

Small RNAs in regulating temperature stress response in plants^{FA}

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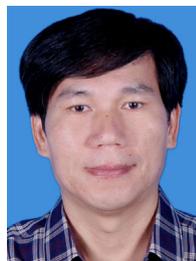
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Invited Expert Review



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Abstract Due to global climate change, temperature stress has become one of the primary causes of crop losses worldwide. Much progress has been made in unraveling the complex stress response mechanisms in plants, particularly in the identification of temperature stress responsive protein-coding genes. Recently discovered microRNAs (miRNAs) and endogenous small-interfering RNAs (siRNAs) have also been demonstrated as

important players in plant temperature stress response. Using high-throughput sequencing, many small RNAs, especially miRNAs, have been identified to be triggered by cold or heat. Subsequently, several studies have shown an important functional role for these small RNAs in cold or heat tolerance. These findings greatly broaden our understanding of endogenous small RNAs in plant stress response control. Here, we highlight new findings regarding the roles of miRNAs and siRNAs in plant temperature stress response and acclimation. We also review the current understanding of the regulatory mechanisms of small RNAs in temperature stress response, and explore the outlook for the use of these small RNAs in molecular breeding for improvement of temperature stress tolerance in plants.

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INTRODUCTION

Temperature is one of the most important environmental factors, affecting the geographical distribution of plant species and their productivity worldwide (Xu et al. 2016). Extreme heat or cold temperatures have an adverse impact on almost all aspects of plant development, growth, reproduction and yield (Sung et al. 2003; Wahid et al. 2007; Mittler et al. 2012). Within the plant life cycle, the reproductive stage is the most sensitive stage to

temperature stress and even a small change in temperature during flowering time can have devastating losses for grain crops (Mittler et al. 2012). According to a report from the Intergovernmental Panel on Climatic Change (IPCC), plant growth will be challenged with warmer environments as the average surface temperature increases 2.0–4.5°C by the end of this century (IPCC 2014). Global warming effects will not only be limited to rising average annual temperatures globally, but also increase the frequency and amplitude of severe temperature events

(Neilson et al. 2010). As temperature stresses become more recurrent, there is an urgent need to identify the genes associated with tolerance to temperature stresses and understand their regulatory mechanisms in order to develop crops with enhancing temperature stress tolerance through genetic manipulation.

Recently, a growing pool of research has revealed the powerful roles of microRNA (miRNA) and small-interfering RNA (siRNA) in plant developmental control at the post-transcriptional level (Liu and Chen 2010). These small RNAs (sRNAs) are shown to negatively regulate genes post-transcriptionally by guiding target mRNAs for degradation or by repressing translation. Compared to siRNAs, the actions of miRNAs are more extensive and remarkable. MiRNAs are involved in almost every aspect of plant growth and development regulation, including plant organ morphogenesis, hormone signal transduction, and responses to various external environmental stresses (Chuck et al. 2009; Liu and Chen 2010; Sunkar et al. 2012; Huang et al. 2016). By exploiting their integral role in plant growth, applications of miRNAs have been used to improve important agronomic traits in plants (Zhang et al. 2013; Zheng and Qu 2015). Recent studies have also established the involvement of miRNAs and siRNAs in regulating temperature stress responses in various plant species, and several reports have highlighted the key roles of these sRNA regulators in plant response to temperature stresses. These results greatly broaden our understanding of the molecular mechanism in plant temperature tolerance and acclimation. In this review, we summarized the diversity of sRNAs in response to temperature stresses in plants, functional confirmation of some important sRNAs associated with temperature stress response and their possible mechanisms in regulating temperature stress tolerance in plants. We also discussed the future directions and potential use of sRNAs for crop improvement.

CHANGES IN miRNA EXPRESSION TO TEMPERATURE STRESS

With the rapid advancement and increasingly affordable usage of high throughput sequencing technology in recent years, genome-wide searches for miRNAs involved in temperature stress response has been conducted in many plant species (Table 1). Many identified miRNAs have been shown to be involved in temperature stress response

in at least two different plant species, suggesting a persistent regulatory role during extreme temperature changes. Three miRNAs (miR156, miR160 and miR168) were identified in up to seven plant species during heat stress response (Table 1). Moreover, these temperature stress-regulated miRNAs were also shown to be involved in almost every aspect of plant growth and development (Table 1). These findings indicate that miRNA development and regulation of cold or heat stress tolerance may be tightly linked and likely use the same mechanism across plants species. Though many temperature stress-regulated miRNAs were shared among different plant varieties, their expression patterns under heat or cold stress appear to be species-dependent to some extent. For instance, miR172 was induced by cold stress in *Brachypodium* and *Prunus persica* (Zhang et al. 2009; Barakat et al. 2012), whereas it was repressed during cold stress in grapevine and wheat (Tang et al. 2012; Sun et al. 2015). Similarly, miR156 was upregulated by heat stress in *Arabidopsis*, wheat, and Chinese cabbage but was downregulated in rice and *Saccharina japonica* (Xin et al. 2010; Yu et al. 2011; Stief et al. 2014a; Kumar et al. 2015; Liu et al. 2015a; Liu et al. 2017). Additionally, it is worth mentioning that miRNAs are also differentially expressed between different tissues or developmental stages under temperature stress. Our recent study indicated that heat-responsive miRNAs at the flowering stage in rice were different from those found during the seedling stage (Liu et al. 2017). Many miRNAs were first documented for their involvement in heat stress response during the flowering stage, including the highly conserved miR528 (Liu et al. 2017). These differences in expression, based on physiological timing, highlight the need for specific and detailed characterizations of temperature-responsive miRNAs in plants.

MiRNA profiles have also been analyzed in genotypes that exhibit contrasting temperature tolerance to investigate the nuances of miRNA expression in plant temperature stress response. A striking difference between miRNA profiles in heat-tolerant and heat-sensitive rice genotypes was observed in a recent study (Liu et al. 2017). A total of 85 heat-responsive miRNAs was identified in the tolerant genotype, whereas only 26 heat-responsive miRNAs were identified in the sensitive genotype. Additionally, among the nine common responsive miRNAs, three miRNAs (miR159a.1, miR159b and miR528-3p) were upregulated in the sensitive genotype while the same set of miRNAs was downregulated in the tolerant genotype (Liu et al. 2017). By contrast, miR396e-5p and

Table 1. Temperature stress-regulated miRNAs in different plant species

miRNA	miRNA targets	Target functions	Cold stress	Heat stress	References
miR156	SPLs	Phase transition; flowering timing	Vvi↓, Ptc↓, Ppe↑	Ath↑, Tae↑, Osa↓, Sja↓	Lu et al. (2008); Xin et al. (2010); Yu et al. (2011); Barakat et al. (2012); Stief et al. (2014a); Kumar et al. (2015); Sun et al. (2015);
miR157	SPLs	Unknown	Ppe↑	Bra↑, Ghi↑↓	Liu et al. (2015a); Wang et al. (2016); Liu et al. (2017)
miR159	MYBs and TCPs	Flowering timing; anther development	Sha↓, Tae↑	Sja↓ Tae↑↓, Osa↑↓, Pvi↑, Csa↓, Ghi↓	Barakat et al. (2012); Liu et al. (2015a) Xin et al. (2010); Cao et al. (2014); Wang et al. (2014a); Kumar et al. (2015); Hivrale et al. (2016); Wang et al. (2016); Liu et al. (2017)
miR160	ARF10, ARF16, ARF17	Seed germination; abiotic stress response	Agr↑, Ptc↑ Ghi↓	Tae↑↓, Pto↓, Sja↑, Hvu↑, Osa↓,	Lu et al. (2008); Xin et al. (2010); Chen et al. (2012); Kruszka et al. (2014); Li et al. (2014a); Kumar et al. (2015);
miR162	DCL1	MIRNA biogenesis	Sha↓	Pvi↑, Ghi↓ Osa↓, Csa↓	Liu et al. (2015a); Hivrale et al. (2016); Wang et al. (2016); Liu et al. (2017) Cao et al. (2014); Li et al. (2016); Liu et al. (2017)
miR164	NAC domain transcription Factors	Root and leaf development floral organ boundary formation	Agr↑, Sha↓ Ppe↑, Ghi↑ Ptc↑	Osa↓, Agr↑, Pvi↑	Lu et al. (2008); Barakat et al. (2012); Cao et al. (2014);
miR166	HD-ZIP transcription factors	Axillary meristem initiation; leaf development; floral organ polarity	Sha↑↓	Tae↑, Osa↓, Hvu↑, Pvi↑	Xin et al. (2010); Cao et al. (2014); Kruszka et al. (2014);
miR167	ARF6 and ARF8	Male and female fertility	Sha↓, Tae↑↓	Pto↑, Osa↓, Hvu↑, Pvi↑	Chen et al. (2012); Tang et al. (2012); Cao et al. (2014); Kruszka et al. (2014); Kumar et al. (2015); Hivrale et al. (2016);
				Tae↑, Ghi↑	Wang et al. (2016); Liu et al. (2017)

(Continued)

Table 1. Continued

miRNA	miRNA targets	Target functions	Cold stress	Heat stress	References
miR168	AGO1	MIRNA biogenesis	Agr↑, Sha↓	Tae↑, Pto↓	Lu et al. (2008); Xin et al. (2010); Chen et al. (2012);
miR169	NF-YA transcription factor	Plant development; flowering timing	Ptc↑, Csn↑	Osa↓, Sja↓, Agr↑, Pvi↑	Cao et al. (2014); Li et al. (2014a); Zhang et al. (2014); Liu et al. (2015a); Hivrale et al. (2016); Liu et al. (2017)
miR171	growth-regulating factor	drought tolerance	Tae↓, Ghi↑	Osa↑↓, Sja↑ Ghi↓	Lu et al. (2008); Zhang et al. (2009); Xin et al. (2010); Chen et al. (2012); Wang et al. (2014a); Liu et al. (2015a); Wang et al. (2016); Liu et al. (2017)
miR172	AP2	Floral development;	Vvi↓, Sha↓	Pto↓, Tae↓	Chen et al. (2012); Kumar et al. (2015); Cao et al. (2014);
miR319	TCPs	abiotic stress response	Bdi↑, Tae↓	Pvi↓	Sun et al. (2015); Hivrale et al. (2016)
miR390	ARF	Flowering timing; floral organ identity	Vvi↓, Ppe↑	Ath↑, Tae↓	Zhang et al. (2009); Lee et al. (2010); Xin et al. (2010); Barakat et al. (2012);
miR393	TIR1, AFB2, AFB3	Leaf development; biosynthesis of JA floral organ size control		Ghi↑	Tang et al. (2012); Sun et al. (2015); Wang et al. (2016)
miR394	F-box proteins	Auxin signaling	Sha↓, Ath↑	Osa↓, Tae↓	Kumar et al. (2015); Hivrale et al. (2016);
miR395	Sulphate transporter	Disease resistance; flowering timing; stress tolerance	Tae↑, Ghi↓	Pvi↑, Csa↓, Ghi↓	Li et al. (2016); Wang et al. (2016); Liu et al. (2017)
		Abiotic stress response	Sha↓, Ptc↑	nd	Lu et al. (2008); Cao et al. (2014)
		Response to sulphate deprivation	Ptc↑, Ppe↑	Osa↓, Pvi↑	Sunkar and Zhu. (2004); Lu et al. (2008); Barakat et al. (2012);
			Ath↑, Tae↓		Tang et al. (2012); Hivrale et al. (2016); Liu et al. (2017)
			Agr↑, Osa↑	Pto↓, Osa↓	Lu et al. (2008); Barrera-Figueroa et al. (2012);
			Ptc↑	Agr↑	Chen et al. (2012); Li et al. (2014a); Liu et al. (2017)
			Agr↑, Vvi↓	Pto↓, Agr↓	Lu et al. (2008); Chen et al. (2012); Li et al. (2014a);

(Continued)

Table 1. Continued

miRNA	miRNA targets	Target functions	Cold stress	Heat stress	References
miR396	GRF transcription factors	Leaf and cotyledon development	Ptc↓	Pvi↑, Tae↑	Kumar et al. (2015); Sun et al. (2015); Hivrale et al. (2016)
miR397	Laccases	Lignin biosynthesis; flowering timing; increase grain yield	Ptc↓, Ppe↓, Ath↑, Tae↓	Osd↓, Pvi↑	Lu et al. (2008); Liu et al. (2008); Zhang et al. (2009); Barakat et al. (2012); Hivrale et al. (2016); Wang et al. (2016); Liu et al. (2017)
miR398	CSDs	Copper homeostasis; oxidative stress	Vvi↓, Sha↑, Ath↑, Bdi↑	Osd↓, Ppy↑	Jeong et al. (2011); Liu et al. (2015b); Liu et al. (2017)
miR399	Phosphate transporter	Response to phosphate starvation; flowering timing	Sha↑, Tae↓	Ath↑, Bra↓, Tae↑, Osd↑	Liu et al. (2017)
miR408	Laccase; TaCLP1	Various abiotic stress responses	Ghi↑	Bra↓, Osd↓	Yu et al. (2011); Cao et al. (2014); Wang et al. (2016);
miR528	Peroxidase	Elimination of ROS	Agr↑, Ghi↑	Pto↓, Osd↓, Sja↓	Liu et al. (2017)
miR827	NAD (P)-binding; SPX- (SYG1/Pho81/XPR) proteins	Involved in signal transduction pathways	Pto↓, Osd↓, Sja↓	Ghi↓	Chen et al. (2012); Li et al. (2014a); Liu et al. (2015a);
miR5175	Acc-like oxidase	Unknown	Agr↓, Pvi↓, Ghi↓	Hivrale et al. (2016); Wang et al. (2016); Liu et al. (2017)	Hivrale et al. (2016); Wang et al. (2016); Liu et al. (2017)

AFB, auxin F-box protein; AGO1, argonaut protein 1; AP2, apetala2; ARF, auxin response factors; CSD, copper/zinc superoxide dismutase; DCL1, diceer like1; GRF, growth-regulating factor; HD-ZIP, class III homeodomain leucine zipper; NAC domain transcription factor, (NAM, ATAF1/2 and CUC2) domain proteins; NF-YA, nuclear factor Y subunit A; SPL, squamosal promoter binding protein-like; TCP, teosinte branched/cycloidea/pcf transcription factor genes; TIR1, transport inhibitor response1; JA, jasmonic acid. Plant species abbreviations: Ath, *Arabidopsis thaliana*; Tae, *Triticum aestivum*; Bra, *Brassica rapa*; Sja, *Saccharina japonica*; Pto, *Populus tomentosa*; Osd, *Oryza sativa*; Hvu, *Hordeum vulgare*; Agr, *Apium graveolens*; Bdi, *Brachypodium distachyon*; Csa, *Cucumis sativus*; Csn, *Camellia sinensis*; Ghi, *Gossypium hirsutum*; Ppe, *Prunus persica*; Ppy, *Pyrus pyrifolia*; Ptc, *Populus trichocarpa*; Pvi, *Panicum virgatum*; Sha, *Solanum habrochaites*; Vvi, *Vitis vinifera*. “↑”, upregulated; “↓”, downregulated; nd, not determined or no change.

miR396f-5p were upregulated in the tolerant genotype but downregulated in the sensitive genotype. Furthermore, an earlier response of miRNAs to heat stress was also observed in tolerant genotypes compared to the sensitive genotype (Liu et al. 2017). Another study involving comparative analysis between two tea plant (*Camellia sinensis*) cultivars differing in cold sensitivity (cold-tolerant “Yingshuang” and cold-sensitive “Baiye 1”) also exhibited differential expression of miRNAs in response to cold stress (Zhang et al. 2014). These discoveries demonstrate that miRNA profiles can be unique in closely related genotypes with contrasting temperature sensitivities. Through differential expression comparison of miRNAs between the two genotypes with contrasting temperature stress tolerance, it may help to identify the tolerance-associated miRNAs and explore their mediated regulatory mechanisms in plants.

THE ROLES OF miRNAs IN COLD TOLERANCE

In the past decade, microarray-based, particularly the genome-wide sequencing analysis led to identifying numerous temperature stress responsive miRNAs in different plants. The actual roles in temperature stresses of a few miRNAs have been investigated.

As one of the highly conserved miRNAs in plants, miR394 has been validated to regulate leaf morphological development and stem cell identity by targeting *leaf curling responsiveness* (LCR) which encodes a putative F-box protein (Song et al. 2012; Knauer et al. 2013). Recently, miR394 was also identified to be involved in regulation of plant responses to cold, salt and drought stresses. The transcription of miR394 and LCR was both significantly induced by cold stress. The miR394 overexpressing (35S::MIR394) and *lcr* mutant plants showed enhanced cold tolerance, while the LCR overexpressing (miR394 cleavage-resistant version) plants exhibited a cold hypersensitive phenotype when compared to the wild-type plants (Song et al. 2016). More proline and total soluble sugars were also identified under cold stress in 35S::MIR394 and *lcr* plants than wild-type plants. According to the previous reports, the C-repeat binding factors (CBFs)/dehydration-responsive element-binding factors 1 (DREB1s)-dependent cold response pathway has been shown to play a predominant role in cold tolerance and acclimation (Chinnusamy et al. 2010). This

attributes to their ability to bind to the C-repeat/dehydration responsive element (CRT/DRE) and activate numerous downstream genes in the cold response pathway (Chinnusamy et al. 2010). Through further analysis, the authors also indicated that the expression levels of *CBF1*, *CBF2* and *CBF3* were remarkably increased in 35S::MIR394 and *lcr* plants compared with wild-type plants after cold stress treatment. In contrast, the transcription of these genes was reduced in LCR overexpressing plants (Song et al. 2016). A similar result was also observed for several downstream genes of CBF, such as *responsive to dehydration 29A* (*RD29A*), *cold-responsive 15a* (*COR15a*) and *cold-induced 1* (*KIN1*). Taken together, these results showed that miR394 positively regulates cold tolerance through the activation of CBF-dependent pathway in plant (Song et al. 2016; Figure 1).

MiR397 is another miRNA which has been also demonstrated to positively regulate cold tolerance in plants via the CBF-dependent pathway (Dong and Pei 2014). Overexpression of miR397a in *Arabidopsis* plants significantly improved plant tolerance to chilling and freezing stresses, accompanied with lower leaf electrolyte leakage compared to wild-type plants. RNA-blot analysis also revealed that the transcript levels of *CBF2* and downstream cold responsive genes (*COR15A*, *COR47A* and *RD29A*) were significantly higher in miR397a overexpressing plants than in wild-type plants (Dong and Pei 2014).

MiR319 is one of the earliest identified and most highly conserved miRNAs in plants, and has been extensively studied in controlling leaf development and leaf senescence by targeting *teosinte branched/cycloidea/pcf* (*TCP*) transcription factors (Palatnik et al. 2003; Schommer et al. 2008). Increasing evidence indicate that miR319 is also involved in cold stress response in plants. When subjected to 4°C for 24 h, miR319 was significantly upregulated in sugarcane plantlet root and shoot tissues (Thiebaut et al. 2012). Oppositely, the expression of miR319 was reduced during cold stress in rice, while its target genes (*OsPCF5*, *OsPCF6*, *OsPCF8* and *OsTCP21*) were dramatically induced (Yang et al. 2013; Wang et al. 2014a; Figure 1). Rice plants overexpressing miR319 showed enhanced cold tolerance, and reduced expression of miR319 target also resulted in enhanced cold tolerance (Yang et al. 2013; Wang et al. 2014b). Wang et al. (2014b) also indicated that the expression levels of cold responsive genes (*DREB1A/1B/1C* and *DREB2A*) were significantly higher in miR319 overexpressing plants than in wild-type plants, and the *OsPCF6* and *OsTCP21* RNAi plants exhibited lower reactive oxygen

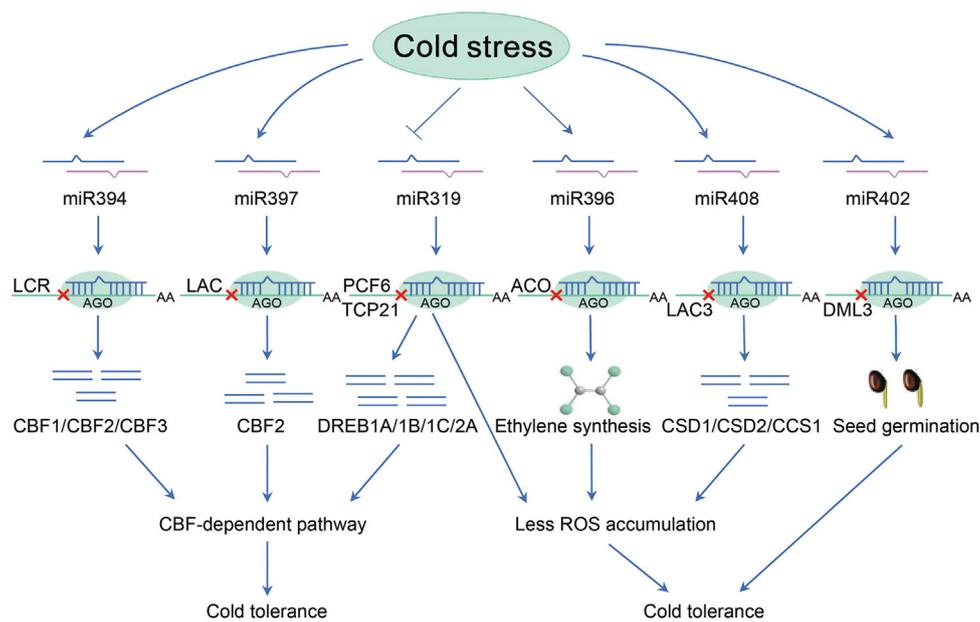


Figure 1. Role of miRNAs in plant cold stress response

Arrows show simultaneous effect in the pathway while nail shape represents repression. ACO, 1-aminocyclopropane-1-carboxylic acid oxidase; CBF, C-repeat binding factors; DML3, demeter-like protein3; DREB1, dehydration-responsive element-binding factors 1; LAC3, laccase 3; LCR, leaf curling responsiveness; ROS, reactive oxygen species.

species (ROS) level than in wild-type plants, suggesting that miR319 confers cold tolerance partially through the activation of CBF-dependent pathway and the elimination of ROS in rice plants. Additionally, miR319 was also reported to be involved in the cold stress responses in *Arabidopsis*, tomato and wheat (Sunkar and Zhu 2004; Tang et al. 2012; Cao et al. 2014), further signifying the importance of miR319 during cold stress response in a wide range of plant species.

Similar to miR319, miR396 has also been demonstrated to positively regulate cold tolerance in plants via the modifying of ROS level. Overexpression of tomato miR396 in tobacco showed enhanced cold tolerance accompanied with decreased production of ROS (Chen et al. 2015). Moreover, the miR396 overexpressing transgenic lemon (*Citrus limon*) plants also displayed improved cold tolerance by reducing the expression of 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), an enzyme involved in ethylene synthesis (Zhang et al. 2016; Figure 1). The reduced ACO transcript resulted in repressed ethylene synthesis while simultaneously promoting polyamine synthesis, leading to enhanced ROS scavenging. This result is consistent with the previous report that ethylene

plays a negative role in cold tolerance by repressing the expression of CBF genes (Shi et al. 2012). Thus, these observations provide new insights into the molecular mechanism underlying the reduction of ethylene production under cold stress.

MiR408 is another miRNA that has been validated to regulate cold stress by controlling the level of ROS in plants. Recently, Ma et al. (2015) has indicated that miR408 overexpressing plants showed enhanced cold tolerance accompanied with lower electrolyte leakage and higher efficiency of photosystem II, whereas the miR408 knock-out plants exhibited enhanced cold sensitivity when compared to wild-type plants. Further analysis revealed that miR408 mediated cold tolerance might be partially due to the enhanced cellular antioxidant capacity, as manifested by the reduced levels of ROS and induced expression of genes associated with antioxidative functions, including copper/zinc superoxide dismutases (CSD1 and CSD2) and glutathione-S-transferase (GST-U25) (Ma et al. 2015). Furthermore, miR402 has been verified to positively regulate seed germination under cold stress condition in *Arabidopsis* via targeting demeter-like protein3 mRNA (DML3) which functions in DNA

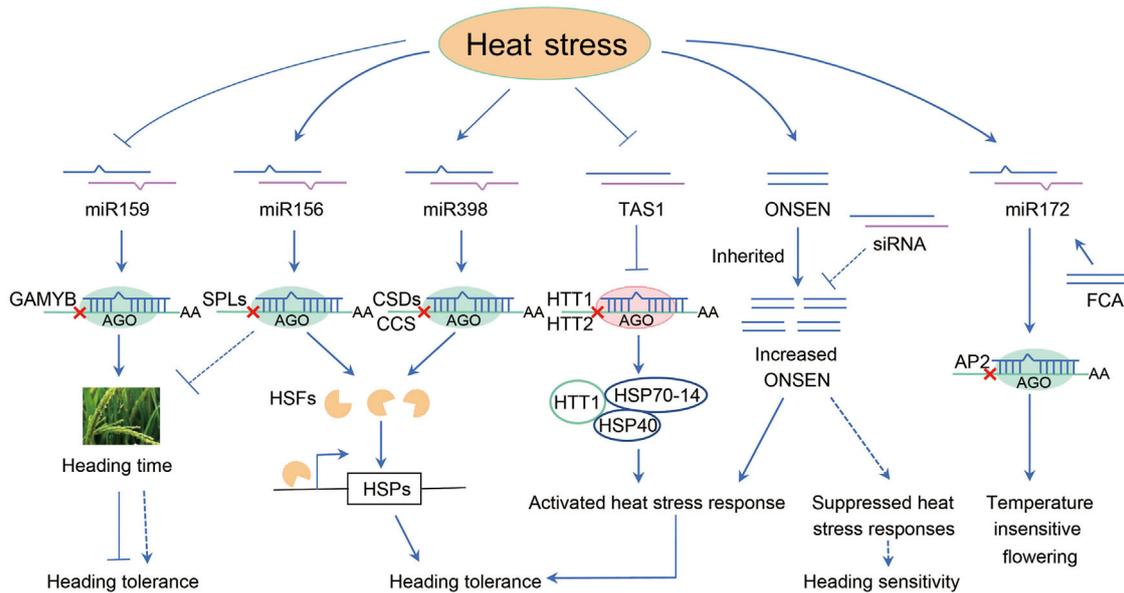


Figure 2. A diagrammatic representation of the small RNA involvement in plant heat stress response

Arrows show simultaneous effect in the pathway while nail shape represents repression. HSFs, heat shock transcription factors; HSPs, heat shock proteins.

methylation. Both the miR402 overexpressing plants and the *dm13* mutant plants showed earlier seed germination than wild-type plants when subjected to 12°C (Kim et al. 2010), indicating the important role of miRNA-mediated regulation of DNA demethylation in plants adapting to cold stress.

THE INVOLVEMENT OF miRNAs IN HEAT TOLERANCE

As one of the most conserved and highly expressed miRNAs in plants, miR156-*squamosa promoter binding protein-like* (SPL) regulatory module has been confirmed to play essential roles in a variety of developmental processes, including phase changes such as the vegetative to reproductive stage. It has been shown that miR156 maintained a higher level in juvenile shoot tissues and its expression decreased at plant maturation, and the decline in miR156 abundance provides a permissive environment for flowering (Wu and Poethig 2006; Wang et al. 2009; Wu et al. 2009). This expression pattern is attributed to the rise in its target SPL levels which directly activates the flowering gene *fruitfull* (*FUL*) (Wang et al. 2009; Wu et al. 2009). Interestingly, two subsequent reports have shown that the miR156-SPL module controlling flowering time is very sensitive

to ambient temperature change (Lee et al. 2010; Kim et al. 2012). Overexpression of miR156 led to delayed flowering at a lower ambient temperature (16°C), whereas overexpression of miR156-resistant *SPL3* caused early flowering, regardless of the ambient temperature (Kim et al. 2012). Intriguingly, Stief et al. (2014a) showed that miR156 was highly induced by heat stress and the activation can persist for several days. Functional analysis has verified that miR156 is required for heat stress memory and that increased miR156 levels enhance and prolong the memory (Stief et al. 2014a, 2014b). The function of miR156 in heat stress memory is also attributed to the repression of *SPL2* and *SPL11* genes in *Arabidopsis* (Stief et al. 2014a). The study also indicated that the higher expression of miR156 can promote sustained expression of heat stress-responsive genes, which may ultimately lead to the enhanced maintenance of acquired thermos-tolerance under heat stress conditions (Stief et al. 2014a; Figure 2).

In contrast to miR156, miR172 displays lower expression during the juvenile phase but high expression during the adult phase (Fornara and Coupland 2009). This expression pattern is attributed to its two target genes, *target of eat 1* (*TOE1*) and *TOE2*, that repress adult characteristics in the leaf epidermis (Fornara and Coupland 2009). Like miR156, miR172

accumulation is also ambient temperature-responsive and its abundance is lower at 16°C than at 23°C (Lee et al. 2010). Overexpression of miR172 leads to temperature-insensitive flowering, suggesting that miR172 may function in thermosensory flowering (Lee et al. 2010). Recently, a report has indicated that the flowering time control protein *FCA* promotes miR172 accumulation during the flower processing phase in response to increases in ambient temperature by directly binding to the primary miR172 transcripts (Jung et al. 2012). This further indicates the key regulatory role of miR172 in flowering time control under heat stress. Notably, the above results have demonstrated that miR156 and miR172 both function in thermosensory flowering. However, they just play opposite roles in thermosensory flowering. Their opposite functions might be due to their different gene targets within the plant (Figure 2).

Another ambient temperature-responsive miRNA, miR398, was discovered to exhibit higher expression level at 23°C than at 16°C, suggesting a role of miR398 in temperature stress response (Lee et al. 2010). More recently, miR398 has been validated as a positive regulator in heat tolerance in *Arabidopsis* (Guan et al. 2013). The study showed that heat stress rapidly induced the expression of miR398, but reduced the transcripts of its target genes *CSD1*, *CSD2* and *copper chaperone for superoxide dismutase (CCS)*. Transgenic plants expressing miR398-resistant forms of *CSD1*, *CSD2* and *CCS* were more sensitive to heat stress, whereas *csd1*, *csd2* and *ccs* mutant plants were more heat-tolerant than the wildtype plants (Guan et al. 2013). Induction of heat shock proteins (HSPs) through the action of heat stress transcription factors (HSFs) is the central component of responses to heat stress in all living organisms (Chauhan et al. 2011). Heat sensitivity changes of these transgenic plants were also accompanied with the changed transcription level of HSPs and HSFs. It was also demonstrated that *HSFA1b* and *HSFA7b* can bind directly to the promoter regions of miR398 to induce its expression, revealing a novel strategy for thermotolerance (Guan et al. 2013).

Contrary to miR156 and miR398 roles, miR159 acts as a negative regulator in regulating heat stress response by targeting *gibberellic acid (GA) regulated Myb (GAMYB)-like* family transcription factors in plants. The rice plants overexpressing *TamiR159* were more sensitive to heat stress compared to the wildtype plants (Wang et al. 2012; Figure 2). Similarly, it was

also demonstrated that the expression of *csa-miR159b* was repressed by heat stress in cucumber plants, and overexpression of *csa-miR159b* led to decreased heat tolerance in *Arabidopsis*, further confirming the negative role of miR159 in heat tolerance (Li et al. 2016).

It is well established that miR396 regulates leaf development by targeting the *growth-regulating factor (GRF)* transcription factors in plants (Das and Nath 2015). Intriguingly, a recent report has indicated that miR396 can mediate the cleavage of *HaWRKY6* in sunflower and it is unique in *Asteraceae* plants (Giacomelli et al. 2012). Transgenic plants expressing the miR396-resistant form of *HaWRKY6* showed increased sensitivity to heat stress response, indicating a role of the recently evolved miR396 regulation of *HaWRKY6* during early responses to high temperature (Giacomelli et al. 2012). These results also reveal that a miRNA that normally regulates development can also be recruited for high-temperature protection in sunflower, a plant particularly well adapted to this type of stress (Giacomelli et al. 2012).

Alternative splicing (AS) is common in many plant species and has been identified as a possible ‘molecular thermometer’, allowing plants to quickly adjust the abundance of functional transcripts to environmental perturbations (Capovilla et al. 2015). Intriguingly, a recent report indicated that AS was also involved in the regulation of miRNA abundance under heat stress condition in *Arabidopsis* (Yan et al. 2012). Intronic miR400 was co-transcribed with its host gene and was downregulated by heat stress, whereas the expression of miR400 primary transcripts was greatly increased. This attributes to an AS event specially induced by heat stress. The AS event occurred in the intron (306 bp) where *MIR400* was located, with that a 100 bp fragment was excised, and the remaining 206 bp intron containing *MIR400* transcripts was retained in the host gene, ultimately leading to the accumulation of miR400 primary transcripts and a low level of mature miR400 (Yan et al. 2012). Furthermore, miR400 overexpressing transgenic plants showed a lower germination percentage, less hypocotyl elongation and less root growth than wild-type plants, indicating that miR400 plays a negative role in plant heat stress response. These results together suggest the important role of AS in linking miRNAs and environmental stress in plants (Yan et al. 2012).

THE ASSOCIATIONS BETWEEN siRNAs, OTHER SMALL RNAs AND TEMPERATURE STRESS

Eukaryotic genomes contain a large number of transposable elements and they are suppressed by host epigenetic mechanisms to prevent their uncontrolled propagation (Ito et al. 2011). Biological organisms have evolved pathways that tightly control their gene expression activity. This regulation involves silencing at the transcriptional and posttranscriptional levels (Stief et al. 2014b). In a recent study, it was shown that a copia-type retrotransposon named *ONSEN* became transcriptionally active in *Arabidopsis* seedlings when subjected to heat stress. The heat-induced *ONSEN* accumulation was stimulated in mutants impaired in the biogenesis of siRNAs (Ito et al. 2011; Figure 2). This indicates that *ONSEN* inactivation requires components of the siRNA-directed silencing pathway. After prolonged heat stress, new *ONSEN* insertions arise through retrotransposition in stressed plants deficient in siRNA biogenesis and are passed on to the next generation (Ito et al. 2011; Stief et al. 2014b). Intriguingly, this transgenerational retrotransposition occurs during flower development and before gametogenesis and may be linked to actively dividing cells (Ito et al. 2011; Matsunaga et al. 2012; Stief et al. 2014b). In addition, *ONSEN* retrotransposition can confer heat-inducibility onto neighboring genes, therefore mobility bursts may generate novel stress-responsive regulatory gene networks (Ito et al. 2011). However, the retrotransposition was not identified in wildtype plants subjected to stress or in non-stressed mutant controls, pointing to a crucial role of the siRNA pathway in restricting retrotransposition triggered by environmental stress (Ito et al. 2011). More recently, the mechanism of the heat-dependent induction of *ONSEN* has been explored. The underlying mechanism attributes to the heat-responsive element (HRE) recognized by plant-derived heat stress defense factors in the long terminal repeat (LTR) of *ONSEN* (Cavrak et al. 2014). Further analysis indicated that *HSFA2* (which is required for heat stress memory) can bind directly to the *ONSEN* LTR element to induce its transcription (Cavrak et al. 2014). However, induction was not fully abolished in the *hsfa2* mutant, suggesting that other HSF proteins may be involved in this process (Cavrak et al. 2014).

Plant genomes also encode a unique class of sRNAs called trans-acting small interfering RNAs (ta-siRNAs) which exert their functions through posttranscriptional gene silencing (Li et al. 2014b). In *Arabidopsis*, there are four families of noncoding precursor genes generating ta-siRNA: *trans-acting siRNA precursor 1* (*TAS1*), *TAS2*, *TAS3* and *TAS4*. Previous studies have demonstrated that *TAS1*-derived siRNAs may play an important role in abiotic stress responses (Sunkar and Zhu 2004; Kumeet al. 2010). The function of *TAS1* in thermotolerance has been validated in *Arabidopsis*. The *heat-induced tas1 target 1* (*HTT1*) and *HTT2* genes were identified to be the target of *TAS1*-derived siRNAs as they were highly induced by heat stress in *Arabidopsis* seedlings (Li et al. 2014b). Overexpression of *TAS1* reduced expression levels of the *HTT* genes and caused weaker thermotolerance, whereas overexpression of *HTT* genes upregulated several HSF genes, leading to stronger thermotolerance (Li et al. 2014b). Moreover, the transcription of *HTT* genes was upregulated in heat-tolerant plants overexpressing *HSFA1a*. It was shown that *HSFA1a* directly binds to *HTT* promoters to activate their expression (Li et al. 2014b). It was also found that *HTT1* interacted with the heat shock proteins HSP70-14 and HSP40 in *Arabidopsis*. These results suggest that *HTT1* mediates thermotolerance pathways targeted by *TAS1a*, mainly activated by *HSFA1a*, and acts as cofactor of HSP70-14 complexes (Li et al. 2014b; Figure 2).

A global analysis of the heat-responsive chloroplast small RNAs (csRNAs) and cis-NATs-derived small interfering RNAs (nat-siRNAs) was also conducted in Chinese cabbage (Wang et al. 2011; Yu et al. 2013). Many csRNAs and nat-siRNAs derived from 12 cis-NATs were responsive to heat stress, suggesting their potential roles in transcriptional or post-transcriptional regulation under heat stress in plants (Wang et al. 2011; Yu et al. 2013).

As for cold stress, Tang et al. (2012) has indicated that the ta-siRNA *TAS3a-5'D6(+)* may play a role in wheat plant adaptation to cold stress by regulating auxin signaling pathway by guiding the cleavage of *auxin response factor* (*ARF*). Moreover, Xia et al. (2014) demonstrated that several ta-siRNAs originating from the three *TAS3* genes were also validated to target *ARFs* under cold stress conditions in cassava, for example, *tasiARF1* targeted both *ARF3* and *ARF4* while *tasiARF3* targeted *ARF3*. These results together suggest that ta-siRNA-mediated temperature stress response may be

partially related to auxin signaling in plants. Two differentially expressed nat-siRNAs were also identified under cold stress in cassava. One of these encoded a NAC (*No Apical Meristem*) domain transcriptional regulator superfamily protein, a superfamily which many members have been implicated in plant cold response (Puranik et al. 2012; Xia et al. 2014).

MECHANISMS OF TEMPERATURE STRESS RESPONSES OF SMALL RNAs

Protection of reproductive tissues from damage by small RNAs

The reproductive phase in flowering plants is the most sensitive stage to heat stress, with even a single hot day sometimes being fatal to reproductive success (Zinn et al. 2010). One of the major effects of heat stress on reproductive tissues that contribute to poor seed set is early or late flowering in plants (Zinn et al. 2010). As indicated above, previous reports have demonstrated that miR156 and miR172 play crucial roles in flowering time control. The two miRNAs were also shown to be induced by heat stress and play important roles in heat tolerance (Lee et al. 2010; Wu et al. 2009; Jung et al. 2012; Stief et al. 2014a). This illustrates that miRNAs may be a crucial player in the signaling mechanism linking temperature sensing to the triggering of flower set. In addition to miR156 and miR172, five other heat-responsive miRNAs (miR159, miR169, miR393, miR397 and miR399) have also been reported to be involved in control of flowering time in plants (Xia et al. 2012; Zhang et al. 2013; Spanudakis and Jackson 2014; Table 1). Moreover, seven heat-responsive miRNAs (miR159, miR164, miR166, miR167, miR171, miR172 and miR319) were discovered to be directly involved in flower development or male and female fertility in plants (Table 1), suggesting their pivotal roles in regulating reproductive tissue development under heat stress conditions. In total, 12 heat-responsive miRNAs have been demonstrated to control flowering time or flower development in plants. Additionally, the heat-induced *ONSEN* retrotransposition occurs during flower development and before gametogenesis, but not in vegetative tissues, further suggesting the importance of regulation at the flowering stage when subjected to heat stress (Ito et al. 2011). The *csd1*, *csd2* and *ccs* mutant plants (equally to the miR398 overexpressing plants)

are more tolerant to heat stress, as indicated by reduced flower damage (Guan et al. 2013). Taken together, we conclude that sRNAs play a significant role in ensuring flower production regardless of heat stress conditions, thereby enhancing transgenerational seed production (Yaish et al. 2011; Figure 3A).

Regulation of HSF/HSP or CBF genes by small RNAs

It is well known that the central component of heat stress responses in all living organisms is *HSP* induction through the activation of *HSFs* (Chauhan et al. 2011). Though many attempts have been made to increase thermotolerance through the overexpression of a single *HSF* or *HSP* gene, limited effects have been achieved. This suggests that *HSFs* and *HSPs* may confer heat tolerance under synergistic pathways in organisms (Qu et al. 2013). Previous studies have indicated that many sRNAs exert their functions on heat stress tolerance through the target and activation of *HSFs* and *HSPs* genes. For example, the expression of *HSP17.6A*, *HSFA2* and *HSP22.0* were over-presented in miR156 overexpressing *Arabidopsis* plants (Stief et al. 2014a). Heat-sensitive transgenic plants expressing miR398-resistant forms of *CSD1*, *CSD2* or *CCS* showed reduced expression of *HSFs* and *HSPs* (such as *HSFA7b* and *HSP90.1*), whereas their expression was enhanced in the heat-tolerant *csd1*, *csd2* and *ccs* plants (Guan et al. 2013). Furthermore, overexpression of *HTT* genes (targets of *TAS1*-derived siRNAs) upregulated several *HSF* genes, and *HTT1* can directly interact with the heat shock proteins Hsp70-14 and Hsp40 during heat stress (Li et al. 2014b). Our latest study in rice demonstrated that *suppressor of the G2 allele of skp1* (*SGT1*), the target gene of miR166e-3p, may bind directly to *HSP90* and *HSP70* to regulate heat stress response at the flowering stage (Liu et al. 2017). These results indicate the downstream functions of *HSFs* and *HSPs* in sRNAs-mediated heat stress response. However, studies also demonstrated that *HSFs* can bind directly to the promoter regions of sRNAs or the target genes to activate their expression, with *HSFA1b* and *HSFA7* inducing the expression of miR398 and *HsfA1a* inducing the expression of *HTT* genes (Guan et al. 2013; Li et al. 2014b). Even in retrotransposition process, *HSFA2* is indispensable to activate the transcription of *ONSEN* whose silence requires components of the siRNA-directed silencing pathway (Cavrak et al. 2014). These discoveries together illustrate that sRNAs achieve their

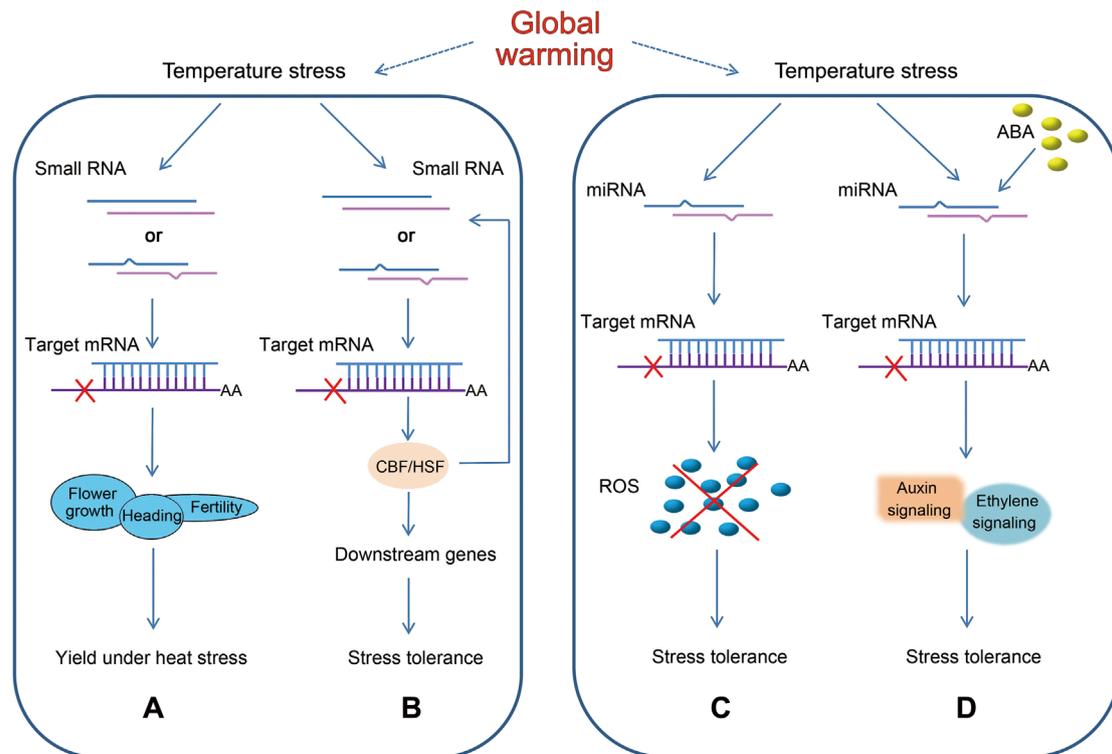


Figure 3. Small RNA-mediated molecular mechanisms of temperature stress responses in plants

Temperature stress regulates the transcription of small RNAs (siRNAs or miRNAs) which then guide the cleavage of their target mRNAs. The target mRNAs function in controlling plant productivity (A), regulating the expression of CBF or HSF genes (B), eliminating the ROS induced by temperature damage (C) and regulating the transcription of genes involved in phytohormone signaling pathway (D), respectively, ultimately leading to stress tolerance in plants.

heat tolerance regulation via the recruitment of HSFs and HSPs in plants (Figure 3B). Interestingly, a feedback loop for *HSFA7b* was also observed in miR398-mediated heat stress response. Heat-induced activation of miR398 requires *HSFA7b*, and miR398 in turn positively affects the heat-induced accumulation of *HSFA7b* and other HSF or HSP genes, further supporting the intimate interactions between sRNAs, HSFs and HSPs in plant heat stress tolerance and acclimation (Guan et al. 2013).

For cold stress, the CBF/DREB1-dependent response pathway has been established to play a predominant role in cold tolerance and acclimation in plants (Chinnusamy et al. 2010). Previous studies have shown that miR394- and miR397- mediated cold tolerance was involved in the activation of CBF and its downstream cold responsive genes in *Arabidopsis* (Dong and Pei 2014; Song et al. 2016). Similarly, miR319 confers cold tolerance via the activation of *DREB1* genes in rice (Wang et al. 2014a). These observations

together indicate that miRNA-mediated cold tolerance may be partially dependent on the modulating of CBF-dependent pathway in plants.

miRNAs-mediated temperature stress tolerance may be partially dependent on the protection of plants from ROS damage

Plants maintain a delicate balance between ROS production and scavenging under normal physiological conditions. However, exposure to stressors such as drought, cold, salinity and heat results in the accumulation of excess ROS, which leads to oxidative damage of membrane lipids, proteins and nucleic acids within the plant cell (Sunkar et al. 2007). In response, plants have developed a highly efficient antioxidant system that includes a variety of enzymatic scavengers to eliminate ROS in stressed conditions (Sunkar et al. 2007). The enzyme *superoxide dismutase* (SOD) constitutes the first line of defense against highly toxic superoxide radicals

(O₂⁻) and functions by converting them into less toxic hydrogen peroxide (H₂O₂). It is well known that miR398-mediated cleavage of *SOD* genes (*CSD1* and *CSD2*) plays a crucial role in oxidative stress response. It has been shown that *Arabidopsis* plants overexpressing miR398-resistant *CSD2* (*mCSD2*) are more tolerant to high light, heavy metals, and other oxidative stresses (Sunkar et al. 2006). It has also been demonstrated that miR398 mediated thermotolerance are due to altered ROS levels under heat stress (Guan et al. 2013). Three miRNAs- (miR319, miR396 and miR408) mediated cold tolerance was also involved in the reduced levels of ROS during cold stress (Wang et al. 2014a; Ma et al. 2015; Zhang et al. 2016). Moreover, Iyer et al. (2012) has identified 12 conserved miRNA families (including miR156, miR159, miR160 and miR166) that are differentially regulated by ozone (a model abiotic elicitor of ROS) in *Arabidopsis* (Iyer et al. 2012). These conserved miRNAs were demonstrated to be involved in cold or heat stress response in many plant species (Table 1). Thus, miRNA-directed gene silencing is considered to be the common regulatory module involved in oxidative stress and temperature stress response. Conversely, miRNA-mediated temperature stress tolerance may be partially dependent on the protection from oxidative stress induced by temperature damage in plants (Figure 3C). The previous study has indicated that heat stress resulted in predominant changes in genes associated with synthesis of scavengers of ROS and ROS homeostasis appeared to play a central role in response to temperature extremes in rice (Mittal et al. 2012). These findings together suggest that miRNAs confer temperature stress tolerance and may at least partially attribute to the elimination of ROS damage in plants.

Temperature stress-responsive miRNAs involved in hormone signaling pathways

Plant hormones have been demonstrated as central regulators of cold stress responses in plants (Eremina et al. 2016). For heat stress, plant hormones such as abscisic acid (ABA), auxin and ethylene have been linked to heat stress signaling in different plant species (Kotak et al. 2007). The genome-wide expression profiling of two wheat cultivars with contrasting heat tolerance under heat stress indicated that many differential expressing genes were involved in phytohormone metabolism or signaling, including ABA, auxin, ethylene and GA reacted to heat stress (Qin et al. 2008). These

findings together indicate the essential roles of hormone signaling in plant heat temperature stress tolerance and acclimation. Intriguingly, many temperature stress responsive miRNAs have been demonstrated to be involved in different hormone signaling pathways, suggesting transcript cross-talk under temperature stress conditions (Wu et al. 2006; Liu et al. 2007; Reyes and Chua 2007; Kruszka et al. 2012; Xia et al. 2012). It has been shown that ABA-induced accumulation of miR159 is a homeostatic mechanism to direct MYB33 and MYB101 transcript degradation to desensitize hormone signaling during seedling stress responses (Reyes and Chua 2007). In addition to miR159, many miRNAs were also regulated by ABA. For instance, miR160, miR319, miR393 and miR397 were upregulated by ABA treatment, whereas miR167, miR169 and miR398 were downregulated by ABA treatment (Kruszka et al. 2012). Interestingly, three of the ABA responsive miRNAs (miR160, miR167 and miR393) have been validated to directly target the components involved in auxin signaling, with miR160 and miR167 modulating ARFs and miR393 modulating *TIR1* and AFBs (Wu et al. 2006; Liu et al. 2007; Xia et al. 2012). The miR393 mediated cleavage of *transport inhibitor response 1 (TIR1)* and *auxin-signaling F-box proteins (AFBs)* have been shown to play crucial roles in both biotic and abiotic stresses in plants (Navarro et al. 2006; Xia et al. 2012). More recently, it has been shown that miR396 confers cold tolerance by directly targeting an ethylene synthesis gene *ACO* (Zhang et al. 2016). Besides, miR5175 has been demonstrated to target *ACC-like oxidase*, another enzyme involved in ethylene biosynthesis, and expression level of *ACC-like oxidase* mRNA was down-regulated in 24 h heat-stressed barley plants (Kruszka et al. 2014). Previous reports have demonstrated that ethylene signaling mutants, such as *ethylene resistant 1 (etr1)* and *ethylene insensitive 2 (ein2)*, are sensitive to heat stress (Kotak et al. 2007). This result can explain the reduced accumulation of *ACC-like oxidase* by miR5175 leading to decreased ethylene levels which would then confer heat sensitivity to barley plants, and is consistent with another study that ethylene may play a negative role in heat stress response (Qin et al. 2008). These results together suggest that miRNAs-mediated temperature stress response is associated with hormone signaling. The interactions between temperature stress responsive miRNAs and the genes in hormone signaling modulate temperature stress tolerance in plants (Figure 3D).

CONCLUSIONS AND PERSPECTIVES

In the past decade, genome-wide studies revealed that many miRNAs and siRNAs change in response to temperature stresses in plants, suggesting that sRNAs play important roles in response to temperature stresses. However, the responses of miRNAs to temperature stresses are complicated, depending on plant species, genotype, tissue, developmental stage, treatment time and stress condition imposed. To understand the miRNA-guided temperature stress regulatory mechanism, these factors should be considered and a specific experiment should be conducted. Even though the responses of miRNAs toward temperature stresses are diverse in plants, some miRNAs are quite conserved, such as that miR167 was induced by heat stress in five plant species and miR164 was induced by cold stress in four plant species, suggesting the persistent regulatory mechanisms of miRNA-guided temperature stress response in plants.

Although many sRNAs have been reported to respond to temperature stresses, only a few temperature stress responsive sRNAs have been validated. There is a significant need for the functional confirmation of temperature stress responsive sRNAs. Particularly, we have very limited knowledge on sRNA-mediated temperature stress response regulatory network and the relationships with sRNA-mediated other important trait regulatory networks in plants. The sRNAs cannot be efficiently used for improvement in plants before their functions are fully understood. With the rapid development of the genome sequencing technology and increasing effort on the study, we can expect that more and more sRNAs associated with temperature stresses would be validated and their regulatory networks would be fully understood in plants in the next few years.

The importance of miRNAs in regulating plant growth, development regulation and responses to various external environmental stresses have been well established. With the functional confirmation of more and more miRNAs, miRNAs have been considered as a newly identified gene resource for the genetic improvement of crops (Zheng and Qu 2015). Although miRNAs provide a good target for efficient improvement in plants, the strategies in selection of miRNAs and techniques should be considered. Generally, there

may be multiple roles of a given miRNA due to its multiple target genes. Some target genes may be pleiotropic and different target genes may have different functions. However, some effects of miRNA may be negative. It is wise to make sure the effects of the miRNAs interest on other important traits in addition to the target trait before it can be used for plant improvement.

Technically, sRNA-based transgenic strategies, such as artificial miRNAs, artificial ta-siRNAs, artificial target mimics and overexpression of miRNA-resistant targets, have been used as a powerful tool for improvement of crop agronomic traits (Liu and Chen 2010; Tiwari et al. 2014; Chen et al. 2016; Djami-Tchatchou et al. 2017). Furthermore, recently emerged Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 technology has also been validated as a non-transgenic RNA-guided genome editing and targeted gene knock-out tool due to its simple structure and its applicability to a variety of organisms (Schiml and Puchta 2016; Djami-Tchatchou et al. 2017). Encouragingly, two recent studies have revealed that by generating CRISPR/cas9 constructs with single-guide RNAs specifically targeting biogenesis processing sites of selected microRNAs, the CRISPR/cas9 can robustly and specifically reduce the expression of microRNAs up to 96% in porcine and human cells (Zhao et al. 2014; Chan et al. 2016). Nevertheless, the application of CRISPR/cas9 in editing sRNAs in plants has not been reported. We believe that with the development of this technology, it can be exploited as a powerful tool in mediating sRNA-guided gene regulation to improve crops temperature stress tolerance.

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REFERENCES

- Barakat A, Sriram A, Park J, Zhebentyayeva T, Main D, Abbott A (2012) Genome wide identification of chilling responsive microRNAs in *Prunus persica*. **BMC Genomics** 13: 481

- Barrera-Figueroa BE, Gao L, Wu Z, Zhou X, Zhu J, Jin H, Liu R, Zhu JK (2012) High throughput sequencing reveals novel and abiotic stress-regulated microRNAs in the inflorescences of rice. **BMC Plant Biol** 12: 132
- Cao X, Wu Z, Jiang FL, Zhou R, Yang Z (2014) Identification of chilling stress-responsive tomato microRNAs and their target genes by high-throughput sequencing and degradome analysis. **BMC Genomics** 15: 1130
- Capovilla G, Pajoro A, Immink RG, Schmid M (2015) Role of alternative pre-mRNA splicing in temperature signaling. **Curr Opin Plant Biol** 27: 97–103
- Cavrak VV, Lettner N, Jamge S, Kosarewicz A, Bayer LM, Mittelsten Scheid O (2014) How a retrotransposon exploits the plant's heat stress response for its activation. **PLoS Genet** 10: e1004115
- Chan H, Yi B, Ma R, Zhang X, Zhao H, Xi Y (2016) CRISPR/cas9, a novel genomic tool to knock down microRNA *in vitro* and *in vivo*. **Sci Rep** 6: 22312
- Chauhan H, Khurana N, Agarwal P, Khurana P (2011) Heat shock factors in rice (*Oryza sativa* L.): Genome-wide expression analysis during reproductive development and abiotic stress. **Mol Genet Genomics** 286: 171–187
- Chen L, Cheng X, Cai J, Zhan L, Wu X, Liu Q, Wu X (2016) Multiple virus resistance using artificial trans-acting siRNAs. **J Virol Methods** 228: 16–20
- Chen L, Luan Y, Zhai J (2015) Sp-miR396a-5p acts as a stress-responsive genes regulator by conferring tolerance to abiotic stresses and susceptibility to *Phytophthora nicotianae* infection in transgenic tobacco. **Plant Cell Rep** 34: 2013–2025
- Chen L, Ren Y, Zhang Y, Xu J, Sun F, Zhang Z, Wang Y (2012) Genome-wide identification and expression analysis of heat-responsive and novel microRNAs in *Populus tomentosa*. **Gene** 504: 160–165
- Chinnusamy V, Zhu JK, Sunkar R (2010) Gene regulation during cold stress acclimation in plants. **Methods Mol Biol** 639: 39–55
- Chuck G, Candela H, Hake S (2009) Big impacts by small RNAs in plant development. **Curr Opin Plant Biol** 12: 81–86
- Das Gupta M, Nath U (2015) Divergence in patterns of leaf growth polarity is associated with the expression divergence of miR396. **Plant Cell** 27: 2785–2799
- Djami-Tchatchou AT, Sanan-Mishra N, Ntushelo K, Dubery IA (2017) Functional roles of microRNAs in agronomically important plants-potential as targets for crop improvement and protection. **Front Plant Sci** 8: 378
- Dong CH, Pei H (2014) Over-expression of miR397 improves plant tolerance to cold stress in *Arabidopsis thaliana*. **J Plant Biol** 57: 209–217
- Eremina M, Rozhon W, Poppenberger B (2016) Hormonal control of cold stress responses in plants. **Cell Mol Life Sci** 73: 797–810
- Fornara F, Coupland G (2009) Plant phase transitions make a SPLash. **Cell** 138: 625–627
- Giacomelli JI, Weigel D, Chan RL, Manavella PA (2012) Role of recently evolved miRNA regulation of sunflower HaWRKY6 in response to temperature damage. **New Phytol** 195: 766–773
- Guan Q, Lu X, Zeng H, Zhang Y, Zhu J (2013) Heat stress induction of miR398 triggers a regulatory loop that is critical for thermotolerance in *Arabidopsis*. **Plant J** 74: 840–851
- Hivrale V, Zheng Y, Puli CO, Jagadeeswaran G, Gowdu K, Kakani VG, Barakat A, Sunkar R (2016) Characterization of drought- and heat-responsive microRNAs in switchgrass. **Plant Sci** 242: 214–223
- Huang J, Yang M, Zhang X (2016) The function of small RNAs in plant biotic stress response. **J Integr Plant Biol** 58: 312–327
- IPCC (2014) IPCC Fifth Assessment Report—Climate Change 2014, Synthesis Report. <http://www.ipcc.ch/>
- Ito H, Gaubert H, Bucher E, Mirouze M, Vaillant I, Paszkowski J (2011) An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. **Nature** 472: 115–119
- Iyer NJ, Jia X, Sunkar R, Tang G, Mahalingam R (2012) microRNAs responsive to ozone-induced oxidative stress in *Arabidopsis thaliana*. **Plant Signal Behav** 7: 484–491
- Jeong DH, Park S, Zhai J, Gurazada SG, De Paoli E, Meyers BC, Green PJ (2011) Massive analysis of rice small RNAs: Mechanistic implications of regulated microRNAs and variants for differential target RNA cleavage. **Plant Cell** 23: 4185–4207
- Jung JH, Seo PJ, Ahn JH, Park CM (2012) *Arabidopsis* RNA-binding protein FCA regulates microRNA172 processing in thermosensory flowering. **J Biol Chem** 287: 16007–16016
- Kim JY, Kwak KJ, Jung HJ, Lee HJ, Kang H (2010) MicroRNA402 affects seed germination of *Arabidopsis thaliana* under stress conditions via targeting DEMETER-LIKE Protein3 mRNA. **Plant Cell Physiol** 51: 1079–1083
- Kim JJ, Lee JH, Kim W, Jung HS, Huijser P, Ahn JH (2012) The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 module regulates ambient temperature-responsive flowering via FLOWERING LOCUS T in *Arabidopsis*. **Plant Physiol** 159: 461–478
- Knauer S, Holt AL, Rubio-Somoza I, Tucker EJ, Hinze A, Pisch M, Javelle M, Timmermans MC, Tucker MR, Laux T (2013) A protodermal miR394 signal defines a region of stem cell competence in the *Arabidopsis* shoot meristem. **Dev Cell** 24: 1–8
- Kotak S, Larkindale J, Lee U, von Koskull-Doring P, Vierling E, Scharf KD (2007) Complexity of the heat stress response in plants. **Curr Opin Plant Biol** 10: 310–316
- Kruszka K, Pacak A, Swida-Barteczka A, Nuc P, Alaba S, Wroblewska Z, Karlowski W, Jarmolowski A, Szweykowska-Kulinska Z (2014) Transcriptionally and post-transcriptionally regulated microRNAs in heat stress response in barley. **J Exp Bot** 65: 6123–6135
- Kruszka K, Pieczynski M, Windels D, Bielewicz D, Jarmolowski A, Szweykowska-Kulinska Z, Vazquez F (2012) Role of microRNAs and other sRNAs of plants in their changing environments. **J Plant Physiol** 169: 1664–1672

- Kumar RR, Pathak H, Sharma SK, Kala YK, Nirjal MK, Singh GP, Goswami S, Rai RD (2015) Novel and conserved heat-responsive microRNAs in wheat (*Triticum aestivum* L.). **Funct Integr Genomics** 15: 323–348
- Kume K, Tsutsumi K, Saitoh Y (2010) TAS1 trans-acting siRNA targets are differentially regulated at low temperature, and TAS1 trans-acting siRNA mediates temperature-controlled At1g51670 expression. **Biosci Biotechnol Biochem** 74: 1435–1440
- Lee H, Yoo SJ, Lee JH, Kim W, Yoo SK, Fitzgerald H, Carrington JC, Ahn JH (2010) Genetic framework for flowering-time regulation by ambient temperature-responsive miRNAs in *Arabidopsis*. **Nucleic Acids Res** 38: 3081–3093
- Li H, Wang Y, Wang Z, Guo X, Wang F, Xia XJ, Zhou J, Shi K, Yu JQ, Zhou YH (2016) Microarray and genetic analysis reveals that csa-miR159b plays a critical role in abscisic acid-mediated heat tolerance in grafted cucumber plants. **Plant Cell Environ** 39: 1790–1804
- Li MY, Wang F, Xu ZS, Jiang Q, Ma J, Tan GF, Xiong AS (2014a) High throughput sequencing of two celery varieties small RNAs identifies microRNAs involved in temperature stress response. **BMC Genomics** 15: 242
- Li S, Liu J, Liu Z, Li X, Wu F, He Y (2014b) Heat-induced *tas1 target1* mediates thermotolerance via heat stress transcription factor *a1a*-directed pathways in *Arabidopsis*. **Plant Cell** 26: 1764–1780
- Liu F, Wang W, Sun X, Liang Z, Wang F (2015a) Conserved and novel heat stress-responsive microRNAs were identified by deep sequencing in *Saccharina japonica* (*Laminariales, Phaeophyta*). **Plant Cell Environ** 38: 1357–1367
- Liu HH, Tian X, Li YJ, Wu CA, Zheng CC (2008) Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. **RNA** 14: 836–8438
- Liu J, Zhang X, Zhang F, Hong N, Wang G, Wang A, Wang L (2015b) Identification and characterization of microRNAs from in vitro-grown pear shoots infected with Apple stem grooving virus in response to high temperature using small RNA sequencing. **BMC Genomics** 16: 945
- Liu PP, Montgomery TA, Fahlgren N, Kasschau KD, Nonogaki H, Carrington JC (2007) Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages. **Plant J** 52: 133–146
- Liu Q, Chen YQ (2010) A new mechanism in plant engineering: The potential roles of microRNAs in molecular breeding for crop improvement. **Biotechnol Adv** 28: 301–307
- Liu Q, Yang T, Yu T, Zhang S, Mao X, Zhao J, Wang X, Dong J, Liu B (2017) Integrating small RNA sequencing with QTL mapping for identification of miRNAs and their target genes associated with heat tolerance at the flowering stage in rice. **Front Plant Sci** 8: 43
- Lu S, Sun YH, Chiang VL (2008) Stress-responsive microRNAs in populus. **Plant J** 55: 131–151
- Ma C, Burd S, Lers A (2015) miR408 is involved in abiotic stress responses in *Arabidopsis*. **Plant J** 84: 169–187
- Matsunaga W, Kobayashi A, Kato A, Ito H (2012) The effects of heat induction and the siRNA biogenesis pathway on the transgenerational transposition of ONSEN, a copia-like retrotransposon in *Arabidopsis thaliana*. **Plant Cell Physiol** 53: 824–833
- Mittal D, Madhyastha DA, Grover A (2012) Genome-wide transcriptional profiles during temperature and oxidative stress reveal coordinated expression patterns and overlapping regulons in rice. **PLoS ONE** 7: e40899
- Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? **Trends Biochem Sci** 37: 118–125
- Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. **Science** 312: 436–439
- Neilson KA, Gammulla CG, Mirzaei M, Imin N, Haynes PA (2010) Proteomic analysis of temperature stress in plants. **Proteomics** 10: 828–845
- Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf morphogenesis by microRNAs. **Nature** 425: 257–263
- Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: Regulation and role in stress tolerance. **Trends Plant Sci** 17: 369–381
- Qin D, Wu H, Peng H, Yao Y, Ni Z, Li Z, Zhou C, Sun Q (2008) Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (*Triticum aestivum* L.) by using Wheat Genome Array. **BMC Genomics** 9: 432
- Qu AL, Ding YF, Jiang Q, Zhu C (2013) Molecular mechanisms of the plant heat stress response. **Biochem Biophys Res Commun** 432: 203–207
- Reyes JL, Chua NH (2007) ABA induction of miR159 controls transcript levels of two MYB factors during *Arabidopsis* seed germination. **Plant J** 49: 592–606
- Schimpl S, Puchta H (2016) Revolutionizing plant biology: Multiple ways of genome engineering by CRISPR/Cas. **Plant Methods** 12: 18
- Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, Nath U, Weigel D (2008) Control of jasmonate biosynthesis and senescence by miR319 targets. **PLoS Biol** 6: 230
- Shi YT, Tian SW, Hou LY, Huang XZ, Zhang XY, Guo HW, Yang S (2012) Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in *Arabidopsis*. **Plant Cell** 24: 2578–2595
- Song JB, Huang SQ, Dalmay T, Yang ZM (2012) Regulation of leaf morphology by microRNA394 and its target LEAF CURLING RESPONSIVENESS. **Plant Cell Physiol** 53: 1283–1294
- Song JB, Gao S, Wang Y, Li BW, Zhang YL, Yang ZM (2016) miR394 and its target gene LCR are involved in cold stress response in *Arabidopsis*. **Plant Gene** 5: 56–64
- Spanudakis E, Jackson S (2014) The role of microRNAs in the control of flowering time. **J Exp Bot** 65: 365–380
- Stief A, Altmann S, Hoffmann K, Pant BD, Scheible WR, Bäurle I (2014a) *Arabidopsis* miR156 regulates tolerance to

- recurring environmental stress through SPL transcription factors. **Plant Cell** 26: 1792–1807
- Stief A, Brzezinka K, Lämke J, Bäurle I (2014b) Epigenetic responses to heat stress at different time scales and the involvement of small RNAs. **Plant Signal Behav** 9: e970430
- Sun XM, Fan GT, Su LY, Wang WJ, Liang ZC, Li SH, Xin HP (2015) Identification of cold-inducible microRNAs in grapevine. **Front Plant Sci** 6: 595
- Sung DY, Kaplan F, Lee KJ, Guy CL (2003) Acquired tolerance to temperature extremes. **Trends Plant Sci** 8: 179–187
- Sunkar R, Kapoor A, Zhu JK (2006) Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in *Arabidopsis* is mediated by downregulation of miR398 and important for oxidative stress tolerance. **Plant Cell** 18: 2051–2065
- Sunkar R, Li YF, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. **Trends Plant Sci** 17: 196–203
- Sunkar R, Zhu JK (2004) Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. **Plant Cell** 16: 2001–2019
- Sunkar R, Chinnusamy V, Zhu J, Zhu JK (2007) Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. **Trends Plant Sci** 12: 301–309
- Tang Z, Zhang L, Xu C, Yuan S, Zhang F, Zheng Y, Zhao C (2012) Uncovering small RNA-mediated responses to cold stress in a wheat thermosensitive genic male-sterile line by deep sequencing. **Plant Physiol** 159: 721–738
- Thiebaut F, Rojas CA, Almeida KL, Grativol C, Domiciano GC, Lamb CR, Engler Jde A, Hemery AS, Ferreira PC (2012) Regulation of miR319 during cold stress in sugarcane. **Plant Cell Environ** 35: 502–512
- Tiwari M, Sharma D, Trivedi PK (2014) Artificial microRNA mediated gene silencing in plants: Progress and perspectives. **Plant Mol Biol** 86: 1–18
- Wahid A, Gclani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: An overview. **Environ Exp Bot** 61: 199–223
- Wang B, Sun YF, Song N, Wei JP, Wang XJ, Feng H, Yin ZY, Kang ZS (2014a) MicroRNAs involving in cold, wounding and salt stresses in *Triticum aestivum* L. **Plant Physiol Biochem** 80: 90–96
- Wang JW, Czech B, Weigel D (2009) miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. **Cell** 138: 738–749
- Wang L, Yu X, Wang H, Lu YZ, de Rooter M, Prins M, He YK (2011) A novel class of heat-responsive small RNAs derived from the chloroplast genome of Chinese cabbage (*Brassica rapa*). **BMC Genomics** 12: 289
- Wang Q, Liu N, Yang X, Tu L, Zhang X (2016) Small RNA-mediated responses to low- and high-temperature stresses in cotton. **Sci Rep** 6: 35558
- Wang ST, Sun XL, Hoshino Y, Yu Y, Jia B, Sun ZW, Sun MZ, Duan XB, Zhu YM (2014b) MicroRNA319 positively regulates cold tolerance by targeting OsPCF6 and OsTCP21 in rice (*Oryza sativa* L.). **PLoS ONE** 9: e91357
- Wang Y, Sun F, Cao H, Peng H, Ni Z, Sun Q, Yao Y (2012) TamiR159 directed wheat TaGAMYB cleavage and its involvement in anther development and heat response. **PLoS ONE** 7: e48445
- Wu G, Park MY, Conway SR, Wang JW, Weigel D, Poethig RS (2009) The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. **Cell** 138: 750–759
- Wu G, Poethig RS (2006) Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. **Development** 133: 3539–3547
- Wu MF, Tian Q, Reed JW (2006) *Arabidopsis* microRN167 controls patterns of ARF6 and ARF8 expression, and regulates both female and male reproduction. **Development** 133: 4211–4218
- Xia J, Zeng C, Chen Z, Zhang K, Chen X, Zhou Y, Song S, Lu C, Yang R, Yang Z, Zhou J, Peng H, Wang W, Peng M, Zhang W (2014) Endogenous small-noncoding RNAs and their roles in chilling response and stress acclimation in Cassava. **BMC Genomics** 15: 634
- Xia K, Wang R, Ou X, Fang Z, Tian C, Duan J, Wang Y, Zhang M (2012) OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. **PLoS ONE** 7: e30039
- Xin M, Wang Y, Yao Y, Xie C, Peng H, Ni Z, Sun Q (2010) Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (*Triticum aestivum* L.). **BMC Plant Biol** 10: 123
- Xu G, Singh SK, Reddy VR, Barnaby JY, Sicher RC, Li T (2016) Soybean grown under elevated CO₂ benefits more under low temperature than high temperature stress: Varying response of photosynthetic limitations, leaf metabolites, growth, and seed yield. **J Plant Physiol** 205: 20–32
- Yaish MW, Colasanti J, Rothstein SJ (2011) The role of epigenetic processes in controlling flowering time in plants exposed to stress. **J Exp Bot** 62: 3727–3735
- Yan K, Liu P, Wu CA, Yang GD, Xu R, Guo QH, Huang JG, Zheng CC (2012) Stress-induced alternative splicing provides a mechanism for the regulation of microRNA processing in *Arabidopsis thaliana*. **Mol Cell** 48: 521–31
- Yang C, Li D, Mao D, Liu X, Ji C, Li X, Zhao X, Cheng Z, Chen C, Zhu L (2013) Overexpression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*Oryza sativa* L.). **Plant Cell Environ** 36: 2207–2218
- Yu X, Wang H, Lu Y, de Rooter M, Carriaso M, Prins M, van Tunen A, He Y (2011) Identification of conserved and novel microRNAs that are responsive to heat stress in *Brassica rapa*. **J Exp Bot** 63: 1025–1038
- Yu X, Yang J, Li X, Liu X, Sun C, Wu F, He Y (2013) Global analysis of cis-natural antisense transcripts and their heat-responsive nat-siRNAs in *Brassica rapa*. **BMC Plant Biol** 13: 208
- Zhang J, Xu Y, Huan Q, Chong K (2009) Deep sequencing of *Brachypodium* small RNAs at the global genome level identifies microRNAs involved in cold stress response. **BMC Genomics** 10: 449

- Zhang X, Wang W, Wang M, Zhang HY, Liu JH (2016) The miR396b of *Poncirus trifoliata* functions in cold tolerance by regulating ACC oxidase gene expression and modulating ethylene-polyamine homeostasis. **Plant Cell Physiol** 57: 1865–1878
- Zhang Y, Zhu X, Chen X, Song C, Zou Z, Wang Y, Wang M, Fang W, Li X (2014) Identification and characterization of cold-responsive microRNAs in tea plant (*Camellia sinensis*) and their targets using high-throughput sequencing and degradome analysis. **BMC Plant Biol** 14: 271
- Zhang YC, Yu Y, Wang CY, Li ZY, Liu Q, Xu J, Liao JY, Wang XJ, Qu LH, Chen F, Xin P, Yan C, Chu J, Li HQ, Chen YQ (2013) Overexpression of microRNA OsmiR397 improves rice yield by increasing grain size and promoting panicle branching. **Nat Biotechnol** 31: 848–852
- Zhao Y, Dai Z, Liang Y, Yin M, Ma K, He M, Ouyang H, Teng CB (2014) Sequence-specific inhibition of microRNA via CRISPR/CRISPRi system. **Sci Rep** 4: 3943
- Zheng LL, Qu LH (2015) Application of microRNA gene resources in the improvement of agronomic traits in rice. **Plant Biotechnol J** 13: 329–336
- Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: Uncovering the weakest links. **J Exp Bot** 61: 1959–1968



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