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REVIEW

Plant small RNAs: advancement in the understanding of biogenesis and role in plant development

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Abstract

Main conclusion **Present review addresses the advances made in the understanding of biogenesis of plant small RNAs and their role in plant development. We discuss the elaborate role of microRNAs (miRNAs) and trans-acting small interfering RNAs (ta-siRNAs) in various aspects of plant growth and development and highlight relevance of small RNA mobility.**

Small non-coding RNAs regulate various aspects of plant development. Small RNAs (sRNAs) of 21–24 nucleotide length are derived from double-stranded RNAs through the combined activity of several biogenesis and processing components. These sRNAs function by negatively regulating the expression of target genes. miRNAs and ta-siRNAs constitute two important classes of endogenous small RNAs in plants, which play important roles in plant growth and developmental processes like embryogenesis, organ formation and patterning, shoot and root growth, and reproductive development. Biogenesis of miRNAs is a multistep process which includes transcription, processing and modification, and their loading onto RNA-induced silencing complex (RISC). RISC-loaded miRNAs carry out post-transcriptional silencing of their target(s). Recent studies identified orthologues of different biogenesis components of novel and conserved small RNAs from different model plants. Although many small RNAs have been identified from diverse plant species, only a handful of them have been functionally characterized. In this review, we discuss the advances made in understanding the biogenesis, functional conservation/divergence in miRNA-mediated gene regulation, and the developmental role of small RNAs in different plant species.

Keywords Small RNA · miRNA · ta-siRNA · Plant development · Root development · Shoot development

Introduction

Small RNAs (sRNAs) are non-coding RNA (ncRNA) fragments, which regulate the post-transcriptional silencing of target genes either through transcript cleavage or by translational inhibition (Axtell 2013). Various classes of sRNAs have been reported which differ from each other on the basis of biogenesis pathways (Chen 2009). Based on the precursor sequence, sRNAs can be classified as: miRNAs, ta-siRNAs and heterochromatin-associated (hc-siRNAs) (Axtell 2013). miRNAs and ta-siRNAs are two important classes of plant sRNAs, which control plant growth and development by negatively regulating the expression of their target genes, mostly through transcriptional cleavage (Chen 2012). On the other hand, hc-siRNAs are involved in carrying out the epigenetic modifications of chromatin in the target loci, thus leading to transcriptional gene silencing (Axtell 2013).

miRNAs are well-studied subset of hairpin RNAs defined by the highly precise excision of one or more functional products, which are called as mature miRNAs. miRNAs were first discovered as regulators of developmental timing in *Caenorhabditis elegans* (Lim et al. 2003). miRNAs are conserved over long evolutionary distances suggesting the

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role of an evolutionarily conserved mechanism of miRNA-mediated gene regulation (Molnar et al. 2007). Another class of endogenous sRNA is ta-siRNAs, which cleaves the targets that are non-identical to them and are therefore referred as trans-acting siRNAs. ta-siRNAs are 21-nucleotide phased sRNAs that are processed from *TAS* genes (Chen 2009).

With the advent of next-generation sequencing technology, a large number of conserved and novel miRNAs, and siRNAs have been identified in various plant species during the last decade (Sunkar et al. 2012; Sun 2012; Jover-Gil et al. 2005). However, only a limited number of developmental roles pertaining to these sRNAs have been characterized. There are reports on functional divergence of conserved miRNAs, which could be a result of critical sequence variation in the mature miRNA and/or its complementary target sequence occurring during the coevolution of miRNAs and their targets (Barik et al. 2014, 2015).

Biogenesis of sRNAs (miRNA and ta-siRNA) is a multistep process involving various components specific for each type. Biogenesis of miRNA differs from that of ta-siRNA due to the formation of the stem loop precursor. In contrast, ta-siRNA biogenesis itself involves miRNA-mediated cleavage of TAS locus (Allen et al. 2005). A wide number of studies have been carried out in *Arabidopsis thaliana* to address the role of sRNAs and various other biogenesis components in plant growth and development. However, with the advent of new technologies and availability of genome sequences, orthologues of sRNA biogenesis pathway components have also been identified in rice, maize, soybean, poplar, etc. (Nagasaki et al. 2007; Chitwood et al. 2009; Husbands et al. 2009). In this review, we summarize the biogenesis and developmental roles of sRNAs, mainly miRNA and ta-siRNA in plants, and the recent advancements made in this area.

miRNA biogenesis

The biogenesis of miRNA is a multistep process involving its transcription, processing and modification and loading onto RISC (Fig. 1a). Similar to protein-coding genes, *MIRNA* genes are also transcribed by RNA polymerase II, which generates primary transcripts of miRNAs (pri-miRNAs) containing both 5′ cap and 3′ poly A tail (Xie et al. 2005). The presence of TATA box in the promoter of miRNA genes suggests that transcriptional regulation of miRNAs is similar to that of protein-coding genes (Zhao et al. 2013; Barik et al.

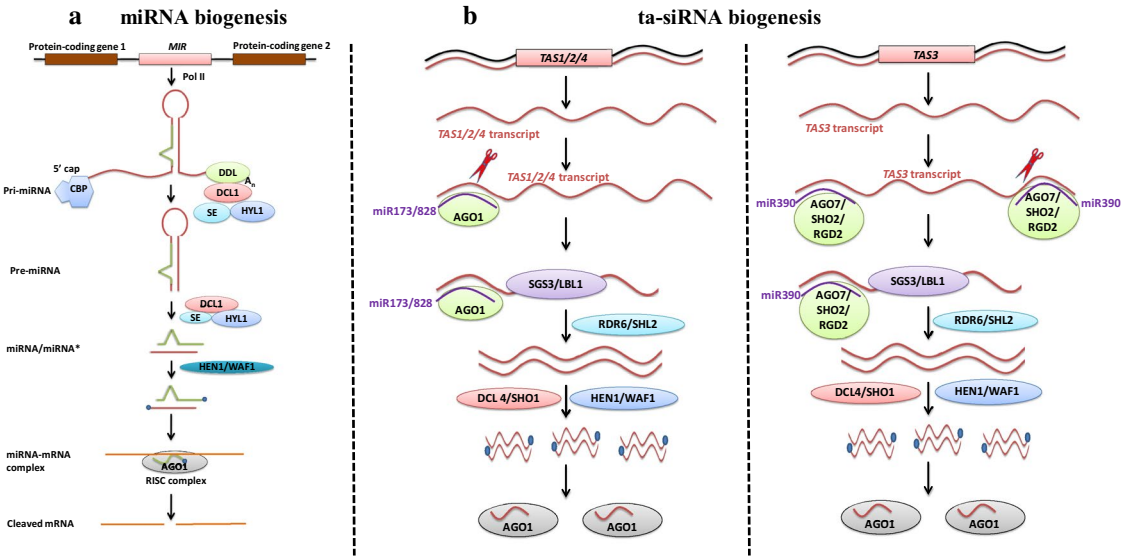

Fig. 1 Biogenesis of sRNAs. **a** Biogenesis of miRNA. RNA Pol II transcribes a *MIRNA* gene into a capped and polyadenylated pri-miRNA. pri-miRNA is further processed into a stem–loop (hairpin) precursor known as pre-miRNA by DCL1 protein in *Arabidopsis*. The pre-miRNA is later processed into a duplex of miRNA–miRNA* by DCL1. During miRNA biogenesis, DCL1 works along with HYL1, which is a double-stranded RNA binding protein. Another protein, HEN1 methylate the 2′ OH of the 3′ terminal nucleotides of miRNA–miRNA* duplex. One strand of the miRNA–miRNA* duplex is loaded into an AGO1 having miRISC. **b** Biogenesis of ta-siRNA. ta-siRNA biogenesis starts from *TAS1*, *TAS2*, *TAS3* and *TAS4* loci. At the *TAS1*, *TAS2*, *TAS4* loci, long noncoding transcripts are cleaved by miR173 or miR828 loaded AGO1. The 3′ cleaved products are bounded by *SGS3/LBL1*, which stabilizes ssRNA and prevents its degradation. ssRNA is copied into the dsRNA by *RDR6/SHL2*. The dsRNA is further processed into ~ 22 nt long siRNAs by the activity of *DCL4/SHO1*. At TAS3 locus, miR390 along with *AGO7/SHO2/RGD2* recognizes noncoding transcripts at 5′ and 3′ site. miR390/AGO7 complex cleaves the transcripts only at 3′ end. The 5′ cleaved products are channeled into ta-siRNA production by the activity of SGS3, RDR6, and DCL4, which targets *ARF2*, *ARF3*, and *ARF4* transcripts in *Arabidopsis* (Chen 2009, 2012; Nagasaki et al. 2007; Nogueira and Timmermans 2007)

2014). miRNA biogenesis involves processing of primary miRNA (pri-miRNA) to precursor miRNA (pre-miRNA) by DICER-LIKE1 (DCL1) protein. DCL1 along with HYPONASTY LEAVES1 (HYL1) and SERRATE (SE) further processes pre-miRNA to produce 21 nt miRNA/miRNA* duplex (Vazquez et al. 2004). In vitro assays showed that HYL1 and DCL1 are required for accurate excision of pri-miRNA during miRNA biogenesis (Dong et al. 2008). Mutation in any of these three genes results in drastic reduction in the level of mature miRNAs also due to an impaired processing the amount of pri-miRNA is increased (Yang et al. 2006; Han et al. 2004; Vazquez et al. 2004). Next step in miRNA/miRNA* processing is the methylation of a duplex on 2´OH of the 3´ terminal nucleotides by HUA ENHANCER1 (HEN1), a miRNA methyltransferase which acts in a sequence–independent and structure-dependent manner (Yu et al. 2005). The final step in miRNA biogenesis is the loading of one miRNA (21–24 nt) strand from the duplex on ARGONAUTE 1 (AGO1), forming miRNA–RISC. Since AGO1 has endonucleolytic activity, it cleaves mRNA–miRNA duplex nearly in the middle of the strand (Baumberger and Baulcombe 2005; Qi et al. 2005).

ta-siRNA biogenesis

In *Arabidopsis*, four *TAS* gene families namely *TAS1*, *TAS2*, *TAS3*, and *TAS4* have been identified which are present at eight genetic loci (Allen et al. 2005; Rajagopalan et al. 2006). ta-siRNA biogenesis is initiated by the cleavage of the *TAS* transcripts by miRNAs and further processing involves the action of various proteins specific to ta-siRNA biogenesis (Fig. 1b). Based on the number of miRNA binding target sites, *TAS* families are divided into two categories: one hit and two hit. The "one hit" targets which include *TAS1, TAS2* and *TAS4* have only one miRNA binding site in the primary-*TAS* transcript, whereas "two hit" target *TAS3* has two miRNA binding sites (Yoshikawa 2013). ta-siRNA is derived from 3´ and 5´ fragment after miRNA cleavage in "one hit" and "two hit" category, respectively. The polarity of fragments derived after miRNA cleavage, which generates ta-siRNA, also differs among these categories. *TAS1* and *TAS2* derived ta-siRNAs are generated by the activity of 22-nt miR173-AGO1 complex and they target members of penta-tricopeptide repeats (Montgomery et al. 2008b; Felippes and Weigel 2009), whereas in case of *TAS3,* the 21-nt miR390 uniquely associates with AGO7 leading to the processing of precursor *TAS3* and finally the formation of functional ta-siRNA. These ta-siRNAs target members of the *AUXIN RESPONSE FACTORs* (*ARF)* gene family (*ARF2*, *ARF3,* and *ARF4)*, and therefore are known as tasiR-ARFs (Fahlgren et al. 2006; Garcia et al. 2006; Marin et al. 2010). The biogenesis of *TAS4*-derived ta-siRNAs requires 22-nt miR828. *TAS4* targets MYB transcription factors *PRODUCTION OF ANTHOCYANIN PIGMENT 1* (*PAP1*), *PAP2* and *MYB113* which regulate anthocyanin biosynthesis (Luo et al. 2012). ta-siRNA biogenesis requires SUPPRESSOR OF GENE SILENCING3 (SGS3), RNA-DEPENDENT RNA POLYMERASE6 (RDR6), AGO1, DICER LIKE4 (DCL4), HYL1, and HEN1 (Allen et al. 2005; Peragine et al. 2004; Rajagopalan et al. 2006; Vazquez et al. 2004; Yoshikawa et al. 2005). SGS3 stabilizes the single-stranded cleaved RNA transcript, and RDR6 converts it to double-stranded RNA. This double-stranded RNA is converted into 21-nt ta-siRNA by DCL4 (Gasciolli et al. 2005; Xie et al. 2005; Yoshikawa et al. 2005). One of the two strands of the phased ta-siRNA is loaded on AGO1 effector complex (Baumberger and Baulcombe 2005). The role of different sRNA biogenesis components across the various plant species is summarized in Table 1.

Role of sRNAs in plant development

Initial genetic screening and loss of function or mis-expression analysis of various sRNA genes and their biogenesis components shed light into the developmental roles of several miRNAs and ta-siRNAs. A wide range of developmental processes are regulated by sRNAs starting from lower plants like moss to the higher plants like *Arabidopsis, Oryza sativa* and *Zea mays* (Nagasaki et al. 2007; Cho et al. 2012; Marin et al. 2010; Yoon et al. 2010; Talmor-Neiman et al. 2006; Juarez et al. 2004b). The roles of miRNA and ta-siRNA in various plant developmental processes are summarized in Table 1.

sRNAs in seed development and germination

Seed is an evolutionary adaptation of land plants which facilitates dispersal and allows germination when the environmental conditions turn favorable (Willmann et al. 2011; Das et al. 2015). Seeds contain miniature new plants as dormant embryos. Studies have shown that miRNA and ta-siRNA pathways regulate seed germination in *Arabidopsis* (Sarkar Das et al. 2018). Mutation in sRNA biogenesis pathway genes, such as *DCL1* leads to severe embryogenesis and seed development defects (Willmann et al. 2011; Das et al. 2015). Overexpression of miR160 is reported to cause hyposensitivity to abscisic acid (ABA) during the seed germination process (Liu et al. 2007). miR160 is known to target *ARF10/16/17* and mutation in *ARF10* leads to defect in seed development (Liu et al. 2007). Both miR156 and miR172 are the master regulators of phase transition and seed germination in plants (Li and Zhang 2015). It was reported that several miRNAs, such as miR165/166, miR160, miR159, miR395, miR417 and miR402 play important roles in seed development, maturation and seed germination processes

Table 1 Role of the small RNA biogenesis component in various species across the plant kingdom

ta-siRNA components	Mutant	Plant species	Role in plant development	References
SGS3	*lbl1*	*Zea mays*	Maize leaf polarity establishment	Dotto et al. (2014)
	Ppsgs3	*Physcomitrella patens*	Gametophyte development	Plavskin et al. (2016)
	sgs3	*Arabidopsis thaliana*	Vegetative phase change	Peragine et al. (2004)
RDR6	*Zmrdr6*	*Zea mays*	Maize leaf and shoot development	Petsch et al. (2015)
	shl2	*Oryza sativa*	SAM maintenance in rice	Nagasaki et al. (2007)
	rdr6	*Arabidopsis thaliana*	Gynoecium development, leaf development	Peragine et al. (2004)
AGO7	*ago7*	*Arabidopsis thaliana*	Defense response to virus, vegetative phase change	Montgomery et al. (2008a)
	rgd2	*Zea mays*	Dorsiventral patterning of maize leaves	Douglas et al. (2010)
	sho2	*Oryza sativa*	SAM maintenance in rice	Nagasaki et al. (2007)
DCL4	*dcl4*	*Arabidopsis thaliana*	Vegetative phase change	Gasciolli et al. (2005)
	sho1	*Oryza sativa*	SAM formation during embryogenesis in rice	Nagasaki et al. (2007)
	Zmdcl4	*Zea mays*	Maize leaf and shoot development	Petsch et al. (2015)
	Ppdcl4	*Physcomitrella patens*	Regulate sporophyte formation	Plavskin et al. (2016)
HEN1	*hen1*	*Arabidopsis thaliana*	Leaf proximal/distal pattern formation	Chen et al. (2002)
	waf1	*Oryza sativa*	Shoot development in rice	Abe et al. (2010)
AG01	*ago1*	*Arabidopsis thaliana*	Leaf proximal/distal pattern formation	Baumberger and Baulcombe (2005)
	ago1a-d	*Oryza sativa*	Pleiotropic developmental phenotype	Wu et al. (2009)

(Das et al. 2015). Overexpression of miR402 enhances seed germination in *Arabidopsis* under salt, osmotic and cold stress conditions (Kim et al. 2010a). Under abiotic stress conditions, miR395 acts as both positive as well as negative regulator of seed germination. miR417 is found to negatively regulate seed germination under salt stress condition in *Arabidopsis* (Jung and Kang 2007; Kim et al. 2010b). The short tandem target mimicry of miR165/166 (STTM165/166) plants is hypersensitive to ABA during seed germination and early seedling development (Yan et al. 2016). Auxin homeostasis is vital for embryo development and is mediated by the action of miR165/166, miR167, miR164, miR158 and miR160 (Martin et al. 2010). The role of different miRNAs in seed development is shown in Fig. 2.

sRNAs in root development

Several sRNAs are known to regulate root growth and patterning by targeting different transcription factors or genes involved in root development (Fig. 3a) (Gautam et al. 2017). For example, miR160 is essential for root growth, branching by negative regulation of its target genes *ARF10, ARF16* and *ARF17* (Wang et al. 2005; Mallory et al. 2005). miR164 regulates lateral root (LR) emergence and branching through the regulation of *NAM/ATAF/CUC1* (*NAC1*) transcription factor (Guo et al. 2005). *ARF6* and *ARF8* are positive regulators of adventitious root growth and both are under tight regulation by miR167 (Gutierrez et al. 2009). miR393 cleaves *TRANSPORT INHIBITOR RESPONSE1* (*TIR1*) and *AUXIN SIGNALING F-BOX2* (*AFB2*) subsequently regulating LR growth (Chen et al. 2012). miR165/166 and its target genes are involved in vasculature differentiation and root growth (Carlsbecker et al. 2010). A recent study shows that miR165/166 regulates root growth through phytohormonal crosstalk (Singh et al. 2017). Like morphogens in animals, some mobile sRNAs also form a gradient and define cell fate boundaries in plants (Benkovics and Timmermans 2014). In *Arabidopsis* root, SHORT-ROOT (SHR) protein moves from stele to endodermis and activates *SCARECROW* (*SCR*) expression. In situ hybridization and miRNA sensor experiments have shown that SCR and SHR transcriptionally activate the expression of *MIR165a* and *MIR166b* in the endodermis. Mature miR165/166 moves radially from endodermis in both inward and outward direction and degrades *Class III HOMEODOMAIN-LEUCINE ZIPPER* (*HD–ZIP III*) transcripts resulting in differential accumulation of target mRNA in the root vasculature (Fig. 3b) (Carlsbecker et al. 2010).

In *Arabidopsis,* miR396 has been found to regulate stem cell niche (SCN) by targeting *GROWTH RESPONSE FACTORS* (*GRFs*) and thus regulate cell division (Bazin et al. 2013; Rodriguez et al. 2015). Recently, it has been reported that miR171 cleaves *HAIRY MERISTEM* (*HAM*) (Llave et al. 2002; Engstrom et al. 2011). Ectopic expression of miR171 affects primary root (PR) length, a mutation in *HAMs* causes defective quiescent centre (QC) and stunted root growth (Wang et al. 2010; Zhou et al. 2015). In *Arabidopsis,* miR847 is important for LR development by regulating the expression of *INDOLE ACETIC ACID 28* (*IAA28*). Down-regulation of *IAA28* leads to increase LR number (Wang and

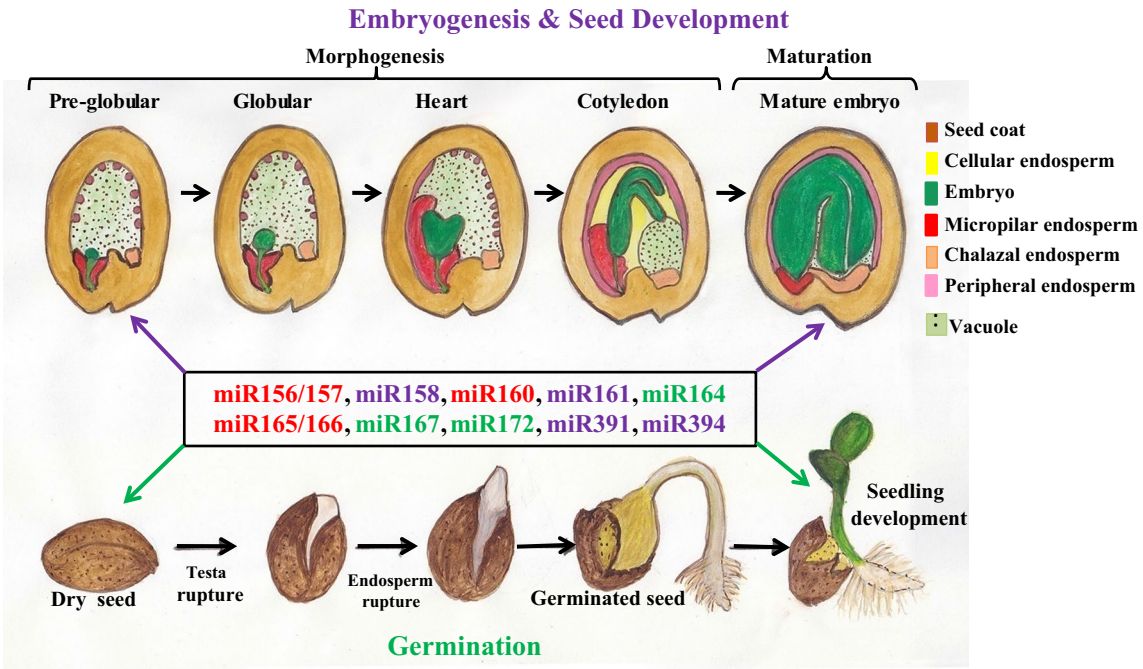

Fig. 2 miRNA-mediated regulation of embryogenesis and seed development, and seed germination. Several miRNAs have been implicated in the embryonic/seed development in plants. Some miRNAs regulate the embryonic development from pre-globular to mature embryonic stages by regulating the various stages of the development in *Arabidopsis.* In addition to the embryonic development, miRNAs also regulate the event of seed growth and germination by regulating the expression of various key target genes, which leads to the formation of the mature seedling. Arrows indicate the regulation. miRNAs involved in embryogenesis and seed development are mentioned in purple color, miRNAs involved in seed germination are mentioned in green color and common miRNAs are mentioned in red color

Guo 2015). miR408 and miR528 target *CUPREDOXIN* and subsequently regulate root cap formation, LR development and root elongation (Liu et al. 2012).

Components of the ta-siRNA pathway are also known to play an important role in *Arabidopsis* root development (Fig. 3a). Studies show that tasiR-ARF regulates LR growth and development by negatively regulating *ARF3* and *ARF4* (Marin et al. 2010; Yoon et al. 2010). Overexpression of *TAS3a* results in increased LR length, whereas mutation in *TAS3a* leads to reduced LR length (Marin et al. 2010). It was found that targets of the tasiR-ARF, *ARF3* and *ARF4* regulate the expression of auxin-induced miR390 by feedback mechanism (Yoon et al. 2010). Thus, LR growth is regulated by the quantitative action of miR390, ta-siRNA, auxin and *ARF*s (Marin et al. 2010). LR density has also been reported to be reduced in *rdr6-11* and *arf4-2* mutants (Yoon et al. 2010). Additionally, a negative feedback loop between tasiR-ARF and *ARF4,* mediates the spatiotemporal expression of *ARF4* (Yoon et al. 2010). miR390 senses the auxin maxima and *TAS3* derived ta-siRNA inhibits expression of *ARF4*, mediating the LR growth in *Arabidopsis* (Yoon et al. 2010; Marin et al. 2010). The miR390 expression is restricted to the lower parts and edges of the LR primordium except at the center during LR development (Marin et al. 2010). miR390 activity is detected in the whole primordium indicating that miR390 acts in a non-cell autonomous manner and moves to few neighboring cells in the root (Marin et al. 2010). A few miRNAs are hypothesized to regulate root development in monocot plants (Fig. 3a).

sRNAs in shoot apical meristem (SAM) maintenance

SAM is responsible for giving rise to the aerial organs of the plant. SAM contains pluripotent stem cells, which are maintained in the undifferentiated state by a negative feedback loop activity between WUSCHEL (WUS) and CLAVATA3 (CLV3) (Aichinger et al. 2012). SAM maintenance is controlled by the activity of several genes and sRNAs (Fig. 4). The effectiveness of *WUS*-dependent stem cell signaling can be increased by *ZWILLE/ARGONAUTE10* (*AGO10*), which competes with *AGO1* to bind miR165/166 and maintains the transcript level of *HD–ZIP III* genes (Aichinger et al. 2012; Zhu et al. 2011) (Fig. 4a).

miR394 regulates SAM maintenance in *Arabidopsis* by targeting and downregulating the expression of *LEAF CURLING RESPONSIVENESS* (*LCR*) gene which affects WUS–CLV3 pathway (Fig. 4a). *pMIR394B:YFP* indicates its expression in the epidermal L1 layer of the SAM; however, miR394 restricts the target *LCR* expression in the L3 layer (Knauer et al. 2013). As evident by in situ localization,

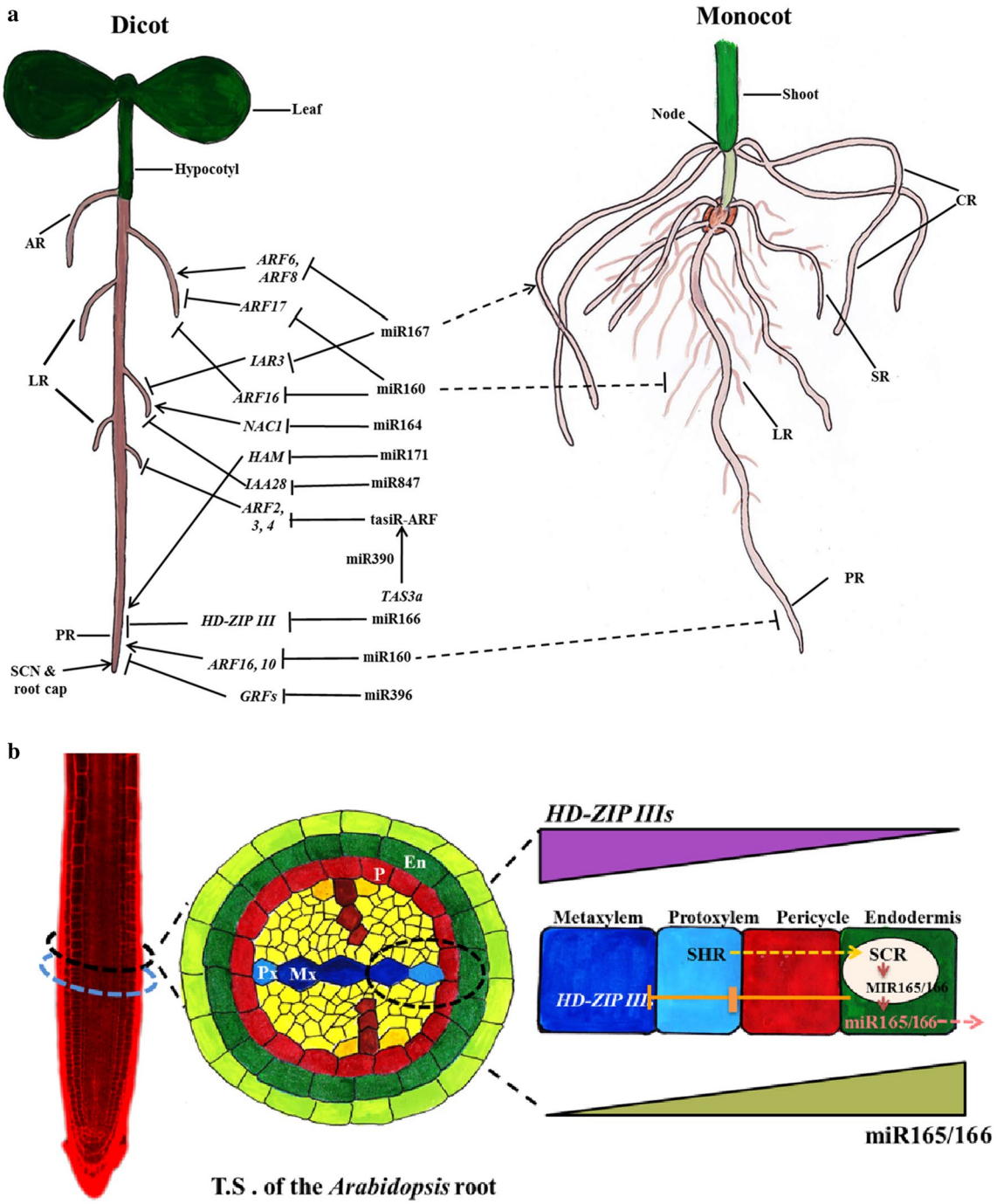

Fig. 3 Role of sRNAs in root development. **a** sRNA-mediated regulation of root development in dicots and monocots. Different root types were shown in dicots (approximately 7-day-old) as, adventitious root (AR), LR, and PR and in monocots (approximately 14-day-old) as crown root (CR), seminal root (SR), PR and LR. The model shows the role of important miRNAs in different root types in monocot and dicot. Dashed lines indicate the potential effect of sRNAs on monocot root development. **b** sRNA movement during xylem cell patterning. Diagrammatic representation of the miR165/166 movement which is a prerequisite for differentiation of xylem cell. The figure represents a transverse section of young *Arabidopsis* root. Cell layers shown in the figure are an outer blue color for endodermis, inner light green for pericycle, yellow are procambial cells, dark green cells are sieve elements, brown cells are companion cells, purple cells are metaxylem and orange is for protoxylem. A mechanistic model for root vascular cell patterning suggested in the figure shows that SHR moves from pericycle to endodermis and forms dimer with SCR. SCR-SHR dimer in endodermis stimulates miR165/166 and later moves towards the stele region and targets *HD–ZIP IIIs*. The differential gradient of miR165/166 and *HD–ZIP IIIs* inside the stele region regulates xylem cell differentiation

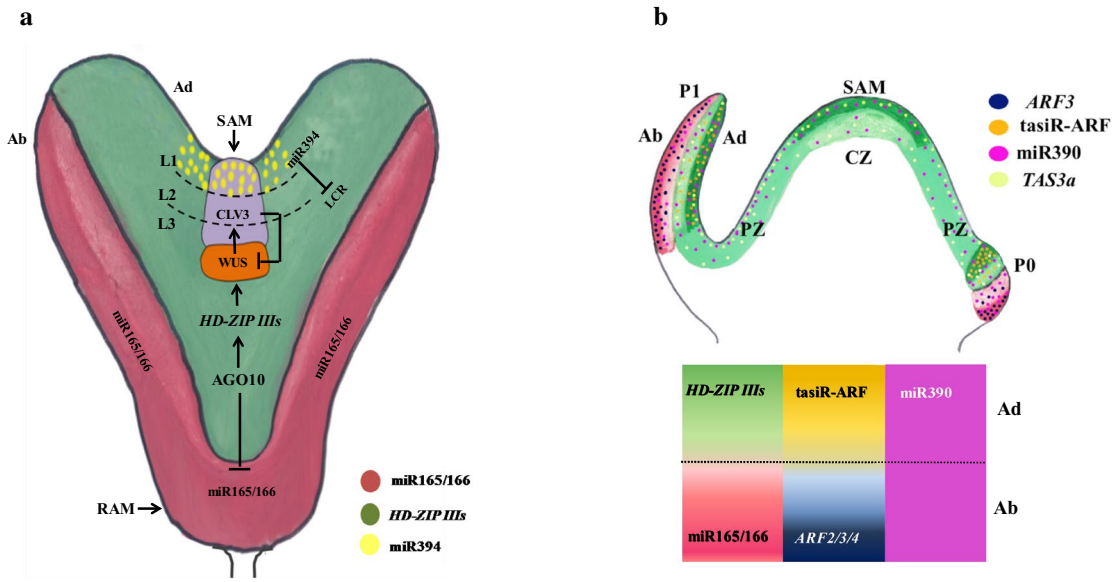

Fig. 4 sRNA-mediated regulation of SAM development in plants. **a** Mobile sRNAs regulates embryonic SAM development. SAM is divided into central zone (CZ) and peripheral zone (PZ), which represents central zone and peripheral zone, respectively. CZ of SAM is further divided into three layers L1–L3. miR394 expresses in the L1 layer of SAM and targets *LCR* gene in the L3 layer. Movement of miR394 in the L3 layer is important for proper SAM development and specification. LCR in L3 layer further regulates *WUS* and maintains SCN. Regulation of *WUS* and *CLV3* is also essential for proper specification of SAM. Upregulation of *HD–ZIP IIIs* due to AGO10 mediated decoying of miR165/166 leads to proper maintenance of SAM (Zhu et al. 2011; Knauer et al. 2013; Aichinger et al. 2012). **b** Mobile miR390 and miR166 regulates post-embryonic SAM development. The balanced activity of miR166 and target *HD–ZIP IIIs,* as well as tasiR-ARF and target *ARFs* is crucial for SAM maintenance in plants. Model here shows the accumulation of miR166 and *ARF3* in the abaxial domain of the leaf primordia (highlighted by red color and dark blue dot, respectively), the adaxial domain is marked by the expression of target *HD–ZIP IIIs* and tasiR-ARF (highlighted by green color and orange dot, respectively). There exists a gradient of *HD–ZIP IIIs* in developing SAM (highlighted by the diffused accumulation of *HD–ZIP III* in green color). miR390 and TAS3a accumulates in the adaxial and SAM region (highlighted by magenta and light green dots, respectively). P1 indicates the first primordia and P0 indicates the incipient leaf primordia. 'Ad' indicate the adaxial surface, 'Ab' indicate the abaxial surface

mature miR394 moves from L1 to L3 layer, where it restricts the expression domain of the target *LCR* and maintains shoot SCN (Knauer et al. 2013). ta-siRNA plays an important role in meristem organization in monocots. It has been shown that mutation in maize *LEAFBLADELESS1* (*LBL1*), a homolog of *SGS3* in *Arabidopsis*, leads to defective meristem (Nogueira et al. 2009). Further, mutation in rice *RDR6*/*SHOOTLESS2* (*SHL2*), *AGO7*/*SHOOT ORGANIZATION2* (*SHO2*) and *DCL4*/*SHO1* leads to lack of SAM (Nagasaki et al. 2007). In rice, defects in SAM formation observed in *shl* mutants were due to the loss of expression of *HD–ZIP IIIs* gene family members (Nagasaki et al. 2007).

sRNAs in leaf development

Leaf develops from a small group of undifferentiated cells and forms defined organ having medio-lateral, proximal–distal and abaxial–adaxial symmetry. Abaxial–adaxial surfaces of leaf are opposite faces of leaf, which are meant for different functions, e.g., adaxial surface is mainly involved in photosynthesis and abaxial is in gaseous exchange (Pulido and Laufs 2010). In *Arabidopsis,* abaxial–adaxial polarity of leaf is also maintained by the coordinated action of sRNAs such as miR165/166, through negative regulation of *HD–ZIP IIIs*. The expression of *HD–ZIP IIIs* in adaxial surface is maintained through negative regulation of miR165/166 which is expressed at abaxial surface of leaf (Pulido and Laufs 2010).

miR394-mediated downregulation of *LCR* is important for regulating leaf morphology and establishing the leaf polarity (Knauer et al. 2013). Like miRNAs, ta-siRNAs such as tasiR-ARF is also hypothesized to move from adaxial L1 layer inwards and regulate dorsiventral leaf polarity in maize and *Arabidopsis* by restricting the expression of target *ARF2/3/4* in the abaxial domain (Chitwood and Timmermans 2010). Interestingly, it is assumed that the expression of tasiR-ARF in the adaxial domain and miR165/166 in the abaxial domain form inwards gradients, which help to establish leaf polarity through downregulation of their respective targets (Chitwood and Timmermans 2010) (Fig. 4b). The formation of final leaf shape and size requires the activity of TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTORS (TCPs), which are targeted by miR319. *TCP* expression

is reduced upon miR319 overexpression resulting in increased leaf serration and altered leaf shape (Palatnik et al. 2003). It has been shown that miR396 regulates leaf shape by targeting *GROWTH-REGULATING FACTORS* (*GRFs*) (Rodriguez et al. 2010). miR396 expression is activated by TCPs suggesting that miR319 and miR396 regulate leaf shape development in a coordinated manner (Schommer et al. 2014). Another miRNA, miR164 regulates leaf serration by negatively targeting *CUP-SHAPED COTYLEDON2 (CUC2)* (Nikovics et al. 2006).

In maize, the roles of components of ta-siRNA pathway are implicated in establishing leaf polarity (Fig. 5). *TAS3*-derived ta-siRNAs require *LBL1/SGS3* for the biogenesis of ta-siRNA from the *TAS3* loci on the adaxial side of the incipient primordia, which guides the cleavage of the *ZmARF3* transcripts (Nogueira et al. 2007; Chitwood et al. 2009; Husbands et al. 2009; Nogueira et al. 2009). *lbl1* displays abaxialized leaf fate due to the complete loss of the adaxial cell identity (Kidner and Timmermans 2007, 2010; Chitwood et al. 2007, 2009; Nogueira et al. 2007; Timmermans et al. 1998). miR166 expressed at the abaxial side of the incipient primordia restricts the expression domain of *HD–ZIP III* genes on the adaxial surface (Husbands et al. 2009). The opposite activities of *TAS3*-derived ta-siRNAs and miR166 specify the polarity in developing maize leaves (Chitwood et al. 2007). *ROLLED1* (*RLD1*), one of the members of *HD–ZIP III* gene family in maize, is expressed on the adaxial side of the leaf. The expression of *RLD1* in adaxial domain is confined by the abaxial specific activity of miR166 (Juarez et al. 2004a). A semi-dominant *Rld1-Original* (*Rld1-O*) mutant results in increased accumulation of *HD–ZIP III* transcripts, leading to adaxialized leaf fate (Juarez et al. 2004a, b).

In *Lotus japonicus*, the role of ta-siRNA has been established in regulating leaf development. *L. japonicus* has a compound arrangement of the leaves, which is different from that of *Arabidopsis*, rice, and maize. In *L. japonicus*, compound leaves are arranged as five leaflets from top to bottom. Leaflets are divided into three categories such as top leaflet (TL), lateral leaflet (LL) and basal leaflet (BL). Two ta-siRNA biogenesis pathway genes have been characterized in lotus, *REDUCED LEAFLET1* (*REL1)* and *REDUCED LEAFLET3* (*REL3*) which are the orthologues of *Arabidopsis SGS3* and *AGO7*, respectively (Yan et al. 2010). *rel1* and *rel3* mutants show altered leaf polarity and reduced number of leaflet. BL is absent in both *rel1* and *rel3* mutants and the leaflets are elongated and pin-shaped (Yan et al. 2010). These mutants depicted the critical role of ta-siRNA pathway in leaflet development and formation.

In *Medicago truncatula*, the mutation in ta-siRNA biogenesis components, *SGS3* and *RDR6,* leads to severe developmental and physiological defects (Bustos-Sanmamed et al. 2014). *M. truncatula sgs3a* and *rdr6.2* mutants show downwardly curled leaves with increased serration, and even lobed margin (Bustos-Sanmamed et al. 2014). The *TAS3* derived ta-siRNA plays a significant role in maintenance of fruit quality and yield in *Vitis vinifera*. The ta-siRNAs derived from *vviTAS3* generally targets *ARF4/5* transcription factor, DIN1a protein, L10 (ribosomal protein), ribosomal protein S1, ferric reductase, RAD24 (a DNA damage checkpoint protein) and many other uncharacterized proteins whose functions have not been clearly identified (Zhang et al. 2012).

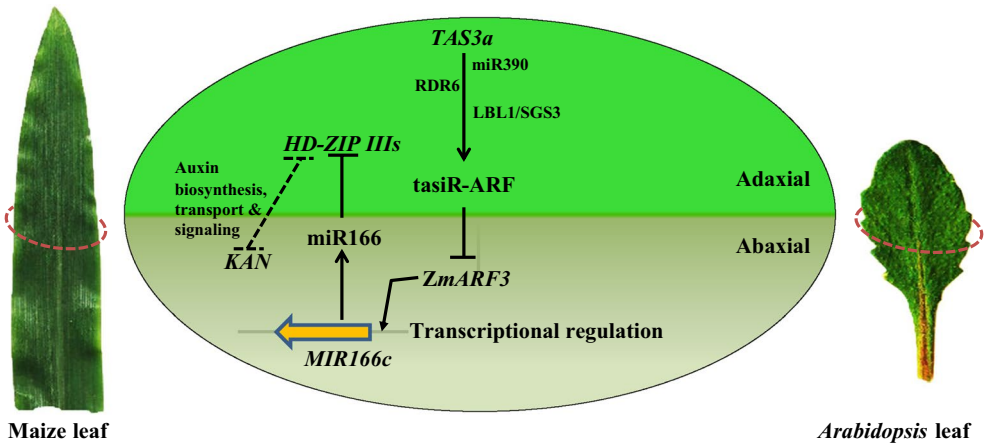

Fig. 5 Model illustrating the leaf polarity through ta-siRNA and miR166 in maize and *Arabidopsis*. Cross section of maize leaf is divided into adaxial and abaxial surfaces. On the adaxial surface, LBL1/SGS3 is required for biogenesis of tasiR-ARFs. tasiR-ARFs cleaves *ZmARF3* transcripts which are accumulated on the abaxial (ab) side. ZmARF3 directly regulates the expression of miR166c, which accumulates on the abaxial surface. miR166 targets members of the *HD–ZIP IIIs* and restricts their expression on the adaxial surface of maize leaf. The opposing activities of miR166 and *HD–ZIP IIIs* regulate leaf polarity in maize and *Arabidopsis* (Nogueira and Timmermans 2007; Nogueira et al. 2007)

sRNAs in flower development and phase transition

Life cycle of a plant involves two phase transitions; juvenile to adult phase transition and adult to reproductive phase transition. The gradient of two miRNAs, miR156 and miR172, is responsible for these phase transitions (Fig. 6a). Ectopic expression of miR156 causes altered vegetative phase transition and delayed flowering (Yu et al. 2010; Xing et al. 2013). Flower development is regulated by a network of genes and sRNAs. In both dicots and monocots, miR156, miR159, miR171, miR172 and miR396 are known to regulate floral identity and timing (Fig. 6b) (Smoczynska and Szweykowska-Kulinska 2016). miR156/157 negatively regulates *SQUAMOSA-PROMOTER BINDING PROTEIN LIKE* (*SPLs*) which subsequently regulate floral timing (Gandikota et al. 2007). *Arabidopsis* genome encodes 17 *SPL* genes of which 11 are post-transcriptionally targeted by miR156 (Rhoades et al. 2002). *SPL3*, *SPL4* and *SPL5* regulate vegetative phase to reproductive phase transition (Schwab et al. 2005; Wang et al. 2008; Wu and Poethig 2006). miR159 controls flowering time by regulating floral meristem identity gene *LEAFY* (*LFY*) by negative regulation of gibberellic acid (GA)-specific transcriptional regulator GAMYB-related proteins (MYB33, MYB65, and MYB101) (Blazquez et al. 1998). These proteins mediate the GA-induced regulation of *LFY*. Overexpression of miR159 causes reduced expression of *LFY* and delays flowering time (Achard et al. 2004). In *Arabidopsis,* miR171a is expressed in the inflorescence and regulates *SCARECROW LIKE* (SCL) SCL6-III and SCL6-IV (Nikovics et al. 2006). In maize, miR172 negatively regulates *AP2*-like gene *GLOSSY15* (*GL15*) in turn regulating juvenile to adult shoot transition (Lauter et al. 2005). miR172 stimulates flowering and is involved in the fate determination of floral meristem by downregulation of its target *AP2* and a small group of *AP2*-like genes; including *TARGET OF EAT1* (*TOE1*), *TOE2*, *TOE3*, *SCHLAFMUTZE* (*SMZ*), and *SCHNARCHZAPFEN* (*SNZ*) (Chen 2004; Aukerman and Sakai 2003).

Floral development is also regulated by miR167, which targets *ARF6* and *ARF8* (Nagpal et al. 2005). A resistant version of *ARF6* and *ARF8* causes sterility suggesting the important role of miR167 in floral development (Wu et al.

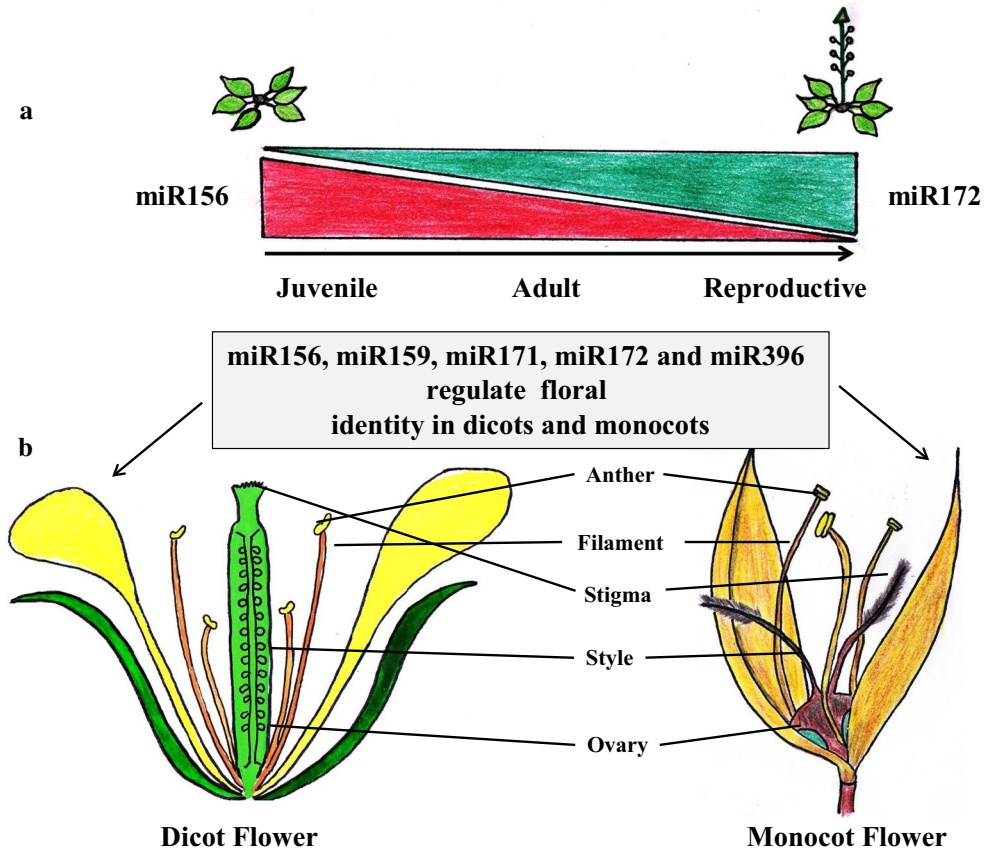

Fig. 6 miRNA-mediated regulation of phase transition and floral development in plants. **a** miR156 and miR172 are important for juvenile to adult phase transition by regulating the activity of their target genes. The cumulative action of the selected miRNAs and target genes regulate the floral development tin both dicot and monocot plants. Arrows indicate the regulation. **b** Regulation of floral development and identity: miR156, miR159, miR171, miR172 and miR396 regulate floral development in both monocot and dicot plants

2006). Many sRNA biogenesis pathway genes are known to be involved in pollen development and a number of miRNAs have been localized to viable pollen cells (Grant-Downton et al. 2013). miRNAs regulated male reproduction have been found to be overlapping among *Arabidopsis* and rice suggesting their conserved function during pollen development. miR156, miR160, miR167 and miR173 are found to be present in pollen tissue (He et al. 2015).

Conclusions and perspectives

Several characterized sRNAs play important roles in modulating the development of seed, root shoot, leaf, and floral organs in both monocot and dicot plants. Mutations in the sRNA biogenesis components lead to the pleiotropic developmental defects in plants, which underlines the functional importance of sRNAs (miRNAs and ta-siRNA) in shaping the various aspects of plant development. Components of sRNA biogenesis pathway and their function appear to be quite conserved among diverse groups of plants. Many sRNAs, such as miR165/166, miR167, miR156, etc. have been implicated in development of both shoot and root. Some miRNAs/ta-siRNAs are also having partially conserved role in shoot and leaf patterning between monocot and dicot plants. Although next-generation sequencing (NGS) approach has identified huge number of sRNAs, only a handful of them have been characterized for their function, even in popular model plants, like *Arabidopsis* and rice. Advanced technology, like laser capture microdissection (LCM) may be applied to identify miRNAs and targets that are enriched in specific developmental tissue of plants (Gautam et al. 2016; Gautam and Sarkar 2015). Often the multigenic origin of a sRNA species and multiple targets makes the functional study of sRNA a difficult one. More exhaustive effort is required to understand the function of many novel sRNAs. As discussed above, some level of functional diversification of miRNA or their targets is predicted to be there, due to their co-evolution. Functional characterization of these sRNAs and their targets will shed light on the evolutionary conservation/divergence of sRNA-mediated regulation of plant development among diverse plant species. Developmental regulation by crosstalk of sRNAs with hormonal signaling, epigenetic regulation is poorly understood and an interesting area to be explored. Besides, traditional loss/gain-of-functional approaches, genome editing of sRNA or target loci may help to foster their functional characterization for role in plant development and physiological responses.

Author contribution statement AS, VG, AKS, designed the outline of the article, composed the manuscript and figures. SS, SV, SD, SM, VM, and AKS provided scientific feedback and critical comments and revised the content. All the authors read and approved the manuscript.

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