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

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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-à-vis 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 juvenile period is in general longer, effective manipulation of this event is desired in modern production system since it influences 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 assist future molecular genetic studies, like linking genes 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-à-vis 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.

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. Mouhut 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 MdMADS1 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).
<table>
<thead>
<tr>
<th>Flowering Gene(s)</th>
<th>Plant</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><em>FT</em> and <em>CiFT</em></td>
<td>Trifoliate orange (<em>Poncirus trifoliata</em> L. Raf.), ‘Moncada’ mandarin, sweet orange (<em>Citrus sinensis</em> (L.)), Satsuma mandarin (<em>Citrus unshiu</em> Marc.).</td>
<td>[35,56,57,13]</td>
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<tr>
<td><em>CsPH5,CsPH5 6</em></td>
<td>Citrus sp.</td>
<td>[68]</td>
</tr>
<tr>
<td><em>TFL, LFY &amp; AP</em></td>
<td><em>Citrus sinensis</em> L.</td>
<td>[63,14,16]</td>
</tr>
<tr>
<td><em>AP3, SOC1, WUS, SPL, miR156, CsAP1,CsLFY, SOC, 3 CiFT, PtFT1,CiFT, Hd3a,SFT</em></td>
<td>Citrus sp.</td>
<td>[69,49,70]</td>
</tr>
<tr>
<td>DNA methylation of <em>CiLFY,AP, FT</em></td>
<td>Citrus sp.</td>
<td>[71]</td>
</tr>
<tr>
<td><em>FT/TFL1 VuMADS1, VuMADS5, VuMADS10 and YAP1</em></td>
<td>Sweet orange (<em>Citrus sinensis</em>)</td>
<td>[105]</td>
</tr>
<tr>
<td><em>FCA, FA, FT,AP3, FLC, FY</em>, protein EARLY FLOWERING, LAR2*</td>
<td>Grapevine (<em>Vitis vinifera</em>)</td>
<td>[72, 73, 15, 20]</td>
</tr>
<tr>
<td><em>FT,Micol, MiFT,MiGA 20-oxy, MiGA3-oxy and MADS-box cDNA</em></td>
<td>Mango (<em>Mangifera indica L.</em>)</td>
<td>[74,59,75]</td>
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<tr>
<td><em>AGAMOUS MADS-box factor</em></td>
<td>Banana (<em>Musa sp.</em>)</td>
<td>[76,77,78]</td>
</tr>
<tr>
<td><em>MuaMADS1,MuaMADS3</em></td>
<td>Wild banana (<em>Musa acuminate</em>)</td>
<td></td>
</tr>
<tr>
<td><em>FLC, FLT, LFY, CO1,Fl</em> &amp; floral organ formation gene</td>
<td>Perennial plants</td>
<td>[5,1]</td>
</tr>
<tr>
<td><em>MdFT1 &amp; MdFT2</em></td>
<td>Apple (<em>Malus domestica</em> Borkh.).</td>
<td>[39]</td>
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<tr>
<td><em>MdFT1-1</em> &amp; <em>MdFT2</em></td>
<td>Apple (<em>Malus domestica</em> cv ‘Pinova’)</td>
<td></td>
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<tr>
<td><em>MdFT1-1</em> &amp; <em>MdFT2</em></td>
<td>Apple (<em>Malus domestica</em> cv ‘Pinova’)</td>
<td>[74,8,79,80]</td>
</tr>
<tr>
<td><em>BpMADS4</em></td>
<td>Apple (<em>Malus domestica</em> cv ‘Pinova’)</td>
<td>[64]</td>
</tr>
<tr>
<td><em>MdMADS4</em></td>
<td>Apple (<em>Malus domestica</em> cv ‘Pinova’)</td>
<td>[38]</td>
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<tr>
<td><em>CiFT, RHV region</em></td>
<td>Pears (<em>Pyrus communis</em> L.)</td>
<td>[36,81]</td>
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<tr>
<td><em>PcFT1-1</em> &amp; <em>PcFT1-2</em></td>
<td>European pear (<em>Pyrus communis</em> subsp. communis),</td>
<td>[8]</td>
</tr>
<tr>
<td><em>New self-incompatibility alleles, S-RNase, F-box, SFB and QTL on G5</em></td>
<td>Apricot (<em>Prunus armeniaca</em> L.)</td>
<td>[82-85]</td>
</tr>
<tr>
<td><em>F-box, MiFT, FY,FPA</em>, flowering-promoting factor, MADS-box protein*</td>
<td>Japanese apricot (<em>Prunus mume</em>)</td>
<td>[86,87,105]</td>
</tr>
<tr>
<td><em>F-box, QTL in G4,G1, G3 &amp; G7; PrdMADS 1,2,3</em></td>
<td>Almond (<em>Prunus dulcis</em>)</td>
<td>[88-90]</td>
</tr>
<tr>
<td><em>PrpMADS 2,4,6</em></td>
<td>Peach (<em>Prunus persica</em>)</td>
<td>[91]</td>
</tr>
<tr>
<td><em>MADS-box gene</em></td>
<td>Peach (<em>Prunus persica</em>)</td>
<td></td>
</tr>
<tr>
<td><em>CoTFL1-1 &amp; CoTFL1-2</em></td>
<td>Quince (<em>Cydonia oblonga</em>)</td>
<td>[8]</td>
</tr>
<tr>
<td><em>EjTF1-1 &amp; EjTF1-2</em></td>
<td>Loquat (<em>Eriobotrya japonica</em>)</td>
<td>[8]</td>
</tr>
<tr>
<td><em>FY-like chromosome LG6, NC_020496.1</em></td>
<td>Wild strawberry (<em>Fragaria vesca</em>)</td>
<td>[92,105]</td>
</tr>
<tr>
<td><em>QTL in LG4, LG6, LG7</em></td>
<td>Prunus sp., peach, apricot and sweet cherry</td>
<td>[93]</td>
</tr>
</tbody>
</table>

Table 1: Flowering Gene and Genomic Region in Fruit Plants.
isolated from sweet orange [14] and grapevine [15] act as floral inhibitors. Expression of *LFY* and *AP1* 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].

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**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 *AP1* 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 *AP1* 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].

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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 MdTFL1-1 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).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>Transcription factor of the FT gene</td>
<td>[94,95]</td>
</tr>
<tr>
<td>FT, PfFT1, CIFT, Hd3a, and SFT</td>
<td>Transition from the vegetative to the reproductive stage. Implicated in the formation of axillary meristems Promoting early flowering in citrus</td>
<td>[69,96]</td>
</tr>
<tr>
<td>MdMAD1 and MdMADS2</td>
<td>Initiation of flower organs in apple</td>
<td>[80]</td>
</tr>
<tr>
<td>SOC1</td>
<td>Enhance the transcription of the floral meristem identity gene LFY</td>
<td>[97]</td>
</tr>
<tr>
<td>FLC</td>
<td>Repressing of the floral pathway integrators CO, LFY and SOC1</td>
<td>[98]</td>
</tr>
<tr>
<td>FLD, FLK and LD</td>
<td>Suppress the transcription of FLC activate the floral induction gene FT</td>
<td>[99]</td>
</tr>
<tr>
<td>FRI and VIP</td>
<td>Up-regulated FLC gene</td>
<td>[100]</td>
</tr>
<tr>
<td>SMZ, SNZ and TFL</td>
<td>Suppress the floral pathway integrator genes and floral meristem identity genes</td>
<td>[101]</td>
</tr>
<tr>
<td>LFY</td>
<td>Repressor of TFL1 and initiation of floral meristems as well as floral organs</td>
<td>[99]</td>
</tr>
<tr>
<td>AP1 and AP2</td>
<td>Activates organ identity genes such as AP3, PI and AG</td>
<td>[102,99]</td>
</tr>
<tr>
<td>TFL1</td>
<td>Inflorescence meristem identity gene and a floral inhibitor</td>
<td>[102,99]</td>
</tr>
<tr>
<td>MIKC-type and MADS-box genes</td>
<td>Transcriptional activation of flowering gene</td>
<td>[103]</td>
</tr>
<tr>
<td>MADS-box and SEP genes</td>
<td>Proper development of petal, stamen and carpel identity in Arabidopsis</td>
<td>[104]</td>
</tr>
<tr>
<td>Ectopic expression of AG, AP3, PI and SEP3</td>
<td>Convert leaves to organs that resemble stamens flower organ development</td>
<td>[104]</td>
</tr>
<tr>
<td>LFY and AP1</td>
<td>Expressed in citrus which drastically reduced the length of the juvenile phase</td>
<td>[63]</td>
</tr>
<tr>
<td>MdTFL1</td>
<td>Down regulation of this gene led to flower induction in apple controlling the transition from the juvenile/vegetative to the reproductive phase in apple.</td>
<td>[40]</td>
</tr>
</tbody>
</table>
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 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 \( FT, SOC1, AP1 \) 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, \( FT, LFY, AP1, TFL \) 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 \( CsFT, CsSL1, CsAP1, \) and \( CsLFY \) were accumulated under controlled conditions. Exposure to water deficit increased the accumulation of \( CsFT \) transcripts, whereas, transcripts of \( CsSL1, CsAP1, \) 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 \( CsSL1, CsAP1, \) 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 \( FT \) 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\( Poncitrus trifoliate \) \( L. \) \( Raf. \), 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 \( AP1 \) 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 \( Poncitrus trifoliate \) \( L. \) \( Raf. \), over-expression of \( LFY \) and \( AP1/orthologues \) 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 \( MdTFL1 \)-Iw 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 understanding 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.

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**References**


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.


