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Plant Dormancy Research: From Environmental Control to Molecular Regulatory Networks

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Annual growth cycles of perennial horticultural and forest woody plant species of temperate and boreal regions can be divided into two phases, namely the growth and dormancy phases. During the dormancy phase, vegetative and reproductive meristems undergo dormancy to withstand sub-zero winter temperatures. The dormant state of the plant organs can be defined classically as a non-growing latent state, where no visible growth occurs in meristem containing plants structures, such as seeds and buds. In the strictest view of the term, dormancy is considered a state of the meristem in which cell divisions cease, and the meristem is unresponsive to growth-promoting cues until dormancy-breaking cues are perceived by the plant (Rohde and Bhalerao, 2007). For more than 30 years, bud dormancy has been distinguished into three types: (i) paradoxically, where growth of the lateral bud is suppressed by actively growing apical bud and is also known as apical dominance; (ii) endo-dormancy, established through environmental cues (low temperature and short day-lengths) and endogenous factors and requires a particular period of low temperature to resume meristematic growth; (iii) eco-dormancy, where plant is competent to resume growth, but prevailing unfavorable environmental conditions arrest its active growth (Lang 1987). As our understanding of the activity-dormancy cycle at the cellular and molecular level has increased, the community’s notions of dormancy have expanded beyond these physiologically-based
categories. For example, new classification and terminology referring to plant dormancy at the cellular level has been proposed by Considine and Considine (2016). At some level, the challenges of defining and classifying the developmental and physiological status of meristem-containing structures through the annual growth cycle have been compounded by the insights into dormancy and dormancy-related processes that have been reported by the community over the last three decades. This becomes particularly evident as we consider dormancy and dormancy-related processes across species.

We illustrate one such example in the Rosaceae, a family that includes many economically important fruit crops such as apple (Malus × domestica) and peach (Prunus persica). In the Rosaceae, the flowering period between the formation of floral meristem (i.e., structural conversion of the shoot apical meristem in terminal, lateral and adventitious buds to the floral meristem with ensuing floral organ primordia formation) and anthesis spans several months over autumn and winter. However, Rosaceae floral buds contain floral meristem and organs that continue to differentiate and develop during the winter. On the other hand, the endodormant vegetative buds of model trees usually contain “developmentally inactive” shoot apical meristems (Singh et al. 2020). In tree phenology, shoot growth cessation is typically the first phenomenon representing tree dormancy induction. Flower meristem formation occurs during and after growth cessation.
Thus, during this dormancy induction phase, floral initiation and floral development continues in floral buds. In other words, flowering and dormancy progress simultaneously in Rosaceae floral buds. This leads to the question as to whether Rosaceae floral buds establish dormancy during tree dormancy phase; or if the term dormancy should not be used in case of winter floral buds of Rosaceae family members. The answer depends on the definition and classification of “dormancy” (Baskin and Baskin, 2004; Cooke et al. 2012; Considine and Considine 2016). Recent molecular and metabolic characterization of Rosaceae floral buds suggest that sweet cherry (P. avium) and peach floral buds stay active during tree dormancy phase (Fadón et al. 2018a; Rothkegel et al. 2020; Yu et al. 2020). However, comparative morphological analysis using Prunus cultivars with different blooming dates suggests that there is a “rest” phase, i.e., temporal suspension or delay of floral development at specific developmental timing (Fadón et al. 2018b; Julian et al. 2011; Yamane et al. 2011). The length of this “rest” phase appeared to be correlated with the genotype-dependent chilling requirements for dormancy release (regain of potential ability to resume growth) and blooming date (Figure 1). Bud dormancy release is similar to vernalization in the family Brassicaceae, in that both are triggered by a prolonged exposure to chilling. Without this chilling, floral organs cannot develop normally in fruit trees, thus chilling conditions are necessary for
the subsequent blooming and fruiting process (Fadón et al. 2018b; Yamane et al., 2011).

The importance of sufficient chilling during the winter dormancy phase was also demonstrated through comparative transcriptomic and DNA methylation-based studies in apple, where sufficient chilling was shown to induce phytohormone-related pathways and post-embryonic development during bud break (Kumar et al. 2017), and promote DNA demethylation with the progressing dormancy period (Kumar et al. 2016b).

The “rest” phase, slow or temporal suspension of flower development even in growth-promoting condition in autumn may be genetically controlled and might play an important role in preventing trees from blooming in autumn and enabling buds to be aligned at a certain developmental stage, thereby ensuring uniform blooming in tree on the onset of spring. Considering that uniform blooming in spring ensures cross-pollination in self-incompatible Rosaceae fruit trees, such as apple and apricot, and also may have merit for humans to simplify cultural practices required for pollination and harvest, floral bud “rest” in Rosaceae could be an acquired trait through natural selection and artificial breeding activity. It is now well established that tree dormancy plays a crucial role in growth and developmental, and in determining fruit quality and yield of the temperate perennial plants. There has been tremendous progress in understanding the regulatory mechanism of seasonal tree growth/dormancy cycle at molecular, physiological and
morphological levels, although further studies will be needed to confirm the existence of
floral bud “rest” phase and its molecular regulatory mechanism in Rosaceae fruit trees.

Potential bud dormancy regulators DAM/SVP2/SVL

Our understanding of molecular regulators of bud dormancy-activity cycles has increased
substantially in the last decade. Most of these studies have been carried out in a limited
number of model species. However, there are some regulators of bud dormancy, such as
the SHORT VEGETATIVE PHASE MADS-box/AGAMOUS LIKE 24 (SVP/AGL24) gene
subfamily, that have been more widely studied across several different taxa. These are
named as DORMANCY-ASSOCIATED MADS-box (DAM) in Rosaceae fruit trees, SVP2
in kiwifruit (Actinidia spp.), and SVP-like (SVL) in poplar (Populus spp.) (Bielenberg et
al. 2008; Kumar et al. 2016a; Falavigna et al., 2019). Genetic studies involving the peach
(Prunus persica) evergrowing (evg) mutant strain incapable of setting terminal buds
indicated that the evg phenotype is caused by a deletion mutation in the peach DAM1–4
genes and low or no DAM5 and DAM6 expression (Bielenberg et al. 2008). Rosaceae
DAMs are down-regulated by a prolonged exposure to cold, which is a key environmental
factor triggering dormancy release (Falavigna et al. 2019), suggesting that DAMs could
be involved in both, dormancy induction and release. Transgenic poplar trees and apple
(Malus × domestica) overexpressing the Japanese apricot (Prunus mume) PmDAM6
gene in vegetative buds, whose expression is up-regulated during dormancy induction (decreased bud break competency in forcing condition) and down-regulated during dormancy release (increased bud break competency in forcing condition), exhibited reduced growth, early bud set and delayed bud break (Sasaki et al. 2011; Yamane et al. 2019). In apple, silencing of \textit{MdDAM1} and \textit{MdDAM4} expression eliminates terminal bud formation and dormancy induction in apple, which is similar to the \textit{evg} mutant phenotype in peach (Moser et al. 2020). Another \textit{DAM} member, \textit{MdDAMb} overexpression caused bud break repression in apple (Wu et al. 2017a). Overexpression of kiwifruit \textit{SVP2} delayed lateral vegetative bud break (Wu et al. 2017b). In poplar, as summarized in this special issue by Singh et al. (2020), SVL mediates the short-day photoperiod-induced abscisic acid (ABA)-dependent vegetative bud dormancy induction and establishment (Singh et al. 2019), as well as represses bud break (Singh et al. 2018). SVL may have multifaceted roles in different phases of dormancy by regulating multiple target factors including ABA and gibberellin biosynthesis and signaling, \textit{TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP)} and \textit{FLOWERING LOCUS T (FT)} genes (Busov 2019). Moreover, Singh et al. (2019) also proposed that poplar SVL may be linked to dormancy-specific cell wall modifications that have a prominent role for vegetative bud dormancy establishment (Paul et al. 2014).
accumulation of ABA induces the expression of callose synthase (*CALS*) gene. The resulting callose aggregation causes plasmodesmata to close because of the formation of a sphincter structure, thereby inhibiting cell–cell communication. Singh et al. (2019) reported that SVL can bind to the *CALS* promoter region and positively regulates *CALS* expression, leading to the formation of plasmodesmata sphincters and the induction of dormancy establishment (Busov 2019). In contrast, molecular pathways targeted by Rosaceae DAM have been partially clarified in some fruit trees and DAMs could affect ABA biosynthesis and signaling in Japanese pear (*Pyrus pyrifolia*) and cytokinin accumulations in apple vegetative buds (Tuan et al. 2017; Yamane et al. 2019). *SVP2* overexpression leads to transcriptome-level changes in the ABA and dehydration response pathways in kiwifruit (Wu et al. 2017b). Chromatin-immunoprecipitation-sequencing (ChIP-Seq) analysis demonstrated that SVP2 target genes negatively regulated plant growth and directly bound to genes associated with ABA and drought response pathways (Wu et al., 2018). Taken together, DAMs/SVP2/SVL may share a common feature of involvement in the regulation of plant growth and ABA activity in vegetative buds (Figure 2). Taking advantage of genome and epigenome sequencing in many different tree crops (for reviews, see Aranzana et al. 2019; Peace et al. 2019) and informatics technology advancement, future works will provide further insights into the
molecular mechanisms underlying the transcriptional control of DAM/SVP2/SVL and their targeting molecular regulatory pathways.

Special issue in commemoration of the 6th Plant Dormancy Symposium

In order to bring together international experts and emerging scientists with an interest in the exciting field of plant dormancy research, the 6th Plant Dormancy Symposium was held in Kyoto, Japan during Oct. 23-26 2018 wherein 134 participants from 23 countries participated. The International Plant Dormancy Symposium has been held once every four to five years since 1995. The first Plant Dormancy Symposium took place in 1995 at Oregon, USA, followed by at Angers, France in 1999; Wageningen, Netherland in 2004; Fargo, North Dakota, USA in 2009; and Auckland, New Zealand in 2013. The plant dormancy symposium was launched by the community of plant seed and bud dormancy researchers. This symposium provides an excellent forum for the exchange of information and ideas on recent developments in plant dormancy research, and the development of new scientific collaborations. In each of the seven sessions of 6th Plant Dormancy Symposium, *viz.*, “Environmental and Signaling”, “Genetics and Epigenetics”, “Ecology (including Climate change)”, “Evolution and Diversity”, “Hormone”, “Applied Aspects”, and “Perspectives from the Basic to the Field”, research topics on both seed and bud dormancy were combined, which effectively enabled the participants to gain the
comparative and integrated insights of both, seed and bud dormancy. The presentations in these sessions encompassed the latest findings related to the mechanisms of plant dormancy in vegetative buds, floral buds, seeds and other meristems from a variety of experimental plant systems ranging from agronomic crops such as rice (*Oryza sativa*), wheat (*Triticum* spp.), and potato (*Solanum tuberosum*), and horticultural and tree crops such as apple, peach, pear, kiwifruit, and grape (*Vitis* spp.) to model plants such as Arabidopsis and poplar. The symposium provided the participants with recent advanced knowledge on various aspects of both seed and bud dormancy. We hereby introduce the special issue on Plant Dormancy in the journal “Tree Physiology” in commemoration of the 6th Plant Dormancy Symposium wherein 10 excellent articles have been compiled and are being made available to the readers. The articles presented in this special issue include eight original research articles, one review article and one methods article. Two research articles reported the changes in phytohormone biosynthesis and signaling pathways during dormancy. Ito et al. (2019) reported that the levels of indole acetic acid (IAA) and trans-zeatin (tZ) remained low during dormancy, but increased towards the flowering stage in the Japanese pear. Gibberellic acid (GA) levels remained high during dormancy induction, then decreased before slightly increasing prior to flowering. Analysis of ABA levels during various stages of dormancy suggested that a reduction in ABA is not needed.
to resume the growth after dormancy. However, in case of sweet cherry (*Prunus avium* L.), Vimont et al. (2020) reported high levels of ABA during the onset of dormancy and reduced level just before the release of dormancy. A *UDP-GLYCOSYLTRANSFERASE* gene, *PavUG71B6* was suggested to be associated with low ABA content in the early cultivars. Three research articles report identification and characterization of genes which play important roles during various phases of winter dormancy. Nishiyama et al. (2019) reported that an apple FLC-like gene, *MdFLC-like*, was upregulated in response to cold exposure and flower primordia development during endodormancy progression. Overexpression of *MdFLC-like* gene in Arabidopsis was found to delay bolting and reduce the plant size, but the number of rosette leaves and flower organ formation remained unaffected. Jia et al. (2020) performed transcriptome and miRNA analysis in the seeds of *Ginkgo biloba* and identified miRNA156 as an important regulator of morphophysiological dormancy. Prudencio et al. (2020) performed RNA-seq analysis in three almond cultivars differing in chilling requirements and flowering times during the endodormancy release of flower buds and identified early and late flowering time candidate genes associated with several important molecular and cellular pathways. In another research paper, Endoh and Fujikawa (2019) investigated the mechanism of freezing resistance in eco-dormant buds of birch (*Betula platyphylla*) using differential
thermal analysis (DTA) and cryo-scanning electron microscopy (cryo-SEM) and reported that the cells in buds respond to subzero temperature through rapid equilibrium dehydration. In an interesting study, Fadón et al. (2020) studied the male meiosis in four cultivars of sweet cherry in relation to the chilling and heat requirements during various stages of dormancy. Their results demonstrated that male meiosis was conditioned by endodormancy, but specially by warm temperature, during the forcing period. This observation is in contrast to the observations reported in other related species, opening a new avenue for further studies to understand the mechanisms of synchronized dormancy with season. Partanen et al. (2020) studied the effect of chilling on the endodormancy release in Norway spruce (*Picea abies* (L.) Karst.) grafts, where twigs used as scions were taken from trees with different age, ranging from 16 to 80 years. The results showed that the environmental regulation of endodormancy release is not related to the age of the tree. In a methods paper, Egea et al. (2020) proposed a dynamic model based on a set of model parameter values to predict the date of endodormancy release in apricot. A review article by Singh et al. (2020) summarized the literature related to the regulation of growth and seasonal dormancy in trees, taking the information related to model tree species *Populus* spp. as a reference.

**Future prospects**
Phytohormones as well as primary and secondary metabolites are also involved in regulation of dormancy in both vegetative and floral buds (Sherif and Liu 2019). Changes to cell-cycle regulation through TOR-kinase signaling (Or et al., 2000; Considine, 2018), lipid bodies (Grimberg et al. 2018; Veerabagu et al. 2020), carbon resources (Tarancón et al. 2017), sugar metabolism (Zhang et al. 2018), DNA methylation (Kumar et al. 2016b) and reactive oxygen species (ROS) (Beauvieux et al. 2018) have been proposed as distinct dormancy-associated characteristics. Additionally, genomic and genetic approaches such as quantitative trait locus (QTL) and genome wide association study (GWAS) are powerful tools to identify genetic regions responsible for natural variation in the regulation of bud phenology (Kitamura et al. 2018; McKown et al. 2018; Shi et al. 2020).

In order to understand complex regulatory network underlying dormancy regulation, latest advance genomic and genetic approaches may prove to be useful. Furthermore, studies not only on model forestry trees and important fruit tree crops, but also on various kinds of trees grown under different climatic conditions, distribution and evolution will be useful to understand the common and distinct mechanisms behind dormancy evolution.

Warming of fall, winter and spring due to climate change is expected to impact timing and intensity of growth resumption and flowering progression, which may threaten stable fruit and timber production, and disrupt the sustainability of ecosystems (Boudichevskaia

A Self-archived copy in Kyoto University Research Information Repository
https://repository.kulib.kyoto-u.ac.jp
et al. 2020). Upon anticipated changes due to global warming, more precise and clear understanding of bud dormancy may help alleviate the negative effects of climate change.

As the editors for this special issue, we thank all the authors who have contributed the interesting articles that appear in the Dormancy special issue. We also thank and appreciate all the reviewers for their generous support and valuable comments that helped to improve the quality of the manuscripts. We sincerely hope that this special issue of Tree Physiology will be useful for scientific community and will help researchers working in the area of plant dormancy to design their future research for the improvement of tree species.

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Figure legend

**Figure 1.** Flower primordia structures in peach floral buds during tree dormancy phase (from autumn to winter). In peach, flower initiation, flower organogenesis and growth cessation (tree dormancy induction) usually occur simultaneously during late summer. By early autumn, flower organ primoria including pistil have usually been generated. During autumn, floral buds tend to show slow or suspension of flower size development before the fulfillment of chilling requirement for blooming. After genotype-dependent chilling requirement is fulfilled, floral buds can bloom in field condition when genotype-dependent heat requirement is fulfilled. Prior to prolonged chilling exposure, such as in November, higher blooming percentages cannot be achieved even trees are grown in forcing condition. However, after prolonged chilling exposure, such as in January, normal blooming can be observed in forcing condition. Therefore, prolonged chilling may have an important environmental cue to promote floral primordia maturation and release the suspension of floral bud development (release of floral bud “rest”). CR: amounts of chilling requirement for dormancy release calculated by chill hour (CH) model (Weinberger, 1950).

**Figure 2.** The common and distinct molecular function of Rosaceae DAM and poplar
SVL on vegetative bud dormancy regulation with special reference to phytohormone regulation. *DAM* is induced by dormancy-inducing environmental factors, short photoperiod and short cold exposure, while *SVL* is induced by short photoperiod-induced increased abscisic acid (ABA). Both *DAM* and *SVL* are repressed by dormancy release-inducing environmental factors, prolonged cold exposure. They have a common function to induce gene encoding key enzyme of ABA biosynthesis, *NCED*, and both are induced by ABA, thus *DAM/SVL* may form ABA positive feedback loop in dormant bud. *DAM* functions as a growth inhibitor, bud set inducer and bud break repressor through the regulation of cytokinin and ABA. On the other hand, *SVL* functions to establish dormancy and also to release dormancy through the regulation of gibberellin (GA) biosynthesis and signaling. Additionally, *SVL* helps to form dormancy-specific cell structure in shoot apical meristem, plasmodesmata sphincter, by promoting *callose synthase (CALS)*. *SVL* also represses *FT* that can induce dormancy release.
Figure 1

Forcing condition

- Oct.
- Nov. (64)
- Dec. (414)
- Jan. (1053)
- Feb. (1831)

Less blooming

Slow or suspension of flowering development (chilling requirement period)

Blooming progression (heat requirement period)

Forcing condition

- ‘Shimizu-Hakuto’ (CR=1100CH)
- ‘Tsukuba-Ichigo’ (CR=500CH)

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Figure 2

Short photoperiod
Short cold

Prolonged cold

Rosaceae DAM

ABA

Positive feedback loop

NCED5

cytokinin

Growth inhibition and bud set

Bud break

Short photoperiod
Prolonged cold

ABA

Poplar SVL

CALS

GA

FT

Dormancy establishment

Dormancy release and bud break

Dormancy release

and bud break

NCED3