

Genetics of flower initiation and development in annual and perennial plants

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Flowering is an integral developmental process in angiosperms, crucial to reproductive success and continuity of the species through time. Some angiosperms complete their life cycle within a year (annual plants), and others have a longer reproductive life, which is characterized by the generation of new flowering and vegetative shoots every year (perennial plants). Despite the differences in their lifespan, the underlying genetics of flower induction and floral organ formation appears to be similar among these plants. Hence, the knowledge gained from the study of flowering mechanism in *Arabidopsis thaliana* can be used to better understand similar processes in other plant species, especially the perennials, which usually have a long generation time and are not amenable to genetic analysis. Using *Arabidopsis* as a model, we briefly discuss the current understanding of the transition from vegetative to reproductive growth and the subsequent formation of individual floral organs, and how this knowledge has been successfully applied to the identification of homologous genes from perennial crops. Although annuals appear to share many similarities with perennials in terms of gene function, they differ in their commitment to flowering. Once an annual reaches the reproductive phase, all meristems are typically converted into either floral or inflorescence meristems. In contrast, each year, each meristem of a mature perennial has the choice to produce either a vegetative or a reproductive shoot. The physiology and genetics of flowering in *Citrus* are used to highlight the complexity of reproductive development in perennials, and to discuss possible future research directions.

Introduction

Continued survival in a hostile and volatile environment often requires complex adaptation on the part of all living organisms. During the course of evolution, plants have developed a highly effective way of ensuring their survival by having a flexible reproductive system, which enables them to reproduce either vegetatively, sexually or via a combination of both. Among these methods, sexual reproduction has the advantage of recombining novel genetic traits, thereby generating phenotypic variation that can be acted on by natural selection.

The largest and most diversified group of plants comes from the angiosperm family, which has approximately 250 000 members (Nabors 2004). This family of plants undergo a dramatic transition from vegetative to reproductive growth once they have attained adulthood. This process is often triggered by environmental cues and coordinated by internal factors, starting with the initiation and development of flowers and culminating in the production of fruit and seeds. By tailoring their reproductive plan to favorable environmental conditions, plants can ensure the success of their progeny. This is especially important for those growing in regions

Abbreviations – GA, gibberellin.

with seasonal changes in day length and temperature. Apart from responding to positive environmental signals, plants are also sensitive to adverse conditions, such as water or nutrient deficiency and heat. Under such conditions, different strategies will be employed by plants in order to survive these potentially life-threatening situations. For woody perennial plants, one of the most obvious responses is profuse, out-of-season blooming.

Progress made in understanding the flowering process in model plant systems has given us an insight into the molecular events surrounding the transition from the vegetative to reproductive phase in angiosperms (e.g. Bernier and Perilleux 2005). This has confirmed a long-held hypothesis, first suggested over 200 years ago (Goethe 1790), that flowers are modified shoots: floral organs, with the exception of seeds, are formed from highly modified leaves (Goto et al. 2001). Hence, the process of flowering involves converting a portion (sometimes all) of what would otherwise be vegetative shoots into flowers. Most of these recent advances in understanding flowering have come from the use of molecular genetic techniques on annual plants, particularly *Arabidopsis thaliana* (commonly known as *Arabidopsis*), which are well suited to this type of investigation. Nevertheless, as the results obtained from these plants have been extended to crop plants, including perennials, it has become increasingly clear that the formation of the structures that comprise a typical flower, such as sepals, petals, stamens and carpels, is largely conserved across all flowering plants, with relatively minor variations in the activity of key developmental genes accounting for the observed variation in flower morphology (Goto et al. 2001, Smyth 2001).

In this review, we discuss our current understanding of the mechanism of flower initiation under favorable environmental conditions and the formation of floral organs, using the annual plant *Arabidopsis* as a model. We then describe how some of this work has been, and will be, used to investigate flowering in perennial trees, particularly *Citrus*.

Floral initiation

Initiation of flowering is a highly regulated process, which involves temporal and spatial interactions between external and internal factors. External signals that are pertinent to the flowering process include day length (in fact the length of the night) and temperature. These, together with endogenous factors, such as developmental stage and floral gene activities, act to promote flower initiation. Most of the recent progress

in this area has concerned annual plants, which flower and set seeds within a single year, whereas perennial plants, in which flowering occurs over multiple years, have been studied in less detail. Critical to the process of floral initiation are meristems. These consist of pools of undifferentiated cells that give rise to lateral organs (such as leaves), axillary shoots (including flowers), internode tissue, and, in indeterminate structures, a continuing supply of new meristem cells. Consequently, the type of cells produced, and their ultimate developmental fate as part of vegetative or reproductive structures, determine whether flowering occurs.

One of the underlying features of the changing seasons is the variation in day length or photoperiod. The regulatory effect of day length on the initiation of flowering was first described by Garner and Allard more than 80 years ago (Garner and Allard 1920). Depending on species, cultivars and geographic location, plants can respond to the changing light and dark period by adjusting their flowering time to be in tune with their surroundings. Some plants require a prolonged period of exposure to long days to flower, whereas others will only flower with short days (Thomas and Vince-Prue 1997). For example, the widely studied model plant *Arabidopsis thaliana* belongs to the former category, whereas rice falls under the latter. However, there are some day-neutral plants whose flowering regime is independent of the duration of light exposure.

Since the seminal work of Garner and Allard, a great deal of progress has been made in understanding the underlying mechanism that controls the initiation of flowering in response to photoperiod. One key discovery is the circadian clock (Hamner 1960), which has been shown to modulate photoperiodic responses in addition to regulating other physiological events such as the daily rhythms of leaf movement, the opening and closing of stomata, and hypocotyl elongation (Samach and Coupland 2000, Miroguchi et al. 2005). This daily timer works together with photoreceptors and flowering genes to bring about the transition from vegetative to reproductive growth (Klejnnot and Lin 2004). Whereas day length is perceived in leaves, the flowering response occurs in meristem(s) that may be some distance away. The nature of this mobile flowering signal, often referred to as florigen, has long been the subject of research. Recent work in *Arabidopsis* has suggested that products of the *FLOWERING TIME (FT)* gene may be at least part of this mobile signal (Abe et al. 2005, Huang et al. 2005, Wigge et al. 2005).

Many plants, including certain temperate wheat and barley cultivars, require an exposure to extended periods of low temperatures (vernalization) in order for

flowering to be promoted (Hay and Ellis 1998). The low-temperature treatment is required to remove floral repressors, whose continuing accumulation would otherwise delay the flowering process (Andersen et al. 2004). This mechanism appears to have evolved independently in many angiosperm lineages to ensure that flowering, particularly for plants growing at high latitudes, will not occur until spring or early summer, when longer days coupled with higher temperatures favor floral bloom, and the subsequent reproductive processes. Although significant progress in understanding the molecular basis of vernalization has been made in the brassicas (e.g. He and Amasino 2005, Michaels et al. 2005, Shindo et al. 2005) and monocotyledons such as wheat and barley (e.g. Dubcovsky et al. 2005, Anderson et al. 2004, Murai et al. 2003, Trevaskis et al. 2003, Yan et al. 2003), it is not clear how these pathways relate to other plant families, particularly woody perennials.

The developmental stage of a plant can also influence the timing of flower initiation. This is particularly true for woody perennial species, which generally have distinguishable juvenile and adult phases. The transition from juvenile to adult phase in woody perennials is accompanied by extreme morphological, physiological and biochemical changes, and the most prominent of all is the emergence of flowering shoots (Lawson and Poethig 1995). However, unlike in many annuals, the conversion to flowering apices apparently only occurs in some meristems, whereas others maintain their indeterminate vegetative state (Jackson and Sweet 1972; see below), essential for continued growth of the plant. Since reproductive development only occurs after the plant has reached the adult phase, the duration of juvenility, which is species dependent, is critical in determining when the plant starts to bear fruit. Most of the economically important fruit crops such as grapes, apple and *Citrus* are subject to this age-related developmental program. For example, *Citrus* trees normally go through a juvenile stage, which can last from 2 to more than 10 years, before starting to flower (Davis and Albrigo 1994).

When plants are at a developmental stage capable of flower initiation, the various environmental cues will be perceived and acted upon by a set of proteins consisting mostly of photoreceptors and transcription factors. It has been shown in *Arabidopsis* that there are defined and sometimes overlapping genetic pathways—photoperiod, vernalization, autonomous and gibberellin (GA)—which integrate the environmental and endogenous signals in deciding the timing of flower initiation (Fig. 1; Simpson and Dean 2002). The individual pathways all converge at a checkpoint for flowering time control,

which consists of at least three genes collectively known as floral pathway integrators: *FLOWERING LOCUS T* (*FT*), *LEAFY* (*LFY*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). These floral pathway integrators activate floral meristem identity genes, such as *APETALA1* (*AP1*), *FRUITFUL* (*FUL*), *CAULIFLOWER* (*CAL*), *LFY* and *SEPALLATA4* (*SEP4*), whose activity causes the transition of vegetative apices to inflorescence meristems. The specification of flowering fate in meristems then triggers the expression of downstream floral organ identity genes, leading to flower formation.

Floral organ specification

Once it is committed to a flowering fate, a shoot apical meristem of an annual plant becomes an inflorescence meristem from which floral meristems emerge on the periphery. The floral meristems then undergo differentiation that causes the sequential development of flower organ primordia, starting with sepals, continuing with petals and stamens, and terminating in carpels. In dicotyledon plants, these different floral organs are generally arranged in four concentric rings called whorls: sepals occupy the first whorl, petals the second whorl, stamens the third whorl, and carpels the fourth whorl.

In 1991, Meyerowitz and Coen proposed a model to describe the determination of floral organ identity based on phenotypic and genetic analyses of homeotic mutants of *Antirrhinum* and *Arabidopsis*. Their model, termed the ABC model (Bowman et al. 1991, Coen and Meyerowitz 1991), hypothesizes that the activity of three classes of genes called A, B and C specifies the type of floral organ in each whorl. The activity of A class genes alone is sufficient to cause sepals to form in the first whorl. However, the formation of petals in the second whorl requires the combined activities of A and B class genes. The development of stamens in the third whorl is similarly promoted by the collective activities of B and C class genes, whereas the formation of carpels in the fourth whorl is dependent solely on the C class gene activity.

The majority of the ABC genes are members of a large transcription factor family called the MADS-box gene family. It derives its name from some of the earliest known homeotic genes: *MCM1* from yeast (Passmore et al. 1988), *AGAMOUS* from *Arabidopsis* (Yanofsky et al. 1990), *DEFICIENS* from *Antirrhinum majus* (Sommer et al. 1990) and *SRF* from *Homo sapiens* (Norman et al. 1988). In plants, this gene family is involved in a myriad of developmental events, including flower initiation, floral patterning, fruit and seed development, and leaf and root formation.

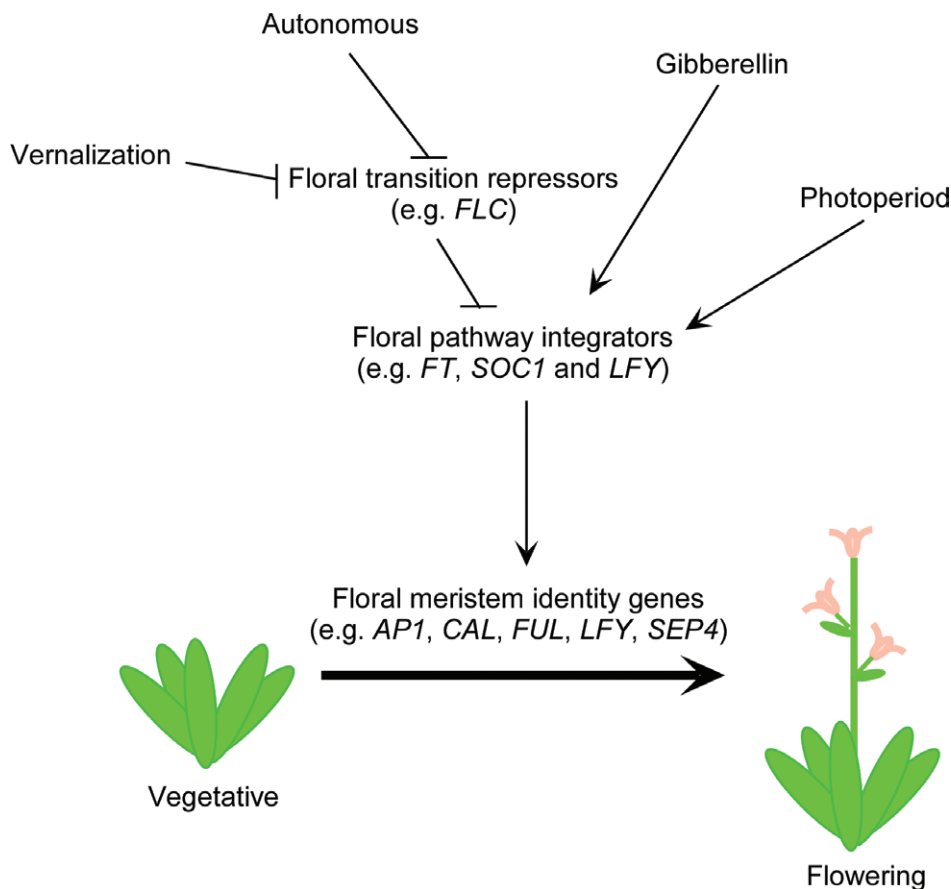


Fig. 1. Flowering pathways of *Arabidopsis*. A simplified diagram showing the various pathways of flower induction in *Arabidopsis*. So far, four independent pathways have been identified: vernalization, photoperiod, autonomic and gibberellin.

Two of the A class genes that have been isolated from *Arabidopsis* include *AP1* (Mandel et al. 1992) and *APETALA2* (*AP2*) (Jofuku et al. 1994). The product of the former, a MADS-box gene, has dual functions in promoting floral meristem identity at the initial stage of flower development, and in subsequently specifying floral organ identity. On the other hand, *AP2*, which is a transcription regulator of the *AP2/ERF* family (Magnani et al. 2004) and does not contain a MADS domain, has been recently implicated as being the target of a microRNA-mediated gene regulatory network (Chen 2004). Strong *ap1* mutants have mostly bract-like structures in the first whorl and no organs in the second whorl, while maintaining the normal development of the inner two whorls (Bowman et al. 1993). In addition, flowers produced by these plants are often partially converted to inflorescence shoots, alluding to the early role of *AP1* in establishing floral meristem identity. In comparison, mutations in the *AP2* gene result in the conversion of sepals to leaves or carpels in the first whorl, and petals to staminoid organs in the second whorl, but do not alter the organ identity of the third and fourth whorls (Bowman et al. 1991).

The B class genes identified so far are *APETALA3* (*AP3*) (Jack et al. 1992) and *PISTILLATA* (*PI*) (Goto and Meyerowitz 1994). Both are members of the MADS-box gene family. The *AP3* and *PI* genes are active in petals and stamens, consistent with their roles in determining the identity of these organs (Jack et al. 1992, Goto and Meyerowitz 1994). Disruption of the normal function of either of these genes causes sepals and carpeloid organs to grow in the second and third whorls respectively (Hill and Lord 1989, Jack et al. 1992).

The most studied member of the C class gene is *AGAMOUS* (*AG*) (Yanofsky et al. 1990). The *AG* gene encodes a MADS-box transcription factor whose function is required for the proper development of stamens and carpels. Mutant *Arabidopsis* plants without functional *AG* produce indeterminate flowers in which stamens are converted to petals and the gynoecium is replaced by a secondary flower that has petals in the third whorl and a tertiary flower in the center. The indeterminate nature of flowers produced in the absence of a functional *AG* shows that, apart from specifying floral organ identity, *AG* is also involved in terminating floral meristem activity towards the end of flower development by repressing the activity of the

meristem-promoting gene, *WUSCHEL* (Lenhard et al. 2001).

Recent studies indicate that other factors are required for specifying the identity of floral organs in conjunction with the ABC genes. Among such factors are the *SEPALLATA* genes (*SEP*) (Pelaz et al. 2000). Like most of the ABC genes, the *SEP* genes are members of the MADS-box gene family. There are four functionally redundant *SEP* genes (Pelaz et al. 2000, Ditta et al. 2004)—*SEP1*, *SEP2*, *SEP3* and *SEP4*—in *Arabidopsis*, and together they are essential for the specification of organ identity in all four whorls of a flower. In addition to specifying floral organ identity, one of the *SEP* genes, *SEP4*, has been shown to play a pivotal role in maintaining flower meristem identity (Ditta et al. 2004). In *sep1 sep2 sep3* triple mutants, the inner three whorls of a flower are converted into sepals (Pelaz et al. 2000). In plants bearing mutations in all four *SEP* genes, floral organs are replaced by leaf-like structures (Ditta et al. 2004). The importance of *SEP* genes in promoting the development of sepals, petals, stamens and carpels has led to the incorporation of these genes, collectively called the E class genes, into the ABC model (Jack 2004).

Flowering genes from woody perennials

The rapid advances made in understanding *Arabidopsis* flowering have allowed researchers to begin similar investigations in perennial crops. This knowledge is greatly accelerating flowering research in perennial trees because, at least in a general sense, the same genes appear to be involved in flower initiation, flower formation and fruit development in all flowering plants. Using the DNA sequence of flowering genes from model plants as a starting point, flowering genes have been successfully isolated from several agriculturally important tree crops, including apples (Yao et al. 1999, Sung et al. 1999, Sung et al. 2000, Kotoda et al. 2000), *Citrus* (Pillitteri et al. 2004a, Pillitteri et al. 2004b, Endo et al. 2005), grapes (Boss et al. 2001, Boss et al. 2002, Boss et al. 2006), eucalypts (Kyo-zuka et al. 1997, Southerton et al. 1998, Dornelas et al. 2004), and other perennials of commercial importance (Dornelas et al. 2005, 2006, Yu et al. 2005). Unfortunately, because of a variety of technical limitations associated with tree crops, usually related to their size combined with long generation times and limited genetic knowledge, it is generally not feasible to confirm the function of these genes through molecular genetic analysis of mutant trees lacking the gene in question. Instead, the function of these genes can usually be broadly predicted based on the extent and

nature of the similarity between the tree genes and the related genes from a model plant (typically *Arabidopsis*) whose function has been defined by molecular genetic analysis. In addition, a common technique is to introduce the tree gene into *Arabidopsis* plants and assess the effect on plant development. If the tree gene is equivalent (orthologous) to an *Arabidopsis* gene, both will be expected to alter *Arabidopsis* development in a similar manner. For example, the two *AP1* orthologues from *Eucalyptus* behaved in a similar way to their *Arabidopsis* counterpart, causing more rapid flowering and the conversion of inflorescence meristems into floral meristems, in addition to causing other developmental changes, when they were ectopically expressed in *Arabidopsis* (Kyo-zuka et al. 1997).

Flowering in many perennial tree crops is significant from a commercial perspective, but this process also represents an important area of plant biology. In particular, as described above, much work has been done on model annual systems, where many and sometimes all meristems will become reproductive before the plant senesces. By contrast, after becoming reproductive, perennial crops must continue to maintain a proportion of vegetative meristems to allow further vegetative growth over multiple seasons. Consequently, much of the research on the flowering physiology of woody perennials has been concerned with seasonal changes in the flowering behavior of mature trees, and this contrasts with the research using annual plants, which has focused on the transition from vegetative to reproductive development and the development of flower organs. To illustrate some of the issues, some of the research on *Citrus* flowering is described below.

Citrus flowering

Citrus flowering has been the subject of ongoing investigation for many years (see Krajewski and Rabe 1995, El-Otmani et al. 2000), but previous research has been limited by the need to work with intact trees, or in some cases, cultured buds. The first approach makes experiments that are simple in annual species difficult and time-consuming, while the second does not fully represent the situation that exists in whole trees (e.g. Garcia-Luis et al. 1989, Koshita et al. 1999). Nevertheless, research on flowering in *Citrus* represents many of the difficulties that are typical of perennial plants, and *Citrus* also illustrates how the knowledge gained in model systems can help us to understand more complex physiological systems. When grown from seeds, *Citrus* seedlings progress through a developmental ontogeny typical for woody perennials, eventually producing a moderately sized tree. After a juvenile period, typically

lasting for several years, *Citrus* trees enter the adult phase, in which they are capable of continuously producing flowers in addition to vegetative shoots. Flowers can potentially be produced throughout the year, but in most oranges and mandarins grown in temperate environments, the majority of flowers are produced during the spring flush. Thousands of flowers are usually produced on established trees, but only a relatively small proportion develop into fruit (Krajewski and Rabe 1995). In some varieties, pollination, fertilization and seed development are required for fruit set, while in others, parthenocarpic fruit development, in some cases stimulated by pollination, can occur (Koltunow et al. 2000).

Citrus shoots develop from initially quiescent buds produced in leaf axils. When released from dormancy, buds on adult trees can grow into leafy shoots (no flowers) or form one of several different types of inflorescence (Sauer 1954, Krajewski and Rabe 1995). These range from single terminal flowers on a leafy shoot to an inflorescence comprised entirely of a single or multiple flowers without developed leaves (Fig. 2). In combination with the number of buds bursting the proportion of the different shoot types present each spring determines the total number of flowers produced. Single terminal flowers, surrounded by leaves on the same shoot, produce fruit of the greatest potential size, presumably because the adjacent leaves can provide additional assimilates for growth (Krajewski and Rabe 1995).

For a given *Citrus* variety, the number of fruit on an individual tree is negatively correlated with final fruit size. Consequently, the tendency for *Citrus* to exhibit a biennial bearing pattern of flower, and hence fruit, numbers has a significant impact on fruit size at harvest. In 'on' years, a relatively large number of flowers are produced, while in 'off' years, relatively few flowers are formed. This cycle tends to repeat itself, and is also observed in a number of other woody perennials, such as apples and stone fruit. In 'on' years, the production of excessive flowers and fruit, although often followed by extensive fruit abscission early in development, has a deleterious effect on final fruit size, which can lead to significant penalties for the price of fresh fruit in the marketplace. In addition to crop load from the previous season, the environment, particularly winter temperatures, can also modify flower production (Garcia-Luis et al. 1992, Garcia-Luis and Kanduser 1995, Garcia-Luis et al. 1995). Because of this environmental effect, trees of a particular variety within a geographic area tend to become synchronized in their biennial bearing pattern. Although this simplifies management to some extent, it greatly exacerbates the overproduction of small fruit in 'on' years.

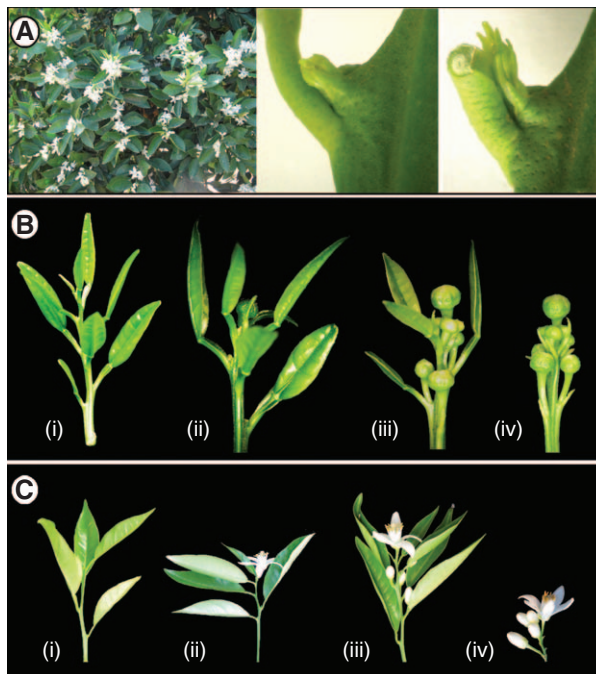


Fig. 2. Flowering in citrus. (A) Mature *Citrus* trees can produce thousands of flowers, although only a small proportion will eventually become fruit (left). Flowers are produced from shoots that develop from axillary buds (centre and right) (B, C) These shoots can exhibit a number of different forms: (i) a vegetative shoot without flowers, (ii) a shoot with a single terminal flower and several leaves, (iii) a shoot with several flowers and several leaves, and (iv) a shoot with one or more flowers and no developed leaves (although undeveloped leaves may be present). Shoots are shown before flower buds have opened (B) and after opening of the most advanced flower (C).

Because of the importance of crop load, methods for reducing the extent of biennial bearing in *Citrus* have been investigated for use in commercial production. Winter GA sprays are one management tool that can be used to regulate flowering, and minimize the effect of biennial bearing. In *Citrus*, as in many other perennial crops, GA application during bud development can inhibit flower production (Monselise and Halevy 1964, Guardiola et al. 1982, Lord and Eckard 1987), and in the following spring lead to a greater proportion of single terminal flowers on leafy shoots, which tend to produce the largest fruit. Despite its observed efficacy, it is not yet clear whether this effect reflects a true physiological role for GA in the regulation of flowering. This uncertainty in part stems from the fact that in many annual plants, such as *Arabidopsis*, GA has a promoting effect on flowering. Thus, either GA has contrasting roles in the flowering of different species, or abnormally high GA levels in woody perennials such as *Citrus*, but not in annuals such as *Arabidopsis*, prevent normal flower formation, presumably by disrupting essential

developmental events. The contrasting effects of GA on flowering in different species raise an important question. How do the genes and flowering pathways described above for annual plants relate to *Citrus* flowering? It is clear that *Citrus* contains genes which are similar, and probably orthologous, to *FT*, *TERMINAL FLOWER1* (*TFL1*), *AP1* and *LFY* (Pillitteri et al. 2004a, Pillitteri et al. 2004b, Endo et al. 2005). Furthermore, the *Arabidopsis* flowering genes *AP1* and *LFY*, under the control of the constitutive 35S promoter from cauliflower mosaic virus, dramatically altered the flowering behavior of transgenic citrange trees (Pena et al. 2001). For example, 35S:*AP1* trees flowered in a few months rather than several years, suggesting that this type of approach has great potential to expedite *Citrus* genetic research. Although these results are exciting, little is known of the molecular events of *Citrus* flowering. However, the results so far suggest that, at least at a fundamental level, flowering genes and presumably pathways are conserved between annuals and perennial trees.

A recent important result is the isolation of the *Citrus* *FT* and the use of this gene to cause early flowering in trifoliate orange (Endo et al. 2005). Since *FT* mRNA appears to be capable of moving from leaves to apex (Abe et al. 2005, Huang et al. 2005, Wigge et al. 2005), it should also be able to move across a graft union in *Citrus*. This is significant because it may be possible to graft other genotypes onto a rootstock ectopically expressing *FT* and dramatically reduce the time to flowering of the scion. This would have major implications for genetic improvement in *Citrus* by conventional breeding, as the normally long juvenile period makes breeding a very time-consuming process.

One potentially instructive approach to comparing flowering physiology between *Arabidopsis* and *Citrus* is to consider the developmental fate of *Citrus* buds produced by mature trees. As described above, these buds can give rise to a range of shoot types, from leaves with no flowers ('vegetative') to clusters of flowers with very limited leaf development (Fig. 2). Since mature trees are defined as having the potential to produce flowers, the meristems present in buds giving rise to 'vegetative' shoots can be considered in one of two ways. In the first scenario, meristems in some buds may be truly vegetative and analogous to meristems produced by non-flowering juvenile *Citrus* trees or vegetative *Arabidopsis* seedlings. In this scenario, individual buds on a single mature tree can be considered to be equivalent to a population of *Arabidopsis* plants, some of which have initiated flowering and some of which are vegetative; in any given season, the proportions of vegetative and reproductive buds will depend

on the proportion of buds in which floral induction occurs. In the second scenario, the meristems in all buds may be potentially competent to produce flowers but, because of the prevailing local physiological conditions, are unable to do so. In this case, these meristems would be analogous, at least early in development, to meristems in other buds that do subsequently produce flowers and to meristems produced by a flowering *Arabidopsis* plant. One possibility is that some meristems undergo 'floral reversion' (Tooke et al. 2005) and become vegetative. If this hypothesis is correct, thorns, which are usually produced in *Citrus* as determinate axillary branches, may in fact be derived from flowers that failed to complete their development (see also Endo et al. 2005). Although microscopic analysis of dormant buds does not reveal meristem identity (Lord and Eckard, 1987), the two scenarios will make distinct and testable predictions of gene expression in dormant winter buds that are responding to signals, such as crop load or temperature, that regulate flower numbers. In the first scenario, expression of genes involved in floral induction will differ between buds, such that some buds express flowering genes, whereas others (which go on to produce vegetative shoots) do not. Alternatively, the second scenario suggests that all buds will express flowering genes, at least during the early stages of development, including those that subsequently fail to produce flowers. Distinguishing between these models must await the identification of *Citrus* flowering genes, but recent work shows that the *Citrus* homologs of *FT*, *TFL1*, *AP1* and *LFY* can readily be isolated by exploiting the functional and sequence conservation of flowering genes among flowering plants (Pillitteri et al. 2004a, Pillitteri et al. 2004b, Endo et al. 2005).

Future prospects

Flowering in higher plants is a complex event involving tightly regulated interactions between external and internal factors. The external factors act as a flowering stimulus that a reproductively competent plant perceives and then responds to with the help of a coterie of transcription regulators, photoreceptors and phytohormones. Genes that regulate flower initiation and development have been identified in *Arabidopsis* and other model annuals, using genetic and molecular approaches. This has subsequently facilitated the discovery of similar genes in a wide range of plant species, including rice, eucalypts, *Citrus*, grapevine and apple, suggesting that they are conserved during the evolution of flowering plants. Studies are ongoing to determine the endogenous function of these genes, which in turn will provide the molecular framework for elucidating

the underlying mechanism of flowering in woody perennials. In this regard, one of the key issues is to determine if buds on mature perennial plants, such as *Citrus*, that do not subsequently produce flowers, can be considered to be 'vegetative' or are in fact 'reproductive' buds that failed to produce visible flowers because of physiological constraints, or 'floral reversion', during bud development prior to shoot growth.

The developmental program of a plant is characterized by two distinct stages: juvenile and adult phases. Herbaceous annuals have a relatively short juvenile phase before progressing into the adult phase, which is typified by the initiation of flowering. In contrast, woody perennials are notoriously late-flowering, due to a prolonged period of juvenility that can continue for decades before the plants acquire the competence to produce flowers. This has been a major hindrance to genetic improvement of these species. A major breakthrough was the discovery that the flowering time of *Arabidopsis* was accelerated by the constitutive expression of flower meristem identity genes such as *LFY* and *AP1* (Mandel and Yanofsky 1995, Weigel and Nilsson 1995). The same technology was then successfully exploited to reduce the generation time of perennials such as hybrid aspen (Weigel and Nilsson 1995) and *Citrus* relatives (Pena et al. 2001; Endo et al. 2005). Following these encouraging developments, it is possible to envisage that this technology will be further refined to make it robust and applicable to other economically important crops.

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