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Reproductive competence from an annual and a perennial perspective

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Abstract

Plants at early stages of development undergo a juvenile phase during which they are not competent to flower in response to environmental stimuli. The length of this phase varies among species and is extended in perennial plants particularly. In annuals, temporal changes in expression of microR156 (miR156), miR172, and their targets are correlated with the transition from the juvenile to the adult phase and flowering. This developmental transition in perennials is probably more complex than in other plants and the molecular mechanisms are less well understood. In addition, once perennials become adult and capable of reproduction they still keep some meristems in the vegetative state that contribute to their polycarpic growth habit. Juvenility and polycarpy, although considered as two different processes in perennials, might be related.

Key words: Annual, flowering, juvenility, life strategies, miR156, perennial, polycarpy, reproductive competence, SPLs.

Introduction

Flowering time is an important trait as it determines the reproductive power of a plant. During evolution plants have adapted several mechanisms to synchronize flowering and seed set with favourable environmental conditions. Prolonged exposure to cold temperatures (vernalization), long/short photoperiods or even a combination of temperature and day length promote or repress flowering, dependent upon the species and their habitat. Genetic and physiological studies in Arabidopsis thaliana have led to the identification of several genes that regulate flowering through a complex network of genetic pathways. The vernalization, ambient temperature, and photoperiod pathways regulate flowering in response to environmental cues, whereas the autonomous, gibberellin, and age pathways regulate flowering in response to endogenous signals (Samach and Wigge, 2005; Kobayashi and Weigel, 2007; Farrona et al., 2008; Turck et al., 2008; Kim et al., 2009; Mutasa-Gottgens and Hedden, 2009; Wang et al., 2009a).

The presence of the correct environmental conditions does not always ensure flowering of an individual plant. This phenomenon is particularly evident in woody perennials that do not flower for several years, although they are exposed to seasonal environmental cues every year. During vegetative development, plants need to gain the ability to respond properly to the environmental stimuli that induce flowering. In general, plants that are reproductively competent are capable of flowering and are referred to as adult, whereas plants that are incompetent to flower are referred to as juvenile.

So far, studies in annual and perennial species have looked at different stages of development to address the molecular mechanisms that regulate reproductive competence. Annuals are fast cyclers and progress quickly from the stage of vegetative development to flowering in order to complete their life cycle within one growing season. The vegetative phase in annuals is very short, which makes it difficult to track when plants acquire the potential to flower. To understand the events that occur in the shoot prior to flowering, studies in annuals have looked at changes in the leaf morphology during the early stages of development. This approach was chosen because certain leaf traits can only be found in leaves that are produced before flowering, which hints that changes in leaf morphology might be associated with an increase in the capability of the shoot to flower.
flower. Studies in annuals have provided valuable information that indicates that factors that regulate leaf morphological traits are also involved in flowering and that these two developmental transitions are related (Wang et al., 2009a; Wu et al., 2009; Yamaguchi et al., 2009).

Perennials have a prolonged vegetative phase that can last from a few weeks to several years. Once a plant has gained competence, the right inductive stimuli may commit some meristems to reproductive development. The majority of perennials maintain vegetative growth after flowering and can cycle between phases of reproductive and vegetative development from one year to the next (Albani and Coupland, 2010). Studies performed in perennials on the molecular mechanisms that regulate the juvenile to adult transition have used the presence of reproductive organs to indicate the adult phase of a plant and tested the involvement of genes shown previously in A. thaliana to regulate flowering.

These experimental approaches in annuals and perennials targeted different phases of development, which is probably due to differences in their life cycles. Generally, studies in annuals have focused on leaf morphological changes, which probably occur prior to the acquisition of reproductive competence, whereas those studies in perennials monitored flowering-related events that followed the acquisition of reproductive competence. In this review, our present knowledge of the transition from the juvenile to the adult phase in annual and perennial species has been compared and important insights from these studies are discussed.

**The juvenile to adult transition in annuals**

*Regulation of developmental changes in leaf morphology*

During vegetative growth, the shoot apical meristem gives rise to the above-ground part of the plant, which mainly consists of leaves and shoot branches. Several leaf traits, such as shape, size, the presence and density of trichomes, cell size, and cell number, may change during development. In A. thaliana, early leaves at the basal nodes are normally small, round, have long petioles, and lack trichomes on their abaxial (lower) side (Fig. 1). These leaves are considered juvenile. The larger adult leaves arise at higher nodes, have more elongated blades with serrated margins, short petioles, develop trichomes on both sides and consist of more cells compared with juvenile leaves (Telfer and Poethig, 1994; Telfer et al., 1997; Usami et al., 2009). The transition from juvenile-type leaves to adult-type leaves is gradual and, several transition leaves are formed during the progression from one phase to another (Willmann and Poethig, 2011). The development of leaves on the plant is regulated endogenously (Poethig, 2003). In addition, environmental conditions have been shown to influence traits such as leaf shape and trichome distribution (Njoku, 1956; Chien and Sussex, 1996; Telfer et al., 1997). For example, plants grown in short photoperiods developed leaves with abaxial trichomes at later nodes compared with plants grown in continuous light conditions (Chien and Sussex, 1996; Telfer et al., 1997).

The plant hormones gibberellins can also influence leaf traits. In A. thaliana, mutants with reduced endogenous levels of bioactive gibberellins or gibberellin-insensitive mutants delay the appearance of abaxial trichomes, whereas application of exogenous gibberellins accelerates the formation of adult leaf traits (Chien and Sussex, 1996; Telfer et al., 1997). These effects of gibberellins on age-related vegetative traits are also conserved in maize (Evans and Poethig, 1995).

Mutagenesis screens in A. thaliana have resulted in the identification of several mutants that accelerated the formation of adult leaves in combination with other phenotypes. Most of the underlying genes were involved in RNA silencing pathways, which can explain their pleiotropic mutant phenotypes. A large class of genes such as *SERRATE, SUPPRESSOR OF GENE SILENCING3*, and *SUPPRESSOR OF GENE SILENCING2/SILENCING DEFECTIVE1/RNA-DEPENDENT POLYMERASE6* were found to regulate microRNA (miRNA) and small interfering RNA (siRNA) biogenesis (Clarke et al., 1999; Peragine et al., 2004). Moreover, *ARGONAUTE1*, *ZIPPY*, *SQUINT*, and *HASTY* have been shown to be responsible for miRNA activity by the promotion of miRNA target
cleavage or translational repression (Bohmert et al., 1998; Telfer and Poethig, 1998; Berardini et al., 2001; Hunter et al., 2003; Park et al., 2005; Yang et al., 2006; Smith et al., 2009). Mutants in these genes have a reduced accumulation of several miRNAs (Park et al., 2005; Lobbes et al., 2006; Wu and Poethig, 2006; Smith et al., 2009). It has been further demonstrated that one of these RNAs, the microRNA 156 (miR156), plays a key role in the regulation of leaf characteristics. Transgenic plants that overexpress different precursors of the miR156 have leaves with typical juvenile characteristics (Wu and Poethig, 2006). This effect is specific for miR156, because plants that have reduced miR156 activity by artificial target mimicry constructs, produce only adult type leaves (Franco-Zorrilla et al., 2007; Wu et al., 2009).

miR156, probably together with the closely related miR157, targets a moderately sized family of plant-specific transcription factors named SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL). In A. thaliana, miR156 regulates 11 of the 17 members of the SPL family by both post-transcriptional gene silencing and translational inhibition (Rhoades et al., 2002; Schwab et al., 2005; Wu and Poethig, 2006; Gandikota et al., 2007; Wang et al., 2008). miR156 and SPLs are expressed in leaves and meristem and their temporal expression patterns have been shown to be complementary during development. miR156 expression is high during the early stages of seedling development and declines as the plant gets older, whereas expression of SPLs increases (Cardon et al., 1999; Schmid et al., 2003; Schwab et al., 2005; Wu and Poethig, 2006; Wang et al., 2009a). Plants that carry loss-of-function mutations in single SPL genes do not show a strong developmental phenotype, which indicates that they act redundantly in the regulation of developmental traits. However, overexpression of individual members of the SPL family has shown that some of these genes have specialized functions in the regulation of leaf traits. For instance, overexpression of SPL3 accelerates the production of trichomes at the abaxial side of leaves and increases cell number. Also, overexpression of SPL9 increases leaf size and reduces the rate of leaf initiation. These results indicate that SPL3 is involved in the regulation of abaxial trichome formation and the number of the cells in the leaf, whereas SPL9 regulates leaf shape and plastochron (Wu and Poethig, 2006; Schwarz et al., 2008; Wang et al., 2008; Usami et al., 2009). The role of SPL15 is also associated with cell number and size. Mutations in the miR156 cleavage site of SPL15 stimulated the production of leaves with increased numbers of small cells at an earlier stage (Usami et al., 2009).

How miR156 is regulated during development is unknown. The application of gibberellins or other hormones did not influence miR156 accumulation (Schwarz et al., 2008; Wang et al., 2009a). However, it was recently demonstrated in A. thaliana that factors that act in the leaf primordia regulate the expression of miR156 and the formation of leaf abaxial trichomes (Yang et al., 2011). Environmental conditions such as ambient temperature have an effect on miR156 expression, but the increase in miR156 accumulation is not mediated by genes known to act in the ambient temperature pathway (Lee et al., 2010).

Regulation of developmental changes in reproductive capability

The transition to flowering is known to require competence of the shoot apical meristem. A. thaliana is a long-day plant and can respond to long photoperiods within four days of germination (Mozley and Thomas, 1995). Sensitivity to photoperiodic induction increases as the plant gets older, which indicates that the acquisition of the competence to flower is a gradual process and not a sharp developmental switch (Mozley and Thomas, 1995). Factors that control reproductive competence have been shown to be developmentally regulated and are related to the activity of the shoot apical meristem (Poethig, 1990). In A. thaliana, gibberellins accumulate at the meristem prior to flowering (Eriksson et al., 2006). The involvement of gibberellins in reproductive competence has not been demonstrated. However, in A. thaliana they are considered to be part of an endogenous pathway that promotes flowering in the absence of inductive stimuli. Application of exogenous gibberellins can bypass all environmental pathways for flower induction and can activate the expression of major flowering regulators in the shoot apical meristem (Moon et al., 2003).

Plants that express miR156 constitutively flower late, whereas plants with inhibited miR156 activity flower early (Schwab et al., 2005; Wu and Poethig, 2006; Franco-Zorrilla et al., 2007). This difference indicates that miR156 plays an additional role in development, repressing the transition to flowering. Most miR156 targets are up-regulated during flowering and some of them interact with genes that act in the meristem during the floral transition (Fig. 2) (Cardon et al., 1999; Schmid et al., 2003; Wang et al., 2009a; Yamaguchi et al., 2009). SPL9 binds to the promoters of the floral identity genes SUPPRESSOR OF OVEREXPRESSION OF CONSTANS I (SOC1), FRUITFULL (FUL), and their closely related gene AGAMOUS-LIKE 42 (AGL42) whereas SPL3 regulates FUL and the meristem identity genes LEAFY (LFY) and APETALAI (API) (Wang et al., 2009a; Yamaguchi et al., 2009). The major evidence that relates miR156 with reproductive competence is that 35S:mir156 plants show a reduced response to transient exposure to inductive photoperiods (Schwarz et al., 2008), miR156 might regulate reproductive competence through SPL9 because SPL9 mRNA has been detected in the vegetative shoot apices (Cardon et al., 1999; Schmid et al., 2005; Wang et al., 2008, 2009a). However, mutations in SPL9 have a flowering phenotype only when combined with mutations in its parologue SPL15. Similar to miR156 overexpressor plants, spl9 spl15 double mutants have reduced response to photoperiodic shifts (Schwarz et al., 2008). Redundancy within the SPL family hampers genetic studies and SPL9 might act redundantly with other SPLs. In addition, exposure to long photoperiods up-regulates SPL mRNA levels but does not change miR156 expression, which adds a complexity when
Fig. 2. The regulation of the juvenile to adult transition in annuals and perennials. Temporal expression patterns of two microRNAs correlate with the juvenile to adult transition in the annual model A. thaliana. miR156 levels are down-regulated as the plants get older, whereas miR172 levels are up-regulated during development. This finding suggests that miR156 represses and miR172 promotes flowering. miR156 targets 11 out of 17 members of the SPL family of the DNA-binding transcription factors. Among them, SPL9 and SPL3 are better characterized, although they probably act redundantly with other members of the family. SPL9 binds to the promoters of the floral integrators SOC1 and FUL and to the SOC1 paralogous gene, AGL42. SPL3 binds to the promoter of FUL and to the floral identity genes AP1 and LFY. MiR156 regulates the expression of miR172 through SPL9, which binds to the promoter of one of the MIR172 loci. miR172 promotes flowering by down-regulating the expression of the AP2-like family of floral repressors. TFL1 in perennials is involved in the juvenile to adult transition and also contributes to the polycarpic growth habit. The yellow box indicates the genes identified in A. thaliana that are involved in the competence to flower and flowering. The pink box indicates genes identified in perennials that are involved in the regulation of competence to flower in perennials. The asterisks indicate genes that are also involved in the regulation of leaf traits. The dotted lines represent interactions not experimentally demonstrated. AGL42, AGAMOUS-LIKE 42; AP1, APETALA 1; AP2-like, APETALA 2-like; FUL, FRUITFUL; LFY, LEAFY; miR156, microRNA 156; miR172, microRNA 172; SOC1, SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS 1; SPL, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE; SPL3, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3; SPL9, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9; TFL1, TERMINAL FLOWER 1.

addressing the role of the miR156-SPL pathway in reproductive competence (Wang et al., 2009a).

The relationship of miR156 with flowering has also been demonstrated through another well-conserved microRNA. miR172 is expressed in a temporal manner opposite to that of miR156 and it is up-regulated as the plant ages (Aukerman and Sakai, 2003; Jung et al., 2007; Wu et al., 2009). In A. thaliana, miR172 promotes flowering, but its involvement in reproductive competence has not been demonstrated. miR172 targets the APETALA2-like (AP2) family of floral repressors. AP2, TARGET OF EAT1, 2, and 3, SCHLAFMUTZE, and SCHNARCHZAPFEN that also play a role in the regulation of leaf traits (Aukerman and Sakai, 2003; Schmid et al., 2003; Schwab et al., 2005; Yant et al., 2010). The relationship of miR156 with miR172 is regulated through SPL9, which binds to the promoter of a MIR172 locus (Wu et al., 2009).

The juvenile to adult transition in perennials

Woody perennials take many years before they are able to reproduce. The formation of reproductive organs has always been used as an indication of the shift to the adult phase although it describes the end-point of this development (Zimmerman, 1972). Similar to annuals, the vegetative phase in perennials is marked with different types of leaves or vegetative structures (Zimmerman, 1972; Spiegel-Roy and Goldschmidt, 1996; Wang et al., 2011a). Crosses in several woody species have demonstrated that the duration of the juvenile phase is inherited genetically and can be under a simple Mendelian inheritance or under polygenic control that is dependent upon the species (Hackett, 1985).

The ability to respond to flower inductive stimuli is considered to be regulated endogenously. However, environmental conditions can also influence the length of the juvenile period. In birch, camellia, and azalea, for example, long photoperiods and high light intensity shorten the juvenile phase by accelerating the plant’s growth (Zimmerman, 1972). These results indicated that plants need to reach a certain size before they are able to respond to flower inductive signals.

In contrast to A. thaliana, gibberellins do not always promote flowering in perennials. Application of exogenous gibberellins in several perennial species can even cause reversion from reproductive to vegetative development, which includes the appearance of juvenile traits of newly formed leaves (Hackett, 1985; Zimmerman et al., 1985). Measurements of endogenous levels of gibberellins have shown that juvenile shoot apices contained higher levels of gibberellins than did adult shoot apices (Hackett, 1985).

The molecular mechanisms that regulate reproductive competence have not been much explored in perennials, largely due to a lack of availability of molecular resources. Most studies have focused on genes known to regulate flowering in A. thaliana. It has been shown that over-expression of downstream genes in the floral pathway can overcome several years of juvenile period in diverged woody species. For example, transgenic citrus plants that express the A. thaliana LFY and API genes constitutively flower within one to two years instead of after seven years (Pena et al., 2001). Interestingly, when API was expressed constitutively in citrus, plants flowered early but also accelerated the production of adult-type vegetative traits, which indicated that there might be a link between reproductive maturation and vegetative development as observed in A. thaliana.

The A. thaliana gene TERMINAL FLOWER 1 (TFL1) has made a major contribution to our understanding of the molecular mechanisms that regulate reproductive competence in perennials. Constitutive expression of TFL1 in A. thaliana delays flowering and prolongs vegetative development, which
indicates that TFL1 is a floral repressor (Ratcliffe et al., 1998). In addition, TFL1 has been found to play a regulatory role after floral induction to maintain inflorescence development by the prevention of LFY and API expression in the inflorescence meristem (Ratcliffe et al., 1998; Liljegren et al., 1999). tf1 mutants flower early and develop inflorescences that terminate prematurely with a flower that is consistent with the role of TFL1 before and after the floral transition (Ratcliffe et al., 1998). Studies in other species have demonstrated several roles for TFL1 in flowering. In perennials, it has been suggested that TFL1 homologues regulate the length of the juvenile period. Transgenic Malus domestica and Populus trichocarpa plants with reduced TFL1 function accelerated flowering by shortening the length of vegetative growth before first flowering (Kotoda et al., 2006; Mohamed et al., 2010). Recently it has been demonstrated that TFL1 in A. alpina regulates reproductive competence. A. alpina plants flower in response to prolonged exposure to cold, but only if they had grown for five weeks in long days before they were vernalized. Transgenic lines with reduced AaTFL1 function do not flower without vernalization but can respond to vernalization treatment at an earlier age compared with non-transgenic lines, which indicates that AaTFL1 regulates competence to flower (Wang et al., 2011b). In addition, 35S:AaTFL1 dsRNAi lines responded to shorter period of vernalization, which suggested that AaTFL1 also regulates the response to environmental cues that induce flowering (Wang et al., 2011b).

TFL1 is a member of the CETs family of phosphatidylethanolamine-binding proteins and is a close relative of the floral promoter FLOWERING LOCUS T (FT) (Bradley et al., 1997). In A. thaliana, the FT protein interacts with the bZIP transcription factor FD in the meristem to promote flowering and also plays an antagonistic role to TFL1 (Abe et al., 2005; Wigge et al., 2005; Ahn et al., 2006). Leaves in adult shoots have higher expression levels of FT than leaves in juvenile shoots (Hsu et al., 2006; Hättasch et al., 2009). In addition, overexpression of FT homologues can induce premature flowering in many perennial species (Bohlenius et al., 2006; Hsu et al., 2006; Kotoda et al., 2010; Trankner et al., 2010; Zhang et al., 2010). This effect of the constitutive expression of FT on the juvenile phase could be explained by overcoming TFL1 repression, although it is not known if the antagonism between FT and TFL1 is conserved in other species.

The relationship between juvenility and polycarpy

Most perennials follow a polycarpic growth habit and undergo several cycles of reproduction during their lifetime. The main characteristic of polycarpicity is to commit some meristems to reproductive development and maintain vegetative growth in the remaining meristems (Battey and Tooke, 2002; Amasino, 2009; Albani and Coupland, 2010). Grafting experiments in several perennials have demonstrated that apical meristems of individual shoot branches undergo a transition from reproductively incompetent to reproductively competent phase independently (Robinson and Wareing, 1969; Hackett, 1985). In addition, the position of a branch on the plant determines its reproductive maturity. Shoot apical meristems from branches at the basal and interior part of a tree always remain juvenile whereas branches at the upper and peripheral parts of a tree can gain reproductive competence (Hackett, 1985). Detailed studies of flowering and vegetative growth patterns in A. alpina and M. domestica demonstrated that vegetative growth is maintained by axillary meristems close to the inflorescence/flower meristems (Foster et al., 2003; Wang et al., 2009b; Albani and Coupland, 2010).

PERPETUAL FLOWERING 1 (PEP1) in A. alpina confers the return to vegetative development during each annual cycle (Wang et al., 2009b). PEP1 is the orthologue of the A. thaliana MADS box transcription factor FLOWER-ING LOCUS C (FLC) and it is a floral repressor that prevents flowering before vernalization. In A. alpina, PEP1 mRNA levels are down-regulated during prolonged exposure to cold temperatures and flowers are initiated during vernalization in the shoot apical meristems of adult shoot branches. PEP1 expression is up-regulated again after vernalization to repress flowering in those shoots that did not become reproductive, through a mechanism that involves histone methylation in the PEP1 chromatin (Wang et al., 2009b). pep1 mutants commit more axillary branches to flowering but remain polycarpic, which indicates that PEP1 is not the only factor that regulates polycarpicity in A. alpina. PEP1 plays a redundant role with the A. alpina TFL1 orthologue to regulate flowering in axillary shoot branches (Wang et al., 2011b). 35S:AaTFL1 dsRNAi plants have more flowering axillary shoot branches than wild-type plants and pep1 35S:AaTFL1 dsRNAi plants enhance the number of flowering shoot branches compared with single mutants. Moreover, TFL1 is expressed in the juvenile shoots to repress flowering. The role of TFL1 in polycarpy has also been demonstrated in other perennial species (Jensen et al., 2001; Kotoda et al., 2006; Mohamed et al., 2010). Transgenic Malus domestica, Populus trichocarpa, A. alpina, and Lolium perenne plants with reduced TFL1 function commit more axillary shoots to flowering. As previously mentioned, transgenic plants also have shortened their period of vegetative growth, which indicates a possible relationship between juvenility and polycarpy.

Conclusion

An understanding of the mechanisms that regulate competence to flower is important, especially in perennial plants. A shortened juvenile phase could greatly facilitate breeding programmes that are aimed at the genetic improvement of trees. Studies in annual models related the temporal expression patterns of miR156, miR172, and their targets, with two developmental processes, leaf morphology and flowering. The function of these two microRNAs in leaf morphology is known to be conserved between A. thaliana, maize and several woody perennial species (Chuck et al., 2007; Wang et al., 2011a). However, a role for these microRNAs in
reproductive competence and/or flowering in perennials still needs to be shown. Every meristem in a perennial plant undergoes autonomously the transition from a juvenile to an adult state. Plants can accumulate meristems at different developmental stages during prolonged vegetative growth, and, in the presence of the inductive stimuli only, competent meristems will commit to flowering whereas the rest will remain vegetative. We suggest here that because TFL1 in many perennials contributes to the length of juvenile phase and to the polycarpic growth habit these processes are related and probably juvenility might play a general role in perennialism.

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