

Flowering Gene and Genomic Region in Fruit Crops: A Tool for Future Breeding

Nimisha Sharma^{1*}, Sanjay Kumar Singh¹, Jai Prakash¹, Ajay Kumar Mahato², Manish Srivastav¹, Awtar Singh¹, Nagendra Kumar Singh²

¹Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

²National Research Centre on Plant Biotechnology, ICAR-Indian Agricultural Research Institute, New Delhi, India

*Corresponding author: Nimisha Sharma, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India. Tel: +911125843214; Fax: +9125843214; E-Mail: nims17sharma@gmail.com

Citation: Sharma N, Singh SK, Prakash J, Mahato AK, Srivastav M, et al. (2017) Flowering Gene and Genomic Region in Fruit Crops: A Tool for Future Breeding. Int J Genom Data Min 01: 108. DOI: 10.29011/2577-0616.000108

Received Date: 12 May, 2017; **Accepted Date:** 19 May, 2017; **Published Date:** 29 May, 2017

Abstract

Flowering in fruit trees, is of immense importance in the reproductive success and enhancing crop productivity. The ability to control the timing of flowering is a key strategy for planning production in perennial fruit crops. A thorough understanding of floral transition with complex genetic network, regulated by multiple environmental and endogenous signals is the primary requirement. With the availability of the draft genome sequences of some fruit crops, it is now possible for undertaking molecular genetic studies on this aspect. This paper reviews the current understanding of the molecular mechanisms of flowering in fruit crops and their possible manipulation for economic gains.

Keywords: Fruit Crops; Flowering Gene; Gene Regulation; Model Plant

Abbreviations:

AG : Agamous
AFL : Apple Floricaula/Leafy
AGL : AgamousLike
AP : Apetala
CO : Constans
FBP : Floral Binding Protein
FHA : Forkhead
FLC : Flowering Locus C
FLD : Flowering Locus D
FLK : Flowering Locus K
FT : Flowering Locus T
FRI : Frigida
GI : Gigantea
LFY : Leafy

LD : Luminidependens
PI : Pistillata
SOC1 : Supressor of Constans 1
SQUA : Squamosa
SVP : Short Vegetative Phase
TFL : Terminal Flower
SMZ : Schlafmutze
SNZ : Schnarchzapfen
TFL : Terminal Flower Locus
VID : Vernalization Independence

Introduction

Flowering in fruit trees is of immense importance in the reproductive success and enhancing crop productivity. The reproductive success and yield depends on the number and quality of flower buds formed on a tree. Development of flower bud is a complex phenomenon comprising of morphological and physiological processes under the control of numerous factors including external and internal signals [1]. The factors controlling the floral transition determined by certain complex growth correlations [2]. Therefore,

many external and internal factors controlling flowering behavior have been worked out. Major pathways related to flowering in fruit trees include environmental induction through photoperiod, vernalization, autonomous floral initiation, interaction of gibberellins, auxins and abscisic acid, and aging by sequentially operating miRNAs (typically miR156 and miR172) responding to endogenous signals. The balance of signals from these pathways is integrated by a common set of flowering genes (*FLC*, *FT*, *LFY*, and *COI*) that determine the flowering time [3,4]. Recent studies have indicated that epigenetic modifications, alternative splicing, antisense RNA and chromatin silencing regulatory mechanisms play an important role in this process by regulating related flower induction gene expressions [2-5]. Dynamic changes between chromatin states facilitating or inhibiting DNA transcription, regulate the expression of floral induction pathways in response to environmental and developmental signals [4]. The ability to control the timing of flowering is a key strategy for regulation of flowering and vis-avis fruiting in perennial fruit crops. A thorough understanding of floral transition achieved through by understanding the complex genetic network and regulation by multiple environmental and endogenous signals is the primary requirement. This paper reviews the current understanding of the regulatory factors related to flowering in fruit crops and the possible impact on manipulation of juvenility and flowering time.

Genetic Control of Flowering

Like any other ontogenic event, flowering in both seedling and vegetatively propagated plants occur after a vegetative pre-requisite is over. However, in perennial fruit crops where the juvenility period is in general longer, effective manipulation of this event is desired in modern production system since it influences production and productivity. Recent studies have highlighted that regulatory mechanisms play an important role in flowering of perennial fruit crops [2,3,5]. Genome sequencing of some fruit crops would precisely assist future molecular genetic studies, like linking genes to biological processes and traits along with their functions [6,7]. Understanding the key interactions between environmental factors and genetic mechanisms controlling the induction and development of inflorescences, flowers, and fruits, juvenility/ precocity are also important areas that require increased emphasis, especially given on the large seasonal fluctuations in flowering and yield experienced *per se* by the crop. This becomes more pertinent under increasing concern for the effects of climate change, erratic weather patterns on existing fruit producing regions. Under these changed situations, it can be expected that extensive genomic and transcriptome analyses would allow identification of the complete gene set for each class of regulatory genes, the sub-sets of genes involved in every regulatory process, related signal transduction systems, and the corresponding downstream metabolic networks, focusing the selection of candidate gene(s) for the final analyses of

biological functions [3-6].

However, there are still several experimental bottlenecks, and novel approaches which are needed to be developed for deciphering gene function assignment in fruit crops. The insight achieved on these aspects on *Arabidopsis* and other model plant species represents important resource to study flowering in fruit crops to uncover similarities and differences [5-7]. Flowering involves the sequential action of two groups of genes: those that switch the fate of the meristem from vegetative to floral (floral meristem identity genes), and those that direct the formation of the various flower parts (organ identity genes) [6,7]. Research attempts on trees are quite expensive, slow owing to long juvenility and thus have often been major bottleneck in successful production of several perennial horticultural species. Recently, the development of genomic and transcriptomic tools has contributed to the better understanding of the metabolic and molecular processes involved in floral biology and the related mechanisms.

Sequence Homology of Flowering Genes

Most of the present understanding of flower induction process have come from studying flowering regulatory genes in *Arabidopsis thaliana* [6]. In general, perennial flowering gene orthologues have been shown to function akin to their *Arabidopsis* namesakes. Mouhu, et al. [7] searched homologs for 118 *Arabidopsis* flowering time genes from *Fragaria* ssp. by EST sequencing and bioinformatics analysis and identified 66 gene homologs that by sequence similarity, putatively correspond to genes of all known genetic flowering pathways. Some of the first homeotic genes designated (*MdMADS1-MdMADS4*) of floral development in apple (*Malus domestica* Borkh.) has been isolated from the cultivar 'Fuji'. These genes are expressed in the inflorescence and floral meristem. The expression of both *MdMAD1* and *MdMADS2* genes was higher during the early stages of flower development, suggesting their role in the initiation of flower organs. The gene *MdTFL1* is expressed in apple vegetative tissue, such as apical buds, seedling stems and roots but not in reproductive tissue such as floral organs. Recently, two different types of cDNA for *LFY* homologues were isolated from six maloid species, namely, *AFL1-Fuji* and *AFL2-Fuji* for apple, *PpLFY-1* and *PpLFY-2* for Japanese pear, *PcLFY-1* and *PcLFY-2* for European pear, *CoLFY-1* and *CoLFY-2* for quince, *CsLFY-1* and *CsLFY-2* for Chinese quince, and *EjLFY-1* and *EjLFY-2* for loquat [8]. The presence of two different *LFY* homologues in maloid plants may reflect the polyploidy origin of Maloideae. Regardless of the types and species, the two *LFY* homologues were expressed in buds, where flower primordia are formed, suggesting that both homologues could play an important role in floral bud formation in the sub-family, i.e., Maloideae of the Rosaceae. *TFL* homologues were transcribed mainly in buds before floral differentiation. Details of flowering gene for homology search have been shown (Table 1) & (Table 2).

Flowering Gene(s)	Plant	Reference
<i>FT</i> and <i>CiFT</i>	Trifoliolate orange (<i>Poncirus trifoliata</i> L. Raf.), 'Moncada' mandarin, sweet orange [<i>Citrus sinensis</i> (L.)], Satsuma mandarin (<i>Citrus unshiu</i> Marc.).	[35,56,57, 13]
<i>CsPH5</i> , <i>CsPH5 6</i>	<i>Citrus</i> sp.	[68]
<i>TFL</i> , <i>LFY</i> & <i>AP</i>	<i>Citrus sinensis</i> L.	[63,14,16]
<i>AP3</i> , <i>SOC1</i> , <i>WUS</i> , <i>SPL</i> , <i>miR156</i> , <i>CsAP1</i> , <i>CsLFY</i> , <i>SOC</i> , 3 <i>CiFT</i> , <i>PtFT1</i> , <i>CiFT</i> , <i>Hd3a</i> , <i>SFT</i>	<i>Citrus</i> sp.	[69,47,70]
DNA methylation of <i>CiLFY</i> , <i>AP</i> , <i>FT</i>	<i>Citrus</i> sp.	[71]
<i>FCA</i> -like chromosome 9, NC_023054.1	Sweet orange (<i>Citrus sinensis</i>)	[105]
<i>FT/TFL1 VuMADS1</i> , <i>VuMADS5</i> , <i>VvMADS10</i> and <i>VAP1</i>	Grapevine (<i>Vitis vinifera</i>)	[72, 73, 15, 20]
<i>FCA</i> , <i>FA</i> , <i>FT</i> , <i>AP3</i> , <i>FLC</i> , <i>FY</i> , protein EARLY FLOWERING, <i>LAR2</i>	Wine grape (<i>Vitis vinifera</i>)	[105]
<i>FT</i> , <i>MiCOL</i> , <i>MiFT</i> , <i>MiGA 20-ox</i> , <i>MiGA3-ox</i> and <i>MADS</i> -box cDNA	Mango (<i>Mangifera indica</i> L.)	[74,59,75]
<i>AGAMOUS MADS</i> -box factor	Banana (<i>Musa</i> sp.)	[76,77,78]
<i>MuaMADS1</i> ; <i>MuaMADS3</i>	Wild banana (<i>Musa acuminata</i>)	
<i>FLC</i> , <i>FLT</i> , <i>LFY</i> , <i>CO1</i> , <i>FI</i> & floral organ formation gene	Perennial plants	[5,1]
<i>MdFT1</i> & <i>MdFT2</i>	Apple (<i>Malus domestica</i> Borkh.).	[58]
35S:: <i>LFY</i>	Apple (<i>Malus domestica</i> cv 'Pinova')	[39]
<i>MdTFL1-1</i> <i>MdTFL1-2</i> , <i>MdCOL1</i> , <i>MdCOL2</i> , <i>MdGA20ox1a</i> , <i>MdGA3oxb</i> , <i>MdGA20ox8a</i> , <i>MdAFB6</i> , <i>Md-MADS</i> , <i>ARF</i> , <i>AFL2</i> , <i>MdFT</i> , <i>MdAP1</i> and <i>MdTFL1</i>	Apple (<i>Malus domestica</i> Borkh.).	[74,8,79,80]
35S:: <i>BpMADS4</i>	Apple (<i>Malus domestica</i> cv 'Pinova')	[64]
35S:: <i>MdFT</i>	Apple (<i>Malus domestica</i> cv 'Pinova')	[38]
<i>CiFT</i> , RHV region	Pears (<i>Pyrus communis</i> L.)	[36,81]
<i>PpTFL1-1</i> & <i>PpTFL1-2</i>	Japanese pear (<i>Pyrus pyrifolia</i>)	[8]
<i>PcTFL1-1</i> & <i>PcTFL1-2</i>	European pear (<i>Pyrus communis</i> subsp. <i>communis</i>),	[8]
New self-incompatibility alleles, <i>S-RNase</i> , <i>F-box</i> , <i>SFB</i> and QTL on G5	Apricot (<i>Prunus armeniaca</i> L.)	[82-85]
<i>F-box</i> , <i>MiFT</i> , <i>FY</i> , <i>FPA</i> , flowering-promoting factor, <i>MADS</i> -box protein <i>FLC</i>	Japanese apricot (<i>Prunus mume</i>)	[86,87,105]
<i>F-box</i> , QTL in G4, G1, G3 & G7; <i>PrdMADS 1,2,3</i>	Almond (<i>Prunus dulcis</i>)	[88-90]
<i>PrpMADS 2,4,6</i>	Peach (<i>Prunus persica</i>)	
<i>MADS</i> -box gene	Peach (<i>Prunus persica</i>)	[91]
<i>CoTFL1-1</i> & <i>CoTFL1-2</i>	Quince (<i>Cydonia oblonga</i>)	[8]
<i>EjTFL1-1</i> & <i>EjTFL1-2</i>	Loquat (<i>Eriobotrya japonica</i>)	[8]
<i>FY</i> -like chromosome LG6, NC_020496.1	Wild strawberry (<i>Fragaria vesca</i>)	[92,105]
QTL in LG4, LG6, LG7	<i>Prunus</i> sp., peach, apricot and sweet cherry	[93]

Table 1: Flowering Gene and Genomic Region in Fruit Plants.

S.No.	Plant Name	Number of sequences in NCBI (GENE) database
1	<i>Arabidopsis thaliana</i>	385
2	<i>Silene noctiflora</i>	199
3	<i>Glycine max</i>	61
4	<i>Oryza sativa</i>	27
5	<i>Oryza sativa japonica</i> Group	26
6	<i>Solanum tuberosum</i>	26
7	<i>Setaria italica</i>	21
8	<i>Solanum lycopersicum</i>	21
9	<i>Cucumis sativus</i>	19
10	<i>Oryza brachyantha</i>	18
11	<i>Prunus mume</i>	18
12	<i>Cicer arietinum</i>	17
13	<i>Medicago truncatula</i>	15
14	<i>Populus trichocarpa</i>	15
15	<i>Fragaria vesca</i>	14
16	<i>Citrus sinensis</i>	14
17	<i>Vitis vinifera</i>	14
18	<i>Zea mays</i>	12
19	<i>Physcomitrella patens</i>	12
20	<i>Brachypodium distachyon</i>	10
21	All other taxa	108

Table 2: Flowering Gene Submitted in NCBI GENE Database (2017).

Floral Signal Pathway

In several species, flowering ability has been demonstrated to be influenced by the integration of environmental signals from the photoperiod and vernalization pathways [9-11]. Horticultural trees generally initiate flowers in response to either an environmental stimulus or autonomously. There is some evidence that the mechanisms through which environmental stimuli act are similar between annual plants and horticultural trees. Vernalization acts on the meristem and leaves in *Arabidopsis thaliana* to suppress floral repressors, but in mango cool temperatures are sensed in the mature leaves that generate a signal that is exported to the meristem to promote flowering. Mango appears to be more analogous to photoperiodic induction in *Arabidopsis*, or to the effects of ambient temperature on genes of the autonomous flowering pathway [12]. Satsuma mandarin *FT* orthologue mRNA levels increased with the seasonal onset of cool temperatures during the time of floral induction [13]. There is evidence that *LFY* and *API* orthologues isolated from sweet orange [14] and grapevine [15] act as floral

promoters; and evidence that *TFL1* orthologues isolated from citrus [16] and grapevine [15] act as floral inhibitors. Expression of *LFY* and *API* homologues in perennials is also associated with floral and inflorescence buds. Expression of these genes appears to follow a bimodal pattern related to the two seasons that are needed to flower. This has been studied in detail in the case of grapevine, apple and citrus. For the *TFL1*-like genes of apple and citrus, constitutive expression in *Arabidopsis* has been shown to cause a late flowering phenotype, similar to that of plants over expressing the *Arabidopsis TFL1* gene. These events and their expression patterns, suggests a role for the *TFL1*-like genes of these perennials in maintaining indeterminacy of the shoot meristems within the developing bud (Table 3) [17-20]. Molecular genetic analysis of seasonal patterns of flowering in diverse annual and perennial species has demonstrated some common features. In particular, vernalization-response pathways have evolved independently in different plant species as repressors of photoperiodic pathways until plants have been exposed to winter temperatures. Furthermore, the activation of transcription of *FT*-like genes by day length is a feature of photoperiodic response with different regulatory mechanisms. Indeed, CETS proteins, particularly, like but also *TFL1* like proteins, have important role in all species examined, and in perennials, the importance of the repressive function of *TFL1* like genes appears to be increased. In addition, though *FT* like genes are characteristically involved in floral promotion, they can control other seasonal responses, such as repressors of vernalization response or induction of tuberization and growth [21]. The environmentally responsive transcription factors converge on a small number of floral integrator genes that initiate the early stages of flowering, and this convergence creates a coordinated response to seasonal cues. The genes *GI*, *FKF1*, *CO* and *FT* have major regulatory roles in this pathway [22,23].

In plants, initiation of the reproductive phase is regulated by an elaborate network of floral signaling pathways, which include the photoperiodic, vernalization, autonomous, light-quality and ambient temperature pathways [24-26]. This is mainly modulated by two floral integrators, the *FT* and the *SOC1* genes [27-30]. Both genes have been described as floral promoters and their overexpression induce early-flowering phenotypes [31-33]. These ultimately regulate expression of the *FT* gene. Flowering is promoted when *FT* protein is produced in permissive photoperiods and moves through the phloem to the apex where it forms a complex with *FD* and activates expression of the floral meristem identity genes (Figure 1). The fact that plants are incapable of initiating flowering during juvenility even when environmental growth conditions are conducive suggests that inhibitory mechanisms may suppress induction of *FT* during juvenility and hence prevent premature flowering [34].

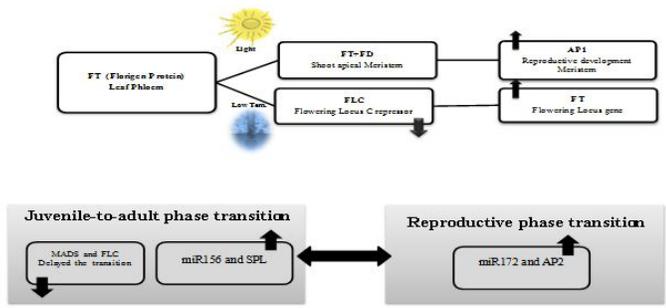


Figure 1. A model for the transition from vegetative to reproductive phase in Citrus for FT gene and transcription factors. FT-Flowering Locus gene, FD-Flowering Locus D Protein, AP1- Floral meristem identity gene, FLC- Flowering Locus C repressor, SPL- Squamosa promoter binding protein.

Several studies have demonstrated that modification of the genes involved in floral induction by a transformation approach successfully shortens the juvenile period. For example, overexpression of *AtFT*-homologous genes accelerates flowering time in apple, plum, poplar, citrus and pear [33,35-38], while repression of *TFL1*-like genes has a similar effect in apple and pear [39-40]. Overlaid on this general pattern of age-related phase change, *TEM* can be considered as a floral repressor that acts on multiple points in the photoperiod and GA flowering pathways. *TEM* may have a more general role in regulating juvenility in a range of herbaceous and woody species [34]. Yamagishi, et al. [41] reported a novel technology that simultaneously promotes expression of *Arabidopsis AtFT* and silencing of apple *MdTFL1*-Using an ALSV vector to accelerate flowering time and life cycle in apple seedlings. When apple cotyledons were inoculated with *ALSV-AtFT/MdTFL1* immediately after germination, more than 90% of infected seedlings started flowering within 1.5-3 months, and almost all early-flowering seedlings continuously produced flower buds on the lateral and axillary shoots. Cross-pollination between early-flowering apple plants produced fruits with seeds, indicating that *ALSV-AtFT/MdTFL1* inoculation successfully reduced the time required for completion of the apple life cycle to 1 year or less. Apple latent spherical virus was not transmitted via seeds to successive progenies in most cases, and thus, this method will serve as a new breeding technique that does not pass genetic modification to the next generation. Some other examples of ectopic expression of flower inducing genes in woody perennial fruit trees are shown in (Table 1) and (Table 3). Gene *MdTFL1* has a key role in the regulation of juvenility, flower induction and development in apple. *TFL1* has an opposite function to *LFY* and *AP1* and belongs to the group of PEBP proteins. Plant PEBP proteins can be grouped into three main clades: the *MFT*-, *FT*- and *TFL1*-like subfamilies [42,43]. Those *TFL1*-like genes for which a function has been found have role in the control of plant development, usually in flowering. *TFL1* in woody perennials and *TFL1* homologues have been studied in few perennial dicots; species such as orange tree (*Citrus sinensis*) [16], apple (*Malus domestica*) [44], *Metrosideros excels* [19] and grapevine [20] (Table 3).

Gene	Function	Reference
<i>CO</i>	Transcription factor of the <i>FT</i> gene	[94,95]
<i>FT, PtFT1, CiFT, Hd3a, and SFT</i>	Transition from the vegetative to the reproductive stage. Implicated in the formation of axillary meristems Promoting early flowering in citrus	[69,96]
<i>MdMAD1</i> and <i>MdMADS2</i>	Initiation of flower organs in apple	[80]
<i>SOCI</i>	Enhance the transcription of the floral meristem identity gene <i>LFY</i>	[97]
<i>FLC</i>	Repressing of the floral pathway integrators <i>CO</i> , <i>LFY</i> and <i>SOCI</i>	[98]
<i>FLD</i> , <i>FLK</i> and <i>LD</i>	Suppress the transcription of <i>FLC</i> activate the floral induction gene <i>FT</i>	[99]
<i>FRI</i> and <i>VIP</i>	Up-regulated <i>FLC</i> gene	[100]
<i>SMZ</i> , <i>SNZ</i> and <i>TFL</i>	Suppress the floral pathway integrator genes and floral meristem identity genes	[101]
<i>LFY</i>	Repressor of <i>TFL1</i> and initiation of floral meristems as well as floral organs	[99]
<i>AP1</i> and <i>AP2</i>	Activates organ identity genes such as <i>AP3</i> , <i>PI</i> and <i>AG</i>	[102,99]
<i>TFL1</i>	Inflorescence meristem identity gene and a floral inhibitor	[102,99]
<i>MIKC</i> -type and <i>MADS</i> -box genes	Transcriptional activation of flowering gene	[103]
<i>MADS</i> -box and <i>SEP</i> genes	Proper development of petal, stamen and carpel identity in <i>Arabidopsis</i>	[104]
Ectopic expression of <i>AG</i> , <i>AP3</i> , <i>PI</i> and <i>SEP3</i>	Convert leaves to organs that resemble stamens flower organ development	[104]
<i>LFY</i> and <i>AP1</i>	Expressed in citrus which drastically reduced the length of the juvenile phase	[63]
<i>MdTFL1</i>	Down regulation of this gene led to flower induction in apple controlling the transition from the juvenile/vegetative to the reproductive phase in apple.	[40]

<i>AFL1</i> and <i>AFL2</i> Upregulation led to flower induction in apple	Upregulation led to flower induction in apple	[40]
<i>FLC</i> , <i>TFL1</i> and <i>SVP</i>	Repressors of the floral pathway integrators	[98]

Table 3: Flowering Gene(S) and their Function(S) in Fruit Crops.

A remarkable increase in the expression of genes encoding proteins associated with calcium-dependent auxin polar transport resulted into reduction in bud endogenous auxin levels [45], and an increase in ABA-metabolizing genes, accompanied by a decrease in ABA levels and those of its catabolizes in buds following de-fruiting were identified. Fruit removal resulted in relatively rapid changes in global gene expression, including induction of photosynthetic genes and proteins [46]. There is now some understanding of how the expression of flowering genes integrates with the environment and flowering time in horticultural trees.

‘On’ and ‘Off’ Regulatory Mechanisms

Genomic analysis resulted in numerous Differentially Expressed Genes (DEGs), allowing the partial identification of mechanisms that convert ‘ON’ into ‘OFF’ buds [47]. In citrus, there are four highly *CAX* homologous genes and the expression of a *CAX3* homologue was highly induced following de-fruiting. Transduction of Ca^{2+} signals is carried out by specific calcium-binding proteins, containing a common structural motif called the ‘EF-hand’, a helix–loop–helix structure that binds a single Ca^{2+} ion [48]. A significant up-regulation of a few genes encoding EF-hand proteins in ‘OFF’ and DEF buds compared with their level in ‘ON’ buds. Four of the up-regulated EF-hand genes show remarkable homology to the genes encoding PBP1 that interacts physically with PID protein kinase, regulating its activity in response to changes in calcium levels [49]. Gene PID regulates the polarity of PIN proteins [50], which are known to direct auxin flow [51]. NPH3-like proteins have recently been shown to affect PIN localization [52,53]. The Citrus *NPH3*-like gene induced in ‘OFF’ and DEF buds compared with ‘ON’ buds. Higher levels of IAA in ‘ON’ buds reflect their inability to distribute IAA efficiently via the Ca^{2+} -dependent PIN-based polar auxin transport mechanism. In addition, efficient auxin removal from the bud appears to be a key component in transforming the ‘ON’ bud into an ‘OFF’ bud. The involvement of auxin in flowering inhibition following an ‘ON’-Crop year was recently suggested [45,54]. The application of auxin polar transport inhibitors resulted in flowering induction in a number of fruit trees [54]. The parallel reduction in endogenous ABA and IAA levels in the bud would suggest cross-talk between the ABA and IAA signaling pathways. Such cross-talk interactions were suggested in embryo axis elongation and root development [55], but not in flowering control processes.

The study of the expression pattern of flowering-genes of ‘ON’ (fully loaded) and ‘OFF’ (without fruits) trees revealed that

homologues of *FT*, *SOC1*, *API* and *LFY* were negatively affected by fruit load. Thus, *CiFT* expression showed a progressive increase in leaves from off trees [56]. The expression of flowering control genes, *FT*, *LFY*, *API*, *TFL* and *miR156*-regulated *SPL5* in leaves and buds of citrus, mango and apple is affected by fruit load [47,56-59]. The expression pattern of *SPL-like*, *miR156* and other flowering control genes suggested that fruit load affects bud fate, and therefore development and metabolism, a relatively long time before the flowering induction period [47]. So, despite the rapid progress in flowering transcriptomic and genetic studies a number of mechanisms are still not clear and need more concerted efforts by combining molecular tools as well as possible horticultural interventions. The possible horticultural interventions to understand the flowering mechanism in its roots. Water deficit can also be the primary stimulus of floral induction for many other species growing in tropical and subtropical climates [60]. Increasing accumulation of *CsFT* transcripts in leaves of trees exposed to water deficit (Figure 1) indicated that the mechanism regulating *CsFT* expression is responsive to signals initiated by water deficit and cool temperature as has been reported elsewhere [13]. Cool ambient temperatures (5 to 20°C) and water deficit are the only factors known to induce flowering in sweet orange (*Citrus sinensis*). A very little information is available on the mechanisms underlying floral induction by water deficit in sweet orange (and other tropical and sub-tropical species) are scarce. During water deficit conditions transcripts of four flower-promoting genes namely *CsFT*, *CsSL1*, *CsAPI*, and *CsLFY* were accumulated under controlled conditions. Exposure to water deficit increased the accumulation of *CsFT* transcripts, whereas, transcripts of *CsSL1*, *CsAPI*, and *CsLFY* were reduced. However, when water deficit was interrupted by irrigation, accumulation of *CsFT* transcripts returned rapidly to pre-treatment levels and accumulation of *CsSL1*, *CsAPI*, and *CsLFY* increased. These results suggest that water deficit induces flowering through the upregulation of *CsFT* and that *CsFT* is the leaf integrator of flower-inducing signals generated by the exposure to water deficit and cool temperatures in sweet orange [61].

Transgenic for Flower Induction

The biotechnological manipulation of endogenous, genetic flowering pathways can be useful for reducing the length of the juvenile phase. This can be achieved through up regulating additional flowering genes, use of inducible promoters to drive transgene expression, and approaches to transmit the transgenic stimulus through grafting/ trans grafting. One of the potential applications for breeding involves the use of a transgenic, early-flowering genotype as a donor to promote flowering in a selected genotype through graft transmission. This strategy would exploit the potential of the *FT* protein to translocate, probably within the phloem stream, across a graft union. This would eliminate the need to genetically modify genotypes on a case-by-case basis [2]. Flachowsky, et al. [62] engineered ‘European plum (*Prunus domestica* L.) BlueByrd’

plum trees with the *FT1* gene from *Populus trichocarpa* under the control of the 35S promoter. Transgenic plants expressing higher levels of *FT* flowered and produced fruits in the greenhouse within 1 to 10 months. *FT* plums did not enter dormancy after cold or short day treatments. This study demonstrates the potential for a single transgene event to markedly affect the vegetative and reproductive growth and development of an economically important temperate woody perennial crop [37]. In transgenic hybrid citrus, *Citrus sinensis* L. Osbeck3 *Poncirus trifoliata* L. Raf., flowering appeared to be under both environmental and endogenous control because it occurred only once a year in the spring [63].

In *Malus domestica* [38,64] used 35S promoter for *Bp-MADS4*, *MdFT* and *API* gene, respectively for early flowering. Similarly, Matsuda, et al. [36] used 35S: *CiFT* construct in *Pyrus communis* cv. Lafrance and Balade that showed early flowering. Kotoda and Wada [44] cloned *Malus domestica TFL1* (*MdTFL1*), a gene highly homologous to the *Arabidopsis TFL1* and *Antirrhinum CEN*, which maintain the identity of inflorescence meristem. *MdTFL1* is expressed in apple vegetative tissue, such as apical buds, seedling stems and roots but not in reproductive tissue such as floral organs. In transgenic hybrid citrus, *Citrus sinensis* L. Osbeck3 *Poncirus trifoliata* L. Raf., over-expression of *LFY* and *API* orthologues substantially reduced the juvenile phase [63]. Other useful floral induction approaches include plant virus vector-based methods, such as those that promote expression of endogenous genes, and Virus-Induced Gene Silencing (VIGS). Plant virus vector system can be used to add new traits to plants without altering the host genome [65,66]. An Apple Latent Spherical Virus (ALSV) vector containing the *AtFT* was used for inoculating the 30% of apple seedlings. These seedlings produced flowers 1.5-2 months after inoculation (7-9 leaf stage) [67] and 10% of apple seedlings produced early flowers when *MdTFL1-1* was silenced by VIGS using an ALSV vector. When apple cotyledons were inoculated with ALSV-*AtFT/MdTFL1* immediately after germination, more than 90% of infected seedlings started flowering within 1.5-3 months, and almost all early-flowering seedlings continuously produced flower buds on the lateral and axillary shoots. Cross-pollination between early-flowering apple plants produced fruits with seeds, indicating that ALSV-*AtFT/MdTFL1* inoculation successfully reduced the time required for completion of the apple life cycle to 1 year or less. Apple latent spherical virus was not transmitted via seeds to successive progenies in most cases, and thus, this method will serve as a new breeding technique that does not pass genetic modification to the next generation [41].

Conclusion

There is an urgent need to meet the challenges in fruit production since human population is increasing day by day and with the limited land resources, hence pressure is too high to the requirement of the people. Geno-Horti concept should be employed so that flowering genes, genomic region etc. can give better under-

standing and practically can be utilized with possible horticulture interventions. Further efforts are needed to uncover key regulators and/or regulatory mechanisms that determine the widespread translation enhancement in response to light treatment, juvenility, and hormonal effect.

Acknowledgments

Authors are thankful to DST-SERB and ICAR-NPTC for providing the financial assistance and Director ICAR-IARI and ICAR-NRC on Plant Biotechnology, New Delhi for research facilities.

References

1. Tan FC, Swain SM (2006) Genetics of flower initiation and development in annual and perennial plants. *Physiol Plant* 128: 8-17.
2. Nocker S, Gardiner SE (2014) Breeding better cultivars faster: applications of new technologies for the rapid deployment of superior horticultural tree crops. *Horticulture Research* 1: 1-8.
3. Khan MR, Ai XY, Zhang JZ (2014) Genetic regulation of flowering time in annual and perennial plants. *Wiley Interdisciplinary Reviews: RNA* 5: 347-359.
4. Meijón M, Feito I, Valledor L, Rodríguez R, Cañal MJ (2010) Dynamics of DNA methylation and Histone H4 acetylation during floral bud differentiation in azalea. *BMC Plant Biology* 10: 10.
5. Sharma N, Singh SK, Singh NK, Srivastav M, Singh BP, et al. (2015) Differential gene expression studies: a possible way to understand bearing habit in fruit crops. *Transcriptomics: Open Access* 3: 1-3.
6. Komeda Y (2004) Genetic regulation of time to flower in *Arabidopsis thaliana*. *Annu Rev Plant Biol* 55: 521-535.
7. Mouhu K, Hytönen T, Folta K, Rantanen M, Paulin L, et al. (2009) Identification of flowering genes in strawberry a perennial SD plant. *BMC Plant Biology* 9: 122.
8. Esumi T, Tao R, Yonemori K (2005) Isolation of *LEAFY* and *TERMINAL FLOWER 1* homologues from six fruit tree species in the subfamily *Maloideae* of the *Rosaceae*. *Sexual Plant Reproduction* 17: 277-287.
9. Onouchi H, Igeño MI, Périlleux C, Graves K, Coupland G (2000) Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among *Arabidopsis* flowering-time genes. *The Plant Cell* 12: 885-900.
10. Amasino RM (2005) Vernalization and flowering time. *Current Opinion in Biotechnology* 16: 154-158.
11. Sheldon CC, Jean Finnegan E, James Peacock W, Dennis ES (2009) Mechanisms of gene repression by vernalization in *Arabidopsis*. *The Plant Journal* 59: 488-498.
12. Blázquez MA, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nature Genetics* 33: 168-171.
13. Nishikawa F, Endo T, Shimada T, Fujii H, Shimizu T, et al. (2007) Increased *CiFT* abundance in the stem correlates with floral induction by low temperature in Satsuma mandarin (*Citrus unshiu* Marc.). *Journal of Experimental Botany* 58: 3915-3927.

14. Pillitteri LJ, Lovatt CJ, Walling LL (2004) Isolation and Characterization of LEAFY and APETALA1 Homologues from *Citrus sinensis* L. Osbeck Washington. *Journal of the American Society for Horticultural Science* 129: 846-856.
15. Boss PK, Sreekantan L, Thomas MR (2006) A grapevine TFL1 homologue can delay flowering and alter floral development when over expressed in heterologous species. *Functional Plant Biology* 33: 31-41.
16. Pillitteri LJ, Lovatt CJ, Walling LL (2004) Isolation and characterization of a TERMINAL FLOWER homolog and its correlation with juvenility in citrus. *Plant Physiology* 135: 1540-1551.
17. Carmona MJ, Cubas P, Martínez-Zapater JM (2002) VFL the grapevine FLORICAULA/LEAFY ortholog is expressed in meristematic regions independently of their fate. *Plant Physiology* 130: 68-77.
18. Calonje M, Cubas P, Martínez-Zapater JM, Carmona MJ (2004) Floral meristem identity genes are expressed during tendril development in grapevine. *Plant Physiology* 135: 1491-1501.
19. Sreekantan L, Clemens J, McKenzie MJ, Lenton JR, Croker SJ, et al. (2004) Flowering genes in *Metrosideros* fit a broad herbaceous model encompassing *Arabidopsis* and *Antirrhinum*. *Physiologia Plantarum* 121: 163-173.
20. Carmona MJ, Calonje M, Martínez-Zapater JM (2007) The FT/TFL1 gene family in grapevine. *Plant Molecular Biology* 63: 637-650.
21. Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. *Nature Reviews Genetics* 13: 627-639.
22. Nelson DC, Lasswell J, Rogg LE, Cohen MA, Bartel B (2000) FKF1 a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* 101: 331-340.
23. Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, et al. (2007) An Electronic Fluorescent Pictograph browser for exploring and analyzing large-scale biological data sets. *PLoS one* 2: e718.
24. Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, et al. (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311: 91-94.
25. Blázquez MA (2000) Flower development pathways. *Journal of Cell Science* 113:3547-3548.
26. Searle I, Coupland G (2004) Induction of flowering by seasonal changes in photoperiod. *The EMBO Journal* 23: 1217-1222.
27. Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, et al. (1999) Activation tagging of the floral inducer FT. *Science* 286: 1962-1965.
28. Borner R, Kampmann G, Chandler J, Gleißner R, Wisman E, et al. (2000) A MADS domain gene involved in the transition to flowering in *Arabidopsis*. *The Plant Journal* 24: 591-599.
29. Lee H, Suh SS, Park E, Cho E, Ahn JH, et al. (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. *Genes & Development* 14: 2366-2376.
30. Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, et al. (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. *Science* 288: 1613-1616.
31. Lee JH, Hong SM, Yoo SJ, Park OK, Lee JS, et al. (2006) Integration of floral inductive signals by flowering locus T and suppressor of over-expression of Constans 1. *Physiologia Plantarum* 126: 475-483.
32. Sreekantan L, Thomas MR (2006) VvFT and VvMADS8 the grapevine homologues of the floral integrators FT and SOC1, have unique expression patterns in grapevine and hasten flowering in *Arabidopsis*. *Functional Plant Biology* 33:1129-1139.
33. Zhang H, Harry DE, Ma C, Yuceer C, Hsu CY, et al. (2010) Precocious flowering in trees: the FLOWERING LOCUS T gene as a research and breeding tool in *Populus*. *Journal of Experimental Botany* 61: 2549-2560.
34. Sgamma T, Jackson A, Muleo R, Thomas B, Massiah A (2014) TEMPRA-NILLO is a regulator of juvenility in plants. *Scientific Reports* 4: 3704.
35. Endo T, Shimada T, Fujii H, Kobayashi Y, Araki T, et al. (2005) Ectopic expression of an FT homolog from *Citrus* confers an early flowering phenotype on trifoliolate orange (*Poncirus trifoliata* L. Raf.). *Transgenic research* 14: 703-712.
36. Matsuda N, Ikeda K, Kurosaka M, Takashina T, Isuzugawa K, et al. (2009) Early flowering phenotype in transgenic pears (*Pyrus communis* L.) expressing the CiFT gene. *Journal of the Japanese Society for Horticultural Science* 78: 410-416.
37. Srinivasan C, Dardick C, Callahan A, Scorza R (2012) Plum (*Prunus domestica*) trees transformed with poplar FT1 result in altered architecture dormancy requirement and continuous flowering. *PLoS One* 7: e40715.
38. Tränkner C, Lehmann S, Hoenicka H, Hanke MV, Fladung M, et al. (2010) Over-expression of an FT-homologous gene of apple induces early flowering in annual and perennial plants. *Planta* 232: 1309-1324.
39. Flachowsky H, Le Roux PM, Peil A, Patocchi A, Richter K, et al. (2011) Application of a high-speed breeding technology to apple (*Malus domestica*) based on transgenic early flowering plants and marker-assisted selection. *New Phytologist* 192: 364-377.
40. Kotoda N, Iwanami H, Takahashi S, Abe K (2006) Antisense expression of MdTFL1, a TFL1-like gene, reduces the juvenile phase in apple. *Journal of the American Society for Horticultural Science* 131: 74-81.
41. Yamagishi N, Kishigami R, Yoshikawa N (2014) Reduced generation time of apple seedlings to within a year by means of a plant virus vector: a new plant-breeding technique with no transmission of genetic modification to the next generation. *Plant Biotechnology Journal* 12:60-68.
42. Carmel-Goren L, Liu YS, Lifschitz E, Zamir D (2003) The SELF-PRUNING gene family in tomato. *Plant Molecular Biology* 52: 1215-1222.
43. Chardon F, Damerval C (2005) Phylogenomic analysis of the PEBP gene family in cereals. *Journal of Molecular Evolution* 61: 579-590.
44. Kotoda, Nobuhiro, and Masato Wada (2005) MdTFL1 a TFL1-like gene of apple, retards the transition from the vegetative to reproductive phase in transgenic *Arabidopsis*. *Plant Science* 168: 95-104.
45. Smith HM, Samach A (2013) Constraints to obtaining consistent annual yields in perennial tree crops. I: Heavy fruit load dominates over vegetative growth. *Plant Science* 207: 158-167.
46. Shalom L, Samuels S, Zur N, Shlizerman L, Doron-Faigenboim A, et al. (2014) Fruit load induces changes in global gene expression and in Abscisic Acid (ABA) and Indole Acetic Acid (IAA) homeostasis in citrus buds. *Journal of Experimental Botany* 65: 3029-3044.
47. Shalom L, Samuels S, Zur N, Shlizerman L, Zemach H, et al. (2012) Alternate bearing in citrus: changes in the expression of flowering control genes and in global gene expression in on-versus off-crop trees. *PLoS One* 7: e46930.

49. Benjamins R, Ampudia CS, Hooykaas PJ, Offringa R (2003) PINOID-mediated signaling involves calcium-binding proteins. *Plant Physiology* 132:1623-1630.
50. Friml J, Yang X, Michniewicz M, Weijers D, Quint A, et al. (2004) A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* 306: 862-865.
51. Wiśniewska J, Xu J, Seifertová D, Brewer PB, Růžička K, et al. (2006) Polar PIN localization directs auxin flow in plants. *Science* 312: 883.
52. Furutani M, Sakamoto N, Yoshida S, Kajiwara T, Robert HS, et al. (2011) Polar-localized NPH3-like proteins regulate polarity and endocytosis of PIN-FORMED auxin efflux carriers. *Development* 138: 2069-2078.
53. Wan Y, Jasik J, Wang L, Hao H, Volkmann D, et al. (2012) The signal transducer NPH3 integrates the phototropin1 photosensor with PIN2-based polar auxin transport in *Arabidopsis* root phototropism. *The Plant Cell* 24: 551-565.
54. Bangerth F (2005) Flower induction in perennial fruit trees: still an enigma? In X International Symposium on Plant Bioregulators in Fruit Production 727: 177-196.
55. Wang L, Hua D, He J, Duan Y, Chen Z, et al. (2011) Auxin Response Factor2 (ARF2) and its regulated homeodomain gene HB33 mediate abscisic acid response in *Arabidopsis*. *PLoS Genet* 7: e1002172.
56. Muñoz-Fambuena N, Mesejo C, González-Mas MC, Primo-Millo E, Agustí M, et al (2011) Fruit regulates seasonal expression of flowering genes in alternate-bearing Moncadamandarin. *Annals of Botany* 108: 511-519.
57. Muñoz-Fambuena N, Mesejo C, González-Mas MC, Iglesias DJ, Primo-Millo E, et al. (2012) Gibberellic acid reduces flowering intensity in sweet orange [*Citrus sinensis* (L.) Osbeck] by repressing CiFT gene expression. *Journal of Plant Growth Regulation* 31: 529-536.
58. Kotoda N, Hayashi H, Suzuki M, Igarashi M, Hatsuyama Y, et al. (2010) Molecular characterization of FLOWERING LOCUS T-like genes of apple (*Malus domestica* Borkh.). *Plant and Cell Physiology* 51: 561-575.
59. Nakagawa M, Honsho C, Kanzaki S, Shimizu K, Utsunomiya N (2012) Isolation and expression analysis of FLOWERING LOCUS T-like and gibberellin metabolism genes in biennial-bearing mango trees. *Scientia Horticulturae* 139:108-117.
60. Albrigo LG, Saúco VG (2002) Flower bud induction, flowering and fruit-set of some tropical and subtropical fruit tree crops with special reference to citrus in XXVI International Horticultural Congress Citrus and Other Subtropical and Tropical Fruit Crops. *ISHS Acta Horticulture* 632: 81-90.
61. Chica EJ, Albrigo LG (2013) Expression of flower promoting genes in sweet orange during floral inductive water deficits. *Journal of the American Society for Horticultural Science* 138: 88-94.
62. Flachowsky H, Hanke MV, Peil A, Strauss SH, Fladung M (2009) A review on transgenic approaches to accelerate breeding of woody plants. *Plant Breeding* 128: 217-226.
63. Peña L, Martín-Trillo M, Juárez J, Pina JA, Navarro L, et al. (2001) Constitutive expression of *Arabidopsis* LEAFY or APETALA1 genes in citrus reduces their generation time. *Nature Biotechnology* 19: 263-267.
64. Flachowsky H, Peil A, Sopanen T, Elo A, Hanke V (2007) Overexpression of BpMADS4 from silver birch (*Betula pendula* Roth.) induces early-flowering in apple (*Malus domestica* Borkh.). *Plant Breeding* 126:137-145.
65. Gleba Y, Marillonnet S, Klimyuk V (2004) Engineering viral expression vectors for plants: the full virus and the deconstructed virus strategies. *Current opinion in Plant Biology* 7: 182-188.
66. Purkayastha A, Dasgupta I (2009) Virus-induced gene silencing: a versatile tool for discovery of gene functions in plants. *Plant Physiology and Biochemistry* 47: 967-976.
67. Yamagishi N, Sasaki S, Yamagata K, Komori S, Nagase M, et al. (2011) Promotion of flowering and reduction of a generation time in apple seedlings by ectopical expression of the *Arabidopsis thaliana* FT gene using the Apple latent spherical virus vector. *Plant Molecular Biology* 75: 193-204.
68. Shi CY, Song RQ, Hu XM, Liu X, Jin LF, et al. (2015) Citrus PH5-like H⁺ATPase genes identification and transcript analysis to investigate the possible relationship with citrate accumulation in fruits. *Front. Plant Sci* 135.
69. Xu F, Rong X, Huang X, Cheng S (2012) Recent advances of *Flowering Locus T* gene in higher plants. *International Journal of Molecular Sciences* 13: 3773-3781.
70. Tan FC, Swain SM (2007) Functional characterization of AP3, SOC1 and WUS homologues from citrus (*Citrus sinensis*). *Physiologia Plantarum* 131: 481-495.
71. Zhang JZ, Mei L, Liu R, Khan MR, Hu CG (2014) Possible involvement of locus-specific methylation on expression regulation of LEAFY homologous gene (CiLFY) during Precocious trifoliolate orange phase change process. *PLoS one* 9: e88558.
72. Boss PK, Vivier M, Matsumoto S, Dry IB, Thomas MR (2001) A cDNA from grapevine (*Vitis vinifera* L.), which shows homology to AGAMOUS and SHATTERPROOF, is not only expressed in flowers but also throughout berry development. *Plant Molecular Biology* 45:541-553.
73. Boss PK, Sensi E, Hua C, Davies C, Thomas MR (2002) Cloning and characterization of grapevine (*Vitis vinifera* L.) MADS-box genes expressed during inflorescence and berry development. *Plant Science* 162: 887-895.
74. Davenport TL, Ying Z, Kulkarni V, White TL (2006) Evidence for a translocatable florigenic promoter in mango. *Scientia Horticulturae* 110:150-159.
75. Zhang X, Garreton V, Chua NH (2005) The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation. *Genes & Development* 19: 1532-1543.
76. Elitzur T, Vrebalov J, Giovannoni JJ, Goldschmidt EE, Friedman H (2010) The regulation of MADS-box gene expression during ripening of banana and their regulatory interaction with ethylene. *Journal of Experimental Botany* 61: 1523-1535.
77. Choudhury SR, Roy S, Nag A, Singh SK, Sengupta DN (2012) Characterization of an AGAMOUS-like MADS box protein, a probable constituent of flowering and fruit ripening regulatory system in banana. *PLoS One* 7: e44361.
78. Inaba A, Liu X, Yokotani N, Yamane M, Lu WJ, et al. (2007) Differential feedback regulation of ethylene biosynthesis in pulp and peel tissues of banana fruit. *Journal of Experimental Botany* 58: 1047-1057.
79. Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. *Trends in Plant Science* 5: 523-530.
80. Mimida N, Kidou SI, Iwanami H, Moriya S, Abe K, et al. (2011) Apple FLOWERING LOCUS T proteins interact with transcription factors implicated in cell growth and organ development. *Tree Physiol* 31: 555-566.

81. Zisovich AH, Stern RA, Sapir G, Shafir S, Goldway M (2004) The RHV region of S-RNase in the European pear (*Pyrus communis*) is not required for the determination of specific pollen rejection. *Sexual Plant Reproduction* 17:151-156.
82. Halász J, Hegedűs A, Hermán R, Stefanovits-BányaiÉ, Pedryc A (2005) New self-incompatibility alleles in apricot (*Prunus armeniaca* L.) revealed by stylar ribonuclease assay and S-PCR analysis. *Euphytica* 145: 57-66.
83. Halász J, Hegedűs A, Pedryc A (2005) Molecular background of self-incompatibility in apricot. *Acta Biol. Szeged* 49: 21-22.
84. Ikeda K, Igic B, Ushijima K, Yamane H, Hauck NR, et al. (2004) Primary structural features of the S haplotype-specific F-box protein, SFB, in *Prunus*. *Sexual Plant Reproduction* 16: 235-243.
85. Romero C, Vilanova S, Burgos L, Martinez-Calvo J, Vicente M, et al. (2004) Analysis of the S-locus structure in *Prunus armeniaca* L. Identification of S-haplotype specific S-RNase and F-box genes. *Plant molecular biology* 56: 145-157.
86. Entani T, Iwano M, Shiba H, Che FS, Isogai A, et al. (2003) Comparative analysis of the self-incompatibility (S-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. *Genes to Cells* 8: 203-213.
87. Esumi T, Kitamura Y, Hagihara C, Yamane H, Tao R (2010) Identification of a TFL1 ortholog in Japanese apricot (*Prunus mume* Sieb. et Zucc.). *Scientia horticulturae* 125: 608-616.
88. Silva C, Garcia-Mas J, Sánchez AM, Arús P, Oliveira MM (2005) Looking into flowering time in almond (*Prunus dulcis*(Mill) DA Webb): the candidate gene approach. *Theoretical and Applied Genetics* 110: 959-968.
89. Ushijima K, Sassa H, Tamura M, Kusaba M, Tao R, et al. (2001) Characterization of the S-locus region of almond (*Prunus dulcis*): analysis of a soma clonal mutant and a cosmid contig for an S haplotype. *Genetics* 158: 379-386.
90. Ushijima K, Sassa H, Dandekar AM, Gradziel TM, Tao R, et al. (2003) Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. *The Plant Cell* 15: 771-781.
91. Tadiello A, Pavanello A, Zanin D, Caporali E, Colombo L, et al. (2009) A PLENA-like gene of peach is involved in carpel formation and subsequent transformation into a fleshy fruit. *Journal of Experimental Botany* 60: 651-661.
92. Mouhu K, Hytönen T, Folta K, Rantanen M, Paulin L, et al. (2009) Identification of flowering genes in strawberry, a perennial SD plant. *BMC Plant Biology* 9: 122.
93. Dirlwanger E, Quero-Garcia J, Le Dantec L, Lambert P, Ruiz D, et al. (2012) Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. *Heredity* 109: 280-292.
94. Teper-Bamnlolker P, Samach A (2005) The flowering integrator FT regulates SEPALLATA3 and FRUITFULL accumulation in *Arabidopsis* leaves. *The Plant Cell* 17: 2661-2675.
95. Liu L, Farrona S, Klemme S, Turck FK (2014) Post-fertilization expression of FLOWERING LOCUS T suppresses reproductive reversion. *Frontiers in Plant Science* 5: 164.
96. Huang X, Ding J, Effgen S, Turck F, Koornneef M (2013) Multiple loci and genetic interactions involving flowering time genes regulate stem branching among natural variants of *Arabidopsis*. *New Phytologist* 199: 843-857.
97. Moon J, Suh SS, Lee H, Choi KR, Hong CB, et al. (2003) The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. *The Plant Journal* 35: 613-623.
98. Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. *The Plant Cell* 16: S18-S31.
99. Vijayraghavan U, Prasad K, Meyerowitz E (2005) Specification and maintenance of the floral meristem: interactions between positively-acting promoters of flowering and negative regulators. *Current Science* 89:1835-1843.
100. Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES (2000) The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). *Proceedings of the National Academy of Sciences* 97: 3753-3758.
101. Roux F, Touzet P, Cuguen J, Le Corre V (2006) How to be early flowering: an evolutionary perspective. *Trends in Plant Science* 11: 375-381.
102. Soltis DE, Soltis PS, Albert VA, Oppenheimer DG, Ma H, et al. (2002) Missing links the genetic architecture of flower and floral diversification. *Trends in Plant Science* 7: 22-31.
103. Jack T (2004) Molecular and genetic mechanisms of floral control. *The Plant Cell* 16:S1-S17.
104. Pelaz S, Gustafson-Brown C, Kohalmi SE, Crosby WL, Yanofsky MF (2001) APETALA1 and SEPALLATA3 interact to promote flower development. *The Plant Journal* 26: 385-394.
105. NCBI database 2017.