Chapter 5
Recent Advances in Genetics and Molecular Control of Bud Dormancy in Pipfruits

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Introduction

Temperate fruit crops have great economic importance worldwide and their production is closely related to bud dormancy, given that a well-adjusted dormancy cycle is crucial for the achievement of their full genetic potential. This process is regulated by environmental inputs, mainly chilling temperatures and photoperiodic changes, which are required for dormancy establishment and release (Horvath et al. 2003; Rohde and Bhalerao 2007). Bud dormancy is usually divided into paradormancy, endodormancy and ecodormancy, which refers to a failure of meristem growth under favorable conditions caused by signals derived from outside of the bud (but from the same plant), from the bud itself or from the environment, respectively (Lang et al. 1987). Dormancy entrance is characterized by growth cessation, bud set and leaf senescence. Once dormant, plants often need to be exposed to extended periods of cold (temperatures below 7.2 °C) to overcome it and the fulfillment of this chilling requirement (CR) culminates in ecodormancy (Horvath et al. 2003). The mechanisms regulating dormancy release are highly heritable and finely tuned, with each genotype being strongly influenced by its region of origin, suggesting a

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strong genetic control of this trait (Dennis 1987; Howe et al. 2000; Labuschagné et al. 2002; Jackson 2003; Campoy et al. 2011).

The direct relationships between bud dormancy and cold exposure gain importance when considering the recently proposed models for global warming. These models predict a rise in global mean temperatures and milder winters, which could result in difficulties for the production of temperate fruit crops (Arora et al. 2003; Campoy et al. 2011; Kirtman et al. 2013). Thereby, the importance of understanding the regulation of dormancy progression is gaining momentum with the main objective of maintaining sustainable crop yields in a changing environment. In this context, a wide range of approaches, from the genetic to the genomic perspective, are being used in several perennial crops as study models. In fact, although the main controlling mechanisms are still unknown or only partially explained, research advances in plant dormancy, especially in peach and poplar, are unveiling key regulators of this process.

Despite worldwide efforts applied to studying the dormancy process, only recently have two of the most economically important temperate fruit crops, apples and pears (FAO 2012), been explored for this agronomic trait. These pipfruits gain their name because of the small hard seeds (pips) in the center of the fruit (Palmer 2012), which differ from seeds of other Rosaceae species, such as peaches and strawberries. In addition, pipfruits also diverge in bud dormancy regulation because instead of being triggered by photoperiodic changes the main regulator of this process is exposure to low temperatures (Heide and Prestrud 2005). The Central and Western Asian origin of the pipfruits could explain their partial insensitivity to photoperiod, given that temperature in these regions varies more strongly than day length in comparison with other latitudes. Therefore, temperature would be a more reliable marker of the cold season than light quality to synchronize their phenology to the environment (Campoy et al. 2011).

Several advanced molecular models for bud dormancy control have been proposed (Horvath 2009; Campoy et al. 2011; van der Schoot and Rinne 2011; Rinne et al. 2011). However, they are based on species in which photoperiodic changes play a major role in dormancy induction and the peculiarities of this process in pipfruits are not addressed by these models. This review intends to help fill this gap, discussing the recent findings in genetics and genomics of bud dormancy control in pipfruits. The better understanding of this process may permit the development of new strategies that could help the generation of cultivars better adapted to each regional cultivation scenario.

Linkage Mapping of Dormancy-Related Traits

A major approach in the discovery of genes controlling phenological characteristics, such as bud dormancy, is to determine the association between the presence or absence of the trait of interest (phenotypes) and the profiles of molecular markers (genotypes) across individuals of a segregating population, a strategy known as linkage mapping (Mackay et al. 2009). Linkage mapping from experimental populations is very widespread in herbaceous crops, such as wheat and rice, but this is

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not the case for tree crops, such as apple and pear (Troggio et al. 2012). The main reasons are the high costs of maintaining a population of trees suitable for linkage mapping and their long juvenile period, especially when working with adult traits such as fruit quality or dormancy of reproductive buds (Flachowsky et al. 2009; Grattapaglia and Kirst 2008; Neale and Kremer 2011; Myles 2013).

One of the first attempts to assess the heritable components of tree bud phenology was done using populations of *Populus* sp. hybrids (Bradshaw and Stettler 1995). At the time, the consensus among geneticists was that characters with broad phenotype distributions, such as time of bud flush, were controlled by a large number of genes, each one with small effects. The authors found that most of the variation for bud phenology observed in their experimental population (84.7 %) was explained by five quantitative trait loci (QTL) distributed in five linkage groups. However, it remained an open question whether each identified QTL represented one gene with a major effect or a cluster of genes with minor effects. This question was addressed by the refinement of the QTL analysis and the mapping of candidate genes for the control of bud phenology (Frewen et al. 2000). The authors found two genes potentially related to dormancy regulation to be coincident with the confidence intervals of two major QTLs, namely *PHYTOCHROME B* (*PHYB*) and *ABSCISIC ACID INSENSITIVE* (*ABI*) homologs. Both were shown to be involved in timing of bud set and bud development (Olsen et al. 1997; Rohde et al. 2002). These first studies demonstrated that most of the genetic control of bud phenology could be mapped to a few genomic intervals.

Dormancy-associated traits, due to their quantitative nature, are often a subject of quantitative genetics disciplines. Bud dormancy-related phenotypes exhibiting a classical Mendelian segregation, which are more straightforward to map than QTLs (Mackay 2001), are very rare. An invaluable research opportunity was explored from the mapping of the *evergrowing* (*evg*) locus of peach (Bielenberg et al. 2008). The *evg* mutants are non-dormant, i.e., they do not stop growing even when exposed to short photoperiods or low temperatures, and the *evg* trait segregates as a single recessive gene. Sequencing of the *evg* locus revealed a cluster of six MIKC-type MADS-box genes, thereafter called *Dormancy-Associated MADS-box* (*DAM*) genes.

When the genetic control of bud dormancy in peach was characterized by quantitative genetics approach, two major QTLs (explaining more than 30 % of the phenotypic variation) were found, and one of them overlapped with the *evg* locus on linkage group one (LG1, Fan et al. 2010). Further high-resolution mapping of this QTL and next-generation resequencing of the genomes from extreme phenotype individuals indicated *DAM* genes as the most probable genetic elements underlying the effects of the LG1 QTL (Zhebentyayeva et al. 2014).

Both peach and poplar are self-compatible and fast-growing trees; hence, true F2 populations can be established in relatively short timeframes (Fan et al. 2010; Faria et al. 2011). Linkage mapping in F2 generation is virtually impossible for self-incompatible species; therefore, alternative cross strategies are needed to obtain segregant populations. The high level of heterozygosity commonly found in self-incompatible species can be used as leverage for the generation of linkage maps by

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the two-way pseudo-testcross approach (Grattapaglia and Sederoff 1994). The main idea behind this strategy is to follow the 1:1 segregation of genotypes from markers that are heterozygous in only one parent. It follows that two linkage maps are constructed, one for each parent, and the maps can be integrated through markers that are present in both parental lines. The two-way pseudo-testcross is a convenient, simple-to-implement and robust strategy for linkage mapping of tree species in the F1 generation and does not depend on prior genetic information from the parental lines.

Apple and pear are self-incompatible species with a long juvenile period, and these limitations have hampered genetic understanding and improvement of both crops (Jackson 2003). The first controlled crosses of apple trees for breeding purposes date from 1806, and apple breeders usually select genotypes carrying desired traits from the F1 progenies (Kellerhals 2009). Many of the target traits to be introgressed to apple cultivars are related to disease resistance, tree architecture, flowering and fruit quality (Korban and Tartarini 2009). Pear breeding also typically involves generation of genetic variation by crossing, aiming to improve fruit quality, disease resistance, storage ability, among other traits (Yamamoto and Chevreau 2009).

Breeding and academic research of slow growing trees, such as apple and pear, can benefit greatly from the knowledge obtained using molecular markers linked to heritable traits. For apple, a considerable range of molecular and genetic data is publicly available, as well as a high-quality whole genome draft (Velasco et al. 2010). Among the many tools and databases available, a noteworthy resource is the apple 8K single nucleotide polymorphism (SNP) array developed by the International RosBREED Consortium (Chagné et al. 2012). The SNPs that compose the chip were chosen after analyzing the resequencing data from 27 cultivar accessions, representing most of the genetic variation available for apple germplasm. Afterwards, due to the lack of SNP markers described for pear and the high collinearity between apple and pear genomes, approximately 1000 newly discovered SNPs from pear were added to the chip, collectively totaling nearly 9000 markers (Montanari et al. 2013). One limitation of this platform, however, is the unexpected segregation patterns for a large number (more than half) of markers (Troggio et al. 2013). The reason for this anomaly is the high level of paralogy exhibited by the apple genome, probably caused by a recent whole genome duplication event (Velasco et al. 2010). In practical terms, a great number of probes anneal in paralogous sites, resulting in distorted genotype proportions in the experimental population. This can be minimized by the use of stringent quality filtering of observed genotype distributions, in order to select only reliable markers.

In apple, several linkage mapping studies have already been done specifically for the characterization of dormancy traits. The experimental population for dormancy-related QTL analysis in apple is set up from the offspring of a crossing between individual cultivars differing in CR. There are many apple cultivars with various ranges of CR, and this trait, as for *Populus* sp., is largely genetically controlled, most likely as a single dominant gene for the low CR trait (Hauagge and Cummins 1991). In an early QTL identification attempt following the two-way pseudo-

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testcross strategy, Conner et al. (1998) found eight regions distributed in seven linkage groups as highly associated with timing of bud break. However, the linkage map constructed did not include markers that could be transferred to the reference apple genetic map and, hence, the numbering of linkage groups is not the standard for apple genetic studies.

In a more recent study, van Dyk et al. (2010) performed map construction and QTL analysis for dormancy traits from populations in South Africa derived from crosses between individuals from 'Anna' (very low CR) and 'Golden Delicious' (high CR) and from 'Anna' and 'Sharpe's Early' (high CR). The maps, constructed from F1 genotypes employing 320 simple sequence repeat (SSR) markers, were composed of 17 linkage groups (LGs), corresponding to the number of apple chromosomes. The single QTL found was positioned on LG9 and explained around 40% of the variation in the timing of both vegetative and floral bud break (van Dyk et al. 2010). In a similar approach, Celton et al. (2011) constructed maps from crossings between 'Starkrimson' and 'Granny Smith' and between X3263 and 'Belrène,' the last consisting of a population of more than 300 individuals. The QTL analysis of timing of bud break revealed several associations for this trait across the genome, the major one being on LG9, in close agreement with the confidence interval found by van Dyk et al. (2010). The region of interest was defined as the first 4 million base pairs from chromosome 9 in the apple genome, a region identical to the one found in an independent linkage mapping of dormancy-related traits performed by our own group (Tessele et al. manuscript in preparation). Candidate gene analysis of this region revealed enrichment for functional classes such as stimulus, biological regulation, signaling, programmed cell death and cell cycle control (Celton et al. 2011). These segregant populations were established in very divergent climatic conditions, yet shared the same genomic region as containing most of the genetic control of the timing of bud break. These findings suggest that variation in dormancy-related traits in apple has a strong genetic component. In addition, the overlap of genomic intervals for QTLs identified from different progenies suggests a common underlying genetic mechanism as responsible for the variation of the trait. The next step, therefore, is to further characterize the major QTLs for apple bud dormancy-related traits, as already carried on for peach (Zhebentyayeva et al. 2014). A consensus approach among molecular geneticists is to genotype the same population used for QTL identification using a high number of markers located in the region of interest, which is often called fine mapping or high-resolution mapping (Mackay 2001).

The availability of the next-generation sequencing technologies and high-quality genomes now allows the discovery of new molecular markers with low relative cost. DNA resequencing was carried out for parental individuals from the population segregating for dormancy traits established by our group, and as a result, more than 80,000 SNPs were discovered (Alencar et al. 2011). After validation, these new markers will be fundamental for the fine characterization of the apple dormancy-related traits QTLs.

Despite its significant economic importance, pear does not benefit from the same range of genetics and genomics resources as apple. Molecular markers have been

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used in pear for the determination of genetic diversity, association with genes of agronomical interest, and construction of linkage maps (Yamamoto and Chevreau 2009). The first pear genetic map was constructed from a cross between Japanese (*P. pyrifolia*) cultivars using random amplified polymorphic DNA (RAPD) markers (Iketani et al. 2001). Yamamoto et al. (2002) assembled a pear map including simple sequence repeats (SSR) markers shared between apple and pear. The transferability of these markers allowed the comparison of maps from apple and pear, and indicated a high level of synteny between the two genomes. The close evolutionary relationship between the two species was clearly demonstrated with the recent publication of the genomes of the Japanese (Wu et al. 2013) and the European (Chagné et al. 2014) pears. In fact, the high transferability of molecular markers between pear and apple allowed a combination of SNPs from both species to be arrayed in the same platform for the genotyping of the two crops interchangeably (Montanari et al. 2013).

Various QTL identification attempts have been made in pear and yielded DNA markers closely associated with disease resistance, fruit storage and leaf traits (Yamamoto and Chevreau 2009; Sun et al. 2009). However, to the best of our knowledge, no QTL mapping for dormancy-related traits were performed in pear to date. Indeed, due to their genetic similarities, much of what is being discovered in apple may be applied to dormancy in pear. This statement is in agreement with the findings reported by Celton et al. (2009), which confirmed the ready transferability of SSR markers from *Malus* to *Pyrus*.

Molecular Control of Bud Dormancy Progression

Bud dormancy is a complex process that includes a range of states, degrees of development and the outgrowth that is tightly synchronized with seasonal changes. The elucidation of molecular networks responsible for the control of bud dormancy progression has been almost exclusively done on systems induced by photoperiodic changes (Böhlenius et al. 2006; Li et al. 2009; Jiménez et al. 2010; Doğramaci et al. 2010). Some components of photoperiod perception are known to play roles in dormancy regulation, such as PHYA (PHYTOCHROME A), CONSTANS (CO) and FT (FLOWERING LOCUS T). In annual plants such as *Arabidopsis thaliana*, flowering occurs in response to long-day photoperiods, with CO and FT controlling photoperiod perception and flowering time, respectively (Amasino and Michaels 2010). In *Populus* trees, Böhlenius et al. (2006) reported that *PtFT1* also controls the short-day-photoperiod-induced growth cessation and bud set. In an independent study, Hsu et al. (2011) identified two *FT* paralogs (*FT1* and *FT2*) in poplar and indicated that their expressions are temporally and spatially separated. These authors demonstrated that *FT1* expression during winter coincides with the transition of vegetative to reproductive phases, whereas *FT2* promotes vegetative growth and inhibition of bud set in response to warm temperatures and long days. In agreement to these findings, Kotoda et al. (2010) reported that apple also has two *FT* genes,

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and Srinivasan et al. (2012) showed that the overexpression of a poplar *FT* in plum (*Prunus* x *domestica*) impaired dormancy entrance.

The expression of genes regulated by photoperiod is interconnected in a cascade of events, where PHYA, along with other circadian clock components, regulates CO, which in turns induces *FT* transcription leading to flowering. Furthermore, the signaling cascade regulated by photoperiod perception is closely connected to the cold temperature perception pathway, involving several related transcription factors (Amasino and Michaels 2010). However, the role of temperature perception in bud set and in induction of bud dormancy is still poorly understood. Some genes that play key roles in photoperiod perception involved in crosstalk with the temperature pathway could act as temperature sensors, such as the phytochromes (Franklin 2009). In *A. thaliana*, temperature regulates flowering through the vernalization pathway, which is mediated by the FLOWERING LOCUS C (FLC). FLC is a MADS-domain transcriptional regulator that represses two floral integrators, *FT* and *SOC1*, inhibiting flowering at low temperatures (Helliwell et al. 2006). Interestingly, there is a feedback loop involving SOC1 and FLC regulation, which may prevent premature flowering under cold conditions (Seo et al. 2009). Hereupon, SOC1 negatively regulates the cold response pathway through the direct repression of C-repeat binding factor/dehydration-responsive element-binding protein (CBF/DREB1) transcription factors, which are responsible for most of the cold-induced gene expression in plants (Seo et al. 2009; Thomashow 2010). On the other hand, the expression of *CBF/DREB1* increases *FLC* expression that in turn represses *FT* and *SOC1*, thereby delaying flowering (Seo et al. 2009). The crosstalk between temperature and photoperiod pathways in dormancy regulation was markedly demonstrated by Wisniewski et al. (2011), which reported that the ectopic expression of a peach *CBF* in apple triggered dormancy induction by short-day photoperiod. The same transgenic plants were further evaluated over three growing seasons demonstrating increased cold tolerance, delayed growth and altered dormancy phenology under field conditions (Artlip et al. 2014).

Horvath (2009) proposed a schematic model of how cold temperatures putatively mediate dormancy induction, suggesting that CBF transcription factors promote expression of *DAM* genes, possibly by chromatin remodeling (Horvath 2009). *DAM* genes are classified as belonging to the SVP/StMADS11 clade of MADS-box transcription factors, and due to protein sequence similarities, genes closely related to *DAM* are sometimes referred to as *SVP*-like genes. In *A. thaliana*, *SVP* is a *MADS-box* gene that regulates floral transition and contributes to the specification of floral meristems (Gregis et al. 2013). The *DAM* genes were first described in peach and presented distinct seasonal expression patterns (Bielenberg et al. 2008). From the six genes described, only *PpDAM5* and *PpDAM6* were regulated by cold exposure (Li et al. 2009). Moreover, the transcript accumulation pattern identified for these genes, e.g., induction during autumn and declining through the winter, suggests a growth repressing role (Li et al. 2009; Yamane et al. 2011). Additionally, it was recently shown that the silencing of *PpDAM6* is preceded by changes in the methylation status of H3K27 residues of histones bound to its chromatin (Leida et al. 2012), as well as occurs in the silencing of *FLC* and other genes that regulate vernalization

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in *A. thaliana* (Angel et al. 2011). Although putative *DAM* orthologues were identified in pear (Ubi et al. 2010; Saito et al. 2013), apple (Falavigna et al. 2014), raspberry (Mazzitelli et al. 2007), kiwifruit (Wu et al. 2012), leafy spurge (Horvath et al. 2010) and apricot (Sasaki et al. 2011), a complete functional characterization of *DAM* genes remains to be reported.

Among efforts made to elucidate the involvement of *DAM* genes in bud dormancy and flowering, Horvath et al. (2010) reported that the overexpression of a leafy spurge *DAM* gene in *Arabidopsis* delayed flowering, as was also observed in *SVP* overexpressing lines (Gregis et al. 2013). Furthermore, Horvath et al. (2010) demonstrated that *DAM* genes are preferentially expressed in response to cold temperatures, causing a negative-regulation of *FT* or *FT*-like genes, leading to growth cessation and dormancy entrance. Interestingly, Sasaki et al. (2011) reported that overexpressing *PmDAM6* in poplar resulted in variable *FT* transcript levels, induction of growth cessation and precocious bud formation. On the other hand, Bai et al. (2013) found no correlation between the expression patterns of *DAM* and *FT* genes in pear, suggesting that both genes are not regulated in the Rosaceae family in the same manner as in leafy spurge. Finally, in the perennial kiwifruit, *SVP*-like genes were identified and functionally characterized in *Arabidopsis*. Out of four genes (*SVP1*, *SVP2*, *SVP3* and *SVP4*), only *SVP3* was able to rescue the flowering phenotype in *Arabidopsis svp* mutant lines (Wu et al. 2012). Distinct roles were therefore suggested for kiwifruit *SVP*-like genes in bud dormancy and flowering. Paradoxically, a report from the same authors showed that the ectopic expression of *SVP3* in kiwifruit and tobacco did not have any effect on growth and dormancy (Wu et al. 2014).

Several models have been devised for the regulation of dormancy induction and release. For example, Horvath (2009) proposed a model for bud dormancy induction where *DAM*, *FT* and a *FT*-like gene named CENTRORADIALIS (*CENL*) play key roles. The *DAM* genes would be induced after a short exposure to cold, probably through the action of CBF and chromatin remodeling mechanisms, as well as by the short-day photoperiod output from the circadian clock mediated by PHYA. Once induced, the DAM transcription factors would repress *FT/CENL*, causing growth cessation and dormancy induction. After long-term cold exposure, likely via chromatin modification, the down-regulation of *DAM* genes occurs leading to dormancy release. Similarly, Jiménez et al. (2010) proposed a simple conceptual model to explain the putative roles of DAM5/DAM6 in the endodormancy-to-ecodormancy transition. According to this model, the expression of *DAM5* and *DAM6* is triggered by short photoperiods. On the other hand, chilling exposure disrupts the circadian perception of photoperiodic stimuli, resulting in repression of *DAM5* and *DAM6*, and allowing the expression of the genes required for growth under permissive environmental conditions. Finally, Campoy et al. (2011) proposed a similar model integrating all this information combined with data generated studying dormancy in other species, such as chestnut and hybrid aspen.

An elegant mechanism to explain dormancy cycling was proposed by Rinne et al. (2001) based on low-temperature mediated enhancement of 1-3-ß-D-glucanases production. Removal of 1-3-ß-glucan from the plasmodesmata restores the symplasmic

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communication network, leading to chilling-induced release from dormancy by the assumption of a proliferation-competent state. The same authors identified three groups of genes, members of *GLUCAN HYDROLASE 17* family (*GH17*), that are upregulated by chilling temperatures and GA biosynthesis in *Populus*. The group 1 *GH17* genes are transiently upregulated by short-term photoperiodic exposure in order to maintain the symplasmic paths to facilitate bud formation. On the other hand, group 2 and 3 *GH17* genes are upregulated by GA_3 and long-term chilling exposure, allowing callose removal and, thereby, enabling reopening of signaling conduits for *FT* transport to the apex. After sufficient chilling, growth-related genes are upregulated by elevated temperatures, mediated by GA_4, leading to bud burst (van der Schoot and Rinne 2011; Rinne et al. 2011). The models proposed for peach and poplar helped to better understand the dormancy processes in perennial trees; however they rely on advances made on species for which photoperiodic changes are the main inductor of bud dormancy (Horvath 2009; Campoy et al. 2011; van der Schoot and Rinne 2011; Rinne et al. 2011). Thus, the major findings related to dormancy progression in pipfruits are often neglected and therefore need to be better addressed.

Bud Dormancy in Pipfruits

Pipfruits differ from other plant models used to study bud dormancy, such as peach and poplar, at the physiological level because the most important environmental trigger for dormancy induction is low-temperature exposure (Heide and Prestrud 2005), instead of photoperiodic changes. Thus, it can be expected that different molecular pathways are being influenced during dormancy entrance in pipfruits. In this sense, several studies have been conducted to identify similarities as well as peculiarities of this process in apples and pears.

Pioneering work has been performed in apple exploring the contrasting phenotypes between 'Gala' and its spontaneous mutation 'Castel Gala'. This last cultivar requires only 50 % of the CR for dormancy release in comparison with the original cultivar, resulting in earlier bud break. Using suppression subtractive hybridization as a gene discovery tool and RT-qPCR for validation, Falavigna et al. (2014) identified 17 candidate genes, with transcripts coding for DAM, dehydrins, GAST1, LTI65, NAC, HTA8, HTA12 and RAP2.12-like proteins presenting major differences in gene expression between cultivars through the winter. One of the most noteworthy results was the transcriptional profile obtained for a *DAM*-like gene, whose expression was very similar to peach *PpDAM5* and *PpDAM6* genes (Li et al. 2009; Yamane et al. 2011). In an independent approach, Porto et al. (2015) carried out a transcriptomic assay aiming to analyze changes in apple gene (~57,000) expression in response to chilling accumulation in the field and under controlled conditions using a microarray chip. Cold exposure mainly repressed the expression of transcripts related to photosynthesis, whereas long-term cold exposure repressed flavonoid biosynthesis genes. These results indicate that photosynthesis and

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auxin transport are major regulatory nodes of apple dormancy and unveil strong candidates for the control of bud dormancy. Genes related to the circadian clock, hormonal signaling, and regulation of growth and flower development were annotated, including the *MdFT1* gene. Interestingly, apple trees overexpressing *MdFT1* displayed early flowering despite a lack of any chilling exposure (Tränkner et al. 2010). Several studies overexpressing *FT* homologous genes in apple reported precocious flowering (Kotoda et al. 2010; Flachowsky et al. 2012; Wenzel et al. 2013), but the authors have not addressed its effects of dormancy process. These findings suggest the existence of common pathways (e.g., DAM family, *FT* homologs and hormone signaling) in the regulation of dormancy progression in apple in comparison with other better characterized species, such as peach and poplar. However, the identification of new pathways whose relationships to dormancy still need to be unveiled remains a possibility.

The availability of the pear genome sequence will likely become a very important tool to improve the genomics of many agronomic traits, including bud dormancy (Chagné et al. 2014; Wu et al. 2013). In fact, despite this advance, several efforts were performed trying to discover the molecular mechanisms underlying bud dormancy progression in pear. Two remarkable and independent pear transcriptomes were generated using RNA-seq to explore endo- and eco-dormant flower buds (Liu et al. 2012; Bai et al. 2013). Interestingly, both studies identified pathways already related to dormancy in other species, but also reported, for the first time, other dormancy-related pathways, such as endocytosis, glycerophospholipid metabolism, and biosynthesis of phenylpropanoids, stilbenoids, diarylheptanoids, gingerols and ether lipids. These data, along with those reported in apple (Falavigna et al. 2014; Porto et al. 2015), suggest that we are far from fully understanding bud dormancy in pipfruits and new research approaches must be explored.

Additionally, besides the whole RNA-seq data generated by Liu et al. (2012) and Bai et al. (2013), both authors presented transcript accumulation patterns for *DAM* genes and their results coincided with the first findings reported for this gene family in pear (Ubi et al. 2010; Saito et al. 2013). Two putative *DAM* genes were identified (namely *PpMADS13–1* and *PpMADS13–2*) and their expression pattern was analyzed by RT-qPCR during dormancy. They showed that both genes are gradually down-regulated concomitantly with endodormancy release (Ubi et al. 2010). After that, a third *DAM* gene was also isolated (*PpMADS13–3*), and its transcript levels showed a decrease near and after endodormancy release (Saito et al. 2013).

Two additional reports also investigated dormancy regulation in pear. Nishitami et al. (2012) identified two putatively novel dormancy-related transcription factors, NAC2 and PRR5, using a microarray chip to study the transition from endodormancy to ecodormancy in pear buds. Both genes displayed a sharp increase in the transcript accumulation levels during the end of endodormancy until ecodormancy. Likewise, Takemura et al. (2013) identified several genes that may play a role in regulating endodormancy release, highlighting the transcriptional profile obtained for clone 245 (*Auxin influx carrier component*), which was induced near and after bud break.

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Another approach to investigate bud dormancy in pear was the characterization of carbohydrate metabolism. Marafon et al. (2011) demonstrated that the exposure of branches to cold temperatures affects starch and soluble sugar contents in wood and bud tissues of Japanese pears. Sufficient chilling supply during winter increased the activities of cell wall acid invertase and sucrose-6-phosphate synthase, yielding increased levels of reducing sugars and starch contents in bud tissues that are then used for budburst and blooming in spring (Marafon et al. 2011). Additionally, another study showed that endodormancy release occurred concomitantly with the accumulation of sorbitol in xylem sap, and the increase of sorbitol influx and catabolism in flower buds occurred only after bud break (Ito et al. 2012). Finally, trying to elucidate which physiological events were involved in the seasonal changes of carbohydrate dynamics during winter, the results found by Ito et al. (2013) suggest that carbohydrates in the shoot tissues may be converted to sorbitol and loaded into xylem sap. Therefore, sorbitol accumulation patterns could be synchronized with the progression of dormancy, whereas the total carbohydrate transported into shoots from other storage organs may be related to freezing tolerance acquisition rather than dormancy progression (Ito et al. 2013).

A groundbreaking discovery by Mason et al. (2014) uncovered fundamental roles of sugar signaling in bud dormancy. According to their report, lateral dormant buds under the effect of apical dominance, which is a form of paradormancy, resume growth upon receiving an extra amount of sugar supply. Sugar surplus in the phloem is a direct consequence of shoot decapitation, and this signal is much faster than auxin depletion across the stem. Lateral bud outgrowth induced by sugars is independent from auxin signaling, long regarded as the main regulator of apical dominance. This new and exciting evidence indicates that carbohydrate metabolism will probably have an increasing importance in studies involving bud dormancy progression in perennial species.

Concluding Remarks

Bud dormancy, especially dormancy release, remains one of the less understood processes in plant biology. This delay in relation to other well-characterized plant phenomena can be due to methodological issues inherent to the study of dormancy itself, as it is one of the most hermetic subjects at the experimental point of view. However, current approaches available in the fields of plant physiology and molecular biology may provide significant advances in the genetics and genomics of this trait. New technologies, such as high throughput data generation and functional analysis in heterologous systems, hold promise for unraveling the inner circuits of dormancy regulation. At the moment, quantitative genetics and comparative genomics seem to be the most fruitful paths toward the identification of components of dormancy regulation. Functional characterization of these components in their original species background is the next challenge, which can reveal how independently described nodes assemble into a full regulatory mechanism.

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