CHAPTER NINE

The Control of Bud Break and Flowering Time in Plants: Contribution of Epigenetic Mechanisms and Consequences in Agriculture and Breeding

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Contents

1. Introduction 278
2. Control of Dormancy and Flowering Time 280
   2.1 Chilling Requirement 280
   2.2 QTLs for Bud Dormancy 282
   2.3 Epigenetic Modifications of *FLC* x Dynamic Model of CR 283
3. Epigenetic as a Key Mechanism for Dormancy Regulation 286
   3.1 Chromatin Modifications during Dormancy and Bud Break 286
   3.2 Epigenetic Regulations of *DAM* and *EBB* Genes 288
4. *FT*: A Master Switch Controlling Flowering in Angiosperms 290
   4.1 The Florigen *FT* Integrates Signals from Different Floral Pathways 290
   4.2 Epigenetic Control of *FT* Expression 293
5. Chromatin Remodeling and Hormonal Stimulus: An Inherent Part of the Flowering Process 297
   5.1 Gibberellins 297
   5.2 Jasmonic Acid 299

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5.3 Brassinosteroids 300
5.4 Ethylene 301
5.5 Salicylic Acid 301
5.6 Cytokinins 302
5.7 Abscisic Acid 304
5.8 Auxins 305
6. Bud Break and Flowering: Consequences in Agriculture and Breeding 306
Acknowledgements 310
References 310

Abstract

In perennial plants, the release of bud dormancy, with subsequent flowering, resembles the vernalization process of *Arabidopsis thaliana* and cereals. Especially for perennial crops from temperate regions, dormancy is an important adaptive trait for both survival and growth. Exposure to sufficient chilling during winter dormancy ensures the normal phenological traits in subsequent growing periods. Here, we compile research data on mechanisms controlling the overlapping developmental processes that define dormancy induction, maintenance and release, bud burst and flowering. Recent findings highlight the relevance of genome-wide epigenetic modifications related to dormancy events, and more specifically the epigenetic regulation of *DORMANCY-ASSOCIATED MADS-box*, *FLOWERING LOCUS C* and *FLOWERING LOCUS T* genes, key integrators of vernalization effectors on flowering. The roles of plant growth regulators in controlling bud break and flowering are discussed in relation to epigenetic mechanisms. A growing body of knowledge demonstrates that epigenetic regulation plays a key role in these processes in perennial horticultural and forestry plants. We discuss the most relevant molecular and genomics research that contribute to better understanding of the dormancy process and pave the way to precise manipulation of dormancy-related horticultural traits, such as flowering time. Finally, some of the challenges for further research in bud dormancy and consequences in agriculture are discussed within the context of global climate change.

1. INTRODUCTION

Most of perennial tree species in temperate areas are cultivated in regions with well-differentiated seasons. In addition, perennials differ from annual and biennial lifestyles in their ability to turn on/off growth in response to environmental and seasonal changes. As an adaptation to long periods of unfavorable environmental conditions, including cold winters and low light availability, many perennial plants have developed a mechanism of protection called dormancy. The first definition of dormancy was proposed by Lang, Early, Martin, and Darnell (1987) as "the absence of

visible growth in any plant structure containing a meristem". Later, Rohde and Bhalerao (2007) proposed another definition aiming to accurate the expression "visible growth" and the theory that dormancy is not only absence of growth, but also include the inability to resume it. Dormancy was therefore redefined as "the inability to initiate growth from meristems (and other organs and cells with the capacity to resume growth) under favorable conditions" (Rohde & Bhalerao, 2007). This definition mainly describes the meristem states including axillary dormant buds with correlative inhibition or apical dominance, but does not include physical dormancy or ecodormancy (Rohde & Bhalerao, 2007), one of the three physiological stages of bud dormancy defined by Lang et al. (1987). Indeed, considering ecodormancy, growth inhibition is determined by adverse external stimuli and once environmental conditions become favorable, the meristematic activity restarts. The two other dormancy stages defined by Lang et al. (1987) are paradormancy and endodormancy. In paradormancy, distal organs signalize growth suppression (apical dominance), whereas in endodormacy, plant internal signals are determinant for growth arrest. Endodormant buds are not capable of resuming growth even when exposed to growth-promoting conditions. Visible changes can be observed in plants during dormancy establishment, maintenance and release. These include the cessation of apical growth, bud development, acquisition of cold/desiccation tolerance, and leaf senescence, as observed in deciduous species (Cooke, Eriksson, & Junttila, 2012). Initially, leaf primordia are modified to form hard scales during bud set (Horvath, 2010). When plants start resuming growth, bud burst can be observed as a swelling (green tip), followed by emission of young leaves and could be related to B and C stages of Fleckinger phenological scale (EPPO, 1984).

Within this context, buds are defined as "the primary shoot-producing meristematic organs for dicotyledonous plants" (Horvath, 2010) and are largely responsible for ensuring a new reproductive cycle in perennial species. Complex and orchestrated mechanisms that involve environmental stimulus, molecular pathways, hormonal signaling, and epigenetic modifications are responsible for both bud set and bud break. As a rule of thumb, photoperiod and temperature are the major signals that stimulate bud set, although their relative importance depends on the species (Cooke et al., 2012). In species of *Betula* (birch), *Populus* (aspen) and *Salix* (willow tree), photoperiod is the major stimulus (Howe, Gardner, Hackett, & Furnier, 1996; Junttila, 1976), whereas for *Malus* (apple), *Pyrus* (pear) and *Sorbus* (mountain ash) species, low temperatures are more determinant (Heide, 2011;

Heide & Prestrud, 2005). Dormancy is a cyclic and dynamic process and in some cases, the same stimuli that induce bud set are essential for dormancy release and bud break. In *Malus* species, for example, exposure to low temperatures during the autumn induces bud set, and continuous chilling exposure over the winter triggers the release from dormancy, indicating that the meristem responds to temperature changes during its endogenous development. The molecular mechanisms that control dormancy establishment and release are still far from a complete elucidation, although different models have been proposed (Campoy, Ruiz, & Egea, 2011; Horvath, 2009; Rinne et al., 2011).

Epigenetic modifications play a crucial role during dormancy regulation, from bud set to bud break, and are generally divided into active or repressive modifications. Active modifications include trimethylation of histone H3 lysine 4 (H3K4me3), di- or trimethylation of histone H3 lysine 36 (H3K36me2/me3), and acetylation of histone H3 lysine 9 (H3K9ac). Repressive modifications include histone deacetylation, trimethylation of histone H3 lysine 27 (H3K27me3), methylation of histone H3 lysine 9, and depending on its position relative to a gene, DNA methylation (see chapter 2 of this book). Nucleosome remodeling may also act to activate or repress gene expression (Guo et al., 2015; He, 2012).

In this chapter, we will discuss the already known regulatory mechanisms that regulate dormancy, bud break and flowering, focusing on the epigenetics events that could directly or indirectly influence these pathways. Potential biotechnological applications for breeding purposes are also presented.

2. CONTROL OF DORMANCY AND FLOWERING TIME

2.1 Chilling Requirement

Although the molecular mechanisms responsible for cold perception and rest completion in dormant buds are far from being fully understood, empirical knowledge about the effects of cold exposure on bud phenology is being employed successfully since a long time in dormancy research as well as for fruit production. Early conceptual models that are still in use today postulate that individual buds somehow record the amount of time of exposure to temperatures typically in the range from 0 to 7.2°C (Chandler, Kimball, Philp, Tufts, & Weldon, 1937; Lamb, 1948). These first models already provided a good correlation with bud phenology and proved to

be useful for researchers and growers. Temperature records from meteorological stations, often in hourly intervals, could be used to estimate dormancy status in tree orchards, and the concept of chilling hours (hours of exposure to temperatures that contribute to dormancy progression) became an important tool for bud dormancy research in trees.

Further analysis of the interaction between climate and bud phenology showed that under slightly warmer temperatures, bud dormancy still progresses, although not in the same pace as with lower temperatures. Towards warmer environments (nearly 15°C), bud dormancy remain unchanged, and when temperature is close to 20°C, the effect of cold is negated: the record of cold exposure is progressively erased and further exposure to chilling is necessary to compensate the period of time under warm temperatures (Erez, Couvillon, & Hendershott, 1979; Young, 1992). These discoveries were implemented in new bud dormancy completion models in which, instead of chilling hours, the exposure to cold temperatures is counted as chilling units, whereas the duration under warmer temperature is either considered of no effect or subtracted from the chilling unit count (Richardson, Seeley, & Walker, 1974; Shaltout, Unrath, & Akademiya, 1983). The exact ranges of temperatures are specific to each model.

All the aforementioned models based on ambient temperature data in temperate regions provide a good prediction of bud phenology. However, when applied to sites with warm winters, the predictive power of these models is not accurate enough. With that in mind, Erez, Fishman, Linsley-Noakes, and Allan (1990) developed a more elaborate conceptual model based on a two-step process for the memory of cold exposure. First, chilling (higher efficiencies occurring between 6°C and 8°C) induces the accumulation of a precursor, which is thermally labile. Therefore, this first reaction is reversible after sufficient time of exposure to higher temperatures. Second, when the precursor accumulates to a threshold value, a second reaction takes place that is not reversible. It produces, in conceptual terms, a stable factor that signals a fixed amount of cold exposure. This process is repeated several times during winter, until the number of dormancy-releasing factors (chilling units) reaches a critical level. The performance of this so called Dynamic Model has been shown to be superior to previous models in warm winter regions (Dennis, 2003; Erez et al., 1990).

The total amount of chilling hours (or chilling units) required for bud endodormancy release is referred to as chilling requirement (CR), and is genetically controlled (Hauagge & Cummins, 1991; Labuschagné, Louw, Schmidt, & Sadie, 2002; Ruiz, Campoy, & Egea, 2007). CR is

variable across cultivars of commercial tree species, which is highly valuable for breeding, allowing crops to achieve high yields in diverse climates.

2.2 QTLs for Bud Dormancy

The heritable nature of CR has been extensively used to map loci significantly contributing to this trait (Allard et al., 2016; Celton et al., 2011; Frewen et al., 2000; Graham et al., 2009; Urrestarazu et al., 2017; van Dyk, Soeker, Labuschagne, & Rees, 2010). Bud dormancy, at first glance, appears as a set of discrete phenotypes (*e.g.* endodormant or ecodormant buds) although in genetic research, it is considered as a quantitative trait. The phenotypic data used as input to quantitative trait loci (QTL) mapping is typically the time period, measured in days, from a reference date until half of all buds of each individual burst during the growing season. The reference date used in most cases is the time of bud flush of the early blooming parental genotype, and is directly related to the CR of each sibling.

One of the best characterized loci controlling bud dormancy in trees, however, was not mapped as a quantitative trait, but rather as a categorical trait with two discrete phenotypes: setting or not setting buds before winter. The peach genotype named "evergrowing" is unable to set cold-hardened buds, and the analysis of a population derived from a cross between *evergrowing* and a high CR peach cultivar allowed the identification of the *EVG* locus located in peach chromosome 1 as responsible for the trait (Wang, Georgi, Reighard, Scorza, & Abbott, 2002). Sequencing and annotation of the *EVG* locus revealed the presence of six MADS-box genes, whose sequences are similar to the *A. thaliana SHORT VEGETATIVE PHASE* (*SVP*) gene, which acts as a transcriptional repressor in distinct flowering and hormonal-signaling pathways (see Section 4, Gregis et al., 2013). These genes were named *DORMANCY-ASSOCIATED MADS-box* (*DAM*) genes 1 to 6 (Bielenberg et al., 2008) and their expression patterns were later shown to correlate with several phases of the dormancy-cycle, strongly suggesting them as important players for dormancy regulation in peach (Li, Reighard, Abbott, & Bielenberg, 2009). *DAM* genes were further identified in other perennials such as leafy spurge (Horvath, Sung, Kim, Chao, & Anderson, 2010), apricot (Sasaki et al., 2011; Yamane, Kashiwa, Ooka, Tao, & Yonemori, 2008), pear (Ubi et al., 2010) and apple (Falavigna et al., 2014; Mimida et al., 2015; Porto et al., 2016).

QTL mapping of CR in peach also pointed to the *EVG* locus as contributing to bud dormancy variation (Fan et al., 2010). Fine mapping of the

EVG locus, combined to DNA sequencing of extreme phenotypes from segregating populations, is in agreement with previous data showing that two peach *DAM* genes, *PpeDAM5* and *PpeDAM6*, are playing major roles during bud dormancy (Zhebentyayeva et al., 2014). Furthermore, sequence data showed a tight association between large insertions in *PpeDAM5* and *PpeDAM6* first introns and low CR (Zhebentyayeva et al., 2014).

Besides peach, QTLs for bud dormancy in regions syntenic to the *EVG* locus were found in other *Prunus* species (Castède et al., 2015; Olukolu et al., 2009; Sánchez-Pérez, Dicenta, & Martínez-Gómez, 2012). However, no *DAM* homologs could be found in major QTLs for CR in apple (Celton et al., 2011; Urrestarazu et al., 2017; van Dyk et al., 2010), pear (Gabay et al., 2017) or poplar (Rohde et al., 2011), although minor QTLs were identified coinciding with the position of *DAM* genes in apple and pear (Allard et al., 2016; Gabay et al., 2017). Possibly, *DAM* genes are more conserved in these species, or the role played by the *DAM* homologs in apple, pear and poplar is not the same as in *Prunus* species (Romeu et al., 2014).

2.3 Epigenetic Modifications of *FLC* x Dynamic Model of CR

Similarly to the variation in CR of tree crop cultivars, the model plant *A. thaliana* show natural variation in flowering time (Bloomer & Dean, 2017; Coustham et al., 2012). Interestingly, up to 70% of this variation is due to polymorphisms of only two genes, *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) (Bloomer & Dean, 2017). The role of FLC in the regulation of flowering time in *A. thaliana* is very well demonstrated in the literature. FLC is a floral repressor and is highly expressed in most tissues during the vegetative phase (Choi, Hyun, & Kang, 2009). During winter, if the plant is exposed to sufficient cold, *FLC* expression is permanently silenced, unlocking downstream pathways of floral induction.

In an elegant approach, Angel, Song, Dean, and Howard (2011) proposed that the dynamics of *FLC* silencing by epigenetic modifications is the main mechanism underlying the memory of cold exposure. During winter, the cold-induced plant-homeodomain (PHD) protein VERNALIZATION INSENSITIVE 3 (VIN3) accumulates in a region spanning the first exon and a portion of the first intron of the *FLC* gene, named nucleation region. VIN3 recruits POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) proteins, which modify the chromatin in the nucleation region by adding the H3K27me3 mark (trimethylation of Lysine 27 of Histone H3), a repressive mark stable over cell cycles (Feng, Jacobsen, & Reik, 2010). After returning to warmer temperatures, the remainder of

the *FLC* chromatin is silenced by the same mechanism under a positive feedback process (Angel et al., 2011). The model predicts that *FLC* gene expression in each individual cell is bistable, that is, can either be activated or silenced. The quantitative signal transduction is achieved when in a population of cells, each cell respond autonomously to cold exposure. Later on, cold-induced *FLC* antisense transcripts (*COOLAIR*) were demonstrated to participate in the silencing process during the first weeks of chilling, but the overall model has been shown to be accurate (Fig. 1; Bloomer & Dean, 2017).

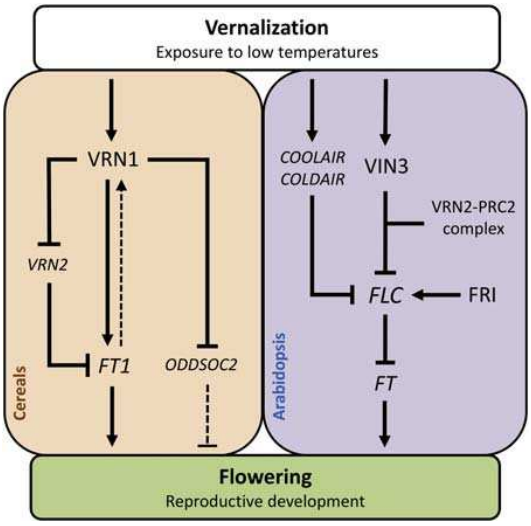

Figure 1 ***Vernalization pathways in Arabidopsis and cereals.*** Vernalization is the process that renders a plant competent to flower after a period of exposure to chilling temperatures. In cereals, such as wheat and barley, cold exposure triggers the accumulation of VRN1 protein, which directly binds to the flower-inducing *FT1* promoter and induces its expression. FT1 might promote the expression of *VRN1* in a feedback loop regulation. In parallel, VRN1 also binds to the promoter and represses two flowering repressors, *VRN2* (a repressor of *FT1* in cereals) and *ODDSOC2* (Andrés & Coupland, 2012; Deng et al., 2015). In *Arabidopsis*, the flower inhibitor *FLC* gene is initially repressed via cold-induced transcription of the *FLC* antisense transcript *COOLAIR*. After 3 weeks of continuous chilling, a *FLC* sense transcript from intron 1 (*COLDAIR*) is also induced. VIN3, a plant homeodomain (PHD) protein, accumulates during continuous cold exposure and associates with the VRN2-containing PRC2 complex to form a PHD-PRC2 complex. This complex promotes trimethylation of histone H3 lysine 27 (H3K27me3) at the *FLC* chromatin to epigenetically repress the *FLC* gene, allowing *FT* to be expressed and induce flowering (Ietswaart, Wu, & Dean, 2012; Li & Cui, 2016; Song et al., 2012a).

A. thaliana accessions differing in flowering time were screened for variation in *FLC* sequence (Li et al., 2014). Interestingly, most variation occurred in noncoding regions of the *FLC* alleles and the predicted FLC protein was identical over all tested genes. Moreover, polymorphism in the nucleation region showed the strongest association with *FLC* silencing phenotypes (Li et al., 2014). One of the early flowering accessions, *Lov-1*, revealed a single nucleotide polymorphism (SNP) that disrupts one of two conserved binding sites for B3 transcriptional regulators (Coustham et al., 2012). This site was later found to be targeted by the transcriptional repressor VIVIPAROUS1/ABI3-LIKE (VAL1), which is necessary for the nucleation of PHD-PRC2 proteins (Qüesta, Song, Geraldo, An, & Dean, 2016).

Epigenetic regulation is regarded as a strong candidate molecular mechanism for bud dormancy progression (Ríos, Leida, Conejero, & Badenes, 2014). Conceptually, the similarities between the Dynamic model and the *FLC*-VIN3-PRC2 regulon are worth noting. The intermediate thermolabile precursor and the stable factor preconized by the model show high resemblance to the VIN3 concentration and histone modifications in the *FLC* locus, respectively. *VIN3* expression is cold-induced, but its protein concentration rapidly declines in the warm (Angel et al., 2011). After the trimethylation of histones at the *FLC* locus, the cold signal is stably and irreversibly transduced in each cell. Individual cells autonomously and stochastically responding to chilling resembles the Dynamic Model of CR, where the independant accumulation of still unknown dormancy-releasing factors is necessary in order to lead to endodormancy release.

From a conceptual point of view, the characterization of epigenetic modifications seems a very promising approach to better understand bud dormancy regulation and flowering at the molecular level. The literature on this subject, especially in recent years, has grown significantly (see next section). *DAM* genes are the main candidates for the role of *FLC* in dormant buds, mainly from studies on *Prunus* species. Although recent findings were able to identify *FLC*-like genes in fruit tree species (Kumar et al., 2017, 2016; Niu et al., 2016; Porto et al., 2016; Takeuchi et al., 2018) to this date, well established homologs of *FLC* remain to be found. On the other hand, homologs of the flowering integrator gene *FLOWERING LOCUS T* (*FT*) were already identified in several tree genomes (Kotoda et al., 2010). An overview of the *FT* role in flowering-time regulation is present on Section 4 of this chapter. Interestingly in poplar, two *FT* paralogs, *FT1* and *FT2*, control dormancy transitions (Böhlenius et al., 2006; Hsu et al., 2011). The first is induced by chilling and signals dormancy release, while

the second one promotes vegetative growth and inhibition of bud set during summer (Hsu et al., 2011). The identification of upstream regulators of each *FT* ortholog can provide valuable information over the control of bud dormancy.

3. EPIGENETIC AS A KEY MECHANISM FOR DORMANCY REGULATION

3.1 Chromatin Modifications during Dormancy and Bud Break

Although bud dormancy is a well-characterized process at the physiological level, the molecular mechanisms controlling this process are only recently starting to be unveiled. Indeed, as demonstrated in the previous section, it is exactly the similarities to other better established phenomena in model plant species such as seed dormancy and flowering that guided the studies over bud dormancy control. As expected, recent studies have provided evidences that bud dormancy is under complex regulatory pathways including, hormonal control, the overlap of cold and photoperiod pathways, miRNAs regulation, and several epigenetic mechanisms. At present, a huge focus is being given to the contribution of epigenetics during plant developmental processes (reviewed in Banerjee, Wani, & Roychoudhury, 2017; Gallusci et al., 2017; Richards et al., 2017), with a special attention to bud dormancy (Ríos et al., 2014). The first clue suggesting that dormancy is epigenetically regulated comes from the study of the major role of cold in this process. Both induction and overcoming of the trait are directly linked to cold exposure, in a way similar to the "vernalization memory" described in *A. thaliana* that involves epigenetic mechanisms (Banerjee et al., 2017). In this context, histone modifications, changes in DNA methylation patterns, and the regulatory role of small non-coding RNAs (sncRNAs) have already been observed during dormancy in several perennial species.

For example, early studies in chestnut using suppression subtractive hybridization were able to identify genes involved in phosphorylation of histone H3 (H3 kinase *CsaAUR3*) during bud burst, and in H2B monoubiquitination (histone mono-ubiquitinase *CsaHUB2*) and acetylation of histone H3 serine (histone acetyltransferase *CsaGCN5L*) in dormant apical buds (Santamaría, Rodríguez, Cañal, & Toorop, 2011). The same group already reported that DNA methylation levels were higher in dormant apical chestnut buds, while H4 histone acetylation levels were higher in

non-dormant ones. Interestingly, the same methylation pattern was not observed in axillary buds, confirming that the DNA methylation levels observed in apical buds is a seasonal trend (Santamaría et al., 2009). In a study using full-genome microarray analysis, the regulation of genes encoding components of the DNA methylation and chromatin remodeling machineries were investigated at distinct stages of the active—dormancy cycle in hybrid aspen (Karlberg et al., 2010). It was demonstrated that short-day and low temperature treatments induced the expression of several histone deacetylases (*HDA14*, *HDA08*, *HDA9*, *SIN3*), a histone lysine methyltransferase (*SUVR3*) and histone ubiquitination (*HUB2*) genes. Simultaneously, a *DEMETER*-like gene, which encodes a DNA glycosylase, was downregulated by short-day treatment, possibly leading to a further increase in DNA methylation and chromatin compaction. On the other hand, long-term low temperature exposure induced a *DML* (*DEMETER*-related) gene, probably leading to demethylation and activation of genes that contribute to dormancy release.

The first evidence demonstrating that changes in cytosine methylation patterns may play a regulatory role in the control of dormancy release was obtained from MSAP (Methylation Sensitive Amplified Polymorphism) and RNA-seq analyses in apple. This study also showed that chilling availability appears to modulate the expression of genes involved in active DNA methylation and demethylation, with a progressive decrease in DNA methylation levels being observed during dormancy release. Using the same approach, high expression levels of the histone acetyltransferase *HCA1* and the histone deacetylases *HDA14* and *HDA19* where identified towards the initiation of active growth, while downregulation of other histone deacetylases like *HDA06* and *HDA08* was observed in the same time points (Kumar et al., 2017). Another study showed that dormancy release triggered the induction of two types of histones, the canonical H2A and an H2A.Z histone variant (Falavigna et al., 2014). In *A. thaliana*, H2A.Z-containing nucleosomes coordinate one of the thermosensory responses (Kumar & Wigge, 2010), being usually more expressed during the S phase of the cell cycle (March-Díaz & Reyes, 2009). The expression of these genes during dormancy release suggests that the dynamic balance in their opposing activities is necessary to regulate the transcriptional levels of target genes.

In poplar, epigenetic changes seem to take place during dormancy in order to control differential gene expression. The balance of epigenetic marks related to low transcriptional activity during winter (i.e. DNA

methylation and histone H4 hypoacetylation) with chromatin modifications compatible with increased gene expression during growth resumption (i.e. DNA hypomethylation and histone H4 acetylation) were observed (Conde, González-Melendi, & Allona, 2013). Further studies demonstrated that growth reactivation is preceded by DNA demethylation mediated by *DML*, thus promoting the necessary changes to apical bud physiology in order to start the bud break process (Conde et al., 2017b, 2017a). The DNA methylation in poplar is a widespread process in non-condensed chromatin and is associated to tissue-specific gene expression. Moreover, in the shoot apical meristem (SAM), this process responds to variations of water availability and affects the expression of genes involved in hormonal pathways (Lafon-Placette et al., 2013, 2018). Taken together, these findings indicate that the dynamics of genomic DNA methylation levels could be involved in the regulation of dormancy—growth cycle.

Recently, the ABA-mediated regulation of dormancy in response to photoperiod has been proposed for growth cessation (Tylewicz et al., 2018). In this model, ABA accumulation in the buds triggers plasmodesmata closure, ensuring bud growth arrest until sufficient chilling has been accumulated. The role of ABA in the induction of several epigenetic marks such as histone modifications, DNA methylation and short interfering RNA pathways was already documented for several development processes and stress responses in plants (Chinnusamy, Gong, & Zhu, 2008). In strawberry, dormancy establishment induced global DNA methylation in young leaves, and these changes were synchronized with endogenous ABA levels (Zhang et al., 2012c). However, how ABA is able to modulate epigenetic changes during the bud dormancy process is yet to be discovered.

3.2 Epigenetic Regulations of *DAM* and *EBB* Genes

Some of the most emblematic genes associated to bud dormancy regulation, the *DAM* genes, are also under strong epigenetic control. Both H3K4me3 and H3K27me3, two epigenetic marks related to activation and repression of transcription, respectively, are recurrently found over *DAM* genes in the different species that have been analyzed. The leafy spurge *EesDAM1* and peach *PpeDAM6* genes showed both an increase of the H3K27me3 and a decrease of the H3K4me3 levels in promoter regions when comparing eco-to endodormancy (Horvath et al., 2010; Leida, Conesa, Llácer, Badenes, & Ríos, 2012). In peach, the same epigenetic changes were also identified at the transcriptional start site (TSS) and in the largest intron of *PpeDAM6* (Leida et al., 2012). Interestingly, these two *DAM* genes

displayed a seasonal expression profile during dormancy, with a peak of transcription during endodormancy that correlates to the levels of the aforementioned epigenetic marks. Additionally, the abundance of acetylation of histone H3 (H3ac), another epigenetic mark associated with gene expression, in the promoter of *PpeDAM6* decreased during dormancy release (Leida et al., 2012). A sequential chain of events affecting *PpeDAM6* chromatin status was identified: loss of H3K4me3 and H3ac modifications during dormancy release would contribute to gene repression, while increase of H3K27me3 at the same time point would enable long-term gene inactivation (Leida et al., 2012). These two pioneering studies have provided the first evidence that epigenetic regulation of *DAM* genes may be operating during dormancy in a manner similar to the regulation of the *Arabidopsis FLC* gene during vernalization.

Further studies in peach were aimed to identify putative regulations of the *DAM* locus, which is responsible for the *EVG* phenotype (Bielenberg et al., 2008). Genes involved in chromatin remodeling were identified in QTLs for nine traits related to bud dormancy, flowering and fruit harvest, emphasizing a prominent role of chromatin modification pathways in this process (Romeu et al., 2014). Thereafter, de la Fuente, Conesa, Lloret, Badenes, and Ríos (2015) identified a significant enrichment of H3K27me3 during dormancy release concomitantly with reduced expression at different regions of the *DAM* locus, and this may be one of the controlling mechanisms responsible for the differential transcriptional profiles observed for *PpeDAM* genes during bud dormancy (Li et al., 2009). In pear, the *DAM* gene *PpyMADS13-1* displayed a seasonal expression pattern that resembles the one identified for *EesDAM1* and *PpeDAM6* (Saito et al., 2013). Besides a reduction of H3K4me3 prior to endodormancy release, the authors identified a tendency for *PpyMADS13-1* promoter to lose the histone variant H2A.Z during dormancy progression (Saito et al., 2015). Within this context, the decrease of *PpyMADS13-1* expression during dormancy progression, along with the removal of this histone variant, add a new layer of complexity to the epigenetic regulation on dormancy-related genes. Finally, recent studies demonstrated that DNA methylation and small interference RNAs (siRNAs) may also participate to the regulation of the sweet cherry *DAM* genes *PavMADS1* and *PavMADS2*. Near dormancy release, *PavMADS1* promoter presented an increase in DNA methylation as well as in the abundance of matching siRNAs (Rothkegel et al., 2017). These findings suggest that both DNA methylation and siRNAs are one of the mechanisms responsible for the seasonal expression profile of these

genes during the dormancy process. Taken together, these results indicate that a complex regulatory network involving epigenetic marks is acting over the *DAM* genes during dormancy.

Another important dormancy regulator gene is called *EARLY BUD-BREAK 1* (*EBB1*), which controls the timing of bud break in poplar by playing an integrative role in the reactivation of the SAM after dormancy (Yordanov, Ma, Strauss, & Busov, 2014). To date, the molecular regulation of bud break and its epigenetic control are poorly understood. Interestingly, transcripts of *DML* gene were localized in poplar apices, similar to that reported for *EBB1* (Conde et al., 2017b; Yordanov et al., 2014). However, the direct target genes of *DML*-dependent DNA demethylation and *EBB1* are not related, indicating that they may act by separate pathways to control bud break. In pear, the orthologous *EBB1* gene *PpyEBB* was highly induced prior to bud break in flower buds, and the analysis of its chromatin status revealed high levels of active histone modifications (H3K4me3) during ecodormancy in relation to endodormancy (Anh Tuan et al., 2016). However, no changes were detected in the levels of H3K27me3 in the same time points. Interestingly, two of the most important dormancy regulators discovered so far, *DAM* and *EBB* genes, are under strong epigenetic control. Taken together, these evidences show that epigenetic mechanisms play an important role in the regulation of bud dormancy, and a better understanding of these processes may help developing a valuable resource to genetically manipulate this trait.

4. *FT*: A MASTER SWITCH CONTROLLING FLOWERING IN ANGIOSPERMS

4.1 The Florigen *FT* Integrates Signals from Different Floral Pathways

The decision to flower is one of the most important steps for plant reproductive success and survival and represents a major life cycle transition in which meristem cells must commit with reproductive development. The flowering pathways have been extensively studied in the model plant *A. thaliana* (Amasino, 2005, 2010; Andrés et al., 2014; Lee et al., 2006; Mateos et al., 2015; Michaels & Amasino, 1999; Moon, Lee, Kim, & Lee, 2005). Five major floral pathways were characterized so far and they finely tune the time of flowering via endogenous (gibberellins, autonomous, and aging), as well as environmental factors (photoperiod and vernalization)

(Blümel, Dally, & Jung, 2015; Khan, Ai, & Zhang, 2014; Lucas-Reina et al., 2016; Song, Ito, & Imaizumi, 2013).

The vernalization and the autonomous pathways control flowering via repression of the *FLC* gene. The photoperiodic pathway involves the perception of the day length by the circadian clock that induces the expression of *CONSTANS* (*CO*). In *Arabidopsis*, flowering is anticipated during long days and delayed during short days, making it a facultative long day plant. The gibberellins (GA) pathway is a hormonal control of flowering and studies have reported that GA levels increase in the leaves and meristems of *Arabidopsis* plants, inducing the expression of the *FT* gene (Andrés et al., 2014; Song et al., 2013). The aging pathway is regulated by the balance of two microRNAs, *miR156* and *miR172*, that regulate transition from juvenile to adult and reproductive phase. All of the flower signaling pathways in *Arabidopsis* are integrated by the *FT* gene, which ultimately regulates the expression of the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA 1* (*AP1*). These major floral pathways are summarized in Fig. 2. Other recently discovered floral pathways include regulation of flowering via ambient temperature, stress, nutrients and sugar balance (for detailed reviews on the flowering pathways see Andrés & Coupland, 2012; Blümel et al., 2015; Cho, Yoon, & An, 2017; Khan et al., 2014; Lucas-Reina et al., 2016; Song et al., 2013).

Different plant species may present different signaling mechanisms for the same endogenous and environmental factors that trigger vegetative-to-reproductive meristem transition, meaning that there is a functional diversification in floral pathways among angiosperms (Blümel et al., 2015; Lucas-Reina et al., 2016). For example, wheat, barley, and *Arabidopsis* all have a cold (vernalization) requirement to induce flowering. However, while in *Arabidopsis* vernalization causes the repression of the flowering-inhibitor *FLC* gene, in wheat and barley vernalization induces the expression of the *VERNALIZATION 1* (*VRN1*) gene that directly binds to *FT* and promotes inflorescence formation (Fig. 1; Andrés & Coupland, 2012; Deng et al., 2015).

The hormone GA seems to affect flower formation differently in *Arabidopsis* compared to several perennial fruit trees including apple, mango, cherry, peach, apricot, almond, and lemon (Khan et al., 2014; Turnbull, Anderson, & Winston, 1996; Upreti et al., 2013; Zhang et al., 2016). While GA is a potent inducer of flowering in *Arabidopsis*, it has been reported that exogenous application of GA usually leads to a reduction of flower formation in fruit trees (Khan et al., 2014; Turnbull et al., 1996; Zhang et al., 2016).

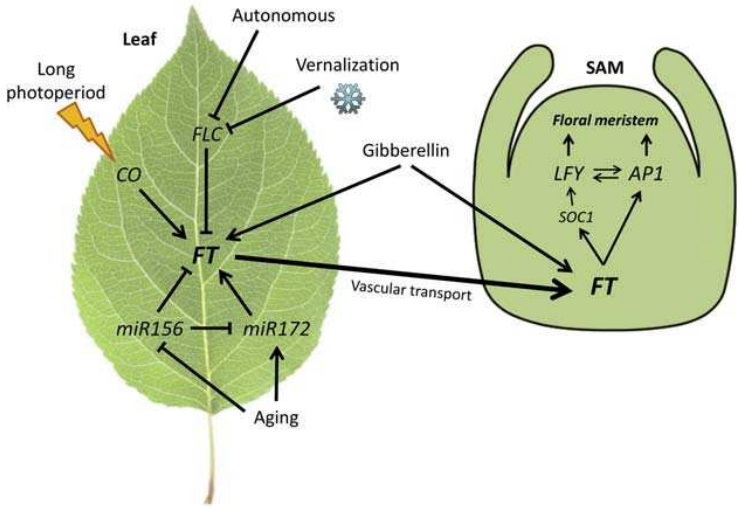

Figure 2 ***Major floral pathways in Arabidopsis.*** Different signaling pathways converge to the *FT* gene, whose protein and mRNA are translocated to the shoot apical meristem (SAM) via vascular transport (Andrés & Coupland, 2012; Jackson & Hong, 2012). The FT protein in the SAM induces the expression of *SOC1*, an activator of *LFY*. At the same time, FT protein also induces the expression of *AP1*, another floral meristem identity gene. The signal initiated by *FT* is amplified via feedforward regulation: *FT* induces *LFY* and *AP1*, and *LFY* and *AP1* continue to induce each other. This mechanism ensures that after *FT* activation, the meristem becomes committed with reproductive development and the process can no longer be reversed.

Moreover, application of paclobutrazol, an inhibitor of GA biosynthesis, was able to even increase the flowering rate of apple and promote early-flowering in mango (Upreti et al., 2013; Zhang et al., 2016). Zhang et al. (2016) have proposed a model in which GA acts by reducing cytokinin (CK) levels in apple shoots, and that a high CK/GA ratio is necessary to promote flower formation. Notwithstanding, the pathways by which GA affects flowering differently in *Arabidopsis* and in most perennials, are not fully understood. This highlights the difficulty of describing a universal model for floral signaling pathways in angiosperms and has led many review papers to focus on the flowering pathways of specific groups of plants (Deng et al., 2015; Guo et al., 2015; Hanke, Flachowsky, Peil, & Hättasch, 2007; Ramírez & Davenport, 2010; Sun et al., 2014; Weller & Ortega, 2015).

Despite all of the efforts aiming at understanding how different species perceive environmental cues and endogenous signals to promote flower

formation, only scarce information concerning the molecular signaling of reproductive meristem formation is available for perennials with a winter dormancy period. This may be due to the fact that differentiation of vegetative meristems into flower organs usually occurs concomitantly with the formation of buds for the next growing cycle (known as flower buds) (Foster, Johnston, & Seleznyova, 2003; Hanke et al., 2007) and also because plants tend to resume flowering soon or immediately after bud break, which makes dormancy the main limiting factor for the cultivation of temperate plants in warmer climates.

Noteworthy, regardless of the pathway that a given plant may utilize to promote floral development, the integration of the flowering pathways seems to converge to the *FT* gene and this appears to be highly conserved among higher plants, between dicots and monocots, and between annual and perennial species (Blümel et al., 2015; Khan et al., 2014; Lucas-Reina et al., 2016; Putterill & Varkonyi-Gasic, 2016). For instance, in both rice and *Arabidopsis*, the *FT* gene is expressed in leaves and the protein and mRNA are translocated to the apical meristem, acting as a strong promoter of flowering (Amasino & Michaels, 2010; Andrés & Coupland, 2012; Jackson & Hong, 2012; Sun et al., 2014). Similarly, studies involving woody perennial dicots and the annual herbaceous plant *Arabidopsis* revealed the conserved role of *FT* genes on flowering (Kotoda et al., 2010; Tränkner et al., 2010). More specifically, the constitutive expression of the apple *MdoFT1* gene was able to induce flowering of *in vitro* shoot cultures of apple and *Populus* (Kotoda et al., 2010; Tränkner et al., 2010). Ectopic expression of the apple *MdoFT1* or *MdoFT2* genes in the annual plant *Arabidopsis* was also able to induce an early-flowering phenotype under long days in wild-type background plants (Kotoda et al., 2010; Tränkner et al., 2010). Besides, several other reports involving the homologous and heterologous expression of *FT* and *FT*-like genes from different plants show that *FT* is able to promote flowering regardless of the species (Blümel et al., 2015; Putterill & Varkonyi-Gasic, 2016; Wickland & Hanzawa, 2015). Thus, the *FT* signaling network appears to constitute a highly conserved convergence hub for the flowering pathways in higher plants (Fig. 2).

4.2 Epigenetic Control of *FT* Expression

The expression of a given gene is affected by different factors, including the genetic information that is contained in the DNA sequence (*e.g. cis*-elements in a promoter), and by epigenetic modifications in the chromatin that can, in some cases, be transmitted to progeny (*e.g.* histone modifications and DNA

methylation; see chapters 1 and 2). Like many other genes, *FT* chromatin is also subjected to epigenetic modifications that participate to the control of gene expression, thereby determining flowering time, as illustrated below.

As part of the ambient temperature flowering pathway, *Arabidopsis* plants growing under long days in temperatures of 27°C show accelerated flowering compared to plants growing at 23°C. This has been attributed to PHYTOCHROME INTERACTING FACTOR 4 (PIF4) binding to the *FT* promoter under high temperatures (27°C). At 23°C, binding of PIF4 to the *FT* promoter is hampered by the occupancy of histone H2A variant H2A.Z at the *FT* locus. Exposure of plants to high temperatures of 27°C triggers the eviction of H2A.Z nucleosomes occupying the *FT* locus. The rate of histone H2A.Z occupancy in the chromatin is one of the factors that mediate temperature responses in *Arabidopsis* and its presence on the *FT* locus decreases accordingly under high temperatures, allowing the binding of PIF4 to the *FT* promoter and flowering (Fig. 3; Lucas-Reina et al., 2016; Song et al., 2013).

The protein AT-HOOK MOTIF NUCLEAR LOCALIZED 22 (AHL22) was described as a repressor of flowering. Overexpression of *AHL22* causes a late-flowering phenotype by reducing the levels of *FT* mRNA. AHL22 binds to AT-rich DNA sequences within the *FT* locus where it recruits histone deacetylases (HDACs), HDA1/HDA19, HDA6, and HDA9. These HDACs remove acetyl groups from the N-terminal tail of histones H3, thus promoting the silencing of the *FT* gene. Evidence also suggests that AHL22 mediates dimethylation of histone H3K9 by recruiting specific histone methyltransferases, as suggested by the increase of H3K9me2 mark abundance in lines overexpressing *AHL22* (Fig. 3; Yun, Kim, Jung, Seo, & Park, 2012).

In *Arabidopsis*, it has been shown that activation of the *FT* gene by CO in the photoperiodic pathway is dependent on the direct interaction between MORF RELATED GENE (MRG) group proteins, MRG1 and MRG2, with the CO protein. The proteins MRG1 and MRG2 recognize H3K4me3 and H3K36me3 histone marks on the *FT* chromatin and allow the binding of CO to the *FT* promoter (Fig. 3). The *mrg1 mrg2* double mutant has reduced *FT* mRNA levels and a late-flowering phenotype associated with impaired binding of CO to the *FT* promoter under long photoperiods (Bu et al., 2014).

Presence of H3K4me3 close by the TSS is generally associated with active transcription (Guo et al., 2015; He, 2012). Absence of two redundant H3K4me3 demethylases, *Jumonji 4* (*AtJmj4*) and *EARLY FLOWERING 6*

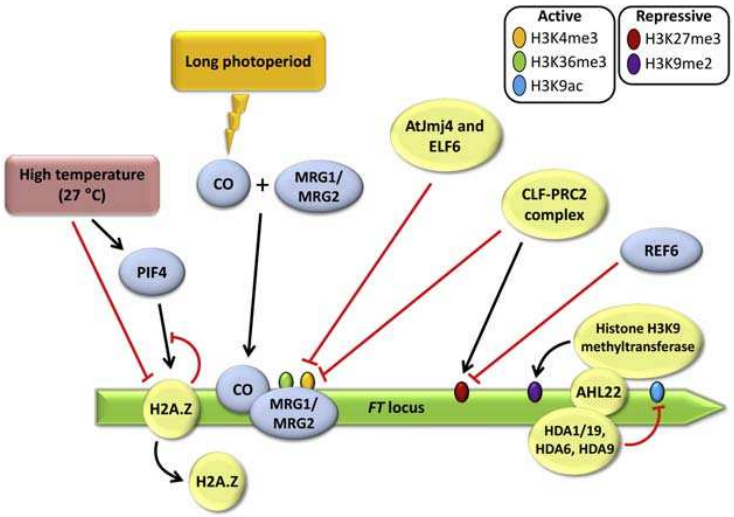

Figure 3 ***Epigenetic mechanisms controlling*** **FT** ***expression in Arabidopsis.*** The binding site of PIF4 to the *FT* promoter is occupied by the histone variant H2A.Z. Exposure to high temperatures causes the eviction of H2A.Z from the *FT* locus, allowing the binding of PIF4. MRG1 and MRG2 recognize methylated H3K4 and H3K36 histone marks, allowing the binding of CO to the *FT* promoter. The binding of CO can be counteracted by the activity of H3K4me3 demethylases (AtJmj4 and ELF6). The CLF-PRC2 complex silences *FT* via trimethylation of histone H3K27 and via demethylation of H3K4me3 marks. REF6 removes H3K27me3 marks, acting against the CLF-PRC2 complex to allow *FT* transcription. AHL22 represses *FT* by recruiting histone deacetylases (HDA1/19, HDA6, and HDA9) and histone H3K9 methyltransferases. Active and repressive histone modifications are indicated in the upper right, respectively. Proteins acting to activate *FT* transcription are depicted in blue, whereas proteins whose function is to silence *FT* expression are pictured in yellow.

(*ELF6*), causes an early-flowering phenotype in *Arabidopsis* under both short and long days (Fig. 3; Jeong et al., 2009). In another study, CURLY LEAF (CLF), an Enhancer of zeste protein constitutive of PRC2, was shown to be a strong repressor of *FT* by mediating the trimethylation of H3K27 and by partially suppressing H3K4me3 marks into the *FT* chromatin (Jiang, Wang, Wang, & He, 2008). In contrast, RELATIVE OF EARLY FLOWERING 6 (REF6) was shown to be an important histone H3K27 demethylase promoting *FT* expression, acting dynamically against the CLF-PRC2 complex (Fig. 3; He, 2012).

Interestingly, it has been demonstrated that deposition of active and repressive histone modifications occur independently of each other

and are mutually antagonistic (Jiang et al., 2008; Shafiq, Berr, & Shen, 2014). In other words, activation or repression of *FT* expression in *Arabidopsis* relies on the relative abundance of active or repressive epigenetic marks. The implications of this finding is that, in order to fully understand how plants control the time of flowering, it may be necessary to look further into the mechanisms that regulate the balance of active and repressive epigenetic marks on the *FT* chromatin.

In rice, chromatin methylation also appears to play an important role in the expression pattern of two *FT* homologs, *Heading date 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*). Knockdown plants with reduced synthesis of S-Adenosyl-l-methionine, a universal methyl group donor, show low levels of histone H3K4me3 and DNA methylation at the *Hd3a* and *RFT1* loci, which was associated with reduced expression of these genes and late-flowering phenotype (Sun et al., 2014). Two genes that are responsible for histone H3K36 di- and tri-methylation, *SET DOMAIN GENE 724* (*SDG724*) and *SDG725*, were also shown to be important for flowering time regulation in rice. A loss-of-function mutant for *SDG724* and a knockdown plant for *SDG725* were associated with a reduction of H3K36 methylation and transcription of *RFT1* and *Hd3a*, and of other flower-related genes (Sun et al., 2014). The presence of H3K9ac around the TSS of *RFT1* was also correlated with active gene transcription and flowering in rice (Sun et al., 2014).

As mentioned above, little information regarding the flowering pathways of perennial plants is available, and even less or no information at all on the epigenetic regulation of *FT* genes of perennials might exist. Nevertheless, a study with sexual dimorphism in poplar has shown that DNA methylation is an important regulatory factor for flower development in *Populus tomentosa* (Song et al., 2012b). In another work using the early-flowering trifoliate orange *Poncirus trifoliate*, the authors treated plants with 5-azacytidine, a DNA methyltransferase inhibitor, and observed several abnormalities related to vegetative development, without noticeable changes in the flowering time of the already precocious trifoliate orange (Zhang, Mei, Liu, Khan, & Hu, 2014). Notwithstanding, it was observed an increase in *CiFT* transcript accumulation with increasing concentrations of 5-azacytidine. The expression level of other flowering genes *CiLFY*, *CiAP1*, *TERMINAL FLOWER 1* (*CiTFL1*), and *CiFLC* was highest at 250 μM of 5-azacytidine and then decreased at higher concentrations (Zhang et al., 2014). Because a decrease in the overall DNA methylation pattern led to an increase in the expression of five major floral genes, the

authors concluded that DNA methylation plays an important role in the regulation of the early-flowering trait of trifoliate orange (Zhang et al., 2014).

The knowledge regarding how plants flower is of utmost importance for breeding programs, especially for perennial fruit trees, in which a long juvenile phase represents a significant hindrance. Activation of *FT* and *FT*-like genes through homologous or heterologous expression in juvenile perennials is sufficient to accelerate entrance into the reproductive phase. *FT* is regarded as the master switch that must be turned on by angiosperm plants to initiate flower formation and complete their reproductive cycle. Epigenetics is a rapidly growing field and we address here that a direct link between epigenetics and the *FT* gene is essential to flowering. As a future perspective, we believe that understanding the genetic mechanisms underlying the epigenetic control of *FT* expression (and how this is linked to repression of *FT* expression in juvenile perennials) could help breeding programs seek important characteristics to shorten the juvenile phase in perennial fruit trees.

5. CHROMATIN REMODELING AND HORMONAL STIMULUS: AN INHERENT PART OF THE FLOWERING PROCESS

The regulation of flowering time depends on many factors including environmental, genetic, and epigenetic mechanisms as detailed in the previous sections. Hormonal regulation plays a central role in this process, integrating internal and external stimulus. In this section, we will explore the importance of well-known phytohormones in the flowering process, connecting them with epigenetic modifications that occur in the chromatin during floral transition (Campos-Rivero et al., 2017; Yamamuro, Zhu, & Yang, 2016).

5.1 Gibberellins

In the model plant *A. thaliana*, the timing of flowering mediated by GA signaling has been extensively studied, leading to the elucidation of some of the underlying molecular mechanisms. The induction of flowering starts with the perception of bioactive GAs and is mediated, among other factors, by nuclear proteins called DELLA. Briefly, DELLA are negative regulators of GA signaling that sequestrate transcriptional activators, inhibiting flowering (Achard & Genschik, 2009; Harberd, 2003). In leaves, the transcriptional activators of *FT*, like CO and PIF4 are sequestered by

DELLA proteins, preventing them from binding to DNA and activating *FT* transcription (Conti, 2017; de Lucas et al., 2008; Tiwari et al., 2010; Wang et al., 2016; Xu et al., 2016). In the SAM, DELLAs bind to SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcriptional regulators and prevent their function as transactivators on target genes (Conti, 2017; Hyun et al., 2016; Yu et al., 2012). When GA is present, the GID1 receptor undergoes conformational changes, increasing its affinity for DELLA proteins (Griffiths et al., 2006; Willige et al., 2007). This interaction (DELLA-GID1) favors the binding of the E3 Ubiquitin ligase SLEEPY1 (SLY1) that marks DELLA for degradation by proteasome 26 (Dill, Thomas, Hu, Camille, & Steber, 2004). Without DELLA proteins, the activators CO and PIF4 are free to promote *FT* expression. In the SAM, SPLs are able to activate *LFY, FRUITFUL* (*FUL*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *AP1* by directly binding to their promoter regions. Expression of these genes contribute to the amplification of the FT-FD signal and causes the activation of the floral meristem identity genes (Abe et al., 2005; Conti, 2017; Melzer et al., 2008; Moon et al., 2003). In contrast to *Arabidopsis*, application of exogenous GAs were shown to inhibit flower formation in some perennial crops like apple (Marcelle & Sironval, 1963; Zhang et al., 2016), peach (Southwick & Glozer, 2000) and citrus (Goldberg-Moeller et al., 2013; Guardiola, Monerri, & Agusti, 1982; Tan & Swain, 2006), as previously described in this chapter. Some works reporting the molecular link between chromatin modifications and GA responses have been already published and will be now more thoroughly discussed.

PKL genes code for an CHD3-family ATP-dependent chromatin remodeling factor, that promote the trimethylation of H3K27, contributing for tissue-specific gene repression in plants (Ogas, Kaufmann, Henderson, & Somerville, 1999; Zhang et al., 2008, 2012b). Interestingly, analyses of *pkl* mutant plants showed a defective ability to respond to GA, demonstrating the important role of methylation in the induction of GA transcriptional network (Rider et al., 2003; Zhang et al., 2008). A recent study showed that PKL is required to promote flowering in Arabidopsis, probably due to its close interaction with DELLA proteins (Park et al., 2017). Another interesting point is that histone modifications are able to regulate genes involved in GA biosynthesis. Some examples as *GA REQUIRING1* (*GA1*), *GA2*, *GA3*, *ENT-KAURENOIC ACID HYDROXYLASE1* (*KAO1*) and *KAO2* have already been reported to undergo H3K27ac modification (Charron, He, Elling, & Deng, 2009).

Dioxygenase genes (*GA2ox7* and *GA3ox2*) are the major targets for light regulation in the GA metabolism (Kamiya & García-Martínez, 1999) and showed to be downregulated by H3K27me3. The higher transcript accumulation of *GA2ox7* and *GA3ox2* correlates with the reduction of H3K27me3 abundance over the promoter regions and first exons of dioxygenase genes, removing the repressive effect of this modification (Charron et al., 2009). On the other hand, genes that are not affected by the GA signaling have no differences in H3K27me3 levels (Charron et al., 2009). These results suggest an import role of epigenetics modifications in GA biosynthesis, signaling and response, influencing flowering time.

5.2 Jasmonic Acid

Jasmonic acid (JA) is a fatty acid-derived molecule that orchestrates different responses to abiotic and biotic stress in plants (Browse, 2009; Stintzi & Browse, 2000) and plays important functions in the regulation of floral development in *Arabidopsis* (Park et al., 2002; Stintzi & Browse, 2000), rice (Cai et al., 2014; Xiao et al., 2014), maize (Acosta et al., 2009; Yan et al., 2012), tomato (Li et al., 2004) and tobacco (Stitz, Hartl, Baldwin, & Gaquerel, 2014). The currently accepted model proposes that, in the absence of JA, repressors of JASMONATE-ZIM domain (JAZ) family interact with the transcriptional factors MYB21 and MYB24, preventing them to activate responsive JA genes. In contrast, the JA signal makes JAZ proteins a potential target for the F-box protein CORONATINE INSENSITIVE PROTEIN 1 (COI1) to ubiquitination and subsequent degradation (Chini et al., 2007). As soon as JAZ is degraded, these factors are released and can activate genes involved in anther development and filament elongation (Song et al., 2011).

Studies developed in *Arabidopsis* have now revealed the importance of HDA6, a RPD3-type histone deacetylase, in JA response, senescence and flowering (Wu, Zhang, Zhou, Yu, & Chaikam, 2008). The interaction of HDA6 with COI1, has been demonstrated (Devoto et al., 2002), suggesting an important connection between JA response and histone modifications. The analysis of *axe1-5*, a splice site mutant of *AtHDA6* (Murfett, Wang, Hagen, & Guilfoyle, 2001) and HDA6-RNAi plants have showed that the JA responsive genes, *PDF1.2*, *VSP2*, *JIN1*, and *ERF1* were downregulated in these plants, reinforcing the idea that HDA6 is necessary to the JA-responsive pathway (Wu et al., 2008). Furthermore, *axe1-5* and HDA6-RNAi plants displayed increased leaf longevity and late flowering compared to wild type plants. These plants also showed an hyperacetylation of histones

at the *FLC* gene and an increase in its expression, suggesting that HDA6 is required for *FLC* deacetylation and repression (Wu et al., 2008). Little is known about epigenetics modification and JA response in perennial plants, revealing a huge gap that remains to be investigated.

5.3 Brassinosteroids

Brassinosteroids (BR) are a class of polyhydroxylated steroidal phytohormones that are involved in many aspects of plant biology, including cell elongation, cell division, root growth, photo-morphogenesis, stomatal and vascular differentiation and seed germination (Gudesblat & Russinova, 2011; Wei & Li, 2016). Phenotype analyses of BRs defective mutants revealed a delayed flowering time, indicating a positive role of BRs in the establishment of flowers (Domagalska et al., 2007; Li, Li, Chen, & An, 2010). A higher level of *FLC* transcripts and increased levels of histone H3 acetylation normally associated with active chromatin at the *FLC* locus were observed in these mutants, suggesting that BRs could contribute to a silenced epigenetic state at the *FLC* promoter, helping to downregulated it and promote flowering (Domagalska et al., 2007).

The transcriptional factor BRI1-EMS suppressor 1 (BES1), one of the major regulators of BR signaling that coordinates several genes in response to BR (Yin et al., 2005), directly interacts with REF6 and its homolog, EARLY FLOWERING 6 (EFL6), both jumonji N/C (JmjN/C) domain-containing proteins essential to catalytic demethylation of histones (Agger, Christensen, Cloos, & Helin, 2008). REF6 and EFL6 (recruited by BES1) have been shown to be involved in BR signaling by affecting histone methylation in the promoters of BR-responsive genes (Noh et al., 2004; Yu et al., 2008). BES1 can also recruit a histone lysine methyltransferase called SET DOMAIN GROUP 8 (SDG8), which is implicated in H3K36 di- and trimethylation (Wang et al., 2014; Xu et al., 2008).

Analysis of the *rice plasticity 1* (*rpl1*) mutant that is impaired in a gene involved in plastic response of rice plants to environmental changes, have also demonstrated an interesting connection between BRs and histone modification (Zhang et al., 2012a). The *rpl1* mutant showed an increased in DNA methylation level and a decrease in overall histone acetylation. Responses to various plant phytohormones including BRs were also negatively affected. Noteworthy, the putative rice BR receptor *OsaBRI1*, a key hormone signaling gene, was extremely downregulated in the *rlp1*

mutant concomitantly to changes in histone marks in the *OsaBRI1* gene locus (Zhang et al., 2012a), reinforcing the importance of epigenetic regulations in BR perception and signaling.

5.4 Ethylene

Ethylene (ET) is a volatile organic chemical compound involved in fruit ripening, senescence of leaves, response to stress and a floral repressor in *Arabidopsis* (Achard et al., 2006). The transcription factor ETHYLENE INSENSITIVE 3 (EIN3) and EIN3-like (EIL) mediate ET transcriptional responses (Guo & Ecker, 2003). EIN3 accumulation delays flowering by activating the *ETHYLENE RESPONSE 1* (*ERF1*) related genes. The negative role of ET in flowering through the EIN3-ERF1 is attributed to reduced bioactive GA levels, causing enhanced accumulation of DELLA proteins (Achard et al., 2007). In the work reported by Zhou, Zhang, Duan, Miki, and Wu (2005), the importance of HDA19, a RPD3-type histone deacetylase, in integrating hormonal stimulus and epigenetic regulations was analyzed in *Arabidopsis*. By overexpressing *HDA19*, an increased expression of *ERF1* was detected, showing that histone deacetylation positively regulates *ERF1*, contributing to an increased delay in flowering (Zhou et al., 2005). These observations contributed to elucidate the roles of histone modification and ET response. However much more efforts need to be done to completely understand this hormonal-epigenetics regulation.

5.5 Salicylic Acid

Salicylic acid (SA) is an important phytohormone that belongs to the group of phenolic compounds and plays distinct roles in many aspects of plant growth and development, as well as in disease resistance (Vicente & Plasencia, 2011). The importance of SA in the flowering process is already described (Cleland & Ajami, 1974; Lee & Skoog, 1965) but the knowledge about its role in the floral regulatory network is still very limited. Arabidopsis SA-deficient plants show a late-flowering phenotype and an increased expression of *FLC*. Low levels of *FT* transcripts were also observed in these mutants compared to wild type, under short day or long day conditions (Martínez, Pons, Prats, & León, 2004).

On the other hand, the *CO* and *SOC1* genes showed a different profile under the same daylength conditions in SA-deficient plants (Martínez et al., 2004). *CO* and *SOC1* expression decreased in long day conditions, while in short day-grown plants, amounts of *CO* transcripts increased and *SOC1* remained stable (Martínez et al., 2004). Given that, *CO* and *SOC1* are

strictly regulated by light changes (Suárez-López et al., 2001), suggesting that SA regulates flowering by interacting with the photoperiod dependent pathway (Martínez et al., 2004; Vicente & Plasencia, 2011).

Sumoylation is a post-translational modification that conjugates small ubiquitin modifier peptides (SUMO) to protein substrates (Mahajan, Delphin, Guan, Gerace, & Melchior, 1997; Matunis, Coutavas, & Blobel, 1996). In mammals and yeasts sumoylation has been included in the list of histone modifications associated to transcriptional repression (Garcia-Dominguez & Reyes, 2009; Nathan et al., 2006; Shiio & Eisenman, 2003). In plants, it is related to biotic and abiotic stress responses, flowering and development (Chosed, Mukherjee, Lois, & Orth, 2006; Jin et al., 2008; Novatchkova, Budhiraja, Coupland, Eisenhaber, & Bachmair, 2004). Analysis of plant mutants in the *SUMO E3 ligase* (*SIZ1*) gene, which play crucial role during SUMO steps, revealed an early flowering time phenotype under short day (Jin et al., 2008). It has also showed elevated SA levels, suggesting an important connection between SUMO modification and SA response (Jin et al., 2008).The authors concluded that under low levels of SA an increased *SIZ1* expression is observed, contributing to sumoylation of *FLOWERING LOCUS D* (*FLD*) and its consequent low expression. The *FLD* gene encodes a plant homolog to an important protein in mammals histone deacetylase complexes (He, Michaels, & Amasino, 2003). Mutations in *FLD* and its consequent low expression promote hyperacetylation of histones in *FLC* chromatin, leading to its transcriptional activation and delayed flowering time (He et al., 2003). Nevertheless, when high levels of SA are perceived, *SIZ1* is repressed, which increases FLD levels and results in lower *FLC* transcript amounts, allowing floral transition (Campos-Rivero et al., 2017; Jin et al., 2008).

5.6 Cytokinins

CK are plant hormones involved, in cell proliferation, differentiation and many other related processes (Werner et al., 2008; Werner & Schmülling, 2009). Their role in the progress of dormancy to flowering is still unclear, although studies showed that cell division is higher in SAM during floral transition (Jacqmard, Gadisseur, & Bernier, 2003). The literature about epigenetic regulation and the role of CK in flowering induction is still sparse, but some insights have been described (Godge, Kumar, & Kumar, 2008; Li et al., 2008; Meijón, Jesús Cañal, Valledor, Rodríguez, & Feito, 2011; Tanaka et al., 1997). The study of Li et al. (2008) analyzed *A. thaliana* plants mutated for the *S-adenosyl-L-homocysteine hydrolase* (*SAHH*) gene, which

codes for a key enzyme in the stabilization of methylation potential in cells (Palmer & Abeles, 1979; Tanaka et al., 1997). They also investigated RNAi lines with reduced *AtSAHH2* gene expression and observed a global reduction of DNA methylation levels and high levels of endogenous CK, even when compared to CK overproducers (Catterou et al., 2002; Chang, Jones, Banowetz, & Clark, 2003).

Similarly, increased levels of CK were observed in *sahh1-1* tobacco mutants (Tanaka et al., 1997). In tobacco plants, it was also observed that CK upregulate genes encoding Cytosine DNA methyltransferases (Li et al., 2008). The overexpression of *SAHH1* and *Adenosine kinase 1* (*ADK1*) in response to CK was similarly detected (Li et al., 2008; Pereira et al., 2007). Taken together, these results indicate a 'feedback-regulatory loop between cytokinin and DNA methylation: reduced DNA methylation potential in plant cells seems to lead to an increased level of CK, which in turn stimulates the genes encoding enzymes of the DNA methylation machinery' (Li et al., 2008). Moreover, the delayed flowering time found in Arabidopsis mutants knockdown for *SAHH* could be related to the accumulation of CK in response to reduction of global DNA methylation (Campos-Rivero et al., 2017; Li et al., 2008; Rocha et al., 2005). In the same way, the treatment with 9-(S)-(2,3-dihydroxypropyl)-adenine (DHPA), an inhibitor of SAHH in tobacco plants resulted in global DNA hypomethylation, flower morphology alterations, reduced fertility and upregulation of floral organ identity genes (Fulneček et al., 2011). The analysis of a gene coding for a CK binding protein, *PETUNIA CYTOKININ BINDING PROTEIN (PETCBP)* from *Petunia hybrida* cv. Mitchell, revealed a highly similar sequence (85%–90%) to the *SAHH* gene from several plant species (Godge et al., 2008). Authors suggest that PETCBP could be a positive regulator of CK response, mainly by modulating CK signal transduction. They also report that antisense expression of this gene resulted in plants with delayed flowering time (Godge et al., 2008).

Another work reported by Meijón et al. (2011) investigated if the treatment with GA inhibitors applied to improve flower production in ornamental azaleas plants could also influence DNA methylation and other phytohormones (as CK) during floral transition. They found that before flower set, low DNA methylation levels were seen, but when floral organs formation was achieved, genomic DNA became hypermethylated. They also observed low levels of GAs (as expected) and high endogenous CK amounts during floral transition. The authors suggested that CK could

induce DNA demethylation, thereby contributing to the induction of gene expression during flowering transition (Meijón et al., 2011).

5.7 Abscisic Acid

ABA is a phytohormone associated mostly with drought stress and important to coordinate an adaptive response during water deprivation (Shinozaki & Yamaguchi-Shinozaki, 2007). Moreover, ABA plays important roles in plant development, even without stress stimuli, like induction of seed and bud dormancy (Barrero et al., 2005). During the transition of flowering, both positive and negative effects of ABA have been reported (Riboni, Test, Galbiati, Tonelli, & Conti, 2016, 2013; Shu et al., 2016; Wang et al., 2013; and reviewed by; Shu, Luo, Meng, & Yang, 2018). Briefly, the delay of *A. thaliana* floral transition is strictly related to *ABSCISIC ACID-INSENSITIVE* (*ABI*) genes. Studies analyzing the *Arabidopsis abi3*, *abi4* and *abi5* mutants revealed an early flowering phenotype; consistently to that, transgenic plants overexpressing those genes (*ABI3*, *ABI4* and *ABI5*) showed delayed floral transition (Shu et al., 2016; Wang et al., 2013). *ABI4* and *ABI5* directly induces *FLC* transcription, while FLC further represses expression of *FT* in Arabidopsis, regulating the flowering process in a negative way (Shu et al., 2018; Wang et al., 2013).

Recently, Shu et al. (2016) proposed that ABI4 negatively controls GA biogenesis, suggesting that it may repress flower formation through the GA flowering pathway. Positive regulation of flowering mediated by ABA response occurs especially under environmental stress conditions, such as drought (Riboni, Galbiati, Tonelli, & Conti, 2013, 2016). It is already described that drought stress causes early flowering in various plant species and high level of ABA biosynthesis (Budak, Hussain, Khan, Ozturk, & Ullah, 2015; Munemasa et al., 2015; Riboni, Test, Galbiati, Tonelli, & Conti, 2014). Molecular mechanisms required for ABA response in order to promote flowering and overpass the drought stress is still under evaluation, but some key factors were already described. Riboni et al. (2016) proposed that in Arabidopsis, ABA induces the drought escape response by promoting *FT* expression, for which *CO* and flower-promoting gene *GIGANTEA* (*GI*) genes are required in a photoperiod dependent manner. In citrus species, water deficit promotes flowering, and the higher level of ABA was also detected during the floral inductive stage (Li et al., 2017). Furthermore, in litchi species, drought stress combined with low temperature remarkably promoted flowering; however, the detailed relationship

between ABA biosynthesis and/or signaling and flowering in litchi needs further investigation (Shen, Xiao, Qiu, Chen, & Chen, 2016).

In the epigenomic context, the nuclear interaction of SWI/SNF (chromatin remodeling complex) with *ABA HYPERSENSITIVE* (*HAB1*) gene, involved in negative regulation of ABA signaling and flower induction in Arabidopsis, was reported as necessary for ABA-responsive gene regulation (Rodriguez, Leube, & Grill, 1998; Saez, Rodrigues, Santiago, Rubio, & Rodriguez, 2008).

Another work showed that Arabidopsis mutants impaired in the *ABA OVERLY-SENSITIVE* (*ABO4*) gene that encodes an important regulator of ABA plant responses (Liu et al., 2010), showed an early flowering phenotype. *abo4-1* plants showed lower expression of *FLC* and high amounts of *FT* transcripts. An altered histone modification pattern in these two loci were also observed (Yin et al., 2009). Thirunavukkarasu et al., (2014) reported that stress-responsive genes involved in the ABA signaling promote floral transition and are regulated by epigenetic events under water stress condition in maize. Altogether, these findings suggest an important crosstalk between epigenetics, ABA responses, drought stress and flowering.

5.8 Auxins

Auxins are phytohormones involved in many aspects of plant biology such as vascular tissue formation, adventitious root initiation, tropistic responses, apical dominance and fruit development (Goldfarb, Lanz-Garcia, Lian, & Whetten, 2003; Luo, Zhou, & Zhang, 2018; Sundberg & Østergaard, 2009). The first link between auxin and floral transition was reported when the auxin transport mutant *pin1* (*PIN-FORMED1*) was characterized (Gälweiler et al., 1998). The *PIN1* gene encodes for a member of the putative auxin efflux regulator proteins, important in polar auxin transport (Gälweiler et al., 1998) and have been related to plant organ formation by regulating auxin distribution (Benková et al., 2003; Furutani et al., 2004). The most intriguing characteristic of the *pin1* mutant was the formation of inflorescence without flowers. Two other mutants showed very similar phenotypes: *pinoid* (Bennett, Alvarez, Bossinger, & Smyth, 1995) and *monopteros* (*mp*) (Przemeck, Mattsson, Hardtke, Sung, & Berleth, 1996). PINOID is a serine/threonine protein kinase and have been reported to be involved in signaling and polar auxin transport (Benjamins, Quint, Weijers, Hooykaas, & Offringa, 2001). MP/ARF5 is a member of a family of transcription factors called auxin response factors (ARFs), and are essential to auxin signaling and response (Hardtke &

Berleth, 1998; Quint & Gray, 2006). Interestingly, these mutants could progress from vegetative to reproductive phase while the inflorescence is formed, however no flower is observed (Cheng & Zhao, 2007). These findings demonstrate the important role of auxin in flower formation, even if the complete pathways connecting auxins and formation of floral primordial remain unclear (Cheng & Zhao, 2007). The link between epigenetic, auxin response and floral initiation was reported in a recent study which identified an auxin hormone-regulated chromatin state switch in order to promote floral primordial development in *Arabidopsis* (Wu et al., 2015). Based on these findings a model for cell auxin sensing and recruitment of chromatin remodeling factors was proposed. The presence of low auxin levels triggers a repressor system for auxin responsive genes that is orchestrated by monopteros (MP) and auxin sensitive Aux/IAA proteins (ASP). In this way, ASP binds to the MP factor associated with its target loci. ASP directly recruits the TOPLESS (TPL) repressor and the histone deacetylase HDA19, preventing gene expression (Guilfoyle & Hagen, 2012; Szemenyei, Hannon, & Long, 2008; Wu et al., 2015; Yamaguchi et al., 2013). The system also prevent recruitment of SWI/SNF ATPase subgroup BRAHMA (BRM) and SPLAYED (SYD) chromatin remodeling complexes, creating an inactive chromatin state (Wu et al., 2015). However, when high levels of auxin are present, ASPs are degraded and prevent the action of TPL and HD19. The recruiting of BRM or SYD complexes is possible and an open chromatin state is achieved, enabling the activation of the general transcriptional machinery (Wu et al. 2015). In loss of function studies, the authors also showed that the SWI/SNF ATPase activity is essential for flower primordium initiation, revealing a 'simple and elegant mechanism for small-signaling-molecule-regulated chromatin state switch' that respond to auxin signal and is essential for flower development (Wu et al., 2015).

6. BUD BREAK AND FLOWERING: CONSEQUENCES IN AGRICULTURE AND BREEDING

Throughout the evolution, temperate fruit trees have developed bud dormancy as an adaption to seasonality (Campoy et al., 2011; Ionescu, Møller, & Sánchez-Pérez, 2017). Bud break and subsequent flowering happen after chilling and heating requirements have been fulfilled during winter and spring, respectively. Within the context of global climate change, there are a number of evidences that increasing temperatures results in

shifting the timing of phenological events, mainly in springtime, when plants resume growth (Hänninen & Tanino, 2011; Legave, Guédon, Malagi, El Yaacoubi, & Bonhomme, 2015; El Yaacoubi, Malagi, Oukabli, Hafidi, & Legave, 2014). Early bud break and flowering have many implications such as an increased risk of frost damage (Cannell & Smith, 1986; Vitasse, Lenz, & Karner, 2014) affecting the photosynthetic capacity of the trees (Ensminger, Schmidt, & Lloyd, 2007), bud burst delay combined with low burst and poor fruit set (Abbott, Zhebentyayeva, Barakat, & Liu, 2015; Celton et al., 2011; Dirlewanger et al., 2012; Erez, 2000), fertility abnormalities due to mis-synchronization of flowering of self-incompatible cultivars (Dirlewanger et al., 2012), changes in flower size and timing of anthesis (Scaven & Rafferty, 2013) and modifications of fruit harvesting periods and fruit marketing logistics (Dirlewanger et al., 2012). The production of floral scent, nectar and pollen can also be affected by temperature. Altered floral scent emission at higher temperatures affect the detectability of flowers by pollinating insects, such as moths, that rely on long-distance cues to locate floral resources (Kevan & Baker, 1983; Yuan, Himanen, Holopainen, Chen, & Stewart, 2009). Changes in nectar production and composition have immediate effects on pollinators activity and fitness (Burkle & Irwin, 2009), especially for those insects, such as some lepidopterans and wasps, that rely on nectar for amino acids as well as for sugars (Kevan & Baker, 1983). Similarly, decreased pollen production affect the reproductive success of many bees, which may need to collect pollen from a large number of plants to successfully rear their offspring (Müller et al., 2006). Along with the effects of warming on floral traits, elevated temperatures can alter other plant characteristics such as vegetative growth. It has been observed that plants exposed to winter warming of 1.5°C via open top chambers were several centimeters taller than plants in control chambers (Liu, Mu, Niklas, Li, & Sun, 2012). In contrast to the negative effects of the warming temperatures over the floral traits, increased vegetative growth may represent a benefit for agriculture in certain circumstances, such as wood and fruit crop production in sub-optimal environments. For example, the Brazilian apple cultivar 'Castel Gala', which is originated from a spontaneous bud sport mutation from a 'Gala Standard' tree, features a low CR when compared to the original cultivar, exhibits a precocious growth cycle that starts 25 days earlier and produces fruits that are anatomically and nutritionally equivalent (Denardi & Seccon, 2005). Due to its earlier bud burst, 'Castel Gala' also presents taller and more vigorous plants, reaching full production capacity earlier and can be planted in warmer

climate sites than its parental type. Examples like this and the abovementioned information help pave the way to better analyze the challenging context of breeding strategies to deal with bud phenology traits in order to generate adapted cultivars to the new climate scenarios for agriculture.

Bud dormancy can still be considered a biological black box, in which the outputs to certain inputs can be predicted with some degree of certainty, but the internal workings remain obscure. Epigenetic regulation seems to play roles in some central steps of the bud dormancy cycles, including gene expression adjustments that remain stable over cell cycles and the quantification of cold exposure. Although the participation of epigenetic modifications in bud dormancy is suggested by many research data in the literature, the genes responsible for transducing epigenetic marks into phenological behaviour remain to be fully characterized. *DAM* and *EBB* genes, at this time, are the most promising candidates; however, further studies on repressors of *FT* transcription in temperate woody plants may uncover new pieces of this puzzle.

Exploiting epigenetic variations (DNA methylation and histone PTMs) for breeding applications depends on the plant propagation strategies (sexual versus clonal, McKey, Elias, Pujol, & Duputié, 2010). Because DNA methylation patterns can be transmitted after mitosis and meiosis, DNA methylation marks could be useful in all crops, irrespective to their propagation mode. By contrast, histone modifications are reset during meiosis (Ingouff et al., 2010; Xiao & Wagner, 2015), therefore, they would be of little benefit for breeding purposes in sexually propagated crops. However, they could be applicable for clonally propagated crops, such as in perennial fruit crops, because new epigenetic patterns could be maintained in meristems for grafting propagation (Bräutigam et al., 2013; Douhovnikoff & Dodd, 2014). As examples of heritable epigenetic states, epimutations, irrespective of their origin (induced or natural epialleles), are generally stable in plant populations and can be selected as classical phenotypes in breeding schemes. An extensive review about the use of epigenetics for breeding purposes is described by Gallusci et al. (2017).

A successful example of the use of epigenetic research and its application for breeding purposes has been demonstrated in the African oil palm *Elaeis guineensis,* where a flower and fruit abnormality known as 'mantled' can develop in oil palm cultivars derived from tissue culture and the resulting mantled palms can become unproductive (Ong-Abdullah et al., 2015). By performing a genome-wide, unbiased, DNA methylation analysis to look for loci epigenetically associated with the mantled

phenotype, it was discovered that hypomethylation of a single *Karma* family retrotransposon embedded in the intron of the homeotic gene *DEFICIENS* is common to all mantled clones and associated with aberrant splicing and termination of the gene transcript. Loss of methylation — dubbed the *Bad Karma* epiallele — predicts a loss of oil palm yield and this property enable screening for higher-performing clones at the plantlet stage. This finding is likely to provide a way to detect unproductive palms much earlier than was previously possible, enabling their timely replacement in plantations, which besides the obvious economic importance, also benefits the environment. The results found for the 'mantled' phenotype in *E. guineensis* have also other key implications. For instance, a well-planned and performed genome-wide methylation mapping can pinpoint precise spots in the genome of a non-model organism that are responsible for a trait of interest.

Unveiling the molecular mechanisms responsible for the regulation of dormancy completion and flowering time in woody perennials is an important front for both basic and applied research. The compatibility between CR and the local climate is one of the main factors determining the success of a temperate fruit production enterprise. Conventional tree breeding, especially for dormancy traits, is costly and highly time-consuming because of protracted generation times. The advances in genomic research in woody perennials has now opened the opportunity to assist the breeders with tools generated through extensive sequencing, genome wide association studies and high density genotyping arrays that lead to the discovery of highly predictive molecular markers for traits of horticultural interest (Laurens et al., 2018). Therefore, markers linked to genes proved to participate in dormancy and flowering can be an invaluable asset for assisted breeding strategies. In combination with a biotechnological approach, master controllers of flowering time and bud dormancy could be used as tools to shorten the juvenile phase of target plant materials in order to accelerate breeding programs. This type of approach is already being suggested with the employment of transgenes that have the ability to induce flowering, such as *FT* (Yamagishi, Kishigami, & Yoshikawa, 2014), *TFL* (Freiman et al., 2012) and *BpMADS4* (Flachowsky et al., 2011; Weigl, Wenzel, Flachowsky, Peil, & Hanke, 2015) genes, but the use of dormancy regulators in similar strategies is still to be proposed. A different and potentially game changer strategy would be to modify the expression of dormancy controlling genes by means of state of the art technologies such as CRISPR/Cas9 (Bortesi & Fischer, 2015). This could potentially be done in already grown plants

employing viral vectors, a plant-breeding technique with no transmission of genetic modification to the next generation (Yamagishi et al., 2014). In fruit production, the application of chemical compounds is already used as a tool to modulate flowering time and increase yields (see review in Ionescu et al., 2017). Because epigenetic mechanisms participate in gene expression control and hormonal signaling, an alternative for plant breeding approaches is to engineer flowering time by combining efforts on modern genetics and chemicals via the external application of flower-inducing compounds. This is an illustration of how the understanding of flowering time and dormancy control can be important to applied research and to agriculture.

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