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) para-dormancy, 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

37 categories. For example, new classification and terminology referring to plant dormancy
38 at the cellular level has been proposed by Considine and Considine (2016). At some
39 level, the challenges of defining and classifying the developmental and physiological
40 status of meristem-containing structures through the annual growth cycle have been
41 compounded by the insights into dormancy and dormancy-related processes that have
42 been reported by the community over the last three decades. This becomes particularly
43 evident as we consider dormancy and dormancy-related processes across species.

44 We illustrate one such example in the Rosaceae, a family that includes many
45 economically important fruit crops such as apple (*Malus* × *domestica*) and peach (*Prunus*
46 *persica*). In the Rosaceae, the flowering period between the formation of floral meristem
47 (i.e., structural conversion of the shoot apical meristem in terminal, lateral and
48 adventitious buds to the floral meristem with ensuing floral organ primordia formation)
49 and anthesis spans several months over autumn and winter. However, Rosaceae floral
50 buds contain floral meristem and organs that continue to differentiate and develop during
51 the winter. On the other hand, the endodormant vegetative buds of model trees usually
52 contain "developmentally inactive" shoot apical meristems (Singh et al. 2020). In tree
53 phenology, shoot growth cessation is typically the first phenomenon representing tree
54 dormancy induction. Flower meristem formation occurs during and after growth cessation

55 in Rosaceae fruit trees. Thus, during this dormancy induction phase, floral initiation and
56 floral development continues in floral buds. In other words, flowering and dormancy
57 progress simultaneously in Rosaceae floral buds. This leads to the question as to whether
58 Rosaceae floral buds establish dormancy during tree dormancy phase; or if the term
59 dormancy should not be used in case of winter floral buds of Rosaceae family members.
60 The answer depends on the definition and classification of "dormancy" (Baskin and
61 Baskin, 2004; Cooke et al. 2012; Considine and Considine 2016). Recent molecular and
62 metabolic characterization of Rosaceae floral buds suggest that sweet cherry (*P. avium*)
63 and peach floral buds stay active during tree dormancy phase (Fadón et al. 2018a;
64 Rothkegel et al. 2020; Yu et al. 2020). However, comparative morphological analysis
65 using *Prunus* cultivars with different blooming dates suggests that there is a "rest" phase,
66 i.e., temporal suspension or delay of floral development at specific developmental timing
67 (Fadón et al. 2018b; Julian et al. 2011; Yamane et al. 2011),. The length of this "rest"
68 phase appeared to be correlated with the genotype-dependent chilling requirements for
69 dormancy release (regain of potential ability to resume growth) and blooming date
70 (Figure 1). Bud dormancy release is similar to vernalization in the family Brassicaceae,
71 in that both are triggered by a prolonged exposure to chilling. Without this chilling, floral
72 organs cannot develop normally in fruit trees, thus chilling conditions are necessary for

73 the subsequent blooming and fruiting process (Fadón et al. 2018b; Yamane et al., 2011).
74 The importance of sufficient chilling during the winter dormancy phase was also
75 demonstrated through comparative transcriptomic and DNA methylation-based studies in
76 apple, where sufficient chilling was shown to induce phytohormone-related pathways and
77 post-embryonic development during bud break (Kumar et al. 2017), and promote DNA
78 demethylation with the progressing dormancy period (Kumar et al. 2016b).

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

91 morphological levels, although further studies will be needed to confirm the existence of
92 floral bud “rest” phase and its molecular regulatory mechanism in Rosaceae fruit trees.

93 **Potential bud dormancy regulators DAM/SVP2/SVL**

94 Our understanding of molecular regulators of bud dormancy-activity cycles has increased
95 substantially in the last decade. Most of these studies have been carried out in a limited
96 number of model species. However, there are some regulators of bud dormancy, such as
97 the S*HORT VEGETATIVE PHASE MADS-box/AGAMOUS LIKE 24* (*SVP/AGL24*) gene
98 subfamily, that have been more widely studied across several different taxa. These are
99 named as *DORMANCY-ASSOCIATED MADS-box* (*DAM*) in Rosaceae fruit trees, *SVP2*
100 in kiwifruit (*Actinidia* spp.), and *SVP-like* (*SVL*) in poplar (*Populus* spp.) (Bielenberg et
101 al. 2008; Kumar et al. 2016a; Falavigna et al., 2019). Genetic studies involving the peach
102 (*Prunus persica*) *evergrowing* (*evg*) mutant strain incapable of setting terminal buds
103 indicated that the *evg* phenotype is caused by a deletion mutation in the peach *DAM1–4*
104 genes and low or no *DAM5* and *DAM6* expression (Bielenberg et al. 2008). Rosaceae
105 *DAMs* are down-regulated by a prolonged exposure to cold, which is a key environmental
106 factor triggering dormancy release (Falavigna et al. 2019), suggesting that DAMs could
107 be involved in both, dormancy induction and release. Transgenic poplar trees and apple
108 (*Malus* × *domestica*) overexpressing the Japanese apricot (*Prunus mume*) *PmDAM6*

109 gene in vegetative buds, whose expression is up-regulated during dormancy induction
110 (decreased bud break competency in forcing condition) and down-regulated during
111 dormancy release (increased bud break competency in forcing condition), exhibited
112 reduced growth, early bud set and delayed bud break (Sasaki et al. 2011; Yamane et al.
113 2019). In apple, silencing of *MdDAM1* and *MdDAM4* expression eliminates terminal bud
114 formation and dormancy induction in apple, which is similar to the *evg* mutant phenotype
115 in peach (Moser et al. 2020). Another *DAM* member, *MdDAMb* overexpression caused
116 bud break repression in apple (Wu et al. 2017a). Overexpression of kiwifruit *SVP2*
117 delayed lateral vegetative bud break (Wu et al. 2017b). In poplar, as summarized in this
118 special issue by Singh et al. (2020), SVL mediates the short-day photoperiod-induced
119 abscisic acid (ABA)-dependent vegetative bud dormancy induction and establishment
120 (Singh et al. 2019), as well as represses bud break (Singh et al. 2018). SVL may have
121 multifaceted roles in different phases of dormancy by regulating multiple target factors
122 including ABA and gibberellin biosynthesis and signaling, *TEOSINTE*
123 *BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTOR* (*TCP*) and *FLOWERING*
124 *LOCUS T* (*FT*) genes (Busov 2019). Moreover, Singh et al. (2019) also proposed that
125 poplar SVL may be linked to dormancy-specific cell wall modifications that have a
126 prominent role for vegetative bud dormancy establishment (Paul et al. 2014). The

127 accumulation of ABA induces the expression of *callose synthase* (*CALS*) gene. The
128 resulting callose aggregation causes plasmodesmata to close because of the formation of
129 a sphincter structure, thereby inhibiting cell–cell communication. Singh et al. (2019)
130 reported that SVL can bind to the *CALS* promoter region and positively regulates *CALS*
131 expression, leading to the formation of plasmodesmata sphincters and the induction of
132 dormancy establishment (Busov 2019). In contrast, molecular pathways targeted by
133 Rosaceae DAM have been partially clarified in some fruit trees and DAMs could affect
134 ABA biosynthesis and signaling in Japanese pear (*Pyrus pyrifolia*) and cytokinin
135 accumulations in apple vegetative buds (Tuan et al. 2017; Yamane et al. 2019). *SVP2*
136 overexpression leads to transcriptome-level changes in the ABA and dehydration
137 response pathways in kiwifruit (Wu et al. 2017b). Chromatin-immunoprecipitation-
138 sequencing (ChIP-Seq) analysis demonstrated that SVP2 target genes negatively
139 regulated plant growth and directly bound to genes associated with ABA and drought
140 response pathways (Wu et al., 2018). Taken together, DAMs/SVP2/SVL may share a
141 common feature of involvement in the regulation of plant growth and ABA activity in
142 vegetative buds (Figure 2). Taking advantage of genome and epigenome sequencing in
143 many different tree crops (for reviews, see Aranzana et al. 2019; Peace et al. 2019) and
144 informatics technology advancement, future works will provide further insights into the

145 molecular mechanisms underlying the transcriptional control of DAM/SVP2/SVL and
146 their targeting molecular regulatory pathways.

147 **Special issue in commemoration of the 6th Plant Dormancy Symposium**

148 In order to bring together international experts and emerging scientists with an interest in
149 the exciting field of plant dormancy research, the 6th Plant Dormancy Symposium was
150 held in Kyoto, Japan during Oct. 23-26 2018 wherein 134 participants from 23 countries
151 participated. The International Plant Dormancy Symposium has been held once every
152 four to five years since 1995. The first Plant Dormancy Symposium took place in 1995 at
153 Oregon, USA, followed by at Angers, France in 1999; Wageningen, Netherland in 2004;
154 Fargo, North Dakota, USA in 2009; and Auckland, New Zealand in 2013. The plant
155 dormancy symposium was launched by the community of plant seed and bud dormancy
156 researchers. This symposium provides an excellent forum for the exchange of information
157 and ideas on recent developments in plant dormancy research, and the development of
158 new scientific collaborations. In each of the seven sessions of 6th Plant Dormancy
159 Symposium, *viz*., "Environmental and Signaling", "Genetics and Epigenetics", "Ecology
160 (including Climate change)", "Evolution and Diversity", "Hormone", "Applied Aspects",
161 and "Perspectives from the Basic to the Field", research topics on both seed and bud
162 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

181 to resume the growth after dormancy. However, in case of sweet cherry (*Prunus avium*
182 L.), Vimont et al. (2020) reported high levels of ABA during the onset of dormancy and
183 reduced level just before the release of dormancy. A *UDP-GLYCOSYLTRANSFERASE*
184 gene, *PavUG71B6* was suggested to be associated with low ABA content in the early
185 cultivars. Three research articles report identification and characterization of genes which
186 play important roles during various phases of winter dormancy. Nishiyama et al. (2019)
187 reported that an apple FLC-like gene, *MdFLC-like*, was upregulated in response to cold
188 exposure and flower primordia development during endodormancy progression.
189 Overexpression of *MdFLC-like* gene in Arabidopsis was found to delay bolting and
190 reduce the plant size, but the number of rosette leaves and flower organ formation
191 remained unaffected. Jia et al. (2020) performed transcriptome and miRNA analysis in
192 the seeds of *Ginkgo biloba* and identified miRNA156 as an important regulator of
193 morphophysiological dormancy. Prudencio et al. (2020) performed RNA-seq analysis in
194 three almond cultivars differing in chilling requirements and flowering times during the
195 endodormancy release of flower buds and identified early and late flowering time
196 candidate genes associated with several important molecular and cellular pathways. In
197 another research paper, Endoh and Fujikawa (2019) investigated the mechanism of
198 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

217 Phytohormones as well as primary and secondary metabolites are also involved in
218 regulation of dormancy in both vegetative and floral buds (Sherif and Liu 2019). Changes
219 to cell-cycle regulation through TOR-kinase signaling (Or et al., 2000; Considine, 2018),
220 lipid bodies (Grimberg et al. 2018; Veerabagu et al. 2020), carbon resources (Tarancón et
221 al. 2017), sugar metabolism (Zhang et al. 2018), DNA methylation (Kumar et al. 2016b)
222 and reactive oxygen species (ROS) (Beauvieux et al. 2018) have been proposed as distinct
223 dormancy-associated characteristics. Additionally, genomic and genetic approaches such
224 as quantitative trait locus (QTL) and genome wide association study (GWAS) are
225 powerful tools to identify genetic regions responsible for natural variation in the
226 regulation of bud phenology (Kitamura et al. 2018; McKown et al. 2018; Shi et al. 2020).
227 In order to understand complex regulatory network underlying dormancy regulation,
228 latest advance genomic and genetic approaches may prove to be useful. Furthermore,
229 studies not only on model forestry trees and important fruit tree crops, but also on various
230 kinds of trees grown under different climatic conditions, distribution and evolution will
231 be useful to understand the common and distinct mechanisms behind dormancy evolution.
232 Warming of fall, winter and spring due to climate change is expected to impact timing
233 and intensity of growth resumption and flowering progression, which may threaten stable
234 fruit and timber production, and disrupt the sustainability of ecosystems (Boudichevskaia

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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383

384 **Figure legend**

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

400

401 **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

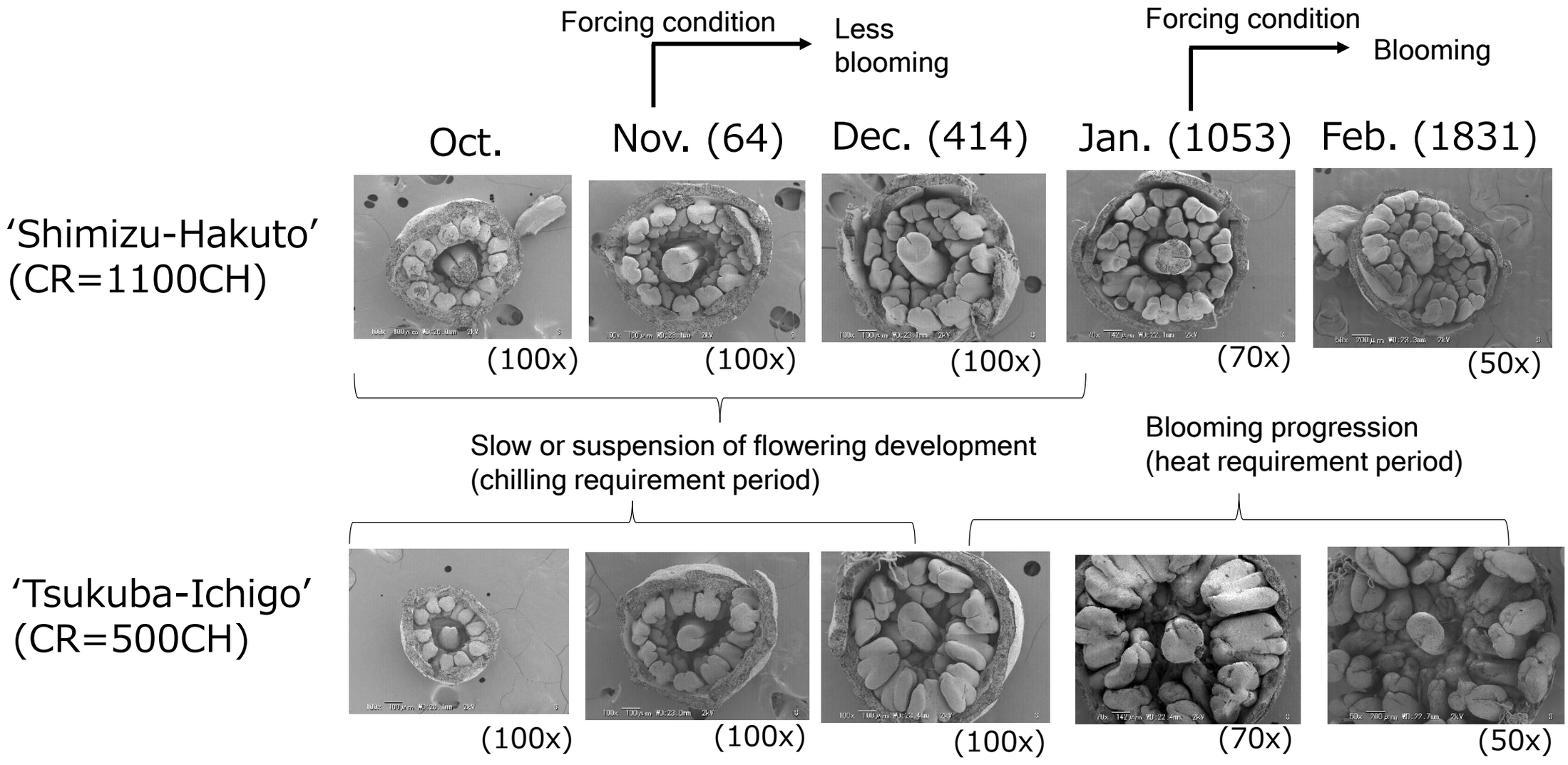
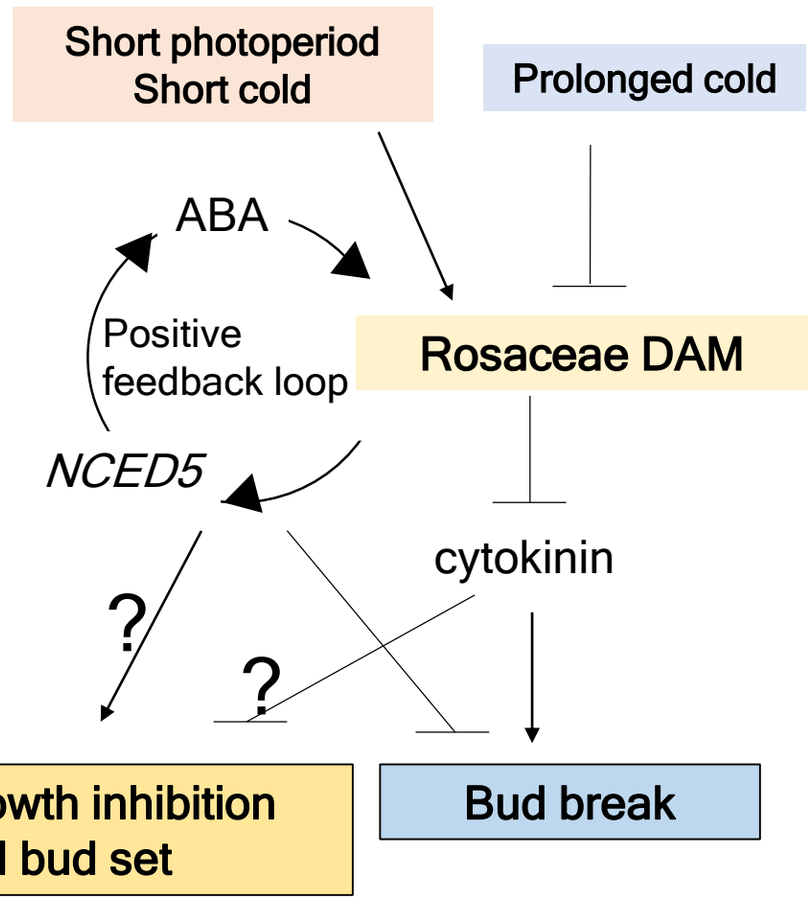
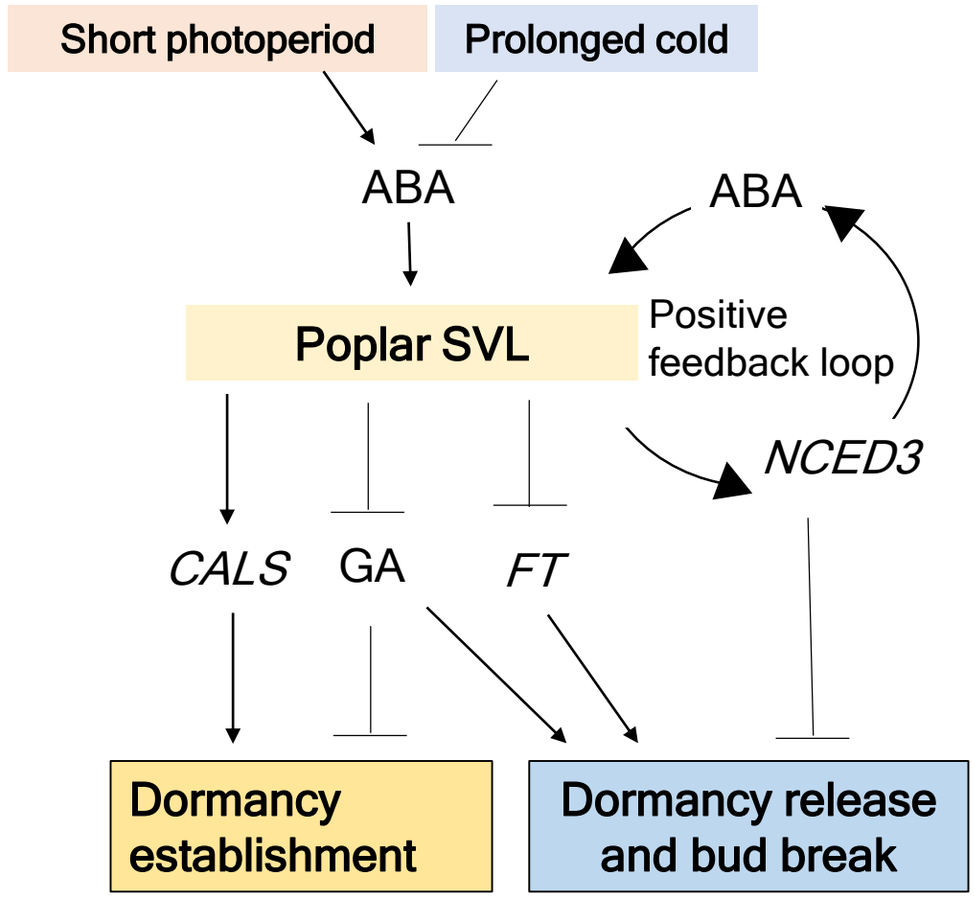

Forcing condition
Less blooming
Forcing condition
Blooming
Oct.
Nov. (64)
Dec. (414)
Jan. (1053)
Feb. (1831)
'Shimizu-Hakuto'
(CR=1100CH)
(100x)
(100x)
(100x)
(70x)
(50x)
Slow or suspension of flowering development
(chilling requirement period)
Blooming progression
(heat requirement period)
'Tsukuba-Ichigo'
(CR=500CH)
(100x)
(100x)
(100x)
(70x)
(50x)

Figure 2


Short photoperiod
Short cold
Prolonged cold
ABA
Positive
feedback loop
Rosaceae DAM
NCED5
cytokinin
?
?
Growth inhibition
and bud set
Bud break


Short photoperiod
Prolonged cold
ABA
ABA
Poplar SVL
Positive
feedback loop
NCED3
CALS
GA
FT
Dormancy
establishment
Dormancy release
and bud break