

Review Article

Role of MicroRNAs in Abiotic and Biotic Stress Resistance in Plants

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Plants in their natural habitat are exposed to adverse environmental conditions such as drought, salinity, temperature extremes, heavy metals toxicity, etc. as well as numerous pathogens. Along with stress-responsive genes, plant microRNAs (miRNAs) play a crucial role in fundamental processes such as growth, development and maintenance of genome integrity in response to abiotic and biotic stresses. MicroRNAs are small 22-24 nt, non-coding regulatory RNAs involved in sequence-specific complex regulatory mechanisms of gene silencing in plants. Role of miRNAs has been implicated in the regulation of plant resistance to abiotic stresses as well as immunity against pathogens. During environmental or biotic stress, defense responses are mediated by changes in both genes and miRNAs expression levels which in turn are involved in the regulation of stress response and resistance in plants. Recent evidences suggest that high-throughput sequencing technologies have significantly contributed towards the identification and functional characterization of numerous miRNAs in plants. It has also been recently reported that primary miRNA transcripts (pri-miRNAs) code for regulatory peptides, roles of some of which have been implicated in improving agronomic traits such as primary root length and nodulation in legumes. Understanding the complex molecular mechanism regulated by miRNAs during abiotic and biotic stresses can potentially offer new approaches to improve plant growth and productivity. Thus, this review summarizes the recent findings and highlights on the contribution of miRNAs as molecular regulators in abiotic stresses and plant-pathogen interactions.

Keywords: Abiotic; Biotic; Mirnas; MIR Genes; Mipep; High-Throughput Sequencing, Regulation

Introduction

Crop growth and productivity have largely been vulnerable to several environmental stress factors such as drought, salinity, extremes of temperature, increasing CO₂ concentration and pathogens. These stress factors thus pose serious threats to sustainable agriculture. The adverse effects of these stresses on crop plants are further compounded due to changing climate worldwide (Siegenthaler *et al.*, 2005; Spahni *et al.*, 2005; Anderson and Bows, 2008). It has been estimated that the average earth temperature may increase by about 2.4°C as a consequence of which there may be a 2.5-30 % reduction in crop yield by 2020 across the globe (Challinor, 2009). Therefore, climate change mitigation is one of the greatest challenges of 21st century and hence, obtaining a key

to getting potential good yields of crop plants under abiotic and biotic stresses has always been a major goal of crop researchers. Modification of endogenous plant pathways, often by manipulation of important regulatory factors, for instance, transcription factors (TFs) and microRNAs (miRNAs) may help in achieving tolerance to abiotic stresses (Sunkar 2010; Lata *et al.*, 2015). Abiotic stress tolerance has been achieved by altering the expression levels of regulatory proteins in the model plant *Arabidopsis* (Sakuma *et al.*, 2006; Ding *et al.*, 2009) as well as in several other crop species (Oh *et al.*, 2005; Nelson *et al.*, 2007). Further, along with other mechanisms, the genome integrity and development in plants are also known to be facilitated by as RNA silencing, which is sequence-specific gene regulatory mechanism mediated by small RNAs (Younis *et al.*, 2014).

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Small RNAs are 19-30nt, non-coding regulatory RNAs that regulate the expression of protein-coding genes at either transcriptional or post-transcriptional levels in plants and animals (Trindade *et al.*, 2010; Sunkar *et al.*, 2012). Small RNAs were first discovered from *C. elegans* (Lee *et al.*, 1993) and are known to be involved in RNA interference (RNAi), co-suppression, gene silencing, or quelling (Napoli *et al.*, 1990; Romano Macino 1992; de. Carvalho *et al.*, 1992; Hannon, 2002). Numerous biological processes including development, maintenance of genome integrity, metabolism, immunity against pathogens, and abiotic stress responses are a crucial attribute for plant growth and development, regulated by small RNAs. On the basis of biogenesis, precursor structure and mode of action, the small RNAs are divided into two main classes: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Small interfering RNAs originate from long, perfectly double standard RNA (dsRNA) derived from transcription of sense-antisense gene pairs, inverted repeat sequences, synthesis by RNA-dependent RNA polymerase (RDRs), or virus-derived transcripts. However, miRNAs are encoded by MIR genes and transcribed by RNA Pol II. Beside cellular, biological and developmental processes (Li and Zhang, 2016), miRNAs also have been reported to play important roles in biotic (Khraiwesh *et al.*, 2012) and abiotic stresses such as drought (Ferdous *et al.*, 2017; Wu *et al.*, 2017; Aravind *et al.*, 2017; Liu *et al.*, 2017; Jatan *et al.*, 2018, 2019), salinity (Li *et al.*, 2013; Sun *et al.*, 2015; Chen *et al.* 2017; Fu *et al.*, 2017; Yin *et al.*, 2017; Jatan *et al.*, 2018; Mondal *et al.*, 2018), heat (Liu *et al.*, 2015; Wang *et al.*, 2016; Mangrauthia *et al.*, 2017; Pan *et al.*, 2017), cold (Barakat *et al.*, 2012; Shu *et al.*, 2016; Yang *et al.*, 2017; Song *et al.*, 2017; Zeng *et al.*, 2018), oxidation (Sunkar *et al.*, 2006), nutrients (Fujii *et al.*, 2005; Pant *et al.*, 2009; Liang *et al.*, 2010) and heavy metals (Zeng *et al.*, 2012; Zhou *et al.*, 2012a). This review thus highlights the importance of miRNAs for crop improvement.

MicroRNAs: Biogenesis and Mechanism of Action

miRNAs are small 22-24 nt, non-coding regulatory RNAs that are produced by the transcription of endogenous MIR genes. Nuclear-encoded MIR genes are transcribed by Pol II to produce transcripts with a hairpin structure called primary miRNAs (pri-

miRNAs) (Voinnet 2009). The RNA-binding protein DAWDLE (DDL) is thought to stabilize the hairpin structure of pri-miRNAs and also involve in the formation of precursor miRNAs (pre-miRNAs). In plants, Dicer-like 1 (DCL1), HYPOPLASTIC LEAVES 1 (HYL1), SERRATE (SE) proteins and the cap-binding proteins complex (CBC) are involved in the processing of pri-miRNAs into pre-miRNAs with a characteristic hairpin structure (Vionnet 2009; Iwata *et al.*, 2013; Dolata *et al.*, 2016). The pre-miRNA hairpin is finally processed into 20- to 24-nt miRNA/miRNA* duplexes by DCL1, HYL1, and SE. Then the methylation of the miRNA/miRNA* duplexes at the 3' terminus takes place by HUA ENHANCER 1 (HEN1) and the methylated miRNA/miRNA* duplexes are exported into the cytoplasm by an exportin protein, HASTY (HST1). In the cytoplasm, guide strand of the duplex is incorporated into an ARGONAUTE (AGO), the catalytic component of RNA-induced silencing complex (RISC), and guides RISC to bind to target transcripts by sequence complementarity. Besides, post-transcriptional controls of target genes, miRNAs also regulate the gene expression by epigenetic modifications such as DNA and histone methylation (Bao *et al.*, 2004; Khraiwesh *et al.*, 2010; Wu *et al.*, 2010).

Functional analysis of miRNAs revealed that miRNAs play an important role in the growth and development of plants. Most of them target transcription factors that successively regulate various biological, physiological and metabolic processes in plants. miRNAs are not only involved in the regulation of developmental processes but also involved in the regulation of plant responses to biotic and abiotic stresses. miRNAs are known to play an important role in response to various abiotic stresses, heavy metal stress and nutrient deprivation (Table 1). It has been previously reported that small RNAs are involved in antiviral defense responses (Ding and Voinnet 2007), however, the role of miRNAs in response to bacteria, nematode, oomycetes and herbivores are also reported (Voinnet 2008; Huang *et al.*, 2016; Islam *et al.*, 2017).

Role of miRNAs in Response to Drought and Salt Stress

Plant growth and yield are dramatically affected by

Table 1: Abiotic and biotic stress-responsive miRNAs in major crops

Types of stress	miRNA families	Response of miRNAs	Putative/validated targets	References
Drought	miR156, miR160,	<i>Gma</i> ↑, <i>Tae</i> ↑ <i>Osa</i> ↑, <i>Tae</i> ↑	SPL TFsARF	(Li <i>et al.</i> , 2011; Akdogan <i>et al.</i> , 2015)(Barrera-Figueroa <i>et al.</i> , 2012; Akdogan <i>et al.</i> , 2015)
	miR164, miR166,	<i>Tae</i> ↑, <i>Osa</i> ↓, <i>Ssp</i> ↑, <i>Tae</i> ↑	NACHD-ZIPIII	(Pandey <i>et al.</i> , 2014)(Barrera-Figueroa <i>et al.</i> , 2012); Gentile <i>et al.</i> , 2015; Akdogan <i>et al.</i> , 2015)
	miR167, miR168,	<i>Gma</i> ↑, <i>Osa</i> ↑, <i>Tae</i> ↑, <i>Osa</i> ↓	ARF AGO1	(Li <i>et al.</i> , 2011; Barrera-Figueroa <i>et al.</i> , 2012; Akdogan <i>et al.</i> , 2015)
	miR169, miR170,	<i>Ssp</i> ↓, <i>Osa</i> ↓ <i>Gma</i> ↑, <i>Hvu</i> ↓,	NFY SCL	(Barrera-Figueroa <i>et al.</i> , 2012), (Gentile <i>et al.</i> , 2015) (Zhou <i>et al.</i> , 2010)
	miR171,	<i>Ssp</i> ↓, <i>Tae</i> ↑	GRAS	(Li <i>et al.</i> , 2011; Kruszka <i>et al.</i> , 2014; Gentile <i>et al.</i> , 2015; Pandey <i>et al.</i> , 2014)
	miR172,	<i>Osa</i> ↓, <i>Ssp</i> ↓, <i>Tae</i> ↓	bZIP, AP2	(Zhou <i>et al.</i> , 2010; Gentile <i>et al.</i> , 2015; Akdogan <i>et al.</i> , 2015)
	miR393,	<i>Osa</i> ↑	TIR1, AFB	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR395,	<i>Gma</i> ↑, <i>Tae</i> ↑	APS/AST	(Li <i>et al.</i> , 2011; Akdogan <i>et al.</i> , 2015)
	miR396,	<i>Gma</i> ↑, <i>Osa</i> ↑, <i>Ssp</i> ↓, <i>Tae</i> ↑	GRF	(Li <i>et al.</i> , 2011; Barrera-Figueroa <i>et al.</i> , 2012; Gentile <i>et al.</i> , 2015; Akdogan <i>et al.</i> , 2015)
	miR408,	<i>Osa</i> ↓	Plantacyanin	(Zhou <i>et al.</i> , 2010)
	miR444,	<i>Hvu</i> ↓	MADS-box	(Kruszka <i>et al.</i> , 2014)
	miR474,	<i>Osa</i> ↑	Protein kinase, PPR SBP-box	(Zhou <i>et al.</i> , 2010)
	miR529,	<i>Osa</i> ↑	Glutaredoxin 2	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR809,	<i>Osa</i> ↓	Cytochrome c	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR1428,	<i>Osa</i> ↓	AAA ATPase	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR1884,	<i>Osa</i> ↓	Wpk4 protein kinase	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR5049,	<i>Tae</i> ↑		(Akdogan <i>et al.</i> , 2015)
Salt	miR156,	<i>Ssp</i> ↑, <i>Tae</i> ↓	SPL TFs	(Bottino <i>et al.</i> , 2013; Pandey <i>et al.</i> , 2014)
	miR159,	<i>Osa</i> ↓, <i>Ssp</i> ↑	MYB TFs	(Barrera-Figueroa <i>et al.</i> , 2012; Bottino <i>et al.</i> , 2013)
	miR160,	<i>Osa</i> ↓, <i>Tae</i> ↓	ARF	(Barrera-Figueroa <i>et al.</i> , 2012; Pandey <i>et al.</i> , 2014)
	miR164,	<i>Tae</i> ↓	NAC	(Pandey <i>et al.</i> , 2014)
	miR166,	<i>Ssp</i> ↑	Class III HD-Zip	(Bottino <i>et al.</i> , 2013)
	miR167,	<i>Ssp</i> ↑, <i>Tae</i> ↓	ARF	(Bottino <i>et al.</i> , 2013; Pandey <i>et al.</i> , 2014)
	miR168,	<i>Hvu</i> ↓, <i>Ssp</i> ↑	AGO1HAP12-CCAAT- box	(Deng <i>et al.</i> , 2015; Bottino <i>et al.</i> , 2013)(Bottino <i>et al.</i> , 2013)
	miR169,	<i>Ssp</i> ↑		
	miR171,	<i>Hvu</i> ↑, <i>Tae</i> ↓	Protein FAN, Scarecrow- like TF	(Deng <i>et al.</i> , 2015; Pandey <i>et al.</i> , 2014)
	miR319,	<i>Osa</i> ↓	TCP family	(Barrera-Figueroa <i>et al.</i> , 2012)
miR393,	<i>Osa</i> ↑	TIR1, AFB	(Barrera-Figueroa <i>et al.</i> , 2012)	
miR394,	<i>Osa</i> ↓	F-box protein	(Barrera-Figueroa <i>et al.</i> , 2012)	
miR396,	<i>Ssp</i> ↑, <i>Tae</i> ↓	GRF1,3	(Bottino <i>et al.</i> , 2013; Pandey <i>et al.</i> , 2014)	
miR444,	<i>Hvu</i> ↓	MADS-box	(Deng <i>et al.</i> , 2015)	
Cold	miR393,	<i>Osa</i> ↑	TIR1, AFB	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR394,	<i>Osa</i> ↑	F-box protein	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR396,	<i>Osa</i> ↑	GRF	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR529,	<i>Osa</i> ↑	SBP-box gene	(Barrera-Figueroa <i>et al.</i> , 2012)
	miR809,	<i>Osa</i> ↓	Glutaredoxin 2	(Barrera-Figueroa <i>et al.</i> , 2012)
Heat	miR156,	<i>Tae</i> ↓	SPL	(Xin <i>et al.</i> , 2010)
	miR159,	<i>Tae</i> ↑	MYB domain	(Pandey <i>et al.</i> , 2014)
	miR160,	<i>Hvu</i> ↑, <i>Tae</i> ↑	ARF 13, 17	(Hackenberg <i>et al.</i> , 2015; Pandey <i>et al.</i> , 2014)
	miR164,	<i>Tae</i> ↓	NAC	(Xin <i>et al.</i> , 2010)
	miR166,	<i>Hvu</i> ↑, <i>Tae</i> ↑	HD-ZIPIII	(Hackenberg <i>et al.</i> , 2015; Xin <i>et al.</i> , 2010)
	miR167,	<i>Tae</i> ↓	ARF	(Xin <i>et al.</i> , 2010)
	miR168,	<i>Tae</i> ↑	AGO	(Xin <i>et al.</i> , 2010)
	miR169,	<i>Tae</i> ↑	NF-YA	(Xin <i>et al.</i> , 2010)

	miR172,	<i>Tae</i> ↓	AP2-like	(Xin <i>et al.</i> , 2010)
	miR396,	<i>Tae</i> ↓	GRF3	(Xin <i>et al.</i> , 2010)
	miR827,	<i>Tae</i> ↑	NLA	(Xin <i>et al.</i> , 2010)
	miR5175	<i>Hvu</i> ↑	ACC-like oxidase	(Hackenberg <i>et al.</i> , 2015)
Heavy metals	miR156,	<i>Osa</i> ↓	SPL TFs	(Ding <i>et al.</i> , 2011)
	miR162a,	<i>Osa</i> ↓	DCL1	(Ding <i>et al.</i> , 2011)
	miR166,	<i>Osa</i> ↓	HD-ZIPIII	(Ding <i>et al.</i> , 2011)
	miR168,	<i>Osa</i> ↓	AGO	(Ding <i>et al.</i> , 2011)
	miR171,	<i>Osa</i> ↓	Scarecrow-like TF	(Ding <i>et al.</i> , 2011)
	miR390,	<i>Osa</i> ↓	LRR-RLK	(Ding <i>et al.</i> , 2011)
	miR396,	<i>Osa</i> ↓	GRF	(Ding <i>et al.</i> , 2011)
	miR444,	<i>Osa</i> ↓	MADS-box	(Ding <i>et al.</i> , 2011)
	miR528	<i>Osa</i> ↑	DCL1	(Ding <i>et al.</i> , 2011)
Bacteria	miR169,	<i>Mtr</i> ↓	HAP2/NF YA	(Reynoso <i>et al.</i> , 2013)
	miR171,	<i>Mtr</i> ↑	GRAS (NSP2)	(Reynoso <i>et al.</i> , 2013)
	miR172,	<i>Mtr</i> ↑	AP2 TF	(Reynoso <i>et al.</i> , 2013, Nova-Franco <i>et al.</i> , 2015)
	miR2118	<i>Mtr</i> ↑	NBS LRRs	(Reynoso <i>et al.</i> , 2013)
Viruses	miR169,	<i>Grapevine</i>	Zinc finger	(Singh <i>et al.</i> , 2012)
	miR398,	<i>Grapevine</i>	Terpene synthase	(Singh <i>et al.</i> , 2012)
	miR1885,	<i>Bra</i>	TIR-NBS-LRR	(He <i>et al.</i> , 2008)
	miR3623,	<i>Grapevine</i>	TIR-NBS-LRR	(Singh <i>et al.</i> , 2012)
	miR6019	<i>Nbe</i>	TIR-NBS-LRR	(Li <i>et al.</i> , 2012)

↑, up regulation; ↓, down regulation.

Bra (*Brassica rapa*), *Glycine max* (*Gma*), *Hordeum vulgare* (*Hvu*), *Medicago truncatula* (*Mtr*), *Nicotiana benthamiana* (*Nbe*), *Oryza sativa* (*Osa*), *Saccharum sp.* (*Ssp*), *Triticum aestivum* (*Tae*), and *Vitis vinifera* (*Vvi*)

both drought and salt stresses. Besides expression of stress-responsive genes, several miRNA families have been found to be regulated by these two abiotic stresses. These stress-responsive genes and miRNAs are known to regulate the expression of several other genes related to growth, development and physiology of plants. A large number of miRNAs related to drought stress were identified in *Arabidopsis* (Liu *et al.*, 2008), rice (Zhou *et al.*, 2010), barley (Ferdous *et al.*, 2017), common bean (Wu *et al.*, 2017), Maize (Aravind *et al.*, 2017), tea (Guo *et al.*, 2017) and tomato (Liu *et al.*, 2017) through small RNAs sequencing. In maize, 65 drought-associated miRNAs belonging to 13 families were identified from microarray data (Aravind *et al.*, 2017). In a tolerant genotype of maize, miR159, miR160, miR164, miR166, miR169, miR390, miR395, miR396, and miR399 family members were significantly up-regulated by drought stress while miR156 and miR159 members were found to be significantly down-regulated (Aravind *et al.*, 2017). On the other hand, in a sensitive genotype of maize, members of miR164, miR169, miR393, miR396, miR399, miR529, and miR2275 families were significantly up-regulated under drought stress while those of miR156, miR159, miR166 and miR395 were significantly down-regulated (Aravind *et al.*, 2017).

The study reported that upregulated expression of miR396c, d down regulates the expression of its target gene, *GRF1* in maize and provides tolerance to drought stress. A total of 105 conserved and 179 novel miRNAs were identified by small RNA profiling of tomato upon drought stress using Illumina sequencing technology (Liu *et al.*, 2017). Out of these, 54 conserved and 98 novel miRNAs were differentially expressed upon exposure to drought stress. Small RNAs sequencing of tea plant exposed to drought stress using the Illumina platform revealed that 299 conserved and 46 novel miRNAs were identified from four small RNA libraries (Guo *et al.*, 2017). The expression of 14 conserved (miR156, miR159a, miR165a-3p, miR854, miR166a, miR166g-3p, miR167d, miR2199, miR398, miR408b-5p, miR435a, miR6170, miR894, and miR395a) and 7 novel (csi-miR4, csi-miR7, csi-miR12, csi-miR18, csi-miR24, csi-miR26, and csi-miR28) drought-responsive miRNAs from *C. sinensis* under drought stress were validated. It was speculated that the target genes of miR854 such as *divalent metal transporter 1 (DMT1)* and copper transporters (*CTR*) are involved in the mineral absorption pathway and maintain nutrient homeostasis in tea plants during the drought stress. Small RNA profiling from drought tolerant and sensitive varieties

of common bean was carried out with or without drought stress using Illumina platform and a total of 120 conserved and 49 novel miRNAs were identified (Wu *et al.*, 2017). Under drought stress 9 and 7 conserved miRNAs showed down and up-regulation, respectively in their expression levels, while 5 and three novel miRNAs exhibited up and down-regulation, respectively. In common bean, 16 miRNAs (gma-miR159c, gma-miR169u, gma-miR169b, gma-miR396f, gma-miR398d, gma-miR1511, gma-miR1527, gma-miR5770a, conserved.CM002296.1_39693, conserved. CM002294.1_26745, novel_Pvm14, novel_Pvm15, novel_Pvm29, novel_Pvm41, novel_Pvm44, and novel_Pvm45) were found to be drought-responsive. Transgenic *Arabidopsis* overexpressing gma-miR394a resulted in repression of an F-box gene (*At1g27340*) and thereby improved drought stress tolerance as compared to wild-type (Ni *et al.*, 2012). Similarly, overexpression of ath-miR408 in chickpea repressed the expression of plantacyanin leading to copper accumulation and enhanced drought tolerance in transgenic plants (Hajyzadeh *et al.*, 2015). On the other hand, overexpression of miR156 in *Arabidopsis* and rice down regulated the expression of *SPL9* genes which affected anthocyanin metabolism pathway by regulating the downstream genes such as *PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1)* and *DIHYDROFLAVONOL-4-REDUCTASE (DFR)*. The transgenic plants thus showed improved tolerance to drought and salt stresses through miR156-SPLs-DFR network (Cui *et al.*, 2014).

Like drought stress, salinity also interferes with the normal growth and physiology of the plants. Numerous salt stress-associated miRNAs have been identified from different plants including rice (Macovei and Tuteja 2012; Mondal *et al.*, 2018), populus (Li *et al.*, 2013; Chen *et al.* 2017), radish (Sun *et al.*, 2015), *Salicornia europaea* (Feng *et al.*, 2015), cotton (Yin *et al.*, 2017) and maize (Fu *et al.*, 2017). Identification and expression profiling of salt-responsive miRNAs from radish under salt stress was performed by small RNAs sequencing using solexa platform which led to the identification of 136 conserved and 68 novel miRNAs (Sun *et al.*, 2015). Out of these, 49 conserved and 22 novel miRNAs were found to be salt responsive and showed differential expression under salt stress. Down regulation of miR172c and miR166g-3p induced the expression of AP2-like

ethylene-responsive transcription factor *SNZ* and SAM-dependent methyltransferase gene in radish under salt stress. However, functional characterization of both genes in salt tolerance mechanisms remains elusive. A total of 201 conserved and 24 novel miRNAs were identified from small RNAs sequencing of cotton under salt stress by deep-sequencing using Illumina platform (Yin *et al.*, 2017). Among them, a total of 78 conserved and 10 novel miRNAs were found to be differentially expressed under salt stress. In cotton, miR780 was strongly down-regulated by salinity while miR7498 was significantly up-regulated during salt stress. The study also reported that the expression of *ARF10*, *ARF16* and *ARF17* were regulated by miR160 and miR167 in cotton resulting in enhanced levels of auxin leading to improved development of plants under salt stress. A total of 224 conserved and 60 novel miRNAs were identified under drought and salt stresses from the root apex of chickpea (Khandal *et al.*, 2017). Recently, 150 conserved and 348 novel miRNAs were identified from a salt tolerant genotype of rice under salt stress (Mondal *et al.*, 2018). Among them, 29 conserved and 32 novel miRNAs were found to be differentially expressed under salinity stress. *Gma-miR172c* overexpressing *Arabidopsis* plants showed enhanced drought and salt tolerance by targeting *Glyma01g39520* and increased survival rate and reduced leaf water loss under stress conditions (Li *et al.*, 2016). The transgenic creeping bentgrass overexpressing osa-miR319a exhibited improved drought and salt tolerance in transgenic plants by targeting TCP (Zhou *et al.*, 2013). Numerous biological mechanisms such as those related with leaf wax synthesis, salt uptake and water retention capacity were found to be altered in miR319 overexpressed transgenic bentgrass upon exposure to drought and salt stresses.

miRNAs in Response to Cold and Heat Stress

The expression of several cold-associated miRNAs has been studied in different plants such as *Prunus persica* (Barakat *et al.*, 2012), *Medicago* (Shu *et al.*, 2016), eggplant (Yang *et al.*, 2017), wheat (Song *et al.*, 2017) and brassica (Zeng *et al.*, 2018). In *Medicago*, 173 known and 24 novel miRNAs were identified from small RNAs sequencing of *Medicago* under cold (4°) and freezing (-8°) stress (Shu *et al.*, 2016). Out of these, 35 miRNAs were associated with cold- and/or freezing-stress. Small RNA

sequencing from the young spikes of wheat under cold stress revealed 192 conserved and 9 novel miRNAs using the Illumina HiSeq™ analysis (Song *et al.*, 2017). Of these, 34 conserved and 5 novel miRNAs were differentially expressed in the cold-stressed samples as compared to the control. In a recent study, 269 conserved and 84 novel miRNAs were identified from the leaves and roots of cold-tolerant and cold-sensitive varieties of *Brassica rapa* under cold stress (Zeng *et al.*, 2018). Eight conserved (miR156a-5p, miR166h-3p-1, miR166u, miR396h, miR398b-3p, miR398b-3p-1, miR408d and miR845a-1) and two novel miRNAs (Bra-novel-miR3153-5p and Bra-novel-miR3172-5p) were differentially expressed in leaves of a cold-tolerant variety expressed under cold stress. The overexpression of *OsmiR156k* in transgenic rice reduced tolerance to cold by regulating *SPL3*, *SPL14* and *SPL17* genes and inhibited the growth at the very early seedling stage by decreasing survival rates, chlorophyll and proline content (Cui *et al.*, 2015). On the other hand, *Osa-miR319b* overexpression enhanced cold stress tolerance by regulating *OsPCF6* and *OsTCP21* leading to accumulation of proline that repressed ROS accumulation and also played vital roles in leaf morphogenesis in rice (Yang C. *et al.*, 2013). The expression of *LEAF CURLING RESPONSIVENESS (LCR)* was regulated by overexpression of miR394 in transgenic Arabidopsis and showed enhanced tolerance to cold stress by increased accumulation of proline and total soluble sugars as compared to wild-type under cold stress (Song *et al.*, 2016).

As like cold stress, the expression of miRNAs under heat stress have been identified in numerous plants including *Saccharina japonica* (Liu *et al.*, 2015), cotton (Wang *et al.*, 2016), *Betula luminifera* (Pan *et al.*, 2017) and rice (Mangrauthia *et al.*, 2017). Identification and expression analysis of small RNAs sequencing from *Saccharina japonica* revealed that 49 conserved and 75 novel miRNAs were identified under heat stress using the Illumina sequencing platform (Liu *et al.*, 2015). Of which seven conserved and 25 novel miRNAs revealed differential expression under heat stress. Deep sequencing was used to identify 115 conserved and 84 novel miRNAs from *B. luminifera* under heat stress (Pan *et al.*, 2017). Among them, 57 conserved and 41 novel miRNAs were found to be heat-responsive under heat stress. Genome-wide profiling and identification of miRNAs

from heat-tolerant and -susceptible rice cultivars was executed under heat stress using Illumina sequencing which led to the identification of 162 miRNA families from the root and shoot tissues of 13-day-old rice seedlings (Mangrauthia *et al.*, 2017). The significant expression of miR1436, miR5076, miR5161, and miR6253 was detected only in the heat-stressed tissue of both genotypes demonstrating their general role against heat stress. However, miR1439, miR1848, miR2096, miR2106, miR2875, miR3981, miR5079, miR5151, miR5484, miR5792, and miR5812 were expressed only in a tolerant genotype under heat stress, indicating these miRNAs play a crucial role in response to heat stress. Overexpression of miR156 in Arabidopsis revealed enhanced tolerance to heat stress and exhibited heat stress memory for plants (Stief *et al.*, 2014). Overexpression of *Ta-miR159* in transgenic rice exhibited more sensitive to heat stress as compared to wild-type (Wang *et al.*, 2012).

miRNAs in Response to Heavy Metals

Natural, anthropogenic and industrial activities have prompted to severe heavy metals pollution in different areas. Among abiotic stresses, heavy metal (HM) toxicity also leads to deleterious effects on the growth, development and productivity of plant by modifying the physiological and metabolic processes (Gupta *et al.*, 2014; Noman and Aqeel, 2017). HMs can be categorized into essential and non-essential HMs. In plant cells, low concentrations of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) plays important roles in enzymatic and biochemical reactions, while higher concentrations become toxic (Rascio and Navari-Izzo, 2011; Gielen *et al.*, 2012). However, aluminum (Al), cadmium (Cd) and mercury (Hg) are toxic to plants even at minute concentrations (Gielen *et al.*, 2012).

It has been reported that the expression of miRNAs is also regulated by Cd, Hg and Al in *Medicago* (Zhou *et al.*, 2008). The study also indicated up-regulated expression levels for miR171, miR319, miR393, and miR529 in response to Al, Cd and Hg while expression of miR319 was up-regulated by Al and Cd but not affected by Hg. In addition, the expression of miR393 was up-regulated by Hg and Cd but not affected by Al (Zhou *et al.*, 2008). A total of 19 Cd-responsive miRNAs have been identified from rice seedlings using microarray-based analysis

(Ding *et al.*, 2011). Genome-wide identification revealed a total of 84 conserved and non-conserved miRNAs were identified from small RNAs sequencing of *Brassica napus* upon exposure to Cd by deep-sequencing using Illumina platform (Zhou *et al.*, 2012a). Out of 19 miRNAs, only miR528 was found to be significantly up-regulated and remaining 18 miRNAs were found to be down-regulated upon exposure to Cd. Genome-wide identification and expression analysis revealed 15 conserved and 8 novel miRNA families found to be differentially expressed in *Raphanus sativus* upon exposure to Cd by using Solexa sequencing technology (Xu *et al.*, 2013). Genome-wide expression profiling of miRNAs was performed from Cd-sensitive and -tolerant genotypes of tobacco (He *et al.*, 2016). The result indicated that 72 conserved and 14 novel miRNAs were differentially expressed in both genotypes. Of these, 28 conserved and 5 novel miRNAs were found to be Cd-responsive, which involved in cell growth, stress defense, ion homeostasis, antioxidant and hormone signaling (He *et al.*, 2016). Further, miR390 overexpression in transgenic rice revealed reduced Cd tolerance with higher accumulation of Cd as compared to wild-type (Ding *et al.*, 2016).

Genome-wide sequencing of small RNAs led to the identification of 201 miRNAs belonging to 63 miRNA families from *M. truncatula* under Hg treatment (Zhou *et al.*, 2012b). Out of 63, 28 miRNA families showed differential expression including miR2111, miR2592, miR2593, miR2595, miR2633 and miR2661 were found to be up-regulated upon Hg exposure. High-throughput sequencing of *M. truncatula* seedlings under Al treatment led to the identification of 326 conserved and 21 novel miRNAs and 23 miRNAs were found to be Al-responsive (Chen *et al.*, 2012). High-throughput sequencing of soybean under Al treatment led to the identification of 97 conserved and 31 novel miRNAs using the Illumina platform (Zeng *et al.*, 2012). Among these, 30 miRNAs showed differential expression under Al stress and considered as Al responsive miRNAs.

Role of miRNAs Against Bacterial Pathogens

The first miRNA was reported as key players during plant-bacterial interactions when *Arabidopsis* treated with bacterial pathogen-associated molecular pattern (PAMP) peptide flg22 that leads to the induced expression of miR393 (Navarro *et al.*, 2006). The

high-throughput sequencing of small RNAs from *Arabidopsis* leaves infected with non-pathogenic *Pseudomonas syringae* pv. tomato strain DC3000 *hrc* (*Pst* DC3000 *hrc*, mutated in type III secretion system) at 1 h and 3 h post-inoculation (hpi) revealed that three miRNAs (miR160, miR167, and miR393) were highly up-regulated and miR825 was down-regulated at 3 hpi (Fahlgren *et al.*, 2007). Overexpressing transgenic lines of miR393 led to the restricted growth of *Pst* DC3000 by the suppression of auxin signaling pathways by regulating TIR1 and other related proteins. Besides miR393, miR160 and miR167 were also induced upon bacterial infection at 3 hpi in the *Arabidopsis* leaves. These miRNAs target the mRNAs of the auxin-response factor (ARF) family genes which are involved in auxin signaling (Yang *et al.*, 2013). Interestingly, it was previously reported that the expression of miR162 and miR168 were also down-regulated upon bacterial infection, which targets *DCL1* and *AGO1*, respectively (Fahlgren *et al.*, 2007). The expression of *DCL1* and *AGO1* genes regulated by miR162 and miR168 suggested that the modulation of miRNA pathway by these genes is a crucial step during the bacterial defense. Similarly, miR825 was down-regulated at 3 hpi of bacterial infection, which is predicted to target the mRNA of remorin, frataxin-related and zinc finger homeobox family encoding genes (Fahlgren *et al.*, 2007).

Expression profiling of *Arabidopsis* miRNAs in response to *Pseudomonas syringae* pv. tomato (*Pst*) infection showed that 15, 27 and 20 miRNA families were differentially expressed upon *Pst* DC3000 *hrcC* (non-pathogenic), *Pst* DC3000 EV (Virulent) and *Pst* DC3000 *avrRpt2* (avirulent) infections, respectively (Zhang *et al.*, 2011). Particularly, most of bacterial-regulated miRNAs target genes were involved in hormone biosynthesis and signaling pathways including auxin, jasmonic acid (JA) and abscisic acid (ABA) pathways, suggested that they play a crucial role in plant defense signaling. RNA sequencing used to analyse the expression of miRNAs from cassava (*Manihot esculenta*) tissue during the infection by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) (Perez-Quintero *et al.*, 2012). A total of 56 conserved and 12 novel cassava-specific miRNAs families have been reported upon infection by *Xam*. During bacterial infection, some up-regulated miRNAs mediate defense by regulating auxin-

responsive factors. However, some down-regulated miRNAs were involved in the regulation of disease resistance-associated genes.

Role of miRNAs Against Fungal Pathogens

miRNAs also play a crucial role in defense mechanisms against fungal infection besides bacterial infections. Lu *et al.* (2007) investigated the miRNAs expression profiling of loblolly pine (*Pinus taeda*) upon infection with the rust fungus *Cronartium quercuum* f. sp. *Fusiforme* and identified 26 miRNAs. This fungus causes fusiform rust disease, resulting formation of spindle-shaped galls on branches or stems of pine. *C. quercuum* infection triggered the down-regulation of some miRNAs in the galls while other miRNAs were up-regulated in the surrounding tissues of the infection site. The target genes of these miRNAs encode defense-related genes such as nucleotide-binding site leucine-rich repeat (NB-LRR) receptors, laccases, ubiquitin ligases and peroxidases (Bamer and Mani, 2013). However, the exact role of miRNAs in defense mechanism upon *C. quercuum* infection remains to be elusive. Twenty-four miRNAs were identified from wheat (*Triticum aestivum* L.) upon *Erysiphe raminis* f. sp. *tritici* (*Egt*) infection (Xin *et al.* 2010). Interestingly, the expression profile of miRNAs was found to be different in susceptible and near-isogenic lines having an R-gene. For example, miR156 was down-regulated in both cultivar and miR164 was down-regulated only in resistant lines. Similarly, miR393 was down-regulated in resistant lines, which targets auxin response factors, suggesting that auxin signaling pathway modulated by miRNAs could play a role in defense mechanism against *E. graminis* infection. Sequencing of miRNAs from rust-susceptible and resistant soybean [*Glycine max* (L.) Merrill] upon infection with *Phakopsora pachyrhizi* revealed the involvement of miRNAs in response to rust fungus (Kulcheski *et al.*, 2011). Profiling of miRNAs and other small non-coding RNAs was investigated in resistant (*Gossypium barbadense*: *Verticillium*-tolerant) and sensitive (*G. hirsutum*: *Verticillium*-sensitive) cotton upon *Verticillium dahliae* infection and found that more than 65 miRNA families revealed different expression pattern in tolerant and sensitive cultivars (Yin *et al.*, 2012). The expression of miR1917 and miR2118 was suppressed upon fungal infection and the target genes of miR1917 are involved in ethylene signaling while miR2118

modulates a putative NB-LRR protein, indicating that miRNAs are involved in defense response in cotton roots against *V. dahliae* infection. Deep sequencing of small RNA from eggplant (*Solanum melongena* L.) upon *V. dahliae* infection led to the identification of 99 known miRNAs families and eggplant ESTs were used to predict two novel putative miRNAs (Yang *et al.*, 2013). Small RNAs sequencing from the upland cotton variety KV-1 seedlings infected with *V. dahliae* strains D07038 (moderate virulence) and V991 (high virulence) led to the identification 443 conserved and 37 novel miRNAs (He *et al.*, 2014). Forty-nine potential target genes for 24 novel miRNAs were predicted and these target genes were found to be involved in plant-pathogen interactions, the mitogen-activated protein kinase (MAPK) signaling pathway etc. The down-regulation of miR8163, miR8165, miR8170, miR8175, and miR8178 involved in defence responses through complex regulatory network mechanism needs to be elucidated.

High-throughput sequencing analysis of *G. hirsutum* and *G. barbadense* root tissues upon infected with *V. dahliae* showed that 140 known miRNAs and 58 novel miRNAs were identified (Zhang *et al.*, 2015). A total of 83 and 24 target genes for the 31 known and 14 novel miRNAs families were identified by degradome analysis. The expression levels of miR419 and miR2118 were up-regulated while miR1886, miR3509, and miR3515 were down-regulated in *G. barbadense* root tissues after infection with *V. dahliae*, however, the involvement of miRNAs in defense response remains to be uncovered. The expression profiling of miRNAs was done using next-generation sequencing of susceptible and resistant to *Magnaporthe oryzae* rice varieties at different time point upon fungal infection (Li *et al.*, 2016). A total of 169 and 33 miRNAs were differentially expressed in the susceptible and resistant cultivar, respectively, as compared to their respective controls at 48 hpi suggested that a high number of miRNAs in susceptible cultivar contributed phenotypic difference in response to fungal infection.

Recently, small RNAs profiling of garlic (*Allium sativum* L.) upon infection with *Fusarium oxysporum* f. sp. *cepae* (FOC) revealed 45 conserved miRNAs and six novel miRNAs (Chand *et al.*, 2017). Moreover, miR164a, miR168a and miR393 overexpressing transgenic garlic revealed enhanced resistance against

FOC infection. However, miRNAs overexpressing transgenic lines showed less fungal growth and increased expression of defense-responsive genes, indicating that the miRNAs play a crucial role in resistance to FOC infection.

Role of miRNAs Against Viral Diseases

Previous studies have shown that small RNAs including miRNAs are involved in antiviral defense in plants during viral infection (Niu *et al.*, 2006; Ding and Vionnet, 2007; Qu *et al.*, 2007, Bester *et al.*, 2017, Kundu *et al.*, 2017, Bao *et al.*, 2018). The silencing of viral RNAs takes place by small RNAs, processed from virus-derived dsRNAs via siRNA pathways (Balmer and Mani, 2013). The expression of bra-miR158 and bra-miR1885 were up-regulated in *Brassica rapa* upon infection with Turnip mosaic virus (TuMV) (He *et al.*, 2008). The bra-miR1885 targets the mRNA of the Toll/interleukin-1, nucleotide-binding site leucine-rich repeat (TIR-NBS-LRR) disease-

resistant gene. Deep sequencing method was used to analyse the expression profiling of miRNAs in rice upon infection with *Rice dwarf virus* (RDV) and *Rice stripe virus* (RSV) (Du *et al.*, 2011). The abundance of miRNA*s instead of the corresponding miRNAs was induced by RSV infection, but not by RDV infection. The expression level of *DCL* and *AGO* genes was up-regulated in RSV infected rice while RDV infection led to the enhanced expression level of *RDR* genes in rice. However, the function of elevated expression of *DCL*, *AGO* or *RDR* genes in defense mechanisms remains elusive. Deep sequencing analysis of *N. benthamiana* infected with *TMV* and *TMV-43A* [a mutated *TMV* in which upstream pseudoknot domain (UPD) replaced with an internal poly (A) tract (43 nt)] used to analyse the expression profile of miRNAs (Guo *et al.*, 2017). Differential expression pattern of miRNAs upon *TMV* and *TMV-43A*-infection in plants affected their corresponding putative mRNA targets and involved in moderate host

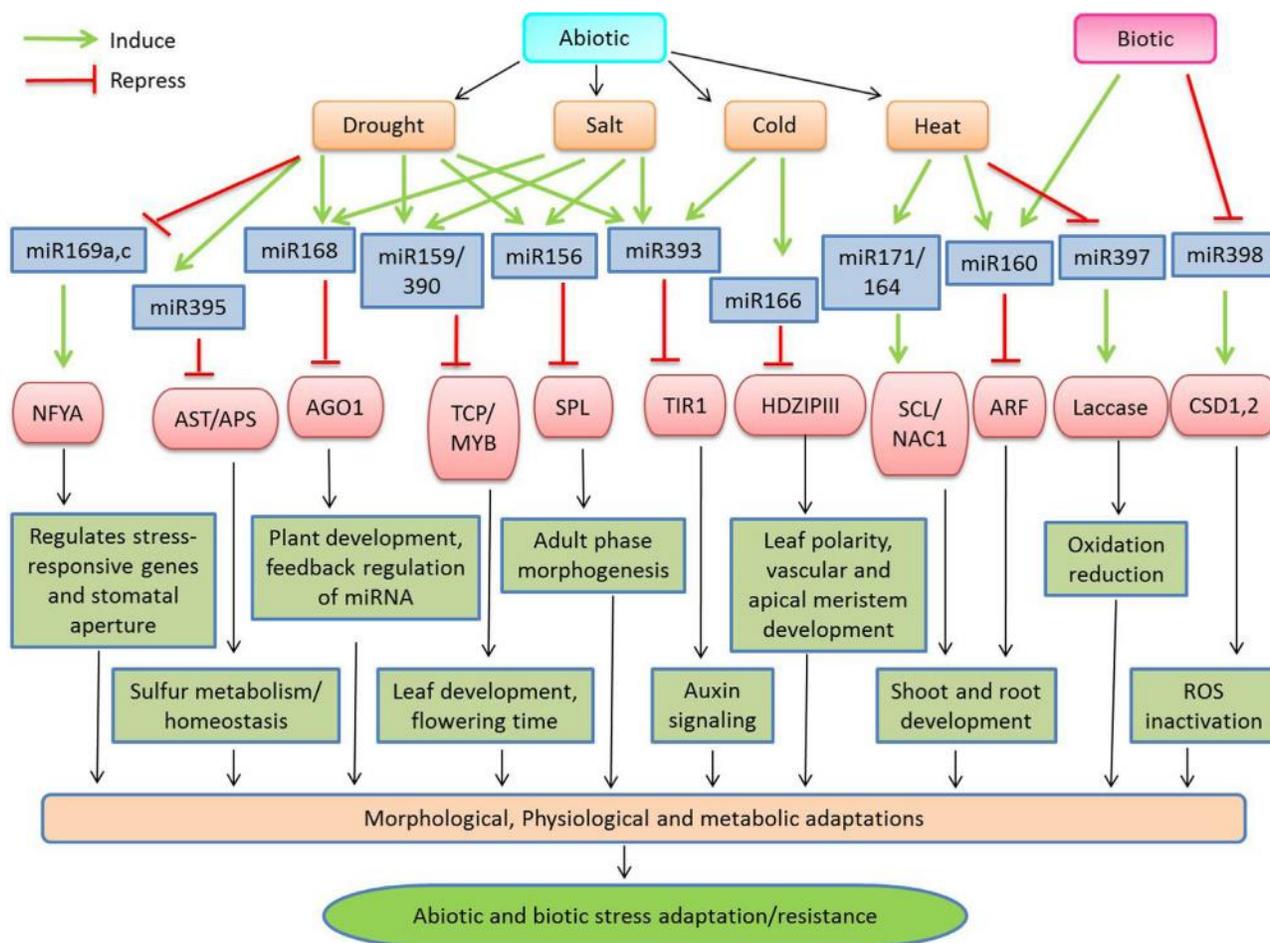


Fig. 1: Network of miRNAs and their regulatory pathways in response to abiotic and biotic stresses in plants

defense response against TMV-43A infection than that to TMV infection (Guo *et al.*, 2017).

Recently, a total of 422 miRNAs were identified from *Phaseolus vulgaris* upon infection with mungbean yellow mosaic India virus (MYMIV) and mock-treated samples (Patawa *et al.*, 2018). Expression of miR482 was reported to be down regulated during MYMIV infection in the resistant genotype and thus up regulation of NB-LRR encoding resistance gene involved in improved resistance to MYMIV (Patawa *et al.*, 2018). Overall, miRNAs transcriptome analysis of numerous plants upon viruses' infections revealed changes in miRNAs expression. Despite several novel studies conducted to explore the role of miRNAs in antiviral resistance, the exact mechanisms against antiviral defense remain to be elucidated.

Conclusion and Future Perspectives

Increasing number of studies establish that numerous host miRNAs are induced or suppressed via various environmental and pathogen challenges that play a crucial role in the reprogramming of gene expression and fine-tuning plant responses. Adaptive response to environmental stress is a complex phenomenon. Various signalling pathways and plant hormones affect the miRNA-mediated gene network. The stress regulatory mechanism through miRNA-mediated gene network has been elaborated in Fig. 1. While, the query remains at which extent miRNAs are modulating protective responses, or if they are fine-tuners of defense responses and stress tolerance by

regulating hormone signaling pathways and transcription factors. Majority of studies revealed a stress-specific change in expression of miRNAs, while, their role in defense responses remains elusive. However, the role of miRNAs in plant-pathogen interactions and abiotic stresses were illustrated in a few well-defined cases. The study of miRNA-mediated regulatory mechanisms in plant defense responses against environmental stresses and pathogens seems to be well-established area, and by using high-throughput sequencing technologies, numerous stress-responsive miRNAs were identified from different plants. However, functional characterization and effective regulation of these miRNAs with their respective targets in plants will provide a better understanding of the molecular role of miRNAs during plant-abiotic stress and plant-pathogen interactions. Further advances in these studies will elucidate the molecular mechanism underlying stress tolerance and resistance and will lead to develop and deploy miRNAs-mediated crop improvement strategies against global-warming in various agricultural crops.

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