

# Exogenous and Endogenous Cues Regulating Genes for Flowering in Subtropical Fruits an Over View

Nayan Deepak, G<sup>1</sup>, Ashok Yadav<sup>2</sup>, K. Usha<sup>3</sup>, M.K. Verma<sup>4</sup> and Sanjai Sirohi<sup>5</sup>

<sup>1,2,3,4,5</sup>Division of Fruits and Horticultural Technology, ICAR- Indian Agricultural Research Institute, New Delhi-12

E-mail: <sup>1</sup>nayan.iari@gmail.com, <sup>2</sup>ashokiari1@gmail.com, <sup>3</sup>kalidindi.usha3@gmail.com,

<sup>4</sup>mahenicar10@gmail.com, <sup>5</sup>sanjaisirohi@gmail.com

---

**Abstract**—The subtropical fruits categorised as fleshy fruits (mango, guava, papaya, grapes, banana, citrus, avocado, litchi, pineapple, loquat, kiwi fruit etc.) and nuts (pecan, walnuts, almonds etc) are having high nutraceutical value (vitamins, minerals, protein, oils and fats) and play a pivot role in maintaining the human health in the present era. Subtropical fruits require warmer conditions and are sensitive to frost and fluctuations in temperature conditions. The information regarding exogenous and endogenous cues that regulate flowering genes in subtropical fruits would help in understanding the major pathways, genomic regions for the reproductive success and fruit crop productivity. Due to increase in population day by day, along with the limited available natural resources like land, water, high pressure and drastic climate change, there is an urgent need to meet challenges by fulfilling the nutritional requirement. To know the problems of flowering in fruit trees understanding tree architecture and impacts of climate change is a key for suggesting the possible horticultural interventions. *Geno-Horti* concept, phosphoproteome mapping, differential gene expression analysis, genetic engineered enzymes and many other biotechnological tools will be helpful for understanding the mechanism regulating flowering genes and their function. This paper gives an overview of the exogenous and endogenous cues that control flowering genes in subtropical fruit crops and the impact of horticultural interventions on flowering.

## 1. INTRODUCTION

The Subtropical fruits require warmer conditions and are sensitive to frost and fluctuations in temperature conditions. Fruits are having high nutraceutical value (vitamins, minerals, protein, oils and fats) and play a pivot role in maintaining the human health. Subtropical fruits are categorised as fleshy fruits (mango, guava, papaya, grapes, banana, citrus, avocado, litchi, pineapple, loquat, kiwi fruit etc.) and nuts (pecan, walnuts, almonds etc). Flowering is a major event in life cycle of subtropical fruit crops. The MADS-box family of genes has a significant role in the development of flowering organs in fleshy and dry fruits. The most dramatic changes occur during floral transition in life cycle of plant and regulated by bulk number of genes. The number and quality of flower buds leads to flowering and fruit yield, which depends on control of

numerous external and internal signals. These genes include flower meristem identity genes and floral organ genes. Environment factors like photoperiod, gibberlins, vernalization, autonomous floral initiation and aging determines the flowering period. Some common set of genes (FLOWERING LOCUS C, FLOWERING LOCUS T, LEAFY, and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1) determines flowering time. Certain complex growth correlations determine reproductive transition and difficult to know the certainty of branch that will produce flowering buds [28]. This is main handicap to understand the mechanism of flowering in fruit trees. There is need to understand the impacts of exogenous, endogenous factors and climate change directly or indirectly by regulating flowering genes. This paper helps in understanding the genes, environment and other factors responsible for flowering in subtropical fruit crops.

## 2. FACTORS RESPONSIBLE FOR FLOWERING

Factors affecting flowering in subtropical fruits are juvenility, age of wood, tree architecture, position of buds, dormancy, water stress, temperature, photoperiod, hormone levels, alternate bearing, fruit load, Carbon: Nitrogen levels and climate change. A number of biochemical changes like differences in peroxidase, esterase isozymes protein phosphorylation and endogenous hormone levels of auxin, abscisic acid, cytokinin and ethylene are involved in the juvenile-to-adult phase transition. The timing of flush development for flowering is important because bud release for vegetative or reproductive growth can only occur from mature flush [29]. The higher and lower axialization leads to vegetative and floriferous axils respectively [20]. Litchi tends to produce inflorescences from terminal buds, whereas avocado produces inflorescences from both terminal and axillary buds [30]. The proteins of photosynthetic machinery represent the majority of leaf nitrogen which is directly related to photosynthetic capacity in C/N theory. In grapevine floral

initiation occurs in uncommitted primordia of developing latent buds, destined for dormancy and subsequent release in the spring [1]. Moderately low water potentials delay shoot initiation through reduced turgor thus extending the age of stems. Despite the role of water stress, a period of cool and dry weather for flowering and long stress period reduce flower size and fruit set. In avocado, temperatures of 10/7°C (day/night) were effective in inducing flower buds, whereas at 25/20°C (day/night) regime was not. In kiwifruit buds enter dormancy in response to shortening day length [31]. Heavy fruit load prevents recognition of the low-temperature flowering inductive signal and/or blocks later stages of inflorescence, such as bud break.

### 3. FUNCTIONS OF SOME FLOWERING GENES

Co gene is responsible for transcription factor of the *FT* gene [23]. Formation of axillary meristems and early flowering in citrus was dependable by the genes *FT*, *PtFT1* and *SFT* [13], whereas, genes *LFY* and *API* reduces vegetative phase [31]. *SMZ*, *SNZ* and *TFL* suppress the floral pathway integrator genes and floral meristem identity genes [34]. Transcriptional activation of flowering gene by the genes *MIKC*-type and *MADS*-box genes [16].

### 4. GENES IDENTIFIED IN SOME SUBTROPICAL FRUIT CROPS AND THEIR ROLES FOR FLOWERING

Flowering in mango is a complex process and not photoperiodic. Mango flowers in cool inductive temperatures under subtropical conditions. By manipulating through environment and at gene expression level we can get of-season flowering and yield. Climate plays a major role in growth and development of mangoes. By girdling and complete defoliation causes zero flowering [33], whereas five leaves in the donor stems produced 100% flowering shoots in all donors and receivers. When “Keitt” trees exposed to cool temperatures with 1/2 leaf per stem resulted in 100% reproductive shoots and 1/4 leaf per stem caused 95% flowering of lateral shoots [7]. Florigenic promoter is synthesized in leaves and transported to buds and *Fp* is carried in the phloem with sugars from the donor leaves to the receiver stems to facilitate floral induction [38]. Low levels of expression under warm temperature was observed in buds of gene encoding protein, similar to *APETALA1* (flower initiation) while, at low temperature high levels of expression [25]. Levels of *FT* were slightly lower in the apical buds of high-fruit-load trees.

Under subtropical conditions lemon trees produces flower round the year. Citrus expressed sequence tag (*CitEST*) database for expressed sequence tags (*ESTs*) showing sequence homology with known elements of flowering-time pathways [24]. *CONSTANS (CO)* and *FLOWERING LOCUS C (FLC)* are two genes responsible for flowering and ultimately lead to the induction of a set of genes called floral meristem

identity (*FMI*) genes and responsible for the fate change of the meristems emerging on the flanks of the shoot apex. Expression pattern of flowering-genes in buds from on (fully loaded) and off (without fruits) trees revealed that homologues of *FLOWERING LOCUS T (CtFT)*, *TWIN SISTER OF FT (TSF)*, *APETALA1 (CsAP1)* and *LEAFY (CsLFY)* were negatively affected by fruit load. By contrast, expression of the homologues of the flowering inhibitors of *TERMINAL FLOWER 1 (CtTFL)*, *TERMINAL FLOWER 2 (TFL2)* and *FLOWERING LOCUS C (FLC)* was generally lower in off trees. Absence of leaves completely abolished blossoming and severely affected the expression of most of the flowering-related genes, particularly decreasing the activity of floral promoters and of *CsAP1* at the induction stage [27].

**Grapevine** - The *Vitis vinifera* flowering is complex phenomena and is greatly affected by environment, genetic and cultural practices from induction to anthesis, which require 12-month period [37]. The Critical stages in grape flowering are induction, initiation, and early differentiation in first season and late differentiation at budburst in second season. In spite of many research on the influence of cultural and environmental factors on grape flower development, still the genetic factor controlling grapes remains poorly understood. Synchronous flowering development is advantageous for grape production whereas non-synchronous is viewed as undesirable, so to overcome the problem of non synchronous flowering it is essential to have the prior knowledge of flowering gene.

#### a. Floral development in grapevine

The flower development in grape occurs through a series of sequential manner under strict genetic control [26]. In Model plant *i.e.* *Arabidopsis*, gene analysis have been carried out by developing *Arabidopsis* mutants to understand the molecular and genetic mechanisms which are involved in floral induction to flower development [2]. Due to long life cycle of grapevine, difficulty in development of the mutant and transgenic plant, the studies on floral initiation and development at molecular level are still not complete because the genes identified need to be characterized functionally [3]. The genetic change involving in the flower development is as follows

**i. Switch from vegetative to the floral state:** It occurs mainly due to difference in environmental and developmental signals by activity of floral meristem identity genes.

**ii. Floral meristem to whorls of organ primordia:** It occurs mainly due to activity of floral-organ identity genes.

**iii. Floral organ primordia to different floral structures:** The genes activate downstream effectors that affect the various tissues which constitute different floral structures [5].

#### b. Floral induction in grape vine

In Grapevine, with the help of grape genome [2] data the three members of the SUPPRESSOR OF CONSTANS1/

AGAMOUS LIKE 20 (SOC1/AGL20) MADS box gene subfamily have been identified. For flowering *V. vinifera* MADS8 (VvMADS8), has been characterized and its expression level is very high in the early stages of inflorescence development and decreases in later stages of floral development whereas, in mature flower or fruit its expression was not found. In grapevine characterization of the floral signal integrator FLOWERING LOCUS T (FT) has also been done [3]. Out of six possible TFL1- FT homologues from whole genome sequence of grape five genes are categorized into three categories *i.e.* FT, MFT (MOTHER OF FT AND TFL1), and TFL1 [13]. The VvFT, is associated with seasonal floral induction and formation of inflorescences, flowers, and fruits [3].

### c. Floral meristem identity in grapevine

Bud bursting occurs in spring where VFL expression reaches to maximum in the floral meristem and also expressed in petal, stamen primordia, leaf primordia and leaf margins responsible for cell proliferation in specific leaf tissues in *Vasconcella* sps. VFUL-L transcripts are restricted to the central part of flower meristem, which plays role in floral transition, carpel and fruit development [2].

### d. Floral organ identity:

Development of floral organs is controlled by a complex genetic regulatory. A, B, C, D and E models were identified for flowering in model plants [19]. In grapes, identified three B-function genes VvMADS9/VvPI, VvAP3, and VvTM6 responsible for petals and stamens but VvTM6 also expressed in carpels. Higher expression of VvPI is during pollen maturation and decreases at process of pollination and fertilization. At last stage of anther development VvTM6 is expressed.

In avocado, PaFT protein was more closely related to other FT and TSF proteins than to BFT or TFL proteins and PaFT is annotated as gene bank accession number KM023154. In case of other crop trees, fruit-load might affect flowering by repressing the expression of PaFT in the leaves [6].

The flowering homologs, FT and LFY, isolated from *Carya illinoensis* contain the domains previously characterized in FT and LFY genes in other plant species [15]. In pineapple, ethylene production leads to reproductive phase due to ACC synthase gene ACACS2 [39]. Nine non-redundant kiwifruit MADS-box genes were identified on the basis of similarity to Arabidopsis floral MADS-box genes, and named Actinidia FUL-like, FUL, AP3-1, AP3-2, PI, AG, SEP1, SEP3 and SEP4 [10].

Kiwifruit MADS-box genes with homology to a floral pathway integrator gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)* and floral repressors *SHORT VEGETATIVE PHASE (SVP)* and *FLOWERING LOCUS C (FLC)* were identified [10]. CARgbox sequence binding AGAMOUS MADS-box protein

in banana flower and fruit nuclear extracts in DNA-protein interaction assays [35]. The deduced protein sequence showed 95% amino acid sequence homology with MA-MADS5, a MADS-box protein.

## 5. DIFFERENT MANAGEMENT STRATEGIES FOR FLOWERING

For flowering transition in subtropical fruit crops require reduced vegetative activity. Alterations include water stress, drought, branch manipulation, mechanical shoot and root pruning, application of hormones, chemical growth regulators, manipulating crop load, fertilizer management and regulated deficit irrigation [4]. Winter pruning increases vegetative growth and decreases flowering. The effects of pruning also vary with the architectural level, the complete removal of 1-year-old short lateral shoots tends to stimulate growth of the remaining shoots and fruit set of adjacent inflorescences. The pruning strategies should be based on the removal of flowering shoots at a young stage of growth, with less pruning of old branches [22] which increases leaf area of remaining shoots and light interception by the tree canopy through a decreased shoot density and a better distribution of shoots in space. A range of rest-breaking chemicals like dinitro-ocresol (DNOC), thiourea, gibberellic acid (GA3), various surfactants, various oils, potassium nitrate, hydrogen cyanamide and combinations of chemicals [9] were using. Dormex (H2HCN), Volk oil and potassium nitrate are few other examples of commercial dormancy breaking chemicals [32]. Hydrogen cyanamide is highly effective in breaking the dormancy of grape buds and kiwi vines. Thinning agents such as the benzyladenine (BA) or the Naphtalene acetic acid (NAA) are used in mango during heavy bearing season. Paclobutrazol is substitute provided by mild water stress or low nitrogen to obtain flowering on younger stems. There is an increase both endogenous cytokinin levels and floral initiation in 'Japanese pear' after application of the growth retardant maleic hydrazide [14]. The role of C/N ratio for floral initiation has often been investigated by measuring levels of stored carbohydrates or imposing treatments such as fruit thinning, girdling that modify the levels of stored carbohydrates, and correlating these with flowering intensity in many woody perennials [12]. Urea enhances initiation of citrus flowering [8]. In Strawberry number of flowers and inflorescences increased when plants were applied with potassium nitrate [17]. When a plant of mango, citrus or litchi is exposed to warm temperatures (30° C day/25° C night) [36] at the time of shoot initiation, the resulting shoot growth is purely vegetative and if maintained in cool conditions (18 °C day/10 °C night), it produces generative shoots. Mild water stress applied during the period of slow fruit growth control excessive vegetative growth while maintaining or even increasing yields. Moderate and severe deficit irrigation advances flowering date while light water-stress caused reduction in watering along the season, failed to modify flowering date in loquat [5].

## 6. CONCLUSION

Floral initiation and flowering in fruit trees is most important event for the reproductive success and crop productivity. For successful fruit production key strategy is ability to control the timing of flowering. It should also be noted that utilizing a QTL-based approach may uncover novel genes/proteins that control flowering via heavy fruit load. These will shed much light on the conservation and differentiation of function of genes predicted to be involved in floral development that leads to the unique phenology of flowering in fruit crops. Although leaves are required for flowering to occur, their absence strongly affects the activity of floral promoters and identity genes. The salient features and differences between the phenologies of these two groups are necessary to understand the problems of flowering and for suggesting possible horticultural interventions. This provides a description of flower development in some fruit crops at the molecular level.

## REFERENCES

- [1] Boss P. K., Buckeridge E. J., Poole A, Thomas M. R, “New insights into grapevine flowering” *Functional Plant Biology*, 2003, 30 pp 593–606.
- [2] Carmona, M. J., J. Chaib, J. M. Martinez-Zapater and M. R. Thomas., “A molecular genetics perspective of reproductive development in grapevine”, *J. Exp. Bot.* 2008, 2579.
- [3] Carmona, M., M. Calonje, and J. Martínez-Zapater., “The FT/TFL1 gene family in grapevine”, *Plant Mol. Biol.* 2007, 63: pp 637-650.
- [4] Chalmers, D. J., Mitchell, P. D. & Jerie, P. H, “The physiology of growth control of peach and pear trees using reduced irrigation”, *Acta Horticulturae*, 1984, 146, pp 143-149. *PLoS ONE* 9(10): e110613.
- [5] Cuevas, J., Cañete, M.L., Pinillos, V., Zapata, A.J., Fernández, M.D., González, M., Hueso, J.J., “Optimal dates for regulated deficit irrigation in ‘Algerie’ loquat (*Eriobotrya japonica* Lindl.) cultivated in southeast of Spain”, *Agric. Water Manage*, 2008, 89, pp 131–136.
- [6] Dafna Ziv, Tali Zviran, Oshrat Zezak, Alon Samach, Vered Irihimovitch, “Expression Profiling of FLOWERING LOCUS T-Like Gene in Alternate Bearing ‘Hass’ Avocado Trees”,
- [7] Davenport, T. L., “Pruning strategies to maximize tropical mango production from the time of planting to restoration of old orchards” *HortScience*, 2006, 41, pp 544–548.
- [8] Davenport, T.L., “Mango: reproductive physiology”, in: DaMatta, F., (ed.), *Ecophysiology of Tropical Tree Crops*, Nova Science Publishers, Inc. New York. 2010, pp. 217-234.
- [9] Erez, A, “Means to compensate for insufficient chilling to improve bloom and leafing” *Acta Hort.*, 1995, 395: pp 81-95.
- [10] Erika Varkonyi-Gasic, R. Wu, S. Moss and R.P. Hellens, “Genetic Regulation of Flowering in Kiwifruit” in *Proc. VIIIth IS on Kiwifruit*. Acta Hort. 913, 2011, 221-227.
- [11] Erika Varkonyi-Gasic, Sarah M Moss, Charlotte Voogd, Rongmei Wu, Robyn H Lough, Yen-Yi Wang and Roger P Hellens, “Identification and characterization of flowering genes in kiwifruit: sequence conservation and role in kiwifruit flower development”, *BMC Plant Biology*, 2011, 11: 72.
- [12] Goldschmidt, E.E. “The evolution of fruit tree productivity: a review”. *Economic Bot.*, 2013, 67, pp 51–62.
- [13] Huang, X., Ding, J., Effgen, S., Turck, F., Koornneef, M, “Multiple loci and genetic interactions involving flowering time genes regulate stem branching among natural variants of *Arabidopsis*”, *New Phytol.* 2013, 199, pp 843–857.
- [14] Ito, A., Hayama, H., Kashimura, Y., Yoshioka, H., “Effect of maleic hydrazide on endogenous cytokinin contents in lateral buds, and its possible role in flower bud formation on the Japanese pear shoot”, 2001, *Scientia Hort*, 2001, 87, 199–205.
- [15] J. J. Randall, A. Rascon, R. J. Heerema and M.T. Potter, “Molecular Mechanisms of Pecan Flower Induction” in *Proc. 1st IS on Pecans and Other Carya in Indigenous and Managed Systems*. *Acta Hort.* ISHS, 2015, pp 89-99.
- [16] Jack, T, “Molecular and genetic mechanisms of floral control”, *Plant Cell*, 2004, 16, pp 1-17.
- [17] Khayat M, Rajae S, Shayesteh M, Sajadinia A, and Moradinezhad F, “Effect of potassium nitrate on breaking and dormancy in strawberry plants”, *J Plant Nutr.* 2010. 33(11):pp 1605-1611.
- [18] Krizek, B. A and J. C. Fletcher, “Molecular mechanisms of flower development”: An armchair guide, *Nat. Rev. Genet.* 2005, 6: pp 688-698.
- [19] Krizek, B., “Molecular biology of floral organogenesis in The Molecular Biology of Flowering”, *B. Jordan (ed.)*, CAB International, King’s Lynn, UK, 2006, pp 100-123.
- [20] Lauri, P. E and Kelner, J. J, “Shoot type demography and dry matter partitioning: a morphometric approach in apple (*Malus domestica*)” *Canadian J. Bot.* 2001, 79, pp 1270–1273.
- [21] Lionakis S. M and Schwabe W. W, “Bud dormancy in the kiwi fruit, *Actinidia chinensis*”, *Annals of Botany*, 1984, 54, pp 467–484.
- [22] Lauri, P.É., Willaume, M., Larrive, G., Lespinasse, J.M., 2004. The concept of centrifugal training in apple aimed at optimizing relationship between growth and fruiting. *Acta Hort.* 636, 35–42.
- [23] Liu, L., Farrona S, Klemme, S., Turck, F.K, Post-fertilization expression of *FLOWERING LOCUS T* suppresses reproductive reversion. *Front. Plant Sci.* 2014.00164
- [24] Marcelo Carnier Dornelas, Raquel Luciana Boscaroli Camargo, Luciana Harumi Morimoto Figueiredo and Marco Aurélio Takita, “A genetic framework for flowering-time pathways in *Citrus* spp.” *Genetics and Molecular Biology*, 2007, 30, 3 (sup), pp769-779.
- [25] Masahiro Nakagawaa, Chitose Honshob, Shinya Kanzakia, “Isolation and expression analysis of FLOWERING LOCUS T-like and gibberellins metabolism genes in biennial-bearing mango trees”, *Sci. Horti.* 139, 2012, pp 108–117.
- [26] Meneghetti, S., M. Gardiman and A. Calo, “Flower biology of grapevine-A review.” *Adv. Hort. Sci.*, 2006, 20: pp317-325.
- [27] Natalia Munoz-Fambuena, Carlos Mesejo, M. Carmen Gonzalez-Mas, Eduardo Primo-Millo, Manuel Agusti and Domingo J. Iglesias, “Fruit load modulates flowering-related gene expression in buds of alternate-bearing ‘Moncada’ mandarin” *Annals of Botany*, 2012, 110: pp1109–1118.
- [28] Nocker, S. V., Gardiner, S. E., “Breeding better cultivars, faster: applications of new technologies for the rapid deployment of superior horticultural tree crops -Mini review”. *Hort. Res.* 2014. 22.

- [29] Nunez-Elisea, R and Davenport, T. L., "Effect of leaf age, duration of cool temperature treatment, and photoperiod on bud dormancy release and floral initiation in mango", *Scientia Hort.* 1995, 62, pp 63–73.
- [30] Olesen, T., "The timing of flush development affects the flowering of avocado (*Persea americana*) and macadamia (*Macadamia integrifolia tetraphylla*)", *Aust. J. Agric. Res.* 2005, 56, pp 723–729.
- [31] Pelaz, S., Gustafson-Brown, C., Kohalmi, S.E., Crosby, W.L., Yanofsky, M.F., "APETALA1 and SEPALLATA3 interact to promote flower development". *The Plant Journal*, 2001. 26, pp 385-394.
- [32] Rahemi M, and Asghari H, "Effect of hydrogen cyanamide, volk oil and potassium nitrate on bud break, yield and nut characteristics of pistachio", *J Hort Sci. Biotech*, 2004, 79(5), pp 823-827.
- [33] Ramirez, F., Davenport, T. L., Fischer, G., "The number of leaves required for floral induction and translocation of the florigenic promoter in mango (*Mangifera indica* L.) in a tropical climate". *Sci Hort.* 2010, 123, pp 443–453.
- [34] Roux, F., Touzet, P., Cuguen, J., LeCorre, V., "How to be early flowering: an evolutionary perspective" *Trends in Plant Science*, 2006, 11, pp 1360-1365.
- [35] Swarup Roy Choudhury, Sujit Roy, Anish Nag, Sanjay Kumar Singh, Dibyendu N. Sengupta, "Characterization of an AGAMOUS-like MADS Box Protein, a Probable Constituent of Flowering and Fruit Ripening Regulatory System in Banana" *Plos one*, 2012. Vol 7: 9, e44361.
- [36] Sukhvibul, N., Hetherington, S.E., Vithanage, V., Wiley, A.W., Smith, M.K., "Effect of temperature on inflorescence development and floral biology of mango (*Mangifera indica* L.)", *Acta Hort.* 2000, 509, pp 601–607.
- [37] Vasconcelos, M. C., Greven, M., Winefield, C. S., Trought, M. C and Raw, V., "The flowering process of *Vitisvinifera*: a review" *American Journal of Enology and Viticulture*, 2009, 60(4), pp 411-434.
- [38] Thomas L. Davenport, Zhentu Ying, Vinod Kulkarni, Thomas L. White, "Evidence for a translocatable florigenic promoter in mango", *Scientia Horticulturae*, 110, 2006, pp 150–159.
- [39] Yuri Trusov and Jose' Ramo'n Botella, "Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple [*Ananas comosus* (L.) Merr.]", *Journal of Experimental Botany*, 2006, 57, pp. 3953–3960.