

MOLECULAR MECHANISM OF MICRORNA-MEDIATED MORPHOGENESIS IN PLANT REPRODUCTIVE ORGANS: A REVIEW

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Abstract

MicroRNAs (miRNAs) are widely involved in plant morphogenesis and play an important role, especially in economic crop production. To elucidate the important role of miRNAs in the morphogenesis of plant reproductive organs, the current study evaluated the main miRNA types and their functions that regulate the morphogenesis of plant leaves, flowers, fruit, and seeds. This study also introduced the typical cases of molecular reprogramming of miRNAs through the long-distance transfer of grafting position to regulate the economic traits of grafting hybrids, providing a scientific basis for the formation mechanism and molecular design breeding of important traits, for example, the economic yield of crops regulated by miRNA epigenetic modification.

Key words: MiRNAs, Reproductive organ development of plant, Epigenetic inheritance and modify.

Introduction

MicroRNAs (non-coding small RNA) are a kind of negative regulator of transcripts and have a length of 18–24 nucleotides. It regulates gene expression after transcription by epigenetic gene silencing, which affect morpho-physiological traits. In recent studies, microRNAs (miRNAs) have been identified in *Glycine max* (Wang *et al.*, 2019), *Cryptomeria fortune* (Zhang *et al.*, 2021), *Cicer arietinum* (Garg *et al.*, 2019), *Prunus sibirica* (Niu *et al.*, 2016), *Solanum lycopersicum* (Lin *et al.*, 2021), *Zea mays* (Aydinoglu & Lucas, 2019), *Elettaria cardamomum* (Nadiya *et al.*, 2017), *Nicotiana tabacum* (Han *et al.*, 2022), *Arabidopsis thaliana* (Zheng *et al.*, 2019a), and *Oryza sativa* (Qiao *et al.*, 2021), showing biological functions in morphogenesis, signal transduction, hormone secretion, growth, development, and stress response (Mallory & Vaucheret, 2006; Damodharan *et al.*, 2018; Tang *et al.*, 2020; Fang & Wang, 2021). Furthermore, these previous studies on miRNAs have focused on transcriptome analysis, target genes, and functions prediction, while there are few reports on the regulation of downstream target genes by miRNAs and the interaction of miRNAs in plant growth processes. Current studies have shown that the main function of miRNAs is to regulate gene expression and play a regulatory role in plant organs, such as the leaves, flowers, fruit, and seeds.

Effects of miRNAs on leaf growth and development:

The development of leaves includes the processes of protoplast differentiation from meristem tissue at the top of the stem, leaf morphology (such as polarity, size, and shape) development, stomata, and leaf senescence. Therefore, in this review, we examined the regulatory of miRNAs in plant leaf growth (Fig. 1).

MiRNAs control leaf morphological development:

MiR165/166 on the back of leaves inhibits the members of the HD-ZIP III family, which results in *HD-ZIP III* gene expression only on the front, leading to polarity in leaf

development (Dong *et al.*, 2022a). MiR319 has been demonstrated to promote leaf cell proliferation by targeting TCP transcription factors and participating in leaf polarity formation (Dong *et al.*, 2022a). Pn-miRNA319a is related to leaf margin development morphology, and the downregulation of target gene *PnTCP2* leads to lobed leaves, suggesting that pnc-miRNA319a and *PnLBD41* play a vital role in the formation of lobed leaves in *P. neurantha* var. *lobophylla* (Sun *et al.*, 2022).

MiR393 mediates the expression of *TIR1* and *AFB1/2/3*, which regulates the leaf shape and size (Chen *et al.*, 2011). Similarly, miR156 targets *SPL10* and binds to *AGAMOUS-LIKE79*, which also affects the shape and size of the blade (Gao *et al.*, 2018). At the same time, miR319a/b, miR393, miR394, and miR166 regulate leaf shape and size. For example, miR319a/b mediates *PCF5/8* to regulate leaf shape and size in rice (Yang *et al.*, 2013); *OSmiR166* regulates *OsHB4* and promotes the development of epidermal vesicular cells and thick-walled cells, thereby affecting the shape and size of leaves (Zhang *et al.*, 2018a). MiR396 can regulate blade size by targeting the *basic Helix-Loop-Helix 74* and *GRF2* (Debernardi *et al.*, 2012). Because *CUC2* is necessary to form the original boundary of the blade, it is also regulated by *miR319* and *miR164* (Dong *et al.*, 2022a).

In *Arabidopsis*, miR319 and *TCP4* genes participate in leaf development, which affects the formation of the cotyledon boundary and leaf sawtooth (Koyama *et al.*, 2017; Li *et al.*, 2022a). In addition, in strawberries, miR164A regulates *CUC2* to wrinkle the leaves and deepen serrations, resulting in changes in leaf size (Zheng *et al.*, 2019b).

MiRNAs mediate leaf stomatal development:

MiRNAs are a key factor regulating stomatal development and play a key role in stomatal development. In *Arabidopsis thaliana*, miR399 regulates *PHO2* and participates in phosphate homeostasis, and miR399 promotes stomatal development (Zhu *et al.*, 2020). In poplar, PumiR172d targets *GT-2-like1* regulatory factors and binds to *STOMATAL DENSITY AND DISTRIBUTION1* to regulate stomatal development, resulting

in PumiR172d overexpression, which significantly reduces stomatal density (Liu *et al.*, 2021). MiR824 and its target *AGL16* are involved in the stomatal development of *Arabidopsis thaliana* leaves. MiR824 overexpression results in decreased stomatal density. However, when miR824 inhibits the regulation of *AGL16*, the stomatal density increases (Kutter *et al.*, 2007). Stu-miR827 negatively regulates the expression of the *StWRKY48* transcription factor, resulting in increased leaf stomatal density, which enhances the drought adaptability of potatoes (Yang *et al.*, 2022).

MiRNAs regulate the aging process of leaves: In tomatoes, SlmiR208 targets *SIIPT2*, and *SIIPT4* negatively regulates cytokinin biosynthesis, resulting in a decrease in the cytokinin concentration. This is demonstrated by the fact that SlmiR208 overexpression causes premature leaf senescence, consistent with the phenotype of the *SIIPT4* silent strain (Zhang *et al.*, 2020b). In addition, *LfNAC1* expression, which regulates the leaf senescence gene, was detected by Lfo-miR164b in the transient expression study of formosan sweet gum leaf tissue. Therefore, miR164b plays a regulatory role in the leaf senescence process (Wen *et al.*, 2020). In *Arabidopsis thaliana*, miR840 targets the downstream genes *WHY3* and *PPR* of the leaf senescence factor, and the phenotypes of the *PPR* and *WHY3* double mutant are consistent with the leaf senescence phenotypes of miR840-overexpressing transgenic lines (Ren *et al.*, 2022). Regulation of the *GPLa* transcription factor by miR827 affects the accumulation of or decrease in phosphate in leaves, and the overexpression or inhibition of miR827 leads to the acceleration or delay of the aging process, respectively (Ma *et al.*, 2021).

Function of miRNAs in regulating floral organ morphogenesis: The expression of target genes is regulated by miRNAs in the form of epigenetic modification after transcription, which affects the reprogramming of whole floral organ development, such as stamen development, petal formation, and ovule development (Damodharan *et al.*, 2018; Tang *et al.*, 2020). As a result, the flowering time, crop yield, and other economic traits are profoundly affected by miRNAs (Fig. 2).

MiRNA-mediated development of plant stamens: During plant flowering, anther and pollen development are important processes. MiRNAs function in anther and pollen development regulation in different species. In *Arabidopsis thaliana*, miR159 regulates *GAMYB* to induce the expression of MYB domain transcription factors R2 and R3 of gibberellins in the seed paste layer and the linseed felt layer, leading to anther and pollen development defects (Millar *et al.*, 2019). In rice, Zma-miR164h-5p regulates the expression of male sterility involved in lipid metabolism during anther development in *ZmABCG26* and *ZmFAR1*. The mutation of these two genes leads to a decrease in anther cuticle content and increase in wax content, which results in male fertile and promotes anther lipid synthesis (Jiang *et al.*, 2021). MiR164 similarly regulates the expression of *OsCUC1* and *OsCUC3* and induces a reduction in stamen number (Wang *et al.*, 2021a). The pollen wall is an important factor in pollen development and flowering. MiR528 regulates the formation of the inner membrane of pollen by regulating the *OsUCL23* gene. *OsUCL23* interacts with proton-

dependent oligopeptide transporters (POT) in the pre-vacuolar compartment and multivesicular bodies to regulate the formation of the pollen inner wall (Zhang *et al.*, 2020c). The absence of miR2118 in rice mutants leads to male and female infertility, which is related to the developmental defects of the anther wall caused by the high expression of miR2118 in the anther wall due to *OsAGO1b/OsAGO1d* regulation (Araki *et al.*, 2020).

Similarly, overexpressing Phe-miR159 results in low expression levels of the target genes *PheMYB98* and *PheMYB42*, eventually leading to the failure of anther dehiscence (Cheng *et al.*, 2020). The overexpression of miR319a regulates *TCP24* gene expression, which destroys the thickening of the in-wall secondary cell wall of the anther, thereby preventing anther cracking and pollen release and leading to male sterility (Wang *et al.*, 2015). MiR167 negatively regulates *ARF6/8* in *Arabidopsis thaliana*, and a mutated miR167 target site leads to anther cleavage in *mARF6* and *mARF8* transgenic lines, indicating that miR167 limits anther growth (Zheng *et al.*, 2019c). In citrus, miR399 negatively regulates its target gene *PHOSPHATE2*, which physically interacts with the floral organ development factor SEP and anther lyses factor CBF, regulates phosphate homeostasis in plants, interferes with the stomatal function of the anther surface, and inhibits anther lyses, leading to abnormal floral organ development and pollen grain collapse (Wang *et al.*, 2020a). Similarly, miR156, miR159, miR160, miR164, miR172, and miR319 determine anther development in maize (Li *et al.*, 2019b).

MiRNAs affect plant petal development: *SEPALLATA* (*SEP*), *CUP-SHAPED COTYLEDON* (*CUC*), and *PETALA* comprehensively regulate plant petal development; these factors are regulated by miRNAs. MiR319a regulates *TCP4* and binds with *SEP3* to control the size of arabidopsis petals (Chen *et al.*, 2018). In strawberries, FvemiR164a regulates the downstream expression of *FveCUC2a*, leading to leaf smoothness and Cardium deformity, overexpressing FvemiR164a restores the sawtooth shape of leaves and petals; thus, FvemiR164a affects the shape of petals (Zheng *et al.*, 2019b). Some miRNAs, such as miR167a-*ARF6/8* (Liu *et al.*, 2014), miR160-*ARF10/16/17* (Damodharan *et al.*, 2016), and miR172-*AP2*, regulate petals in tomatoes (Lin *et al.*, 2021).

MiRNAs affect plant ovule development: MiRNAs also have functions in the plant ovule-forming process. MiR156 regulates *AGL15*-induced somatic embryogenesis; in addition, miR156 regulates somatic embryogenesis in coordination with Histone Deacetylase6 and Histone Deacetylase19 histone deacetylase (Nowak *et al.*, 2020). In cotton, *AtAGL6* and *SITDR8* genes are highly homologous to the *MADS*-box, and miR157 overexpression inhibits *AtAGL6* and *SITDR8*, resulting in floral organ size reduction, ovule reduction, and seed yield decline, suggesting that miR157/*SPL* induces the formation and growth of the floral organ and ovule through regulation of the *MADS*-box gene and auxin signal pathway (Liu *et al.*, 2017). MiR166 enables the development and formation of microspore by ensuring the expression of *SPL/NZZ* and inhibiting the key gene *PHB* in ovule formation in the inner layer of *Arabidopsis thaliana* (Li *et al.*, 2019a).

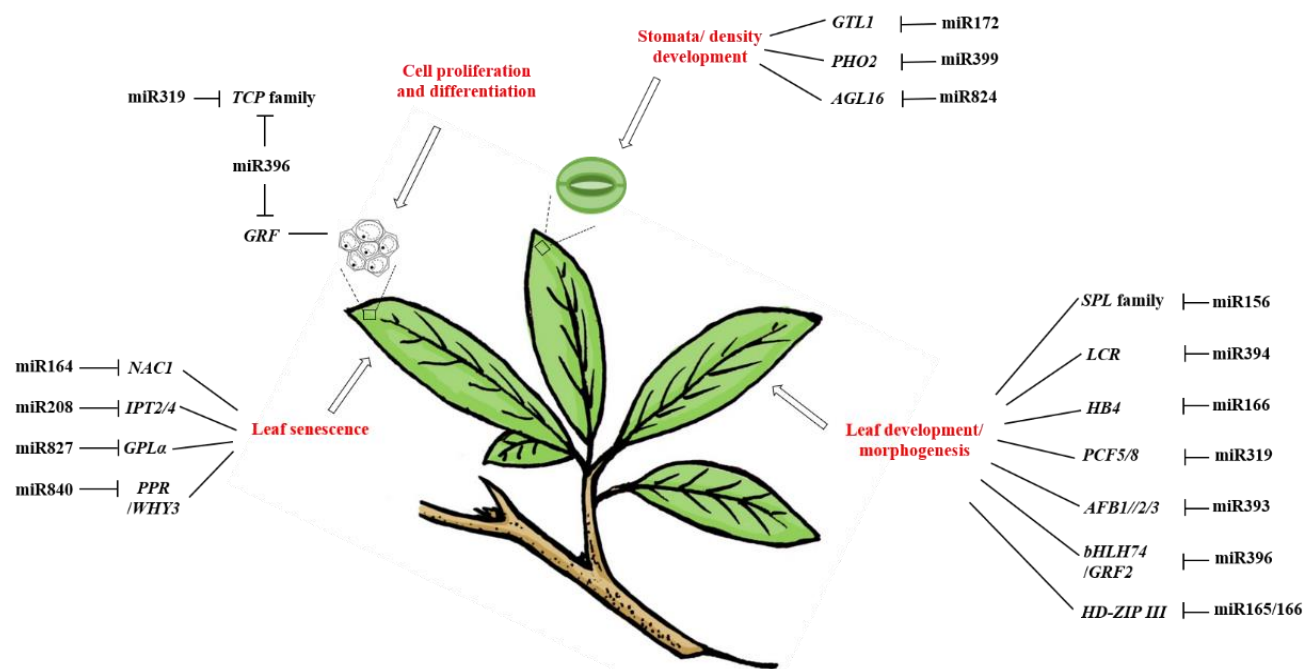


Fig. 1. Regulated model of miRNAs in leaf growth and development of plants. All of the miRNAs with verified functions in cell proliferation and differentiation, leaf senescence, stomata/density development, and leaf development/morphogenesis as well as their respective main targets are represented. Symble "⊣" is suppression.

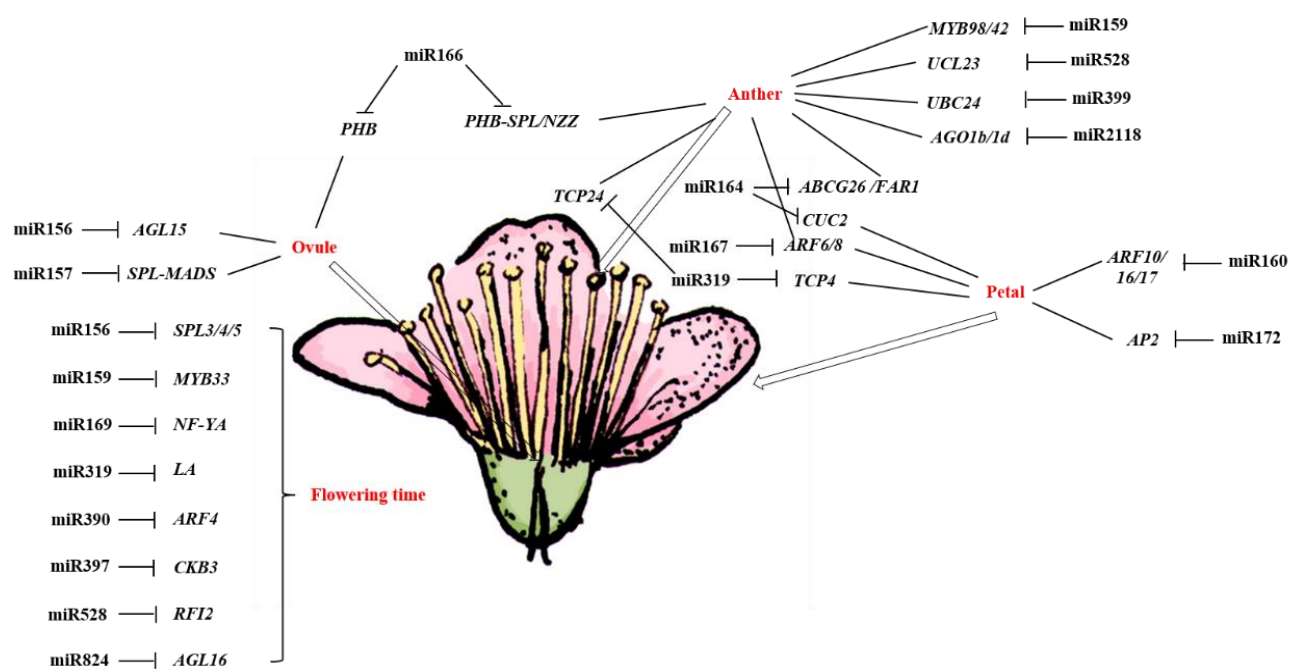


Fig. 2. Regulated model of miRNAs in flower organization morphogenesis of plants. All of the miRNAs with verified functions in ovule, anther, petal, and flowering time as well as their respective main targets are represented. Symble "⊣" is suppression.

MiRNAs affect the flowering time of plants: Flowering is an important symbol of a plant's transition from infancy to maturity. MiRNAs are widely involved in regulating flowering. MiR156 and miR172 are the main regulatory members of flowering, and they antagonize each other (Ahmad *et al.*, 2023). For instance, miR156 overexpression in switch grass, arabidopsis, and tobacco can delay flowering and prolong the seedling stage (Zhang *et al.*, 2015; Johnson *et al.*, 2017; Xie *et al.*, 2020), whereas miR172

overexpression promotes flowering in arabidopsis (Aukerman & Sakai, 2003). The expression of miR156 is highest in seedlings and decreases before floral induction, while the expression of miR172 is lower in the early flowering stage and then increases in the transition stage to flowering (Ahsan *et al.*, 2019b). In *Arabidopsis thaliana*, *PhyA* can directly interact with *SPL3/4/5*, the three important members of miR156, to repress the transcription of *FUL*, *LFY*, *API*, and miR172C, the key flowering regulatory

genes of *SPL3/4/5* (Xie *et al.*, 2020). MiR172 regulates the downstream gene of miR156, and miR156 regulates the expression of miR172 indirectly (Teotia & Tang, 2015).

MiR169 regulates the *NF-YA* family, which can regulate the flowering stage under biotic stress with multiple genes. MiR169d overexpression results in early flowering, whereas *rNF-YA2* overexpression delays the flowering time, indicating that miR169 induces flowering in plants by inhibiting *AtNF-YA* expression during stress (Xu *et al.*, 2014). In the gibberellin (GA) signaling pathway, miR159 encodes the MYB transcription factor to promote short-day flowering. MiR159 overexpression in arabidopsis, rice, and peach delays flowering (Waheed & Zeng, 2020). MiR397 regulates LAC transcription factor, which affects seed and fruit development in plants, such as pear, rice and corn. In the flowering of *Arabidopsis thaliana*, miR397b regulates *CKB3*, and *CKB3* bind to the *CIRCADIAN CLOCK ASSOCIATED 1* promoter to delay flowering in arabidopsis (Huang *et al.*, 2021). *AGL16* performs a function under the regulation of FLOWERING LOCUS T (FT), and *AGL16* interacts with *SHORT VEGETATIVE PHASE* and *FLC*. Meanwhile, the FLC protein inhibits the expression of FT, which causes miR824 to negatively regulate *AGL16* and delay the flowering time in arabidopsis (Hu *et al.*, 2014). In tomatoes, miR319 regulates *LANCEOLATE* by increasing the GA concentration and inactivates *SINGLE FLOWER TRUSS* and *API/MACROCALYX*, thus inhibiting tomato flowering (Silva *et al.*, 2019). FvemiR390 indirectly regulates the *FveARF4* gene in coordination with *FveAPI/FveFUL* to induce the flowering time of strawberry, and FvemiR390a overexpression delays the flowering time by 17 days (Dong *et al.*, 2022b). In rice, miR528 regulates *RED AND FAR-RED INSENSITIVE 2* to promote flowering under long days (Yang *et al.*, 2019).

Role of miRNAs in controlling fruit morphogenesis:

MiRNAs play an important role in fruit development, fruit size, fruit ripening, fruit coloring, and fruit quality (Hussain *et al.*, 2020), as well as important agronomic traits (Fig. 3).

MiRNAs regulate the fruit development process:

Seedless grape development is induced by GA, and during this process, VvmiR31-3p and VvmiR8-5p negatively regulate *VvCCoAOMT* and *VvDCAF1*, which inhibits nuclear sclerosis and embryo development; thus, seedless grapes are produced (Wang *et al.*, 2021b). In tomatoes, SlmiR159 targets *SIGAMYB1/2* and promotes fruit formation by regulating AUX and GA concentrations. SlmiR159 overexpression results in the abnormal development of plant ovules, which is similar to the phenotype of the monatomic fruiting mutant, and its downregulation results in the formation of seedless fruit due to *SIGAMYB1* in the ovary (Da Silve *et al.*, 2017). MiR393 regulates *CsTIR1* and *CsAFB2* auxin-signaling F-box proteins involved in cucumber fruit development (Xu *et al.*, 2017). However, miR396a regulates the expression of *GRFs*, growth regulator genes that increase tomato fruit weight (Cao *et al.*, 2016). In *Camellia oleifera*, miR156, miR390, and miR395 are involved in carbohydrate accumulation, while miR477 is involved in fatty acid synthesis to regulate fruit growth and the development process (Liu *et al.*, 2019).

MiRNAs control fruit size: The functions of miRNAs in fruit size showed that miR172 targets the AP2 transcription factor and binds directly to *FUL* and *AGAMOUS*. *FUL* activates miR172 expression through promoter binding. MiR172 overexpression reduces apple fruit size but performs different functions in the growth of arabidopsis cuttle fruits (Yao *et al.*, 2015). In rice, the OsmiR408-regulated *OsUCL8* gene can affect the chloroplast pigment protein and photosynthesis. *OsUCL8* knockout can improve grain yield, while *OsUCL8* overexpression can lead to the opposite phenotype, indicating that miR408-*OsUCL8* regulates photosynthesis and grain yield (Zhang *et al.*, 2017). OsmiR530 expression is activated by phytochrome-interacting factor like 15 (*OsPIL15*); *OsPIL15* is directly combined with the G-box element in the promoter. The knockout of OsmiR530 increases the grain yield, while OsmiR530 overexpression significantly reduces the grain size and number of ear branches, resulting in a loss of grain yield (Sun *et al.*, 2020). In addition, in cucumber, Csa-miR160d is expressed only in the mature fruit, and Csa-miR160d negatively regulates target gene *CsARF5/11/13*, an auxin response factor, indicating that Csa-miR160d plays an important role in the control of cucumber fruit size (Li *et al.*, 2022b). Yang *et al.* (2020) studied the effects of miR1917-SICTR4 on tomato fruit development by regulating multiple genes in the ethylene pathway. STTM1917 mutant plants showed a series of developmental phenotypes, including higher biomass, longer terminal leaflets, bigger floral organs, and increased fruit and seed sizes. Cao *et al.* (2016) found that STTM396a overexpression in tomatoes leads to an increase in sepal length and fruit size and revealed that miR396 plays a regulatory role in tomato flower and fruit development.

MiRNAs control fruit maturity: In kiwifruit, miR164 regulates the expression of *AdNAC6* and *AdNAC7* after exogenous ethylene application, and miR164 binds to *AdACS1*, *AdACO1*, *AdMAN1*, and *AaTPS1* promoters to regulate fruit ripening. MiR164 also regulates fruit ripening in other fruits, such as citrus and grape (Wang *et al.*, 2020b). Under exogenous abscisic acid (ABA) treatment, we can promote fruit ripening by differentially regulating *NCED1*, *PYR1*, *ABI1*, and *SnRK2.2* genes, which are involved in ABA metabolism and signal transduction, and the reduction of Fan-miR73 expression promotes the accumulation of *ABI5* transcripts in ripening strawberry fruit (Li *et al.*, 2016b).

Fruit color is also one of the phenotypes used to measure fruit maturity. In litchi, miR156a targets transcription factors *LcSPL1/2* and interacts with *LcMYB1*, a key regulatory gene for anthocyanin biosynthesis, to regulate anthocyanin biosynthesis in litchi fruit (Liu *et al.*, 2016; Ahsan *et al.*, 2019a). In apple, *mdm-miR828* overexpression inhibits anthocyanin synthesis, and negative regulation of *mdm-miR828* expression changes the accumulation of anthocyanins due to *MdMYB1*, leading to pigmentation in the mature apple pericarp (Zhang *et al.*, 2020a). MiR858 affects anthocyanin biosynthesis in tomato fruit by regulating the expression of transcription factors *SIMYB7* and *SIMYB48*. Similarly, miR858a and *ELONGATED HYPOCOTYL 5* inhibit the expression of *MYB-LIKE 2*, which results in the decrease in anthocyan content in arabidopsis (Wang *et al.*, 2016; Meng *et al.*, 2023).

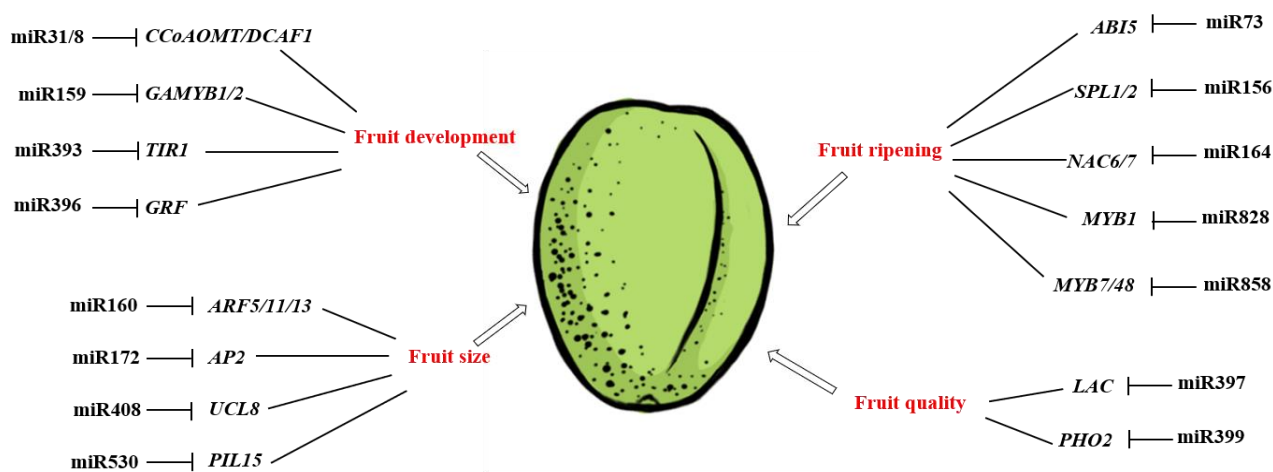


Fig. 3. Regulated model of miRNAs in fruit morphogenesis of plants. All of the miRNAs with verified functions in development, size, ripening, and quality as well as their respective main targets are represented. Symble "⊣" is suppression.

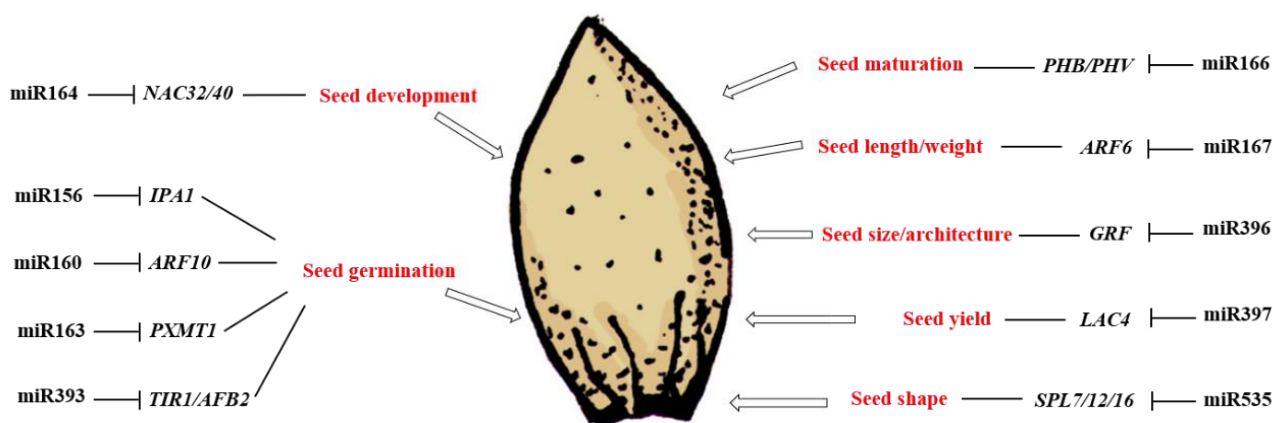


Fig. 4. Regulated model of miRNAs in seed morphogenesis of plants. All of the miRNAs with verified functions in development, germination, maturation, length/weight, size/architecture, yield, and shape as well as their respective main targets are represented. Symble "⊣" is suppression.

MiRNAs regulate fruit quality: MiR397a can regulates the lignin biosynthetic enzyme in stone cells of pear growth fruit by inhibiting the expression of *PbrLACs*, thus reducing the lignin content and the number of fruit nucleus cells and improving pear fruit quality (Xue *et al.*, 2019). Strawberry fruit quality is positively correlated with the phosphorus content, and the phosphorus content in the fruit is increased 1.1–2.1 times due to miR399a overexpression, indicating that miR399 is involved in the regulation of phosphate homeostasis (Wang *et al.*, 2017). Moreover, miR399 targeting of *PHO2* plays a key role in plant phosphate signal transduction. Knockout of *TaPHO2-A1* significantly increases the phosphorus uptake and production of wheat under low phosphorus conditions (Ouyang *et al.*, 2016).

Role of miRNAs in controlling plant seed morphogenesis: MiRNAs are involved in seed development, maturity, phenotypic characteristics (length, size, and weight), and dormancy. Recent research has shown the identification and functional analysis of miRNAs (Yu *et al.*, 2019) (Fig. 4).

MiRNAs regulate seed development in plants: Md-miR156h regulates the heterotypic expression of its target gene *AtSPL9/15* in transgenic *Arabidopsis thaliana*, this causes partial infertility in transgenic plants (Sun *et al.*, 2013). In maize, miR164 regulates the expression of *NAC32/NAC40* and downstream genes *EXPB14* and *EXPB15* related to seed development, which may be a concern in maize seed development (Zheng *et al.*, 2019d). Similarly, in wheat, the miR164-target *TaPSK5* gene is a positive regulator that regulates grain number and grain weight (Geng *et al.*, 2020). In *Arabidopsis thaliana*, AGO1 and miR166 inhibit the expression of *PHB/PHV*, and *PHB/PHV* promotes seed maturation by activating *LEAFY COTYLEDON2* expression, directly and indirectly activating other positive regulators (Tang *et al.*, 2012).

MiRNAs regulate plant seed phenotype changes: The phenotypic characteristics of plant seeds are a key factor influencing grain yield. Common phenotypic characteristics, such as length, size, and weight, are

influenced by miRNAs. MiR167a regulates auxin-responsive factor *OsARF6*, which interacts with auxin-signaling negative regulator *OsIAA8* or *OsIAA820* and binds to auxin-transport vector gene *OsAUX3* promoter response elements, thereby changing the auxin content and distribution in rice seed-shell cells, affecting the size of rice seed-shell cells, and further regulating rice grain length and grain weight (Qiao *et al.*, 2021). MiR396-*GRF* is an important regulator of rice grain size and plant type. The length, width, and thickness of rice grain are significantly increased in mir396ef mutant, and the weight of rice grains is increased by 40% (Miao *et al.*, 2020). OsmiR535 overexpression inhibits the expression of *OsSPL7/12/16* and *OsSPLs* downstream spike-related genes *OsPIN1B*, *OsDEP1*, *OsLOG*, and *OsSLR1Os* and increases the grain length in rice (Sun *et al.*, 2019). The miR397b-targeted laccase gene *LAC4* regulates lignin synthesis and seed yield in *Arabidopsis thaliana*. MiR397b overexpression results in the production of more than two inflorescence branches, which increases the number and length of keratinocytes and hence the number of seeds (Wang *et al.*, 2014).

MiRNAs regulate seed dormancy and germination:

Whether the seeds can sprout normally is a precondition for plant growth and development. Interactions between plant hormones can regulate miRNAs involved in seed development. ABA inhibits germination, and GA promotes germination during seed germination. MiR156 inhibits the GA pathway by regulating the expression of *Ideal Plant Architecture 1* and promotes seed dormancy (Miao *et al.*, 2019). Similarly, miR156 also participates in the dormancy of *Ginkgo* seeds through gene regulation related to ABA/GA hormone and embryo development (Jia *et al.*, 2021). In rice, miR393 negatively regulates *OstIR1* and *OsAFB2* auxin receptor genes, which inhibit germination sheath elongation and stomatal development, leading to seed dormancy (Guo *et al.*, 2016).

In maize, miR160b-5p regulates *ARF10* and inhibits seed germination under ABA signal transduction conditions, while miR417 and miR395c promote seed germination under GA conditions (Liu *et al.*, 2020a). In *Arabidopsis thaliana*, miR163 is negatively correlated with methyltransferase encoding gene *PXMT1*, and germination of the miR163 mutant or *PXMT1*-overexpressing plants is delayed under continuous illumination (Chung *et al.*, 2016). In *Arabidopsis thaliana*, miR156 downregulates the expression of *AtSPL9* and *AtSPL15*. Overexpression of miR156 results in the prolongation of the growth stage, short keratinous fruit, and partial seed abortion; increases the number of leaves, and causes germination failure (Sun *et al.*, 2013).

Molecular mechanisms underlying the regulation of graft heterozygous morphogenesis by miRNA long-distance transport:

Grafting is a technique that co-integrates two or more plants into one plant. It has been used for over 2000 years (Turnbull & Carrington, 2022; Zhang *et al.*, 2022). Current studies have shown that

grafting can cause the exchange of genetic material between rootstocks and scions and leads to changes in the characteristics of chimeras and stable inheritance to offspring. This phenomenon is known as grafting hybridization; the formation of grafting chimeras is called grafting hybrid (Stegemann & Bock, 2009; Liu *et al.*, 2010; Zhao *et al.*, 2018). Grafting can accelerate the formation of target traits in the process of economic forest breeding, which has been widely supported in breeding practice in recent years for phenotypic traits (such as leaf color, leaf type, leaf villi, height of the tree crown, and fruit size) (Xanthopoulou *et al.*, 2019; Dong *et al.*, 2021), flowering and seedling (Ahsan *et al.*, 2019a), tolerance to biotic and abiotic stress enhancing (Zhang *et al.*, 2018b; Li *et al.*, 2021), yield and quality (Riga *et al.*, 2016; Musa *et al.*, 2020; Qiao *et al.*, 2021), and physiological metabolic process (He *et al.*, 2020). This has realized the artificial control of fruit size, yield, and other quantitative characteristics (such as fruit size and yield), and has become a new way to innovate germplasm (Fig. 5).

Role of miRNAs in the long-range regulation of grafted hybrid traits:

The reconnection of functional tissue between the rootstock and scion mainly lies in the reconnection of the functional tissue of the callus (Miao *et al.*, 2021). Successful grafting between two different species lies in the molecular exchange of proteins, hormones, mRNAs, and miRNAs at the grafting site; it is an important molecular mechanism regulating the communication between rootstock and scion (Rasool *et al.*, 2020). For example, miR395, miR398, miR399, and miR156 are involved in the physiological response from the root of sweet cherry rootstock and are transported a long distance from the sweet cherry scion to the chinese cherry rootstock (Wen *et al.*, 2022). In avocados, miR172, miR156, and their target gene *SPL4* are controlled by the physiological age of the scion, which determines whether the grafting hybrids will pass from the seedling stage to the flowering stage (Ahsan *et al.*, 2019a). 603 unreported miRNAs were found in the rootstocks of two different genotypes, high and low, they may regulate the regulation and transport of IAA, cell viability, and inorganic phosphate (Pi) uptake in the long-distance grafting hybrids, which may affect the growth and development of grafted pecan (Liu *et al.*, 2020b). The biosynthesis of gibberellins in the ear is controlled at miR171f_3 in ‘Nantongxiaofangshi’ (*Diospyros kaki* Thunb., *D. kaki* Thunb.), which results in the dwarfing of persimmon (Dong *et al.*, 2021). MiR159, miR164, miR171 expression are significantly higher in heterogonous grafted cucurbit than in homologous rootstock, resulting in a statistically significant difference in fruit traits and size between grafted hybrids (Xanthopoulou *et al.*, 2019). ClmiR86 can enhance root growth and phosphate uptake of grafted hybrids under phosphate deficiency by inhibiting *CICIPK5*, which promotes the flowering elongation of watermelon grafted hybrids (Wu *et al.*,

2021). MiR166a and miR395b are significantly upregulated in grafted hybrid tomato, which enhances the ability of inoculated plants to transport sulfate across the membrane, significantly reducing the accumulation of heavy metal cadmium in the grafted hybrid and improving the fruit quality (He *et al.*, 2020). A total of 976 unknown miRNAs have been identified in Fuji, M9, and Fuji/M9. Among them, miR156, miR172 are expressed in different levels of grafted Fuji/M9 and self-rooted M9. These miRNAs are involved in cell biosynthesis and metabolism, as well as in the regulation of plant flowering (An *et al.*, 2018). MiR2111 is produced in the leaves and transported to the roots under low nitrogen stress, promoting the formation of root nodules by inhibiting the accumulation of Too Much Love (TML) expression in the roots (Gautrat *et al.*, 2020). Furthermore, a large number of current studies have focused on the change in miRNA expression after the scion response to grafting and have shown that some key miRNAs affect the expression of miRNAs in grafted tomato (Khaldun *et al.*, 2016), cucumber (Li *et al.*, 2016a), and watermelon (Wu *et al.*, 2016). These results further indicate that miRNAs play a pivotal role in the formation of grafted hybrids.

Discussion

Plant morphogenesis involves many factors in many regulatory pathways, including structural gene-induced functional changes, many transcription factors, and non-

coding RNAs. The regulation of the development of floral organs, fruit size, seed size, and other quantitative traits is difficult in economic forest species breeding. Among these factors, changes in the expression of one regulator can lead to the reshaping of the entire development process, which is beneficial for the breeding of high heterozygous genes in the economic forest.

According to recent studies, miRNAs affect plant development by negatively regulating downstream targets. This study provides new insights into the functions of miRNAs and target genes in the regulation of reproductive organs and provides new approaches and candidate factors for the molecular regulation of important economic traits in woody plants. For example, miR159, miR164, miR165/6, miR319, miR390, and miR396 are involved in leaf development; miR167, miR169, miR171, miR399, and miR824 are involved in floral organ development; miR164, miR167, miR172, and miR390 are involved in fruit development. MiRNAs are widely involved in the development of plant reproductive organs.

Based on the study of miRNA function, virus-induced gene silencing technology can be used to verify the function of miRNAs in woody plants that lack stable genetic systems. The CRISPR/Cas genome editing method is a molecular modification technology for plant morphology construction (Scheben *et al.*, 2017). The methods to determine whether miRNAs interact with target proteins include CHIP, FISH, luciferase, and RLM-RACE. In the future, these methods can be used to analyze the key miRNAs regulating the economic traits of crops and provide theoretical support for molecular design breeding using miRNAs, which is an epigenetic modification.

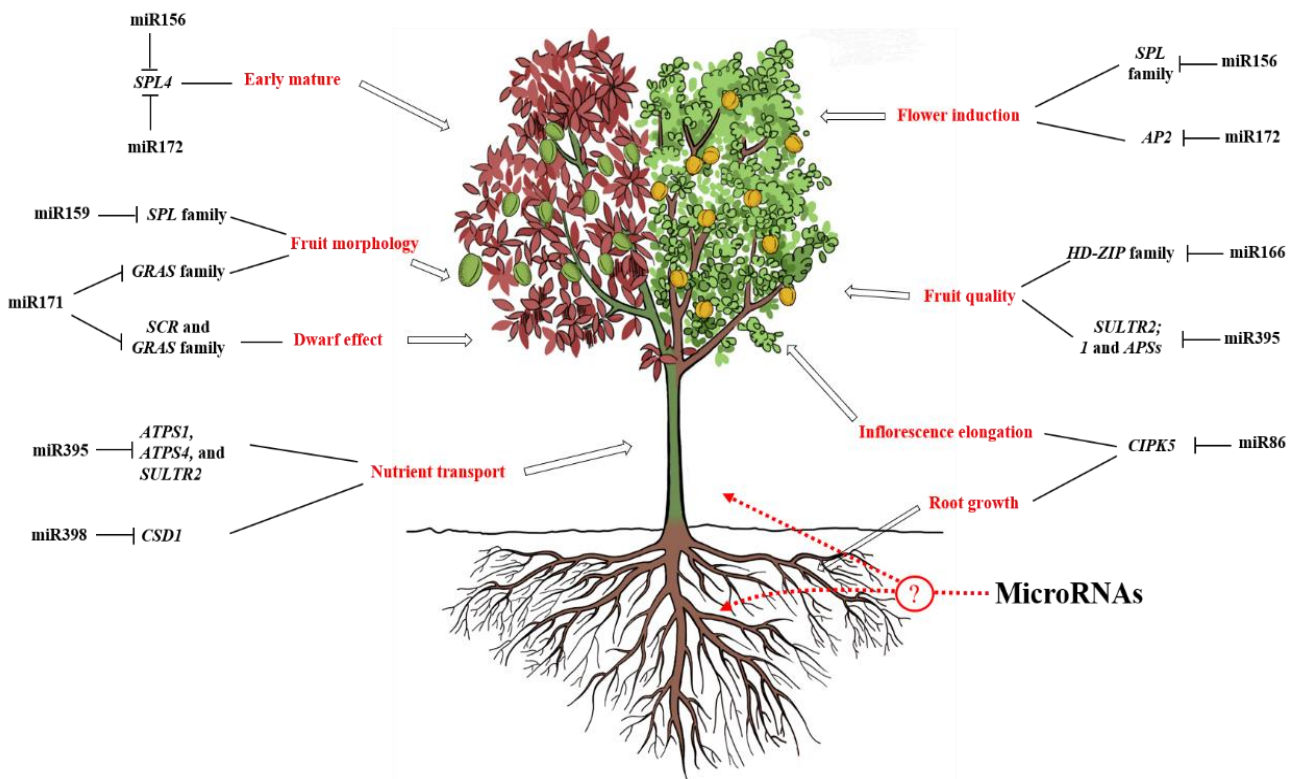


Fig. 5. Regulated model of miRNAs in grafting hybrid of plants. All of the miRNAs with verified functions in early mature, fruit morphology, dwarf effect, flower induction, fruit quality, inflorescence elongation, and root growth as well as their respective main targets are represented. Symbol "⊥" is suppression.

Acknowledgments

This work was supported by the National Key R&D Program of China (2019YFD1001200).

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(Received for publication 22 February 2023)