Involvement of miR156 in the Regulation of Vegetative Phase Change in Plants

Lu Zhang¹ and You-biao Hu²

College of Earth Science and the Environment, Anhui University of Science and Technology Huainan, 232001, China

Hua-sen Wang¹, Sheng-jun Feng¹, and Yu-ting Zhang¹

Zhejiang Province Key Laboratory for Quality Improvement of Agricultural Products, College of Agriculture and Food Science, and the Research Center of Bio-Breeding Industry, Zhejiang Province Key Laboratory for Wood Science and Technology, Zhejiang A & F University, Lin'an 311300, Zhejiang, China

ADDITIONAL INDEX WORDS. juvenile stage, adult stage, microRNA, SPLs, epigenetic modification

ABSTRACT. Plant growth and development are determined by complex exogenous and endogenous cues. A plant follows several temporally distinct developmental stages, including embryonic, vegetative, and reproductive. The vegetative stage, which is usually the longest stage, can be subdivided into juvenile and adult phases. The transition from the juvenile to the adult phase, also called the vegetative phase change, is characterized by anatomical, morphological, and physiological changes in the vegetative parts of the shoot. Recent studies in several systems have identified the genetic temporal mechanisms of this process, which is regulated by an endogenous age cue (i.e., microRNA156/157) and its targeted genes (i.e., *Squamosa promoter binding protein-box transcription factors*). This review summarizes the recent advances in the study of the underlying regulatory mechanisms of vegetative phase change. This review also describes the modes of miRNA action and the functions of their targeted genes in this highly conserved developmental process.

Plant growth is characterized by increases in volume and weight. Plant development is the formation and completion of organs and physiological functions. Both processes show sequential qualitative changes. Different plant species have different growth and development characteristics. Annual plants complete the progression of germination to death within 1 year, whereas biennial and perennial plants take at least 2 years or more to finish their life cycles. Despite their different lengths of life cycles, these plants undergo the same growth stages. The development of a mature flowering plant usually follows several temporally distinct stages, such as embryonic, vegetative, and reproductive. The vegetative stage, which is usually the longest stage, can be subdivided into juvenile and adult phases. The transition to the adult phase is indicated by the flowering competence of a plant. Before a plant acquires its reproductive competence, it usually undergoes evident anatomical, morphological, and physiological changes in the vegetative parts of the shoot; such changes involve leaf shape and size, branch pattern, wax layer appearance, trichome distribution, cell shape, leaf vein pattern, phyllotaxy, cell staining capability, anthocyanin and phytochemical accumulation, adventitious root production, and disease or herbivore resistance (Lawson and Poethig, 1995). These changes yield distinct features during the juvenile and adult phases (Telfer et al., 1997). However, research on the changes during vegetative phase is limited to morphological and physiological aspects because these are not harder to detect. Therefore, the mechanisms underlying the changes in the genetic system-mediated vegetative phase in

plants remain unknown. This review summarizes the advances in understanding the molecular basis of vegetative phase change (juvenile to adult phases), especially miRNAs involvement, which was testified as the master regulators of vegetative phase change (Poethig, 2013).

Plant Morphological Characteristics of Vegetative Phase Change

The length of the juvenile phase varies depending on the plant species. Poethig (2009) considered leaf morphological variation to indicate juvenile-to-adult vegetative transition. Woody perennials display prominent phenotypical changes in their long juvenile phase, which lasts anywhere from a few weeks to several years. For example, 1-month-old canadian poplar (*Populus × canadensis*) have small, oval leaves, whereas 1-year-old trees have large and deltoid leaves (Wang et al., 2011). Knight (1795) found through grafting experiments that old and young stems in the same plant grafted under the same conditions show different development characteristics; old stems flower in the second year, whereas young stems do not blossom. This phenomenon can be attributed to the fact that the juvenile-to-adult transition progresses from the stem base to the tip; thus, different plant parts are in different stages of maturity. In general, the base of the tree is in the juvenile stage, the tip is in the adult, and the middle part is somewhere in between. Herbaceous annuals also have subtle phenotypic changes during this phase transition. For example, arabidopsis (*Arabidopsis thaliana*) and maize (*Zea mays*) have a short juvenile phase that lasts for only a few days to several weeks. In arabidopsis, early leaves are small and round, have long petioles, and have a simple rosette shape. Adult leaves are slightly elongated, with serrated margins and short petioles. The two phases are usually marked by the production of trichomes, which cover both sides of adult blades but only the adaxial side of juvenile leaves (Telfer and Poethig, 1998; Telfer et al., 1997). In maize, obvious differences

Received for publication 27 Mar. 2015. Accepted for publication 30 July 2015. We gratefully acknowledge the financial support by the National Natural Science Foundation of China (31101554, 31300997) and the Natural Science Foundation of Zhejiang province (Y3110340 and LQ12C15002).

¹These authors contributed equally to this work.

²Corresponding author. E-mail: whsyh66@163.com.

between the juvenile and adult phases can be found in the leaf epidermis. Juvenile leaves are covered with epicuticular wax, whereas adult leaves have epidermal hairs without wax (Bongard et al., 1996; Freeling and Lane, 1994; Lawson and Poethig, 1995). In soybean (*Glycine max*), juvenile and adult traits are distinguished by the complexity and phyllotaxy of their leaves (Yoshikawa et al., 2013). In rice (*Oryza sativa*), the structure of the stem, the size of the shoot apical meristem, the rate of photosynthesis, and the presence of the midrib are the markers of the juvenile-to-adult transition (Asai et al., 2002). A previous study on *Brassica rapa* ssp. *pekinensis* revealed that the direction of leaf curvature reflects the vegetative phase change (Wang et al., 2014). However, this transition is not solely marked by morphological changes. Different physiological characteristics also appear during development. The juvenile phase is marked by rapid growth, respiration, nucleic acid metabolism, and protein synthesis. After entering the adult phase, plants demonstrate matured organs and gradually decreased metabolic and physiological activities.

The growth of vegetative organs is highly important to humans, since growth of vegetative organs directly affects crop yield. If reproductive organs are the ones to be harvested, then the growth of vegetative organs considerably influences the growth of the reproductive organs because most of the nutrients needed for the formation and development of reproductive organs are supplied by vegetative organs.

Molecular Regulatory Mechanisms Underlying the Juvenile-to-adult Transition

The possible triggers of juvenile-to-adult transition are endogenous cues and environmental conditions, including daylength, light intensity, light quality (phytochrome B), temperature, and gibberellic acid concentration (Willmann and Poethig, 2005). In 1990, Poethig emphasized that vegetative phase change is mainly regulated by genetic temporal programs. Previous studies reported that *Teopod* mutations *Tp1*, *Tp2*, and *Tp3* in maize prolong the expression of juvenile traits while still allowing subsequent completion of the vegetative phase change. Poethig (1990) suggested that the juvenile and adult phases in plants may be regulated by two independent pathways. This finding laid the foundation for further studies on the molecular mechanisms of vegetative phase change.

Small RNA miR156 regulates the vegetative transition

miRNAs are non-coding RNAs in eukaryotes (Bartel, 2004). These RNAs regulate gene expression through transcript cleavage and translational inhibition. In arabidopsis, *HST* is the ortholog of *exportin 5* (*EXP5*), which functions in exporting pre-microRNAs (pre-miRNAs) and tRNAs into the cytoplasm in mammalian cells (Park et al., 2005). In arabidopsis, *Hst* mutants consistently produce fewer juvenile leaves than wild type and promote flowering without influencing the development of adult leaves (Telfer and Poethig, 1998). These phenomena implied that plant miRNAs are involved in vegetative phase change. To identify the function of *HST* in phase change, scientists analyzed the expression levels of various microRNAs in *Hst* mutants and found that a highly conserved microRNA (i.e., miR156) is crucial in vegetative phase change. Constitutive overexpression of miR156 prolongs the juvenile phase and moderately delays vegetative phase change (Chuck et al., 2007; Wang et al., 2009; Wu and Poethig, 2006; Wu et al., 2009). By contrast, reduced

miR156 level with target mimicry or in loss-of-function mutations of miR156A and miR156C (Yang et al., 2013; Yu et al., 2013) promotes vegetative phase change and early flowering (Franco-Zorrilla et al., 2007).

miR156 regulates vegetative phase change by repressing the expression of special transcription factors called squamosa promoter binding protein-like (SBP/SPL) proteins. In the arabidopsis genome, 11 of the 17 *SPL* genes are targeted by miR156. These 11 genes can be grouped into four clades, namely, *SPL3/4/5*, *SPL9/15*, *SPL2/10/11*, and *SPL6/SPL13a/b*. *SPL3/SPL4/SPL5* as shown in Table 1 induce the appearance of adult leaf characteristics, but exert minimal or no influence on leaf shape. *SPL9/10/11/15* promotes the development of adult leaf morphology, retard leaf initiation rate, and accelerate flowering (Wang et al., 2008). Initially, miR156 is highly expressed in plants. Vegetative phase change starts when miR156 expression levels decline, leading to a reduced abundance of SBP/SPL proteins (Wu and Poethig, 2006). The transition also involves another miRNA, namely, miR172, which plays an equally important role in plant development. miR172 exhibits a complementary expression pattern with miR156; i.e., it increases with decreasing miR156; moreover, miR172 overexpression suppresses the level of its target genes *AP2* (*apetala 2*), *TOE1* (*target of EGR1 protein 1*), and *TOE2* to accelerate vegetative phase change and flowering (Aukerman and Sakai 2003; Chen, 2004; Jung et al., 2007; Wu et al., 2009). The complex regulatory mechanism underlying miR156/*SPLs*/miR172 expression in arabidopsis can be described as follows: miR156 regulates miR172 expression via *SPL9* which, along with *SPL10*, directly promotes miR172b transcription. miR172b and miR156a are positively regulated by their target transcription factors, suggesting that the expression of these targets is modulated by a negative feedback loop (Wu et al., 2009) as shown in Fig. 1. The sequences and functions of miR156/*SPLs* are evolutionarily conserved in various plants. The overexpression of miR156 causes similar morphologies in arabidopsis (Wang et al., 2009; Wu and Poethig, 2006; Wu et al., 2009), maize (Chuck et al., 2007), tomato [*Solanum lycopersicum* (Zhang et al., 2011)], canadian poplar (Wang et al., 2011), *Torenia fournieri* (Shikata et al., 2012), rice (Xie et al., 2006, 2012), *Panicum virgatum* (Chuck et al., 2011; Fu et al., 2012), soybean (Yoshikawa et al., 2013), chinese cabbage [*Brassica rapa* var. *pekinensis* (Wang et al., 2014)], and potato [*Solanum tuberosum* (Bhogale et al., 2014)]. Chinese cabbage, which has a high homology with arabidopsis, must undergo two further steps, namely, folding and heading, after the seedling and rosette stages. Transgenic plants that overexpress *Brp-miR156a* have prolonged juvenile and early adult phases. By contrast, overexpression of a mutated miR156-resistant form of *BrpSPL9-2* shortens the juvenile and early adult phases (Wang et al., 2014). Maize dominant mutant *Corngrass* (*Cg*) has slender leaves, increased tillers, and exuberant vegetative phase (Singleton, 1951). Recently, Chuck et al. (2007) have reported this phenomenon is caused by the increased abundance of miR156b and miR156c in *Cg* (Chuck et al., 2007). *Tp1* and *Tp2* also share analogous phenotypes with *Cg* (Poethig, 1988). Increased expression of miR156 is responsible for these traits. In addition, another type of maize with a mutated *Glossy15* undergoes an abbreviated juvenile stage because of the loss of the miR172 target locus in the *AP2* region (Lauter et al., 2005). Similar phenotypes can also be observed in rice with overexpressed miR156 (Xie et al., 2006, 2012).

Table 1. Genes involved in vegetative phase change.

Gene name	Abbreviation
MicroRNA156/157	miR156/157
MicroRNA172	miR172
<i>Squamosa promoter binding protein-box transcription factors 2/3/4/5/6/9/10/11/13/15</i>	<i>SPL 2/3/4/5/6/9/10/11/13/15</i>
<i>Teopod1/2</i>	<i>TP1/2</i>
<i>Hasty</i>	<i>HST</i>
<i>Apetala 2</i>	<i>AP2</i>
<i>Target of EGR1 protein 1/2</i>	<i>TOE1 /2</i>
<i>Corngrass</i>	<i>CG</i>
<i>Glossy15</i>	<i>GL15</i>
<i>Peter pan syndrome</i>	<i>PPS</i>
<i>Gibberellin1</i>	<i>GA1</i>
<i>Rubisco</i>	<i>RBCS</i>
<i>Chlorina1</i>	<i>CHI</i>
<i>Hexokinases1</i>	<i>HXK1</i>
<i>Double-stranded RNA-binding protein 1</i>	<i>HYL1</i>
<i>Dicer-like1</i>	<i>DCL1</i>
<i>Serrate</i>	<i>SE</i>
<i>ARGONAUTE1</i>	<i>AGO1</i>
MicroRNA168	miR168
<i>Squint</i>	<i>SQN</i>
<i>Suppressor of gene silencing2/3</i>	<i>SGS2/3</i>
<i>Silencing defective1</i>	<i>SDE1</i>
<i>RNA-dependent polymerase6</i>	<i>RDR6</i>
<i>Meaea</i>	<i>MEA</i>
<i>Swinger</i>	<i>SWN</i>
<i>Trichomeless1</i>	<i>TCL1</i>
<i>Triptychon</i>	<i>TRY</i>
MicroRNA171	miR171
<i>Lost meristems</i>	<i>LOMs</i>
<i>Cup-shaped cotyledon</i>	<i>CUC</i>
<i>Teosinte branched1/cycloidea/pcf</i>	<i>TCP</i>
MicroRNA319	miR319
MicroRNA164	miR164
<i>Constans</i>	<i>CO</i>
<i>Flowering locus t</i>	<i>FT</i>
<i>Flowering locus d</i>	<i>FD</i>
<i>Leafy</i>	<i>LFY</i>
<i>Fruitfull</i>	<i>FUL</i>
<i>Suppressor of overexpression of constans1</i>	<i>SOC1</i>
<i>Agamous-like 24</i>	<i>AGL42</i>
<i>Flowering locus c</i>	<i>FLC</i>

Research in rice vegetative phase change identified a unique mutant, *Mori1*, which is defective in vegetative phase change. This mutant maintains dwarf architecture and small leaves, such as the second leaves in wild type during the entire life cycle (Asai et al., 2002). *PPS* in rice delays the vegetative phase by retarding the miR156–miR172 regulatory module and inhibiting the expression of gibberellin (GA)-related genes. Double mutation experiments demonstrated that *Mori1PPs* displays *Mori1* unique phenotype; this result indicates that *MORI1* is the epistatic gene of *PPS* (Tanaka et al., 2011). Previous studies uncovered the conserved function of miR156 in the regulation of tuber development in Solanaceae, such as

tomato (Eviatar-Ribak et al., 2013; Salinas et al., 2012; Zhang et al., 2011) and potato (Bhogale et al., 2014; Eviatar-Ribak et al., 2013). However, whether miR156 is involved in developmental phase transitions of them remains unclear. Quantitative polymerase chain reaction (q-PCR) and gel shift assays indicate the regulation of miR172 by miR156 through *StSPL9*, which supports miR172 regulation via the miR156–*SPL9* module. Potato plants that overexpress miR156 were speculated to have a prolonged vegetative phase change and decreased levels of miR172 and *StSP6*, thereby affecting the production of tubers. In a recent study, Bhogale et al. (2014) have overexpressed the potato miR156a gene in tobacco (*Nicotiana tabacum*) and found that miR156-overexpressing plants exhibit a bushy appearance that is similar to other plants (Cho et al., 2012; Chuck et al., 2011; Fu et al., 2012; Wang et al., 2011; Wu and Poethig, 2006; Wu et al., 2009; Xie et al., 2006, 2012). These findings provided new insights into the function of miR156 in the Solanaceae and prompted us to further investigate whether miR156 regulates vegetative phase change in Solanaceae plants. Wang et al. (2011) showed that overexpressed miR156 prolongs the expression of the juvenile phase in the tree canadian poplar. Their results integrated the information about vegetative phase change across species and strongly suggest that the mechanism underlying the juvenile-to-adult transition is conserved throughout flowering plants.

Upstream regulatory network of miR156/SPLs

The mechanisms of miR156–SPLs in the vegetative phase change pathway were studied in various plants, but the upstream regulatory network remains poorly understood. In arabidopsis, the abundance of miR156 decreases with age. For example, the expression of pri-MIR156A in the third leaves is reduced by ≈ 5 -fold compared with the first and second leaves; in the seventh leaves, the level drastically drops by ≈ 100 -fold (Yang et al., 2013). Similarly, the expression of miR156 also declines in maize (Chuck et al., 2007), rice (Tanaka et al., 2011), soybean (Yoshikawa et al., 2013), chinese cabbage (Wang et al., 2014), and canadian poplar (Wang et al., 2011) as shown in Fig. 1. Recent studies have demonstrated that the regulatory mechanism underlying miR156 level variation is involved in endogenous molecular signal responses and exogenous biotic and abiotic stress stimuli.

GIBBERELLIN REGULATES VEGETATIVE PHASE CHANGE. Previous studies have indicated that GA either promotes or inhibits vegetative phase change and flowering in a species-dependent manner (Zimmerman et al., 1985). Exogenous GA causes rejuvenation in woody plants, such as *Hedera helix* and *Acacia farnesiana* (Rogler and Hackett, 1975; Borchert, 1965). However, the opposite phenomenon is observed in arabidopsis (Evans and Poethig, 1995; Telfer et al., 1997) and maize (Wilson et al., 1992). Mutations of genes involved in GA biosynthesis and response in arabidopsis prolongs juvenile phase (Poethig, 2009; Telfer et al., 1997). For example, *Gal-3* prevents transition of seedlings to the adult phase. However, GA exerts no direct effect on miR156 expression (Jung et al., 2012; Wang et al., 2009). Other studies provided insights into the GA-mediated upregulation of *SPLs* (Jung et al., 2012). Exogenous spraying of GA can also accelerate the appearance of abaxial trichomes in both miR156-overexpressing plants and *Spl9* and *Spl15* double mutants (Schwarz et al., 2008). Furthermore, GA promotes vegetative phase change in rice (Asai et al., 2002) and maize (Wilson et al., 1992).

Fig. 1. Interplay of miR156 and targets [squamosa promoter binding protein-box transcription factors (SPLs)] in plants development phase change. In the miR156/SPLs-mediated vegetative phase pathway, miR156 level is reduced with age, triggered by increasing levels of sugars, stress and epigenetic modify during vegetative growth. As a result, the level of miR156-targeted SPLs increases, leading to accelerated adult phase, which cause corresponding changes in vegetative-specific traits. Otherwise, miR156/SPLs also integrated the embryonic phase and reproductive phase to influence embryo development and flowering.

GLUCOSE METABOLISM REGULATES miR156 EXPRESSION. Sugar plays important roles in vital plant processes (Moore et al., 2003). In particular, exogenous sugar prolongs the juvenile phase of *Physcomitrella patens* (Lorenz et al., 2003). This finding indicates that sugar metabolism is involved in the vegetative phase change of phycophyta. Gibson (2005) also revealed that increased sugar content can delay germination, stimulate floral induction, and promote plant senescence. Rubisco is the pivotal enzyme of photosynthetic carbon assimilation, which also directly affects the amount of photosynthetic product. Tsai et al. (1997) revealed that transforming the antisense gene *RBCS* in tobacco can reduce the photosynthetic rate of transgenic seedlings. The leaf initiation rate and blade life span of transgenic tobacco can also be increased. These results show the importance of sugar in vegetative phase change in flowering plants. Previous studies proved that reduced miR156 level clearly regulates vegetative phase change. However, the source and identity of the signals that initiate this transition remain unknown. A recent study has reported that arabidopsis mutant (*Ch1* to *Ch4*) plants that are deficient in chlorophyll show delayed vegetative phase change. Ectopic experiments on *Ch1* with the suppression of miR156 (35S::MIM156) demonstrate that the deferred vegetative phase change of *Ch1* depends on the activation of miR156 (Yang et al., 2013; Yu et al., 2013). These results indicate that photosynthesis products can promote vegetative phase change by lowering miR156 levels. Moreover, sugar may be an

upstream regulator of miR156. The results of leaf ablation experiments demonstrated that miR156 level is elevated in arabidopsis, *Nicotiana benthamiana*, and maize; consequently, the expression of SPL genes regulated by miR156 decreases (Yang et al., 2011a). These data suggest that the signal(s) produced by the leaf primordia may be the cause of the decreased level of miR156. Recent reports have revealed that exogenous sugar can repress the expression of miR156 as effective as an absent leaf primordium. Researchers also found a gene called *HXXI*, which encodes a glucose-signaling protein that contributes to the glucose-induced decrease in the expression of two miR156 genes [miR156A and miR156C (Yang et al., 2013; Yu et al., 2013)]. These results strongly demonstrated that sugar is a component of the leaf signal that promotes vegetative phase change.

EXOGENOUS BIOTIC AND ABIOTIC STRESS STIMULI CONTROL THE EXPRESSION OF miR156. Extreme conditions, such as low temperature stimulation, upregulate the miR156a and miR156g levels in arabidopsis but downregulate the target genes of *SPL2*, *SPL3*, *SPL5*, *SPL9*, *SPL10*, and *SPL13* (Lee et al.,

2010). In addition, the level of miR156 in wheat (*Triticum aestivum*) and chinese cabbage can be significantly upregulated in heat stress (HS), particularly after 0.5 h of exposure to this stimulus (Xin et al., 2010; Yu et al., 2012b). However, the expression of miR156 in wheat can be downregulated by powdery mildew (*Erysiphe graminis* f. sp. *tritici*) infection. In consequence, the expression levels of target genes (*Ta3711* and *Ta7012*) increase (Xin et al., 2010). A recent study has established that HS induces the expression of miR156, thereby affecting the expression of HS memory-related genes through SPL genes (Stief et al., 2014). This study implies that the miR156/SPL regulatory module integrates stress responses with development. The essential macronutrient phosphorous contributes to plant growth, development, and reproduction (Kuo and Chiou, 2011). Hsieh et al. (2009) conducted small RNA gel analyses of Pi starvation-regulated miRNAs and found that miR156 is upregulated while SPLs are downregulated in the roots. Furthermore, miR156 is commonly regulated by nitrogen deficiency (Zhao et al., 2012) or various metal stresses (Yang and Chen, 2013).

Biotic and abiotic stresses are known to influence the expression of miR156. However, the molecular mechanisms and the response pathway remain poorly understood. In a recent issue, under stress conditions (salt and drought), miR156 is induced to maintain the plant in the juvenile state for a relatively long period, whereas under favorable conditions, miR156 is suppressed to accelerate the developmental transition.

Overexpression of miR156 can increase stress tolerance, whereas silent the level of miR156 (35S::MIM156) increased the sensitivity to stress treatment (Cui et al., 2014). These results uncovered a molecular mechanism for plant adaptation to the environment through the miR156/SPLs pathway, which coordinates development and abiotic stress tolerance.

REGULATION OF VEGETATIVE PHASE CHANGE IN miRNA BIOGENESIS AND FUNCTION PATHWAY. miRNAs are first transcribed to primary miRNAs (pri-miRNAs), which possess stem-loop structures of long primary transcripts (Vaucheret et al., 2006). After undergoing processing and modification, these pri-miRNAs become functional and mature miRNAs. An increasing number of studies identified new components and regulatory mechanisms involved in miRNA biogenesis and effect (Wu, 2013). Both of the components play important roles in miRNA-mediated gene regulation. The arabidopsis *HYL1* gene encodes a nuclear double-stranded RNA-binding protein with two RNA-binding domains for cooperative binding most likely to the miRNA/miRNA* duplex region (Chaabane et al., 2013; Han et al., 2004). A loss-of-function mutation of the *HYL1* gene causes defects in the timing of the juvenile phase. *HYL1* controls the expression levels of miR156-targeted *SPL* genes and enables plants to undergo the juvenile phase, which ensures maximum growth and productivity during plant development (Li et al., 2012) as shown in Fig. 2. The RNase III endonuclease *Dicer-like1* (*DCL1*) is essential in pri-miRNA and pre-miRNA processing (Dong et al., 2008). Arabidopsis

Dcl-4 mutants have long and decurved rosette leaves that have early abaxial trichomes (Xie et al., 2005) as shown in Fig. 2. *SERRATE* is a zinc-finger protein that enhances the accuracy of *DCL1*-dependent pri-miRNA processing together with *HYL1* (Lobbes et al., 2006; Yang et al., 2006). *Serrate* causes precocious vegetative development, prolonged plastochron length, serrated leaf margin, and abnormal phyllotaxy (Wu, 2013) as shown in Fig. 2.

Mature miRNAs are incorporated into an RNA-induced silencing complex with proteins for the direct cleavage, translational repression, and epigenetic modification of partially complementary mRNA target transcripts (Wu, 2013). Arabidopsis *ARGONAUTE1* (*AGO1*) encodes the RNA slicer enzyme necessary for the inhibition of the target mRNA, and the target genes are upregulated in loss-of-function *Agol* mutants; however, miRNA levels are not reduced, suggesting that *AGO1* acts downstream to *DCL1* and *HYL1* (Wu, 2013). In addition, *AGO1* is regulated by miR168-programmed, *AGO1*-catalyzed mRNA cleavage, implying that these miRNAs control the activity of the miRNA pathway through feedback regulation. The regulation of *AGO1* homeostasis maintains miRNA pathway functioning and normal plant development (Vaucheret et al., 2006). *SUO* is a component of the translation repression machinery in *arabidopsis*. The loss-of-function mutant *Suo* upregulates *SPL* proteins and accelerates the expression of adult vegetative traits by reducing the activities but not the accumulation of *AGO1* and miR156/miR157 (Yang et al., 2011) as

Fig. 2. A diagram of the regulatory factor involved in plant vegetative phase change in miRNA biogenesis and function pathway in plants. miRNAs are transcribed into primary miRNAs (pri-miRNAs) by DNA-dependent RNA polymerase II (Pol II). Pri-miRNAs are then processed into precursor miRNAs (pre-miRNAs) by the combinatorial action of DCL1 (*dicer-like1*), SE (*serrate*), HYL1 (*double-stranded RNA-binding protein 1*). Pre-miRNAs or mature miRNAs processed by DCL1 are transported from the nucleus to the cytoplasm by HST (*hasty*). The guide strand of the RNA duplex is then incorporated into the RNA-induced silencing complex (RISC) complex with argonaute (AGO) protein to play its function. These factors involved in miRNA biogenesis and function pathway have indirect influence of vegetative phase change by regulating the abundance or activity of miR156.

shown in Fig. 2. *Squint* (*SQN*), which encodes the arabidopsis ortholog of cyclophilin 40 (Smith et al., 2009), promotes the activity of miR156 by promoting the function of *AGO1*. In *Sqn* mutants, the reduced activity of *AGO1* influences the effect of miR156, which leads to dwarfing and precocious phenotype by increased *SPLs*. Transgenic experiments indicated that the overexpression of miR156 or the suppression of *SPLs* can restore the phenotype of *sqn* (Yang et al., 2011b) as shown in Fig. 2. Peragine et al. (2004) revealed that *SGS3*, *SGS2*, *SDE1*, and *RDR6* are required for post-transcriptional gene silencing. In *Sgs3-11* and *Rdr6-11* mutants, the abundance of miR156 exerts no effect, but the transcript of *SPL3* is distinctly elevated in this silencing pathway. Semiquantitative reverse transcription-PCR analysis demonstrated that the antisense transcript of *SPL3* is unaffected in *Rdr6-11* and *Sgs3-11*. These results suggest that *SPL3* upregulation is not involved in the miR156/*SPL* regulatory module (Wu and Poethig, 2006).

EPIGENETIC MODIFICATIONS INFLUENCE VEGETATIVE PHASE CHANGE. Epigenetics is a study of the reversible function and heritable changes of genes with no nuclear DNA sequence change. It includes DNA modification, histone modification, and nucleosome positioning. MEA, SWN, and CLF are the core components of the histone methylation modification complex. A previous study reported that *Swn/Clf* double mutants can only survive in culture medium, with tufted products and no capability to differentiate into other organs. This finding suggests that histone methylation modification is necessary for normal plant differentiation and development. Phenotypic observations of *Swn* mutants show that *SWN* inhibits the rate of leaf production and promotes the juvenile-to-adult phase transition (Thorpe, 2009). Therefore, histone methylation modification is necessary in the juvenile-to-adult phase transition. An in vivo study showed that *Swn/Clf* double mutants downregulate euchromatin H3k27me3 and upregulate heterochromatin H3k27me3. A recent study has demonstrated that H3k27me3 modification exists in miR156/157 loci, but their target *SPL* genes are not directly modified by H3k27me3. Therefore, histone methylation modification indirectly regulates the transcription factor *SPLs* through miR156 (Marcel et al., 2011). Partial endogenous genes involved in the biosynthesis of miRNAs and inhibition of target genes have been found, but their effects on plant growth and their own regulation mechanisms remain unknown. Epigenetics can provide new insights into the juvenile-to-adult transition and open up new directions for future research.

miR156/*SPLs* control downstream genes to regulate plant development

miR156 acts as an endogenous age cue in conjunction with its targeted genes, SBP-box transcription factors, to regulate vegetative phase change, which is involved with a series of phenotypic, physiological, and biochemical changes. However, the downstream controlling mechanisms regulated by miR156/*SPLs* are poorly understood.

miR156 SCULPTS GENE EXPRESSION IN EMBRYONIC DEVELOPMENT. The embryonic phase is the early developmental stage before vegetative phase. In a recent issue, Nodine and Bartel (2010) using genome-wide transcript profiling to analyzed the changes in gene expression in early *Dcl1* mutants embryos of arabidopsis, which are developmentally defective at the globular stage of embryogenesis and exhibit abnormal divisions throughout the extraembryonic suspensor. They found higher expression

levels for ≈ 50 putative miRNA targets in early *dcl1* embryos when compared with wild-type embryos. Interestingly, two of miR156 targets: *SPL10* and *SPL11* are partly responsible for the early onset of the maturation program in the mutant. In vivo, reducing the levels of *SPL10* and *SPL11* in the *Dcl1* background rescues early defects in *Dcl1* mutants, but not later aspects of development. On the contrary, disrupting miRNA156 regulation of *SPL10* and *SPL11* caused defects during early embryonic morphogenesis. These results implied that, by preventing precocious expression of partial differentiation-promoting transcription factors, miRNA156 enable proper embryonic patterning as shown in Fig. 1.

miR156/*SPLs* INFLUENCE TRICHOME DISTRIBUTION. In arabidopsis, trichome distribution is spatially and temporally regulated, and the distribution pattern serves as a hallmark of different developmental stages. Yu et al. (2010) uncovered that miR156-targeted *SPLs* temporally repress trichome distribution on stem and inflorescence by activating *TCL1* and *TRY*. Another study found that miR171-targeted *LOMs* functionally interfere with selected *SPLs* through protein-protein interactions to relieve the repression of *SPLs* in trichome distribution. Moreover, miR171 gene expression is regulated by its targeted *LOMs*, forming a homeostatic feedback loop (Xue et al., 2014).

miR156/*SPLs* COMMAND LEAF COMPLEXITY BY MIRNA-REGULATED LICENSING OF PROTEIN COMPLEXES. In arabidopsis, the progression from the juvenile to the adult phase is characterized by increased leaf serration. Rubio-Somoza et al. (Ignacio et al., 2014) found that miR319-targeted *TCP* transcription factors interfere with the functions of miR164-dependent and miR164-independent CUC proteins, preventing the formation of serrations in early leaves. The results of Y2H and BiLC assays demonstrated that *SPL9* interacts with *TCP4*, which disturbs the activity of *TCP* in CUC. As plants age, the increased abundance of *SBP/SPL* proteins acts as a cue that destabilizes *TCP*-CUC interactions.

miR156/*SPLs* INFLUENCE FLOWERING. Vegetative and reproductive phases are independent plant growth stages that work with each other. The miR156/*SPLs* regulatory module represents a major regulatory axis that promotes flowering in the absence of photoperiodic cues (Wang et al., 2009). Schmid et al. (2003) found that *SPL* genes affect flowering as downstream targets of *CO* and *FT* at the shoot apex in the photoperiodic pathway. Wang et al. (2009) showed that *SPL* genes play important *FT*-independent roles in regulating flowering. *SPLs* and the *FT/FD* complex share several direct targets, including the *Apetalal1* (*API*) flowering factor. A recent study has suggested that *SPL3* is a direct upstream activator of some flowering factors, such as *LFY* (*leafy*), *FUL* (*fruit full*), and *API*, which are directly activated by *SPL3* (Yamaguchi et al., 2009). The results of chromatin immunoprecipitation (ChIP) assay with GFP antibodies and β -glucuronidase staining indicated that *SOC1* and *AGL42* are direct *SPL9* targets and that *FUL* is transcriptionally regulated by *SPLs* in the leaves and at the shoot apex (Wang et al., 2009) as shown in Fig. 1.

Kim et al. (2012) disclosed that the overexpression of miR156-resistant *SPL3* triggers early flowering, regardless of the ambient temperature, which is associated with *FT* upregulation and *FUL* expression. ChIP assay showed that the *SPL3* protein directly binds to GTAC motifs within the *FT* promoter. The researchers inferred that the miR156-*SPL3* module and *FT* are part of the regulatory mechanism that controls flowering time in response to ambient temperature.

GA promotes early flowering and short-day flowering in a long-day plant. Studies in *Arabidopsis* have shown that GA exerts no effect on the expression of miR156 but promotes *SPL* genes transcription. Short-day flowering may be caused by the accumulation of the SPL protein or their enhanced activity via GA function (Wu et al., 2009). Yu et al. (2012a) found that DELLA proteins, which function as flowering repressors in the GA pathway, directly interact with miR156-targeted *SPLs*. Their combination suppresses the expression of downstream flowering genes. This work is the supplement to the cross talk between miR156/SPLs and GA pathways.

Zhou et al. (2013) and Bergonzi et al. (2013) found that the biennial *Cardamine flexuosa* and the perennial *Arabis alpina* have a common mechanism of age-dependent response to winter temperature in flowering. This mechanism downregulates two inhibitory factors, namely, *FLC* (*flowering locus c*) (Willmann and Poethig, 2005) and *TOE1*, to complete flowering. Decreased miR156 levels and increased miR172 levels in the age pathway relieve the suppression of AP2-like protein to promote flowering. With the inhibition of *FLC*, the two pathways regulate *SCO1* expression to activate the transcription of downstream flowering genes (Bergonzi et al., 2013; Zhou et al., 2013).

Conclusion

Genetic analyses of vegetative phase change in various plants have produced significant advances in the past two decades, which have broaden our horizons in understanding of the molecular mechanisms of this process. These studies have powerfully demonstrated that miR156/157 is the master regulator of vegetative development in some plant species. This regulatory mechanism of miR156/157 and their repressive SBP/SPL transcription factors by post-transcriptionally explains the progressive of vegetative phase change, which also involves a lot of vegetative-specific complex changes at the level of morphology, physiology, biochemistry, and cell biology. Growing evidence suggests that the molecular mechanism of miR156/157/SPLs not only control the independent processes of vegetative phase change, but also integrated the other developmental stage (embryonic phase and reproductive phase). These complex genetic pathways that underlie these phase transitions will lead to a better understanding of what drives plant diversification and distribution in relation to ecological factors. Another important question is how the timing of vegetative phase change is regulated. Although several cures have been found to be a component of this upstream signal, it remains to be determined if they play the direct or indirect role of regulating vegetative phase change. Moreover, the influence of other factors, especially epigenetics, on plant growth and development needs further exploration. This complex mechanism still warrants further analysis. Therefore, in-depth research is crucial to elucidate the mechanism and clarify other plant processes.

Literature Cited

Asai, K., N. Satoh, H. Sasaki, H. Satoh, and Y. Nagato. 2002. A rice heterochronic mutant, *mori1*, is defective in the juvenile–adult phase change. *Development* 129:265–273.

Aukerman, M.J. and H. Sakai. 2003. Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. *Plant Cell* 15:2730–2741.

Bartel, D.P. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116:281–297.

Bergonzi, S., M.C. Albani, E.V.L. Themaat, M.K.J.V. Nordstr, R.H. Wang, K. Schneeberger, P.D. Moerland, and G. Coupland. 2013. Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. *Science* 340:1094–1097.

Bhogale, S., A.S. Mahajan, B. Natarajan, M. Rajabhoj, H.V. Thulasiram, and A.K. Banerjee. 2014. miRNA156-A potential graft-transmissible microRNA that modulates plant architecture and tuberization in potato (*Solanum tuberosum ssp andigena*). *Plant Physiol.* 164:1011–1027.

Bongard, P.D.K., M.M.S. Evans, and R.S. Poethig. 1996. Heteroblastic features of leaf anatomy in maize and their genetic regulation. *Intl. J. Plant Sci.* 157:331–340.

Borchert, R. 1965. Gibberellic acid and rejuvenation of apical meristems in *Acacia melanoxylon*. *Naturwissenschaften* 52:65–66.

Chaabane, B.S., R. Liu, V. Chinnusamy, Y. Kwon, J.H. Park, S.Y. Kim, J.K. Zhu, S.W. Yang, and B.H. Lee. 2013. STA1, an *Arabidopsis* pre-mRNA processing factor 6 homolog, is a new player involved in miRNA biogenesis. *Nucleic Acids Res.* 41:1984e.

Chen, X. 2004. A microRNA as a translational repressor of APETALA2 in *Arabidopsis* flower development. *Science* 303:2022–2025.

Cho, S.H., C. Coruh, and M.J. Axtell. 2012. miR156 and miR390 regulate tasiRNA accumulation and developmental timing in *Physcomitrella patens*. *Plant Cell* 24:4837–4849.

Chuck, G.S., C. Tobias, L. Sun, F. Kraemer, C. Li, and D. Dibble. 2011. Overexpression of the maize *Corngrass1* microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass. *Proc. Natl. Acad. Sci. USA* 108:17550–17555.

Chuck, G., A.M. Cigan, K. Saetern, and S. Hake. 2007. The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. *Nat. Genet.* 39:544–549.

Cui, L.G., J.X. Shan, M. Shi, J.P. Gao, and H.X. Lin. 2014. The miR156-SPL9-DFR pathway coordinates the relationship between development and abiotic stress tolerance in plants. *Plant J.* 80:1108–1117.

Dong, Z., M.H. Han, and N. Fedoroff. 2008. The RNA-binding proteins HYL1 and SE promote accurate in vitro processing of pri-miRNA by DCL1. *Proc. Natl. Acad. Sci. USA* 105:9970–9975.

Evans, M.M. and R.S. Poethig. 1995. Gibberellins. vegetative phase change and reproductive maturity in maize. *Plant Physiol.* 108:475–487.

Eviatar-Ribak, T., A. Shalit-Kaneh, L. Chappell-Maor, Z. Amsellem, Y. Eshed, and E. Lifschitz. 2013. A cytokinin-activating enzyme promotes tuber formation in tomato. *Curr. Biol.* 23:1057–1064.

Franco-Zorrilla, J.M., A. Valli, M. Todesco, I. Mateos, M.I. Puga, I. Rubio-Somoza, A. Leyva, D. Weigel, J.A. Garcia, and J. Paz-Ares. 2007. Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* 39:1033–1037.

Freeling, M. and B. Lane. 1994. The maize leaf, p. 17–28. In: M. Freeling and V. Walbot (eds.). *The maize handbook*. Springer-Verlag, New York, NY.

Fu, C., R. Sunkar, C. Zhou, H. Shen, J.Y. Zhang, J. Matts, J. Wolf, D.G. Mann, C.J. Stewart, Y. Tang, and Z.Y. Wang. 2012. Overexpression of miR156 in switchgrass (*Panicum virgatum* L.) results in various morphological alterations and leads to improved biomass production. *Plant Biotechnol. J.* 10:443–452.

Gibson, S.I. 2005. Control of plant development and gene expression by sugar signaling. *Curr. Opin. Plant Biol.* 8:93–102.

Han, M.H., L. Song, and N. Fedoroff. 2004. The *Arabidopsis* double stranded RNA-binding protein HYL1 plays a role in microRNA mediated gene regulation. *Proc. Natl. Acad. Sci. USA* 101:1093e1098.

Hsieh, L.C., S.I. Lin, A.C. Shih, J.W. Chen, W.Y. Lin, C.Y. Tseng, W.H. Li, and T.J. Chiou. 2009. Uncovering small RNA-mediated responses to phosphate deficiency in *Arabidopsis* by deep sequencing. *Plant Physiol.* 151:2120–2132.

- Ignacio, R.S., C.M. Zhou, C. Ana, M. Claudia, V.B. Patrick, B.G. Elena, J.W. Wang, and D. Weigel. 2014. Temporal control of leaf complexity by miRNA-regulated licensing of protein complexes. *Curr. Biol.* 24:2714–2719.
- Jung, J.H., Y.H. Seo, P.J. Seo, J.L. Reyes, J. Yun, N.H. Chua, and C.M. Park. 2007. The GIGANTEA-regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in arabidopsis. *Plant Cell* 19:2736–2748.
- Jung, J.H., Y. Ju, P.J. Seo, J.H. Lee, and C.M. Park. 2012. The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in arabidopsis. *Plant J.* 69:577–588.
- Kim, J.J., J.H. Lee, W. Kim, H.S. Jung, P. Huijser, and J.H. Ahn. 2012. The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 module regulates ambient temperature-responsive flowering via *FLOWERING LOCUS T* in arabidopsis. *Plant Physiol.* 159:461–478.
- Knight, T.A. 1795. Observations on the grafting of trees. In: A letter from Thomas Andrew Knight, Esq. to Sir Joseph Banks, Bart. *Philos. Trans. R. Soc. Lond.* 85:290–295.
- Kuo, H.F. and T. Chiou. 2011. The role of MicroRNAs in phosphorus deficiency signaling. *Plant Physiol.* 156:1016–1024.
- Lauter, N., A. Kampani, S. Carlson, M. Goebel, and S.P. Moose. 2005. microRNA172 down-regulates glossy15 to promote vegetative phase change in maize. *Proc. Natl. Acad. Sci. USA* 102:9412–9417.
- Lawson, E.J.R. and R.S. Poethig. 1995. Shoot development in plants: Time for a change. *Trends Genet.* 1:263–268.
- Lee, H., S.J. Yoo, J.H. Lee, W.H. Kim, S.K. Yoo, H. Fitzgerald, J.C. Carrington, and J.H. Ahn. 2010. Genetic framework for flowering-time regulation by ambient temperature-responsive miRNAs in arabidopsis. *Nucleic Acids Res.* 38:3081–3093.
- Li, S.X., X. Yang, F.J. Wu, and Y.K. He. 2012. *HYL1* controls the miR156-mediated juvenile phase of vegetative growth. *J. Expt. Bot.* 63:2787–2798.
- Lobbes, D., G. Rallapalli, D.D. Schmidt, C. Martin, and J. Clarke. 2006. *SERRATE*: A new player on the plant microRNA scene. *EMBO. Rpt.* 7:1052–1058.
- Lorenz, S., S. Tintelnot, R. Reski, and E.L. Decker. 2003. Cyclin D-knockout uncouples developmental progression from sugar availability. *Plant Mol. Biol.* 53:227–236.
- Marcel, L., K. Phillip, L.H. Mareike, L.T. Frazer, C. Oliver, and S. Daniel. 2011. Dynamic regulation of H3K27 trimethylation during arabidopsis differentiation. *PLoS Genet.* 7:e1002040.
- Moore, B., L. Zhou, F. Rolland, Q. Hall, W.H. Cheng, Y.X. Liu, I. Hwang, T. Jones, and J. Sheen. 2003. Role of the arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* 300:332–336.
- Nodine, M.D. and D.P. Bartel. 2010. MicroRNAs prevent precocious gene expression and enable pattern formation during plant embryogenesis. *Genes Dev.* 24:2678–2692.
- Park, M.Y., G. Wu, A. Gonzalez-Sulser, H. Vaucheret, and R.S. Poethig. 2005. Nuclear processing and export of microRNAs in arabidopsis. *Proc. Natl. Acad. Sci. USA* 102:3691–3696.
- Peragine, A., M. Yoshikawa, G. Wu, H.L. Albrecht, and R.S. Poethig. 2004. *SGS3* and *SGS2/SDE1/RDR6* are required for juvenile development and the production of trans-acting siRNAs in arabidopsis. *Genes Dev.* 18:2368–2379.
- Poethig, R.S. 1988. Heterochronic mutations affecting shoot development in maize. *Genetics* 119:959–973.
- Poethig, R.S. 1990. Phase changes and the regulation of shoot morphogenesis in plants. *Science* 250:923–930.
- Poethig, R.S. 2009. Small RNAs and developmental timing in plants. *Curr. Opin. Genet. Dev.* 19:374–378.
- Poethig, R.S. 2013. Vegetative phase change and shoot maturation in plants. *Curr. Top. Dev. Biol.* 105:125–152.
- Rogler, C.E. and W.P. Hackett. 1975. Phase change in *Hedera helix*: Induction of the mature to juvenile phase change by gibberellin A3. *Plant Physiol.* 34:141–147.
- Salinas, M., S.H. Xing, S. Hmann, R. Berndtgen, and P. Huijser. 2012. Genomic organization, phylogenetic comparison and differential expression of the SBP-box family of transcription factors in tomato. *Planta* 235:1171–1184.
- Schmid, M., N.H. Uhlenhaut, F. Godard, M. Demar, R. Bressan, D. Weigel, and J.U. Lohmann. 2003. Dissection of floral induction pathways using global expression analysis. *Development* 130:6001–6012.
- Schwarz, S., A.V. Grande, N. Bujdoso, H. Saedler, and P. Huijser. 2008. The microRNA regulated SBP-box genes *SPL9* and *SPL15* control shoot maturation in arabidopsis. *Plant Mol. Biol.* 67:183–195.
- Shikata, M., H. Yamaguchi, K. Sasaki, and N. Ohtsubo. 2012. Overexpression of arabidopsis miR157b induces bushy architecture and delayed phase transition in *Torenia fournieri*. *Planta* 236:1027–1035.
- Singleton, W.R. 1951. Inheritance of Corn grass, a macromutation in maize, and its possible significance as an ancestral type. *Amer. Naturalist* 305:81–96.
- Smith, M.R., M.R. Willmann, G. Wu, T.Z. Berardini, B. Moller, D. Weiers, and R.S. Poethig. 2009. Cyclophilin40 is required for miRNA activity in arabidopsis. *Proc. Natl. Acad. Sci. USA* 106:5424–5429.
- Stief, A., S. Altmann, K. Hoffmann, B.D. Pant, W.R. Scheible, and I. Bäurle. 2014. Arabidopsis miR156 regulates tolerance to recurring environmental stress through SPL transcription factors. *Plant Cell* 25:1792–1807.
- Tanaka, N., H. Itoh, N. Sentoku, M. Kojima, H. Sakakibara, T. Izawa, J.I. Itoh, and Y. Nagatoka. 2011. The *COP1* ortholog *PPS* regulates the juvenile-adult and vegetative-reproductive phase changes in rice. *Plant Cell* 23:2143–2154.
- Telfer, A. and R.S. Poethig. 1998. *HASTY*: A gene that regulates the timing of shoot maturation in *Arabidopsis thaliana*. *Development* 125:1889–1898.
- Telfer, A., K.M. Bollman, and R.S. Poethig. 1997. Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*. *Development* 124:645–654.
- Thorpe, F. 2009. The roles of Polycomb-group proteins in the development of *Arabidopsis thaliana*. *Biol. Sci. Diss., University of Edinburgh, Edinburgh, UK.*
- Tsai, C.H., A. Miller, M. Spalding, and S. Rodermel. 1997. Source strength regulates an early phase transition of tobacco shoot morphogenesis. *Plant Physiol.* 115:907–914.
- Vaucheret, H., A.C. Mallory, and D.P. Bartel. 2006. AGO1 homeostasis entails coexpression of miR168 and *AGO1* and preferential stabilization of miR168 by *AGO1*. *Mol. Cell* 22:129–136.
- Wang, J.W., C. Benjamin, and W. Detlef. 2009. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* 138:738–749.
- Wang, J.W., M.Y. Park, L.J. Wang, Y.J. Koo, X.Y. Chen, D. Weigel, and R.S. Poethig. 2011. MiRNA control of vegetative phase change in trees. *PLoS Genet.* 7:e1002012.
- Wang, J.W., R. Schwab, B. Czech, E. Mica, and D. Weigel. 2008. Dual effects of miR156-Targeted SPL genes and CYP78A5/KLUH on plastochron length and organ size in *Arabidopsis thaliana*. *Plant Cell* 20:1231–1243.
- Wang, Y.L., F.J. Wu, J.J. Bai, and Y.K. He. 2014. BrpSPL9 (*Brassica rapa* ssp. *pekinensis* SPL9) controls the earliness of heading time in chinese cabbage. *Plant Biotechnol. J.* 12:312–321.
- Willmann, M.R. and R.S. Poethig. 2005. Time to grow up: The temporal role of smallRNAs in plants. *Curr. Opin. Plant Biol.* 8:548–552.
- Wilson, R.N., J.W. Heckman, and C.R. Somerville. 1992. Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Plant Physiol.* 100:403–408.
- Wu, G. 2013. Plant MicroRNAs and development. *J. Genomics Genet.* 40:217–230.
- Wu, G. and R.S. Poethig. 2006. Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target *SPL3*. *Development* 133:3539–3547.

- Wu, G., M.Y. Park, R.C. Susan, J.W. Wang, W. Detlef, and R.S. Poethig. 2009. The sequential action of *miR156* and *miR172* regulates developmental timing in arabidopsis. *Cell* 138:750–759.
- Xie, K.B., C.Q. Wu, and L.Z. Xiong. 2006. Genomic organization, differential expression, and interaction of *SQUAMOSA promoter-binding-like transcription factors* and microRNA156 in rice. *Plant Physiol.* 142:280–293.
- Xie, K.B., J.Q. Shen, X. Hou, J.L. Yao, X.H. Li, J.H. Xiao, and L.Z. Xiong. 2012. Gradual increase of miR156 regulates temporal expression changes of numerous genes during leaf development in rice. *Plant Physiol.* 158:1382–1394.
- Xie, Z.X., A. Edwards, A. Wilken, and C.C. James. 2005. DICER-LIKE 4 functions in trans-acting small interfering RNA biogenesis and vegetative phase change in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 102:12984–12989.
- Xin, M., Y. Wang, Y.Y. Yao, C.J. Xie, H.R. Peng, Z.F. Ni, and Q.X. Sunday. 2010. Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (*Triticum aestivum* L.). *BMC Plant Biol.* 10:23.
- Xue, X.Y., B. Zhao, L.M. Chao, D.Y. Chen, W.R. Cui, Y.B. Mao, L.J. Wang, and X.Y. Chen. 2014. Interaction between two timing MicroRNAs controls trichome distribution in arabidopsis. *PLoS Genet.* 10:e1004266.
- Yamaguchi, A., M.F. Wu, L. Yang, G. Wu, R.S. Poethig, and D. Wagner. 2009. The MicroRNA-regulated SBP-Box transcription factor *SPL3* is a direct upstream activator of *LEAFY*, *FRUITFULL*, and *APETALA1*. *Dev. Cell* 17:268–278.
- Yang, L., S.R. Conway, and R.S. Poethig. 2011a. Vegetative phase change is mediated by a leaf-derived signal that represses the transcription of *miR156*. *Development* 138:245–249.
- Yang, L., Z.Q. Liu, F. Lu, A.W. Dong, and H. Huang. 2006. *SERRATE* is a novel nuclear regulator in primary microRNA processing in arabidopsis. *Plant J.* 47:841–850.
- Yang, L., G. Wu, and R.S. Poethig. 2011b. Mutations in the GW-repeat protein *SUO* reveal a developmental function for microRNA-mediated translational repression in arabidopsis. *Proc. Natl. Acad. Sci. USA* 109:315–320.
- Yang, L., M. Xu, Y. Koo, J. He, and R.S. Poethig. 2013. Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of *MIR156A* and *MIR156C*. *eLife* 2: e00260.
- Yang, Z.M. and J. Chen. 2013. A potential role of microRNAs in plant response to metal toxicity. *Metallomics* 5:1184–1190.
- Yoshikawa, T., S. Ozawa, N. Sentoku, J.I. Itoh, Y. Nagato, and S. Yokoi. 2013. Change of shoot architecture during juvenile-to-adult phase transition in soybean. *Planta* 238:229–237.
- Yu, N., W.J. Cai, S. Wang, C.M. Shan, L.J. Wang, and X.Y. Chen. 2010. Temporal control of trichome distribution by microRNA156-targeted SPL genes in *Arabidopsis thaliana*. *Plant Cell* 22:2322–2335.
- Yu, S., L. Cao, C.M. Zhou, T.Q. Zhang, H. Lian, Y. Sun, J. Wu, J. Huang, G. Wang, and J.W. Wang. 2013. Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. *eLife* 2: e00269.
- Yu, S., C.G. Vinicius, Y.C. Zhang, D. Horrer, T.Q. Zhang, Y.H. Hao, Y.Q. Feng, S. Wang, M. Schmid, and J.W. Wang. 2012a. Gibberellin regulates the arabidopsis floral transition through miR156-targeted squamosa promoter binding-like transcription factors. *Plant Cell* 24:3320–3332.
- Yu, X., H. Wang, Y.Z. Lu, M.D. Ruitter, M. Cariaso, M. Prins, A.V. Tunen, and Y.K. He. 2012b. Identification of conserved and novel microRNAs that are responsive to heat stress in *Brassica rapa*. *J. Expt. Bot.* 63:1025–1038.
- Zhang, X.H., Z. Zou, J.H. Zhang, Y.Y. Zhang, Q.Q. Han, T.X. Hua, X.G. Xu, H. Liu, H.X. Li, and Z.B. Ye. 2011. Over-expression of sly-miR156a in tomato results in multiple vegetative and reproductive trait alterations and partial phenocopy of the sft mutant. *FEBS Lett.* 585:435–439.
- Zhao, M., H. Tai, S. Sun, F. Zhang, Y. Xu, and W.X. Li. 2012. Cloning and characterization of maize miRNAs involved in responses to nitrogen deficiency. *PLoS One* 7:e29669.
- Zhou, C.M., T.Q. Zhang, X. Wang, S. Yu, H. Lian, H.B. Tang, Z.Y. Feng, Z.L. Judita, and J.W. Wang. 2013. Molecular basis of age-dependent vernalization in *Cardamine flexuosa*. *Science* 340:1097–1100.
- Zimmerman, R.H., W.P. Hackett, and R.P. Pharis. 1985. Hormonal aspects of phase change and precocious flowering. *Encycl. Plant Physiol.* 11:79–115.