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No Flower no Fruit – Genetic Potentials to Trigger Flowering in Fruit Trees

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ABSTRACT

The development of flower buds and a sufficient fruit set are basic requirements for fruit growers to generate a marketable crop. However, fruit trees remain in a juvenile (nonflowering) phase for years, and after a transition period of getting reproductively competent they enter the adult phase of tree life. The reproductive phase is associated with the ability to alternate between the production of vegetative and reproductive buds. Efficient breeding is limited in fruit trees due to the long period of juvenility. Therefore, it is important to accelerate flowering by reducing the juvenile phase of the tree. In fruit production, precocious flowering of the tree is also favoured to reduce the vegetative phase of tree development after planting in order to obtain the earliest fruit crop. Additional critical aspects of flower development in fruit trees, such as alternate bearing, accentuate the necessity to improve our understanding on genetic factors controlling floral initiation as well as flower and fruit development in perennial fruit trees. Most of what we know about regulating floral development is based on research in annual plants, like *Arabidopsis thaliana*. In this review, we summarize floral transition, meristem development and flower bud formation in *Malus domestica*, one of the most important representatives of temperate fruit trees. We also focus on current findings of the transition from vegetative to reproductive growth obtained in *Arabidopsis* and how this knowledge can be applied to fruit trees, particular to apple. We discuss state-of-the-art and future research to manipulate maturation and flower initiation in apple.

Keywords: apple, floral initiation, flower development, fruit tree, *Malus domestica*, transition
Abbreviations: ***AG***, *AGAMOUS*; ***AFL***, *APPLE FLORICAULA/LEAFY*; ***AGL***, *AGAMOUS* like; ***AP***, *APETALA*; **BA**, benzylamino acid; **bp**, base pair; **C**, carbohydrate; ***CAL***, *CAULIFLOWER*; **CCC**, chlorocholine chloride; **cDNA**, copy DNA; ***CO***, *CONSTANS*; ***DEF***, *DEFICIENS*; **EST**, expressed sequence tag; ***FBP***, *FLORAL BINDING PROTEIN*; ***FHA***, *FORKHEAD*; ***FLC***, *FLOWERING LOCUS C*; ***FLD***, *FLOWERING LOCUS D*; ***FLK***, *FLOWERING LOCUS K*; ***FLO***, *FLORICAULA*; ***FRI***, *FRIGIDA*; ***FT***, *FLOWERING LOCUS T*; ***FUL***, *FRUITFULL*; **GA**, gibberellic acid; ***GI***, *GIGANTEA*; ***GLO***, *GLOBOSA*; ***LD***, *LUMINIDEPENDES*; ***LFY***, *LEAFY*; **mRNA**, messenger RNA; **N**, nitrogen; ***PI***, *PISTILLATA*; ***PLE***; *PLENA*; **RACE**, rapid amplification of complementary ends, **RNA**, ribonucleic acid; **SAM**, shoot apical meristem; ***SEP***, *SEPALATA*; ***SHP***, *SHATTERPROOF*; ***SMZ***, *SCHLAFMUTZE*; ***SNZ***, *SCHNARCHZAPFEN*; ***STK***, *SEEDSTICK*; ***SOC1***, *SUPRESSOR OF CONSTANS 1*; ***SQUA***, *SQUAMOSA*; ***SVP***, *SHORT VEGETATIVE PHASE*; ***TFL***, *TERMINAL FLOWER*; ***VIP***, *VERNALZATION INDEPENDENCE*; ***VRN***, *VERNALIZATION*

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Special Feature

INTRODUCTION

Flowering in fruit crops, such as apple, is of great economical importance. Yield depends on the number and quality of flower buds formed. Flowering is a complicated developmental process of physiological and morphological stages under the control of a number of external signals and internal factors. The first and main important part towards crop formation is flower initiation, followed by flower differentiation, fertilization, fruit set and fruit development. Each of these processes may be a limiting factor for crop formation. Flower set and fruit set are the main components of yield in apple. Instability of flower formation from year to year during the main cropping period of an apple tree is defined as the main reason for instability in fruit production (Schmidt *et al*. 1989). Adverse environmental conditions can lead to a specific phenomenon in temperate fruit trees known as alternate (biennial) bearing, which is characterized by large yields of small-sized fruit in the "on-year" and low yields of oversized fruit in the "off-year". Alternate bearing is caused by the adverse relationship between fruit development and flower bud differentiation, i.e. differentiation of flower buds in apple coincides with embryo development in the fruit. In the case if there is a high amount of developing fruits on the tree, the flower bud development is inhibited by hormones and the endogenic cycle of alternative bearing will be initiated (Schmidt 1973; Schmidt 1974; Jonkers 1979; Monselise and Goldschmidt 1982; Handschack and Schmidt 1985, 1986).

Beside the network of processes involved in flower formation during season which effects yield, there are also other specific problems in fruit trees which have to be taken into account. Yield will be only produced during the adult phase of tree development, i.e. there is a period of several years prior to cropping, the juvenile phase. Detailed understanding of flower formation mechanisms during tree development is necessary to develop appropriate techniques to shorten the juvenile phase which is especially important for breeders. Compared to other woody species, in fruit tree species yield is also specifically influenced by the relationship between two genetically different parts of the tree, the rootstock and the scion, which may or may not be in balance.

All these phenomena stress the necessity to improve our knowledge on genetic factors controlling the processes of flower formation in fruit tree species. The knowledge gained from the study of flowering mechanism in *Arabidopsis thaliana* can be used to better understand similar processes in other plants species, especially in perennials, which usually have a long generation time and are not amendable to genetic analysis (Tan and Swain 2006).

Using *Arabidopsis* as a model, we briefly discuss current understanding on transition from vegetative to reproductive growth and floral development and how this knowledge may be successfully applied to the identification of similar genetically determined processes in fruit tree crops. This review will focus mainly on floral development in apple, the most important fruit tree species in Europe.

WHAT WE DO KNOW FROM *ARABIDOPSIS* ABOUT FLORAL INITIATION AND FLOWER DEVELOPMENT?

What are the factors controlling floral transition in *Arabidopsis*?

Arabidopsis thaliana, the little annual plant of the *Brassicaceae* family, became to plant biology what *Drosophila melanogaster* and *Caenorhabditis elegans* are to animal biology. While it has no commercial value as it is considered as a weed, it has proven to be an ideal organism for studying plant development. Although there are differences between annual and perennial plants the genetics of flower induction and floral organ formation seems to be similar among these plants (Tan and Swain 2006). Therefore, the knowledge gained by the model plant *Arabidopsis* can also be used as basis for perennial plants.

The life cycle of a plant can be divided into two parts: the vegetative phase determined by the inability of a seedling to flower, and the generative phase determined by the ability of a seedling to flower. The change from the vegetative to the generative stage is named transition phase. The time of floral transition is influenced by endogenous and environmental factors which trigger or repress the change of the shoot meristem from generating leaves to the development of reproductive organs (a simplified model is illustrated in **Fig. 1**).

Stimulators of floral transition can induce promoting pathways, activating the expression of genes which cause floral transition (floral pathway integrators) and enabling pathways. Repressors antagonizing the activation of floral transition are regulated by the enabling pathways (Boss *et al*. 2004). This complex interaction of multiple pathways ensures the transition of a plant into the generative phase during favourable environmental conditions. Genetic analysis of *Arabidopsis* flowering time mutants resulted in four major pathways controlling the time of floral transition (Martinez-Zapater *et al*. 1994).

Whereas the photoperiod and the vernalization pathways mediate the response to environmental factors, the autonomous and gibberellin pathways are largely independent from environmental influences (Parcy 2005). The two main pathways promoting the expression of floral integrators are photoperiod and gibberellins (GA). Whereas GA acts directly on floral integrators, photoperiod mediated response is mainly directed via the expression of the gene *CONSTANS* (*CO*). Since *Arabidopsis* is a facultative long day plant, exposure of plants to long day conditions results in a higher accumulation of *CO*. The transcription of *CO* is enhanced and the gene product is stabilized by blue and dark red light (Suarez-Lopez *et al*. 2001). At a certain level *CO* works as a transcription factor of the floral integrator gene *FLOWERING LOCUS T* (*FT*) (Kardailsky *et al*. 1999; Kobayashi *et al*. 1999; Samach *et al*. 2000; Teper-Bamnolker and Samach 2005). Besides *CO*, *FHA* and *GIGANTEA* (*GI*) are activated in the rosette leaves of *Arabidopsis* in a circadian rhythm (Koornneef *et al*. 1998; Michaels and Amasino 2000; Soltis *et al*. 2002; Yoo *et al*. 2005) under long day conditions. The promoting effect of GA to flowering of *Arabidopsis* particularly under short days has been proven by applications of exogenous GA (Chandler and Dean 1994; Langridge 1957). Grafting experiments showed that also endogenous GA applied to the grafting donor initiates flowering in *Arabidopsis*. In addition, mutants in GA biosynthesis or signalling fail to flower under short day conditions and show delayed flowering under long day conditions (Sun and Kamiya 1994; Wilson *et al*. 1992). GA regulates the expression of *suppressor of overexpression of constans 1* (*soc1*) (Moon *et al*. 2003), another floral integrator, and enhance the transcription of the floral meristem identity gene *LEAFY* (*LFY*) independent of the *SOC1* activation (Blazquez *et al*. 1997; Blazquez and Weigel 2000).

An important repressor of flowering is *FLOWRERING LOCUS C* (*FLC*) (Michaels and Amasino 1999) through repressing of the floral pathway integrators *CO*, *LEAFY* and *SOC1* (Kobayashi *et al*. 1999; Blazquez and Weigel 2000; Lee *et al*. 2000; reviewed in Boss *et al*. 2004). *FLC* itself is negatively regulated by the expression of genes induced by the vernalization and the autonomous pathways. In *Arabidopsis* floral initiation can also occur under short day conditions induced by low temperatures (vernalization pathway). Cold is perceived in the shoot apical meristem (SAM) by activation of the cold-response genes *VERNALISATION 1* (*VRN1*), *VRN2* and *VRN3* and by changes in DNA methylation (Finnegan *et al*. 1998). These factors suppress *FLC*, a central inhibitor of the floral induction gene *FT*, and the *FT* downstream floral initiation cascade (Michaels and Amasino 2000; Sheldon *et al*. 2000; Vijayraghavan *et al*. 2005). Thus, vernalization promotes flowering by suppression of *FLC* and activates the *FT* controlled transition. The autono-

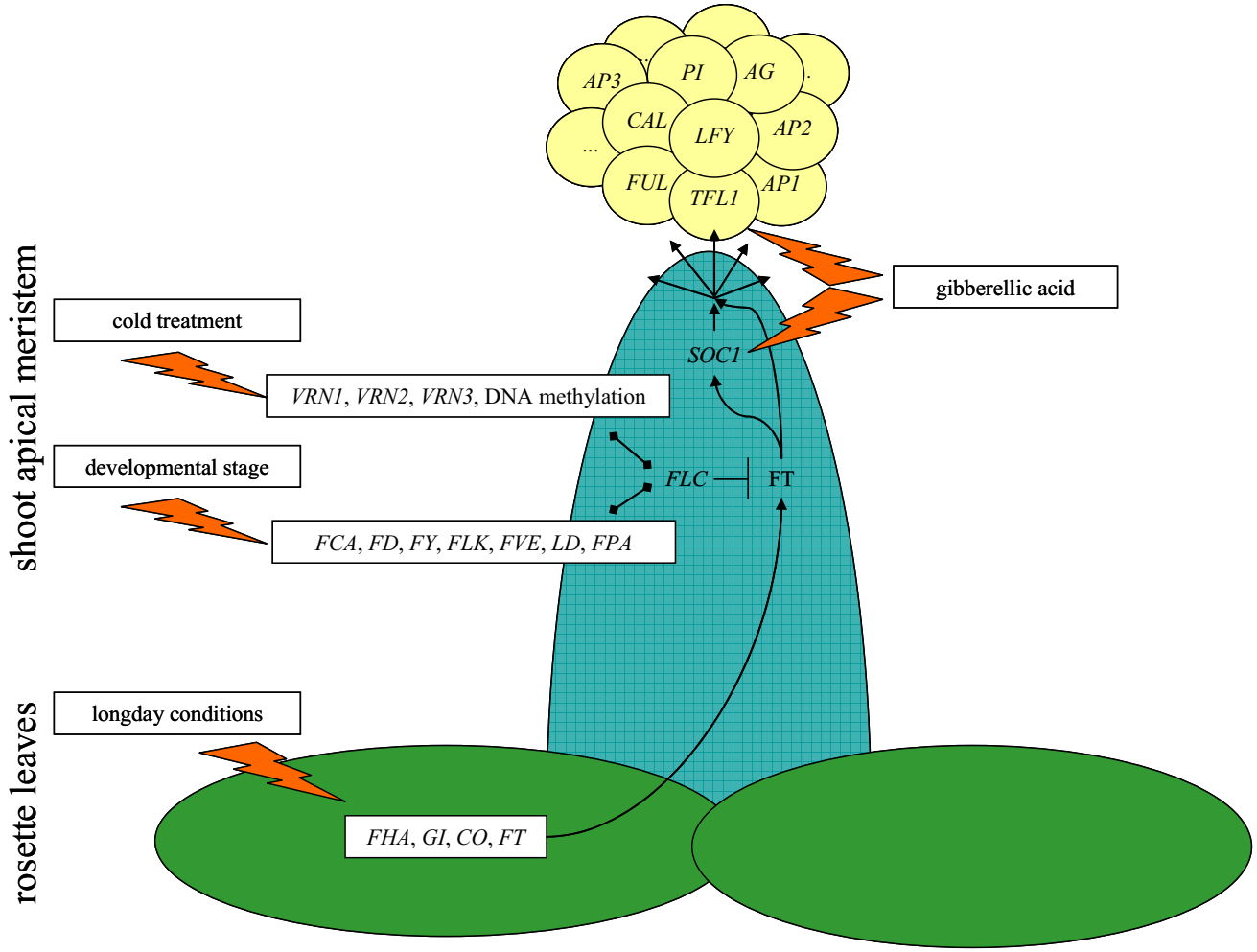

Fig. 1 A simplified model of environmental and genetic factors controlling the floral initiation in *Arabidopsis*. While the photoperiodic pathway (long day) is processed in rosette leaves, the vernalization (cold treatment), the autonomous (developmental stage) and the GA pathways are perceived in the shoot apical meristem (SAM). Signal processing is always carried out by a determined cascade of pathway specific genes. Through certain key genes like *FLC*, *FT* and *SOC1* these four pathways are genetically connected. Thus, each pathway results in the activation of *FT* and/or *SOC1*, two genes responsible for promoting floral meristem identity genes and floral organ identity genes. Black arrows: way of activation, bars: repressive interactions, flash arrows: signal processing; The different genes cited are explained in the text.

mous pathway equally leads to the repression of *FLC*. For example, genes like *FLOWERING LOCUS D* (*FLD*), *FLOWERING LOCUS K* (*FLK*) and *LUMINIDEPENDENS* (*LD*) suppress the transcription of *FLC* and by this means activate the floral induction gene *FT* indirectly under short days (Michaels and Amasino 2000; Sheldon *et al*. 2000; Soltis *et al*. 2002; Vijayraghavan *et al*. 2005). On the other hand, *FLC* is up-regulated by a set of genes, e.g. *FRIGIDA* (*FRI*) and *VERNALIZATION INDEPENDENCE* (*VIP*) (Michaels and Amasino 1999; Sheldon *et al*. 2000; Zhang and van Nocker 2002; Zhang *et al*. 2003). Besides *FLC,* a lot of other floral repressors have been described, for example: *SCHLAFMUTZE* (*SMZ*), *SCHNARCHZAPFEN* (*SNZ*) and *TERMINAL FLOWER LOCUS* (*TFL*), which do not only suppress the floral pathway integrator genes but also the floral meristem identity genes (Henderson and Dean 2004; reviewed in Sung *et al*. 2003; Roux *et al*. 2006). The floral pathway integrators *FT*, *LFY* and *SOC1* (reviewed in Henderson and Dean 2004; Simpson and Dean 2002) act upstream of the floral meristem identity genes (*AP1*, *AP2*, *FUL*, *CAL*, *LFY*).

The floral integrator *FT* is thought to be the long-sought florigen (discussed in Zeevaart 2006). *FT* mRNA produced in the leaf is transported to the shoot apex, where its arrival is correlated with flower formation (Huang *et al*. 2005; Teper-Bamnolker and Samach 2005). *FT* acts via *SOC1* as an activator of floral initiation in the SAM. Thereby the specification of floral meristem occurs in conjunction with floral meristem identity genes such as *LFY* (An *et al*. 2004; Vijayraghavan *et al*. 2005).

LFY plays a key role in the initiation of floral meristems as well as floral organs, and in the formation of the inflorescence architecture. *LFY* acts as a repressor of *TFL1* which is an inflorescence meristem identity gene and a floral inhibitor. Furthermore, *LFY* is a transcription factor of other floral meristem identity genes, like *APETALA1* (*AP1*) and *AP2*. Besides these functions, *LFY* activates organ identity genes such as *AP3*, *PISTILLATA* (*PI*) and *AGAMOUS (AG)* (Huala and Sussex 1992; Bowman *et al*. 1993; Blazquez *et al*. 1997; Liljegren *et al*. 1999; Ratcliffe *et al*. 1999; Ferrandiz *et al*. 2000; Parcy *et al*. 2002; Soltis *et al*. 2002; Vijayraghavan *et al*. 2005). Flower development takes place when all these genes are induced.

Genes and models for flower development in higher plants

Models for flower development in higher plants are based on research performed in two major model plants, *Arabidopsis thaliana* (thale cress) and *Antirrhinum majus* (snapdragon). The flower of a higher plant usually consists of four whorls built by different floral organs: first whorl - sepals, second whorl - petals, third whorl - stamens and fourth whorl - carpels. Homeotic mutants with a change of floral organ identity have been studied for *A. thaliana* (Haugh and Summerville 1988) and *A. majus* (Schwarz-Sommer *et al*. 1990). Three classes of mutants have been found: carpels in the first whorl instead of sepals and stamens in the second whorl instead of petals (class A); sepals in the second whorl and carpels in the third whorl (class B);

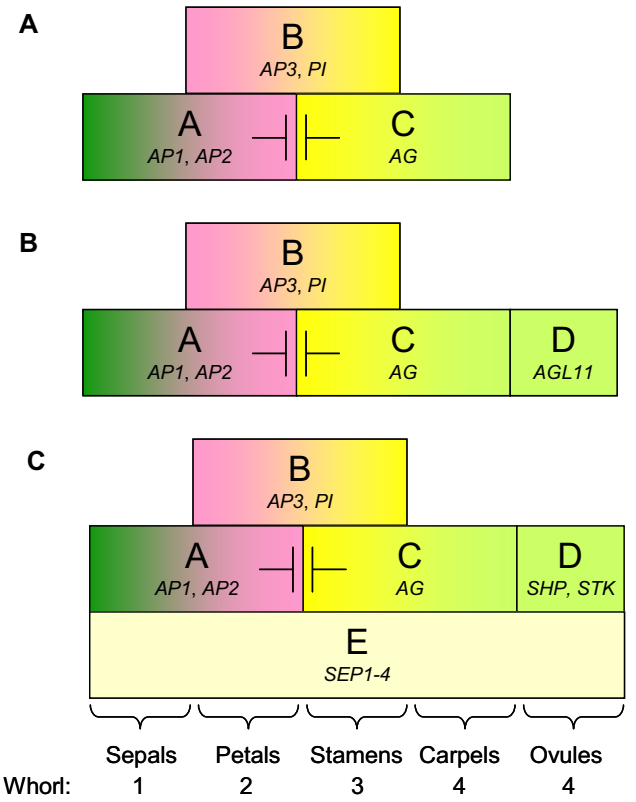

Fig. 2 From the ABC to the ABCDE model of floral organ identity and the corresponding genes of *A. thaliana*. The combined activities of classes of homeotic genes (A-E), indicated by bars lying upon each other, determine organ identity. The activities of A and C are mutually antagonistic. The schematic illustrations of the ABC (a), the ABCD (b) and the ABCDE models (c) were modified from: Coen and Meyerowitz (1991), Angenent and Colombo (1996), Theißen (2001) and Theißen and Melzer (2007).

petals in the third whorl and sepals in the fourth whorl (class C). From these mutants it was concluded that flower development depends on the expression of homeotic floral organ identity genes forming the different floral organs. Early ABC models were proposed for A. *thaliana* (Haugh and Summerville 1988) and *A. majus* (Schwarz-Sommer *et al*. 1990) before the classical ABC model (**Fig. 2**) (Coen and Meyerowitz 1991) was designed in 1991. The ABC model suggests the function of three different classes of gene activities A, B and C acting alone or together to determine floral organ identity. The function of A alone specifies sepals, petals are formed by the expression of A and B together, the combination B and C leads to male reproductive organs (stamens) and carpel identity is caused by action of C alone. The corresponding genes of *A. thaliana* (**Fig. 2**) have been identified and characterized by Bowman *et al*. (1989, 1991). *APETALA1* (*AP1*) and *APETALA2* (*AP2*) were identified for class A, class B function is contributed also by two genes, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), and one gene *AGAMOUS* (*AG*) was isolated for class C function. *AP1-3*, *PI* and *AG* and the corresponding genes of *A. majus*, *SQUAMOSA* (*SQUA*) an ortholog of *AP1*, *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*) contributing B function, and *PLENA* (*PLE*) C function (Sommer *et al*. 1990; Huijser *et al*. 1992; Schwarz-Sommer *et al*. 1992; Tröbner *et al*. 1992; Bradley *et al*. 1993), were shown to be transcription factors (for reviews see Theißen and Saedler 1999; Theißen *et al*. 2000). This kind of transcription factors belong to a family called MADS box genes. The name MADS was deduced from the first 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). All ABC genes, except *AP2*, belong to the so called **MIKC**-type MADS-box genes. They encode for proteins with the same characteristic structural features. The N-terminus starts with a highly conserved region named **M**ADS box (encoding DNA binding, nuclear localization, and dimerization functions), followed by an **I**ntervening-region, the conserved **K**eratin-like-domain (responsible for protein interaction and dimerization), and the **C**-terminus-domain (involved in transcriptional activation) (reviewed in Jack 2004). Riechman *et al*. (1996a, 1996b) stated that the products of the relevant MADS-box genes build dimers and only certain combinations are capable of binding to conserved DNA sequences called CArG-boxes. The function of the MADS-box transcription factors in plants seem to be generally conserved as shown by analyses of homologs of *A. thaliana* and *A. majus*, and other core eudicots ABC genes (for reviews see Irish and Kramer 1998 and Becker and Theißen 2003).

The generally accepted ABC model was extended to an ABCD model after MADS-box genes *FBP7* (*FLORAL BINDING PROTEIN 7*) and *FBP11* have been identified in *Petunia* to specify placenta and ovule identity (Angenent and Colombo 1996; Colombo *et al*. 1995). For *Arabidopsis* the *FBP11* ortholog *AGL11* (*AGAMOUS-LIKE 11*) (Rounsly *et al*. 1995), recently renamed *SEEDSTICK* (*STK*) (Pinyopich *et al*. 2003), and *SHATTERPROOF1* (*SHP1*) and *SHP2* could be considered as class D genes (Favaro *et al*. 2003; Jack *et al*. 2004).

Later on it was demonstrated that a fourth class of MADS-box genes, the *SEPELLATA* (*SEP*) genes, are necessary for proper development of petal, stamen and carpel identity in *Arabidopsis* (Pelaz *et al*. 2000, 2001). First, in *A. majus* the formation of ternary protein complexes between SQUAMOSA, DEFICIENS and GLOBOSA, showing higher DNA-binding affinity than the individual dimers, was reported (Egea-Cortines *et al*. 1999). Honma and Goto (2001) revealed that ectopic expression of *AG*, *AP3*, *PI* and *SEP3* is sufficient to convert leaves to organs that resemble stamens, and suggested the tetramer complexes PI-AP3-AP1-SEP3 and PI-AP1-SEP3-AG for the second and third whorls of *A. thaliana*, respectively. The necessity of *SEP* genes for proper flower organ development (Pelaz *et al*. 2000, 2001) and the finding that MADS-box protein form ternary complexes (Egea-Cortines *et al*. 1999) led to the floral quartet model (Theißen 2001; Theißen and Saedler 2001), proposing that tetrameric complexes of floral homeotic proteins control flower organ identity. Theißen (2001) suggested the term E function for the SEP genes providing another floral homeotic function than A-D genes, thus extending the former ABC-model to an ABCDE model or A-E model (reviewed in Teixeira da Silva and Nhut 2003). In contrast to the quartet model, the A-E model did not specify the protein complexes, which are maybe involved in specification of organ identity.

For *Arabidopsis* up to now four functionally redundant transcription factors of class E, *SEPELLATA1-4* (*SEP*) were reported which are essential for the specification of organ identity in all four whorls of a flower (Ditta *et al*. 2004; Pelaz *et al*. 2000). Triple mutants in *SEP1-3* lead to the conversion of the inner three whorls of a flower in to sepals (Pelaz *et al*. 2000), the loss of function of the fourth *SEP* replaces all flower organs by leaf-like structures (Ditta *et al*. 2004). The assignment of loss of function of *SEP4* (Ditta *et al*. 2004) and the proof of MADS-box genes required for ovule identity (Favaro *et al*. 2003; Pinyopich *et al*. 2003) were integrated in an improved floral quartet model, recently been published (Melzer *et al*. 2006). This new floral quartet model extents the floral quartet model given by Theißen and Saedler (2001) by the ovule identity quaternary complex, thus reflecting the ABCDE-model (for review see Theißen and Melzer 2007), and the corresponding CArG-boxes.

In *Arabidopsis* an abundant number of MADS-box genes (<100) have been identified. Due to molecular evolutionary criteria the MADS genes can be divided in two classes, the type I and type II MADS genes. Most type II

MADS genes are MIKC-type genes, whereas type I MADS genes do not encode the K-domain (Jack 2004).

FLORAL INITIATION AND FLOWER DEVELOPMENT IN APPLE

Juvenile phase of tree development

A period of juvenility is characteristic to all higher plants. Juvenility was defined as the period during which a plant cannot be induced to flower (Goldschmidt and Samach 2004). The juvenile period is the period elapsing between seed germination and first flowering of the seedling. During the juvenile phase of plant development meristems acquire reproductive competence, becoming able to sense and respond to signals that induce flowering. Within a species, the onset of flowering can vary tremendously, either because of differences in the environment or because of genetic differences. Even annual herbaceous plants are not competent to flower unless a short juvenile phase of reproductive incompetence was completed (Martin-Trillo and Martinez-Zapater 2002).

Perennial plants, such as trees, generally display long juvenile phases (Hackett 1985). In the domesticated apple (*Malus domestica*) the juvenile phase can last seven to eight years, but in certain *Malus* species flowering can be delayed substantially more than eight years (Zimmerman 1972). Fruit tree seedlings in their juvenile period show a number of anatomical and morphological characters which disappear or change with time. Fritzsche (1948), Murawski (1955) and Karnatz (1963) described juvenile characteristics in apple and pear. Leaves differ from those in the adult phase as to size (smaller), width (narrower), serration (sharper), cell size (larger). Beside this, thorns are present during the juvenile period of the tree and the angle between side shoots and main stem is wide. However, the attainment of the flowering stage and the disappearance of juvenile symptoms do not necessarily occur simultaneously in all seedlings of a given progeny. There are several observations obtained on apple seedling progenies and their parents which are of practical value in breeding and fruit production (Visser 1965). Seedlings which attain the flowering stage sooner also show a faster rate of modification of juvenile characteristics toward adult, i.e. there is a highly significant correlation between the length of the juvenile period and the degree to which seedlings show juvenile symptoms. There is also a significant correlation between length of the juvenile period and parent characteristics, such as season of flowering, ripening, and length of growth period of the fruits from flowering to picking. In this respect much greater proportion of summer apples is to be found among seedlings with a relative short juvenile period and visa versa with regard to winter apples. Varieties with fruits developing in a relatively short period produce seedlings flowering in a relatively short period and visa versa for varieties the fruits of which need a longer developmental period. Visser (1965) stated also a highly significant correlation between the unproductive period of the parent and that of the seedling, i.e. length of the juvenile period and length of the vegetative period of the parents from grafting on rootstock to first bearing. From these studies it was presumed that the unproductive phase of juvenile seedlings and of adult varieties are similar physiological phenomena in which the attainment of the flowering stage is governed by the same or similar factors. This means: the juvenile period of the seedling is quantitatively determined by the length of the vegetative period of the parents; measures that reduce/promote growth will prolong/shorten the unproductive period of both seedlings and varieties; interaction between rootstock and scion is similar for seedlings/varieties as in both cases the scion bears sooner or later depending on which rootstock is used. Observations on several fruit tree species have shown that seedling vigour and juvenile period are inversely related (Visser 1964). Stem diameter of apple seedlings is inverse correlated with the duration of the juvenile period (the thicker, the shorter) and initial productivity of the seedling (the thicker, the higher) (Visser 1970). This inverse correlation exists also for trees on rootstocks, i.e. between stem diameter and unproductive period (Visser and de Vries 1970). It was shown that adult seedlings after having budded on a rootstock flowered sooner when the juvenile period has been shorter (Visser and Schaap 1967; Visser and de Vries 1970).

Zimmerman (1973) proposed a transition period between juvenile and adult period. The end of the juvenile period is indicated by the attainment of the ability to flower and the actual production of flowers is the first evidence that plant is in the adult phase. However, the end of the juvenile period and the first appearance of flowers may not coincide. Thus, seedlings do not flower because of other factors; even through the seedlings have attained the ability to flower. This period of transition is also defined as the adult vegetative phase (Poething 1990). The adult vegetative phase is the most important phase for breeders as during this phase most floral-inducing techniques are applied successfully.

Evaluating the juvenile phase of seedlings, Zimmerman (1973) found that the lowest bud of a seedling which may be an indicator of the point of transition to the adult phase occurred at a height of 1.8-2m on greenhouse plants in crab apple. Later on, he stated that the stage of development is better measured by node number than by height of the seedling, thus the transition occurred at about 75th to 80th node. Aldwinckle (1975, 1976) estimated that apple seedlings attained the height at which flower buds were formed 9-12 months after germination. To force flowering in apple progenies, seedlings were grown as single-shoot plants under optimum greenhouse conditions, manually defoliated and planted in the field after chilling. Fischer (1994) proposed another agro-technical approach to fasten seedling growth by cultivation at long-day conditions (16 h daylight) in the greenhouse for two years. At the height of 1.8m graftsticks were taken and grafted in April in the field on Hibernal interstem/M9 rootstocks. A few grafted seedlings flowered almost in the year of grafting, i.e. 28 months after germination of seeds. The determination of the transitional point to the reproductive phase is difficult to determine. In order to identify potential biochemical markers that can be used as indicators for the change from the juvenile to the adult period, Zhang *et al*. (2007) studied the dynamics of polyphenolic compounds. The lowest flowering node on seedlings was found at around 122nd node under natural conditions, in other words the seedlings had reached the reproductive phase. However, in response to application of plant growth regulators, such as BA and ethephon, flowering was induced at the lowest node, node 77, indicating that the seedlings had been in the adult vegetative phase, i.e. transition phase according to Zimmerman (1973). Based on these studies it was concluded that the transition points from juvenile to adult vegetative and from adult vegetative to reproductive phase would be around node 77 and 122, respectively. A schematic description of current knowledge on ontogenic phases of development in apple is given in **Fig. 3**.

The acquisition of reproductive competence in plants is under genetic and environmental controls. The most extensive genetic analysis of factors affecting shoot maturation and the juvenile phase has been performed in *Arabidopsis* (Martin-Trillo and Martinez-Zapater 2002). However, the genetic basis of juvenility has not been fully understood up to now and it remains unclear how genetic factors cause an incompetent meristem to flower. Tree juvenility phase could be reduced through genetic engineering as shown first by Weigel and Nilsson (1995) for the constitutive expression of the *Arabidopsis* gene *LFY* in an aspen clone. More recently, *LFY* and *AP1* have been expressed in citrus which drastically reduced the length of the juvenile phase (Peña *et al*. 2001). Progress in understanding the regulatory mechanisms of meristem competence in *Arabidopsis* will provide additional gene sequences to be tested in trees. Kotoda *et al*. (2006) reported that the down-regulation of

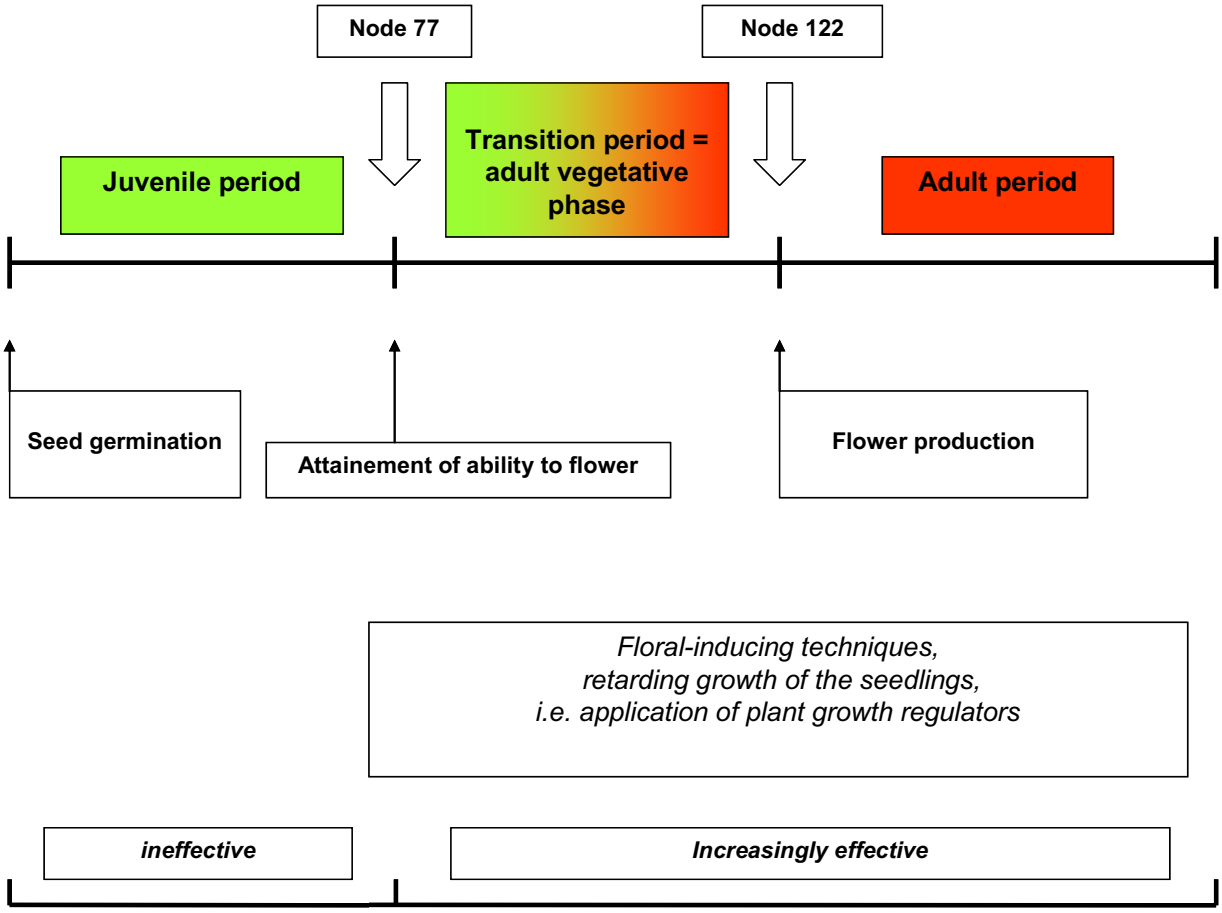

Fig. 3 Schematic illustration of the ontogenesis in apple seedlings based on observations of Zimmerman (1973) and Zhang *et al.* (2007).

MdTFL1 might trigger the up-regulation of *AFL1* and *AFL2* in apical buds resulting in flower induction in apple. Based on the results obtained in *MdTFL1* antisense transgenic apple plants it was stated that *MdTFL1* must be one of the factors controlling the transition from the juvenile/vegetative to the reproductive phase in apple. Shortening the juvenile phase in fruit trees, like apple, can drastically accelerate the breeding cycle of new cultivars.

Repetitive seasonal flower formation

Once fruit trees have passed the juvenile phase and reached an adult phase of reproductive competence, a portion of shoot meristems will initiate flowers each year. Upon the transition from the juvenile phase to the reproductive phase, apple shoots begin producing flower buds that contain inflorescences with bracts and floral meristems. Shoots develop in a defined pattern that has specific vegetative and floral bud locations. Fruit buds in apple are borne terminally on fruiting spurs and/or terminally or laterally on long shoots. Whether the mechanism regulating competence in adult tree meristems is the same as that responsible for the acquisition of competence in the juvenile to adult transition remains to be elucidated (Martin-Trillo and Martinez-Zapater 2002).

Flowering in apple consists of several stages including flower induction, flower initiation, flower differentiation and anthesis (blooming).

Flower induction is the transition of the meristem development from the vegetative to the reproductive phase. During this period, flower signals are received by the apical meristem and the genes required for flower development are turned on. *Flower initiation* is the period, when a series of histological changes are underway but no visible morphological differences can be observed. During this phase buds are sensitive to stimuli which determine their fate. When a bud is induced to be reproductive, it will irreversibly undergo the process of floral organ development, regardless of the internal/external conditions that could affect flower induction (Miller 1982). *Flower differentiation* is characterized by morphological changes of apple buds. It starts by the appearance of floral primordia in the bud (Abbott 1977; Hirst and Ferree 1995, 1996) and ends with the development of the primordia of floral organs. Flower differentiation is always marked by the appearance of the dome-shape apex in the bud that takes place about 12 weeks after full bloom (Abbott 1977). As a result, the central part of the apex becomes a 'King' flower surrounded by four lateral flowers and sepals, petals, stamens and carpels are differentiated subsequently. Flower buds increase in weight and enter maturation during winter dormancy. The growth of flower organs and the development of flowers last until the following spring when blooming takes place. The rate of development and the quality of flower buds will decrease by water deficits, high temperatures, nutrient deficiencies, defoliation, inadequate chilling temperatures during dormancy, and freezing injury. Apple trees growing in regions where root zone temperatures are lower than 15°C have delayed bud break and up to 20% fewer clusters than apple trees exposed to higher root zone temperatures (Greer *et al.* 2006). However, raising the air temperature after full bloom favoured flowering in apple but flower quality based on the number of well-developed flowers per cluster tended to decrease with increasing temperature (Zhu *et al.* 1997). Fertigated trees exhibit increased node development, axillary flower-bud densities, extension shoot growth and trunk increments (Dencker and Hansen 1994a, 1994b). Some apple cultivars produce low yields when grown in regions with inadequate winter chilling. Their unsatisfactory development is attributed to the lack of climatic adaptation which causes some abnormalities in bud differentiation (Oukabli *et al.* 2003). Defoliation in apple trees was found to have a negative effect on flower bud formation as well as flowering (Stampar *et al.* 1999).

Topography of flower bud formation in apple and floral morphogenesis

The tree and shoot architecture in apple has evolved to accommodate both vegetative and reproductive growth. The juvenile apple shoots form only vegetative buds, adult shoots form vegetative and generative (floral) buds in a sequential manner. The shoot apex includes the growing point of the plant, the shoot meristem, surrounded by young primordia, which before floral induction develop into leaves, and afterwards into flowers. Apple shoots form terminal buds on shoots in addition to axillary buds in leaf axils. Depending on the cultivar, age, and vigour of the tree fruit, buds are formed on fruit spurs, which are shortened shoots with a length less than 5 cm, and/or terminally or axillary on one-year old shoots (**Fig. 4**). The shoot meristem produces in an ordered sequence leaf-like primordia, which further differentiate into budscales, transition leaves and leaf primordia prior commitment to floral development. One of the first description of an apple bud was given by Abbott (1970), who stated that the winter bud consists of nine bud-scales, three transition leaves, six true leaves and three bracts. The axis of the bud is terminated by a flower primordium (the 'King flower') and lateral flower primordia are formed in the axils of the three bracts and the three distal leaves. The floral primordia and subtending bracts on the flanks of the terminal meristem are initiated before initiation of a terminal floral meristem. Differentiation of the lateral floral meristems does not occur until after bractlets and sepals are initiated on the terminal floral meristem (Foster *et al*. 2003). By the time of leaf fall the terminal floral meristems have differentiated sepals, stamen and carpels (Bergh 1985). Subsequent vegetative growth is from a 'bourse' shoot that develops from the axils of one of the leaf primordia in the floral bud (Foster *et al*. 2003). Flowers from which fruit develop were initiated during the previous growing season. Most of studies describing the bud architecture in apple are focused on terminal buds.

Starting from the second half of the last century, flower bud formation in fruit tree species, especially apple, was studied intensively (Zeller 1960a, 1960b, 1960c, 1962, 1964; Fulford 1965, 1966a, 1966b, 1966c; Abbott 1977; Luckwill and Silva 1979; Landsberg and Thorbe 1985; McLaughlin and Greene 1991a; Hirst and Ferree 1995; Huang 1996). These studies were aimed on the identification of factors involved into transition of the vegetative meristem into a reproductive one. However, much of the work has focused on later stages of floral morphogenesis, i.e. floral organ differentiation (Pratt 1988). Hanke (1981) examined the development of terminal and lateral meristems in apple using a histological approach and determined five ontogenic stages of shoot apex development during growing season (**Fig. 5**): (I) Vegetative stage which is characterized by a completely flat and narrow shoot apex. The meristem produces leaf primordia in a stable rhythm (plastochron). (II) Intermediate stage which is characterized by swelling and broadening of the shoot apex based on increase of the cell number in different cell layers of the apex. The apex is still producing leaf primordia. (III) Prefloral stage which is the starting point of the reproductive differentiation characterized by doming of the apex. (IV) Formation of the inflorescence primordia. (V) Differentiation of floral organs in the inflorescence. Foster *et al*. (2003) studied the progression from vegetative to floral development in apple bourse shoot buds using scanning electron micrographs and images of sectioned shoot apices. Eight morphologically distinct stages of shoot apex development prior to winter dormancy were defined. Based on measurements of the meristem diameter, two stages of vegetative development were recognized (stage 0-narrow, flat apex; stage 1-broad apex). Pronounced doming of the apex marked stage 2. Stages 3 to 7 were applied to lateral and subsequent terminal floral meristem formation at the domed apex, and to floral organ formation (bractlets and sepals). The results suggested that broadening of the apex (stage 1) is the first morphological sign of commitment to flowering. From the morphological characterization of early floral development in apple described by Hanke (1981) and Foster *et al*. (2003) it can be summarized that the vegetative stages I and II are comparable to stages 0 and 1, and stage III (doming of the apex) is identical to stage 2. Besides, both reports stated morphological features that mark the beginning of floral development stages. Foster *et al*. (2003) found a diameter of <130 µm for the flat vegetative apex and >130 µm for the vegetative apex committed to floral development, whereas Hanke (1981) stated for the flat apex a diameter less than 120 µm and 19 cells in the upper tunica layer and for the broadened apex more than 130 µm and 21 cells in the upper tunica layer during three consecutive years of experiment.

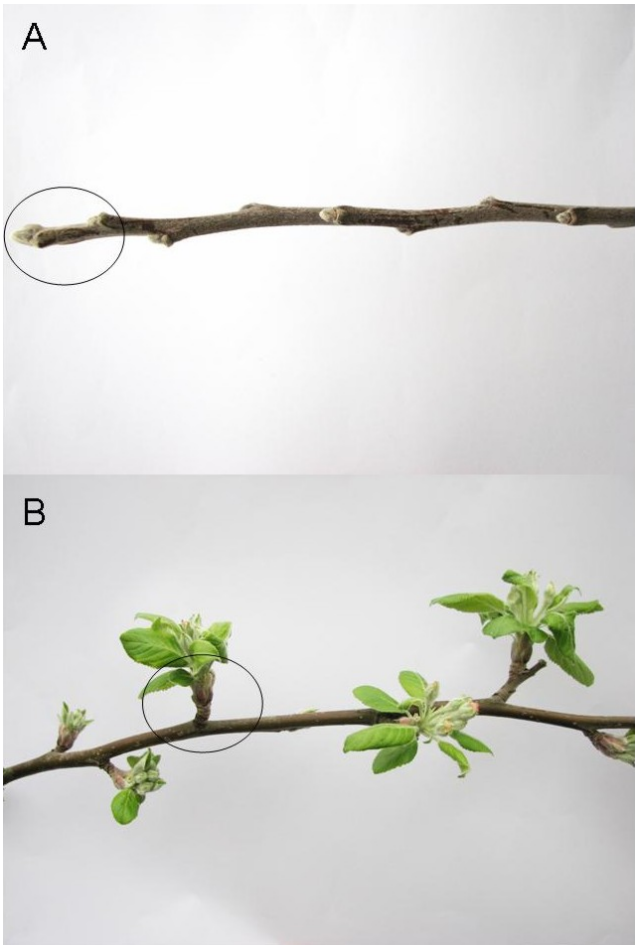

Fig. 4 In apple, flower buds are formed mainly in the terminal position on one-year-old shoots (A) and on spurs of older shoots (B).

Plastochron and time of flower induction/initiation

Flower induction in temperate fruit trees, especially in apple, is still a relatively unclear phase of development as from the time when it takes place as from the physiological preconditions. However, experimental results indicate that the induction of flower formation in buds which occurs much later in the year is realized still in the vegetative phase of bud development (**Fig. 6**).

Fulford (1965, 1966a) reported that the formation of successive primordia at the apex is realized in stable phases of meristem activity, which may extent 5, 7 or 18 days of plastochron. In cases of high apical dominance produced by young leaves and fruits, an 18-days plastochron can be found in buds and the differentiation of floral organs will fail. When the shoot growth is ceased, the most outer leaf primoridia at the apex turn into budscales. At fruit spurs which differentiate flower buds more frequently and which do not show elongation growth, this process starts im-

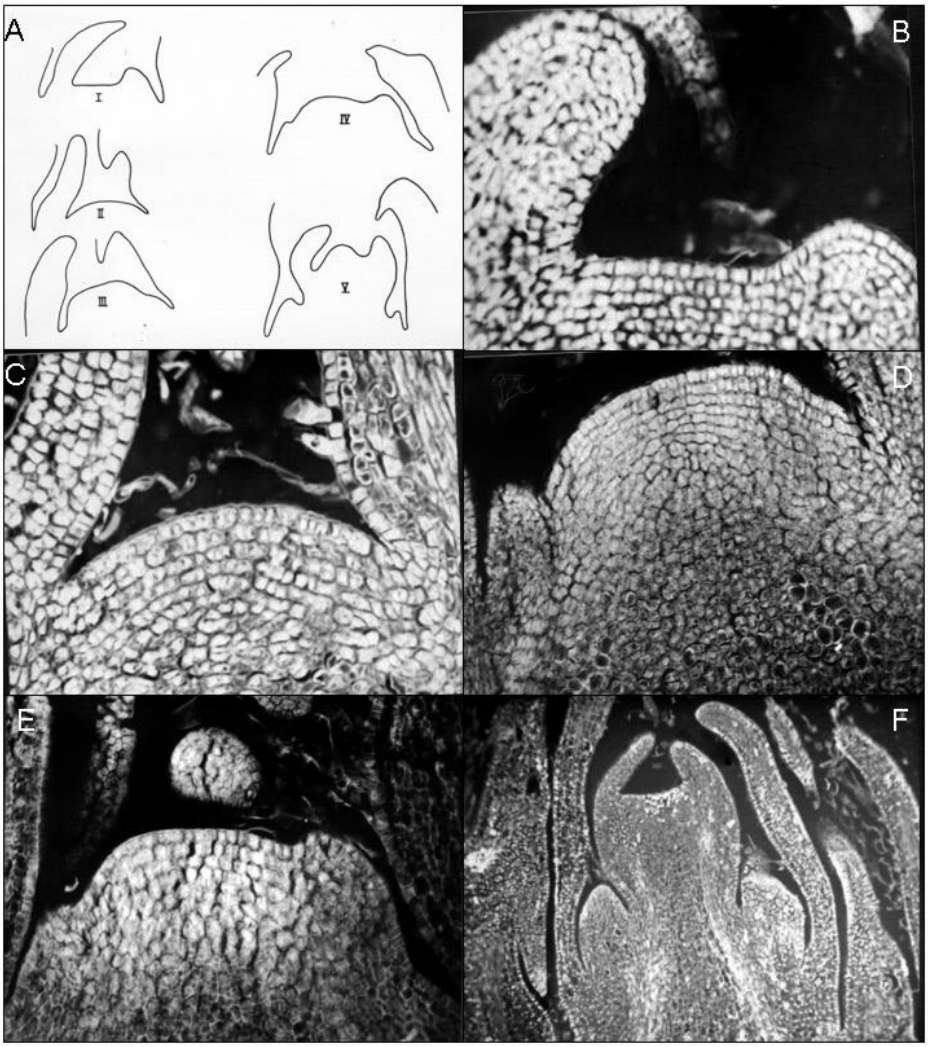

Fig. 5 Histological images from sectioned vegetative and inflorescence meristems in apple. Schematic application of five (I to V) developmental stages of the shoot apical meristem (**A**). Shoot apical meristem in stage I (vegetative) – narrow, flat vegetative meristem (**B**), in stage II (intermediate)- broad, swollen vegetative meristem (**C**), in stage III (pre-floral)- domed apex (**D**), in stage IV- formation of the inflorescence primordia (**E**) and stage V- differentiation of the inflorescence (**F**). The classification is analogous to Hanke (1981).

mediately long before leaves of the previous year are unfolded (Schmidt and Hofmann 1988). In terminal buds and in lateral buds of the one-year-old shoots the bud development is delayed. Schmidt and Hofmann (1988) also found a relatively constant plastochron in apple buds during the vegetative growth period which started at the beginning of April and was not influenced by environmental factors, like air temperature. As shown by Luckwill and Silva (1979) the node number in vegetative and floral buds resulted from one or two phases of node formation and differences in flower initiation can not be explained by modified plastochron. Hoover *et al*. (2004) found a cultivar-specific rate of appendage formation which was highest at the beginning of the growing season and declined thereafter.

Since the work of Fulford (1965, 1966a, 1966b, 1966c) it is believed that the appendage initiation rate at the shoot apex is a critical factor in determining flower initiation within the bud. He described the maturation of apple buds prior entering floral development and found that after formation of a certain number of budscales (8 to 10) at the vegetative apex eight leaf primordia have to be formed before flower organs may be differentiated. Abbott (1970) and Luckwill (1975) also proposed that in apple before flower initiation occurs, the node number in a bud must have reached a certain critical value. The critical node number seems to be genotype-dependent and independent from environmental conditions (Fulford 1966a). This was also supported by Hirst and Ferree (1995, 1996) who found that rootstock and year of investigation had little effect on critical node number. Zhu *et al*. (1997) found a value of about 18 for spur buds and 15-16 for terminal and lateral buds of one-year old shoots in Summered. The node number was obviously not affected by temperature. McLaughlin and Greene (1991a, 1991b) also stated that a critical node number has to be reached by a certain time before the meristem may acquire the capacity to initiate flowers. In contrary to the theory that a certain threshold level of node number has to be reached before flower initiation takes place, Lauri *et al*. (1996) found that transition to flower formation might occur as soon as the first nodes are formed. Costes (2003) studied winter bud content with respect to bud position on annual shoots, and according to branching order and shoot age. He found that a certain number of organs must be initiated before floral differentiation occurred. The minimum number of organs was about 15, including scales. Total number of lateral organs formed was shown to vary with both bud position and meristem age, increasing from newly formed meristems to one- and two-year-old meristems on different shoot types. These results correspond with data published by other authors who found that the total number of appendages appears to be highly variable, depending on the position of the inflorescence in the tree or on the shoot, but in the mature fruit bud it is around 20 (Barritt and Konishi 1993; Volz *et al*. 1994).

Histological investigations of the shoot apex indicated further results on timing of floral commitment of the apple bud. Foster *et al*. (2003) suggested that broadening of the apex in vegetative stage 1 which occurs between 39 and 53 days after full bloom, is the first morphological sign of floral commitment. This is in context with other reports. Buban and Faust (1982) described histological changes at the shoot apex between three to six weeks after full bloom indicating on floral commitment. Investigating different types of buds collected from one-year-old shoots and bourse shoots in three consecutive years, Hanke (1981) found that the occurrence of ontogenic stages of shoot meristems is independent from the date of full bloom but seems to be

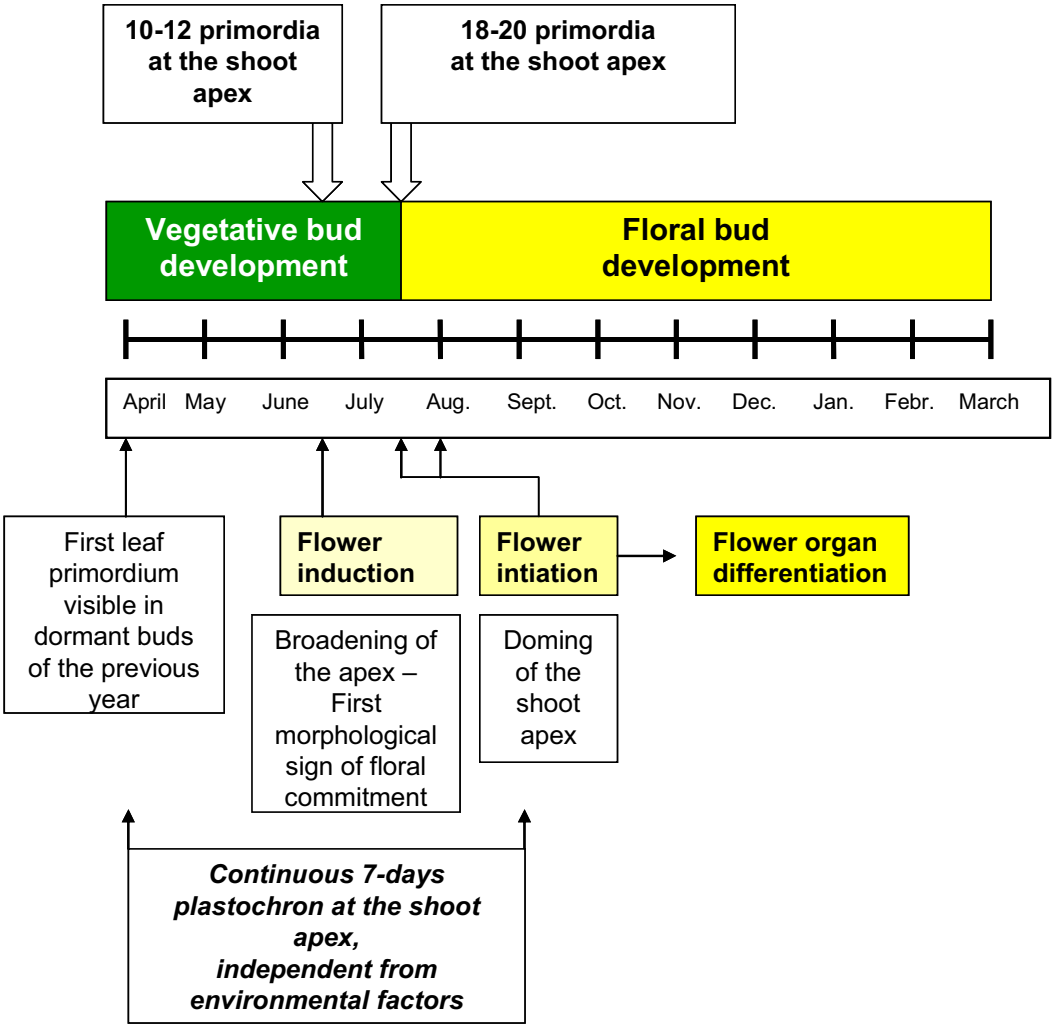

Fig. 6 Schematic illustration of seasonal changes in the development of terminal spur buds based on observations of Abbott (1977), Fulford (1965, 1966a, 1966b), Luckwill and Silva (1979), Schmidt and Egerer (1982), Schmidt and Hofmann (1988) in apple.

stable according to the calendar, which means there might be a correlations to the daylight length. Thus vegetative stage II of the shoot apex occurs mainly from mid June and stage III from mid July. This observation coincides with results from practical application of floral-inducing plant growth regulators, like ethephon, which are effective at the time between stages II and III. According to Hoover *et al.* (2004) floral commitment as indicated by doming of the apex was observed during the period from 60 to 150 days after bloom and was initiated and completed in dependence on the cultivar investigated. This timing agrees with stage 2 of shoot meristem development described by Foster *et al.* (2003) and stage III described by Hanke (1981) who suggested floral commitment much earlier. According to Hoover *et al.* (2004) the timing of floral commitment seems not to be related to the time of flowering, nor to the time of fruit maturity of the cultivar. It was found that the timing of specific events during flower morphogenesis differed between cultivars. A series of experiment involving defoliation and water stress at different dates indicated that these treatments can make apple trees to flower a second time in one year as long as the treatments are given near the end of July (Jones 1987). These results suggest that reflowering was dependent on the flower buds destined to open after the treatment having been already differentiated at the time of treatment. Dissections confirmed that flower primordia were first clearly detectable in the next year's buds on about 21 July in south-east England.

Recently it was reported on seasonal-dependent expression of a *TFl-1* like gene, termed *MdTFL1*, which is involved in the maintenance of juvenile/vegetative phase in apple, in apices of apple (Kotoda and Wada 2005). It was shown that mRNA was expressed strongly in July (about eight weeks after full bloom), approximately two weeks prior to the initiation of floral bud formation, thereafter expression decreased gradually to late July. According to this report, *MdTFL1* is possibly involved in the regulation of flower induction from late June to late July since this period is thought to be critical for the determination of meristem identity in apple (Hanke 1981; Buban and Faust 1982; Kotoda *et al*. 2000; Foster *et al*. 2003).

Biochemical changes during flower formation

Beside morphological studies, the process of flower bud initiation and differentiation was also studied using physiological and biochemical approaches. Schmidt and Buban (1971) reported on a relationship between changes of the phosphate metabolism and flower bud differentiation in apple. In several photoperiodically inducible plants which save as objects for flower differentiation experiment, one of the earliest inductive stimuli was found to be an increase of RNA synthesis. In apple, it was shown that the transition of the bud from the vegetative stage to the reproductive one is accompanied directly with changes in the nucleic acid metabolism, i.e. the flower differentiation is based on synthesis of a specific ribonucleic acid. If the synthesis of this specific nucleic acid is inhibited by specific compounds, flower bud differentiation will not take place (Hess 1961a, 1961b). Schmidt (1964, 1965, 1971) performed studies on RNA content of flower buds before flower initiation and reported on a relationship between the amount of RNA and the frequency of flower bud differentiation. The same result was obtained for lateral buds in one-year-old shoots in apple when the growth regulator ethephon was applied which stimulates flower bud initiation and differentiation (Schmidt and Egerer 1982). There was also obtained a variation of the RNA content in buds in the course of time. The first peak of RNA content was obtained during flower induction until mid of June, followed by a period of rather

small increase and a second peak of increase at the beginning of flower differentiation in August (Schmidt 1978; Schmidt and Egerer 1990). The nucleic acid content was reported to be higher in buds of fruitless spurs than that of spurs bearing fruits, and the content of histone proteins was lower in fruitless spurs (Buban and Hesemann 1979; Buban and Simon 1978). Schmidt (1964) suggested that the processes which cause flower bud differentiation in one-year-old shoot and in spurs are generally the same. However, differences in the development of axillary or terminal buds allow the assumption that the physiological processes are different. Thereby, in some cases buds in leaf axils remain vegetative and in other cases they are enabled to form flower organs.

Internal factors affecting flower formation

Following flower induction in monocarpic plants mostly all buds are forming flowers. This is not the case in polycarpic plants, like apple. More or less buds remain vegetative (Lang 1965). As this is also the case in fruit trees, the phenomenon was explained by a deficit of nutrients. Based on practical experience in fruit growing, the C/N-ratio hypothesis was used to explain differences in flower bud differentiation in fruit trees. High C/N ratio favours flower formation and excessive N fertilisation inhibits it. It seems that nutrients are not the limiting factor for flower formation when a threshold level is reached.

Chailakhyan (1937) proposed that flowering is caused by a flower-inducing hormone, called florigen. Searle (1965) stated in general that flower induction of photoperiodically sensitive plants is controlled by florigen which is produced in leaves and transported to buds. Florigen was described to play a positive role activating genes or a negative one, blocking gene-repressors. There were several attempts to find an evidence for a florigen-specific mRNA (Hess 1961a, 1961b). A range of experiments reported on the regulating function of inhibitors for flower formation which are produced in non-inducing leaf tissue and transported to buds as the locus of action. It was Denffer (1950) who combined first the theory of the universal flowering hormone with the theory of flowering inhibition. Also Wellensiek (1962) assumed that the flower induction in buds is influenced by flower stimulating compounds as well as by inhibiting compounds.

In the 1970's the modern hormone theory was developed which abandoned the C/N hypothesis and the florigen concept. The process of flower formation in fruit trees was studied more complex as a system of different interactions in the tree leading to morphological changes in the meristem. Following this way, Fulford (1965, 1966a, 1966b, 1966c) described in details the developmental process of apple buds under the influence of leaves of different ages and of fruits as well. He stated that leaves and fruits do not effect the meristems directly via supply of nutrients, but there should be other unclear at that time regulating mechanisms. He also assumed that the flower formation in apple buds can be rather explained by elimination of factors limiting the reproductive development than by synthesis of specific flower-inducing compounds. His concept is based on interactions of natural plant growth regulators and correlative inhibition of flower bud and vegetative bud formation due to the so-called apical dominance. In this context great attention was given to hormones produced in seeds of developing fruits as fruit development occurred at the same time as floral development in buds. Luckwill *et al*. (1969) found gibberellin activity in apple seeds to be highest in the ninth week after full bloom. A rather strong circumstantial evidence was suggested that gibberellins translocated from the seeds to the bourse may inhibit flower initiation in the bourse bud (Luckwill 1970). Lang (1965) stated three factors which are responsible for buds remaining in a vegetative stage: maturation of the bud, inhibition based on apical dominance and endogenic dormancy of the buds.

The term 'apical dominance' is usually taken to refer to correlative inhibition of lateral buds by the terminal buds or growing apex of the shoot. In woody plants, the term is also used in a different sense to refer to the stronger growth made by the upper or leading shoot on a branch, in comparison with the weaker growth of the lateral shoots (Luckwill 1968). Which are the regulating mechanisms of apical dominance remains still unclear. The theory of apical dominance was described by Phillips (1969). The phenomenon was initially discusses using the theory of nutrients by an unequal distribution of nutrients amongst competing shoots (Smith and Wareing 1964; Wareing and Nasr 1961). However according to Libbert (1964) the plant hormone auxin is directly and/or indirectly involved. Besides, it is assumed that cytokinins as plant hormones are acting as correlative inhibitors (Sachs and Thimann 1964, 1967). Luckwill (1968) stated that gibberellin is produced in the young expanding leaves near the apex which migrates to the apex where it stimulates either the production or downward movement of auxin. As apical dominance may be explained mainly by interaction of several plant hormones, the distribution of nutrients into the places of meristematic activity should not be underestimated. Actively growing parts of the plant which are the source of correlative inhibition are also the main sinks for accumulation of nutrients. It seems to be difficult to decide what are the cause and the effect in this relationship. The phenomenon of correlative inhibition based on distribution of hormones and nutrients is broadly discussed by Schmidt (1973).

Different hormones regulate flower formation interactively (Tu 2000). Auxins are both promoting and inhibiting flower formation. Cytokinins are associated with promotion of flowering in apple. The effect of abscisic acid remains unclear. Ethylene affects flowering in a stimulating manner. Of all currently known hormones, gibberellins (GA) are most strongly associated with flowering (Pharis and King 1985). GA treatments have been shown to inhibit flower bud formation in fruit tree species and at the same time GA seem to be indispensable for floral development (Goldschmidt *et al*. 1997). Evidently, GA play a regulatory role in vegetative and reproductive plant development (Goldschmidt and Samach 2004) and they are closely related to alternate bearing (Tu 2000).

Flower formation and interaction with other organs

Kobel (1954) reported that specific factors inducing flower bud development are concentrated in the appropriate leaves attached to the bud. According to Bünning (1952) the developmental stage of the leaves is essential for the formation of flower organs. The unique role of leaves in the formation of flowers can be explained by the facts that leaves as organs of assimilation provide carbohydrates needed in flower induction, that leaves are an important site of hormone synthesis and that they are the receptor of environmental signals. In the post-genomic era when genes have been identified that control floral development, it became evident that important floral-inducing genes are active in leaves. It should also be mentioned that removing leaves by defoliation techniques before flower induction can greatly inhibit flower formation in apple. The end of flower induction was studied by removing leaves at different time after full bloom (Li *et al*. 1995). When defoliation has no inhibitory effect on flowering, the implication is that meristems have passed flower induction period. According to Davis (2002) defoliation in early July caused the least amount of flowering the following year, as the defoliation timing and severity was delayed there was less suppression of flowering and fruit set. Lauri *et al*. (1996) suggested that the leaf number generally had a stronger influence on fruit set in apple than flower number.

There seems to be a correlation between cessation of terminal shoot growth and flower formation in apple. Flower induction can take place either before or after shoot elongation ceases (Zeller 1960b; Tromp 1968; Williams

1973). However, it is mostly accepted that flower bud differentiation occurs after cessation of shoot growth (Luckwill 1970). Shoot growth may inhibit flower formation. However, the observation that growth vigour and flower-bud formation are often antagonistic does not imply a causal relationship between the two. Zhu *et al*. (1997) found that generative development in lateral buds of long shoots already started 12-13 weeks after full bloom, whereas growth of these shoots continued until week 19. An independent control of shoot growth and flowering was also suggested by (Luckwill 1970; Tromp 1973; Luckwill and Silva 1979; Forshey 1989).

Despite the difficulty in predicting flowering probability, recently new features were highlighted regarding the within-tree flowering occurrences (Costes *et al*. 2003). Newly developed long shoots have a lower flowering ability than the older shoots. Flowering probability depends on both shoot and tree age. There was found a decline corresponding to a growth reduction and an increase in the probability of flowering from the centre of the tree towards the periphery. This centrifugal gradient found in apple is also consistent with results found in other tree species (Costes *et al*. 1992; Sabatier and Barthelemy 2000) and with the definition of a within-tree 'physiological age' of buds (Gatsuk *et al*. 1980).

Fruits also effect flower formation in apple as development of fruits coincides with the time of flower induction. Fruits represent a strong sink for assimilates which may suppress flower induction by competing for carbohydrates. Chan and Cain (1967) demonstrated that the presence of seeds is the crucial element in fruit inhibition of apple flower induction. These experiments were repeated by Neilson (1998, cited by Tu 2000) who indicated that control of apple flowering was not as simplistic as it was believed for almost 30 years. The length of the bourse shoot as well as the presence of seeds was suggested as controlling factors. There is less inhibition of flowering by seeds when the bourse shoot is longer.

Thus heavy crop load is inducing alternative bearing in apple. Alternative (or biennial) bearing, i.e. the negative correlation between fruit development and flower bud differentiation, is one of the most investigated causes of flower set variability in apple. Practical approaches in fruit production aimed on the reduction of this negative correlation rely mainly on fruit thinning reducing flower formation in the 'on-year' which was induced in the 'off-year'. There should be also mentioned the stimulating effect of the growth regulator ethephon in apple that temporarily can compensate the correlative inhibition between fruit development and flower bud differentiation. The application of growth regulators is still a practical approach to reduce the effect of alternative bearing resulting in 'on-year' and 'off-year' fruit production (Schmidt *et al*. 1989). Most of the orchard techniques for controlling apple flowering will be effective only if applied during the period of flower initiation. Genotype is probably the most important cause for alternative bearing. There are regular bearing cultivars and cultivars whose trend is to be very biennial bearing. However, based on the analysis of cropping variability between years it was found that more or less a part of this variability can not be explained by an endogenically induced, i.e. genetically determined, alternative bearing. Environmental factors, like air temperature, are also involved (Handschack and Schmidt 1986, 1988, 1990).

Environmental factors affecting flower formation

Most of the studies on flower initiation were carried out on photoperiodic sensitive annual plants or plants in which flowering is induced by vernalization. According to Alleweldt (1964a, 1964b, 1964c) fruit trees are long day plants as the flower induction takes place during a period of natural long day conditions. The photoperiod and specific temperature conditions are important factors that regulate blooming of trees as they represent inductive conditions for commencement and cessation of dormancy and thereby influencing vegetative growth as well as development of flowers (Lang 1965).

There are several environmental conditions that may influence flower-bud formation in apple, whereas a relatively large amount of attention has been given to temperature. Tromp (1976) compared two different temperatures (24° and 17°C) and found that flowering was stimulated at the lower temperature when applied from full bloom, but was reduced when temperature was raised from 17 to 24°C seven weeks before harvest, i.e. before flower bud differentiation. Later Tromp (1980) suggested that the first four to five weeks after full bloom are of especially great importance for flower formation, i.e. during flower initiation. Zhu *et al*. (1997) studied the effect of temperature on flower bud formation, shoot growth and bud morphogenesis at different time after full bloom. In this study it was found that flowering was stimulated by increasing temperature when applied for the whole season as well as when applied six or seven weeks after full bloom, on spurs as well as on one-year-old shoots, but the effect was most pronounced in the range of 13-20°C. The effect of temperature on node number did not show any consistent pattern. Tromp (1973) studied also the effect of light, soil temperature and other environmental factors on flower formation.

Reflowering in any one year was shown to be initiated by water stress and by defoliation in apple. The results suggested that the reflowering after a period of water stress was primarily a result of the loss of leaves that occurred when the plants were subsequently rewatered and that the reflowering was the greatest when the treatments were imposed in mid-to late July (Jones 1987).

Agrotechnical approaches to affect flower formation

The interest of breeders (and growers) was always in precocious flowering of seedlings by shortening the juvenile period. Shortening of the juvenile period of seedlings would greatly improve the efficiency of breeding. In fruit tree species, the juvenile period was shortened initially by selecting and propagating naturally occurring early flowering genotypes or mutants. Various practical techniques have been considered to accelerate flowering of seedlings. Seedlings of the cultivated apple rarely flower in the field before they are three years old and often not until they are aged eight years or more (Aldwinckle 1976). The basic idea is to grow seedlings rapidly from the germination stage to the transition to flowering (Visser 1964; Aldwinckle 1975). According to Visser (1964, 1965, 1970) the juvenile period can be shortened genetically by the choice of early-productive parents or physiologically by favourable cultural practices and by grafting on a precocious rootstock. A longer growing season will also reduce the length of the juvenile period (Jonkers 1971).

Since ancient times fruit growers have practiced a range of agrotechnical manipulations to force the induction of flowers in fruit trees. All factors that reduce apical dominance in the shoot can promote flower formation. Vegetative growth of fruit trees can be inhibited by a range of practical approaches which increase flower formation, i.e. choosing the appropriate rootstock, trunk ringing, scoring, bark inversion, root pruning, and fertilization (Way 1971). Defoliation is also used to stimulate flowering in apple (Taylor *et al*. 1984). Seedling scions grafted onto dwarfing rootstocks flowered two to four years earlier than the seedlings from which they were taken (Hackett 1985). Besides, gravimorphism plays an important role in fruit trees. Vegetative growth is affected negatively by gravity (Wareing and Nasr 1961; Smith and Wareing 1964). Shoot growth and flower-bud formation is obviously affected by shoot orientation, i.e. placing shoots in a horizontal position increases flower-bud formation and reduces growth (Longman *et al*. 1965; Tromp 1967, 1968). Tromp (1973) found that shoot growth was reduced and flowering was increased

Table 1 Ectopical expression of flowering genes in fruit tree species.

Gene/construct	Expression in	Vector	Number of lines	Early flowering
35S::LFY	*Citrus sinensis* × *Poncirus trifoliata*	pROK II	22[a]	yes*
	Malus domestica cv. 'Pinova'	pDW151	7[b]	no
	Malus domestica cv. 'Gala'	pDW151	10[c]	no
35S::AP1	*Citrus sinensis* × *Poncirus trifoliata*	pROK II	12[a]	yes
	Malus domestica cv. 'M26'	pROK II	4[d]	no
35S::BpMADS4	*M. domestica* cv. 'Pinova'	pAKE 1	25[e]	yes*

[a]Peña *et al*. 2001; [b]Flachowsky *et al*. (unpublished); [c]Schaart *et al*. (unpublished), [d]Zhu *et al*. (unpublished), [e]Flachowsky *et al*. 2007, **in vitro* flowering.

by shoot bending and by application of a chemical growth inhibitor. In all cases a promotion of shoot growth increases apical dominance and vice versa an inhibition of shoot growth supports lateral flower-bud formation. Early manual or chemical flower/fruit thinning will also increase flower bud differentiation, avoiding the negative effect of gibberellins produced by seeds of the developing fruits on flower formation. Plant growth regulators can greatly effect both shoot growth and flower initiation (Zimmerman 1972; Luckwill 1973). There is a plenty of literature reporting on different applications of various growth regulators aimed on their utilization in fruit production for crop improvement. In this review some basically results will be cited. Gibberellic acid applied at the critical time in the year preceding flowering will reduce or inhibit flower initiation, whereas growth retardants such as CCC and aminozide will promote it (Luckwill 1973). Spraying gibberellic acid on the trees during flower induction significantly suppresses flowering and has little effect on flower organ differentiation (Luckwill and Silva 1979). Application of the growth regulator ethephon also promoted flower differentiation in apple when applied in June. This promotion of flower formation was not accompanied by growth retardation or fruit thinning (Katzfuss *et al*. 1975; Schmidt *et al*. 1975; Katzfuss and Schmidt 1977, 1986).

WHAT WE DO KNOW ABOUT THE GENES INVOLVED IN FLORAL TRANSITION AND FLOWER DEVELOPMENT OF APPLE?

Ectopical expression of flowering genes in fruit trees

In the early nineties of the last century several key genes were identified which regulates floral induction and flower development in *Arabidopsis*. In this context two genes, *LFY* and *AP1*, were identified as necessary for the determination of the flower meristem identity (Yanofsky 1995). Using genetically modified plants overexpressing the *LFY* and *AP1* gene, respectively, it was shown that these genes are sufficient to promote flower initiation and development in *Arabidopsis* (Weigel and Nilsson 1995; Mandel and Yanofsky 1995). Based on these findings both genes were transferred into different plant species. Thereby, it was found that a constitutive overexpression of the *Arabidopsis LFY* gene resulted in precocious flowering in rice, tobacco and hybrid aspen (Weigel and Nilsson 1995; Nilsson and Weigel 1997; He *et al*. 2000). Further, the *LFY* gene was successful used to accelerate the juvenile phase in citrus (Peña *et al*. 2001). After constitutive overexpression of the *Arabidopsis AP1* gene early flowering was obtained in transgenic citrus (Peña *et al*. 2001) and tomato (Ellul *et al*. 2004) whereas in transgenic hybrid aspen no effect was ascertained (Nilsson and Weigel 1997). With the work published by Peña and Co-workers (Peña *et al*. 2001) the proof of principle was adduced that genes coming from an herbaceous plant as *A. thaliana* could be used to manipulate specific traits in fruit tree species. Starting from this fact several studies using the *AP1* and *LFY* genes of *Arabidopsis* were performed on apple but up to now no early flowering was described (**Table 1**). More successful was the work using the *BpMADS4* of silver birch. This gene is similar to *FUL* of *Arabidopsis* and after constitutive overexpression of *BpMADS4* precocious flowering was found in transgenic tobacco (Elo *et al*. 2001) as well as in transgenic apple (Flachowsky *et al*. 2007). In *BpMADS4* transgenic apple plants the juvenile stage was dramatically reduced. Several lines set up their first flowers during *in vitro* cultivation.

Isolation and characterization of flowering genes of fruit trees

In parallel to the ectopical expression of genes coming from phylogenetically distant plant species much effort has been made to isolate native orthologs/homologs of known *Arabidopsis* genes involved in the transition from the juvenile to adult stage as well as in the development of flowers and floral organs. In this context two *CONSTANS*-like cDNA clones (*MdCOL1* and *MdCOL2*) were isolated from a fruit specific cDNA library of 'Fuji' apple as described by Jeong *et al*. (1999). Both genes were identified as members of *CO*-like proteins. Further, it was found that in addition to *MdCOL1* and *MdCOL2* there are several genes in the apple genome that share homology to the *CO* gene. This is similar to other plant species. Coupland *et al*. (1997) identified 12 EST's from *Arabidopsis* and five from rice with significant homology to *CO*. It is assumed that *CO*-like genes are members of a large gene family in the plant kingdom (Jeong *et al*. 1999). Subsequently, based on an expression study Jeong and colleagues assumed that the *MdCOL* genes may also play a role in regulating floral organ development but their roles could possibly be different in apple and *Arabidopsis*. To clarify the real function of *MdCOL1* and *MdCOL2* in apple additional experiments are necessary.

Putative native homologs to genes that enable floral transition

Different genes like *FLC*, *TFL1* and *SVP* are supposed to be repressors of the floral pathway integrators (Boss *et al*. 2004). These repressor genes are of particular interest in fruit tree breeding because they are responsible for the maintenance of juvenility. The down-regulation of floral repressor genes results mostly in the activation of floral integrators and subsequently in the development of inflorescences and flowers.

One of the best characterized floral repressor genes in fruit trees is *TERMINAL FLOWER 1*. The presence of a putative *TFL1* homolog in the domesticated apple *Malus domestica* was firstly described by Kotoda *et al*. (2003). They isolated the coding region of *MdTFL1* from cDNA of the apple cv. 'Jonathan'. Based on the results of a Southern hybridization, the authors assumed the presence of multiple copies of *MdTFL1* in the apple genome. This assumption was confirmed by Esumi *et al*. (2005) who found two types of cDNA for *TFL1* homologs in six investigated Maloid species. Based on the deduced amino acid sequence Esumi *et al*. (2005) classified the *TFL1* homologues genes into two distinct clades. The TFL1-like genes were attributed to their corresponding group and designated with *MdTFL1-1* and *MdTFL1-2* for apple, *PpTFL1-1* and *PpTFL1-2* for Japanese pear, *PcTFL1-1* and *PcTFL1-2* for European pear, *CoTFL1-1* and *CoTFL1-2* for quince, *CsTFL1-1* and *CsTFL1-2* for Chinese quince and *EjTFL1-1* and *EjTFL1-2* for loquat. Further, it was found that genes of the *TFL1-1* and *TFL1-2* clades are quite similar and that they are placed very close in a phylogenetic tree. Based on the obtained

results Esumi *et al*. (2005) assumed that the two Maloid types of *TFL1* homologues can be attributed to the polyploid nature of Maloid species, and that they have the same function. The expression of *TFL1* homologs of Japanese pear and quince was studied by Esumi *et al*. (2006). A high level of expression was found in the apical meristem but it significantly recede just before floral differentiation. This could be an indication that a decrease in expression of these *TFL1*-like genes induces floral initiation in Maloid species. In parallel Kotoda and Wada (2005) studied the function of *MdTFL1* in *CaMV35S::MdTFL1* transgenic *Arabidopsis* plants. They found that the constitutive overexpression of *MdTFL1* retards the transition from the vegetative to the reproductive phase as known for *TFL1*. Further, it was shown that the suppression of *MdTFL1* in transgenic apple reduces the juvenile stage and induces early flowering (Kotoda *et al*. 2006). First solitary flowers were detected on transgenic glasshouse plants of the apple cv. 'Orin' eight months after grafting. Similar results were found in citrus. Pillitteri *et al*. (2004) isolated a *TFL1* homologues gene (*CsTFL*) from the Washington navel orange (*Citrus sinensis* L. Osbeck). *C. sinensis* is a hybrid perennial tree crop which has a relatively heterozygous genome like apple. In contrast to apple, hybridization pattern of restricted genomic *C. sinensis* DNA were consistent with *CsTFL1* being a single copy gene. Ectopic expression of *CsTFL* in *Arabidopsis* resulted in a significant delay in flowering. Furthermore, it was found that *CsTFL* expression is correlated with juvenility in *Citrus* (Pillitteri *et al*. 2004). It is assumed that down-regulation of *CsTFL* using a transgenic approach could be a powerful tool to reduce the juvenile stage and to induce early flowering. In grapevine, a *TFL1* homolog (*VvTFL1*) was isolated by Boss *et al*. (2006). The expression of this gene was studied in different time and tissue (Joly *et al*. 2004; Boss *et al*. 2006). Ectopic expression of *VvTFL1* in transgenic *Arabidopsis* and tobacco generally delays flowering (Boss *et al*. 2006). The obtained results indicated that *VvTFL1* is a repressor of flowering like *TFL1*. Based on the findings obtained from apple, orange and grapevine it could be ascertained that *TFL1*-like genes are present in perennial fruit tree crops, and that they have a comparable function to *TFL1* in *Arabidopsis*.

Another very interesting gene is *MdJOINTLESS*. The predicted amino acid sequence of *MdJOINTLESS* is very similar to that of *LeJOINTLESS* of tomato and *SVP* of *Arabidopsis*. The primary role of *LeJOINTLESS* in tomato is to maintain the inflorescence state by suppressing the sympodial program of development in inflorescence meristems (Szymkowiak and Irish 2006). The *SVP* gene acts in contrast to *LeJOINTLESS* as a floral repressor. This gene acts in a dose dependent manner to delay flowering. Furthermore, it does not alter the effects of photoperiod or vernalization on flowering time (Boss *et al*. 2004). Whether the function of *MdJOINTLESS* is more like *LeJOINTLESS* or more like *SVP* is unknown up to now. A detailed evaluation of its expression will help to understand its function in apple. Furthermore, it is interesting to note that several apple EST's (EB114714, EB137980, CO723380, CO9017 97, CO899324, DT042645, CV658081, CN997148, DR99 1348) found in the "Genome Database of Rosaceae" (http://www.bioinfo.wsu.edu/gdr/) show also similarity to *SVP* and *JOINTLESS*.

The most well characterized enabling pathways in *Arabidopsis* are those which regulate the floral repressor *FLC*. A putative apple homolog of *FLC* could not yet been isolated up to now, but several apple EST's with similarity to genes like *VRN1* (GI: 71921493, GI: 71919480) and *FCA* (GI: 46601189, GI: 71822441) were found, which are known to affect *FLC* in *Arabidopsis*.

Isolation and characterization of native floral pathway integrators

Considerably more is known about genes which are putative homologs of the floral pathway integrators *LFY*, *FT* and *SOC1*. In 2000, Kotoda and colleagues reported about the isolation of a *FLORICAULA/LEAFY* like gene from 'Jonathan' apple (Kotoda *et al*. 2000). Using primers corresponding to the conserved domains in the coding region of *FLO* in *Antirrhinum* and *LFY* in *Arabidopsis* they were able to amplify a 447 bp fragment. The sequence of the *AFL* (Apple *FLORICAULA/LEAFY*) gene was quite similar to the sequences of *FLO* and *LFY* and at the amino acid level identities of 91% (*FLO*) and 89% (*LFY*) were found. Using the same primers Wada *et al*. (2002) isolated a 440 bp (*AFL400*) fragment from 'Jonathan' apple, too. Based on this fragment specific primers were designed and used for a 5′/3′ RACE. As a result of the 5′/3′ RACE two kinds of 1.4 kbp cDNA fragments were obtained and named *AFL1* and *AFL2*. Both genes had high homology (90%) in their coding regions, but they were expressed in distinct tissues and times in floral and vegetative development. Genomic analyses and the evaluation of expression patterns indicated clearly the presence of two *FLORICAULA/LEAFY* homologues in apple (Wada *et al*. 2002). Similar results were reported by Esumi *et al*. (2005) who found two distinct clades of *LFY* homologues (*LFY-1* and *LFY-2*, respectively) in six different Maloid species. Esumi *et al*. (2005) observed an amino acid sequence identity of about 95% and 97% among homologues within the same group and about 90% identity between homologues in different groups. The function of *AFL1* and *AFL2* was evaluated by ectopically expression in transgenic *Arabidopsis* plants. The constitutive overexpression of these genes in *Arabidopsis* caused early flowering and phenotypes comparable with *CaMV35S::LFY* plants (Wada *et al*. 2002). Based on the obtained results Wada *et al*. (2002) assumed that *AFL1* also had the transition ability like *AFL2*, but the effects were weaker by overexpressing *AFL1* than *AFL2*. In contrast, no precocious flowering was obtained by overexpressing the *AFL* genes in apple (Kotoda *et al*. 2003). Based on the results obtained after overexpression of the *Arabidopsis LFY* gene (**Table 1**) as well as the *AFL* genes in apple it could be assumed that *LFY* and *LFY*-like genes are not the trigger for the switch from juvenility to the adult stage in apple. Putative *LFY* homologs were also isolated from other fruit tree species. In *Citrus sinensis* L. Osbeck 'Washington' Pillitteri and colleagues (2004) found a *LFY*-like gene which was designated as *CsLFY*. This gene had 68% amino acid identity with the *Arabidopsis LFY* gene and its genomic organization with three exons and two introns is similar to that observed for other known *LFY* genes. The overexpression of *CsLFY* in transgenic *Arabidopsis* plants resulted in precocious flowering. The observed phenotypes were similar to those that were known for *Arabidopsis* plants constitutively overexpressing the *Arabidopsis LFY* gene (Pillitteri *et al*. 2004). Other *LFY*-like genes (*VFL*, *ALF*) were isolated from grapevine and kiwifruit (Carmona *et al*. 2002; Walton *et al*. 2001). The expression of these genes was studied in different time and tissue. Thereby, the highest levels of *ALF* and *VFL* expression were found at the time of flower meristem formation (Carmona *et al*. 2002). Consequently, it was concluded that these genes play an important role in this process.

Less is known about *FT*- and *SOC1*-like genes in apple. Up to now only the presence of one *FT*-like sequence (*MdFT*) was reported by Kotoda and Wada (2005). Nothing is known about the expression of *MdFT*. In citrus, a *FT* homolog designated as *CiFT* was found by screening of an EST catalogue of a cDNA library of *Citrus unshiu* Marc. (Hisada *et al*. 1997). The ectopical overexpression of *CiFT* in *Arabidopsis* resulted in early flowering (Kobayashi *et al*. 1999). Similar results were obtained by overexpression of *CiFT* in trifoliate orange (*Poncirus trifoliate* L. Raf.) (Endo *et al*. 2005). Recently it was reported by Matsuda *et al*. (2006) that the overexpression of *CiFT* in pear (*Pyrus communis* L.) led to *in vitro* flowering on transgenic shoots. In grapevine, the *VvFT* gene which is a homologues gene to *FT* was isolated and its expression was characterized (Sree-

Table 2 Classification of MADS-box genes originating from different fruit tree species.

Gene	GI number	Class	References
Malus domestica			
MdMADS1	3290209	E	Sung and An 1997
MdMADS2	3947985	A	Sung *et al*. 1999
MdMADS3	5777904	E	Sung *et al*. 2000
MdMADS4	5777906	E	Sung *et al*. 2000
MdMADS5	3646320	A	Yao *et al*. 1999; Kotoda *et al*. 2000, 2002
MdMADS6	3646322	E	Yao *et al*. 1999
MdMADS7	3646324	E	Yao *et al*. 1999
MdMADS8	3646334	E	Yao *et al*. 1999
MdMADS9	3646336	E	Yao *et al*. 1999
MdMADS10	3646326	C/D	Yao *et al*. 1999
MdMADS11	3646340	G	Yao *et al*. 1999
MdMADS12	32452882	A	van der Linden *et al*. 2002
MdMADS13	16973294	B-AP3	van der Linden *et al*. 2002
MdMADS14	16973296	C/D	van der Linden *et al*. 2002
MdMADS15	16973298	C/D	van der Linden *et al*. 2002
MdJOINTLESS	122056647	T	Heo *et al*.*
MdPI	12666533	B-PI	Yao *et al*. 2001
MdSOC1	114386386	F	Mahna *et al*.*
MdAGL	33308109	C/D	Sung*
Prunus persica			
PrpMADS2	70955228	E	Xu *et al*.*
PrpMADS4	52219460	C/D	Wu *et al*.*
PrpMADS6	52219462	A	Wu *et al*.**
PrpMADS	73990913	A	Rasori *et al*.*
PrpSHP	110559304	C/D	Tani *et al*.*
Prunus dulcis			
PrdMADS1	63094569	C/D	Silva *et al*. 2005
PrdMADS2	63094571	A	Silva *et al*.*
PrdMADS3	63094573	E	Silva *et al*. 2005
Pyrus* x *bretschneideri			
PbMADS2	121309556	E	Inaba *et al*.*
Musa acuminata			
MuaMADS1	66735452	C/D	Inaba *et al*. 2007
MuaMADS3	115520907	E	Inaba *et al*. 2007
Citrus unshiu			
CitMADS1	116078095	C/D	Endo *et al*. 2006
CitMADS3	116078097	E	Endo *et al*. 2006
CitMADS5	116078099	A	Endo *et al*. 2006
CitMADS6	116078101	C/D	Endo *et al*. 2006
CitMADS8	116078103	B-AP3	Endo *et al*. 2006
Citrus sinensis			
CiAP1	37703724	A	Pillitteri *et al*. 2004
Vitis vinifera			
VvMADS1	14279306	C/D	Boss *et al*. 2001
VvMADS2	20385584	E	Boss *et al*. 2001
VvMADS3	20385586	G	Boss *et al*. 2001
VvMADS4	30171289	E	Boss *et al*. 2001
VvMADS5	20385590	C/D	Boss *et al*. 2001
VvMADS6	30526323	A	Sreekantan *et al*.*
VvMADS9	67764083	B-PI	Sreekantan *et al*. 2006
VvAP1	46949180	A	Calonje *et al*. 2004
VFUL	46949182	A	Calonje *et al*. 2004
VvSOC1	95116634	F	Sreekantan and Thomas 2006
VvTM6	115492982	B-AP3	Frederici *et al*.*

* unpublished data, ** article in Chinese, only the abstract is written in English, GI – gene identification number of the NCBI database

kantan and Thomas 2006). Furthermore, this gene was overexpressed in transgenic *Arabidopsis*. Because of the early flowering genotype of the transgenic *Arabidopsis* plants it was assumed that the *VvFT* gene acts as a promoter of flowering when ectopically expressed in a heterologous plant. Based on the results obtained for *CiFT* and *VvFT* it could be assumed that the overexpression of *FT*-like genes in apple could also lead to precocious flowering.

Recently the sequence of a *SOC1*-like gene found in apple was cited in the NCBI database (**Table 2**). But up to now nothing is known about the function and the expression of *MdSOC1*. In grapevine, a *SOC1*-like gene (*VvMADS8*) was isolated by Sreekantan and Thomas (2006). The *VvMADS8* gene was ectopically overexpressed in transgenic *Arabidopsis* and the transgenic plants flowered precociously. Interestingly the flowering was more accelerated in transgenic *Arabidopsis* plants overexpressing the *VvFT* gene.

Summarizing results, it could be stated that putative homologs for the three known floral pathway integrators of *Arabidopsis* can be found in different perennial fruit tree species. However, their function can be different in other species compared to *Arabidopsis*.

Isolation and characterization of native floral meristem identity genes

Beside the work on floral pathway integrators much effort has been made on isolation of genes which are homolog to known meristem identity genes. It was possible to isolate putative homologs of *AP1*, *FUL* and *LFY* (see above). Whereas *AP1* is merely known as a meristem identity gene, recent expression data have indicated that *FUL* may also act as a floral integrator (Schmid *et al*. 2003). The same is true for *LFY* which could be considered as a flowering time gene and a meristem identity gene (reviewed by Parcy 2005).

The first report about the isolation of an *AP1*-like gene from apple was published in 1999. Yao and colleagues isolated seven MADS-box genes from RNA of the apple cv. 'Granny Smith' two days after pollination (Yao *et al*. 1999). Based on the deduced amino acid sequence of the MADS-box genes a phylogenetic analysis was performed. The apple MADS-box genes were compared to known MADS-box genes of *Arabidopsis* and classified into two of three MADS-box gene groups (*AP1*, *AG* and *PI/AP3*), which were known at that time. Six of the apple MADS-box genes (*MdMADS5*, *MdMADS6*, *MdMADS7*, *MdMADS8*, *MdMADS9*, *MdMADS11*) were grouped into the *AP1* (A function) group. Only the *MdMADS10* gene was classified into the *AG* (C/D function) group. Furthermore, it was found that the *MdMADS5* gene showed the highest similarity to *AP1*. This gene is expressed specifically in sepals (Kotoda *et al*. 2000) and different parts of the fruit (Yao *et al*. 1999). The expression pattern of this gene was similar to *AP1* and *SQUA* (Kotoda *et al*. 2000). Since that time more and more MADS-box genes of different plant species were isolated and classified into several phylogenetic groups. A phylogenetic tree which was build by comparing the predicted peptide sequences of known MADS-box genes of fruit trees (listed in **Table 2**) and *Arabidopsis* shows clearly that only *MdMADS5/MdAP1* could be classified into the *AP1/CAL* group (**Fig. 7**). In contrast *MdMADS6*, *MdMADS7*, *MdMADS8* and *MdMADS9* are more related to genes of the *SEPALLATA* group (E function) whereas *MdMADS11* is similar to *AGL6*. The function of *MdMADS5/MdAP1* was studied on transgenic *Arabidopsis* plants (Kotoda *et al*. 2002). The constitutive overexpression of *MdMADS5/MdAP1* resulted in plants which flowered earlier, had a shorter inflorescence, and a reduced number of rosette leaves. Based on their results Kotoda *et al*. (2002) concluded that the *MdMADS5/MdAP1* gene might have a similar function to that of *AP1*. Contrary results were found on transgenic apple plants overexpressing the *MdMADS5/MdAP1* gene. Whereas no precocious flowering was found on transgenic plants of the apple cv. 'Orin' (Kotoda *et al*. 2003), Kim *et al*. (2006) reported recently that *MdMADS5/MdAP1* transgenic 'Fuji' plants set up their first flowers during *in vitro* cultivation.

Pillitteri *et al*. (2004) isolated an *AP1*-like gene (*CsAP1*) from the orange cv. *Citrus sinensis* L. Osbeck 'Washington'. A cDNA fragment of 462 bp amplified using primers designed on the basis of an alignment of four *AP1* homologues was used for a genome walking procedure. The obtained genomic sequence of *CsAP1* spanned 5.5 kb and contained eight exons and seven introns. The coding sequence of *CsAP1* was constitutively overexpressed in *Arabidopsis* driven by the *CaMV35S* promoter. Fifteen out

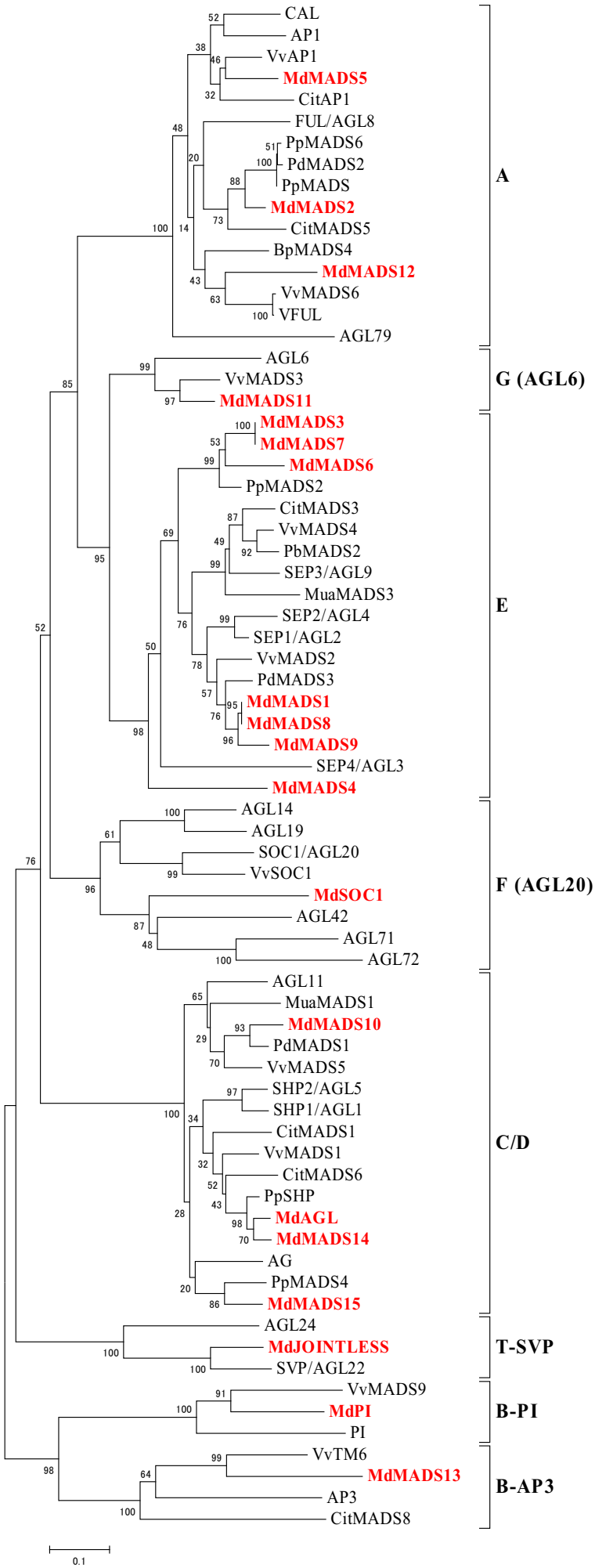

Fig. 7 Phylogenetic tree of 60 MADS-box genes from *Arabidopsis* and different fruit tree species. The phylogenetic analysis was performed on protein sequences listed in **Table 2**. The tree was constructed by the neighbour-joining method with Poisson-correction (PC) distance. The number for each interior branch is the percent bootstrap value (1000 resamplings), and only values >50% are shown. The notation of the classes was done as described by Nam *et al*. (2004). The flowering genes of apple are red coloured.

of 36 T_1 plants showed an extreme early flowering phenotype (Pillitteri *et al*. 2004). Therefore it was concluded that the function of *CsAP1* was similar to that of *AP1*. Further *AP1*-like genes (*VAP1*, *AAP1*) were isolated from grapevine and kiwifruit (Walton *et al*. 2001; Calonje *et al*. 2004). Based on their deduced amino acid sequence as well as the expression pattern in different time and tissue they were classified as putative orthologs of previously described *AP1*-like genes in other plant species.

In 1999, Sung *et al*. described the isolation of *MdMADS2* from 'Fuji' apple. This gene was classified as a member of the *SQUA* subfamily because the *MdMADS2* protein showed more than 60% amino acid identity to different genes of this subfamily including the *FUL/AGL8* gene of *Arabidopsis*. The expression pattern of *MdMADS2* was studied by RNA *in situ* localization. Because of the assumption that the transcription pattern of *MdMADS2* does not reflect its protein level if the gene is posttranscriptional regulated, an additional examination by *in situ* immunolocalization was performed. Thereby the *MdMADS2* mRNA as well as the protein could be localized in the inflorescence meristem, the bud procambium, and the adjacent leaf appendages in the flower bud (Sung *et al*. 1999). In leaf buds with a vegetative apex, the protein was not detectable. In contrast to *SQUA*, *AP1* and *FUL* the *MdMADS2* gene is expressed in apple flower buds in both, the inflorescence meristem and the floral meristems. Ectopic expression of *MdMADS2* in transgenic tobacco resulted in early flowering. A phylogenetic analysis depicted in **Fig. 7** led to the conclusion that *MdMADS2* is closely related to *FUL* of *Arabidopsis* and *CitMADS5* of *Citrus unshiu*. The *MdMADS12* gene described by van der Linden *et al*. (2002) is encoding also a *FUL*-like protein. This gene is more similar to *VFUL* a *FUL*-like gene of grapevine and the *BpMADS4* gene of silver birch (**Fig. 7**). Surprisingly the expression data obtained for *MdMADS12* suggest that this gene has no regulatory function in the floral transition (van der Linden *et al*. 2002). The real functions of *MdMADS2* and *MdMADS12* in apple are not known at the moment. It is assumed that *MdMADS2* is involved in the early development of the floral meristem and inflorescence (van der Linden *et al*. 2002).

Isolation and characterization of native floral organ identity genes

Beside genes described above, different MADS-box genes of apple that maybe involved in the development of floral organs were isolated (Sung and An 1997; Yao *et al*. 1999; Sung *et al*. 2000; Yao *et al*. 2001; van der Linden *et al*. 2002). Based on the deduced amino acid sequence of these genes, they could be assigned to the gene classes B, C/D, E, F and G (**Table 2; Fig. 7**).

CONCLUSIONS FOR FUTURE RESEARCH

Summarizing the results cited above, a reduction of the juvenile phase in fruit trees using transgenic approaches is feasible by constitutive or induced overexpression of native floral pathway integrator genes as well as by down-regulation of floral suppressors, like *TFL1*. Especially the evaluation of systems leading to a systemic acquired silencing of suppressor genes would be of particular interest. From several studies in transgenic tobacco it became obviously that there is a systemic transport of silencing inducing signals from a transgenic silencing transmitter genotype to a non-transgenic scion, grafted onto the transmitter genotype (Palauqui *et al*. 1997; Sonoda and Nishiguchi 2000). If a systemic transport of silencing signals does exist in perennial woody plants is unknown up to now. Further research is necessary to clarify, whether grafting of non-transgenic commercially used apple scion cultivars onto transgenic rootstocks which express signals for silencing of economically important traits, like early flowering could be a successful tool for fruit tree breeding. Furthermore, it is of interest whether the overexpression of *CO*-like genes in apple rootstocks induces a graft-transmissible, phloem-mobile signal (most likely *FT*) that accelerate flowering as described by Ayre and Turgeon (2004) in *Arabidopsis*.

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