miRNomes involved in imparting thermotolerance to crop plants

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Abstract

Thermal stress is one of the challenges to crop plants that negatively impacts crop yield. To overcome this ever-growing problem, utilization of regulatory mechanisms, especially microRNAs (miRNAs), that provide efficient and precise regulation in a targeted manner have been found to play determining roles. Besides their roles in plant growth and development, many recent studies have shown differential regulation of several miRNAs during abiotic stresses including heat stress (HS). Thus, understanding the underlying mechanism of miRNA-mediated gene expression during HS will enable researchers to exploit this regulatory mechanism to address HS responses. This review focuses on the miRNAs and regulatory networks that were involved in physiological, metabolic and morphological adaptations during HS in plant, specifically in crops. Illustrated examples including, the miR156-SPL, miR169-NF-YA5, miR395-APS/AST, miR396-WRKY, etc., have been discussed in specific relation to the crop plants. Further, we have also discussed the available plant miRNA databases and bioinformatics tools useful for miRNA identification and study of their regulatory role in response to HS. Finally, we have briefly discussed the future prospects about the miRNA-related mechanisms of HS for improving thermotolerance in crop plants.

Keywords MicroRNA · Heat stress · Crops · Target genes · Regulatory mechanisms

Introduction

Elevated temperature resulted due to global warming poses a serious threat to growth and productivity of crop plants. Increasing frequency of solar flares has also contributed to the heat stress (HS) faced by crop plants. HS generally impairs the photosynthetic activity, increases the degree of lipid peroxidation, and reduces the relative water content thus affecting plant survival, growth, and development. Together, these all lead to decreased plant yield and biomass production (Wang et al. 2003; Mittler 2006; Song et al. 2014; Narayanan et al. 2016). In the years 1980–2008, HS has caused an estimated decline of almost 5.5% and 4.0% in the global wheat and maize production (Lobell et al. 2011; Asseng et al. 2015). Further, it is also estimated that the increase of every 1 °C temperature resulted in a decline of

0.6–8.9% global yields of the six most widely grown staple food crops including barley, maize, rice, sorghum, soybean and wheat (Lobell and Field 2007; Zhao et al. 2017). Thus, the increasing occurrence of extreme heat events, which affect crop productivity, are becoming a serious issue for agriculture. It is important to identify the underlying molecular mechanisms that drive the response to HS and developing new methods to enhance HS tolerance in crop plants (Scharf et al. 2012). Previous studies on plant HS response were focused on the genes (*HSPs*, *LEA*, *MIP*, *ROS*, *NADPH*, etc.) and transcription factors (HSFs, DREB, TIFY, NAC, bZIP, etc.) that regulates the expression of heat-responsive genes (Huang and Xu 2008; Hasanuzzaman et al. 2013; Zhang et al. 2015a; Liu and Chu 2015; You et al. 2015). Recently, it is reported that microRNAs (miRNAs) play an important role in HS responses in plants via post-transcriptional gene expression regulation (Zhao et al. 2016). The post-transcriptional regulations are essential for plants to reestablish their cellular homeostasis during and in recovery from HS phases (Sunkar et al. 2012).

The miRNAs constitute a class of small non-coding RNAs (ncRNAs), with 20–24 nucleotides (nts) in length. The miRNA negatively regulates gene expression of corresponding mRNA either by translation inhibition or

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degradation (Rogers and Chen 2013) depending upon the similarity level. Due to their smaller sizes, precision target and ease of understanding, miRNA-mediated abiotic and biotic trait improvement is gaining increasing appeal in the science community. A successful example that demonstrated utilization of miRNAs for trait improvement wherein over-expression of *os-miR397* enhanced rice yield by increasing grain size and promoting panicle branching (Zhang et al. 2013b). Usually, HS-up-regulated miRNAs down-regulate their target mRNAs, whereas their suppression in response to HS leads to up-regulation of their target mRNAs and function of positive regulators (Chinnusamy et al. 2007; Shriram et al. 2016). A number of studies have shown that HS conditions modulate the expressions (either up-regulated or down-regulated) of miRNAs in plants including several crops (Table 1). Till now, for the identification of miRNAs in different plant species, both the experimental as well as bio-informatic approaches have been used. However, it was challenging to identify a large number of miRNAs, because of their small sequence size, multiple occurrences and different methylation status. But recent advance in high-throughput sequencing, different bioinformatic pipelines, and development of several computational tools and databases resulted in a rapid increase in number of miRNAs and their functional annotations in plants (Tripathi et al. 2015; Shriram et al. 2016). Several HS-related miRNAs were also identified using high-throughput sequencing and computational approaches from stress tissues. These findings suggested that above HS-responsive miRNAs can be utilized as plausible targets for genetic manipulations to engineer thermal stress tolerance in important crops.

In this review, we briefly summarized recent updates on important miRNAs, target genes, cross-talks and their regulatory roles in thermotolerance in crop plants. At some places, we have taken examples from the Arabidopsis model system which are worked out well and are properly documented in relevant context. Also, we have discussed the role of different databases and computational tools in the discovery and study of the regulatory roles of miRNAs. Eventually, we have highlighted the prospect of miRNA-mediated thermotolerance in crop plants for agricultural applications.

The miRNAs and regulatory networks involved in thermotolerance

Recent researches on miRNAs have revealed their integral role in post-transcriptional regulation of genes which are necessary for HS responses (Zhang 2015; Alptekin et al. 2017). In crop and/or model plants, various abiotic stress-regulated miRNAs including HS have been identified and characterized (Gao et al. 2011; Goswami et al. 2014; Kruszka et al. 2014; Zhao et al. 2016). To identify

these stress-responsive miRNAs, large-scale library screening, computational prediction or deep sequencing methods have been adopted. HS-responsive miRNAs were identified through screening of small RNA libraries isolated from model system and crop plants, including Arabidopsis (Lu et al. 2013; May et al. 2013; Barciszewska-Pacak et al. 2015; Lin et al. 2018), tomato (Zhou et al. 2016), *Brassica rapa* (Yu et al. 2012), barley (Kruszka et al. 2014), rice (Liu et al. 2017), and wheat (Xin et al. 2010; Goswami et al. 2014; Kumar et al. 2015) and the count is still increasing. Mostly miRNA target genes, which encode top tier regulatory genes including *NFY-A5*, *APS*, *AST*, *WRKY*, *CSD*, *HSF*, *PPR*, *NAC*, *SPL*, *MYB*, *ARF*, etc., have important modulating roles in abiotic stress response. Important families of miRNAs that were involved in thermotolerance along with their targets are discussed briefly. These miRNA families represent a possible substantial opportunity for crop improvement for HS.

miRNAs involved in physiological and metabolic adaptations

In plants, certain miRNA families (miR169, miR395, miR396, miR397, miR398, miR400 and miR408) were targeting those genes [i.e., nuclear transcription factor Y subunit alpha (*NFY-A5*), sulfate transporter (*AST*), *WRKY*, *LACCASE*, copper/zinc superoxide dismutases (*CSD*), pentatricopeptide repeats (*PPR*)] which modulated the physiological and metabolic adaptations in response to HS (Fig. 1; Table 1). Each one of the possible pair and their implications in mediating HS response discussed below.

The miR169-NF-YA5 regulatory pathway

The miR169 family is one of the largest and conserved family. This family has a significant role in the physiological adaptations (e.g., controlling stomatal aperture opening and closing, stomatal conductance, transpiration rate, etc.) of plants in response to environmental stresses (Zhang et al. 2011; Ni et al. 2013). The miR169 targets NF-YA5, a factor that was shown to regulate several abiotic stress responses in the Arabidopsis and crop plants (Stephenson et al. 2007; Zhao et al. 2007a, b; Ni et al. 2013; Ferdous et al. 2015; Quach et al. 2015). In several studies, it was shown that the down-regulation of miR169 allowed the expression of the mRNA of NF-YA5, which play an essential role in response to HS in important crops (Li et al. 2008; Chen et al. 2012; Sailaja et al. 2014; Zhou et al. 2016; Hivrale et al. 2016). In contrast to the above observations, up-regulation of miR169 in wheat under the HS condition was also reported. This suggests that miR169 with contrasting patterns of expression in different plants under HS probably due to their involvement in coordination of up- and down-regulation of a set of target genes in different crop plants. Further, it is hypothesized that

Table 1 List of miRNAs involved in heat stress response in different plant species including important crops

miRNA Family	miRNA	Regulation	Target	References
I. Physiological and metabolic adaptations				
miR169	<i>tae-miR169a-d</i>	U	NF-YA5	Xin et al. (2010)
	<i>pto-miR169n-t</i>	D	NF-YA	Chen et al. (2012)
	<i>osa-miR169d</i>	D	NF-YA	Sailaja et al. (2014)
	<i>pvi-miR169</i>	D	NF-YA	Hivrale et al. (2016)
miR395	<i>tae-miR395a, b</i>	U	APS1/AST	Goswami et al. (2014) and Kumar et al. (2015)
	<i>agr-miR395</i>	U	APS/AST	Li et al. (2014a)
	<i>pvi-miR395</i>	U	APS/AST	Hivrale et al. (2016)
	<i>spi-miR395d</i>	U	APS/AST	Zhou et al. (2016)
miR396	<i>han-miR396</i>	D	WRKY6	Giacomelli et al. (2012)
	<i>osa-miR396a-c,f</i>	D	WRKY	Liu et al. (2017) and Mangrauthia et al. (2017)
miR397	<i>ath-miR397a</i>	D	LACCASES	Mahale et al. (2014)
	<i>mes-miR397a</i>	D	LACCASES	Ballén-Taborda et al. (2013)
	<i>osa-miR397b</i>	D	LACCASES	Sailaja et al. (2014) and Liu et al. (2017)
	<i>sly-miR397</i>	D	LACCASES	Pan et al. (2017)
miR398	<i>tae-miR398</i>	U	CSD/CCS	Xin et al. (2010); Goswami et al. (2014) and Kumar et al. (2015)
	<i>bra-miR398</i>	D	CSD1, CSD2	Yu et al. (2012)
	<i>ath-miR398</i>	U	CSD1, CSD2, CCS	Guan et al. (2013) and Lu et al. (2013)
	<i>osa-miR398</i>	D	CSD	Sailaja et al. (2014)
	<i>han-miR398</i>	U	CSD	Ebrahimi Khaksefidi et al. (2015)
miR400	<i>ath-miR400</i>	D	PRR	Yan et al. (2012) and Barciszewska-Pacak et al. (2015)
miR408	<i>tae-miR408</i>	U	Plastocyanin	Kumar et al. (2015)
	<i>spi-miR408a</i>	U	Plastocyanin	Zhou et al. (2016)
	<i>osa-miR408</i>	U	Plastocyanin	Mangrauthia et al. (2017)
II. Morphological adaptations				
miR156	<i>tae-miR156f</i>	U	SPL	Xin et al. (2010)
	<i>bra-miR156g/h</i>	U	SPL2	Yu et al. (2012)
	<i>ath-miR156a-f</i>	U	SPL2, SPL3, SPL11	Stief et al. (2014)
	<i>osa-miR156a,g,h</i>	D	SPL	Sailaja et al. (2014) and Liu et al. (2017)
	<i>spi-miR156blf</i>	U	SPL2, SPL9	Zhou et al. (2016)
miR159	<i>tae-miR159a,b</i>	D	GAMYB1, GAMYB2	Wang et al. (2012)
	<i>mes-miR159a</i>	D	Myb like HTHs	Ballén-Taborda et al. (2013)
	<i>ath-miR159a,b</i>	D	MYB	Zhong et al. (2013)
	<i>pvi-miR159</i>	D	MYB	Hivrale et al. (2016)
	<i>osa-miR159a.1,b</i>	D	MYB	Liu et al. (2017)
miR160	<i>tae-miR160</i>	U	ARF	Xin et al. (2010) and Goswami et al. (2014)
	<i>ath-miR160a-c</i>	U	ARF	Zhong et al. (2013) and Lin et al. (2018)
	<i>agr-miR160</i>	U	ARF	Li et al. (2014a)
	<i>hvu-miR160a</i>	U	ARF13, ARF17	Kruszka et al. (2014)
	<i>spi-miR160a</i>	U	ARF10, ARF16, ARF17	Zhou et al. (2016)
miR164	<i>ath-miR164a,b</i>	U	NAC22, NAC100	May et al. (2013)
	<i>tae-miR164</i>	U	NAC1	Pandey et al. (2014)
	<i>pvi-miR164</i>	U	NAC	Hivrale et al. (2016)
miR166	<i>tae-miR166d</i>	U	HD-ZIP-III	Xin et al. (2010)
	<i>ath-miR166a</i>	U	HD-Zip (PHV, REV, HOX9)	May et al. (2013)
	<i>hvu-miR166a</i>	U	HD-Zip (PHV, REV, HOX9)	Kruszka et al. (2014)
	<i>pvi-miR166</i>	U	HD-ZIP-III	Hivrale et al. (2016)
miR167	<i>tae-miR167</i>	U	ARFs, ABP1	Xin et al. (2010) and Ragupathy et al. (2016)
	<i>pto-miR167c,d,f</i>	U	ARFs	Chen et al. (2012)
	<i>bra-miR167</i>	U	ARF6	Yu et al. (2012)

Table 1 (continued)

miRNA Family	miRNA	Regulation	Target	References
miR168	<i>hvu-miR167h</i>	U	ARF8	Kruszka et al. (2014)
	<i>spi-miR167a,b</i>	U	ARF8	Zhou et al. (2016)
	<i>tae-miR168</i>	U	AGO1	Xin et al. (2010)
	<i>pto-miR168a,b</i>	D	AGO1	Chen et al. (2012)
	<i>agr-miR168</i>	U	AGO1	Li et al. (2014a)
	<i>osa-miR168</i>	D	AGO1	Sailaja et al. (2014)
	<i>bra-miR168</i>	U	AGO1	Bilichak et al. (2015)
	<i>pvi-miR168</i>	U	AGO1	Hivrale et al. (2016)
miR172	<i>tae-miR172a, b</i>	D	AP2 like TFs	Xin et al. (2010) and Gupta et al. (2014)
	<i>ath-miR172b</i>	D	AP2 like TFs (SMZ, SNZ, TOE1, TOE2)	May et al. (2013)
miR319	<i>han-miR172</i>	D	AP2 like TFs	Ebrahimi Khaksefidi et al. (2015)
	<i>osa-miR172</i>	D	AP2 like TFs	Mangrauthia et al. (2017)
	<i>ath-miR319a,b</i>	U	TCP2, TCP3, TCP24	Li et al. (2014b) and Barciszewska-Pacak et al. (2015)
	<i>pvi-miR319</i>	U	TCP	Hivrale et al. (2016)
	<i>osa-miR319a</i>	U	TCP	Mangrauthia et al. (2017)
miR390	<i>pvi-miR390</i>	U	ARFs	Hivrale et al. (2016)
miR393	<i>tae-miR393</i>	U	TIR1/AFB	Xin et al. (2010)
	<i>pvi-miR393</i>	U	TIR1	Hivrale et al. (2016)
	<i>spi-miR393b</i>	U	TIR1	Zhou et al. (2016)
	<i>osa-miR393b</i>	U	TIR1	Mangrauthia et al. (2017)
miR399	<i>tae-miR399a</i>	D	PHO2	Xin et al. (2010)
	<i>ath-miR399b</i>	D	PHO2	Kim et al. (2011)
	<i>bra-miR399b</i>	D	PHO2	Yu et al. (2012)
	<i>osa-miR399a-c</i>	D	PHO2	Liu et al. (2017)

ath: *Arabidopsis thaliana*; agr: *Apium graveolens*; bra: *Brassica rapa*; han: *Helianthus annuus*; hvu: *Hordeum vulgare*; mes: *Manihot esculenta*; osa: *Oryza sativa*; pto: *P. tomentosa*; pvi: *Panicum virgatum*; sja: *Saccharina japonica*; spi:; tae: *Triticum aestivum*

U up-regulated, D down-regulated

AGO1 ARGONAUTE1, AP2 Apetala2, APS/AST ATP sulfurylase/affinity sulfate transporter, ARF auxin response factor, CSD/CCS copper/zinc superoxide dismutases/copper chaperone for SOD, GAMYB gibberellic acid MYB, HD-Zip homeodomain-leucine zipper, HOX9 homeobox leucine zipper protein HOX9-like, NAC NAM, ATAF, and CUC, NF-Y nuclear transcription factor Y, PHO2 phosphate2, PHV phavoluta, REV revoluta, PPR pentatricopeptide repeats, SMZ schlafmütze, SNZ schnarchzapfen, SPL squamosa promoter binding, TCP teosinte branched/cycloidea protein, TIR1/AFB, transport inhibitor response1/auxin-related F-box, TOE target of eat

NF-YA5 in guard cells controls stomatal aperture, whereas NF-YA5 expressed in other important cells regulate the expression of stress-associated genes (Zhao et al. 2009). Therefore, miR169-NF-Y-A5 regulatory pathway could be utilized for improving plant tolerance to HS.

The miR395-APS/AST regulatory pathway

The expression of miR395 family has been observed to be up-regulated in response to HS in different plant species, i.e., *Arabidopsis* (Jones-Rhoades and Bartel 2004), wheat (Kumar et al. 2015), *Populus* (Chen et al. 2012), *Celery* (Li et al. 2014a) and switchgrass (Hivrale et al. 2016). The miR395 regulate several downstream genes which ultimately regulate pathways controlling complex processes of physiology and metabolism. Up-regulation of miR395 is involved

in sulfur metabolism via its targets ATP sulfurylases (APS1, APS3, and APS4), an important group of enzymes catalyzing the initial activation step of sulfate assimilation (Lapartient et al. 1999). The miR395 also targets affinity sulfate transporter (AST), a sulfate transporter. At metabolism level, these miR395-regulated genes (APS and AST) were involved in accumulation of glutathione thus ultimately have increased plant stress resistance against various stresses including HS (Kawashima et al. 2011).

The miR396-WRKY regulatory pathway

The miR396 family was found to involved in cell proliferation and leaf development by targeting the growth-regulating factor (GRF) and basic helix loop helix (bHLH) TFs (Rodriguez et al. 2010; Debernardi et al. 2012). Another

Fig. 1 The miRNA-mediated regulatory networks involved in different physiological, metabolic and morphological adaptations during heat stress (based on the literature given in Table 1; for abbreviations see Table 1)

diverse role of this miRNA is shown in sunflower (*Helianthus annuus*) where it has been involved in high-temperature stress tolerance. Further, it has been shown that miR396 also targets a WRKY TFs in addition to GRF and bHLH TFs (Giacomelli et al. 2012). WRKY TFs have been associated with HS regulation and other abiotic stresses via transient elevation of stomatal conductance and lower leaf temperature (Rushton et al. 2010; Macková et al. 2013; Gahlaut et al. 2016). It has been shown that miR396 in sunflower was repressed by HS, which results in the up-regulation of the *HaWRKY6*, which is a putative target of miR396. But, overexpressing miR396-resistant plants of *HaWRKY6* were hypersensitive to HS, indicating that *HaWRKY6* is involved in a fine modulation in response to HS (Giacomelli et al. 2012).

The miR397–LACCASE regulatory pathway

Another environmental stress-regulated miRNA family is miR397. The members of this family were shown to be down-regulated in response to heat and drought stresses in plants (Shinozuka et al. 2012; Ballén-Taborda et al. 2013; Mahale et al. 2014; Liu et al. 2017; Pan et al. 2017). During HS, miR397 negatively regulate its target gene, i.e., LACCASE (LAC). LAC encodes a copper-containing

glycoprotein (McCaig et al. 2005), and have shown to be involved in strengthening of cell–cell adhesion and maintaining the cell wall structure during HS conditions (McCaig et al. 2005; Roy et al. 2017). Thus, under HS condition, down-regulation of miR397 might play an important role to increase the transcript levels of LAC that ultimately helps in mitigation of HS. Furthermore, up-regulation of LAC gene has been also reported during high NaCl concentrations in both maize and tomato. This suggests an increase in transcript level of LAC under stress might be a widespread response in plants (Wei et al. 2000; Liang et al. 2006), thus making miR397 a good target for engineering HS tolerance.

The miR398–CSD/CCS–HSFs/HSPs regulatory pathway

Members of miR398 family have been shown to be involved in HS response in model plant *Arabidopsis* and in some important crop plants including wheat, rice and *B. rapa* (Xin et al. 2010; Chen et al. 2012; Yu et al. 2012; Guan et al. 2013; Goswami et al. 2014; Sailaja et al. 2014; Kumar et al. 2015). In *Arabidopsis*, the miR398 family is represented by three members namely miR398a, miR398b and miR398c. These family members have four target genes including *CSD1* (copper/zinc superoxide dismutases), *CSD2*, *CCS1* (copper chaperone for SOD), and *COX5b-1* (cytochrome-c

oxidase) (Sunkar and Zhu 2004; Zhu et al. 2011; Guan et al. 2013). HS condition leads to up-regulation of miR398 and down-regulation of its target genes (*CSD1*, *CSD2* and *CCS*) that control ROS accumulation in *Arabidopsis* (Guan et al. 2013). The altered redox status contributes to accumulation of HSFs (heat stress TFs) and HSPs (heat shock proteins) that play a critical role during thermotolerance. Further, it was shown that HSFs induce the expression of miR398 during HS. The transgenic plant expressing miR398-resistant forms of *CSD1*, *CSD2* and *CCS* are more sensitive to HS. In contrast, *csd1*, *csd2* and *ccs* mutant plants are more tolerant to HS than the wild-type, with higher HSF and HSP levels (Guan et al. 2013). Similarly, in wheat *tae-miR398* showed up-regulation under differential HS conditions, whereas its target gene Cu/Zn-SOD showed down-regulation under HS (Goswami et al. 2014; Kumar et al. 2015). Further, miR398 and its target genes *CSDs* and *CCSs* were also found to be associated with HS responses of rice, *B. rapa* and *Populus tomentosa* (Kotak et al. 2007; Yu et al. 2012; Sailaja et al. 2014). However, in rice and *B. rapa*, HS down-regulated the expression of the miR398, which guides heat stress response of their target gene *CSD* (Yu et al. 2012). Thus, the above studies indicate that the miR398-CSD/CCS-HSFs/HSPs is an essential regulatory mechanism that is involved in thermotolerance and could be an effective target to engineer HS tolerance in crop plants.

The miR400-PRR regulatory pathway

It is known that HS-induced alternative splicing (AS) also regulates the expression of intronic miRNAs in plants (Yan et al. 2012; Zhao et al. 2016). For instance, intronic *miR400* is co-transcribed with its host gene *Atlg32583*. During HS, a specific AS occurred at the intron of above gene containing the *miR400* hairpin, which causes down-regulation of mature *miR400*, but did not influence the expression of the host gene. This AS event may be congenial for thermotolerance in plants, as over-expression of *miR400* made the plants more sensitive to HS (Yan et al. 2012). Further, miR400 target *pentatricopeptide repeat* (*PPR*) gene, which is involved in abiotic stress and plant development, induced its expression upon HS in *Arabidopsis* (Li et al. 2014a; Park et al. 2014). Generally, the PRRs (PPR1 and PPR2) were localized in mitochondria and regulate the ROS metabolism in the mitochondria, which results in more H₂O₂ accumulation upon HS in plants (Park et al. 2014). Thus, this intronic miRNA mitigates HS in plants (Yan et al. 2012).

The miR408-plastocyanin regulatory pathway

The miR408 family has been found to be associated with several abiotic stresses (HS, drought, salt, cold, oxidative stress) in previous studies. The members of this family act

in various plants species, including important crops, barley (Kantar et al. 2010), *Populus* (Chen et al. 2012), rice (Mutum et al. 2016; Mangrauthia et al. 2017), Celery (Li et al. 2014a), wheat (Kumar et al. 2015) and switchgrass (Hivrale et al. 2016). The miR408 target includes a group of genes belonging to the phytochrome family (i.e., plantacyanin, cupredoxin and uclacyanin). These proteins function as electron-transfer shuttles among different proteins and regulate the cellular redox state in response to HS and drought stress (Choi and Davidson 2011; Ma et al. 2015; Hivrale et al. 2016; Mutum et al. 2016). Thus, this miR408 family provides a distinct mechanism to regulate various stress responses besides HS. This family could be effectively targeted to control HS responses of crop plants.

miRNA families involved in morphological adaptations

Another group of miRNAs is associated with morphological adaptations (leaf, shoot, lateral root development, leaf shape and size, vegetative phase change, etc.) of plants in response to HS. During HS, several conserved miRNAs families target the genes that were engaged in the regulation of different morphological adaptations of plants. For example, miR156, miR159, miR160/167/390, miR166, miR164, miR166, miR168, miR172 target following TFs, i.e., SPLs, MYBs, ARFs, NACs, HD-ZIPs, AGO1, AP2s, AFBs and PHO2, respectively (Fig. 1; Table 1). The expression of these miRNAs and their target TFs appears to be regulated during HS, suggesting that growth and development of plants were modulated during HS. Engineering the crop plants for such miRNAs provides a structure-based protection measure.

The miR156-SPL regulatory pathway

The miR156 family members influence flowering time, which control the juvenile to adult phase transition (Yamaguchi and Abe 2012). This family targets and down-regulate the expression of Squamosa Promoter Binding-Like (SPL) TFs, which further induce the expression of *FLOWERING LOCUS T* (*FT*), *FRUITFULL* (*FUL*) and *LEAFY* (*LFY*) genes (Huijser and Schmid 2011; Yamaguchi and Abe 2012; Stief et al. 2014; Hong and Jackson 2015). The miR156-SPL regulatory pathway was also shown to modulate HS-responsive flowering and upregulate the expression of *FT* and *FUL* genes (Yamaguchi et al. 2009; Kim et al. 2012; Zhong et al. 2013; Stief et al. 2014). Furthermore, the members of the miR156 family in crop plants were also induced in response to HS in *B. rapa* (Yu et al. 2012), and wheat (Xin et al. 2010; Kumar et al. 2015). However, contrasting expression pattern, i.e., repression by HS in two important crops including cassava (Ballén-Taborda et al. 2013) and rice (Sailaja et al. 2014; Liu et al. 2017) have been reported.

In *B. rapa*, two members of miR156 family (*Bra-miR156h* and *bra-miR156g*) were up-regulated in response to HS and their target *BracSPL2* was down-regulated (Yu et al. 2012). The *tae-miR156* up-regulation and down-regulation of its target *SPL* genes (*Ta3711* and *Ta7012*) in response to HS were also reported in wheat (Xin et al. 2010). The miR156 family was also important for HS memory in Arabidopsis. This family was reported to promote the sustained expression of HS-responsive genes through *SPL* genes, especially *SPL2* and *SPL11*, and is critical only after HS (Stief et al. 2014; Cui et al. 2014). So, owing to the conserved nature of miR156 family and its target genes (*SPLs*), the function of miR156–*SPL* regulatory pathway in HS memory may also be conserved in crop plants (Stief et al. 2014) and poised to be a good target for engineering HS tolerance in crop plants.

The miR159-MYB regulatory pathway

miR159 family in Arabidopsis and crop plants negatively regulates the expression of its target MYB TF genes at the post-transcriptional level (Xin et al. 2010; Wang et al. 2012; Li et al. 2014a; Kumar et al. 2015; Hivrale et al. 2016). It was reported that miR159 negatively regulates the gibberellic acid MYB (*GAMYB*) TF genes, which plays an important role in flower development and seed germination (Reyes and Chua 2007). In Arabidopsis and other crop plants, some of the MYB genes are expressed in the anthers and seeds, where miR159 is less accumulated (Millar and Gubler 2005). Thus, this negative correlation in expression pattern provides evidence for antagonistic relation of miR159 and *GAMYB*. The role of the miR159-MYB pathway in the HS response is also known. The expression level of miR159 was down in wheat seedling after HS for 2 h and while its target *TaGAMYB* was up-regulated. Further, the *tae-miR159* overexpressing rice transgenic lines and *Arabidopsis myb-33myb65* double mutants were found to be more sensitive to HS relatively in comparison to the wild-type, suggesting that miR159-MYB regulatory pathway was also involved in responding to HS (Xin et al. 2010; Wang et al. 2012).

The miR160/167/390-ARF regulatory pathway

Three miRNA families including miR160, miR167 and miR390 have their role established in HS-related auxin signaling by modulating the expression of auxin response factor (ARF) TF genes. The ARFs are pleiotropically involved in root, shoot, flower and root development, and stress response (Meng et al. 2010; Kruszka et al. 2014; Hivrale et al. 2016). The miR160 targets the following three ARF genes *ARF10*, *ARF16*, and *ARF17* (Meng et al. 2010; Lin et al. 2018). During HS, the expression of miR160 was found to be up-regulated, while the

expression of its target ARFs was down-regulated in Arabidopsis (Mallory 2005; Lin et al. 2018) and in following crop plants, wheat (Xin et al. 2010), barley (Kruszka et al. 2014) and rice (Mangrauthia et al. 2017). This increase in expression of miR160 and down-regulation of ARFs during HS affects many plant cell activities including cell elongation and division, and thus affecting overall plant growth (de Wit et al. 2014). In addition, several HSP genes (*HSP17*, *HSP21*, *HSP70B*) were also regulated by via ARFs (*ARF10*, *ARF16*, and *ARF17*). This suggested that there is a plausible role of miR160-induced regulation mechanisms in improved thermotolerance of plants (Lin et al. 2018). Similarly, miR167 targets *ARF6* and *ARF8*, which regulates floral organ (gynoecium and stamen) development. In response to HS, the expression of these ARFs was down-regulated, while expression of miR167 was up-regulated significantly in different crops like barley (Kruszka et al. 2014), wheat (Xin et al. 2010; Kumar et al. 2015; Ragupathy et al. 2016) and rice (Saibo et al. 2009; Sailaja et al. 2014). In addition to above two miRNAs, miR390 were also involved in the auxin signaling and in the HS response in several crop plants via miR390-TAS3-ARF3/-4 regulatory pathway (Xin et al. 2010; Vidal et al. 2010; Guan et al. 2013; Hivrale et al. 2016). The miR390 indirectly represses two ARF TF genes (*ARF3* and *ARF4*) by promoting the production of another type of small RNA known as *trans*-acting short-interfering RNA (*tasiRNAs*) (Garcia 2008; Endo et al. 2013). The above findings reveal that several hormones related to miRNAs were involved in regulation of plant development and growth during HS through miRNA-target gene networks.

The miR164-NAC regulatory pathway

The miR164 family has been shown to target stress-associated NAC TF family members, which were involved in the regulation of shoot and root development in several crop plants and Arabidopsis (Gupta et al. 2014; Bhardwaj et al. 2014; Pandey et al. 2014; Fang et al. 2014; Qiu et al. 2016). In Arabidopsis, miR164 was up-regulated and *NAC1* gene was down-regulated after HS treatment (May et al. 2013; Li et al. 2014b). In rice, it was reported that miR164 target six NAC TF genes (*OMTN1–OMTN6*), four of which (*OMTN2*, *OMTN3*, *OMTN4*, and *OMTN6*) had negative effects on drought stress tolerance (Fang et al. 2014). Further, in *Brassica*, miR164 was shown to target NAC TF gene, and expression of this NAC gene was negatively correlated with miR164 under different abiotic stresses like drought stress, salinity stress, and HS (Bhardwaj et al. 2014). These studies support that the miR164-NAC regulatory pathway is widely involved in plant development and growth during the HS in different crop plants.

The miR166-HD-Zip regulatory pathway

The miR166 family targets homeodomain-leucine zipper (HD-Zip) TFs, and these TFs were involved in regulation of auxiliary meristem initiation and leaf morphology (Rhoades et al. 2002). Increase in the transcript level of miR166 reported in response to HS in Arabidopsis (Zhong et al. 2013), wheat (Xin et al. 2010; Kumar et al. 2015), barley (Kruszka et al. 2014) and rice (Liu et al. 2017). In barley, *PHV* (*PHAVOLUTA*), *REV* (*REVOLUTA*), and *HOX9* (homeobox leucine zipper protein HOX9-like), the members of the HD-Zip TF family were down-regulated in heat-stressed plants as compared to control plants (Kruszka et al. 2014). This indicates that miR166-HD-Zip regulatory pathway involved in regulation of flower development and leaf morphology during HS and could be an important target to control HS mitigation in crop plants.

The miR168-AGO1 regulatory pathway

Under HS, members of miR168 family (miR168a-3p and miR167a-3p) were down-regulated, which caused a high level of its target gene *AGO1* (Millar and Gubler 2005; Chen et al. 2012; Barciszewska-Pacak et al. 2015). *AGO1* plays an essential role in plant development and as well as in the production of miRNAs (Vaucheret 2009). Differential expression of miR168 in response to HS was also observed in various crop species, including wheat (Xin et al. 2010), rice (Sailaja et al. 2014) and *B. rapa* (Bilichak et al. 2015). This suggests the important role of miR168 during HS in crop plants. In *B. rapa*, it was observed that bra-miR168 following HS showed a differential expression that was negatively correlated with *AGO1* gene expression levels. This study also suggests that the miR168-AGO1 regulatory pathway have a role in transgenerational stress memory inheritance (Bilichak et al. 2015) and thus could be a target for addressing HS responses crop plants.

The miR172-AP2 regulatory pathway

The miR172 family, which target and regulates the expression of APETALA2 (AP2)-like TFs, were involved in multiple developmental processes (regulation of vegetative phase change, flowering and spike morphogenesis) as well as involved in abiotic stress including HS (Wu et al. 2009; Li et al. 2016; Debernardi et al. 2017). Following, six AP2 type genes were targets of miR172 in *Arabidopsis*: *AP2*, *TARGET OF EAT 1* (*TOE1*), *TOE2*, *TOE3*, *SCHLAFMÜTZE* (*SMZ*), and *SCHNARCHZAPFEN* (*SNZ*). These genes were involved in floral repression. It is reported that AP2-type transcript levels are high in the early seedling stage and decline thereafter, as level of miR172 rise with increasing plant age, thus causes the repression of flowering as the plant matures (Zhu

and Helliwell 2011). The miR172 family showed altered expression and generally down-regulated in response to HS in several plant species including important crops Arabidopsis (May et al. 2013), *H. annuus* (Ebrahimi Khaksefidi et al. 2015), wheat (Giacomelli et al. 2012; Gupta et al. 2014), barley (Hackenberg et al. 2015) and rice (Mangrauthia et al. 2017), and while its target genes (*TOE1*, *TOE2*, *TOE3*, and *SMZ*) were up-regulated in response to HS (May et al. 2013; Gupta et al. 2014; Li et al. 2014b). These findings indicate that miR172-AP2 regulatory pathway has an important role in HS-induced flowering, which modulate plant growth and development during HS.

The miR319-TCP regulatory pathway

The miR319 family targets the teosinte branched/cycloideae protein (TCP) TFs (Rubio-Somoza and Weigel 2013). The TCP TFs have been shown to play roles in flowering time, leaf and gametophyte development (Schommer et al. 2012). Initially, the role of TCPs and their regulation by miR319 was reported from in jaw-D mutants in Arabidopsis. These mutants overexpressed miR319 and showed late-flowering phenotype (Jones-Rhoades et al. 2006; Schommer et al. 2012). Contrariwise, loss-of-function of the miR319 target TCP4 also generated a late-flowering phenotype (Schommer et al. 2012). In addition to the role of miR319-TCP pathway in the regulation of flowering time in plants, it was also found to be responsive to several abiotic stresses including thermal stress (Barciszewska-Pacak et al. 2015). The miR319 was found to be up-regulated and its targets of TCP family TFs (for e.g., TCP2, TCP3, and TCP24) were down-regulated under HS condition in Arabidopsis (Li et al. 2014b; Barciszewska-Pacak et al. 2015), rice (Mangrauthia et al. 2017), and switchgrass (Hivrale et al. 2016). These findings indicate that miR319-TCP regulatory pathway was also involved in responding to HS.

The miR393-TIR1/AFB regulatory pathway

Compared to miR160, miR167 and miR390 that are involved in auxin signaling and HS discussed above, miR393 is distinct in a sense that miR393 indirectly participate in the HS response and auxin signaling (Vidal et al. 2010; Zhang 2015). Further, the expression of miR393 is also reported to alter by many environmental stresses (HS, cold, dehydration, NaCl and ABA treatments) in Arabidopsis (Gao et al. 2011; Guan et al. 2013), wheat (Xin et al. 2010), rice (Gao et al. 2011; Mangrauthia et al. 2017) and switchgrass (Hivrale et al. 2016). The mechanism of action is understood as miR393 targets and down-regulates the expression of the auxin receptors *Transport Inhibitor Response 1* (*TIR1*) and *Auxin Signaling F-BOX* (*AFB1-3*) genes (Jones-Rhoades and Bartel 2004; Sunkar et al. 2012). Both the genes (*TIR1* and

AFB) were involved in root development and response to heat stress treatment via their participation in auxin signaling (Meng et al. 2010). Thus, miR393-TIR/AFB regulatory pathway could be another plausible target for improving crop plant tolerance to HS.

The miR399–PHO2 regulatory pathway

The miR399–PHO2 pair represents another important regulatory pathway that was reported in both flowering time control and abiotic stress responses (Kim et al. 2011; Kruszka et al. 2014; Gao et al. 2015). The miR399 governs phosphorus homeostasis by targeting and regulating the expression of a ubiquitin-conjugating E2 enzyme *PHOSPHATE 2* (*PHO2*) in Arabidopsis (Chiou 2006; Kruszka et al. 2014). It is reported that during the phosphate starvation miR399-dependent regulation of *PHO2* is attenuated (Chiou 2006). Further, the miR399 activity in targeting *PHO2* is quenched by *IPS1* (Induced by Phosphate Starvation 1), which is a long non-coding RNA (Franco-Zorrilla et al. 2007). *IPS1* functions as a target mimic, thus protecting cleavage of *PHO2* by miR399. The role of miR399 during HS-responsive flowering time was reported in crop plants: *B. rapa* (Yu et al. 2012), wheat (Xin et al. 2010; Zhao et al. 2013) and rice (Liu et al. 2017). It was shown that miR399 has its role in ambient temperature-responsive flowering in Arabidopsis (Kim et al. 2011). When mutant plants with miR399 overexpression/*PHO2* loss-of-function grown at 23 °C they flowered early, whilst, there is no change in flowering time in these mutants when grown at 16 °C. It indicates that the miR399–*PHO2* regulatory pathway governs the ambient temperature-dependent flowering pathway (Kim et al. 2011) and it could be targeted for improving HS tolerance responses of various crops.

The miRNA–miRNA cross-talk regulations during HS

The miRNAs are largely recognized for their roles in regulation of specific target mRNA. However, miRNAs targeting more than one gene or conversely different miRNAs targeting more than one specific gene are also reported (Xu et al. 2016). This indicates that miRNAs exhibit functional, complexity and thus a cross-talk of miRNAs takes place during regulation of a particular biological function in plants including abiotic stresses like HS response. For example, in case of tomato during HS, three miRNAs (miR160 and miR167 and miRNA393) were reported to target the ARFs which in turn regulate plant growth and development under stress via auxin signaling (Pan et al. 2017). The above study by (Pan et al. 2017) reported the regulatory network and cross-talks of miRNAs participating in stigma exertion under HS in tomato using integrated high-throughput sequencing and molecular biology approaches. The following miRNA target

pairs, miR398/CSD1, miR393/TIR1, miR160/ARF10/16, and miR156/SPL15 were strongly correlated and thus work in coordination for the regulation of stigma exertion under HS condition (Fig. 2). Similarly, in Arabidopsis, at least four miRNAs (miR156, 157, miR159 and miR172) are known to regulate flowering time during environmental stress conditions (Jin et al. 2013). Out of these four miRNAs, miR156 and miR157 target the SPL3 (squamosa promoter binding-like protein 3), miR159 targets the mRNA-encoding GA-specific transcriptional regulator GAMYB-related proteins (MYB33, MYB65 and MYB101) and miR172 targets the floral integrator FLOWERING LOCUS T (Fig. 2) (Chen et al. 2010). Besides, miR172 also targets APETALA2 (AP2) (Chen 2004) and AGAMOUS (AG) (Zhao et al. 2007a, b), both of these genes are known to be involved in flower development. The above-mentioned few examples of miRNAs cross-talks during stress condition pave the possibilities of future research on miRNA-responsive regulation and cross-talks and their utilization to enhance plant stress tolerance for crop improvement.

Role of bioinformatics in establishing miRNA and plant HS tolerance interactions

In various plant species, the identification and functional annotation of miRNA families were exponentially increased in the past decade (Tripathi et al. 2015). Coupled with next-generation sequencing, improved algorithm has made it possible. This rapid increase is also due to the advancement in cloning techniques and analysis methods (Jagadeeswaran et al. 2010; Rosewick et al. 2013). Further, utilization of different bioinformatics databases and tools have revolutionized the study of miRNAs and their regulatory roles during different abiotic stresses including HS.

Most of the identified miRNAs have been found to be evolutionarily conserved and have homologs and orthologs in relative plant species. Applying this corollary, bioinformatics tools predicted the miRNAs by sequences which were showing a perfect match to miRNAs reported in earlier studies (i.e., data available at different miRNA databases). For instance, in *Brassica napus*, 35 miRNA families were identified in heat-stressed condition in comparison with available *A. thaliana* miRNAs data, thus, the conserved miRNA sequences could be used for annotating new stress-responsive miR genes in different crops (Wang et al. 2004; Adai 2005; Zhang et al. 2006; Yu et al. 2012; Lukasik et al. 2013). Similarly, the regulatory targets of miR genes could be identified that are conserved among the different plants. For instance, Jones-Rhoades and Bartel (2004) identified miRNAs and their target mRNAs that are conserved among Arabidopsis and rice. They utilized the MIRcheck algorithm and predicts the miRNAs genes could target the superoxide

Fig. 2 An overview of miRNA–miRNA cross-talks in response to heat stress in plants (based on available literature). *ARFs* auxin response factors, *CSD1* copper/zinc superoxide dismutases 1, *MYBs*

myeloblastosis transcription factors, *SPL* squamosa promoter binding, *TIR1* transport inhibitor response1

dismutases (SOD), laccases, and ATP sulfurylases, which were also involved in different stress responses. This leads to identification of participation of miR398 family in the ROS pathway via targeting the SOD during abiotic stresses (drought, heat, cold and salt stresses) (Sunkar and Zhu 2004; Jones-Rhoades and Bartel 2004; Guan et al. 2013). Structure vector machine-based application has also been developed which ‘learn’ the parameters from previously known sequences and apply the same for de novo prediction of miRNAs (Gomes et al. 2013; Vitsios et al. 2017).

A detailed list of different databases and computational tools, that were involved in miRNA identification, miRNA target prediction, validated miRNA finding, miRNA expression analysis, miRNA and TF interplay is provided in Table 2. Some of the popularly used databases and tools that contain data of crop plants are discussed below in a contextual manner.

In recent years, utilizing different biological and bio-informatic approaches, thousands of miRNAs in plants,

animals, and viruses were reported. This information of miRNA sequences and annotation are now collected in different databases like miRbase, miREST, PMRD, PASmiR etc. (for more details, the list of different databases is given in Table 2). For instance, miRBase was one of the important online repositories of miRNA sequences and annotation. The current version (release 22) of miRBase contains 38,589 precursor miRNAs, 48,885 mature miRNA products from 270 species, including 1346 miRNA precursors in important crop plants (wheat, rice, maize, brassica, sorghum, barley; Fig. 3) that produce 1677 mature miRNAs (Kozomara and Griffiths-Jones 2014). Similarly, miRNEST database has the data of about 40,000 miRNA sequences of different 544 species (Szcześniak et al. 2012). The latest version (miRNEST2.0) contains data of miRNAs from > 200 species including several important crop plants. Interestingly, this database utilized deep sequencing libraries for miRNA predictions, and contains data from different degradome analyses and results of pre-miRNA classification (Szcześniak and Makalowska

Table 2 Details of major databases and bioinformatic tools available for the prediction and analysis of plant miRNAs and their targets

S. no.	Database/tool	Organism	Available data	Weblink	References
I. Major plant databases					
1.	PMRD	Plants	Published miR sequences, target information, miRNA expression data	http://bioinformatics.cau.edu.cn/PMRD/	Zhang et al. (2010)
2.	miRBase	Plants, animals, viruses	Published miR sequences and annotation, target information, target expression data	http://www.mirbase.org/	Kozomara and Griffiths-Jones (2014)
3.	miRNEST	Plants, animals	Published miR sequences, target information, miRNA expression data	http://rhesus.amu.edu.pl/mirnest/copy/home.php	Szczęśniak and Makalowska (2014)
4.	PNRD	Plants	Published miR sequences, target information, miRNA expression data	http://structuralbiology.cau.edu.cn/PNRD	Yi et al. (2015)
5.	PMTED	Plants	miRNA target expression data	http://pmted.agrinome.org/	Sun et al. (2013)
6.	DIANA-TarBase	Plants, animals, viruses	Experimentally validated miR-target interactions data	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index	Vlachos et al. (2015)
7.	miRTarBase	Plants, animals, viruses	Experimentally validated miR-target interactions data	http://mirtarbase.mbc.nctu.edu.tw/	Chou et al. (2016) and Chou et al. (2018)
8.	mirEX 2.0	Plants	miRNA expression data	http://www.combio.pl/mirex2	Zielezinski et al. (2015)
9.	PmiRExAd	Plants (wheat, rice and maize)	miRNA expression data	http://pmirexat.nabi.res.in	Gurjar et al. (2016)
10.	TransmiR	Plants, animals	TF-miRNA regulation	http://www.cuilab.cn/transmir	Wang et al. (2010)
11.	PlantTE-MIR DB	Plants	Details of TE-related miRNAs	http://bioinfo-tool.cp.utfpr.edu.br/plantemirdb/	R Lorenzetti et al. (2016)
12.	PASmiR	Plants	Details of miRNA involved in abiotic stress tolerance	http://pcsb.ahau.edu.cn:8080/PASmiR/f	Zhang et al. (2013a)
13.	WMP	wheat	Details of miRNA involved in development and abiotic stress tolerance	http://wheat.bioinfo.uqam.ca/index.php?action=citation	Remita et al. (2015)
II. Major computational tools					
1.	semirna	Plants	miRNA and target identification	http://www.bioinfocabd.upo.es/node/5	Muñoz-Mérida et al. (2012)
2.	C-mii	Plants	miRNA and target identification	http://www3a.biotech.or.th/c-mii/	Nummark et al. (2012)
3.	iMir	Plants, animals	Novel and known miRNA identification, computes differential expression, target prediction, isomiRs identification	http://www.labmedmolge.unisa.it/fnglese/research/imir	Giurato et al. (2013)
4.	mirTools 2.0	Plants, animals	Novel and known miRNA identification, computes differential expression, target prediction, target annotation	http://www.wzgenomics.cn/mr2_dev/	Wu et al. (2013)
4.	miRPlant	Plants	Novel and known miRNA identification, precursor prediction	http://www.australianprostatecentre.org/research/software/mirplant	An et al. (2014)
5.	miR-PREfer	Plants	Novel and known miRNA identification	https://github.com/hangelwen/miR-PREfer	Lei and Sun (2014)

Table 2 (continued)

S. no.	Database/tool	Organism	Available data	Weblink	References
6.	mTide	Plants	Novel and known miRNA identification, computes differential expression, target prediction, target annotation	http://bis.zju.edu.cn/MTide/	Zhang et al. (2015b)
7.	BioVLAB-MMIA-NGS	Plants, animals	Novel and known miRNA identification, computes differential expression, target prediction, target annotation	http://epigenomics.snu.ac.kr/biovlab_mmia_ngs/	Chae et al. (2015)
8.	miRge	Plants, animals	Known miRNA and isomiRs identification	http://atlas.pathology.jhu.edu/baras/miRge.html	Baras et al. (2015)
9.	miRA	Plants	Novel miRNA identification	https://github.com/mhuttner/miRA	Evers et al. (2015)
10.	TAPIR	Plants	Prediction of plant miRNA targets, including target mimics	http://bioinformatics.psb.ugent.be/webtools/tapir/	Bonnet et al. (2010)
11.	psRNATarget	Plants	miRNA target prediction	http://plantgrm.noble.org/psRNATarget/	Dai and Zhao (2011)
12.	sPARTA	Plants	miRNA target prediction	https://mpss.danforthcenter.org/tools/mirna_apps/download.php	Kakrana et al. (2014)
13.	MFSN	Plants	miRNA function prediction	–	Meng et al. (2016)
14.	P-SAMS	Plants	Designs artificial miRNAs and synthetic trans-acting small interfering RNAs	http://p-sams.carringtonlab.org/	Fahlgren et al. (2016)
15.	miRCat2	Plants, animals	miRNA prediction from NGS data	http://srna-workbench.cmp.uea.ac.uk/	Paicu et al. (2017)

Fig. 3 Total available miRNAs in model plant *Arabidopsis* and in major crops in two important miRNA databases (miRBase and PNRD)

2014). A plant-specific miRNA database was also built which is known as Plant miRNA database (PMRD). PMRD is a holistic database which utilizes GBrowse to display data including plant–miRNA sequences, their secondary structure, their target genes, and their expression profiles across vegetative and reproductive stages (Zhang et al. 2010). About 8400 miRNA entries from more than 120 plants, which also includes major crops, i.e., barley, maize, rice, soybean, sorghum, and wheat are available at PMRD. Recently, a latest version of PMRD has been released as Plant Non-coding RNA Database (PNRD) with additional miRNA data. The PNRD also contain broad spectrum RNA types including lncRNAs, rRNA, snRNA, snoRNA, tRNA, and tasiRNA (Yi et al. 2015). A total of 28,212 entries of non-coding RNA (ncRNA) from around 166 plant species, including important food crops. This version also offers various search options and analysis tools. The utility of such holistic database lies in its diversification and it is of immense help for crop plants where the miRNome knowledge is fragmented.

Beside the above databases, there are some specific databases, such as DIANA-TarBase (Vlachos et al. 2015) and miRTarBase (Chou et al. 2018) that provides information about experimentally validated miRNA–target interactions (MTIs). Typically, these MTIs were verified by

following experimental techniques that involve Quantitative RT-PCR, luciferase reporter assay and Western blot, microarray and NGS (CLIP-seq, CLASH and PAR-CLIP) (Chou et al. 2016). Similarly, mirEX 2.0 (Zielezinski et al. 2015) and PmiRExAt (Gurjar et al. 2016) databases integrate data of miRNAs expression (based on qRT-PCR and NGS experiments) in multiple tissues and developmental stages in plants including important crops (wheat, rice and maize).

In addition to above databases, more than 160 bioinformatics tools and online resources that were involved in miRNAs identification, prediction of their precursors and targets, the elucidation of miRNAs functions, and gathering various relevant biological information were available (Akhtar et al. 2016). Of the above tools, some important ones that were specific to plant are listed in Table 2. Further, due to the availability of a large amount of literature or existing online resources, it is very difficult to choose an appropriate bioinformatic tool for miRNA-related research. To overcome this problem, recently a user-friendly and manually curated open-access platform known as ‘tools4miRs’ was developed that gathers all the available bioinformatic tools (over 160) dedicated to the broad miRNA analysis (Lukasik et al. 2016). The collected tools in tools4miR were classified into several categories in which the users can utilize the available

methods according to their specific miRNA research needs (Lukasik et al. 2016).

Despite the availability of numerous databases and computational tools that were involved in miRNA identification, annotation, expression data and target prediction, very few abiotic stress-related miRNAs databases exist. Only two such databases including PASmiR (Zhang et al. 2013a) and WMP (Remita et al. 2015) are available, that has data on plant abiotic stress-related miRNAs. The PASmiR database is a comprehensive repository, that was developed to provide detailed, searchable descriptions of role of miRNA in the molecular regulation of different abiotic stresses in plants. This database includes more than 1000 regulatory relationships between 682 miRNAs and 35 abiotic stresses in 33 plant species including important crop plants (Zhang et al. 2013a). Similarly, WMP (Wheat MiRNA web-Portal) database was developed, this database is exclusively for wheat miRNAs, with emphasis on stress-responsive miRNAs from different genotypes and tissues. This database stores and shows differential gene expression data compiled from numerous small RNA libraries (Remita et al. 2015).

miRNA-based markers and their utilization in plant breeding

Evolutionarily, most of the families of miRNA were highly conserved in both animals and plants (Voinnet 2009). Hence, one could explore the possible use of miRNA-based functional markers to study genetic diversity in crop plants which could be ultimately exploited for molecular breeding. Such miRNA-based markers have been largely developed in animals, but in case of plants there are only few miRNA-based SSR and SNP markers linked to different traits that are reported in *Brassica* species (Fu et al. 2013; Singh et al. 2017), rice (Mondal and Ganie 2014), foxtail millet (Yadav et al. 2014), and *Medicago truncatula* (Min et al. 2017). Further, for abiotic stress, miRNA-SSR/SNPs were identified only in case of *Brassica* (for heat stress; Singh et al. 2017) and rice (for salt stress; Mondal and Ganie 2014). In *Brassica*, six miRNA-SSR and 34 miRNA-SNP markers were identified. Markedly, one of the SNPs (C/T) was found on mature region of miR2926. This C/T transition led to unstable hairpin structure of miR2926 leading to complete loss-of-target function, i.e., heat stress response (Singh et al. 2017). In rice, Mondal and Ganie (2014) identified 12 polymorphic miRNA-SSRs. Out of which only one (miR172b-SSR) could distinguish between salt-tolerant and susceptible genotypes and thus could be used as a biomarker for salinity stress (Mondal and Ganie 2014). Therefore, these miRNA-SSR/SNP markers could be utilized further in marker-assisted breeding program in plants.

Utilization of miRNA knowledge in crops' thermotolerance

These information on different miRNA targets regulatory networks involved in regulation of HS responses in plants pave a path for the development of several miRNA-derived approaches for crop improvement for HS tolerance. The miRNA-based genetic manipulations were most promising because miRNA regulates gene expression at transcriptional or post-transcriptional levels. We can employ several methods that manipulates miRNAs including overexpressed/repressed mutant lines of HS-responsive miRNAs, their target genes, miRNA-resistant target genes, use of target mimics and artificial miRNAs (amiRNAs), which may confer the HS tolerance in crops (Zhou and Luo 2013). Several examples in different crop plants were available which utilized miRNA-based manipulations for crop improvement for abiotic stresses. For example, the miR156, which regulates the expression of SPL TFs, has an important role in plant development and flowering time. The miR156-overexpressing rice mutant showed reduced cold tolerance (Cui et al. 2014). In rice, wheat and *Arabidopsis* miR159 negatively regulates the expression of GAMYB TF genes, that is involved in flower development, gibberellin (GA) signaling in cereal aleurone cells and abiotic stress tolerance (Wang et al. 2012). The transgenic rice lines overexpressing *TamiR159* were more sensitive to HS relative to the wild type, so this down-regulation of *TamiR159* after HS might participate in a heat stress tolerance in wheat (Wang et al. 2012). The miR172, which is involved in regulation of AP2-like TFs, was known to be involved in the regulation of flowering time as well as abiotic stress responses. Transgenic lines overexpressing gma-miR172 in *Arabidopsis* revealed the enhanced water deficit and salt tolerance (Li et al. 2016). Transgenic rice overexpressing miR319 showed enhanced cold tolerance. Similarly, transgenic tomato plants overexpressing sly-miR169c showed reduced stomatal opening, decreased transpiration rate, lower leaf water loss and enhanced drought tolerance in tomato (Zhang et al. 2011).

Further, where some miRNAs function as negative regulators of the abiotic stresses including HS, a overexpressing miRNA-resistant form of the target genes that could be developed that escapes cleavage by its miRNA might be very a useful strategy for developing crops that are HS-tolerant. Additionally, one could also have developed artificial miRNAs to specifically silence any given genes that were involved in thermotolerance in crop plants.

The natural genetic variations in miRNAs and their respective target genes are important sources that could be utilized for thermotolerance in crop plants. There are some examples that showed an association between genetic

variation in miRNA and their target genes, and following traits' grain length, seed size, plant height, inflorescence architecture formation and domestication, etc., but there are no such examples that deal with traits which are associated with HS. For example, in rice, a G/A polymorphism in the miRNA osa-miR2923a precursor was associated with grain length (Wang et al. 2013). Similarly, alternative polyadenylation miRNA osa-miR156h was associated with following traits, reduced plant height, increased tiller numbers and increased grain yield in rice (Zhao et al. 2015).

Conclusions and perspectives

Thermal stress greatly affects the growth, development, and final grain yield of important crop plants. Here we discussed the biological significance of some important miRNA–target regulatory pathways that were involved in thermotolerance in crop plants. The miRNA-based genetic manipulations of miRNAs and their target genes (i.e., overexpressed/repressed mutant lines miRNAs and their target genes), development of miRNA-resistant target genes and use of target mimics/artificial miRNAs (amiRNAs), which may confer the HS tolerance in crops could be used for development of thermotolerant crops. We can also utilize the available natural genetic variations in miRNAs for thermotolerance in crop plants. Recently, a breakthrough technology for genome editing known as CRISPR–Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) is developed for genome editing (Shan et al. 2014). This technology rapidly creates heritable and desirable changes in crops in an efficient and precise manner. These changes were indistinguishable from the natural variation/mutations as selected during conventional breeding methods. Although using CRISPR–Cas9 method, several single/multiple protein-coding gene knockouts have been reported in many plant species (Li et al. 2017; Scheben et al. 2017; Gao et al. 2017; Karkute et al. 2017), but literature related to use of CRISPR–Cas9 editing of miRNA genes, in plants were not available. Therefore, we can also utilize this genome editing method that targets plant miRNA loci and thus, expand the range of miRNA applications for crop breeding in thermotolerance. The availability and development of various bioinformatics databases and tools (discussed in “Role of bioinformatics in establishing miRNA and plant HS tolerance interactions” of this review) have revolutionized the study of miRNAs and made them pertinent for genetic engineering programs for enhancing thermal stress tolerance. Further, an in-depth understanding of the function of these miRNAs will broaden our understanding of plant biological processes during HS and allows us to select appropriate targets for improving crop plant thermotolerance.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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