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REVIEW PAPER

Emerging roles of microRNAs in the mediation of drought stress response in plants

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Abstract

Drought is a major environmental stress factor that limits agricultural production worldwide. Plants employ complex mechanisms of gene regulation in response to drought stress. MicroRNAs (miRNAs) are a class of small RNAs that are increasingly being recognized as important modulators of gene expression at the post-transcriptional level. Many miRNAs have been shown to be involved in drought stress responses, including ABA response, auxin signalling, osmoprotection, and antioxidant defence, by downregulating the respective target genes encoding regulatory and functional proteins. This review summarizes recent molecular studies on the miRNAs involved in the regulation of drought-responsive genes, with emphasis on miRNA-associated regulatory networks involved in drought stress response.

Key words: Drought, gene regulation, microRNA, stress response, target.

Introduction

MicroRNAs (miRNAs) are a class of 21-nucleotide non-coding small RNAs, playing negative regulatory roles in gene expression at the post-transcriptional level (Carrington and Ambros, 2003; Bartel, 2004). In *Arabidopsis*, after transcription by Pol II or Pol III enzyme into primary miRNA (pri-miRNA), the miRNA gene is processed by Dicer-like (DCL) into a stem-loop miRNA::miRNA* duplex. Subsequently, the miRNA::miRNA* duplex is processed by DCL1, with assistance from the double-stranded RNA-binding protein HYL1. The 3′ ends of miRNA duplexes are methylated by HEN1 and loaded onto AGO1. The miRNAs are then exported to the cytoplasm by a HASTY protein and cleaved into mature miRNAs. Mature miRNAs are incorporated into the RNA-induced silencing complex (RISC), where the mature single-stranded miRNA guides the RNA slicing activity of AGO1 to partially complementary mRNA. In plants, miRNAs generally interact with their targets through perfect or near-perfect complementarity and lead to the cleavage of target mRNA (Aukerman and Sakai, 2003; Mallory and Vaucheret, 2006; Voinnet, 2009) (Fig. 1). Large amounts of data have indicated that miRNAs are involved in numerous processes in plants, including development, hormone regulation, nutrient homeostasis, stress response, and even self-regulation of the miRNA biogenesis pathway (Carrington and Ambros, 2003; Sunkar, 2010; Khraiwesh *et al.*, 2012).

Drought stress is a common adverse environmental condition. It has deleterious effects on plant metabolic processes including stomatal movement, nutrient uptake, and production of photosynthetic assimilates, and ultimately causes crop losses (Shinozaki *et al.*, 2003; Neumann, 2008; Jaleel *et al.*, 2009). Plants employ mechanisms of drought avoidance and/or tolerance to cope with drought. Drought avoidance is usually achieved through morphological changes in plants, such as decreased stomatal conductance, reduced leaf area, and development of extensive root systems (Levitt, 1980). On the other hand, drought tolerance is achieved by physiological and molecular mechanisms, including osmotic adjustment, and the production of antioxidant and scavenger compounds (Bartels and Sunkar,

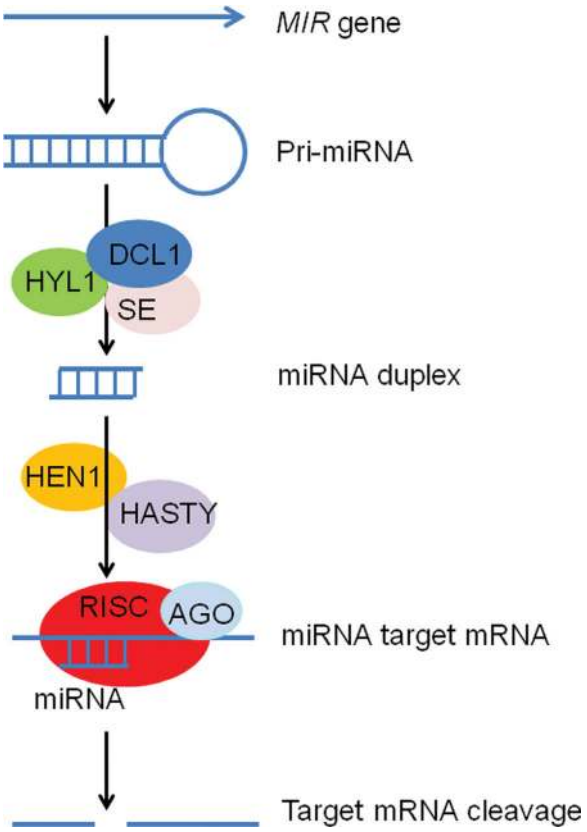

Fig. 1. The miRNA biogenesis pathway in plants. Factors involved in miRNA biogenesis and messenger RNA cleavage specified by miRNA are indicated. Pri-miRNA, primary miRNA.

2005). Both strategies involve the induction of specific gene expression and the accumulation of proteins such as dehydrins (dehydration-induced proteins), key enzymes for osmolyte biosynthesis, and detoxification enzymes (Reddy *et al*., 2004; Shinozaki and Yamaguchi-Shinozaki, 2007). Transcriptional regulatory mechanisms controlling the expression of drought-inducible genes have been discussed elsewhere (Shinozaki and Yamaguchi-Shinozaki, 2007; Golldack *et al*., 2011). With the discovery of small RNAs, increased attention has been focused on the importance of post-transcriptional gene regulation by miRNAs in response to drought stress (Carrington and Ambros, 2003; Sunkar, 2010).

Recently, miRNAs have emerged as important modulators in drought tolerance and avoidance via control of the expression of drought-responsive genes (Covarrubias and Reyes, 2010; Martin *et al*., 2010; Shuai *et al*., 2013). Drought-induced miRNAs downregulate their target mRNAs, which may be negative functional proteins involved in drought response. Conversely, other miRNAs are downregulated, leading to the accumulation of their target mRNAs that contribute positively to stress adaption. It is essential to note that most of these miRNAs target genes encoding transcription factors, which place miRNAs at the centre of gene regulatory networks (Fig. 2 and Table 1). This review focuses on the miRNA-associated regulatory networks involved in drought stress response, thereby helping decipher the molecular events triggered in plants under drought conditions.

miRNAs involved in ABA response

Abscisic acid (ABA), a key plant stress hormone, is produced *de novo* under water-deficit conditions and plays a central role in mediating the expression of stress-related genes and in the initiation of stomatal closure (Koornneef *et al*., 1998; Wilkinson and Davies, 2002). Indications that miRNAs participate in the ABA response were first provided by the isolation of ABA-hypersensitive mutants impaired in any of several key genes of the miRNA biogenesis pathway, such as *HYL1*, *DCL1*, *HEN1*, *SE*, and *HASTY*. The *hyl1* mutant was shown to be hypersensitive to ABA during *Arabidopsis* germination (Lu and Fedoroff, 2000). In another study, mutant alleles for *dcl1* and *hen1* increased ABA sensitivity during germination. Furthermore, *se* and *hasty* mutants exhibited enhanced sensitivity to ABA, salt, and high osmoticum (Zhang *et al*., 2008). Thus, it is possible that these mutants reveal a defect in a particular miRNA that is rightfully involved in the regulation of the ABA response process. Additionally, *AGO1*, a key factor for miRNA processing, was found to be targeted by miR168, which in turn displayed differential expression under drought stress by miRNA microarray analysis in *Arabidopsis* and rice (Liu *et al*., 2008; Zhou *et al*., 2010); this suggests that feedback regulation might play a role in miRNA activity in plants under drought stress and might induce further changes in numerous miRNA activities (Figs. 1 and 2). Thus far, a few examples of drought-responsive miRNAs have been identified, which may explain why mutations of miRNA biogenesis factors such as *HYL1*, *DCL1*, and *HEN1* alter ABA sensitivity (Lu and Fedoroff, 2000; Reyes and Chua, 2007; Covarrubias and Reyes, 2010). The crucial roles of miRNAs in ABA signalling and response are exemplified in this review by case studies, providing information on which miRNAs participate in drought responses in diverse plant species and on the mechanisms behind this participation (Figs. 2 and 3).

miR159 was induced by ABA and drought treatments in germinating *Arabidopsis* seeds (Reyes and Chua, 2007). In *Arabidopsis*, miR159a mediates the cleavage of *MYB33* and *MYB101* transcripts (Reyes and Chua, 2007; Allen *et al*., 2010). Overexpression of miR159a suppressed *MYB33* and *MYB101* mRNA levels and rendered plants hyposensitive to ABA, whereas transgenic plants overexpressing cleavage-resistant forms of *MYB33* and *MYB101* were hypersensitive to ABA treatment (Reyes and Chua, 2007). MYB transcription factors were found to bind *cis*-elements in the dehydration-responsive gene response to dehydration 22 (*RD22*) promoter and cooperatively activate *RD22*. Overexpression of both *MYC2* and *MYB2* improved osmotic stress tolerance of the transgenic plants (Abe *et al*., 2003). These results suggest that *MYB* are positive regulators of ABA signalling, and miR159 may play a key role in ABA response by directing the degradation of *MYB* mRNAs in *Arabidopsis*.

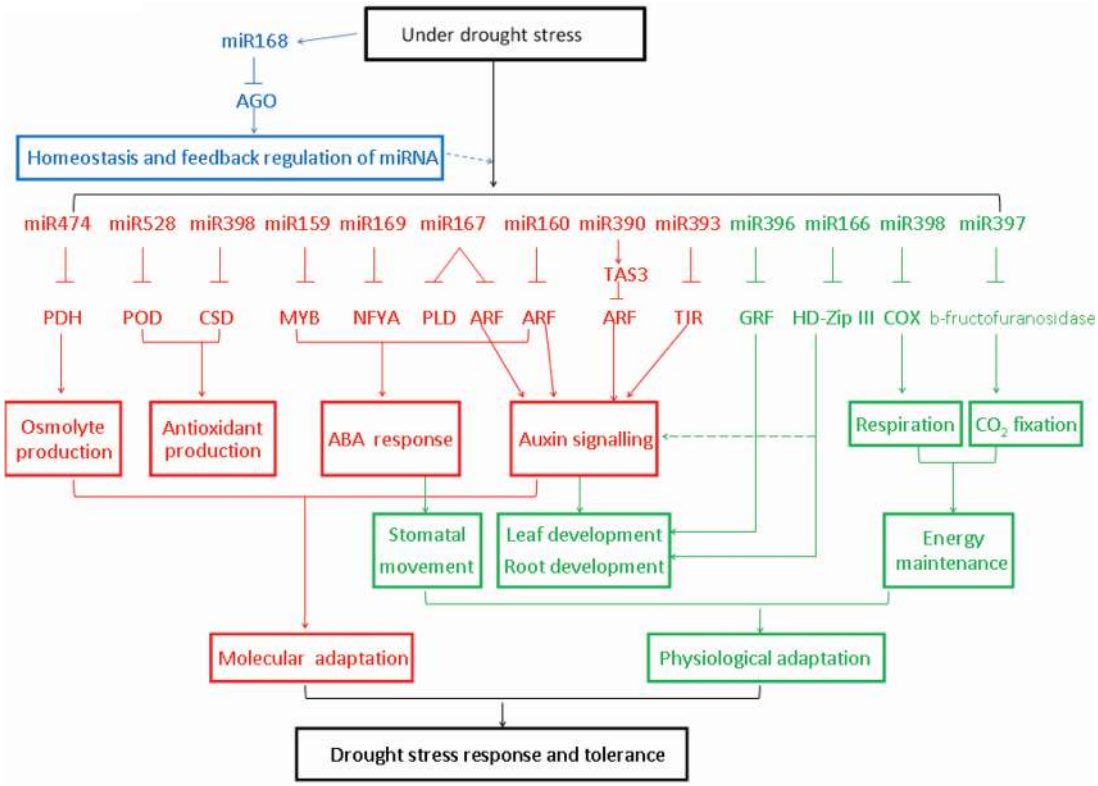

Fig. 2. Regulatory networks involving miRNAs and their target genes in drought response of plants. Blue letters and lines refer to the feedback regulation of miRNAs under drought. Red indicate miRNA-associated molecular adaptation. including osmolyte production, antioxidant production, ABA response. and auxin signalling. Green indicates physiological adaptation such as stomatal movement, leaf and root development, and energy maintenance. Dashed lines indicate potential paths that have not been demonstrated but are suggested here. ARF, auxin response factor; COX, cytochrome C oxidase; CSD, Cu/Zn-superoxide dismutase; GRF, growth-regulating factor; HD-Zip, homeodomain-leucine zipper; NFY, nuclear factor Y; PDH, proline dehydrogenase; PLD, phospholipase D; POD, peroxidase; TIR, transport inhibitor response.

miR167 was downregulated by ABA treatment in rice seedlings (Liu *et al*., 2009a), while it was upregulated by drought stress in *Arabidopsis* (Liu *et al*., 2008). Phospholipase D (*PLD*), a positive regulator of drought stress resistance, was predicted as a target of miR167d. The expression of miR167 was inhibited by drought stress in maize (*Zea mays*), which was important for the accumulation of *PLD* mRNA (Wei *et al*., 2009). *PLD* was reported to direct ABA response and affect stomatal movement in guard cells (Zhang *et al*., 2005). Thus, it implied that miR167-mediated *PLD* activation is important in the processes of ABA signalling and/or response.

miR169a targets the *NFYA5* mRNA, encoding a subunit of the nuclear factor Y (NF-Y) transcription factor (Li *et al*., 2008). NF-Y proteins are plant-specific transcription factors, with important roles in plant development and responses to environmental stresses (Kumimoto *et al*., 2008). miR169a was downregulated by drought stress and ABA treatments in *Arabidopsis*, and this downregulation of miR169a contributed to the strong induction of the *NFYA5* transcript. Furthermore, miR169a overexpression and *nfya5* knockout plants were drought sensitive, whereas transgenic lines overexpressing *NFYA5* were drought tolerant (Li *et al*., 2008). Promoter:β-glucuronidase analyses showed that *NFYA5* was highly expressed in vascular tissues and guard cells. *NFYA5* expressed in guard cells controlled stomatal aperture, while *NFYA5* expressed in other cells was crucial for the expression of a number of drought stress-responsive genes, such as glutathione transferase (*GT*) or peroxidase (*POD*) based on microarray analysis (Li *et al*., 2008). These results suggest that miR169a-guided regulation of *NFYA5* plays an important role in drought resistance in *Arabidopsis*.

High-throughput sequencing showed that miR169 was also downregulated under drought stress in *Medicago truncatula* (Wang *et al*., 2011a). In contrast to findings on *Arabidopsis and M. truncatula*, miR169g was upregulated by drought in rice, and this induction was more prominent in roots than in shoots. The existence of two dehydration responsive elements (DREs) in the promoter of *MIR169g* further supported the role for miR169g in drought stress response (Zhao *et al*., 2007). Additionally, SlmiR169 accumulation was induced by drought stress in tomato (*Solanum lycopersicum*). Compared with wild-type plants, transgenic tomato lines overexpressing miR169c showed enhanced drought tolerance, attributed to the significant reduction in stomatal conductance and water loss (Zhang *et al*., 2011). Interestingly, while miR169 is a conserved miRNA family that regulates a homologous target, it appears to behave in contradictory ways in different plant species, because of differences in plant developmental

Table 1. *miRNAs responsive to drought stress in plants*

MicroRNA family	Species	Response under drought	Target	Process involved in drought resistance	Reference
miR168	*Arabidopsis thaliana*	Upregulated	AGO	miRNA processing	Liu *et al*. (2008)
	Oryza sativa	Downregulated			Zhou *et al*. (2010)
miR159	*Arabidopsis thaliana*	Upregulated	MYB	ABA signalling and osmotic stress tolerance	Abe *et al*. (2003); Reyes and Chua (2007)
miR167	*Arabidopsis thaliana*	Upregulated	ARF	ABA response	Liu *et al*. (2008)
	Oryza sativa				Liu *et al*. (2009a)
	Zea mays	Downregulated	PLD	ABA response and controlling stomatal movement	Wei *et al*. (2009)
miR169	*Arabidopsis thaliana*	Downregulated	NFYA	ABA response and controlling stomatal aperture	Li *et al*. (2008)
	Medicago truncatula	Downregulated			Wang *et al*. (2011a)
	Oryza sativa	Upregulated			Zhao *et al*. (2007)
	Solanum lycopersicum	Upregulated			Zhang *et al*. (2011)
miR160	*Arabidopsis thaliana*		ARF	ABA response	Liu *et al*. (2007)
miR393	*Arabidopsis thaliana*	Upregulated	TIR1	Auxin signalling	Sunkar and Zhu (2004); Liu *et al*. (2008)
	Oryza sativa	Upregulated			Zhao *et al*. (2007)
miR390	*Vigna unguiculata*	Upregulated	TAS3-ARF	Auxin signalling and lateral root development	Barrera-Figueroa *et al*. (2011)
miR396	*Arabidopsis thaliana*	Upregulated	GRF	Leaf development	Liu *et al*. (2008)
	Nicotiana tabacum	Upregulated			Yang and Du (2009)
	Oryza sativa	Downregulated			Zhou *et al*. (2010)
miR166	*Medicago truncatula*	Upregulated	HD-Zip	Root and nodule development	Boualem *et al*. (2008); Trindade *et al*. (2010)
	Oryza sativa	Downregulated			Zhou *et al*. (2010)
	Triticum dicoccoides	Downregulated			Kantar *et al*. (2011)
miR474	*Zea mays*	Upregulated	PDH	Proline accumulation	Wei *et al*. (2009)
	Oryza sativa	Upregulated	PPR	Controlling organelle gene expression	Zhou *et al*. (2010)
	Medicago truncatula	Upregulated			Trindade *et al*. (2010)
miR528	*Zea mays*	Downregulated	POD	ROS detoxification	Wei *et al*. (2009)
miR398	*Medicago truncatula*	Upregulated	CSD	ROS detoxification	Trindade *et al*. (2010)
	Triticum dicoccoides	Upregulated	COX	Respiration pathway	Kantar *et al*. (2011)
miR397	*Oryza sativa*	Downregulated	β-Fructofuranosidase	CO_2 fixation	Zhou *et al*. (2010)
	Arabidopsis thaliana	Upregulated	Laccase	Unknown	Sunkar and Zhu (2004)

ARF, auxin response factor; COX, cytochrome C oxidase; CSD, Cu/Zn-superoxide dismutase; GRF, growth-regulating factor; HD-Zip, homeodomain-leucine zipper; NFY, nuclear factor Y; PDH, proline dehydrogenase; PLD, phospholipase D; PPR, protein kinase, kinesin, leucine-rich repeat; POD, peroxidase; TIR1, transport inhibitor response 1.

stages, growth conditions, and the duration and strength of the applied stress.

miRNAs involved in auxin signalling

Drought stress significantly affects plant growth and development (Zhu, 2001). Decreased cell growth is suggested as an adaptive feature for plant survival under stress, because it allows plants to divert assimilates and energy intended for plant growth into protective molecules to resist stress (Zhu, 2002; Chaves and Oliveira, 2004). Auxin is a key hormone that is responsible for modulating many aspects of plant growth, including root and leaf architecture, organ patterning, and vascular development (Millner, 1995). Several miRNA families have been demonstrated to be involved in controlling auxin signalling to regulate plant growth and development under drought stress (Fig. 3 and Table 1).

It is known that miR393 was commonly upregulated during drought stress in *Arabidopsis* (Sunkar and Zhu, 2004), rice (Zhao *et al*., 2007), and sugarcane (*Saccharum* spp.) (Ferreira *et al*., 2012). The target of miR393 encodes TIR1 (transport inhibitor response 1), an auxin receptor in *Arabidopsis*. The TIR1 enzyme is a positive regulator of auxin signalling by promoting the degradation of Aux/IAA proteins through ubiquitination (Dharmasiri and Estelle, 2002). Xia *et al*. (2012) reported that the growth of miR393-overexpressing rice seedlings was repressed by 1-day drought treatment, compared with the control plants. miR393-overexpressing rice also demonstrated

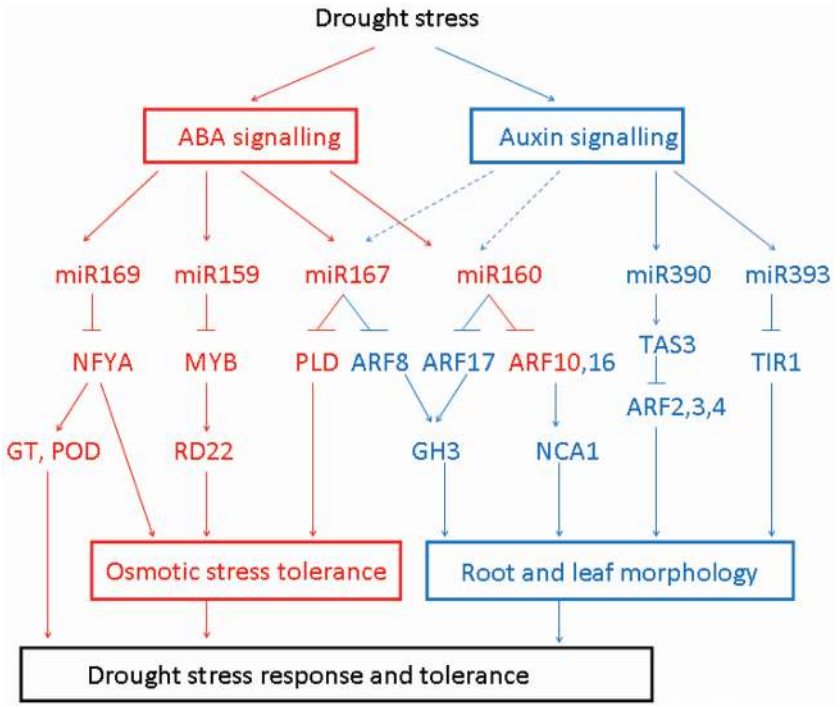

Fig. 3. A schematic representation to demonstrate that miRNAs modulate ABA and auxin regulatory pathways under drought stress in plants. miR169 and miR159, which guide ABA response, are shown in red; miR390 and miR393, which are important for determining the cellular auxin level, are shown in blue. Both miR167 and miR160 are involved in ABA and auxin signalling under drought stress.

hyposensitivity to synthetic auxin analogue treatments (Xia *et al*., 2012). Thus, increased levels of miR393 would down-regulate auxin signalling and may reduce plant growth under drought stress.

Auxin response factors (ARFs) are important transcription factors involved in auxin signal transduction by binding to specific *cis*-elements in the upstream regions of auxin-inducible genes. Several *ARF* gene family members have been confirmed as target genes for miRNAs. *ARF10*, *ARF16*, and *ARF17* are targeted by miR160, while miR167 guides the regulation of *ARF6* and *ARF8*, which apparently negatively regulate free indole acetic acid levels by controlling *GH3*-like gene expression (Mallory *et al*., 2005; Teotia *et al*., 2008). Both miR160 and miR167 have been reported to play major roles in drought and ABA response in plants. Microarray analysis showed that miR167 was induced by drought in *Arabidopsis* (Liu *et al*., 2008). Northern blot analysis showed that miR167 was downregulated by ABA treatment in rice seedlings (Liu *et al*., 2009a), suggesting that ABA might cause an increase in *ARF* mRNA accumulation. Since ARF is an auxin response factor, the change in miR167 expression indicated an intersection between ABA and auxin signalling. Besides, negative regulation of *ARF10* by miR160 modulated the response to ABA during *Arabidopsis* germination. Transgenic seeds overexpressing miR160 exhibited ABA insensitivity or tolerance during germination, while transgenic plants expressing a miR160-resistant form of *ARF10* exhibited an ABA-hypersensitive phenotype during germination. These observations suggest that negative regulation of *ARF10* by miR160 affects ABA sensitivity and may play a role in auxin-ABA cross-talk (Liu *et al*., 2007).

In *Arabidopsis*, miR390 was reported to mediate the novel regulatory pathway miR390-TAS3-ARF2/ARF3/ARF4 involved in auxin signalling (Marin *et al*., 2010; Yoon *et al*., 2010). miR390 does not target a protein-coding mRNA but rather triggers the production of tasiRNA (TAS3-derived *trans*-acting small interfering RNA), which regulates lateral root emergence and organ polarity establishment by targeting transcription factors such as *ARF2*, *ARF3*, and *ARF4* (Meng *et al*., 2010). miR390 was upregulated under drought stress in cowpea (*Vigna unguiculata*) (Barrera-Figueroa *et al*., 2011) and *Brachypodium distachyon* (Budak and Akpinar, 2011), indicating the role of miR390-dependent *trans*-acting siRNA pathway in drought response.

miRNAs involved in cell growth

Plant responses to drought are regulated by complex networks leading to rapid reprogramming of plant cell growth. miRNAs have been emerged as important players in the regulation of growth and development (Carrington and Ambros, 2003; Mallory and Vaucheret, 2006). The role of miRNAs in drought response was investigated in young leaves of *B. distachyon* by next-generation sequencing. Sixty-six annotated miRNAs showed a high expression level, consistent with their involvement in early leaf development and cell identity (Bertolini *et al*., 2013). These results provide evidence for an miRNA regulatory network controlling cell division in both normal and stressed conditions.

In search of potential miRNAs involved in drought response, miR396 was found to be downregulated by drought in rice (Zhou *et al*., 2010) and cowpea (Barrera-Figueroa *et al*., 2011), but upregulated in drought-stressed *Arabidopsis* (Liu *et al*., 2008) and tobacco (Yang and Du, 2009; Frazier *et al*., 2011). miR396 has been shown to target six growth-regulating factor (*GRF*) transcription factors with roles in the coordination of cell division and differentiation during leaf development in *Arabidopsis* (Jones-Rhoades and Bartel, 2004; Wang *et al*., 2011b). Transgenic *Arabidopsis* plants overexpressing miR396a displayed narrow-leaf phenotypes because of reduction in cell number, achieved through repression of the expression of *GRF* genes (Liu *et al*., 2009b) and cell cycle-related genes including *CYCD3;1*, *histone H4*, *CYCA2;1*, and *CYCB1;1* (Wang *et al*., 2011b). Furthermore, miR396a-overexpressing transgenic plants were more tolerant to drought than wild-type plants, likely because of the lower stomatal density observed in the former (Liu *et al*., 2009b). Overexpression of miR396 also conferred increased tolerance to drought stress in tobacco (Yang and Du, 2009). These observations suggest that miR396 plays important roles not only in leaf development but also in drought tolerance of plants.

miR166 is another example of many drought-responsive miRNAs that were previously characterized as crucial for cell development. It post-transcriptionally regulates class-III homeodomain-leucine zipper (*HD-Zip III*) transcription factors, which were demonstrated to be important for lateral root development, axillary meristem initiation, and leaf polarity (Hawker and Bowman, 2004; Chen, 2005; Boualem *et al*., 2008). miR166 was downregulated in response to drought in barley (Kantar *et al*., 2010) and in *Triticum dicoccoides* (Kantar *et al*., 2011). Conversely, in *M. truncatula*, miR166 was upregulated by water deficit and showed the highest expression in roots and a lower expression in seedlings and shoots (Trindade *et al*., 2010). Furthermore, overexpression of miR166a reduced the number of lateral roots in *M. truncatula* (Boualem *et al*., 2008). Plant root growth is greatly influenced by drought stress. When the external water supply is limited, root system architecture will be changed to improve water uptake efficiency (Malamy, 2005). These results imply that miR166-mediated post-transcriptional regulation is an important regulatory pathway involved in the regulation of root architecture and drought response.

miRNAs involved in osmotic adjustment

Osmotic adjustment represents a general mechanism to maintain cell turgor and to stabilize protein structure during drought stress. Many plants and other organisms cope with drought stress by producing and accumulating various osmoprotectants or osmolytes, including amino acids such as proline, sugars and sugar alcohols such as mannitol, and amines such as glycine betaine (Bartels and Sunkar, 2005; Seki *et al*., 2007). The synthesis and accumulation of osmolytes varies among plant species as well as among different cultivars of the same species (Reddy *et al*., 2004).

Proline has been reported to play a multifunctional role in defence mechanisms. It acts as an osmolyte, a free radical scavenger, and a stress-related signal (Nanjo *et al*., 1999). Proline accumulation in plants is caused not only by the activation of proline biosynthesis but also by the inactivation of proline degradation. Proline degradation to glutamic acid in higher plants is catalysed by proline dehydrogenase (*PDH*) (Reddy *et al*., 2004). Antisense suppression of *PDH* gene in *Arabidopsis* led to an accumulation of proline (Nanjo *et al*., 2003). Interestingly, miR474 is linked to the process of proline degradation, because it targets the *PDH* gene in maize (Rayapati and Stewart, 1991). It was reported that miR474 was upregulated during drought stress in maize (Wei *et al*., 2009), rice (Zhou *et al*., 2010), and *M. truncatula* (Trindade *et al*., 2010) by using miRNA microarray analysis. The upregulation of miR474 decreased the expression level of *PDH*, leading to the accumulation of proline and thus an improvement in plant stress tolerance under drought-prone conditions (Wei *et al*., 2009). In rice and *Populus trichocarpa*, miR474 also targets *PPR* (protein kinase, kinesin, leucine-rich repeat) with a potential role in the post-transcriptional regulation of organelle gene expression and RNA processing (Lu *et al*., 2005; Zhou *et al*., 2010). However, the precise role of miR474 in osmotic response remains to be verified in future experiments.

miRNAs involved in antioxidant defence

One of the inevitable consequences of drought stress is enhanced reactive oxygen species (ROS) production in the different cellular compartments, namely, in the chloroplasts, the peroxisomes, and the mitochondria (Cruz, 2008). ROS are potentially dangerous to plants (Jiang and Zhang, 2001; Peltzer *et al*., 2002). Plants have evolved defence systems, consisting of antioxidative enzymes and low-molecular-weight antioxidants to scavenge ROS. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) are important parts of the antioxidative enzyme system (Loggini *et al*., 1999; Mittler, 2002).

Recently, the role of miRNAs in the regulation of antioxidant enzymes has been established. Wei *et al*. (2009) reported that miR528 was downregulated by drought in maize seedlings. *POD* is a predicted target of miR528. RT-PCR analysis showed that the expression level of *POD* was upregulated because of the downregulation of miR528. The upregulation of *POD* would promote the removal of excessive H_2O_2 and alleviate the injury caused by ROS. Thus, miR528 was probably a regulator of antioxidant defence under drought conditions in plants. miR398 targets two closely related Cu/Zn SODs (*CSD1* and *CSD2*) and cytochrome C oxidase subunit V (*COX5b*). The CSD enzymes are involved in oxidative stress detoxification (Mittler, 2002), while COX5b functions in electron transport in the mitochondrial respiratory pathway (Jones-Rhoades and Bartel, 2004; Sunkar and Zhu, 2004). miR398 was downregulated under drought stress in *M. truncatula* (Wang *et al*., 2011a) and maize (Wei *et al*., 2009). This

would predictably lead to increases in the activities of CSDs and consequently oxidative stress tolerance. However, the drought-induced downregulation of miR398 in *M. truncatula* was in contrast to the results reported by Trindade *et al.* (2010) and Kantar *et al.* (2011). The differences in the expression of miR398 shown here may result from differences in species, the metabolic states of the individual plants, and variability in the extent and duration of drought stress in different studies.

miRNAs involved in photosynthesis and respiration

Drought stress is known to inhibit photosynthetic activity and photosynthetic electron transport capacity (Schulze, 1986). Photosynthetic activity is shown to be suppressed after drought stress, whereas respiration is enhanced by drought (Rizhsky *et al.*, 2002; Shinozaki and Yamaguchi-Shinozaki, 2007). The fixation of CO_2 and the synthesis of starch are important biochemical processes for plant growth (Reddy *et al.*, 2004). Drought stress progressively reduces photosynthetic activity and CO_2 assimilation in plants. Maintaining a reasonable rate of synthesis of carbon-hydrogen compounds helps to protect against drought stress in plants. The expression of miR397 was downregulated in drought-stressed rice (Zhou *et al.*, 2010), while miR397b was upregulated by drought and ABA treatments in *Arabidopsis*. In soybean, miR397ab were upregulated in the sensitive genotype but downregulated in the tolerant genotype during the water-deficit condition (Kulcheski *et al.*, 2011). miR397 is predicted to target β-fructofuranosidase, which takes part in starch and sucrose metabolism (Zhou *et al.*, 2010). Thus, the change in miR397 expression plays a role in the reductive carboxylate cycle (CO_2 fixation) and energy supply. In addition, miR397 is predicted to target a laccase gene which was reported to reduce root growth under dehydration in a knockout mutant (Cai *et al.*, 2006). However, the specific role of miR397 in drought response requires additional investigation.

miR398 plays a role in the regulation of respiration, as it targets cytochrome C oxidase subunit V (*COX5b*), which functions in electron transport in the mitochondrial respiratory pathway (Jones-Rhoades and Bartel, 2004; Sunkar and Zhu, 2004). miR398 was reported to be upregulated in *M. truncatula* (Trindade *et al.*, 2010) and *T. dicoccoides* (Kantar *et al.*, 2011) under drought stress. The increased level of miR398 led to the downregulation of *COX5b* transcripts, indicating the significance of miR398 in the regulation of mitochondrial respiration under water deficit (Trindade *et al.*, 2010).

Conclusions and perspectives

Changes in gene expression play an important role in plant drought stress response. For many drought-related genes, miRNAs function as critical post-transcriptional modulators controlling their expression. Recently, an increasing number of drought stress-responsive miRNAs have been described by using high-throughput sequencing and miRNA microarray methods (Shuai *et al.*, 2013). High-throughput sequencing of *Populus euphratica* identified 197 conserved miRNAs and 58 new miRNAs. Comparison of high-throughput sequencing with miRNA microarray data showed that 104 miRNAs were upregulated, whereas 27 were downregulated under drought stress in *P. euphratica* (Li *et al.*, 2011). Using a microarray platform, 11 downregulated miRNAs and 8 upregulated miRNAs were revealed under drought stress in rice across different developmental stages (Zhou *et al.*, 2010). Using high-throughput sequencing, 18 miRNAs were found to be differentially regulated by drought stress from rice inflorescences (Barrera-Figueroa *et al.*, 2012). These miRNAs combined with their target genes constitute large regulatory networks that control metabolic pathways in the response to drought stress. In this review, we envisage a regulatory network involving miRNAs and their target genes in drought response, highlighting the functional role of miRNAs in plant drought tolerance or drought avoidance (Fig. 2 and Table 1). This characterization provides a view for future analysis of miRNAs involved in drought stress resistance and will be useful for drought tolerance improvement in plants.

Although a number of drought-related miRNAs have been identified, their precise role remains to be verified. Additional strategies need to be employed to investigate the functions of miRNAs and their associated signalling pathways and gene networks under drought stress. Overexpression of miRNAs and/or miRNA targets or knockout mutants of target genes have been proven to be useful for analysing the function of stress-responsive miRNAs and for uncovering miRNA-regulated transcripts upon application of stress (Zhang *et al.*, 2011; Ni *et al.*, 2012). Further investigation of the regulatory pathways upstream of the drought-responsive miRNAs and regulatory pathways downstream of the miRNA target genes would offer new insights for understanding the regulation of drought adaptation. Besides, a cross-examination of the responses between drought stress and developmental regulation may provide additional information on drought-related miRNAs. miRNAs offer a link between environmental factors and developmental modulation, and the regulatory mechanisms of miRNA-mediated plant development and gene regulation require investigation. Moreover, drought-responsive miRNAs provide evidence for the molecular mechanism of potential crosstalk between auxin and ABA. Many genes in the auxin signalling cascade have been confirmed as targets of miRNAs, which in turn identified to be regulated under drought and ABA response (Fig. 3). The relationship between drought stress and miRNAs-mediated auxin-ABA crosstalk and their associated regulatory networks represents a compelling area of research to pursue in the future.

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