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Current status of microRNA-mediated regulation of drought stress responses in cereals

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Abstract

Drought is one of the most important abiotic stress factors impeding crop productivity. With the uncovering of their role as potential regulators of gene expression, microRNAs (miRNAs) have been recognized as new targets for developing stress resistance. MicroRNAs are small noncoding RNAs whose abundance is significantly altered under stress conditions. Interestingly, plant miRNAs predominantly targets transcription factors (TFs), and some of which are also the most critical drought-responsive genes that in turn could regulate the expression of numerous loci with drought-adaptive potential. The phytohormone ABA plays important roles in regulating stomatal conductance and in initiating an adaptive response to drought stress. miRNAs are implicated in regulating ABA-(abscisic acid) and non-ABA-mediated drought resistance pathways. For instance, miR159-MYB module and miR169–NFYA module participates in an ABA-dependent pathway, whereas several other ABA-independent miRNA-target modules (miR156–SPL; miR393–TIR1; miR160–ARF10, ARF16, ARF17; miR167–ARF6 and ARF8; miR390/TAS3siRNA–ARF2, ARF3, ARF4) collectively regulate drought responses in plants. Overall, miRNA-mediated drought response manifests diverse molecular, biochemical and physiological processes. Because of their immense role in controlling gene expression, miRNA manipulation has significant potential to augment plant tolerance to drought stress. This review compiles the current understanding of drought-responsive miRNAs in major cereals. Also, potential miRNA manipulation strategies currently in use along with the challenges and future perspectives are discussed.

KEYWORDS
artificials, CRISPR/Cas-based MIR editing, drought, gene regulation, miRNA, stress-response

1 | INTRODUCTION

Drought is a major abiotic hurdle constraining crop productivity and has significantly impacted global crop production in the past decades (Bartels & Sunkar 2005; Kim et al. 2019; Matiu et al. 2017). Drought-related yield losses are anticipated to be higher in the future due to global climatic changes and declining precipitation (Kim et al. 2019). In order to sustain the required production level, it is highly desired to enhance the performance of agricultural crops under drought; this can be achieved only with a better understanding of the molecular mechanisms governing drought tolerance (Raza et al. 2019). However, drought tolerance is a complex trait presuming many genes and cellular pathways. During the course of evolution, plants have acquired miRNA regulatory networks to acclimatize to stress conditions (Shriram et al. 2016). MicroRNAs (miRNAs) are 20–24 nucleotides long, endogenous small RNAs that silence or down-regulate gene expression at the posttranscriptional level by targeting mRNAs through imperfect sequence complementarity (Bonnet et al. 2006; Saroha et al. 2017; Ji et al. 2018; Singroha & Sharma 2019). Increasing evidences suggests that miRNAs are important regulators of various plant processes, including growth and development (Waheed & Zheng 2020) as well as both abiotic and biotic stress responses (Sunkar et al. 2006; Sunkar et al. 2012; Sunkar & Zhu 2004). miRNA-mediated drought responses manifest in a range of molecular, biochemical and physiological processes (Balyan et al. 2017; Begum et al. 2019; Ding et al. 2013; Liu et al. 2015).

Among the thousand genes that respond to drought, the most important class of regulatory genes are the transcription factors (AREB/ABF, MYB (myeloblastosis), NF-Y (Nuclear Factor Y), NAC (NAM: no apical meristem; ATAF: *Arabidopsis* transcription activation factor; CUC: cup-shaped cotyledon), HD-ZIP and WRKY), which in turn regulate numerous downstream genes that play essential roles in adaptation to drought (Bartels & Sunkar 2005; Jan et al. 2019; Joshi et al. 2016; Khan et al. 2018; Samad et al. 2017). The overexpression of TFs like DREB2A (Dehydration Responsive Element Binding), NF-YB (Nuclear Factor Y -B subunit), ERF (Ethylene Responsive Factor), and NAC has been reported to enhance drought resilience in various plant species (Tang & Chu 2017). Remarkably, several members belonging to MYB, NF-Y, NAC, and HD-ZIP class of transcription factors are controlled by miRNAs (Sunkar et al. 2012).

miRNAs regulate both ABA-(abscisic acid) and non-ABA-mediated pathways under drought. ABA plays an important role in regulating stomatal conductance and in initiating the adaptive response to drought (Farooq et al. 2020; Pan et al. 2017; Zhou et al. 2019). ABA also coordinates with other phytohormones like salicylic acid (SA), auxin (AUX), ethylene (ETH), brassinosteroid (BR), gibberellins (GA), and jasmonic acid to impart drought tolerance via auxin signaling (Ku et al. 2018; Verma et al. 2016).

The miRNAs induced/upregulated during stress can represss/fine-tune the abundance of target transcript(s) that may function as negative regulators of stress tolerance. On the other hand, the level of some of the miRNAs declines under stress, leading to the increase of their target mRNAs that positively contribute to the adaptation during stress (Sunkar et al. 2007; Fard et al. 2017; Mohsenifard et al. 2017). Therefore, it is essential to understand miRNA regulation during drought in plants. In this review, we have discussed miRNA biogenesis and their modulation under drought stress in plants, with a special emphasis on cereals. In the later sections, we have discussed drought-responsive miRNAs in cereals and the potential biotechnological approaches to modulate miRNA levels for designing stress-resilient crops.

2 | miRNA BIOGENESIS AND PROCESSING IN PLANTS

pri-miRNAs (20–24 nt) fold back on itself generating a double-stranded RNA stem-loop structure which is further acted upon by DICER-LIKE1 (DCL1, an RNase III enzyme), yielding a miRNA: miRNA* duplex with two nucleotides overhangs at its 3′ end (Yu et al. 2017). The active strand that regulates the target gene expression is known as miRNA and the opposite strand is designated as miRNA*. The dicing complex is located in the nucleus and comprises DCL1 (O'Brien et al. 2018) associated with other accessory proteins: RNA-binding protein called HYPONASTIC LEAVES1 (HYL1; Han et al. 2004, Kurihara et al. 2006), SERRATE (SE; Yang et al. 2006, Machida et al. 2011), DOUBLE RNA BINDING PROTEIN (DRB2; Eamens et al. 2012) and C-TERMINAL DOMAIN PHOSPHATASE LIKE1 (CPL1; Manavella et al. 2012). HYL1 is crucial for maintaining the accuracy of miRNA processing, whereas SE stimulates the DCL1 activity (Iwata et al. 2013; Yang et al. 2014) along with a scaffolding function (Dolata et al. 2018). The RNA methyltransferase (located in the nucleus), known as HUA-ENHANCER 1 (HEN1), adds a methyl group to the duplex at the 3′ends (2′-O-methylation) of the miRNA: miRNA* duplex (Yu et al. 2005).

The miRNA: miRNA* duplex formed in the nucleus is exported to the cytoplasm through a nuclear membrane-localized HASTY protein. The mature miRNA is then loaded into the RNA-induced silencing complex (RISC), where the mature single-stranded miRNA serves as a guide molecule, while the miRNA* is rapidly degraded (Bologna et al. 2018). Although the miRNA strand selection for loading onto ARGONAUTE 1 (AGO1) is not thoroughly understood, the duplex structure, identity of the 5′ base and HYL1 appear to play important roles (Fang & Qi 2016). Recent findings suggest that the empty AGO1 is located inside the nucleus, but the loading of miRNA onto the AGO1 induces conformational change exposing the nuclear export signal that signals its way to the cytoplasm (Bologna et al. 2018). The mature miRNA loaded onto the AGO1 guides the RISC to pair with the complementary region on the target mRNAs (Jones-Rhoades et al. 2006). Plant miRNAs interact with their targets via perfect or near-perfect complementarity resulting in target mRNA cleavage between 10th and 11th nucleotides relative to the 5′ end of the miRNA. The translation is repressed when a miRNA with a significant number of mismatches and bulges align to its target mRNA (Brodersen et al. 2008; Lanet et al. 2009).

3 | POTENTIAL ROLES OF miRNAS DURING DROUGHT STRESS

Drought stress is known to alter the expression of numerous genes and gene products (Bartels & Sunkar 2005; Nezhadahmadi et al. 2013; Shinozaki & Yamaguchi-Shinozaki 2007). miRNAs themselves show altered expression in response to drought and regulates the expression of several drought-responsive genes. Drought-responsive miRNAs have been investigated in several plant species such as Arabidopsis (Liu et al. 2008; Sunkar & Zhu 2004), soybean (Kulcheski et al. 2011; Li et al. 2011; Zheng et al. 2016), cowpea (Barrera-Figueroa et al. 2011), *Phaseolus vulgaris* (Arenas-Huertero et al. 2009), sugarcane (Gentile et al. 2015), rice (Cheah et al. 2015), wheat (Akdogan et al. 2016; Giusti et al. 2017), tea (Guo et al. 2017), apple (Niu et al. 2019), and tomato (Candar-Cakir et al. 2016; Zhou et al. 2016; Zhou et al. 2020).

The miRNA response to drought stress is highly intricate, and their altered expression is influenced by treatment methods, tissue, genotype and developmental stage (Ferdous et al. 2015). For instance, water deficit induced the expression of miR393, miR397b and miR402 in

Arabidopsis, while miR319c was downregulated (Sunkar & Zhu 2004). Similarly, the expression of miR164c, miR319b, and miR1861d was downregulated under drought in rice, whereas miR166h, miR172d and miR408 were upregulated (Zhang et al. 2016). miR482 was downregulated in *T. aestivum* cv Sivas 111/33 under drought conditions (Akdogan et al. 2016). Members of the same miRNA families have been found to be differently regulated under drought stress in some plant species reflecting differential regulation of the same miRNA family members (Wang et al. 2011; Zhou et al. 2010). In response to drought stress, miRNAs are regulated differentially between different tissues, organs and at different developmental stages suggesting differences in spatio-temporal regulation. Ferdous et al. (2015) reported higher expression of miR169 in root tissues of rice in comparison to shoot under drought conditions.

In response to drought stress, ROS (reactive oxygen species) are generated in chloroplast and peroxisomes (Cruz de Carvalho 2008; Zhou et al. 2019). Enhanced levels of ROS are harmful to cells; therefore, to nullify their toxic effect, plants produce anti-oxidative enzymes, namely superoxide dismutase (SOD), peroxidase (POD), catalase, glutathione reductase, and ascorbate peroxidase (APX) that facilitate ROS scavenging (Mittler 2002; Zhou et al. 2019). Interestingly, some of the plant miRNAs are known to modulate oxidative stress networks by targeting Cu/Zn superoxide dismutases (miR398) and peroxidase (miR528). Pertinently, the abundances of miRNAs that are targeting the antioxidant genes were often found to be downregulated under stress. For instance, downregulated miR528 under drought stress resulted in the accumulation of their target, peroxidase, which facilitates ROS detoxification and imparts drought resilience (Wei et al. 2009). Similarly, miR398 levels were often shown to be downregulated under stress conditions to enhance the accumulation of Cu/Zn superoxide dismutases that participates in relieving ROS-mediated damage under stress (Sunkar et al. 2006; Jagadeeswaran et al., 2009).

4 | REGULATORY NETWORKS OF miRNAS

The major strategy employed by plants to combat stress conditions is by regulating the transcript level of stress-responsive genes (Pan et al. 2017; Shinozaki & Yamaguchi-Shinozaki 2007). Interestingly, a large number of highly conserved miRNA families in plants target transcription factors that regulate numerous other genomic loci. Although indirectly, this implies that the miRNAs also control transcriptional regulation by regulating the abundance of transcription factors. The interaction among miRNAs and transcription factors coordinates various signaling pathways (ABA-mediated and non-ABA-mediated pathways) in plants.

4.1 | ABA-mediated pathway

When plants encounter drought, dehydrated roots synthesize ABA to repress the growth of lateral roots, thereby redirecting the resources towards the production of deeper roots to enhance water absorption from the soil (Xiong et al. 2006). The phytohormone ABA is known to impart drought tolerance by modulating stomata closure, reducing water loss via transpiration, and improving plants water use efficiency, as well as by activating ABA-dependent gene regulatory networks that confer survival under water stress (Lee & Luan 2012, Daszkowska-Golec & Szarejko 2013, Tian et al. 2015). ABA, through miRNAs, could regulate genes that are important during drought adaptation. The potential involvement of miRNAs in ABA-dependent drought responses became evident with the observation that the *hyl1* mutant showed hypersensitivity to ABA and drought (Lu & Fedoroff 2000). Also, the Arabidopsis mutants isolated for ABA hypersensitivity (*ABA hypersensitive during germination1*; *absg1* - named as *dcl1-11*) and *ABA hypersensitive during germination2* (*absg2*-named as *hen1-16*) were found to be allelic to DCL1 and HEN1, respectively (Zhang et al. 2008). All these three proteins, that is, HYL1, DCL1 and HEN1 are extremely critical for miRNA biogenesis in plants. Various transcription factors such as the MYB and NAC, which are also targeted by miRNAs, are involved in the ABA-mediated pathway of drought tolerance (Figure 1; Zhao et al. 2017a, Khan et al. 2018). For instance, miR159, which targets *MYB33* and *MYB101* transcripts, was upregulated in response to ABA and drought treatments in Arabidopsis (Allen et al. 2010; Reyes & Chua 2007). The overexpression of miR159 repressed the transcript level of *MYB33* and *MYB101* and rendered plants less sensitive to ABA, while transgenic plants overexpressing miR159-resistant forms of MYB33 and MYB101 showed hypersensitivity to ABA (Reyes & Chua 2007). miR159 also acts as a key repressor of the primary root growth through repression of MYB65 (Xue et al. 2017). The enhanced expression of core genes of ABA signaling has been observed when TaNAC29 (a NAC transcription factor from wheat) was overexpressed in Arabidopsis (Huang et al. 2015). Liu et al. (2019) affirmed that miR164 regulates the abundances of MYB and NAC TFs under stress in maize and their expression was regulated by ABA.

The downregulation of miR166 was reported in barley and *Triticum dicoccoides* under water deficit stress (Kantar et al. 2010; Kantar et al. 2011). miR166 regulates the homeodomain-leucine zipper (HD-Zip III) transcription factors, important for axillary meristem initiation and lateral root development (Boualem et al. 2008; Mao et al. 2016; Turchi et al. 2015). An elevated level of HD Zip III transcripts influences root architecture, facilitating water uptake by roots (Guo et al. 2017; Malamy 2005). Recently, Yang et al. (2019) reported that miR165/166-HDZIPIIIs module-mediated drought tolerance through ABA signaling in maize.

miRNAs have been portrayed as key regulators of root development architecture via targeting several TFs, including Auxin Response Factors (ARFs; Khan et al. 2018). Relevantly, miR167 that regulates ARFs, was found to be largely upregulated in the primary root growth zone of soybean and maize under drought (Seeve et al. 2019; Zheng et al. 2016). Similarly, miR167 levels were upregulated in Arabidopsis, wheat, and maize under drought stress (Liu et al. 2016; Pandey et al. 2013). In rice, miR167 was shown to be downregulated under ABA treatment (Liu et al. 2009; Wei et al. 2009). Relatedly, miR167d

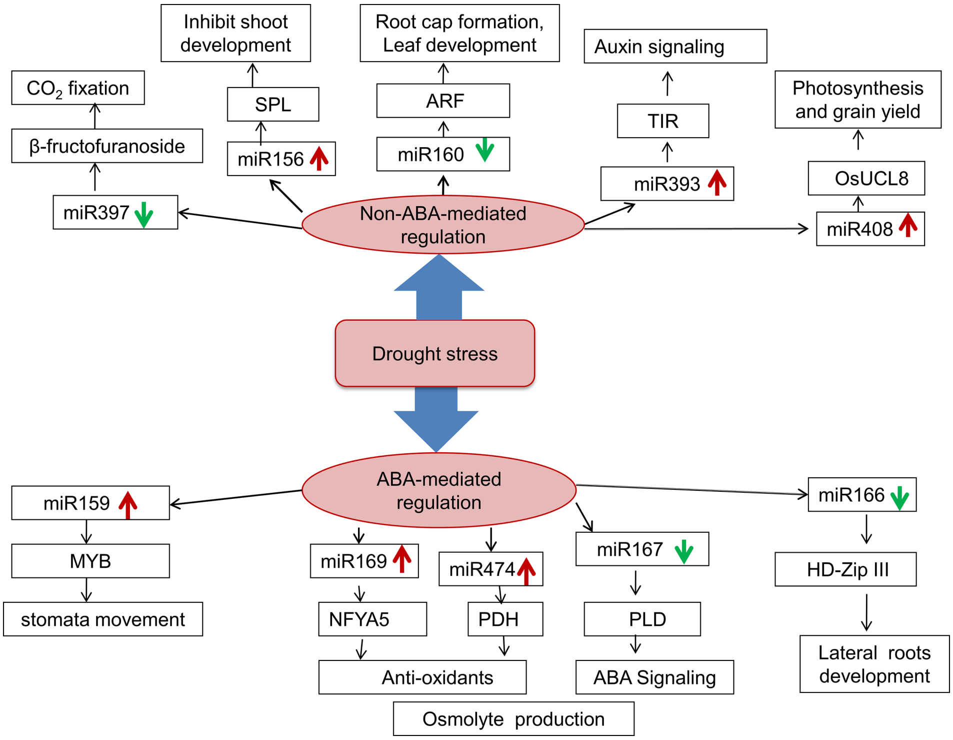

FIGURE 1 miRNA-mediated ABA and non-ABA pathways. Green arrows show downregulated miRNAs and red arrows show upregulated miRNAs. Upregulated miRNAs downregulate their target mRNA and vice versa. ARF, auxin response factor; HD-Zip III, homeodomain-leucine zipper (HD-Zip III); MYB, myeloblastosis; NFYA5, nuclear factor Y; PDH, proline dehydrogenase; PLD, phospholipase D; SPL, squamosa promoter-binding protein-like; TIR, TIR1 transport inhibitor response 1

levels in maize were downregulated under drought, which results in elevating the Phospholipase D (PLD, one of the nonconserved targets of miR167 in maize) levels, and together promotes stress tolerance (Wei et al. 2009). PLD activation is critical for ABA signaling to regulate stomatal movement in several other plant species including rice (Zhang et al. 2009a, 2009b).

4.2 | Non-ABA-mediated pathway

In plants, four miRNA families (miR393, miR160, miR167 and miR390) regulates auxin signaling to regulate plant growth and development. Studies have shown the upregulation of miR393 upon drought imposition in Arabidopsis, rice, switchgrass, maize, soybean and sugarcane (Ferreira et al. 2012; Hivrale et al. 2016; Seeve et al. 2019; Sunkar et al. 2012; Sunkar & Zhu 2004; Zhao et al. 2007; Zheng et al. 2016). miR393 targets *TIR1* (TRANSPORT INHIBITOR RESPONSE 1) an auxin receptor, a positive regulator of auxin signaling (Figure 1), which promotes Aux/IAA proteins degradation through ubiquitination (Dharmasiri & Estelle 2002). The miR160 family is involved in root cap formation through *ARF-10* and *ARF-16*-mediated auxin signaling (Yang et al. 2019). miR167 targets *ARF6* and *ARF8* and was shown to be upregulated in Arabidopsis, wheat, maize, switchgrass, soybean, maize and tomato under water stress (Hivrale et al. 2016; Liu et al. 2019; Pandey et al. 2013; Seeve et al. 2019; Zheng et al. 2016; Zhou et al. 2020). miR390, also a component of auxin signaling through a TAS3 siRNAs (miR390-TAS3-*ARF2*/*ARF3*/*ARF4*; Marin et al. 2010, Yoon et al. 2010). Contrary to the usual mRNA targets, miR390 triggers tasiRNA (TAS3-derived *trans*-acting small interfering RNA) involved in regulating lateral root development and organ polarity establishment by targeting *ARF2*, *ARF3*, and *ARF4* factors (Meng et al. 2010). Under drought, miR390 levels were upregulated in cowpea (*Vigna unguiculata*) and switchgrass, suggesting a role of miR390 dependent *trans*-acting siRNA pathway in drought responses (Barrera-Figueroa et al. 2011; Hivrale et al. 2016).

Mutum et al. (2016) reported the enhanced expression of miR408 transcripts in drought-tolerant rice cultivars in comparison to drought-sensitive lines. miR408 in rice targets *OsUCL8* (a plastocyanin-like protein) and this module was reported to positively regulate photosynthesis and grain yield under drought (Zhang et al. 2017a, 2017b, 2017c). NFY (Nuclear Factor Y) transcription factors are implicated in plant growth and development as well as responses to environmental stresses (Kumimoto et al. 2008; Li et al. 2008). NFY5A mRNAs are exceedingly expressed in stomata and can control the guard cell aperture and several drought-responsive genes, for example, superoxide dismutase (SOD), glutathione transferase (GT) and peroxidases (POD; Li et al. 2008). miR169a was downregulated under drought stress, increasing the level of NFY5A transcripts in Arabidopsis, tomato and rice (Lee et al. 2015; Zhang et al. 2011). These observations suggest that miR169a-controlled *NFYA5* regulation imparts drought resistance via controlling the antioxidant enzymes.

The miR156 is known to play an important role in plant phase transitions by targeting SPL (SQUAMOSA promoter binding protein-like) transcription factors (Chen 2010; Niu et al. 2019). The miR156-SPL module has also been shown to improve drought resistance in Arabidopsis (Han et al. 2013; Wang et al. 2009). miR398, targeting *CSD1* and *CSD2* encoding copper/zinc superoxide dismutase (Cu/Zn-SODs) and *CCS1* encoding the copper chaperone for SOD is another modulator specifically downregulated under drought stress (Cheah et al. 2015; Ding et al. 2013; Li et al. 2010; Sunkar et al. 2006;

Sunkar & Zhu 2004). Liu et al. (2019) has shown that miR398b was specifically downregulated in the roots and leaves of maize genotype H082183 under drought, underpinning its importance during drought stress.

miR528 is involved in ROS detoxification under drought (Zhang et al. 2017a, 2017b, 2017c). miR528 was downregulated in maize seedlings and sensitive rice cultivars under stress, while it was upregulated in tolerant cultivars of rice (Balyan et al. 2017; Wei et al. 2009). miR474 targets *PDH* (*PROLINE DEHYDROGENASE*) gene and plays a role in proline degradation (Wei et al. 2009; Zhou et al. 2010). The drought-triggered miR474 expression in maize resulted in downregulation of *PDH* transcript levels, leading to accumulation of proline under drought (Wei et al. 2009). Similarly, upregulation of miR474 is associated with decreased proline degradation in rice by decreasing the expression of *PDH* levels (Zhou et al. 2010).

Niu et al. (2019) reported a higher miR395 transcript level in the drought-tolerant apple than in the drought-sensitive one under drought stress. miR395 has been shown to target *WRKY33* gene (probably a nonconserved target for miR395 in apple) and the upregulated miR395 has been linked with the downregulation of *WRKY33* under stress, which may contribute to increased drought tolerance. The sucrose synthase gene expression is found significantly higher in drought-tolerant wheat cultivar as compared to drought-sensitive ones (Guo et al. 2018; Nemati et al. 2018). Interestingly, a novel miRNA identified in wheat (NmiRNA15) was found to be downregulated under drought, which is likely to regulate the transcripts of sucrose synthase. Taken together, several miRNA-target modules are functioning in non-ABA-dependent pathways under drought stress in plants.

5 | miRNAS INVOLVED IN IMPARTING DROUGHT TOLERANCE IN ECONOMICALLY IMPORTANT CROP PLANTS

Known miRNAs involved in drought stress tolerance of important cereals are detailed in Table 1.

5.1 | Wheat

Wheat is one of the most important cereal crops known for its contribution to fulfilling the caloric demand of a large part of the population.

TABLE 1 Drought-responsive microRNAs in cereals

miRNA	Target name	Species	Source
miR156	SBP	Rice, *Triticum dicoccoides*, Arabidopsis	Liu et al. 2008, 2008; Kantar et al. 2011
miR159	MYB, TCP, plantacyanins, laccases	Rice, Wheat, Arabidopsis	Balyan et al. 2017; Jones-Rhoades & Bartel 2004; Liu et al. 2008, 2008; Allen et al. 2010
miR164	MYB, NAC	Maize	Liu et al. 2019
miR166	HD-ZIPIII protein	Soyabean	Li et al. 2011
miR167	PLD	Rice, Maize	Liu et al. 2009; Wei et al. 2009
miR168	AGO1	Arabidopsis, Maize	Wei et al. 2009; Zhou et al. 2010
miR169a	NF-YA	Rice, Arabidopsis	Liu et al. 2008, 2008; Zhao et al. 2019
miR170	SCL	Arabidopsis, Rice	Zhou et al. 2010; Sun et al. 2014
miR171	GRAS	Arabidopsis, *Triticum dicoccoides*, Rice	Kantar et al. 2011; Zhou et al. 2010
miR172	APETALA2, bZIP	Arabidopsis, Rice	Zhou et al. 2010
miR319	MYB	Rice	Zhou et al. 2010
miR390	ARF2/ARF3/ARF4	Arabidopsis	Marin et al. 2010; Yoon et al. 2010
miR393	TIR1	Arabidopsis, Rice	Sunkar & Zhu 2004; Zhao et al. 2007
miR396	GRL	Sorghum, rice, Arabidopsis	Hamza et al. 2016; Liu & Yu 2009; Zhou et al. 2010
miR397	Laccase	Arabidopsis, Rice	Sunkar & Zhu 2004; Ding & Zhu 2009
miR398	Cu/Zn-SODs	Maize	Zhu et al. 2011; Leng et al. 2017
miR408	Uclacyanin, Cu/Zn SODs	Rice, Arabidopsis	Balyan et al. 2017; Liu et al. 2008, 2008
miR474	Kinesin	Maize	Zhou et al. 2010
miR827	MYB, NAC	Rice	Singh et al. 2020
miR399e	Ubiquitin conjugating enzymes	Maize	Seeve et al. 2019
miR528	Peroxidase	Maize	Wei et al. 2009
miR827	NAD, SPX	Maize	Zhang et al. 2009a, 2009b
miR1432	ADP-ribose	*Triticum dicoccoides*	Zhang et al. 2009a, 2009b; Kantar et al. 2011
TaMIR1119	CAT, POD, SOD	*Triticum aestivum*	Shi et al. 2018

Kantar et al. (2010, 2011) documented differential expression of 13 drought-responsive miRNAs in *Triticum dicoccoides*. Ma et al. (2015) reported that out of 46 conserved and 321 novel differentially expressed miRNAs between the wheat genotypes Hanxuan10 (drought tolerant) and Zhengyin1 (Drought susceptible), 13 miRNAs exhibited opposite patterns of expression in leaves during dehydration stress. Akdogan et al. (2016) reported that 285 miRNAs (207 upregulated and 78 downregulated) in leaf and 244 miRNAs (115 upregulated and 129 downregulated) in root were differentially expressed in wheat under drought stress. Among the differentially expressed miRNAs, 23 miRNAs were only expressed in the leaf and 26 miRNAs were only expressed in the root of wheat growth under drought stress. Chen et al. (2017) observed that out of the four differentially expressed miRNAs, the expression of tae-nsmR10 and tae-miR9654a-3p was upregulated, while that of tae-csmR5082-1 and tae-nsmR5/tae-nsmR6 showed a down-regulated pattern under drought stress. Shi et al. (2018) functionally characterized TaMIR1119, a miRNA family member of wheat (*Triticum aestivum*), in mediating drought tolerance through regulating the target genes that modulate osmotic stress-associated biological processes, including osmolytes accumulation and ROS homeostasis. Apart from bread wheat, drought-responsive miRNAs have been identified in durum wheat. Fileccia et al. (2017) mapped on each wheat chrosomosomes key miRNAs involved in the response to drought stress which will allow targeting of the miRNAs in genetic improvement schemes assisted by molecular markers. Relatively, less is known about the impact of drought-modulated miRNAs on their target genes in wheat; therefore, future studies should focus on analyzing miRNA targets under stress.

5.2 | Rice

Previously, Zhou et al. (2010) reported miRNA profiles in drought-challenged rice using a microarray platform. Their findings revealed that 16 miRNAs were significantly upregulated under drought stress. Chung et al. (2016) and Bakhshi et al. (2016) reported that the drought-responsive miRNAs play important roles in the regulation of abscisic acid signaling, detoxification, calcium signaling, and lateral root formation. Balyan et al. (2017) identified cultivar-specific drought-responsive miRNAs in the flag leaf of Nagina 22 (drought-tolerant) under drought conditions. Zhang et al. (2017a, 2017b, 2017c) reported 138 novel miRNAs in Dongxiang wild rice (*Oryza rufipogon*, DXWR), of which 67 were significantly altered under drought. Jiang et al. (2019) reported that OsNAC2 positively regulates drought tolerance through the ABA pathway. Recently, Singh et al. (2020) have profiled miRNAs in leaf at panicle initiation stage in drought-tolerant rice cultivar ARC-10372. Their analysis revealed differential regulation of 10 miRNAs (that were not reported previously as drought-responsive miRNAs in rice. Xia et al. (2020) investigated the time course analysis of miRNA profiles and found that 354 conserved miRNAs belonging to 57 families were drought-responsive with genotype- and developmental stage-specific expression patterns.

5.3 | Maize

Initially, drought-responsive miRNAs in maize were identified using computational and cloning strategies. Li et al. (2013) reported differential regulation of 68 miRNAs in drought-tolerant maize and suggested the important role of miR156, miR159 and miR319 families under drought stress. Aravind et al. (2017) identified 13 drought-responsive miRNA families, while Seeve et al. (2019) identified miRNAs in the primary root growth zone under water-deficit stress. Intriguingly, their analysis revealed that the miR399e,i,j-3p, which are responsible for regulating phosphate homeostasis was significantly upregulated under water-deficit stress. Recently, Liu et al. (2019) have compared the miRNA profiles between maize inbred lines with contrasting drought sensitivities. Their analysis revealed differential regulation of several miRNA-target genes (miR164-MYB, miR164-NAC, miR159-MYB, miR156-SPL and miR160-ARF) in these genotypes.

5.4 | Sorghum

Sorghum (*Sorghum bicolor* [L.] Moench) is a major staple food crop of millions of people in Asia and Africa. More than 241 miRNAs from sorghum have been deposited in the miRBase21 (Katiyar et al. 2015). Du et al. (2010) predicted sorghum miRNAs and their targets using computational methods. Small RNA sequencing has been used to identify miRNAs in sweet sorghum (Calviño et al. 2011; Zhang et al. 2011). Katiyar et al. (2015) provided a comparative analysis of drought-responsive miRNAs in drought-tolerant and drought-sensitive sorghum genotypes and reported differences in expression of eight known and 88 novel miRNAs under drought conditions. Similarly, several drought-regulated miRNAs such as sbi-miR-4, sbi-miR-41, sbi-miR-87, sbi-miR-391, and sbi-miR-412, which target ARFs, were upregulated in M35-1 but downregulated in C43 sorghum genotype under drought stress. Hamza et al. (2016) analyzed the miRNA expression profile of sorghum under drought and observed that the sbi-miR396 and sbi-miR398 levels were the highest expressed in sorghum genotypes.

5.5 | Barley

The barley miRNAs were initially predicted using in silico approaches (Colaiacovo et al. 2010; Dryanova et al. 2008). Experimental identification of miRNAs using deep sequencing in barley has been attempted by several research groups (Hackenberg et al. 2015; Kantar et al. 2010; Schreiber et al. 2011). Ferdous et al. (2017) identified 11 miRNAs, out of which only four miRNA (*Ath*-miR169b, *Osa*-miR1432, *Hv*-miRx5 and *Hv*-miR166b/c) had significant expression differences under drought in four barley genotypes. Recently, Qiu et al. (2020) provided the first comparative study on the miRNA transcriptome in the roots of two Tibetan wild barley genotypes differing in drought tolerance and identified 69 conserved miRNAs and 1574 novel miRNAs. Among them, 36 novel miRNAs showed significantly genotype-specific expression patterns in response to drought stress. Additional investigations are required to identify drought-responsive miRNAs and validate their targets in barley.

6 | BIOTECHNOLOGICAL TOOLS TO MODULATE MIRNA EXPRESSION

Artificial miRNAs (amiRNAs), artificial target mimics and CRISPR/Cas mediated MIR gene editing have been exploited for fine-tuning miRNA expression and their target activity (Table 2).

6.1 | Artificial MIR genes

miRNAs expression can be manipulated using an artificial MIR gene (amiRNA) strategy to control a gene of interest (Schwab et al. 2006; Zhang et al. 2018). The authentic miRNA-5p:miRNA-3p sequence is altered by an engineered miRNA to target specific mRNA (Basso et al. 2019). The amiRNAs comprise a conserved secondary fold back structure atypical of a pre-miRNA. Pre-amiRNA processing yields a single amiRNA targeting a known sequence, thus avoiding off-target effects. amiRNAs offer advantages like stable inheritance, with no off-target effects and very limited production of secondary siRNAs from pre-amiRNA (Manavella et al. 2012). This strategy demands a backbone with low sequence similarity to nontarget genes for greater target specificity (Carbonell et al. 2015; Carbonell et al. 2016; Zhang et al. 2018). Initially developed for Arabidopsis, amiRNAs strategy has been employed in several crops like tobacco, rice, wheat and tomato (Carbonell et al. 2014; Carbonell et al. 2015; Gasparis et al. 2017; Sharma & Prasad 2020; Yogindran & Rajam 2021). Wyrzykowska et al. (2016) successfully silenced *CBP80/ABH1* gene in *Solanum tuberosum* using amiRNA. CBP80/ABH1 protein is part of the Cap Binding Complex involved in drought response and its silencing resulted in increased drought tolerance.

6.2 | Endogenous and artificial target mimicry

Endogenous target mimicry (eTMs), also known as miRNA decoys, sponges or competing endogenous RNAs, is a natural strategy employed by plants to regulate miRNA activity (Karakuulah et al. 2016; Lin et al. 2015). These eTMs transcripts are longer than 200 nt and lack open reading frame, and show differential expression under unfavorable conditions (Karakuulah et al. 2016; Liu et al. 2015; Liu et al. 2017; Zhang et al. 2013). eTMs bind to miRNAs via sequence complementarity and prevent miRNA from pairing with the real target transcripts, promoting the accumulation of target mRNAs (Thomson & Dinger 2016). After the first report of eTM (induced by *PHOSPHATE STARVATION 1 [IPS1]*) in Arabidopsis (Franco-Zorrilla et al. 2007),

TABLE 2 miRNA based genetic modifications

Gene modified	Expression strategy	Transgenic plants	Phenotype in transgenic plants	References
Osa-miR535	STTM inhibition and CRISPR-Cas9	*Oryza sativa*	Increased seedling survival rate	Yue et al. (2020)
miR160 and miR165/166	Short Tandem Target Mimic	*Arabidopsis thaliana*	Leaf development and drought tolerance	Yang et al. (2019)
Osa-miR393a	–	*Agrostis stolonifera*	Improved drought tolerance	Zhao et al. 2019
Osa-miR166	Knock-down of miR166 using STTM	*Oryza sativa*	Tolerance to drought	Zhang et al. (2018)
Gma-miR172	CRISPR/Cas9 mediated miR169a and miR827a deletion	*Arabidopsis thaliana*	Improved drought tolerance	Li et al. (2016)
CBP80/ABH1	using amiRNA	*Solanum tuberosum*	Improved drought tolerance	Wyrzykowska et al. (2016)
Ath-miR408	CaMV 35S promoter	*Cicer arietinum*	Enhanced drought tolerance	Hajyzadeh et al. 2015
Ath-miR393	Overexpression of a miR393-resistant TIR1 gene	*Arabidopsis thaliana*	Enhanced osmotic stress tolerance	Chen et al. (2015)
Ath-miR394a/b	CaMV 35S promoter	*Arabidopsis thaliana*	Regulation of leaf morphology, ABA hypersensitivity and improved drought stress tolerance	Song et al. (2012, 2013)
Osa-miR319a	Constitutive overexpression of Osa-miR319a	*Agrostis stolonifera*	Enhanced drought and salt tolerance	Zhou et al. (2013)
Gma-miR394a	CaMV 35S promoter	*Arabidopsis thaliana*	Drought stress tolerance	Ni et al. (2012)
Ath-miR396	CaMV 35S promoter	*Nicotiana tabacum*	Enhanced drought tolerance	Yang and Yu (2009)

Abbreviations: Ath, *Arabidopsis thaliana*; Gma, *Glycine max*; Osa, *Oryza sativa*.

numerous eTMs have been identified using in silico approach in Arabidopsis, rice, soybean and other sequenced plant genomes (Banks et al. 2012; Ye et al. 2014). Karakuulah et al. (2016) developed an online search engine called PeTMbase (http://petmbase.org) utilizing RNA-seq data from 11 plant species. Short tandem target mimic (STTM) strategy is based on the overexpression of an engineered lncRNA with multiple miRNA targeting sites. The short tandem target mimic possesses two or more conserved binding sites for the specific target miRNA with three nucleotide mismatches. STTM targeting the MIR genes have been engineered for functional analysis of miRNAs in crop plants (Peng et al. 2018; Zhang et al. 2017a, 2017b, 2017c; Zhao et al. 2017b). Yang et al. (2019) investigated the interplay between miR160 and miR165/166 and its effect on leaf development and drought tolerance in *Arabidopsis* using STTM and reported that the *ARFs* and *HD-ZIP IIIs* may play opposite roles in the regulation of leaf development and drought tolerance.

6.3 | CRISPR/Cas-mediated MIR gene editing

The clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 nuclease (CRISPR/Cas9) CRISPR/Cpf1 or CRISPR/Csm1systems, guided by RNA (guide RNA) have emerged as a new class of genome editing tools (Osakabe et al. 2016; Wang et al. 2017; Wang et al. 2018). These nucleases introduce double-stranded breaks at the target site. Nucleotides are likely to be inserted or deleted (indels) during the repair process at the target site. Additionally, genome editing can be achieved using engineered donor DNA. Chang et al. (2016) and Zhou et al. (2017) have introduced indels at pre-miRNA sequences or the miRNA processing sites of MIR genes that interfere with the miRNA biogenesis. On the other hand, indels insertion in mature miRNA can hamper pairing between miRNA and target mRNA. Similarly, complete deletion or knock-in of MIR genes or altering their promoter sequences is also possible through CRISPR-Cas9 system (Zhao et al. 2016). However, certain factors, like multiple members within a miRNA family, limit the possibility of knocking down MIR genes. Further, the smaller size of the MIR genes (encoded within intron sequences) hinders MIR gene editing (Barrangou et al. 2015; Basak & Nithin 2015; Jacobs et al. 2015). In addition to MIR genes, the CRISPR/Cas9 system can be used for editing promoter or enhancer sequences (Chang et al. 2016; Shi et al. 2017; Zhou et al. 2017). Recently, Yue et al. (2020) generated a knockout of Osa-miR535 using CRISPR-Cas9 and reported significantly increased seedling survival rate under PEG as well as drought stress. Similarly, OsmiR818 knockout lines were obtained using CRISPR-Cas9 system and the mutants displayed drought sensitivity (Chung et al. 2020).

6.4 | miRNA-based SSR markers for improving drought tolerance

Recently, several researchers have reported polymorphism in miRNAs encoding MIR genes and their target genes, suggesting that miRNA-based polymorphism can be exploited for the development of miRNA-based SSR markers (Pang et al. 2011; Yadav et al. 2014). Based on this, miRNA-based SSR markers are reported in several crops (Iquebal et al. 2019; Mehta et al. 2021; Mondal & Ganie 2014; Pang et al. 2011; Sharma et al. 2021; Yadav et al. 2014). miRNA-based SSRs exhibit a high degree of reproducibility (Mondal & Ganie 2014). Drought-induced miRNAs can be used as markers that can be directly utilized for MAS in drought improvement programs. Identification of drought-induced and species-specific miRNAs in important crop species will increase their future use for the improvement of tolerance to abiotic stresses in cereals. Marker-assisted selection will continue to be the preferred approach for the plant breeders for improving drought resilience, and miRNA-based user-friendly markers will be useful in QTL mapping and association studies in cereals.

7 | CHALLENGES, FUTURE PERSPECTIVES AND CONCLUSIONS

Because miRNAs are central components of the gene regulatory networks critical for plant growth and development and stress responses, their utility to improve crop plants has enormous potential. Although some progress has been made, many challenges still remain. To widen the scope of miRNA-based crop, there is a need for thoroughly dissected functional studies to decipher the complex mechanisms behind miRNA-dependent stress tolerance. Till now, investigations have focused on the role of individual miRNAs associated with stress tolerance; however, there is a need to address the interactions among different miRNAs. Additionally, many miRNAs are targeting multiple TFs belonging to the same family, which may control the expression of overlapping and/or unique sets of downstream genes. Dissecting these aspects will allow us to prioritize specific miRNAs or their targets. It is also essential to characterize the *cis*-regulatory elements in MIR genes. Knowing the corresponding transcription factors and how the miRNA transcription is regulated under drought will offer new insights to engineer enhanced drought stress tolerance. Further, identifying signaling pathways that are critical for miRNA regulation under stress is an important area that needs to be explored in the future. The constitutive overexpression of the MIR genes has been shown to impact various agronomic traits in plants. However, this often results in pleiotropic phenotypes and yield penalties. This can be resolved by developing and using tissue-specific and stress-inducible promoters to offset undesirable effects. Overall, the knowledge of miRNAs and their target genes may be exploited via breeding programs or genetic engineering, specifically by using CRISPR/Cas9 technology to improve drought tolerance in cereal crops.

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AUTHOR CONTRIBUTIONS

Pradeep Sharma conceived the study. Garima Singroha and Pradeep Sharma wrote the first draft. Pradeep Sharma and Ramanjulu Sunkur edited the manuscript. All authors have reviewed and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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