REVIEW / SYNTHÈSE

Flowering transition in grapevine (*Vitis vinifera* L.)[1]

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Abstract: The available information on the regulation of flowering transition in model systems, such as *Arabidopsis* and rice, provides a framework to undertake the study of this process in plant species with different growth strategies. The grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world. Understanding the regulation of flowering transition in this species can be relevant for the improvement of yield and quality of the crop. The grapevine is a representative of the family Vitaceae, whose species mostly grow as vines and have evolved climbing organs, tendrils, which are ontogenetically related to the reproductive organs. Here, we summarize the available information on the flowering transition in the grapevine. With this purpose, we first describe the vegetative and reproductive development of the grapevine as well as the reports on the physiology of flowering induction in this species. As well, we review the recent information on the molecular genetics of flowering signal integrator and flower meristem identity genes in the grapevine and compare the process with what is already known in model systems such as *Arabidopsis*. Finally, we propose a preliminary model to explain the regulation of flower initiation in the grapevine that is useful to identify its differential features and infer future prospects in the understanding of this process.

Key words: Flowering transition, flower initiation, grapevine, flower identity genes.

Résumé : L'information disponible sur la régulation de la floraison dans des systèmes modèles, comme l'*Arabidopsis* et le riz, offre un cadre de travail pour entreprendre l'étude de ce processus chez des espèces végétales ayant des stratégies de croissance différentes. La vigne à raisin (*Vitis vinifera* L.) représente l'espèce de fruit le plus largement cultivé et économiquement important au monde. Une compréhension de la régulation de la transition florale, chez cette espèce, peut s'avérer pertinente pour l'amélioration du rendement et de la qualité de cette culture. Représentative de la famille des Vitaceae, la vigne à raisin comporte des espèces généralement sarmenteuses ayant développé des organes pour grimper au cours de l'évolution, les vrilles, ontogénétiquement reliées aux organes de reproduction. Les auteurs résument l'information disponible sur la transition florale, chez la vigne à raisin. À cette fin, ils décrivent d'abord le développement végétatif et reproductif de la vigne à raisin, ainsi que les rapports sur l'induction florale chez cette espèce. De plus, ils révisent l'information récente sur la génétique moléculaire de l'intégration des signaux floraux et l'identité des gènes des méristèmes floraux, avant de comparer ce processus avec ce que l'on connaît déjà dans des modèles comme l'*Arabidopsis*. Finalement les auteurs proposent un modèle préliminaire pour expliquer la régulation de l'initiation florale chez la vigne à raisin, utile pour identifier ses caractéristiques différentielles et en déduire de nouvelles prospectives pour la compréhension de ce processus.

Mots-clés : transition florale, initiation forale, identité des gènes floraux.

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Introduction

Flowering transition is one of the most important decisions in the life cycle of plants. To achieve reproductive success, wild plants need the most favorable environmental conditions to initiate their reproductive development. Furthermore, in crop species, flowering initiation and flower and fruit developmental decisions can determine the quality and quantity of crop production consisting of either fruits, seeds, or vegetative organs (Boss et al. 2004).

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Extensive genetic and molecular analyses performed in the facultative long-day annual plant *Arabidopsis thaliana* (L.) have generated a complex genetic model explaining how the plant integrates environmental signals (mainly light and temperature-related variables) to regulate the expression of genes specifying the identity of flower meristems and flower organ primordia (Ausín et al. 2005). Parallel studies in other herbaceous species such as rice (*Oryza sativa* L.), with short-day photoperiod requirements, have revealed the conservation of the molecular mechanisms regulating flowering responses to photoperiod in dicots and monocots (Hayama and Coupland 2004). In contrast, responses to temperature could have evolved independently after the separation of monocot and dicot taxa, since wheat (*Triticum aestivum* L.) and *Arabidopsis* have recruited different regulatory genes (Yan et al. 2004; Ausín et al. 2005). Little is known about the regulation of flowering transition in woody perennial species or in species with particular growth habits (Martin-Trillo and Martinez-Zapater 2002). However, the comparative and functional genomic approaches undertaken in some species are starting to provide information on the conservation of flowering regulatory pathways in woody species (Brunner and Nilsson 2004). For instance, recent results indicate that the photoperiod pathway regulating flowering time in herbaceous plants is also functional in aspen (*Populus* sp.) (Bohlenius et al. 2006).

The Vitaceae is a basal family of eudicots (Judd et al. 1999) with a pattern of organ formation and development quite distinct from that previously described for annual herbaceous plants (Mullins et al. 1992). This family, mostly formed by species that grow as vines, includes the widely grown fruit-crop species, *Vitis vinifera* L. When grown in the wild, *Vitis vinifera* subsp. *sylvestris*, the putative ancestor of the cultivated grapevine, is a vigorous climbing plant with pressure-sensitive tendrils that allows it to climb into the forest canopy to a height of 20–30 m, supported by the forest trees. These plants flower once they reach the forest canopy and produce a large number of small bunches of fruits. When grown as a crop, the grapevine *V. vinifera* subsp. *sativa* is severely pruned to reduce bunch number and to increase fruit size and quality. Moreover, cultivars are vegetatively propagated and the observed developmental pattern generally corresponds to that of the adult plant. Understanding the genetic and molecular mechanisms responsible for flowering transition in the grapevine can shed light on the specific mechanisms evolved by the Vitaceae to adapt to their particular growing conditions. Furthermore, knowledge of the regulation of flowering transition and inflorescence and flower development in grapevine can help develop new genetic or management strategies to improve berry production.

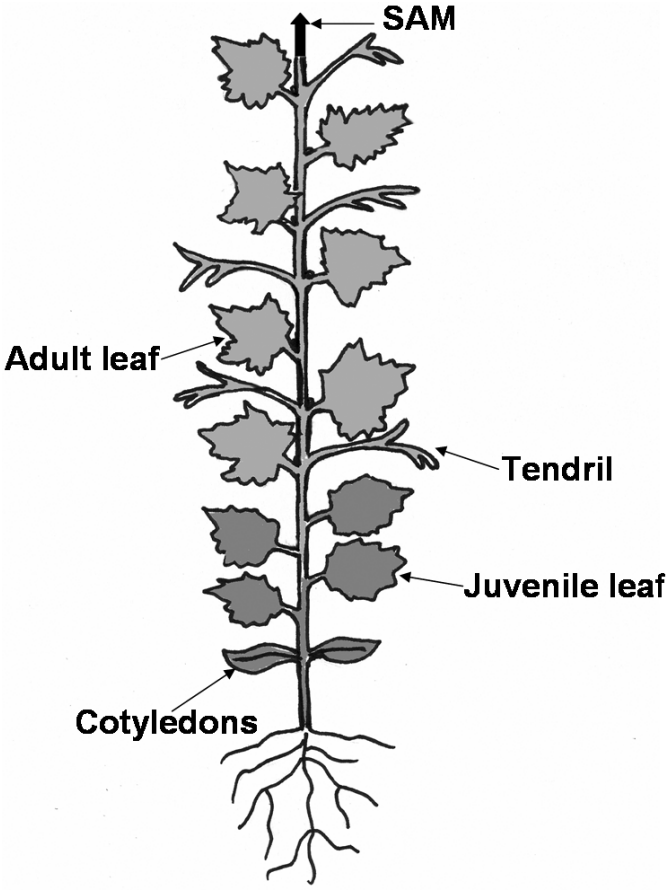
In this review we summarize the current understanding of flowering transition in the grapevine with special emphasis on the differences that can be observed between the grapevine and plant species that have been more thoroughly analyzed. We will start with a description of grapevine development and will summarize the available molecular and genetic information to develop a hypothetical genetic model on the regulation of flower transition in this species. The reproductive development of the grapevine and other Vitaceae species has been described in previous morphological works (Srinivasan and Mullins 1980; Posluszny and Gerrath 1986; Gerrath and Posluszny 1988*b*) and we will only consider those basic aspects that distinguish the Vitaceae and help to understand the flowering transition process. A few previous studies have reviewed the flowering process in the grapevine (Boss and Thomas 2000; Boss et al. 2003) and will be used here as a starting point to consider additional results and interpretations.

Grapevine vegetative development

A description of grapevine development requires consideration of the environmental conditions in which the plant develops in the wild. Grapevine seeds germinate in humid and shady riverbank forests. Upon germination, seedlings display a short-lived juvenile vegetative phase in which the shoot apical meristem (SAM) initiates leaf primordia that will give rise to leaves with a spiral phyllotaxis (Mullins et al. 1992) (Fig. 1). After the production of 6–10 nodes, depending on the genotype, plants undergo major developmental changes that are considered to mark the developmental transition to an adult vegetative phase. These changes are the transition from spiral to alternate phyllotaxis (Lacroix and Posluszny 1989), the modification of leaf morphology, and the initiation of lateral meristems by the SAM. These lateral meristems, historically known as *uncommitted primordia*, alternate with leaf primordia following a characteristic sequence (Tucker and Hoefert 1968; Pratt 1974; Gerrath and Posluszny 1988*a*; Gerrath et al. 1998) and give rise to tendrils, modified shoots that function as a climbing organ in the Vitaceae. Owing to unequal internode elongation, tendrils developed from the lateral meristems become opposed to leaves in the expanded shoot (Fig. 1). The SAM produces a series of two consecutive nodes containing opposed leaf primordia and lateral meristems alternating with one node bearing a solitary leaf primordium (Fig. 1). Both the leaf phyllotaxis and leaf morphological changes and the differentiation of tendrils are related to the acquisition of the climbing habit of the plant (Pratt 1974).

Adult vegetative plants need to compete with other forest trees for access to full-light exposure in the forest canopy. During vegetative growth, the alternate pattern of leaf and tendril development is constantly repeated. This phyllotactic pattern, in which both leaf and uncommitted lateral meristems are produced at the shoot apex, is uncommon in vascular plants, with only few similar exceptions known (Gerrath et al. 1998). Attempts to explain this unusual organ positioning have generated equally plausible interpretations on shoot formation, either sympodial (Snyder 1933; Alleweldt 1963; Alleweldt and Balkema 1965) or monopodial (Tucker and Hoefert 1968; Pratt 1971; Srinivasan and Mullins 1976). For discussion see Pratt (1974), Gerrath and Posluszny (1988*a*), Morrison (1991), and Gerrath et al. (1998). Axillary meristems in the axils of leaves develop into bract-protected structures known as buds (Fig. 2A). These initial buds are known as summer lateral buds and grow out the same year they are produced. However, axillary to their first modified leaf or prophyll, they carry a compound latent bud composed of a primary bud with one or two additional secondary buds (Pratt 1974; Morrison 1991; May 2000). In temperate climates this compound latent bud enters dor-

Fig. 1. Grapevine vegetative development. After germination, the seedling follows a short juvenile phase producing 6–10 juvenile leaves with spiral phyllotaxis. Transition to the adult phase is marked by the production of tendrils and changes in leaf morphology and phyllotaxis. Note that tendrils and leaves become opposed at maturity owing to unequal internode elongation and that tendrils are not produced in the grapevine at every third node.

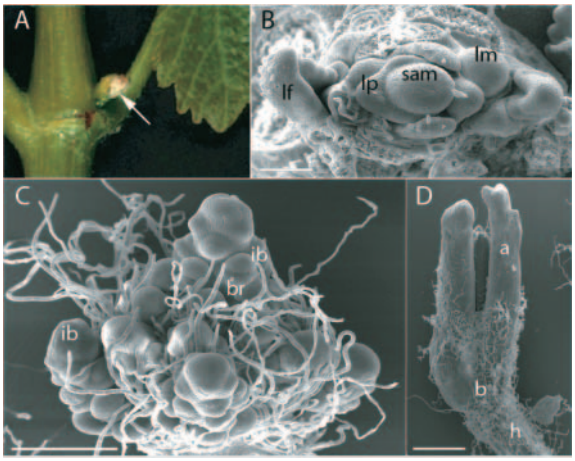


mancy at the end of the summer and the primary bud will produce new shoots the following spring. SAMs in these buds follow a similar pattern of organ differentiation as the main SAM. During their first months of development, the SAM produces three to eight leaf primordia with spiral phyllotaxis (Fig. 1). Afterwards, it begins to produce lateral meristems alternate to leaf primordia. Organ growth takes place after the winter resting period, when dormant buds break and generate new vegetative branches (Srinivasan and Mullins 1980; Posluszny and Gerrath 1986; Gerrath and Posluszny 1988*b*; Mullins et al. 1992).

Flower Initiation in Grapevine

In the wild, adult plants can take several years before producing their first flowers, since flowering induction takes place in canes that reach the canopy (Mullins et al. 1992). However, under particular viticulture practices in warm environments, they can be forced to flower in their second year of growth. Flowering in the grapevine requires two consecutive growing seasons. The flowering induction process takes place in the latent primary buds during the summer. However, flower initiation and development will

Fig. 2. Shoot development within a grapevine latent bud. (A) Grapevine bud (white arrow) in the axil of a young leaf. (B) SEM of the shoot structures that differentiate within the bud during the flowering inductive season (scale bar = 100 μm). (C) SEM of an inflorescence around the end of the first growing season (scale bar = 200 μm). (D) SEM of a developing tendril. sam, shoot apical meristem; lp, leaf primordium; lf, leaves; lm, lateral meristem; ib, inflorescence branch meristem; br, bracts subtending each inflorescence branch meristem; h, hypoclade; b, branching zone of the tendril; a, tendril arm (scale bar = 1 mm). Samples for SEM were fixed (phosphate-buffered saline, 4% paraformaldehyde, 0,1% Triton, 0,1% Tween 20), incubated in a phosphate-buffered saline solution with 2% osmium tetroxide, dehydrated through an ethanol series (50%, 70%, 85%, 95%, and 100% [*v*/*v*]) and critical-point dried. They were then coated with gold particles and observed with SEM.



not take place until the following spring (Mullins et al. 1992). The process of inflorescence and flower initiation has been characterized by scanning electron microscopy (SEM) in several *Vitis* species and *V. vinifera* cultivars (Srinivasan and Mullins 1976, 1981; Posluszny and Gerrath 1986; Gerrath and Posluszny 1988*b*; Carmona et al. 2002). In flowering-induced canes, the SAMs in the primary and secondary buds of the compound buds will initiate two to three lateral meristems that give rise to inflorescences in place of tendrils (Pratt 1971; Srinivasan and Mullins 1981; Posluszny and Gerrath 1986; Gerrath and Posluszny 1988*a*; Morrison 1991). These inflorescence meristems grow rapidly within the bud during the summer. First, a bract is formed at the region farthest from the apex (Fig. 2C). Then, the inflorescence meristem splits into two unequal meristems. The larger, adaxial or inner meristem will give rise to the main body of the inflorescence, while the smaller abaxial (outer) one will form the most basal branch of the inflorescence. Each inflorescence branch meristem, through consecutive branching, will give rise to second and third order inflorescence branch meristems, each of which is subtended by a bract. Within the latent bud there is no internode elongation, so by the end of the summer the bud encloses a compressed shoot with inflorescence branch meristems and tendril and leaf primordia (Figs. 2C and 2D). These buds are protected from desiccation and freezing by bracts, stipules, and epidermal hairs, and become dormant during the fall.

During the following spring, when the environmental conditions permit, bud-growth resumes. Upon reactivation, the SAM of the primary bud produces additional leaf and tendril primordia. The second and third order inflorescence branch meristems can form additional inflorescence meristems in a spiral phyllotaxis. Thus, each racemose inflorescence is formed by multiple branches that prefigure the conical bunch of grapes. At this stage, each inflorescence branch meristem divides into a cluster of three to four flower meristems arranged as a dichasium. The terminal flower develops first, then the lateral ones develop, and the basal-most develops last. Flower development takes place when the bud swells and shoot internodes begin to elongate. Flower meristems sequentially form sepal, common petal-stamen, and carpel primordia, which will differentiate in the corresponding flower organs (see Srinivasan and Mullins (1981), Gerrath and Posluszny (1988*b*) and Gerrath (1993) for a description of grapevine flower development).

As early as 1875, Darwin realized the existence of homology between the tendril and the inflorescence of the Vitaceae and it is generally accepted that both are modified shoots with a common origin (Tucker and Hoefert 1968; Pratt 1974; Morrison 1991). This hypothesis is based on several observations:(*i*) they are derived from the same meristem, the lateral meristem (also known as anlagen or uncommitted primordium); (*ii*) each organ can substitute for the other depending on environmental conditions or hormonal treatments (Srinivasan and Mullins 1976, 1980; Boss and Thomas 2000, 2002); *iii*) intermediate organs between tendrils and inflorescences are frequently formed (Boss et al. 2003). Furthermore, confirming their shoot nature, they can be forced to differentiate as shoots by chemical treatments or under extreme environmental conditions (Srinivasan and Mullins 1976, 1980; Morrison 1991). The production of lateral meristems that give rise to tendrils, inflorescences, or intermediate organs and the differentiation by the SAM of both leaves and lateral meristems that become opposed at maturity are special features of the Vitaceae (Gerrath et al. 1998).

Physiology of flower induction in grapevine

The environmental stimuli that induce flowering in the grapevine are short-term exposures to high temperature and high light intensity (Mullins et al. 1992). In experiments performed under controlled environmental conditions (Butrosse 1969*a*, 1969*b*), a 4 h pulse of high temperature per day was sufficient to induce the maximum number of inflorescence meristems in the apices of latent buds, when applied three weeks before the formation of lateral meristems. Low temperatures did not seem to have major effects on flowering induction. The number and size of inflorescences also increased at higher light intensities (Butrosse 1968), but no effect of light quality on inflorescence formation has been reported. Although there are no photoperiod requirements for flowering induction, some cultivars produce higher number of inflorescences per bud under long-day than under short-day photoperiods (Butrosse 1974). Genetic variation for temperature requirements and responses to light intensity are very frequent among grapevine cultivars (Butrosse 1970; Sánchez and Dokoozlian 2005). Thus, a combination of exposure to high temperature and high light intensity is necessary for maximum fruitfulness of latent buds (Mullins et al. 1992). Interestingly, an increase in light and temperature are the environmental stimuli that a wild grapevine cane encounters when reaching the forest canopy, and this promotes flowering induction and causes flower formation the following season.

At the hormonal level, both gibberellins (GAs) and cytokinins seem to be involved in the control of flowering induction in grapevine. Gibberellins promote the initiation of lateral meristems but inhibit their development as inflorescences favouring tendril development. GA-treated plants show premature sprouting and precocious formation of lateral meristems that differentiate as tendrils. Furthermore, both lateral meristem initiation and tendril elongation are suppressed by chlormequat, an inhibitor of gibberellins biosynthesis (Srinivasan and Mullins 1980). Genetic evidence of the flowering inhibitory role played by GA in the grapevine is provided by the phenotype of gibberellin-insensitive grapevine plants mutated at *VvGAI*, the *Arabidopsis GIBBERELLIN INSENSITIVE* (*GAI*) orthologous gene (Boss and Thomas 2002). These mutant plants are dwarfs with short internodes and all their tendril primordia are transformed into inflorescences, even in young seedlings. This phenotype strongly supports the hypothesis that gibberellins or a gibberellin-dependent signal-transduction pathway inhibit the differentiation of inflorescences (Boss and Thomas 2002).

Cytokinins seem to positively regulate the development of inflorescences from lateral meristems. For instance, when isolated tendril primordia are grown in vitro with either benzylaminopurine (BA), 6-(benzylamino)-9-(2-tetrahydropiranil)-9H-purine (PBA) or zeatin riboside, they actively branch and develop as inflorescences or inflorescence-like structures (Srinivasan and Mullins 1978). Moreover, repeated applications of PBA to shoot tips of intact plants promote newly formed lateral meristems and tendril primordia to develop as inflorescences (Srinivasan and Mullins 1979, 1980) and simultaneous applications of both chlormequat and cytokinins to tendril primordia results in prolific flower formation (Srinivasan and Mullins 1980). Altogether, these results suggest that variation in gibberellin and cytokinin levels could determine the developmental fate of lateral meristems. It is possible that environmental signals, such as light and temperature increases, could regulate the differentiation of lateral meristems through their effects on hormone biosynthesis or response pathways.

Molecular genetics of flower initiation in grapevine

A mutant analysis of flowering induction and flower initiation has not been attempted in the grapevine owing to the complexities of this strategy in a woody species with a long generation time and without the availability of pure lines. One alternative approach for trying to understand the genetic and molecular mechanisms that underlie flowering induction in the grapevine is based on the identification and functional analysis of *V. vinifera* orthologs of *Arabidopsis* genes involved in flowering-signal integration and the establishment of flower-meristem and flower-organ identity (see Putterill et al. 2004; Boss et al. 2004; Ausín et al. 2005 and Parcy 2005 for recent reviews on the *Arabidopsis* genes). Several laboratories have reported the isolation of putative grapevine

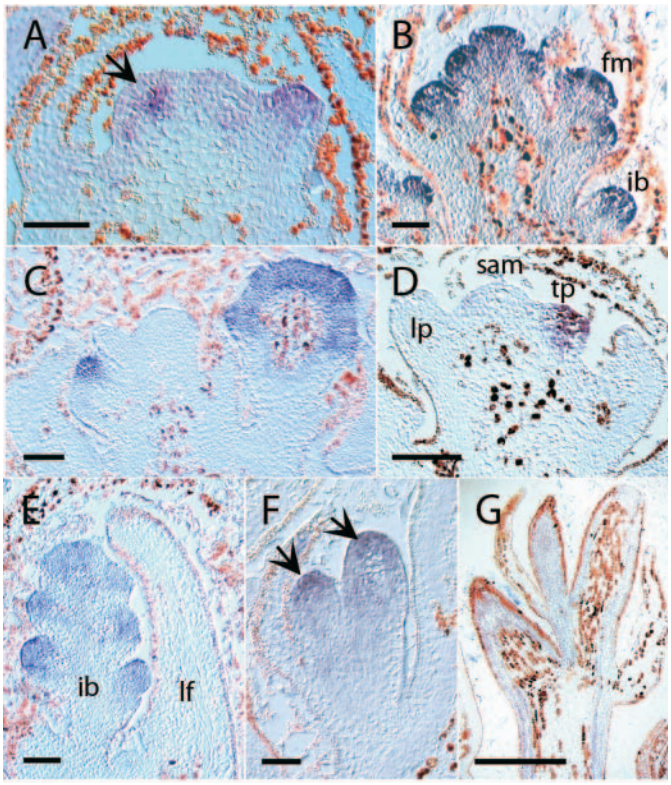
flowering signal integrators and flower meristem identity genes and analyzed their expression along reproductive development (Carmona et al. 2002; Calonje et al. 2004; Joly et al. 2004; Boss et al. 2006; Carmona et al. 2006; Sreekantan and Thomas 2006*a*). However, information about gene function in the grapevine is still scarce, because only the expression information exists for most genes. Regarding grapevine flowering signal integrators, three putative members of the *SOC1/AGL20* MADS-box gene subfamily of MADS transcription factors (Pařenicová et al. 2003) are present in the grapevine genome [ESTs and genomic sequence data, (Carmona et al., unpublished results, 2007)]. One of these, *VvMADS8*, has been partially characterized (Sreekantan and Thomas 2006*a*). *VvMADS8* expression is high during the very early stages of inflorescence development and decreases in later stages of flower development, not being detected in mature flowers and fruits. *VvMADS8* is also slightly expressed during tendril development. Overexpression of *VvMADS8* in transgenic *Arabidopsis* plants promotes early flowering, supporting a similar role for this gene as for the endogenous *Arabidopsis* ones. These results provide evidence for the functional conservation of this MADS-box gene subfamily, but further work is required to establish the role of each one of its members in the grapevine.

The *Arabidopsis* flowering signal integrator gene *FT* belongs to the small gene family (*FT/TFL1*), which encodes proteins similar to mammalian phosphatidylethanolamine-binding proteins with either positive or negative effects on flower initiation (Bradley et al. 1996). Homologous genes have also been characterized in the grapevine (Joly et al. 2004; Boss et al. 2006; Carmona et al. 2006; Sreekantan and Thomas 2006*a*). The grapevine *FT/TFL1* family contains at least five members that can be grouped in three subfamilies (*FT*-like, *MFT*-like, and *TFL1*-like, Carmona et al. 2006), as previously shown in other species (Carmel-Goren et al. 2003; Chardon and Damerval 2005; Ahn et al. 2006). Among them, expression of the most probable *FT* ortholog, *VvFT,* is associated with seasonal flowering induction in latent buds and to the development of inflorescences, flowers, and fruits (Carmona et al. 2006; Sreekantan and Thomas 2006*a*). Furthermore, overexpression of *VvFT* in transgenic *Arabidopsis* causes similar effects as *FT* (Kardailsky et al. 1999; Kobayashi et al. 1999) suggesting that this gene is the *FT* ortholog. Three members of the grapevine *FT/TFL1* subfamily are more related to *Arabidopsis TFL1* (Carmona et al. 2006). These genes are expressed in first-season latent buds and during the initial stages of inflorescence development, but are not detected along flower development. Overexpression of *VvTFL1A* in transgenic *Arabidopsis* seems to delay flowering time (Boss et al. 2006) and the initiation of flower meristems, yielding a phenotype of complex inflorescences with multiple coflorescences (Boss et al. 2006; Carmona et al. 2006). Both its expression pattern in the grapevine and the phenotypic effects caused in transgenic *Arabidopsis* plants are consistent with a role for this gene in maintaining meristem indeterminacy. Whether the additional grapevine *TFL1*-like genes are functionally redundant with *VvTFL1A* or have specific roles in different meristems awaits further characterization. In general, expression patterns of grapevine *FT/TFL1*-like genes are found associated with either meristem proliferation or determination processes. Expression of genes such as *VvFT* and *VvMFT* is associated with meristem determination and differentiation of organs such as inflorescences, flowers, or tendrils, whereas *TFL1*-like gene expression is more associated to meristem proliferation in shoot and root apices (Carmona et al. 2006). These expression patterns are in agreement with the biological function proposed for these genes subfamilies in other species (Bradley et al. 1997; Pillitteri et al. 2004; Ahn et al. 2006), and could suggest a basic role for the gene family in meristem maintenance and determination (Lifschitz et al. 2006).

Concerning the putative grapevine flower meristem identity genes, a single ortholog of the *Arabidopsis* LEAFY (LFY) transcription factor (Weigel et al. 1992), known as VFL, has been found in the grapevine (Carmona et al. 2002; Joly et al. 2004; Boss et al. 2006). *VFL* expression was detected in lateral meristems prior to any commitment, and later in inflorescence and tendril meristems (Fig. 3A). *VFL* expression is soon down-regulated during tendril development. In contrast, *VFL* expression increases in the proliferating inflorescence meristems that are dividing to generate inflorescence-branch meristems in summer latent buds. Furthermore, its expression reaches the highest levels in the floral meristems that develop in bursting buds the following spring (Fig. 3B). *VFL* is also expressed in petal and stamen primordia, where its expression declines as organs expand. Expression of *VFL* in reproductive meristems and developing floral organs suggests that *VFL* plays an important role during reproductive development, as it has been suggested for most *FLO/LFY* like genes analyzed in other species (Maizel et al. 2005). Expression patterns spanning two growing seasons have also been described for the *FLO/LFY* ortholog in kiwi fruit (*Actinidia deliciosa* (A. Chev.) Liang & Ferguson), another woody perennial with winter-bud dormancy (Walton et al. 2001). In both cases, the highest levels of *FLO/LFY* expression correspond to the time of flower meristem formation (first season in the case of kiwi fruit and second season in the case of the grapevine), in agreement with a role of *LFY* orthologous genes in the specification of flower meristem identity in these woody species. The function of *VFL* has not been demonstrated yet in transgenic grapevine plants. However, overexpression of *VFL* in transgenic *Arabidopsis* promotes a rapid transformation of inflorescence to flower meristems similarly to the effect of the overexpression of the endogenous *LFY* gene (Carmona et al., unpublished results, 2007).

Expression of *VFL* is not restricted to reproductive organs. Low transcript levels are detectable in the inner cell layers of the vegetative SAM even in young germinating seedlings as well as in tendril meristems (Fig. 3A) (Carmona et al. 2002; Joly et al. 2004), suggesting that *VFL* expression alone is not sufficient to induce flowering. *VFL* transcripts also accumulate in leaf primordia and developing leaves, as has also been shown for many *FLO/LFY* orthologs analyzed (Kelly et al. 1995; Blázquez et al. 1997; Pouteau et al. 1997; Southerton et al. 1998; Molinero-Rosales et al. 1999; Rottmann et al. 2000; Walton et al. 2001). In developing leaves, *VFL* accumulates at the growing margins. At this region, *VFL* could be involved in maintaining cell proliferation in specific areas generating the palmate shape of

Fig. 3. Expression of flower meristem identity genes in developing shoot structures within the latent buds. (A) Section of a bud during floral transition. *VFL* is expressed in the lateral meristem (arrow). (B) Section of an inflorescence at a more advanced stage than that in Fig. 2C showing accumulation of *VFL* in inflorescence branch meristems (ib) and in newly formed flower meristems (fm). C Expression of *VAP1* in inflorescence meristems. (D) *VFUL-L* expression in a lateral meristems that will give rise to a tendril (tp) but not in the leaf primordia (lp). (E) Expression of *VAP1* in the inflorescence branch meristems (ib) of an inflorescence of a stage similar to that in Fig. 2C. (F) *VAP1* expression is restricted to the arms of the developing tendrils (arrows). (G) *VFUL-L* is expressed throughout developing tendrils. Digoxigenin labeling of RNA probes, tissue preparation, and hybridization were performed as described by Coen et al. (1990). The template for the *VFL* digoxigenin-labeled riboprobes was a 1249 bp fragment, containing the *VFL* complete coding region obtained by reverse transcriptase-PCR and cloned in pBlueScript KS vector. The templates for the *VFUL-L* and *VAP1* riboprobes were a 734 bp and a 720 bp fragments, respectively, containing the 3′ region of the genes. Hybridized sections were visualized with Nomarski optics in a Leica DMR microscope (DMR, Leica, Wetzlar, Germany). A–F: scale bars = 50 μm; G: scale bar = 1 mm.



the grapevine leaves. Such a role for *FLO/LFY*-like genes has been suggested in the pea where *UNIFOLIATA* is required to generate the wild-type compound leaves (Hofer et al. 1997) and in tomato where *falsiflora* mutants have leaves with fewer leaflets than wild-type plants (Molinero-Rosales et al. 1999).

Homologs of *Arabidopsis* flower meristem identity genes such as *APETALA1* (*AP1*) (Mandel et al. 1992) and *FRUITFULL* (*FUL*) (Gu et al. 1998; Ferrándiz et al. 2000) MADS-box genes have also been characterized in the grapevine under the names of *VAP1* and *VFUL-L* (Calonje et al. 2004). *FUL-L* is a *FUL* paralog, likely with a similar function as *FUL* in *Arabidopsis* (Litt and Irish 2003). In contrast to *VFL* expression, *VFUL-L* and *VAP1* transcripts were not detected in the SAM or in leaf primordia, but appeared very early in the uncommitted lateral meristems (Figs. 3C–3E), and their expression was maintained in the modified shoots produced by the lateral meristems; either inflorescences or tendrils. As their *Arabidopsis* homologs, both *VAP1* and *VFUL-L* are expressed throughout flower development suggesting that they could play a role in the specification of flower organ identity. *VAP1* is broadly expressed in the newly formed flower meristems but soon becomes excluded from the sepal-forming region and restricted to the inner part of the meristem that forms the petals, stamens, and carpels. Later, *VAP1* preferentially accumulates at the tips of the flower-organ primordia and becomes restricted to the carpel-forming region of the flower meristem. In contrast, *VFUL-L* is expressed in a small, central region of young floral meristems. During flower development, *VFUL-L* was not detected in sepal, petal, or stamen primordia but was restricted to the prospective carpel-forming region, which would be consistent with a role of this gene in carpel and fruit development. The strong and distinctive expression of *VFUL-L* and *VAP1* in developing tendrils (Figs. 3F and 3G) could be interpreted in two ways: (*i*) it could suggest that both genes have been recruited for a new role, the development of the Vitaceae tendril; (*ii*) their expression throughout tendril development could suggests that this climbing organ would have evolved from the loss of fertility (loss of flowers) of a reproductive organ. Considering this viewpoint, tendrils could be considered as sterile inflorescences.

Particular features of grapevine flowering transition

Genetic and molecular characterization of the flowering process in different species reveals a conservation of the basic genetic mechanisms controlling the early stages of flowering transition and initiation (Theissen and Saedler 1999; Ng and Yanofsky 2001, Hayama and Coupland 2004). However, when homologous genes from species other than *Arabidopsis* have been analyzed, differences regarding expression patterns and mutant phenotypes have been detected that may reflect roles distinct from those described for the *Arabidopsis* genes. Thus, flowering transition may involve regulatory mechanisms common to all the angiosperms as well as species-specific mechanisms whose genetic and molecular bases are yet unknown (Ferrario et al. 2004).

Flowering transition in the grapevine, a woody perennial, shows fundamental differences when compared with herbaceous-annual model systems such as *Arabidopsis* or rice, as well as some peculiarities with respect to what has been described for other polycarpic woody species (Brunner and Nilsson 2004). Most specific features of grapevine flowering transition relate to the fact that the vine uses tendrils, which share a common ontogenetic origin with inflorescences, as climbing organs. This feature determines important differences in the vegetative development of the plant as well as in its flowering transition.

Compared with other woody species, the grapevine juvenile phase is very short (only a few nodes), and transition to

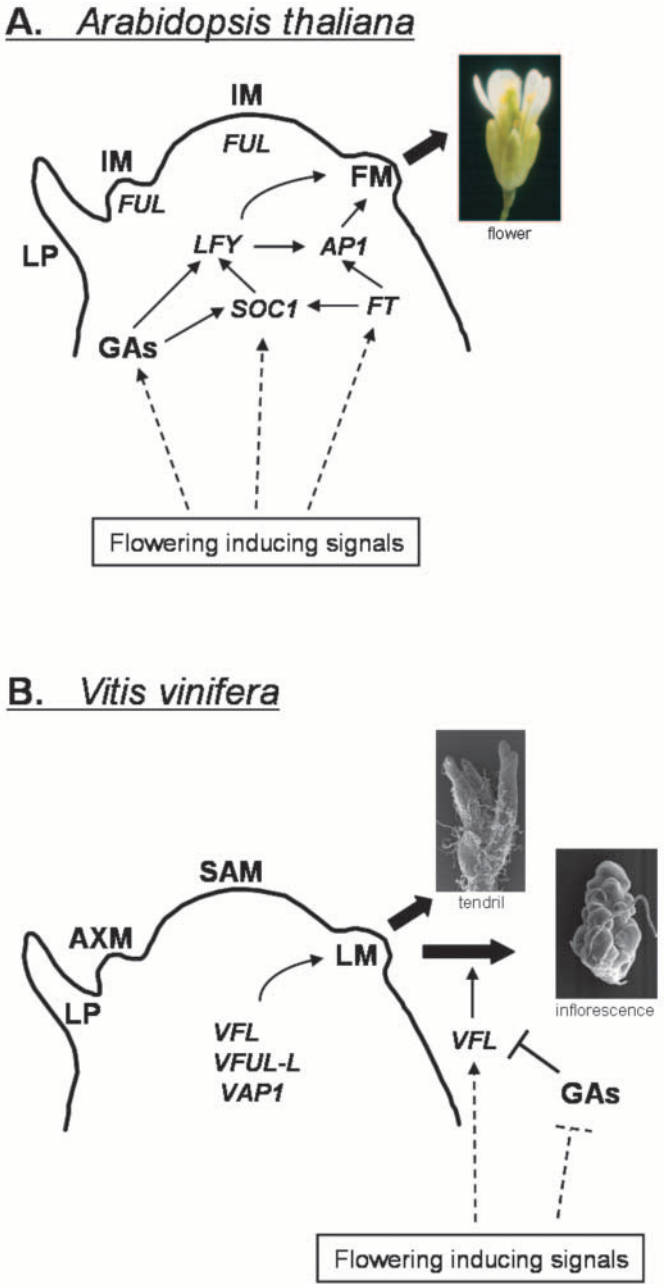


Fig. 4. Schematic comparative models of pathways controlling flower initiation in *Arabidopsis* (A) and grapevine (B). In *Arabidopsis*, different flowering regulatory pathways respond to flowering inducing signals regulating the expression of flowering integrator genes such as *LFY*, *SOC1*, and *FT*. Among the flowering pathways, gibberellins promote the expression of *LFY*. As a result of flowering induction, the SAMs of the plant are transformed into inflorescence meristems (IM) where *FUL* is expressed. Later, expression of *LFY* and *AP1* in the lateral meristems formed by the IM, specify their flower meristem (FM) identity. All lateral meristems produced by the IM are FMs giving rise to flowers. In the grapevine, the SAM produces two types of lateral meristems, the leaf axillary meristems (AXM) and the uncommitted lateral meristems (LM), all through the vegetative development of the adult plant. Expression of *VFL*, *VAP1*, and *VFUL-L* is associated with the initiation of lateral meristems. Throughout vegetative development, LMs follow a default developmental program giving rise to tendrils, a process that could require *VAP1* and *VFUL-L* expression. Flowering inductive signals, through so far unknown pathways, promote a fate-change in the developmental program of the LMs that give rise to inflorescences and flowers. This reproductive developmental program is associated with an increase in *VFL* expression and is repressed by gibberellins.

the adult phase is not only marked by phyllotactic and morphological changes of the leaves, but by the initiation of additional lateral meristems or uncommitted primordia at the SAM, which will give rise to modified shoots. These modified shoots will differentiate as tendrils during the adult vegetative phase and as inflorescences during reproductive phases. Thus, the SAM of adult grapevine plants simultaneously produces two types of lateral meristems, the vegetative leaf axillary meristems that always give rise to vegetative shoots and the lateral meristems that give rise to different types of modified shoot structures (Fig. 4). This developmental pattern allows the concurrent existence of vegetative and reproductive buds on the same shoot and differs with respect to the developmental pattern observed in herbaceous species such as *Arabidopsis*. In *Arabidopsis*, the SAM produces axillary meristems giving rise to vegetative shoots (secondary rosettes) when grown under noninductive flowering conditions, or inflorescences and flowers when flowering is induced (Fig. 4). This developmental pattern observed in the grapevine also contrasts with the pattern observed in other woody species. In trees like aspen, the SAM meristem alternates the production of vegetative and reproductive lateral meristems depending on the season (Brunner and Nilsson 2004), differing significantly from the simultaneous development observed in grapevines.

Flowering transition in adult grapevine plants does not target the fate of the axillary vegetative meristems, as in other species, but the developmental pattern of the modified shoots developing from the uncommitted lateral meristems (Fig. 4). Under noninductive conditions, these lateral meristems would follow a default developmental program to generate the modified climbing shoot or tendril. However, under flowering-inductive conditions, these lateral meristems will follow a reproductive program giving rise to inflorescences. *VFL*, *VFUL-L*, and *VAP1* (Carmona et al. 2002; Calonje et al. 2004; Joly et al. 2004) are expressed in the uncommitted lateral meristems independently of their fate (Figs. 3A, 3C, and 3D). The three genes are also expressed throughout inflorescence development (Figs. 3B, 3C, and 3E). However, in tendril primordia, *VFL* is only expressed in the early stages of its development (Fig. 3A), whereas *VFUL-L* and *VAP1* are maintained until late developmental stages (Figs. 3F and 3G). These observations suggest that *VFL*, *VFUL-L*, and *VAP1* could have a role in the specification of the uncommitted lateral meristem. Furthermore, expression of both MADS-box genes along tendril and inflorescence development (Figs. 3F and 3G) could suggest that they are involved in the development of both organs without determining the developmental fate of the uncommitted lateral meristem (Calonje et al. 2004). The choice of a reproductive developmental program by the grapevine lateral meristems could be associated with an increase in *VFL* expression (Carmona et al. 2002; Boss et al. 2006).

Although *VFL* is initially expressed in the lateral meristems independently of their fate, an increase in *VFL* expression is only correlated with inflorescence and flower development (Fig. 3B). This correlation might suggest that expression of *VFL* is required to be over a given threshold level to establish the reproductive developmental pathway. A threshold level of *LFY* expression has also been shown to be associated to flower initiation in *Arabidopsis* (Blázquez et al. 1997). Thus, the role of *VFL* in the grapevine could parallel what has been described in *Arabidopsis* where *LFY* integrates flowering signals from different pathways and controls the expression of flower organ identity genes (Parcy et al. 1998; Blazquez and Weigel 2000).

As yet, it is unknown what flowering induction pathway(s) could regulate *VFL* expression in the grapevine. However, the correlation observed in the *VvGAI* mutant between inflorescence development and increased *VFL* expression (Boss and Thomas 2002) suggests that gibberellins could function to repress inflorescence development, directly or indirectly affecting *VFL* expression (Fig. 4). Interestingly, in *Arabidopsis*, *LFY* expression is induced by the gibberellin pathway that is required for flowering promotion, independently of photoperiod (Blazquez and Weigel 2000). Thus, a gibberellin-mediated flowering pathway targeting *LFY/VFL* genes could be shared between *Arabidopsis* and the grapevine, although with opposite effects on its expression. This is not an uncommon situation in the regulation of flowering in species with different environmental requirements, as has been shown for photoperiod flowering induction, where long-day species such as *Arabidopsis* and short-day species such as rice share the same regulatory pathways, although with different promotion or repressive effects on the expression of target orthologous genes (Hayama and Coupland 2004).

Future prospects

Comparative analysis of the flowering transition process in the grapevine and other herbaceous and woody species allows the identification of at least two key regulatory switches for this process in the grapevine. First, the specification of the lateral meristem identity that marks the transition between juvenile and adult plants and the acquisition of a climbing habit. Second, control of the fate of lateral meristems that can either follow a default developmental pathway to produce a tendril or a reproductive pathway to give rise to an inflorescence. Identifying what genes are involved in these developmental switches and how they are regulated will contribute significantly towards understanding flowering transition in the Vitaceae.

Availability of the grapevine-genome sequence in the near future will provide a major tool to answer those questions. Comparative genome studies combined with genome-wide expression analyses using microarrays will facilitate the rapid identification of candidate genes and pathways regulating those developmental switches. However, confirmation of the candidate-gene hypotheses will require either forward or reverse genetic approaches, still poorly developed in the grapevine. On one hand, reverse genetic approaches are hampered by the low efficiency of current genetic transformation protocols and the lack of mutant collections or VIGS strategies for *Vitis*. On the other hand, forward genetic approaches based on mutant screens are, to date, difficult in the grapevine given the lack of pure lines and the size and life cycle of the plant. Added to that, the elucidation of the regulatory networks controlling flowering transition poses additional difficulties related to the late expression of the trait. Nevertheless, as more research groups become involved in the task of developing functional genomic tools, these constraints will be reduced, as has been shown for other species (Somerville and Somerville 1999; An et al. 2005). The generation of a rapid cycling grapevine strain derived from an L_1 regenerant of Pinot Meunier provides a useful starting tool in grapevine genetics (Boss and Thomas 2000).

The lack of artificial genetic variation can be partially overcome by the exploitation of natural genetic variation in both forward and reverse genetic approaches. In fact, natural variation has been described for several flowering-related traits such as precocity (timing of fruit production), potential fertility (the number of inflorescences that a bud can produce), or real fertility (number of clusters produced by a bud), etc. All of these traits affect the final yield and quality of the fruit and, therefore, have been characterized in many cultivars. The wide availability of molecular markers and genetic maps (Dalbó et al. 2000; Doligez et al. 2002; Grando et al. 2003; Riaz et al. 2003; Adam-Blondon et al. 2004; Doucleff et al. 2004) allows the quantitative genetic analysis of this variation and the identification of the genomic regions involved. Thus, forward genetic approaches based on natural genetic variation can be good alternatives for identifying genomic regions responsible for the variation in flowering transition, either through segregation or association studies. Several thousand grapevine genotypes are being maintained and phenotypically characterized in germplasm resource centers around the world (Alleweldt and Possingham 1988). Furthermore, F_1 progenies segregating for flowering-related traits and amenable for quantitative genetic analyses are available. In this way, the quantitative genetic analysis of real fertility in an F_1 derived from the cross between table grape cultivars 'Dominga' and 'Autumn Seedless' have identified a QTL explaining 35% of the phenotypic variation for this trait on linkage-group 5 of the grapevine genetic map (J.A. Cabezas, unpublished observations, 2007). These genetic tools, combined with the powerful genomic (Adam-Blondon et al. 2005) tools, will facilitate positional cloning (Barker et al. 2005) and help solve the questions regarding the particular features of grapevine flowering transition. In addition, the genetic analysis of somatic variants arising in the vegetatively propagated cultivars has also been shown to be useful for the identification of loci and responsible gene sequences controlling the process as exemplified by the *VvGAI* mutant identified in L_1 regenerants of Pinot Meunier (Boss and Thomas 2002) as well as other somatic variants for flower development that are being analyzed (Sreekantan et al. 2006*b*).

Understanding the genetic and molecular basis of this developmental process can have major impacts in the management of specific cultivars, as well as in the development of more productive cultivars with improved fruit-quality features and more independance from environmental conditions.

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