Ornamental Plant Research

REVIEW

Open Access

https://doi.org/10.48130/opr-0024-0008
Ornamental Plant Research **2024**, 4: e010

MicroRNA156: a count up timer with potential to enhance horticultural traits

Yunchen Xu[1,2#], Cong Gao[2#], Ziyang Huang[1], Jie Liu[1], Ziming Ren[1], Yong He[3], Yiping Xia[2], Shengjun Feng[3*] and Yun Wu[1*]

[1] *Laboratory of Flower Bulbs, Department of Landscape Architecture, Zhejiang Sci-Tech University, Hangzhou 310018, China*
[2] *Genomics and Genetic Engineering Laboratory of Ornamental Plants, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China*
[3] *College of Horticulture Science, Zhejiang Agriculture and Forestry University, Hangzhou 311300, China*
[#] These authors contributed equally: Yunchen Xu, Cong Gao
* Corresponding authors, E-mail: 20170039@zafu.edu.cn; yunwu@zju.edu.cn

Abstract

MicroRNA156 (miR156), targeting a subset of *SQUAMOSA PROMOTOR BINDING PROTEIN-LIKE* (*SPL*) genes, is one of the most evolutionarily conserved microRNAs (miRNAs) in plants. miR156 is a recognized molecular marker of plant age. In addition to being an age indicator, miR156 has demonstrated strong multifunctionality in the regulation of a broad range of biological processes in horticultural plants, including vegetative growth, floral induction, fruit ripening, stress responses, and secondary metabolism. Due to its versatility and high level of conservation among phylogenetically distinct plant species, miR156 may be a powerful tool for biotechnological modification in horticultural plants. Here, we summarize the functional diversity of miR156 in horticultural plants to provide new insights for further research on the biological function and regulatory mechanism of miR156 and on how to employ miR156 to achieve the enhancement of horticultural traits.

Citation: Xu Y, Gao C, Huang Z, Liu J, Ren Z, et al. 2024. MicroRNA156: a count up timer with potential to enhance horticultural traits. *Ornamental Plant Research* 4: e010 https://doi.org/10.48130/opr-0024-0008

Introduction

MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs of approximately 20–22 nucleotides in length that are widely distributed in animals and plants[1]. They negatively regulate gene expression *via* mRNA cleavage, translational repression, or both. MicroRNA156 (miR156), targeting a subset of *SQUAMOSA PROMOTOR BINDING PROTEIN-LIKE* (*SPL*) genes, is one of the most evolutionarily conserved miRNAs in plants[2]. It was initially found in *Arabidopsis thaliana*[3]. Since then, a large number of miR156 family members have been identified and functionally verified in various horticulture plants. Based on the species registered in the miRBase database (www.mirbase.org)[4], the miR156 sequences of at least 16 horticultural plants have been revealed to have different numbers of miR156 precursors and mature miR156 (Fig. 1). As a key player in balancing plant age and other biological functions, miR156 is involved in the regulation of a wide range of horticultural traits. Moreover, miR156 plays a remarkably conserved role throughout the angiosperms[5]. Hence, miR156 could potentially be an emerging means suited for biotechnological modification in horticultural plants. In this review, we mainly summarize the multifunctional regulation of miR156 in horticultural plants and also discuss perspectives for future studies.

The miR156 family and its targets in horticultural plants

Evolutionary conservation of miR156

Based on 141 sequences of horticultural plant species released from miRBase 21.0, the miR156 sequences from 16 horticultural plants belong to 26 subfamilies (miR156a-z) (Fig. 2). A study using a total of 310 miR156 sequences from 51 plant species for phylogenetic analysis found a significant expansion in the number of miR156 family members from lower to higher plants[6]. In apple (*Malus domestica*), 15 different miR156 mature sequences are derived from 31 miR156 precursors; while there is only one unique miR156 sequence in *Aquilegia caerulea* from two precursors. Though various horticultural plant species exhibit a divergence in terms of the number of the identified mature miR156s, ranging from 1 to 15 (Fig. 1), their sequences show great conservation, according to the alignment of miR156 subfamilies from different horticultural plants (Fig. 2). All the base variations occur at the miR156 skeleton sequence 'TGACAGAAGAGAGTGAGCAC'. Additionally, the variation sites are primarily found in the 11th and 14th base (near the cleavage site) at the 5' end of miR156[6]. These two sites are nearly consistent with the cleavage sites of miRNAs on their target genes, which implies that different members of *SPL* were targeted by miR156s with corresponding base variation, and mismatch caused by point mutation could perturb the miR156-directed transcriptional cleavage and translation repression[7,8]. miR156-3p is produced from the other arm of miR156 precursors. Unlike common miR156 (miR156-5p) sequences, the strands of miR156-3p show a large divergence and are only found in five horticultural plant species, i.e., *Citrus sinensis*, *Fragaria vesca*, *Solanum lycopersicum*, *Solanum tuberosum* and *Vriesea carinata* (Fig. 1). Due to the highly conserved nature, 5p sequences of miR156 have been fairly well-studied compared with 3p sequences. However, it is important to note that evidence has emerged that 3p sequences also serve specific biological functions[9,10].

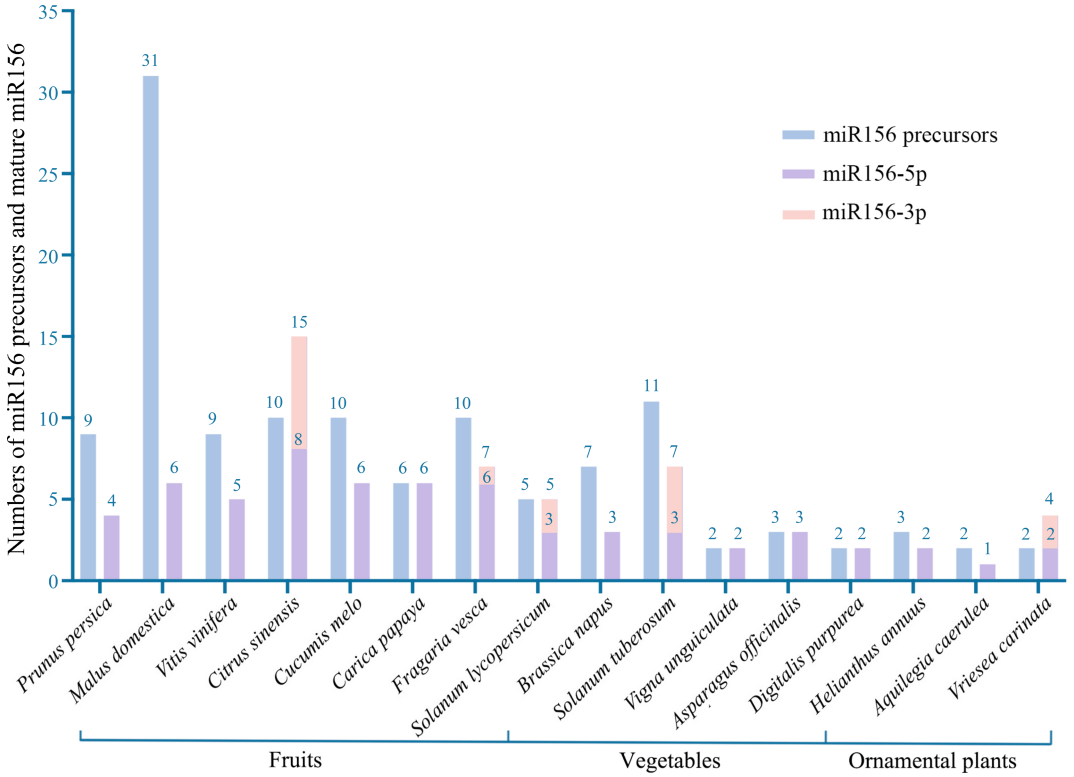

Fig. 1 Number of miR156 precursors and unique mature sequences in horticultural plants published in the miRbase database. Identical mature sequences from different precursors were considered as one sequence.

Despite the conservation of mature miR156, its precursors vary notably in the number and length of members in different horticultural plants and show more significant discrepancies in sequences. Compared with the mature sequences of miR156, the phylogenetic analysis of its precursor sequences can more clearly reflect the evolutionary history of miR156 (Fig. 3). The gene family varies significantly in size and genomic organization in different plant species, which may cause dosage effects and spatial and temporal differences in target gene regulations[11]. Moreover, despite producing the same mature sequences, research has suggested that different *MICRORNAs* (*MIRNAs*) play certain functional roles[12,13]. Studies of the *MIR156* family in horticultural plants have primarily concentrated on *MIR156A*, and it is yet unclear how *MIR156s* function synergistically or redundantly to regulate plant development.

Targets of miR156

The targets of miR156 belong to *SPL* transcription factors containing a highly conserved DNA binding domain (SQUAMOSA promoter binding protein (SBP) domain) with two separate zinc binding sites, while they bear limited conservation outside the SBP domain[5]. *SPL* genes were first identified in *Antirrhinum majus*, where the closely related SBP proteins regulate flower development by binding to the promoter of the MADS-box gene *SQUAMOSA* (*SQUA*)[14]. SPL transcription factors, as a class of plant-specific proteins, have been widely identified in horticultural plants thereafter. In the genomes of apple, grape (*Vitis vinifera*), tomato (*Solanum lycopersicum*), pear (*Pyrus pyrifolia*), and petunia (*Petunia hybrida*), 33, 18, 17, 9, 19, and 21 members, respectively, of the *SPL* gene family were identified. Among them, 20, 12, 7, 7 and 14 *SPLs* contained miR156 binding sites. Notably, the existence of binding sites does not necessarily imply a negative regulatory role of miR156, as the studies on cucumber suggested[15,16]. The *SPL* genes targeted by miR156 can be divided into two major groups, represented by *SPL3* (*SPL3*, *SPL4*, and *SPL5*) and *SPL9* (*SPL2*, *SPL6*, *SPL9*, *SPL10*, *SPL11*, *SPL13*, *SPL13*-like, and *SPL15*)[17], which generally serve different roles in plant development[18]. To date, studies have revealed that the miR156-SPL module could engage in multiple important biological processes in horticultural plants, including but not limited to vegetative phase change[19], flower development[20], fruit ripening[21] and stress responses[9]. Functional conservation of the miR156-SPL module has been found in various horticultural plants, while many new functions are also progressively being discovered.

Multifunctional regulation of miR156 in horticultural traits

Vegetative growth and morphological change

Plants go through a vegetative phase and a reproductive phase in their post-embryonic development, and the vegetative phase could be further divided into a juvenile phase and an adult phase[5]. The transition from the juvenile to the adult stage is defined by the acquisition of reproductive competence, while the transition from the vegetative to the reproductive stage is marked by the production of novel reproductive structures, such as flowers or cones[22]. miR156 is a key intermediary connecting plant age and development. This is demonstrated by the fact that the constitutive expression of miR156 prolonged the expression of juvenile vegetative traits and delayed flowering[23,24]. The expression of miR156 is typically highest in the juvenile stage and declines as plants reach the

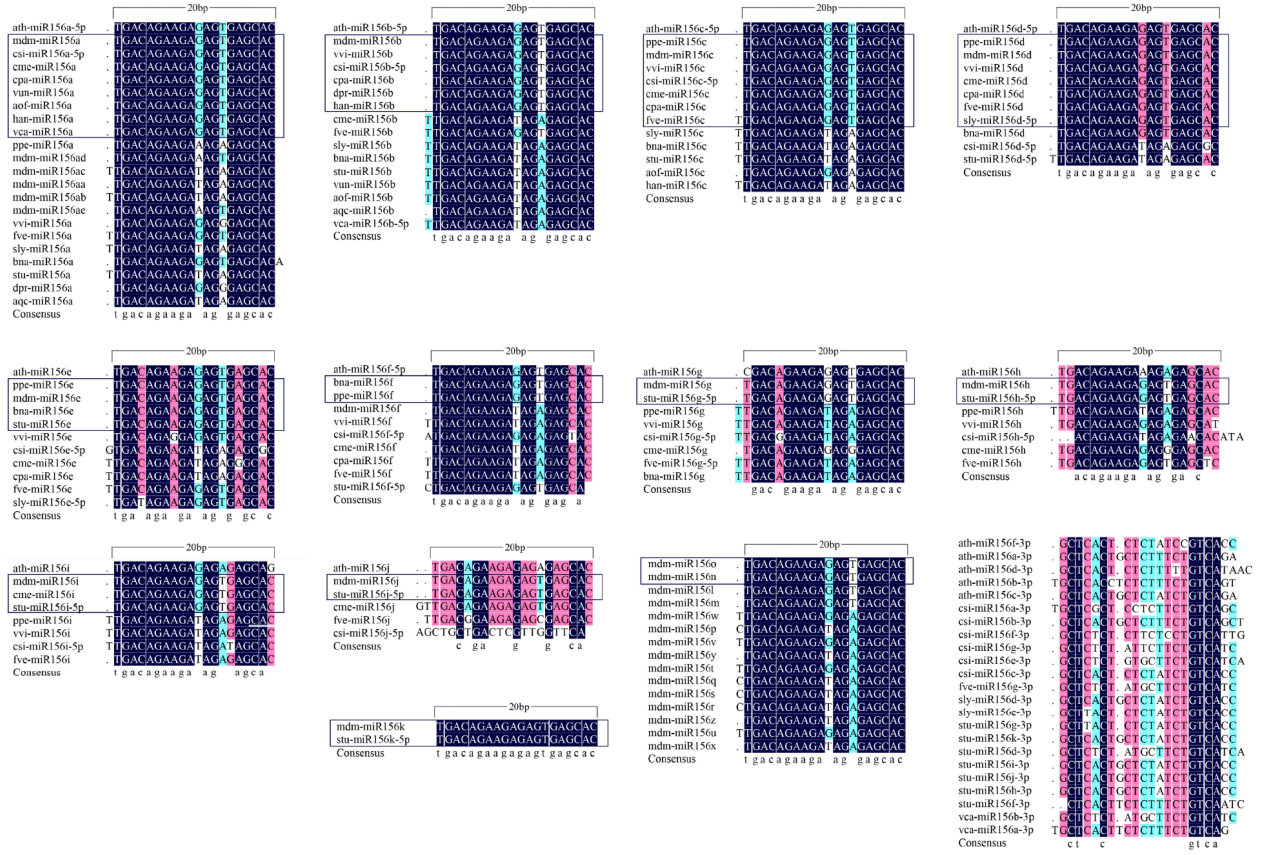

Fig. 2 Alignment of orthologous miR156 subfamilies from *Arabidopsis* and horticultural plants. A total of 138 miR156 mature sequences from 16 different horticultural plants were aligned. The sequences boxed in black are miR156 seed sequences. ath: *Arabidopsis thaliana*; mdm: *Malus domestica*; csi: *Citrus sinensis*; cme: *Cucumis melo*; cpa: *Carica papaya*; vun: *Vigna unguiculata*; aof: *Asparagus officinalis*; han: *Helianthus annuus*; vca: *Vriesea carinata*; ppe: *Prunus persica*; vvi: *Vitis vinifera*; fve: *Fragaria vesca*; sly: *Solanum lycopersicum*; bna: *Brassica napus*; stu: *Solanum tuberosum*; dpr: *Digitalis purpurea*; aqc: *Aquilegia caerulea*. The miR156 sequences were downloaded from the miRbase database. Sequences are listed in Supplemental Table S1.

adult stage. This has been observed in a variety of horticultural plants, such as apple[19], mango (*Mangifera indica*)[24], mulberry (*Morus atropurpurea*)[22], cucumber[13], tree peony (*Paeonia delavayi*)[26], orchid (*Dendrobium catenatum*)[27] and lily (*Lilium* × *formolongi*)[28]. Notably, unlike *Arabidopsis*, whose life cycle can be completed in less than three months, many horticultural plants, such as lilies, have a protracted juvenile stage that can last for years. In this regard, the miR156-mediated age pathway is a promising tool for horticultural plants with a prolonged juvenile period, as it provides the possibility of accelerating the plant vegetative growth process *via* a shortened juvenile stage. Similarly, the juvenile stage and the vegetative phase transition are closely related to biomass accumulation in horticultural plants. Overexpression of miR156 delayed the heading time of Chinese cabbage (*Brassica rapa*), concomitant with prolongation of the seedling and rosette stages; while the overexpression of *SPL9* caused significant heading earliness, which could lead to high yield in Chinese cabbage[29]. Thus, manipulating the vegetative phase transition through an age-dependent pathway is a potential approach to improve the productivity of horticultural plants.

Plants show morphological differences under the regulation of the age pathway[30]. The term heteroblasty refers to the phenomenon in which the same plant exhibits abrupt morphological variations during the transition from the juvenile to the adult stage[31]. The term is also increasingly used to encompass the gradual transitions in a variety of traits of most flowering plant species, particularly leaf size and shape, as well as internode length and trichome distribution[32]. miR156 is both necessary and sufficient for the expression of juvenile traits, while an increase in the level of miR156-targeted *SPLs* is required to trigger heteroblasty[30] by promoting cell growth anisotropy[33]. Many horticultural plants exhibit heteroblastic traits[26,34]. During the seedling stage of cucumber, short and nonfunctional tendrils are formed, while elongated and functional tendrils are formed in the climbing stage. The production of functional tendrils is reported to be age-dependent and regulated by miR156[16]. *Passiflora edulis* undergoes remarkable changes in leaf morphology with a decrease in the level of miR156[35]. The expression of miR156 and its targets could also be linked to glandular trichome development, which provides resistance to insect pests in tomato[36]. Therefore, these heteroblastic traits deserve to be focused on as they are closely linked to the agronomic performance of horticultural plants.

Flowering time and flower pattern

The age pathway is one of the main flowering pathways, together with the photoperiod pathway, the vernalization pathway, the GA pathway, and the autonomous pathway[37,38]. Through the differential expression pattern of the miR156-SPL module, the age pathway regulates flowering time in two ways.

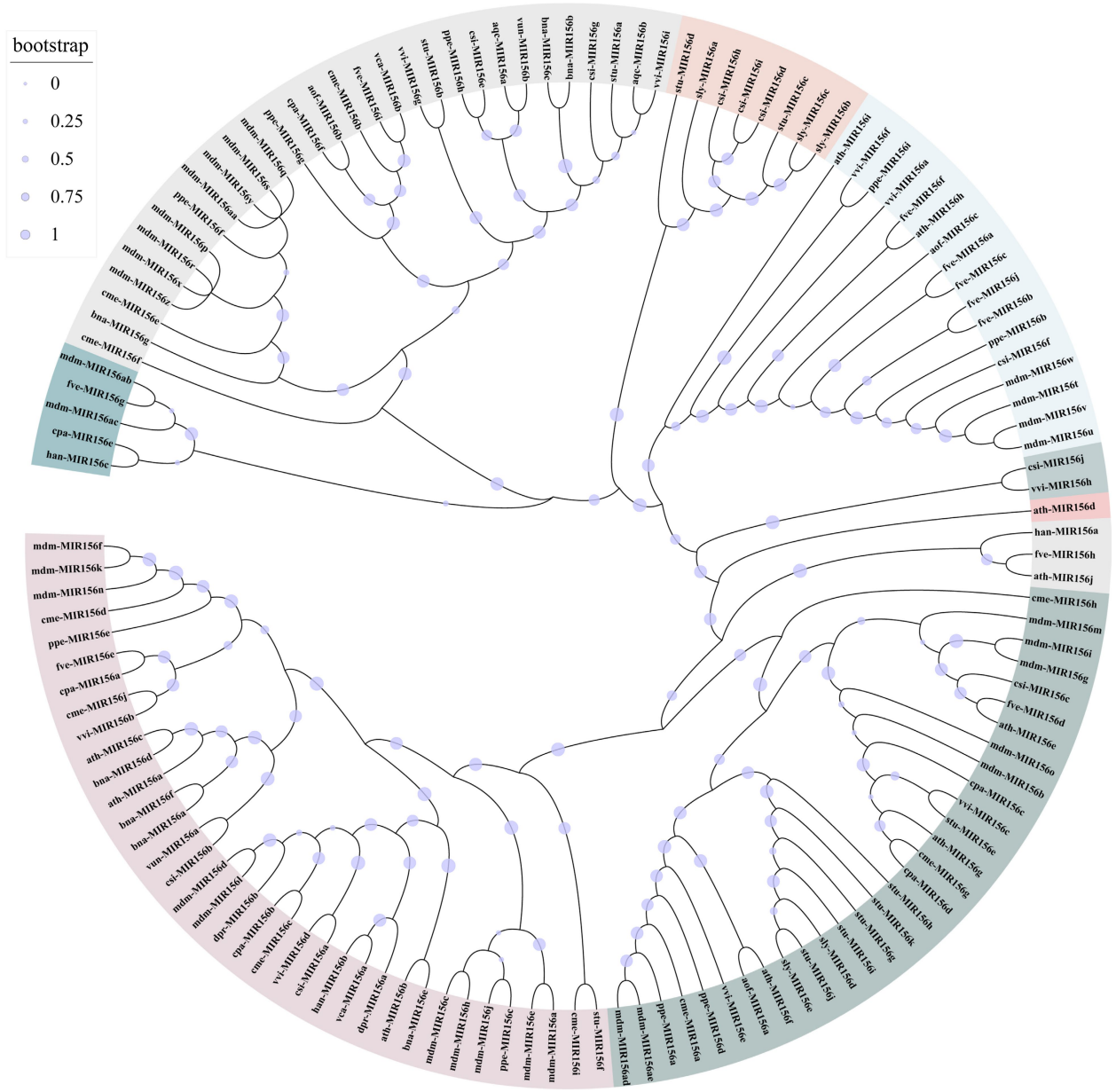

Fig. 3 Phylogenetic relationships between *MIR156* from different plants. A total of 122 *MIR156* sequences were phylogenetically analyzed. ath: *Arabidopsis thaliana*; mdm: *Malus domestica*; csi: *Citrus sinensis*; cme: *Cucumis melo*; cpa: *Carica papaya*; vun: *Vigna unguiculata*; aof: *Asparagus officinalis*; han: *Helianthus annuus*; vca: *Vriesea carinata*; ppe: *Prunus persica*; vvi: *Vitis vinifera*; fve: *Fragaria vesca*; sly: *Solanum lycopersicum*; bna: *Brassica napus*; stu: *Solanum tuberosum*; dpr: *Digitalis purpurea*; aqc: *Aquilegia caerulea*. The *MIR156* sequences were downloaded from the miRbase database. Sequences are listed in Supplemental Table S2.

One is to suppress the activity of flowering repressors derived from the *APETALA2*-like (*AP2*-like) family through microRNA172 (miR172), and the other is to directly induce the expression of floral integrators, such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), to induce flowering (Fig. 4)[37,39,40].

The constitutive expression of miR156 tends to delay flowering, while the miR156-targeted *SPL*s redundantly promote flowering[23,39,41–43]. miR172, a target of *SPL*s, acts downstream of miR156 in an antagonistic expression pattern. In contrast to miR156, the expression of miR172 is low during the juvenile phase and exhibits a subsequent increase toward progression to flowering[7,23,30,44]. Early or late flowering of ornamental gloxinia (*Sinningia speciosa*) was observed in transgenic plants in which miR172 was overexpressed or suppressed, respectively, accompanied by corresponding changes in *AP2*-like[45]. In addition, several floral integrators work directly downstream of *SPL*, including *SOC1*, *FLOWERING LOCUS T* (*FT*), *LEAFY* (*LFY*) and *APETALA1* (*AP1*)[39,46,47]. *SPL* transcription factors promote flowering in tomato by positively regulating the expression of the tomato inflorescence-associated gene *SINGLE FLOWER TRUSS* (*SFT*) in leaves and the MADS-Box gene *AP1*/*MC* in the shoot apex[20,48]. Research on litchi (*Litchi chinensis*) plants also suggests that *SPL* transcription factors promote age-dependent flowering by directly binding to the *FT* promoter[49]. The above findings indicate an effective strategy for regulating flowering time in horticultural plants through the age pathway.

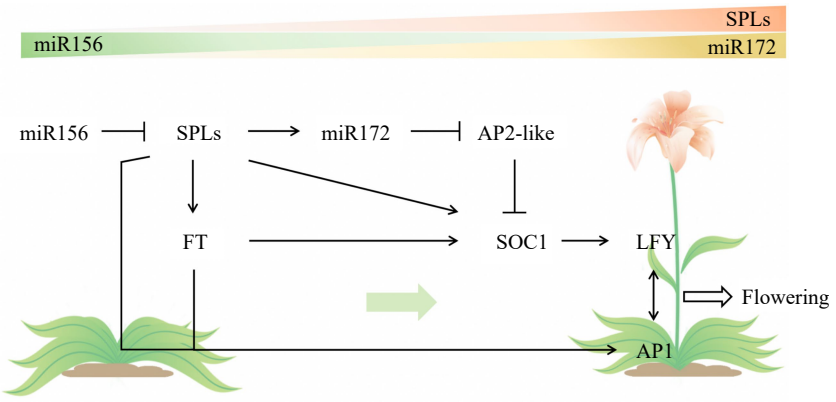

Fig. 4 Age pathway for flowering defined by the miR156-SPL module.

Notably, the miR156-mediated age pathway may define an endogenous mechanism that ensures plants flower in the absence of exogenous inductive cues[39]. This opens the possibility for ornamental flowers to bloom regardless of environmental restrictions. Furthermore, a study on chrysanthemum shows that the expression of the nuclear factor gene *CmNF-YB8* is regulated by age, but not by day length, low temperature, or GA, and CmNF-YB8 is shown to bind to the promoter of the *cmo-MIR156* and influence flowering time through directly regulating the expression of cmo-MIR156 in the age pathway[42]. The unveiling of the specific upstream transcription effectors of miR156 reveals the significance and independence of the age pathway in flower formation.

miR156 also plays a role in petal coloration and floral pattern formation[50,51]. Increased miR156 expression levels promote accumulation of anthocyanins, whereas the target *SPL9* negatively regulates anthocyanin accumulation by directly inhibiting the expression of anthocyanin biosynthetic genes through destabilization of a MYB-bHLH-WD40 (MBW) transcription complex[52]. Similarly, miR156-3p was reported to be involved in yellow flower formation by improving anthocyanin accumulation in herbaceous peony[10,53]. Furthermore, the miR156-SPL module is associated with petal spot rearrangement in *Gorteria diffusa*, resulting in better mimicry of female flies resting on the flower[54]. Notably, analysis of differentially expressed genes also reveals the potential multifunctional role of miR156 in the development of sterile and fertile flowers[55], double flower forms[51] and multi-tepal patterning[56] in various ornamental plants, which suggests a regulatory node contributing to the diversity of floral morphogenesis.

Plant yield and quality

Ample evidence shows the significant role of miR156-SPL in fruit development[24,57,58]. miR156 transgenic tomato exhibited abnormal fruit morphology with extra carpels and ectopic structures as well as small fruit size and reduced fruit numbers[59,60]. miR156 was also found to be an adverse regulator of tuberization in potato[61]. A decrease in miR172 may partially account for this as it was reported to contribute to tuberization[62]. In addition, some evidence has shown that miR156 is instrumental in secondary metabolism, which is closely associated with the quality and nutritional value of horticultural products. As it does in flower petals, the miR156-SPL module serves as an important regulator by participating in anthocyanin biosynthesis in peel coloration during fruit ripening. SPLs in Chinese sand pear interact with MYB10, presumably disrupting the formation of the MBW protein complex[63]. Similar results were also found in horticultural plants such as grape[64,65], litchi[66] and peach[67]. Apart from the involvement in anthocyanin accumulation, miR156 is also engaged in catechin accumulation in tea plants[68,69]. Furthermore, the synthesis of sesquiterpenes, important floral volatiles, has also been reported to be age-regulated. Specifically, the miR156-targeted SPL promotes accumulation of sesquiterpenes in elder plants by upregulating the expression of the patchoulol synthase (*PatPTS*) gene[70]. These findings provide support for miR156-SPL as a feasible approach to enhance yield and product quality in horticultural plants.

Resistance to biotic stress

During the development process, plants are constantly exposed to a variety of biotic stresses, including pest infestation and pathogen invasion. The plant vigor hypothesis suggests greater herbivore abundance on young and vigorous plants[71] and that older plants may display increased resistance to pathogens[72]. Such studies show a tradeoff between plant defense ability and age. This has been partly explained by a study on the age-regulated dynamics of plant insect resistance: the miR156-SPL9 module is responsible for the age-dependent decay of phytohormone jasmonate (JA), a key regulator of plant insect defense, whereas constitutive accumulation of defense compounds such as glucosinolates plays a role in compensating for JA-response attenuation during plant maturation[73].

Research shows that miR156 is involved in the defense against invading pathogens in horticultural plants. High-throughput sequencing shows that miR156 functions in the defense against apple stem pitting virus in pear likely through the regulation of its target gene *pbRPS6*[74]. In a study on the response to *Botrytis cinerea* in tomato, bioinformatics analysis on JA-deficient mutants and wild type plants revealed that miR156e-3p is likely involved in pathogen resistance through the JA pathway[75]. Several studies demonstrate the role of miR156 in the resistance to anthracnose disease, which is caused by *Colletotrichum gloeosporioides*. The expression of miR156 was significantly altered in tea plants after infection[76,77]. Additionally, research on walnut (*Juglans regia*) revealed that miR156 enhanced plant resistance to anthracnose likely through the regulation of *WRKY*[78]. Furthermore, miR156s were found to move across the kingdom boundary to

the invading *C. gloeosporioides* cells in cassava, showing a distinct cross-species defense mechanism against the fungal pathogen[79]. Clearly, miR156 is involved in diverse pathogen invasion responses to ensure successful growth and development of horticultural plants.

The miR156-SPL module is also engaged in the regulation of insect resistance[73,80,81], yet it has been reported only sparingly in horticultural plants. A very specific defense mechanism against herbivores exists in swollen thorn acacias (genus *Vachellia*). In exchange for protection, acacias provide ants with refuge and food in the form of swollen stipular spines, protein-lipid-rich Beltian bodies, and sugar-secreting extrafloral nectaries ('swollen thorn syndrome'). These defense traits are reported to be an age-dependent development correlated with the miR156-SPL pathway rather than a passive developmental pattern arising from developmental constraints[82]. These findings, together with the research on the development of glandular trichome[36] and age-regulated jasmonate response decay and defense metabolite accumulation[73], unveiled the complex mechanisms of miR156-regulated insect resistance during vegetative development (Fig. 5).

Resistance to abiotic stress

Abiotic stresses strongly affect the growth of horticultural plants. When plants encounter adversity, their development is likely to be delayed. miR156s were reported to play an important role in environmental adaption. This is demonstrated by the fact that miR156 showed a larger differential expression in sugarcane (*Saccharum officinarum*) under cold, salt and drought stress treatments compared with other implicated miRNAs[83]. However, the underlying mechanism by which miR156 regulates different abiotic stress responses is still unclear.

The expression level of miR156 is dramatically altered under different abiotic stresses in many horticultural plants. For instance, temperature stress resulted in high expression of miR156 in sugarcane[83], banana[84] and cabbage[85]. In addition to this, ample evidence demonstrated the role of miR156 in drought stress. Drought-induced accumulation of strigolactones in tomato leaves leads to the expression of miR156, which may in turn increase guard cell sensitivity to ABA, resulting in stomatal closure. Moreover, the after-effect of drought, by which stomata do not completely re-open after rewatering, was enhanced by miR156[86]. Additionally, the expression of miR156 was much higher in drought-tolerant apple plants than in drought-sensitive plants under control or drought conditions[89]. Notably, the functional role of miR156 against abiotic stresses in horticultural plants has generally not been experimentally verified. Therefore, performing functional validation and further exploring the miR156-related defense mechanism are crucial in future studies.

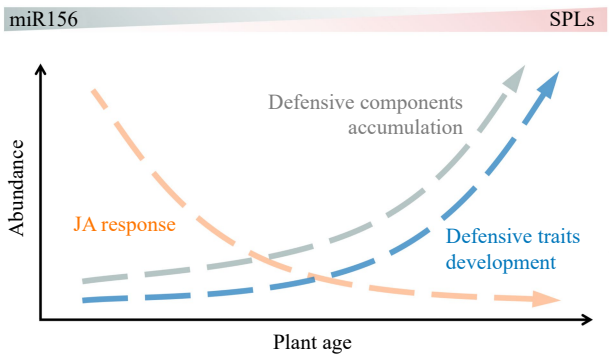

Fig. 5 Age-dependent change in plant resistance to biotic stress.

Generally, expression of miR156 is induced under various abiotic stress conditions to improve stress tolerance[88,89], whereas it is suppressed to accelerate the developmental transition under favorable conditions[90]. This is achieved in part by influencing the anthocyanin biosynthesis through the regulation of the downstream genes *DIHYDROFLAVONOL-4-REDUCTASE* (*DFR*) and *WD40*[90–92]. However, some studies on horticultural plants have reported exceptions. Drought treatment did not regulate miR156 in apple[87,93]. Moreover, expression of miR156 led to weakened salt tolerance in apple, and the overexpression of the miR156-targeted gene *MdSPL13* strengthened salt tolerance by activating the *MdWRKY100* promoter[93]. These adverse roles of miR156 might be partially explained by the differences in the life cycles of woody and herbaceous plants[93]. The findings above suggest that the multiple functional roles of miR156 in stress response in diverse species is worth exploring in horticultural plants.

Other functions

Recent studies have demonstrated a number of novel functions of miR156. Among them, influenced by *DELAY OF GERMINATION1* (*DOG1*), miR156 and miR172 were involved in the regulation of seed dormancy in lettuce (*Lactuca sativa*). This has been verified in *Arabidopsis*, in which higher miR156 expression levels enhanced seed dormancy and delayed flowering while overexpression of miR172 exerted the opposite effect[94]. According to the results, a novel linkage, DOG1-miR156-miR172 interaction, has been revealed between dormancy release and flowering, critical developmental transitions in plant life cycles, and it has subsequently been studied in a variety of plants[95,96]. These studies open the possibility for new pathways in dormancy research in horticultural plants, and study of the integrated mechanism for coordinating these two life cycle transitions would be of great significance to optimize both.

miR156 also comes into play during various stages of somatic embryogenesis, including embryogenic callus differentiation and cotyledon and globular embryo development[97]. Overexpression of csi-miR156a significantly enhanced the capability of somatic embryos in preserved citrus embryogenic callus, with more abundant amyloplasts accumulating in the embryonic cells, leading to a significant increase in starch content[98,99]. In contrast, suppression of csi-miR156a reduced somatic embryo capability and the number of amyloplasts and starch content through the downregulation of the expression of both csi-miR172d and some starch biosynthesis genes[100]. These studies suggest a positive role of miR156 in somatic embryogenesis and provide new insights into enhancing somatic embryo capability in horticultural plants.

miR156 is broadly involved in the regulation of many biological processes in horticultural plants to a remarkable extent (Table 1) and is recognized to be a regulatory hub toward various horticultural traits. Notably, rather than operating independently, miR156-SPL functions in collaboration with other pathways. Hence, cross-talk between age and other signaling pathways is discussed in the next section. Furthermore, studies on miR156 have focused on age-regulated plant growth and development, while the involvement of miR156 in other

biological functions such as stress response have been addressed, but mostly not functionally verified. Therefore, in the final section, we summarize the methods used for functional validation of miR156 with a view to providing useful information for the in-depth study of the function of miR156.

Cross-talk between age and other signaling pathways

Despite showing great functional diversity, the miR156-SPL module does not work in isolation, but rather in concert with other signaling pathways. The juvenile-to-adult phase transition triggered by miR156 is reported to occur following an increase in sugar[112,113]. The glucose-induced repression of miR156 is thought to be conserved in many plants[112] and is partly dependent on the signaling activity of HEXOKINASE1 (HXK1)[113], which, according to a recent study, is required for CURLY LEAF (CLF)- and SWINGER (SWN)-mediated histone H3 lysine 27 (H3K27me3) deposition and glucose-mediated gene repression[114]. Previous studies suggest that CLF and SWN are responsible for the increased level of H3K27me3 at *MIR156A*, which thereby leads to the repressed expression of

Table 1. Biological functions of miR156 in horticultural plants.

Classification	Species	Growth and development	Stress response	Others	Ref.
Vegetables	*Solanum tuberosum*	Tuberization; development of leaves, trichomes, branches, lateral roots and inflorescences		Cytokinin and strigolactone level	[61, 101]
	Solanum lycopersicum	Development of leaves, branches and trichomes; floral induction and flower development; ovary and fruit development	Drought, cold; *Botrytis cinerea* infection		[9, 20, 36, 48 , 59, 60, 75, 86]
	Brassica campestris	Vegetative phase change; heading	Heat		[29, 85]
	Cucumis sativus	Tendril formation; vegetative phase change			[15,16]
	Brassica alboglabra	Seed development			[102]
	Brassica oleracea		*Xanthomonas campestris* infection		[103]
	Lactuca sativa			Seed dormancy	[94]
Fruits	*Malus* spp.	Vegetative phase change; adventitious root formation	Salt, drought	Browning inhibition	[19, 92, 93, 104, 105]
	Citrus sinensis	Vegetative phase change		Somatic embryogenesis	[99, 100]
	Vitis vinifera	Vegetative phase change; flower development in winter; grape berry development and ripening			[65, 106, 107]
	Citrus × *paradise*	Vegetative phase change			[108]
	Morus atropurpurea	Vegetative phase change	Silkworm herbivory		[25, 81]
	Passiflora edulis	Vegetative phase change			[48]
	Saccharum officinarum		Cold, drought and salt		[83]
	Musa acuminata	Fruit ripening	Heat		[58, 84]
	Pyrus spp.	Red peel coloration	Apple stem pitting virus infection	Anthocyanin biosynthesis	[63, 74]
	Vaccinium corymbosum	Fruit coloration		Anthocyanin biosynthesis and chlorophyll degradation	[64]
	Litchi chinensis	Flowering		Anthocyanin biosynthesis	[49, 66]
Ornamental plants	*Paeonia lactiflora*	Flower coloration		Anthocyanin biosynthesis	[10, 53]
	Paeonia delavayi	Vegetative phase change			[26]
	Chrysanthemum morifolium	Flowering time			[42]
	Rosa chinensis	Vegetative phase change; flowering time			[109, 110]
	Vachellia cornigera		Herbivore defense		[82]
	Paulownia fortunei		Paulownia witches' broom (PaWB) infection		[111]
	Pogostemon cablin			Sesquiterpene biosynthesis	[70]
	Gorteria diffusa	Petal spot placement			[54]
	Lilium Oriental Trumpet	Vegetative phase change			[28]
	Viburnum macrocephalum	Differentiation of fertile and sterile flowers			[55]
	Cymbidium goeringii	Reproductive organ development			[56]
	Dendrobium catenatum	Vegetative phase change			[27]
	Petunia spp.	Development of branches and internodes; flowering time			[43]
Tea plants	*Camellia sinensis*	Double flower domestication	*Colletotrichum gloeosporioides* infection	Taste compound (catechin, caffeine, and theanine) biosynthesis	[51, 68, 69, 76, 77]

miR156[115,116]. The above studies indicate that glucose signaling likely regulates miR156 expression through epigenetic modification. In addition, several studies demonstrate that the trehalose 6-phosphate (T6P) pathway acts downstream of sucrose to impact vegetative phase transition. To be specific, T6P, a disaccharide signaling molecule that conveys sucrose status to downstream signaling pathways, is able to regulate the expression level of *SPL3/4/5* through miR156[117]. Additionally, the T6P pathway strongly suppresses the miR156-SPL-mediated age pathway to promote the vegetative phase change[118]. These findings demonstrate that the T6P pathway is identified as signals associated with age to control the miR156/SPL module. Recent research has reported the effect of sucrose treatment on lily juvenile vegetative development. Exogenous sucrose treatment enhanced the growth of seedlings, especially lily bulbs, with a significant decline in miR156 expression and an increasing level of trehalose-6-phosphate synthase (TPS)-coding genes and *LfTPS*s[119]. Bulb flowers, typified by lilies, generally encounter the problem of prolonged breeding cycles due to long juvenile stages and bulb size (and likely carbohydrate accumulation), and miR156 is shown to be pivotal for vegetative phase change in bulb flowers[120]. Uncovering the molecular link of sugar signaling with the miR156-mediated age pathway in bulb flowers will certainly help to address the problem of long juvenile stage in bulb plants.

Strigolactones (SLs), a newly identified class of phytohormones, have also been shown to regulate biological process in horticultural plants in coordination with miR156. A typical example is that exogenous SLs are sufficient for miR156 accumulation in tomato leaves and endogenous SLs act as a molecular link between drought stress and miR156 in tomato[86]. Moreover, several studies have reported the integration of SL signaling and the miR156/SPL module in the regulation of plant architecture[121,122].

The age-dependent pathway also interacts with other flowering pathways at multiple levels to ensure the optimal timing of flowering[123]. The molecular mechanism by which the age pathway is integrated into other flowering time pathways, including the autonomous, GA, photoperiod, and vernalization pathways, has been summarized by a previous review[124]. For instance, TAF15b, an autonomous pathway protein, is revealed to interact with miR156-targeted *SPL1* and both act synergistically to promote early flowering of rose (*Rosa chinensis*)[110]. Notably, the mode of integration between age and other pathways may vary in different species[124].

Functional validation techniques for miR156 in horticultural plants

The techniques for functional validation of miR156 include overexpression and expression suppression. Specifically, overexpression of miR156 is achieved by overexpressing miR156 precursors, and expression suppression could be achieved by two technologies, namely target mimicry (TM) and short tandem target mimicry (STTM). An endogenous long noncoding RNA (lncRNA), *Induced by Phosphate Starvation1* (*IPS1*), was shown to bind to miR399 and cause the abolishment of the cleavage effect of miR399 on *IPS1*. This mechanism of miRNA activity inhibition was termed 'target mimicry'[125]. Artificial target mimics have been shown to be similarly functional in crippling other corresponding miRNAs including miR156[30]. However, IPS1-based TM was also reported to be responsible for the incomplete inactivation of miRNA and work effectively only on a small set of miRNAs[126]. Hence, STTM, which harbors two copies of partially complementary miRNA sequences linked by a short spacer, was developed and demonstrated to be more effective in reducing the levels of targeted miRNAs[127]. As a powerful approach to interrogate the function of miRNAs, STTM has been successfully applied in various horticultural plants to validate the functional role of miR156[92,100]. Despite its effectiveness, STTM still has limitations, exemplified by the fact that STTM targets mature miRNAs and therefore fails to distinguish the functional diversity of each *MIRNA* gene[40]. The precise editing of plant genome sequences has been achieved thanks to the development of CRISPR/Cas9 technology in the past few years and it has been successfully applied to the study of miRNA functions in horticultural plants to create loss-of-function mutagenesis[13,128]. Since it has been recognized that *MIRNA*s coding for the same mature miRNA may still carry out specific functions[13], the application of CRISPR/Cas9 technology has made it possible to examine the functional role of individual *MIR156* genes, which enables the unraveling of a more complex regulatory network of miR156.

Perspectives

Due to its highly conserved nature and significant activity in numerous biological processes, miR156 has the potential to serve as a versatile tool to regulate multiple horticultural traits. Since miR156 is a key regulator of the age pathway, how to shorten the juvenile stage of fruit trees and perennial flowers and achieve the enhancement of quality and yield of horticultural plants through the age-regulated pathway remains a priority for future studies. To achieve the biotechnological modification of horticultural traits, in-depth research is required on several specific issues. First, despite the many important studies reviewed herein, the complex regulatory mechanisms of miR156 in horticultural plants remain largely unknown. Horticultural plants often have characteristics such as special organs and developmental stages that are not present in *Arabidopsis*, such as fruit development in tomato, tuberization in potato, petal spot arrangement in ornamental flowers, tendril formation in cucumber, and heading in Chinese cabbage (Fig. 6). The involvement of miR156 in the development of these special horticultural traits, which are often tightly linked to the economic and ornamental value of horticultural plants, has been reported but has not been fully investigated. It would thus be of great significance to focus on these specific traits for the improvement of horticultural plant quality in the future. Second, due to the diversity of functions performed by miR156, there may be a knock-on effect when it is utilized to improve specific horticultural traits. In this regard, the mechanism by which miR156, as a regulatory hub, manages to balance age with other biological functions is an interesting topic of research. This may be achieved in part through precise regulation of individual genes. Additionally, both *SPL*s and *MIR172*s were reported to be functionally redundant, and may fail to lead to phenotypic differences. Therefore, having a thorough grasp of the specific roles played by individual genes in the age pathway as well as those found downstream of it would be helpful to enable precise engineering. Third, as described above, the age pathway defined by miR156-SPL is

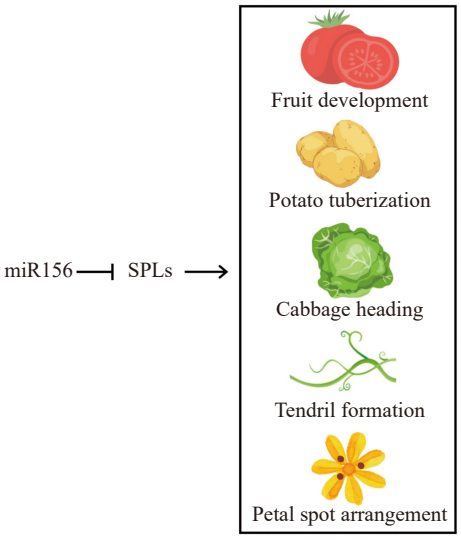

Fig. 6 miR156-SPL module regulates special horticultural traits.

partly tangled with other signaling pathways. To explore the integration mechanism by which the age pathway works synergistically with other signaling molecules in different plant species is a future challenge.

There is considerable variation in the characteristics of different horticultural plants. For example, as mentioned above, fruit trees and certain perennial flowers often undergo a protracted juvenile period, thus it is crucial to speed up the vegetative growth process in order to shorten the breeding cycle and encourage large-scale output. Moreover, given the comparatively short vegetative period of annual vegetables and ornamental plants, extending the vegetative phase may increase production by allowing biomass to accumulate. The coloring of floral and foliar organs, in general, is what confers ornamental plants their desirable characteristics, therefore research into the mechanism of coloration might serve as a foundation for the enhancement of ornamental value and commercial quality. Furthermore, traits such as secondary flowering and petal spots are conducive in increasing the value of ornamental plants further. All of these characteristics are distinct from those of *Arabidopsis*. Therefore, it is pivotal to understand the functional diversity and regulation mechanism of miR156 to improve the quality and benefits of horticultural plants. In future research, the priority is to unveil the regulatory mechanism of miR156, construct its entire regulatory network, and validate its role in regulating life processes with an emphasis on the special developmental phases and organs in horticulture plants.

Author contributions

The authors confirm contribution to the paper as follows: draft manuscript preparation: Xu Y, Gao C; data analysis: Xu Y, Hang Z, Liu J, Ren Z; manuscript revision: Xu Y, Wu Y, Feng S, Xia Y, He Y. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this article and its supplementary information files.

Acknowledgments

We thank Dr. Heng Lian (CAS Center for Excellence in Molecular Plant Sciences, China) for the careful revision of this manuscript. This work was funded by National Natural Science Foundation of China (Grant No. 32002071), National Natural Science Foundation of China (Grant No. 32372743), Zhejiang Sci-Tech University Research Program Start-up Funding (Grant No. 21052103-Y), Zhejiang Province first-class discipline (civil engineering) construction project (Grant No. 11141131282001) and 2022 Hangzhou Agricultural and Social Development Scientific Research Guidance Project (Grant No. 20220919Y1).

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/opr-0024-0008)

Dates

Received 31 October 2023; Accepted 18 February 2024; Published online 2 April 2024

References

1. Voinnet O. 2009. Origin, biogxenesis, and activity of plant microRNAs. *Cell* 136:669–87
2. Song X, Li Y, Cao X, Qi Y. 2019. MicroRNAs and their regulatory roles in plant-environment interactions. *Annual Review of Plant Biology* 70:489–525
3. Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP. 2002. MicroRNAs in plants. *Genes & Development* 16:1616–26
4. Kozomara A, Griffiths-Jones S. 2011. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Research* 39:D152–D157
5. Wang H, Wang H. 2015. The miR156/SPL module, a regulatory hub and versatile toolbox, gears up crops for enhanced agronomic traits. *Molecular Plant* 8:677–88
6. Wang C, Wang Q, Zhu X, Cui M, Jia H, et al. 2019. Characterization on the conservation and diversification of *miRNA156* gene family from lower to higher plant species based on phylogenetic analysis at the whole genomic level. *Functional & Integrative Genomics* 19:933–52
7. Chuck G, Cigan AM, Saeteurn K, Hake S. 2007. The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. *Nature Genetics* 39:544–49
8. Yang L, Conway SR, Poethig RS. 2011. Vegetative phase change is mediated by a leaf-derived signal that represses the transcription of miR156. *Development* 138:245–49
9. Zhang L, Song J, Lin R, Tang M, Shao S, et al. 2022. Tomato *SlMYB15* transcription factor targeted by sly-miR156e-3p positively regulates ABA-mediated cold tolerance. *Journal of Experimental Botany* 73:7538–51
10. Zhao D, Xia X, Wei M, Sun J, Meng J, et al. 2017. Overexpression of herbaceous peony miR156e-3p improves anthocyanin accumulation in transgenic *Arabidopsis thaliana* lateral branches. *3 Biotech* 7:379
11. Li A, Mao L. 2007. Evolution of plant microRNA gene families. *Cell Research* 17:212–18
12. Lin D, Zhu X, Qi B, Gao Z, Tian P, et al. 2022. *SlMIR164A* regulates fruit ripening and quality by controlling *SlNAM2* and *SlNAM3* in tomato. *Plant Biotechnology Journal* 20:1456–69

13. Gupta SK, Vishwakarma A, Kenea HD, Galsurker O, Cohen H, et al. 2021. CRISPR/Cas9 mutants of tomato *MICRORNA164* genes uncover their functional specialization in development. *Plant Physiology* 187:1636–52
14. Klein J, Saedler H, Huijser P. 1996. A new family of DNA binding proteins includes putative transcriptional regulators of the *Antirrhinum majus* floral meristem identity gene *SQUAMOSA*. *Molecular and General Genetics* 250:7–16
15. Wang S, Yang A, Wang H, Xu Y. 2021. Identification and expression analysis of miR156/miR157-SPL pathway genes in cucumber. *Acta Horticulturae Sinica* 48:2227–38
16. Hong Z, Wang X, Fan Z, Wang J, Yang A, et al. 2013. The intrinsic developmental age signal defines an age-dependent climbing behavior in cucumber. *Horticultural Plant Journal* In press
17. Xing S, Salinas M, Höhmann S, Berndtgen R, Huijser P. 2010. miR156-targeted and nontargeted SBP-box transcription factors act in concert to secure male fertility in *Arabidopsis*. *The Plant Cell* 22:3935–50
18. Zhang S, Ling L. 2014. Genome-wide identification and evolutionary analysis of the SBP-box gene family in castor bean. *PLoS One* 9:e86688
19. Jia X, Chen Y, Xu X, Shen F, Zheng Q, et al. 2017. miR156 switches on vegetative phase change under the regulation of redox signals in apple seedlings. *Scientific Reports* 7:14223
20. Cui L, Zheng F, Wang J, Zhang C, Xiao F, et al. 2020. miR156a-targeted SBP-Box transcription factor *SlSPL13* regulates inflorescence morphogenesis by directly activating *SFT* in tomato. *Plant Biotechnology Journal* 18:1670–82
21. Manning K, Tör M, Poole M, Hong Y, Thompson AJ, et al. 2006. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genetics* 38:948–52
22. Poethig RS. 2013. Chapter Five - Vegetative phase change and shoot maturation in plants. *Current Topics in Developmental Biology* 105:125–52
23. Wu G, Poethig RS. 2006. Temporal regulation of shoot development in *Arabidopsis thaliana* by *miR156* and its target *SPL3*. *Development* 133:3539–47
24. Ahsan MU, Hayward A, Powell R, Wilkie J, Beveridge C, et al. 2018. MicroRNA control of flowering and annual crop cycle in tropical/subtropical horticultural. *Acta Horticulturae* 1205:681–86
25. Li H, Luo Y, Ma B, Hu J, Lv Z, et al. 2021. Hierarchical action of mulberry miR156 in the vegetative phase transition. *International Journal of Molecular Sciences* 22:5550
26. Zhu F, Wang S, Xue J, Li D, Ren X, et al. 2018. Morphological and physiological changes, and the functional analysis of *PdSPL9* in the juvenile-to-adult phase transition of *paeonia delavayi*. *Plant Cell, Tissue and Organ Culture* 133:325–37
27. Zheng J, Ma Y, Zhang M, Lyu M, Yuan Y, et al. 2019. Expression pattern of *FT/TFL1* and miR156-targeted *SPL* genes associated with developmental stages in *Dendrobium catenatum*. *International Journal of Molecular Sciences* 20:2725
28. Chen Y, Zhao M, Wang X, Cui J, Ge W, et al. 2022. Key microRNAs and target genes involved in regulating maturation in *Lilium*. *Ornamental Plant Research* 2:9
29. Wang Y, Wu F, Bai J, He Y. 2014. *BrpSPL9* (*Brassica rapa* ssp. *pekinensis SPL9*) controls the earliness of heading time in Chinese cabbage. *Plant Biotechnology Journal* 12:312–21
30. Wu G, Park MY, Conway SR, Wang J, Weigel D, et al. 2009. The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. *Cell* 138:750–59
31. Goebel K. 1889. Ueber die jungendzustände der Pflanzen. *Flora oder Allgemeine Botanische Zeitung* 72:1–45
32. Costa MMR, Yang S, Critchley J, Feng X, Wilson Y, et al. 2012. The genetic basis for natural variation in heteroblasty in *Antirrhinum*. *New Phytologist* 196:1251–59
33. Tang H, Wang J, Wang L, Shang G, Xu Z, et al. 2023. Anisotropic cell growth at the leaf base promotes age-related changes in leaf shape in *Arabidopsis thaliana*. *The Plant Cell* 35:1386–407
34. Pasquet-Kok J, Creese C, Sack L. 2010. Turning over a new 'leaf': multiple functional significances of leaves versus phyllodes in Hawaiian *Acacia koa*. *Plant, Cell & Environment* 33:2084–100
35. Silva PO, Batista DS, Cavalcanti JHF, Koehler AD, Vieira LM, et al. 2019. Leaf heteroblasty in *Passiflora edulis* as revealed by metabolic profiling and expression analyses of the microRNAs miR156 and miR172. *Annals of Botany* 123:1191–203
36. Vendemiatti E, Zsögön A, Silva GFFE, de Jesus FA, Cutri L, et al. 2017. Loss of type-IV glandular trichomes is a heterochronic trait in tomato and can be reverted by promoting juvenility. *Plant Science* 259:35–47
37. Teotia S, Tang G. 2015. To bloom or not to bloom: role of microRNAs in plant flowering. *Molecular Plant* 8:359–77
38. Hyun Y, Richter R, Coupland G. 2017. Competence to flower: age-controlled sensitivity to environmental cues. *Plant Physiology* 173:36–46
39. Wang J, Czech B, Weigel D. 2009. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* 138:738–49
40. Lian H, Wang L, Ma N, Zhou C, Han L, et al. 2021. Redundant and specific roles of individual *MIR172* genes in plant development. *PLoS Biology* 19:e3001044
41. Xie K, Wu C, Xiong L. 2006. Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. *Plant Physiology* 142:280–93
42. Wei Q, Ma C, Xu Y, Wang T, Chen Y, et al. 2017. Control of chrysanthemum flowering through integration with an aging pathway. *Nature Communications* 8:829
43. Zhou Q, Shi J, Li Z, Zhang S, Zhang S, et al. 2021. miR156/157 targets *SPLs* to regulate flowering transition, plant architecture and flower organ size in petunia. *Plant and Cell Physiology* 62:839–57
44. Tanaka N. 2012. Gibberellin is not a regulator of *miR156* in rice juvenile-adult phase change. *Rice* 5:25
45. Li X, Guo F, Ma S, Zhu M, Pan W, et al. 2019. Regulation of flowering time via miR172-mediated *APETALA2-like* expression in ornamental gloxinia (*Sinningia speciosa*). *Journal of Zhejiang University-SCIENCE B* 20:322–31
46. Yamaguchi A, Wu M, Yang L, Wu G, Poethig RS, et al. 2009. The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of *LEAFY, FRUITFULL*, and *APETALA1*. *Developmental Cell* 17:268–78
47. Kim JJ, Lee JH, Kim W, Jung HS, Huijser P, et al. 2012. The *microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3* module regulates ambient temperature-responsive flowering via *FLOWERING LOCUS T* in Arabidopsis. *Plant Physiology* 159:461–78
48. Silva GFF, Silva EM, Correa JPO, Vicente MH, Jiang N, et al. 2019. Tomato floral induction and flower development are orchestrated by the interplay between gibberellin and two unrelated microRNA-controlled modules. *New Phytologist* 221:1328–44
49. Xiao Q, Su Z, Chen H, Shen J. 2019. Genome-wide identification and involvement of litchi *SPL* genes in flowering in response to cold and leaf maturity. *The Journal of Horticultural Science and Biotechnology* 94:428–40
50. Jung JH, Lee S, Yun J, Lee M, Park CM. 2014. The miR172 target TOE3 represses *AGAMOUS* expression during *Arabidopsis* floral patterning. *Plant Science* 215–216:29–38
51. Li X, Li J, Fan Z, Liu Z, Tanaka T, et al. 2017. Global gene expression defines faded whorl specification of double flower domestication in *Camellia*. *Scientific Reports* 7:3197
52. Gou JY, Felippes FF, Liu CJ, Weigel D, Wang JW. 2011. Negative regulation of anthocyanin biosynthesis in *Arabidopsis* by a miR156-targeted SPL transcription factor. *The Plant Cell* 23:1512–22
53. Zhao D, Wei M, Shi M, Hao Z, Tao J. 2017. Identification and comparative profiling of miRNAs in herbaceous peony (*Paeonia lactiflora* Pall.) with red/yellow bicoloured flowers. *Scientific Reports* 7:44926

54. Kellenberger RT, Ponraj U, Delahaie B, Fattorini R, Balk J, et al. 2023. Multiple gene co-options underlie the rapid evolution of sexually deceptive flowers in *Gorteria diffusa*. *Current Biology* 33:1502–1512.e8
55. Li W, He Z, Zhang L, Lu Z, Xu J, et al. 2017. miRNAs involved in the development and differentiation of fertile and sterile flowers in *Viburnum macrocephalum* f. *keteleeri*. *BMC Genomics* 18:783
56. Yang F, Zhu G, Wang Z, Liu H, Xu Q, et al. 2017. Integrated mRNA and microRNA transcriptome variations in the multi-tepal mutant provide insights into the floral patterning of the orchid *Cymbidium goeringii*. *BMC Genomics* 18:367
57. Wang S, Wu K, Yuan Q, Liu X, Liu Z, et al. 2012. Control of grain size, shape and quality by *OsSPL16* in rice. *Nature Genetics* 44:950–54
58. Bi F, Meng X, Ma C, Yi G. 2015. Identification of miRNAs involved in fruit ripening in Cavendish bananas by deep sequencing. *BMC Genomics* 16:776
59. Zhang X, Zou Z, Zhang J, Zhang Y, Han Q, et al. 2011. Over-expression of sly-miR156a in tomato results in multiple vegetative and reproductive trait alterations and partial phenocopy of the *sft* mutant. *FEBS Letters* 585:435–39
60. Silva GFFE, Silva EM, da Silva Azevedo M, Guivin MAC, Ramiro DA, et al. 2014. microRNA156-targeted SPL/SBP box transcription factors regulate tomato ovary and fruit development. *The Plant Journal* 78:604–18
61. Bhogale S, Mahajan AS, Natarajan B, Rajabhoj M, Thulasiram HV, et al. 2014. *MicroRNA156*: a potential graft-transmissible microRNA that modulates plant architecture and tuberization in *Solanum tuberosum* ssp. *andigena*. *Plant Physiology* 164:1011–27
62. Martin A, Adam H, Díaz-Mendoza M, Zurczak M, González-Schain ND, et al. 2009. Graft-transmissible induction of potato tuberization by the microRNA *miR172*. *Development* 136:2873–81
63. Qian M, Ni J, Niu Q, Bai S, Bao L, et al. 2017. Response of miR156-SPL module during the red peel coloration of nagging-treated Chinese sand pear (*Pyrus pyrifolia* Nakai). *Frontiers in Physiology* 8:550
64. Li X, Hou Y, Xie X, Li H, Li X, et al. 2020. A blueberry *MIR156a-SPL12* module coordinates the accumulation of chlorophylls and anthocyanins during fruit ripening. *Journal of Experimental Botany* 71:5976–89
65. Wang B, Wang J, Wang C, Shen W, Jia H, et al. 2016. Study on expression modes and cleavage role of miR156b/c/d and its target gene *Vv-SPL9* during the whole growth stage of grapevine. *Journal of Heredity* 107:626–34
66. Liu R, Lai B, Hu B, Qin Y, Hu G, et al. 2016. Identification of microRNAs and their target genes related to the accumulation of anthocyanins in *Litchi chinensis* by high-throughput sequencing and degradome analysis. *Frontiers in Plant Science* 7:2059
67. Zhou H, Lin-Wang K, Wang H, Gu C, Dare AP, et al. 2015. Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by NAC transcription factors. *The Plant Journal* 82:105–21
68. Zhao S, Mi X, Guo R, Xia X, Liu L, et al. 2020. The biosynthesis of main taste compounds is coordinately regulated by miRNAs and phytohormones in tea plant (*Camellia sinensis*). *Journal of Agricultural and Food Chemistry* 68:6221–36
69. Fan K, Fan D, Ding Z, Su Y, Wang X. 2015. Cs-miR156 is involved in the nitrogen form regulation of catechins accumulation in tea plant (*Camellia sinensis* L.). *Plant Physiology and Biochemistry* 97:350–60
70. Yu Z, Wang L, Zhao B, Shan C, Zhang Y, et al. 2015. Progressive regulation of sesquiterpene biosynthesis in *Arabidopsis* and Patchouli (*Pogostemon cablin*) by the miR156-targeted SPL transcription factors. *Molecular Plant* 8:98–110
71. Price PW. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* 62:244–51
72. Kus JV, Zaton K, Sarkar R, Cameron RK. 2002. Age-related resistance in *Arabidopsis* is a developmentally regulated defense response to *Pseudomonas syringae*. *The Plant Cell* 14:479–90
73. Mao Y, Liu Y, Chen D, Chen F, Fang X, et al. 2017. Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance. *Nature Communications* 8:13925
74. Zhang Q, Zhang Y, Wang S, Hao L, Wang S, et al. 2019. Characterization of genome-wide microRNAs and their roles in development and biotic stress in pear. *Planta* 249:693–707
75. Li L, Jin H, Liu S, Zou J, Li T. 2020. Expression analysis of miRNA with tomato JA deficient mutant response to *Botrytis cinerea* infection. *Acta Horticulturae Sinica* 47:1323–34
76. Jeyaraj A, Elango T, Yu Y, Chen X, Zou Z, et al. 2021. Impact of exogenous caffeine on regulatory networks of microRNAs in response to *Colletotrichum gloeosporioides* in tea plant. *Scientia Horticulturae* 279:109914
77. Jeyaraj A, Wang X, Wang S, Liu S, Zhang R, et al. 2019. Identification of regulatory networks of microRNAs and their targets in response to *Colletotrichum gloeosporioides* in tea plant (*Camellia sinensis* L.). *Frontiers in Plant Science* 10:1096
78. Hao F, Yang G, Zhou H, Yao J, Liu D, et al. 2021. Genome-wide identification and transcriptional expression profiles of transcription factor *WRKY* in common walnut (*Juglans regia* L.). *Genes* 12:1444
79. Pinweha N, Netrphan S, Sojikul P, Viboonjun U, Sae-Lim P, et al. 2022. Cross-kingdom microRNA transfer for the control of the anthracnose disease in cassava. *Tropical Plant Pathology* 47:362–77
80. Ge Y, Han J, Zhou G, Xu Y, Ding Y, et al. 2018. Silencing of miR156 confers enhanced resistance to brown planthopper in rice. *Planta* 248:813–26
81. Li H, Ma B, Luo Y, Wei W, Yuan J, et al. 2022. The mulberry *SPL* gene family and the response of *MnSPL7* to silkworm herbivory through activating the transcription of *MnTT2L2* in the catechin biosynthesis pathway. *International Journal of Molecular Sciences* 23:1141
82. Leichty AR, Poethig RS. 2019. Development and evolution of age-dependent defenses in ant-acacias. *Proceedings of the National Academy of Sciences of the United States of America* 116:15596–601
83. Yang Y, Zhang X, Su Y, Zou J, Wang Z, et al. 2017. miRNA alteration is an important mechanism in sugarcane response to low-temperature environment. *BMC Genomics* 18:833
84. Zhu H, Zhang Y, Tang R, Qu H, Duan X, et al. 2019. Banana sRNAome and degradome identify microRNAs functioning in differential responses to temperature stress. *BMC Genomics* 20:33
85. Ahmed W, Xia Y, Zhang H, Li R, Bai G, et al. 2019. Identification of conserved and novel miRNAs responsive to heat stress in flowering Chinese cabbage using high-throughput sequencing. *Scientific Reports* 9:14922
86. Visentin I, Pagliarani C, Deva E, Caracci A, Turečková V, et al. 2020. A novel strigolactone-miR156 module controls stomatal behaviour during drought recovery. *Plant, Cell & Environment* 43:1613–24
87. Niu C, Li H, Jiang L, Yan M, Li C, et al. 2019. Genome-wide identification of drought-responsive microRNAs in two sets of *Malus* from interspecific hybrid progenies. *Horticulture Research* 6:75
88. Stief A, Altmann S, Hoffmann K, Pant BD, Scheible WR, et al. 2014. *Arabidopsi miR156s* regulates tolerance to recurring environmental stress through *SPL* transcription factors. *The Plant Cell* 26:1792–807
89. Matthews C, Arshad M, Hannoufa A. 2019. Alfalfa response to heat stress is modulated by microRNA156. *Physiologia Plantarum* 165:830–42
90. Cui L, Shan J, Shi M, Gao J, Lin H. 2014. The *miR156-SPL9-DFR* pathway coordinates the relationship between development and abiotic stress tolerance in plants. *The Plant Journal* 80:1108–17
91. Feyissa BA, Arshad M, Gruber MY, Kohalmi SE, Hannoufa A. 2019. The interplay between *miR156/SPL13* and *DFR/WD40-1* regulate drought tolerance in alfalfa. *BMC Plant Biology* 19:434

92. Chen G, Wang Y, Liu X, Duan S, Jiang S, et al. 2023. The *MdmiR156n* regulates drought tolerance and flavonoid synthesis in Apple calli and *Arabidopsis*. *International Journal of Molecular Sciences* 24:6049
93. Ma Y, Xue H, Zhang F, Jiang Q, Yang S, et al. 2021. The miR156/SPL module regulates apple salt stress tolerance by activating MdWRKY100 expression. *Plant Biotechnology Journal* 19:311–23
94. Huo HQ, Wei SH, Bradford KJ. 2016. *DELAY OF GERMINATION1* (*DOG1*) regulates both seed dormancy and flowering time through microRNA pathways. *Proceedings of the National Academy of Sciences of the United States of America* 113:E2199–E2206
95. Miao C, Wang Z, Zhang L, Yao J, Hua K, et al. 2019. The grain yield modulator miR156 regulates seed dormancy through the gibberellin pathway in rice. *Nature Communications* 10:3822
96. Liu M, Shi Z, Zhang X, Wang M, Zhang L, et al. 2019. Inducible overexpression of *Ideal Plant Architecture1* improves both yield and disease resistance in rice. *Nature Plants* 5:389–400
97. Siddiqui ZH, Abbas ZK, Ansari MW, Khan MN. 2019. The role of miRNA in somatic embryogenesis. *Genomics* 111:1026–33
98. Long J, Liu C, Feng M, Liu Y, Wu X, et al. 2018. miR156-*SPL* modules regulate induction of somatic embryogenesis in citrus callus. *Journal of Experimental Botany* 69:2979–93
99. Liu M, Wu X, Long J, Guo W. 2017. Genomic characterization of miR156 and *SQUAMOSA promoter binding protein-like* genes in sweet orange (*Citrus sinensis*). *Plant Cell, Tissue and Organ Culture* 130:103–16
100. Feng M, Lu M, Long J, Yin Z, Jiang N, et al. 2022. miR156 regulates somatic embryogenesis by modulating starch accumulation in citrus. *Journal of Experimental Botany* 73:6170–85
101. Luo H, Yang J, Feng Y, Zhang H, Liu S, et al. 2021. The effect of stu-miR156 silencing by STTM technology on potato lateral root development. *Acta Horticulturae Sinica* 48:531–38
102. Tang W, Zhao Y, Zeng J, Li Z, Fu Z, et al. 2022. Integration of small RNA and transcriptome sequencing reveal the roles of miR395 and ATP sulfurylase in developing seeds of Chinese kale. *Frontiers in Plant Science* 12:778848
103. Santos LS, Maximiano MR, Megias E, Pappas M, Ribeiro SG, et al. 2019. Quantitative expression of microRNAs in *Brassica oleracea* infected with *Xanthomonas campestris* pv. *campestris*. *Molecular Biology Reports* 46:3523–29
104. Chen C, Liu C, Jiang A, Zhao Q, Zhang Y, et al. 2020. miRNA and degradome sequencing identify miRNAs and their target genes involved in the browning inhibition of fresh-cut apples by hydrogen sulfide. *Journal of Agricultural and Food Chemistry* 68:8462–70
105. Xu X, Li X, Hu X, Wu T, Wang Y, et al. 2017. High miR156 expression is required for auxin-induced adventitious root formation via *MxSPL26* independent of PINs and ARFs in *Malus xiaojinensis*. *Frontiers in Plant Science* 8:1059
106. Cui M, Wang C, Zhang W, Pervaiz T, Haider MS, et al. 2018. Characterization of Vv-miR156: *Vv-SPL* pairs involved in the modulation of grape berry development and ripening. *Molecular Genetics and Genomics* 293:1333–54
107. Wang C, Zhang Y, Fang J, Song C, Liu H, et al. 2012. Spatiotemporal expression of microRNA156b and microRNA172c and their target genes during flower development of winter buds growing on cut-back treated shoots of grapevine. *Journal of Nanjing Agricultural University* 35:59–64
108. Yamane H, Mimura S, Tao R. 2018. Precocious flowering of Citrus seedlings and expression analysis of *FT/TFL1* family genes and miR156/172 involved in vegetative phase transition. *II Asian Horticultural Congress, Chengdu*, 2016:25–30
109. Tan J, Yi X, Luo L, Yu C, Wang J, et al. 2021. RNA-seq and sRNA-seq analysis in lateral buds and leaves of juvenile and adult roses. *Scientia Horticulturae* 290:110513
110. Yu R, Xiong Z, Zhu X, Feng P, Hu Z, et al. 2023. RcSPL1–RcTAF15b regulates the flowering time of rose (*Rosa chinensis*). *Horticulture Research* 10:uhad083
111. Fan G, Cao X, Niu S, Deng M, Zhao Z, et al. 2015. Transcriptome, microRNA, and degradome analyses of the gene expression of Paulownia with phytoplamsa. *BMC Genomics* 16:896
112. Yu S, Cao L, Zhou C, Zhang T, Lian H, et al. 2013. Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. *eLife* 2:e00269
113. Yang L, Xu M, Koo Y, He J, Poethig RS. 2013. Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of *MIR156A* and *MIR156C*. *eLife* 2:e00260
114. Liu Y, Bai Y, Li N, Li M, Liu W, et al. 2022. HEXOKINASE1 forms a nuclear complex with the PRC2 subunits CURLY LEAF and SWINGER to regulate glucose signaling. *Journal of Integrative Plant Biology* 64:1168–80
115. Xu M, Hu T, Smith MR, Poethig RS. 2016. Epigenetic regulation of vegetative phase change in *Arabidopsis*. *The Plant Cell* 28:28–41
116. Xu Y, Guo C, Zhou B, Li C, Wang H, et al. 2016. Regulation of vegetative phase change by SWI2/SNF2 chromatin remodeling ATPase BRAHMA. *Plant Physiology* 172:2416–28
117. Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, et al. 2013. Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. *Science* 339:704–07
118. Ponnu J, Schlereth A, Zacharaki V, Działo MA, Abel C, et al. 2020. The trehalose 6-phosphate pathway impacts vegetative phase change in *Arabidopsis thaliana*. *The Plant Journal* 104:768–80
119. Zhang Q, Zhang M, Zhao Y, Hu H, Huang Y, et al. 2022. Identification of trehalose-6-phosphate synthase (TPS)-coding genes involved in flowering induction of *Lilium* × *formolongi*. *Plant Physiology and Biochemistry* 171:84–94
120. Langens-Gerrits M, De Klerk GJ, Croes A. 2003. Phase change in lily bulblets regenerated in vitro. *Physiologia Plantarum* 119:590–97
121. Liu J, Cheng X, Liu P, Sun J. 2017. miR156-targeted SBP-box transcription factors interact with DWARF53 to regulate *TEOSINTE BRANCHED1* and *BARREN STALK1* expression in bread wheat. *Plant Physiology* 174:1931–48
122. Liu Y, Wu G, Zhao Y, Wang HH, Dai Z, et al. 2021. DWARF53 interacts with transcription factors UB2/UB3/TSH4 to regulate maize tillering and tassel branching. *Plant Physiology* 187:947–62
123. Wang J. 2014. Regulation of flowering time by the miR156-mediated age pathway. *Journal of Experimental Botany* 65:4723–30
124. Bergonzi S, Albani MC, Ver Loren van Themaat E, Nordström KJV, Wang R, et al. 2013. Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. *Science* 340:1094–97
125. Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, et al. 2007. Target mimicry provides a new mechanism for regulation of microRNA activity. *Nature Genetics* 39:1033–37
126. Todesco M, Rubio-Somoza I, Paz-Ares J, Weigel D. 2010. A collection of target mimics for comprehensive analysis of microRNA function in *Arabidopsis thaliana*. *PLoS Genetics* 6:e1001031
127. Yan J, Gu Y, Jia X, Kang W, Pan S, et al. 2012. Effective small RNA destruction by the expression of a short tandem target mimic in *Arabidopsis*. *The Plant Cell* 24:415–27
128. Bortesi L, Fischer R. 2015. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances* 33:41–52