showed an increase during high saline conditions while it decreased in conditions of drought. In rice, expression of miR169g and miR169n(o) responses to excessive salt and drought have been studied<sup>29</sup>. The presence of cis-acting abscisic acid response elements (ABRE) upstream of miR169n(o), which may be controlled by ABA and miR169g generated by the osmotic stress brought on by drought via dehydration responsive elements (DRE) <sup>30</sup>.

Another proposed way of adapting to drought is to reduce water usage. It has been determined that miR393 targets the TIR1 gene. The auxin receptor gene codes for this. Autotaxin activity is crucial to plant development. An adaptation process called retarded plant development under stress enables plants to use less water, transfer energy to the creation of defense chemicals, and endure stress <sup>31</sup>.

Another adaptation strategy is the plant's retention and redistribution of water. The miR835-likely targeted genes produce aquaporin, which helps plants retain water in drought conditions <sup>25</sup>. The miR 814 target genes produce glycoproteins high in hydroxyproline. Its family of proteins promotes the formation of proline, which helps plants adapt to drought and other abiotic stressors and normalizes the osmotic pressure inside cells. There are no other instances of miRNAs controlling the production of the family of hydroxyproline-rich proteins in the literature at hand. However, it was shown that miR474 contributed to the proline dehydrogenase (PDH) gene's inhibition of expression in mice <sup>32</sup>.

#### Role of miRNAs in salinity stress:

A key obstacle to the optimal development and production of crop plants worldwide is still salt stress <sup>(33)</sup>. According to reports, salt stress is the biggest issue preventing crops from growing and producing. Plants have developed a variety of gene regulatory profiles to withstand the effects of high salt stress, foster growth, and maintain proper photosynthesis. These regulatory genes influence the physiological, metabolic, and cellular processes necessary for protein synthesis, degradation, signaling, and energy consumption<sup>(34)</sup>. Under salt stress conditions, a

substantial number of gene transcripts are either up- or down-regulated, demonstrating that transcription is tightly regulated in stressed plants. Therefore, post-transcriptional gene regulation plays a crucial role in how plants react to salt stress. It is well known that miRNAs play an important role to regulate gene expression in plants. Numerous studies have been done on how specific miRNAs regulate their transcription in response to salt stress<sup>35</sup>.

Through research, it has been discovered that hundreds of salinity stress-responsive miRNAs are from different crop species <sup>6</sup>. Among them, several microRNAs are upregulated in response to salinity stress, including miR156, miR393, miR159, miR165, miR319, miR168, miR397, miR169, miR167, miR394, miR396, and however miR398' expression is downregulated<sup>36</sup>. miR165 targets Class III HD-ZIP transcription factor genes, miR166 targets Squamosa promoter-binding protein-like factors, miR167 primarily targets ARF6 and ARF8 genes, miR168 targets AGO1, miR319 targets TCP transcription factor, miR393 targets F-box protein; AFB2; Auxin receptor; TIR1 genes, miR394 targets F-box protein, miR396 targets GRL transcription factors, and upregulated in salinity stress while miR398 targets Cu/Zn Supe <sup>16</sup>. Additionally, it has been demonstrated that under salt stress, in *Arabidopsis* miR172c, which controls AP2 like TF for flower induction, is activated <sup>37</sup>. MiR319 has also been observed to control salt exclusion through HKT family transporters <sup>38</sup>. The conservative miRNA family miR393 has members of several plant species. In similar research, ath-miR395e overexpression reportedly accelerates seed germination in salinity via regulating the expression of ATP sulfurylase (APS) and the sulfate transporter SULTR2 <sup>39</sup>. In 2017 Dr. Nirjhar Dasgupta et al worked on mangrove *Bruguiera gymnorrhiza*. They found targeted genes for two miRNA, bg-miR1029 and bg-miR5021 in *Bruguiera gymnorrhiza* are involved in major stress response characteristics<sup>40</sup>.

Additionally, research shows that the miR169 family has strong responses to salt stress in rice <sup>29</sup>. According to studies, these miR169 family members preferentially target one of the NF-YA genes, OS03g29760, a CCAAT box binding transcription factor that participates in the transcriptional control of several genes to enhance in high salinity<sup>29</sup>.

Studies have established that under salinity stress, miR395 expression is downregulated in *Arabidopsis* by targeting sulfate assimilation regulatory networks, but miR395 expression is upregulated in *Populus trumula* <sup>41,42</sup>.

Salt stress was one of many environmental factors that affected the expression of many miRNAs in *Populus trichocarpa*. It is observed that the expression of miR1445, miR530a, miR1447, miR1446a-e, and miR1711-n were downregulated. Targeted genes for miR1445 are currently unclear, while miR1446 primarily targets POPTER, 0001s39950 and miR530 primarily targets the 'F-box domain-containing protein <sup>43</sup>.

Additionally, within salt stress, miR169n, and miR169g, two members of the miR169 family, are substantially



expressed. According to research, it has been found that the stress hormone ABA may regulate miR169n. The target mRNA for miR169g and miR169n is transcription factors subunit-A (NF-YA), and their expression was downregulated in wheat leaves that had experienced drought-affected<sup>44</sup>.

An analysis of the miRNAs miR156, miR164, miR167, and miR396 on salt-sensitive and salt-resistant lines cultivars of maize revealed that these miRNAs are downregulated during salt stress<sup>46</sup>. Oxidoreductase genes and growth regulator factors (GRF1, GRF5, and GRF15) are predicted targets for miR167<sup>45</sup>. Although miR168, miR395, miR474, and miR162 are elevated in salt-affected roots of maize. Assumed targets of the ARGONAUTE1 (AGO1) protein include zma-miR162 and zma-168<sup>46</sup>. The targeted site for miR395 in maize is ATP-sulfurylase<sup>47</sup>.

In rice Osa-miR393a and Osa-miR393b, are belongs to miR393 family members, and target the AFB3, TIR1, F-box domain, AFB2, and MYB family genes. Osa-393b expression remained constant but in reaction to salt and alkaline stress, the osa-miR393a impression drastically changed<sup>48</sup>. ANR1, a MADS-box transcription factor important for root elongation and growth, is the target of rice miR444 in plants. Inhibiting lateral root growth while inducing main root elongation were the effects of overexpressing miR444 in rice<sup>49</sup>.

### Role of miRNAs in Temperature Stress

Temperature is considered one of the environmental factors, which is considered to influence physiological growth, development, and reproduction in plants<sup>47</sup>. Excess temperatures (hot or cold) harm nearly all aspects of plant development, growth, reproduction, and productivity<sup>50</sup>. When a plant is in its reproductive stage, sensitivity to temperature stress is greatest, and even a slight shift in the temperature during the flowering season can cause catastrophic sufferers for grain harvests<sup>51</sup>. The study by Intergovernmental Panel on Climate Change (IPCC) indicates when the average surface temperature rises to 2 to 4.5 °C by the end of the century, the development of plants would be inhibited<sup>52</sup>.

### Role of miRNAs in High temperature

With the aid of miRNAs, plants modify their molecular gene expression patterns as a result of the hot weather. Plants respond to heat stress in crucial ways in conjunction with Heat Shock Transcription Factors (HSFs), which are necessary for Heat Shock Protein activation (HSPs)<sup>53</sup>. According to research, in *Arabidopsis* heat stress activated CCS, CSD1, and CSD2 genes<sup>54</sup>. Transgenic plants which consist of CCS, CSD1, and CSD2 genes expressing miR398 are more sensitive to heat stress<sup>54</sup>. *Arabidopsis*, wheat, and cabbage have been found to upregulate miR156 in response to heat stress by targeting SPLs protein, which regulates phase transition, but rice has been shown to downregulate miR156 by regulating the blooming time<sup>55,15</sup>. It is believed that the role of miR156 in *Arabidopsis* is to regulate the SPI2 and SPI11 genes. On the other hand

miR156, and miR172 downregulate the expression during the juvenile phase and upregulate expression during the adult phase. These miRNAs mainly target TOE1 and TOE2, which prevent the leaf epidermis from developing adult characteristics. It has been claimed that rice miR526 decreases ROS by regulating peroxidase<sup>54</sup>. MiR398 is upregulated through targeting CDSs to reduce oxidative stress, but miR399 is downregulated in response to phosphate deficit<sup>56</sup>.

Additionally, it will be important for researchers to continue to argue that temperature stressors cause miRNA expression to fluctuate between various tissues or developmental stages. According to a recent study, hit-responsive miRNAs in rice differed from those discovered in the seedling stage during the blooming stage<sup>57</sup>.

### Role of miRNAs in cold temperature stress

Cold temperature stress is also an example of abiotic stress which affects a plant's growth, development, and seedling. In recent years, in plants, several cold temperatures stress-responsive miRNAs have been discovered which help to withstand the adverse effect of low temperature and accumulate at the post transcription level.

Recent research has revealed that the expression of, miR393, miR408 miR396, and miR165/166 in *Arabidopsis* are upregulated in cold stress<sup>37</sup>. The targets of miR408 are laccase13 and plant cyanin<sup>58</sup>. MiR398 was discovered to target the Cu/Zn superoxide dismutase-coding genes cytosolic CSD1 and chloroplastic CSD2 in *Arabidopsis* (22). TIR1, AFB1, and AFB3 are the genes that miR393 targets to facilitate auxin signaling<sup>59</sup>. In *Arabidopsis*, miR398, miR156/157, miR159/319, miR164, and miR394 were either temporarily or slightly regulated<sup>22</sup>. CSDs are miR398's target genes, and copper homeostasis is one of its target functions<sup>15</sup>. F Box protein and NAC domain transcription are the two target genes of miR394 and miR164, respectively<sup>60</sup>.

MiR156, miR475, and miR476 family members' expressions were discovered to be downregulated in the *Populus* plant, however, members of the miR168 and miR477 families were found to be upregulated<sup>37</sup>. According to Scientist Chen et al, miR168 primarily targets AGO1 genes and aids in the production of miRNA. MiR156 helps in phase transition by targeting SPLs genes<sup>4</sup>.

Scantiest W.X Li et al discovered 18 miRNAs implicated in the cold response in rice, with the majority of them being suppressed during cold stress, leading to the hypothesis that miRNAs serve as ubiquitous regulators<sup>37</sup>. In a different investigation, rice plants exposed to cold stress showed upregulated expression of miR812q, whereas its targets CIPK10 genes, showed downregulated expression<sup>61</sup>. The calcium-dependent CBL-CIPK signaling pathway utilizes these CIPK transcripts<sup>62</sup>.

Scientist Xu et al created a short RNA and degradome library using wild tomatoes (*Solanum habrochaites*). In this study, 192 miRNAs' expression was found to be





upregulated, whereas 205 miRNAs' expression was found to be downregulated. Additionally, these miRNAs' expected and confirmed target genes were found. The target genes were mostly discovered to be involved in controlling the production of antistress proteins, which results in plant response to cold stress<sup>63</sup>.

#### Role of miRNAs in Heavy metal stress:

Increased heavy metal (HM) pollution has become a severe issue on a global scale. The minerals that plants absorb from the soil are used for a variety of biological processes, including cellular signaling, photosynthesis, water transmission, etc<sup>64</sup>. At an optimum level, essential metals including zinc, copper, and manganese (Zn, copper, Mg) are required for biological procedures; but, at higher concentrations, these metals can be harmful to plants. High concentrations of metals like Cd and Hg impede plant growth due to binding with the free sulfhydryl group of protein. As a result, interruption of homeostasis is observed within the plant body<sup>65</sup>.

To prevent heavy metal toxicity, plants need mechanisms to manage the concentrations of Other nonessential metals like lead, cadmium, and mercury (Pd, Cd, Hg) are very toxic to plants. It has been reported that the two primary characteristics of heavy metal toxicity are Inhibition of the enzyme system and oxidative stress<sup>65</sup>. Essential metals i.e., Cu, Zn, and Mn, inside biological restrictions and restrict the absorption of non-essential metals. Several strategies have been implemented to increase the heavy metals tolerance in plants. By research, many groups of HM stress-inducive miRNAs accompanied by their targeted proteins have been discovered to lower heavy metal toxicity in the plant<sup>66</sup>.

Plants require copper (Cu) necessarily for various physiological activities. Plastocyanin (Pc), Cytochrome C oxidase (COX), and Copper/Zn superoxide dismutase (Cu-Zn sod), the three most prevalent Cu proteins, are vital for plummeting oxidative stress, photosynthesis, and respiratory electron transport<sup>67</sup>. Extreme levels of Cu concentration can alter a variety of physiochemical characteristics. It has been shown that the most dangerous impact may be an obstruction of the electron transport chain of photosynthesis and the elaboration of certain substances that might induction other reactions. The most vital SOD for the dismutation of superoxide radicals is Cu-Zn SOD<sup>68</sup>. Being a Cu protein, CSD may perform as sinks when the amount of Cu is greater than the normal level. Three CSD isoenzymes—CSD1 in the cytoplasm, CSD2 in the chloroplast stroma, and CSD3 in the peroxisome—are found in Arabidopsis<sup>69</sup>. Research has shown that the expression of miR398 in Arabidopsis controls the regulation of CSD1 and CSD2. miR398 expression is downregulated at high Cu concentrations, and this downregulation is the reason for post-transcriptional induction of CSD1 and CSD2 mRNA. The detoxification of superoxide radicals is assisted by the induction of CSD at high Cu concentration. According to studies, transgenic plants have a far higher capacity for tolerating the effects

of heavy metal stress and have accumulated more CSD2 mRNA than ordinary plants<sup>70</sup>.

When a plant face copper (Cu) deficiency, miR398 expression inhibits CSD by promoting CSD mRNA degradation. SOD activity in the plastid is maintained by downregulating CSD-mRNA along with the induction of the FeSOD. The downregulated CSD expression allows efficient transfer of limited Cu to Pc. miR398 is therefore inferred to be Cu's primary regulator to sustain Cu stress homeostasis<sup>19</sup>. By research, it has been discovered that three more miRNAs, namely miR397, miR408, and miR857, play a role in the response at low Cu concentration. Their targeted genes in Arabidopsis were encoded by laccase and Pc. The three miRNAs reciprocally reduced the transcript for Pc and Laccase<sup>70</sup>.

Scientists consider that Cadmium (Cd) is one of the most dangerous pollutants, which caused severe poisonousness to plants. According to researchers, it has been that Cd induces oxidative stress by the formation of ROS. The researchers have found evidence of H<sub>2</sub>O<sub>2</sub> generation and accumulation in some plants with high Cd concentrations. Three distinct miRNAs, miR156, miR171, and miR396a, were shown to be downregulated in high Cd concentrations by scientist Zhou et al<sup>71</sup>. Additionally, he identified a total no of 38 miRNAs in *Medicago truncatula*. Among those miR166 and miR398 were downregulated but miR171, miR319, and miR529 were upregulated. The transcription of CSD1/CSD2 is stimulated by the downregulation of these miRNAs, reducing the toxicity caused by high Cd stress. In *Brassica napus*, it is observed that miR156, miR393, miR171, and miR396a are downregulated when exposed to Cd<sup>72</sup>. These miRNAs target the F-box proteins (TIR1), bHLH transcription factors, SCL transcription factors, GRL transcription factors, Rhodanase-like proteins, and Kinesin-like protein B, which are involved in signaling and development regulation respectively. According to reports, in soybean, miR1535b is responsible for the cleavage of Glyma07g38620.1, which is responsible for isopentenyl transferase (IPT) catalyzing the first stage of de novo cytokinin (CK) synthesis<sup>64,73</sup>.

Huang et al. discovered OSA-miR604, which is expected to target the wall-associated kinase (WAK) protein. Some WAK members are reported to be involved in plant defense and heavy metal stress development. Despite this, osa-miR604 targets the lipid transfer protein gene (LTP). These LTP transcripts are upregulated in rice seedling roots under Cd stress<sup>73</sup>.

Along with other heavy metals, researchers also considered mercury as the top metal toxic pollutant. miRNA is considered to be very useful for controlling post-transcriptional gene regulation which involves Hg toxicity. In 2008 Zohu et al. identified some miRNAs that are sensitive to Hg toxicity. Among them, miR171, miR319, miR393, and miR529 are upregulated, on the contrary, the expression of miR166 is upregulated while miR319, miR160, and miR395 were reportedly unaffected in response to Hg. miR398 was shown to be downregulated



in plants facing essential nutrient deficiency and Cd or Hg toxicity. This indicates that the essential role of miRNA398 is to control the ROS equilibrium under nutritional stress<sup>74</sup>.

Arsenic (As), a common element in the earth's crust and a known carcinogen may be extremely harmful to people. When plants are exposed to As, they may experience symptoms such as reduced photosynthetic rate, irregular glucose metabolism, and increased ROS generation<sup>72</sup>.

Different As responsive miRNAs have been identified by Yu et al in *O. sativa*. Among all miRNAs in *O. Sativa*, 14 seemed to participate in the gene regulation of genes involved in metabolism, signaling, and transportation. These findings further showed the importance of miRNA-based control of lipid metabolism and jasmonic acid (JA) signaling during As exposure in plants. MiR528 targets Cu<sup>2+</sup>-binding proteins (CBPs), which may translocate Zn, Cu, or other dangerous metals out of the ER to control the quantity of free cellular auxin. As a result, miR528 expression preserves conjugated auxins to protect *O. Sativa* seedlings against arsenic-based damage despite the loss of the IAR1 protein<sup>75</sup>.

Expression of miRNAs is identified by scantiest Liu et al in *B. Juncea* root when they expose to As. Several miRNAs have been identified in various developmental processes, nutrition absorption, hormone production, and functions using target prediction for miRNAs. Plant development under as stress was favorably impacted by altered expression of miR167, miR319, and miR854 caused by exogenously provided IAA and JA<sup>76</sup>.

Unanimously, these results supported the idea that the contribution of one miRNA or its variation role in the reaction to metal stress responses is the most advantageous experience in improving plant tolerance to HMs and capacity to detoxify these metals

### Conclusion and Prospects:

Since the identification of plant miRNAs at the beginning of the twenty-first century, substantial research has been done on the role of sRNAs in plant abiotic stress tolerance. The importance of these midget RNAs in the process that controls gene expression under various stress circumstances has been shown by several credible articles from across the world. One of the key factors limiting the growth and output of significant crops is thought to be abiotic stresses. These stresses, which are mostly associated with water and excessive salinity, are made worse by the climate. Gene expression change patterns have a substantial impact on how plants respond to abiotic stress. Amazing transcriptional modulators called miRNAs to control several stress-tolerant genes. We may investigate the function of miRNAs in plant stress responses by comprehending the processes by which miRNAs influence gene expression. Research into how miRNAs control various biotic and abiotic stress has advanced thanks to recent advancements in high throughput sequence technologies and the accessibility of whole genome data for many plant species. Meanwhile, microRNAs are fundamental components of gene

regulatory networks, and a complete knowledge of plant tolerance to abiotic stress is required. Understanding miRNA-guided stress regulatory networks will open up new avenues for the genetic augmentation of abiotic stress tolerance in plants. Several results suggest that gene regulation which is guided by miRNA may help to create stress tolerance in plants. Studying stress-responsive miRNAs and the expression of their target genes in particular cell types will offer significant insights into miRNA target networks that behave in a cell- or tissue-specific manner during stress, even though our knowledge of miRNA evolution is still in its infancy for elucidating their complex regulatory roles. It is still difficult to identify and characterize the series of short RNAs that exhibit changed expression levels in response to abiotic stress in various agricultural plants, as well as to find the targeted genes of these recently discovered miRNAs. By overcoming this obstacle, we learn new aspects of plant stress tolerance and gain insight into the intricate regulatory system that controls the abiotic stress response. If our knowledge of the functions of miRNAs under abiotic stress improves, there will be a tremendous promise for using miRNA-mediated gene regulation to boost plant stress tolerance.

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