Bud dormancy allows most deciduous fruit tree species to avoid injury in unsuitable environments, synchronize their annual growth, and adapt to a temperate zone climate. Because bud dormancy affects next season’s fruit production and vegetative growth, it is considered one of the most important physiological factors that control fruit production. Recent global climate changes require us to better understand the genetic factors regulating bud dormancy, especially those that induce dormancy release and subsequent bud break. In this review, environmental factors that affect the seasonal dormancy depth of Japanese apricot (P. mume Siebold & Zucc.) and peach [P. persica (L.) Batsch] are first outlined. Next, recent progress of genetic, biochemical, and molecular biological studies of Prunus dormancy regulation is described. Recent advances in functional genomics have promoted the discovery of gene function and gene networks associated with bud dormancy regulation. A group of candidate genes for bud dormancy regulation, the DORMANCY-ASSOCIATED MADS-box (DAM) genes in Prunus, are focused. Recently reported expressional analysis suggests a significant role for DAMs in dormancy release and bud break of Japanese apricot and peach vegetative buds. Transformation studies of PmDAM6 have demonstrated that it has an inhibitory effect on the apical growth of poplar (Populus spp.). As bud dormancy is a quantitative polygenic trait, not only DAMs, but also other genes and gene networks appear to regulate bud dormancy. Ongoing and future studies will undoubtedly facilitate the unveiling of the molecular aspects of bud dormancy regulation in temperate fruit tree species of Prunus.

Key Words: chilling requirement, climate change, DORMANCY-ASSOCIATED MADS-box, endodormancy, transcription factor.
meristem to resume growth under favorable conditions (Rohde and Bhlerao, 2007). Lang (1987) and Lang et al. (1987) classified the dormancy states as being paradormancy, endodormancy, and ecodormancy. Both endodormancy and paradormancy can be defined as a state induced by the perception of the promoting environmental or endogenous signaling cue, whether this originated solely within the meristem-containing tissue (endodormant) or in a structure distinct from the structure undergoing dormancy (paradormant). A specific amount of chilling exposure is known to critically induce the shift of endodormancy to ecodormancy. Ecodormancy is a state brought about by the limitation of growth-promoting factors, such as warm temperatures, sufficient water and nutrient supply. Although Lang’s definition has been widely adopted for use in dormancy research papers, recently accumulated knowledge about the molecular mechanisms of dormancy requires us to revisit the use of this terminology. For example, it is difficult to discriminate between paradormancy and endodormancy if the involvement of a mobile signal from leaves to meristem is critical for meristem endodormancy regulation. Furthermore, since chilling exposure can promote bud burst even after endodormancy release, the timing of chilling requirement (CR) fulfillment is difficult to determine. Therefore, it is difficult to distinguish between endodormant buds and ecodormant buds. Indeed, bud dormancy is a dynamic rather than a single state, with interactions between genetic and environmental cues (as reviewed by Cooke et al., 2012). Therefore this review uses the terms deep dormancy, non-deep dormancy, and less dormancy, as proposed by Cooke et al. (2012), where the number of opened buds increases and time to bud burst shortens as the tree phase moves from deep-dormant state to less-dormant state.

Genetic and molecular regulation of bud dormancy has been extensively studied in a model woody plant, poplar (Populus spp.), and much progress has been made, as reviewed by Cooke et al. (2012), Rinne et al. (2010), and Rohde and Bhlerao (2007). In Populus, the photoperiodic control of growth cessation and bud set has been extensively studied as target dormancy traits compared to other dormancy events, such as dormancy maintenance and release. The accumulated evidence suggested that phytochrome- and circadian clock-related genes such as PHYTOCHROME A, LATE ELONGATED HYPOCOTYL, CIRCADIAN CLOCK ASSOCIATED 1, and TIMING OF CAB EXPRESSION were involved in short daylength (SD)-induced bud set in poplar (Ibañez et al., 2010; Kozarewa et al., 2010; Olsen et al., 1997; Ruttkink et al., 2007). An important breakthrough in our understanding of poplar growth cessation and bud set was the finding that the CONSTANS (CO)/FT module, a well-known component playing a critical role in flowering induction, also regulates this SD-induced phase transition (Bohlenius et al., 2006; Hsu et al., 2011; Pin and Nilsson, 2012). Rohde et al. (2011) identified six robust QTLs for time to bud set conserved in four different poplar pedigrees, and FT was co-localized with one of these QTLs. Recently, Rinne et al. (2011) reported that FT is hyperinduced during the chilling-induced dormancy release of poplar, suggesting that FT is involved not only in dormancy induction but also in dormancy release. Mohamed et al. (2010) reported that overexpression of CENTRORADIALIS (CEN)/TERMINAL FLOWER1 (TFL1), another member of the PEBP family to which FT belongs, resulted in altered chilling requirements and delayed bud burst in Populus. As with seed dormancy regulation (for a review, see Finkelstein et al., 2008), plant hormones such as abscisic acid (ABA) and gibberellic acid (GA) seem to be integrated in bud dormancy regulation (Cooke et al., 2012). For example, Rinne et al. (2011) reported that chilling up-regulated a number of GA biosynthesis genes, leading to reopened signaling conduits in the embryonic shoot and resulting in dormancy release. A gene encoding transcription factor involved in ABA signaling, ABSCISIC ACID-INSSENSITIVE3, overexpressors showed altered bud formation (Rohde et al., 2002). In addition, a recent work has highlighted the potential importance of epigenetic regulation in bud dormancy of Populus (Bräutigam et al., 2013; Ruttkink et al., 2007).

The accumulated knowledge about poplar bud dormancy regulation, as briefly described above, is of great use and undoubtedly accelerates molecular and genomic efforts to discover the genes associated with the bud dormancy of temperate fruit trees. However, we must keep in mind that programmed genetic systems of bud dormancy regulation may not necessarily be the same among the diverse plant species that exhibit bud dormancy, even though most perennial woody plants have adapted and evolved dormancy for survival. In addition, primary environmental cues that trigger the bud phenology cycle, such as the induction of bud set (or shoot tip abortion), vary depending on a given plant species (Tanino et al., 2010). Accordingly, the characterization of molecular networks regulating the dormancy of various woody species is being carried out by omics studies that use the target plants themselves. Examples of recently published omics studies using agronomically important fruit tree species include: grapevine (Vitis spp.) (Diaz-Riquelme et al., 2012; Mathiason et al., 2009); Japanese pear (Pyrus pyrifolia) (Bai et al., 2013; Liu et al., 2012; Nishitani et al., 2012); chestnut (Castanea sativa) (Santamaria et al., 2011); raspberry (Rubus idaeus) (Mazzitelli et al., 2007); blackcurrant (Ribes nigrum L.) (Hedley et al., 2010); and Prunus spp. (described in detail below).

This review focuses on the molecular mechanisms of the regulation of bud dormancy release of fruit tree species of Prunus, such as peach and Japanese apricot. The review first describes the growth-dormancy cycle of fruit tree species of Prunus, and environmental cues related to bud phenological changes are presented. Then, the recent progress of genetic and molecular approaches...
used to understand the regulation of bud dormancy release in *Prunus* are outlined. Identification and characterization of *Prunus* MADS-box genes, candidate genes that may play roles in regulating *Prunus* bud dormancy, are highlighted. Finally, the current working hypothesis of the biological function of these genes is discussed based on data obtained from our transgenic and other reported studies.

1. **Seasonal growth-dormancy phase transition of Japanese apricot and peach in the context of environmental changes**

A typical Japanese apricot leaf axil has three separate buds, and these consist of a single vegetative bud subtended by two flower buds. The vegetative bud contains a shoot apical meristem, and this generates an annual shoot for the next growing season, whereas each flower bud contains a floral meristem, which develops into a solitary flower. Following floral meristem formation in early summer, blooming is not usually observed until the next spring, after the passing of winter. Thus, flower buds stay in a dormant state during autumn and winter; however, flower organ differentiation continues during this dormant period (Takamatsu et al., 2004). Continuous flower organ differentiation and development during dormancy are also observed in peach (Yamane, 2013; Yamane et al., 2011b, c). Flower bud development and dormancy are more directly related to fruit production than vegetative (leaf) bud dormancy, and its understanding is therefore of more interest for agriculture use. However, this review mainly focuses on the dormancy of vegetative growth because the shoot apical meristem has been an exclusive target of dormancy studies for many other perennial plants, including poplar. In major fruit tree species other than *Prunus* spp., such as apple, Japanese pear, persimmon, and grape, which normally bear mixed buds, floral buds are often used as materials for bud dormancy studies, this is because these buds contain both the floral meristem and the shoot apical meristem within a single bud. As both flower and vegetative buds commonly require chilling exposure for bud break, knowledge of vegetative bud dormancy regulation would be helpful for understanding flower bud dormancy in *Prunus*.

The seasonal phase transition from active vegetative growth to dormancy in Japanese apricot occurs gradually, and as with other temperate fruit trees, takes a long time. Figure 1 shows a schematic of the annual growth-dormancy phase transition and seasonal vegetative and reproductive development of Japanese apricot. Although blooming is often observed from February to March under field conditions in Kyoto, vegetative bud flushing does not occur until April. Shoot growth cessation of long branches is observed from June, a second flushing sometimes follows, and the majority of long branches have stopped active growth by the end of August. The trees shed their leaves by early December. Heide (2008) investigated the effects of temperature and photoperiod on the growth and growth cessation of three *Prunus* spp., sour cherry (*P. cerasus*), Insititia plum (*P. insititia*), and sweet cherry (*P. avium* L.). Their results demonstrated that there was a pronounced interaction of photoperiod and temperature in the regulation of growth and growth cessation in all three species. On the other hand, apple and pear tended to show continuous growth regardless of photoperiodic conditions and dormancy of these species was induced by lowering the temperature (Heide and Prestrud, 2005). Vegetative growth patterns of Japanese apricot adult trees grown under field conditions suggested that growth cessation of Japanese apricot appeared to respond to the progressively decreasing photoperiod and was further established by lowering temperatures. However, continuous growth was not achieved in controlled long day length and warm temperature conditions in *Prunus* (Kataoka et al., 2002; Samish, 1954); thus, it is proposed that an endogenous mechanism induces growth cessation and environmental factors modulate it.

Seasonal changes of the dormancy level in given genotypes can be measured using repeated sampling of cuttings from trees (Gariglio et al., 2006), or using multiple pot-grown trees (Sugiura et al., 2010). Schematics of the experimental procedures used for estimating dormancy levels are shown in Figure 2A. Either branch cuttings, single node cuttings, or pot-grown trees are incubated under forcing conditions (long daylength, with an optimum temperature of approximately 20–25°C) and the time to bud burst, or the bud burst percentage after a certain period, is measured. Another method of measurement is the comparison of the weight of buds before and after cuttings are exposed to a forcing condition for a
A. Experiments for estimating dormant depth of buds

- Field-grown tree
- Pot-grown tree in field

Forcing condition

Branch cutting

Single node cutting

Counting time to bud burst or bud burst percentage after a certain period of forcing

B. Experiments for estimating CR

Calculation of chilling exposure

- Chilling hours
- Chill unit
- Dynamic model

Repetitive sampling

| Oct | Nov | Dec | Jan | Feb | Mar |

Forcing condition

Time to bud burst decreases with exposure to chilling in field.

More buds have burst with exposure to chilling in field.

Fig. 2. Schematic of experimental procedures used for estimating dormant depth of buds (A) and CR (B). (A) Branch cutting, single node cutting, or pot-grown trees were used as the experimental materials for estimating dormant levels. (B) CR fulfillment is often determined using the following parameters: bud burst percentage after a certain period of time or decreasing rate of time to bud burst in forcing conditions. Daily accumulation of chilling is calculated by three different models: chilling hours (Weinberger, 1950), chill unit (Richardson et al., 1974), or the dynamic model (Fishman et al., 1987a, b).

predetermined period. The assignment of CR to a given genotype is based on the accumulated chilling exposure, at the date of forcing treatment when bud burst is observed beyond a predetermined threshold level [(endo) dormancy release date] (Fig. 2B). Typically, three models are used for the calculation of accumulated chilling exposure: the “chill hour model” involves counting the number of hours at temperatures less than 7.2°C (45°F) (Weinberger, 1950); the “chill unit model” considers negative effects of high (> 16°C) and extremely low (< 0°C) temperatures (Richardson et al., 1974) on the fulfillment of CR; and the “dynamic model” considers the effect of different temperature cycles by assuming the conceptual reversible and irreversible portions (chill portions) required to be accumulated for fulfillment of CR (Fishman et al., 1987a, b). It is empirically assumed that the “dynamic model” is the best method to compare CR among different genotypes, especially those cultivated in warm winter climates (Topp et al., 2008). For Japanese apricot grown in a warm climate in Nanjing, China, the dynamic model proved to be the best of the three models for determining cultivar-dependent CR (Gao et al., 2012). This suggests that temperature sensitivity and the associated signal transduction system that leads to dormancy release is more complicated and dynamic than simple memorization of the cold accumulation experienced. Two separate temperature responses are known to lead to bud break, CR and heat requirement, and it is difficult to differentiate them as one can interfere with the other (Erez, 2000; Harrington et al., 2010). Indeed, when buds were exposed to greater chilling, they required less heat accumulation for bud break to occur; therefore, greater heat accumulation can compensate for insufficient chilling. Therefore, it is quite complicated and difficult to accurately quantify chilling and heat requirements. In Japan, the “developmental index (DVI) model” is often used to predict the rate of chilling exposure, namely dormancy progression and release (Sugiura and Honjo, 1997). DVI is set to zero when chilling exposure begins, and reaches DVI = 1 when CR is fulfilled. The DVI value is a very useful dormancy parameter because DVI values are assigned based on the tree’s temperature response and can be considered as a parameter of the dormancy state at a specific time point from the deep-dormancy state until bud break and blooming (Sugiura and Honjo, 1997). Not only temperature, but daylength, water status, and other uncharacterized factors may affect bud break through, at least partly, enhancing dormancy release in parallel with temperature effects (Erez, 2000; Erez et al., 1998).

In Japanese apricot ‘Nanko’, seasonal changes of the bud dormancy depth in the Kyoto climate were measured by the cutting method over several different seasons. Figure 3 shows the seasonal changes of bud development and dormancy depth along with annual changes in temperature and daylength. When branches cut from trees were incubated in forcing conditions, bud burst was observed in long branches collected in early June. Bud burst then became unstable, and fluctuated depending on the branches and the year collected after late June, and during summer. This suggests that these buds are facultatively non-deep dormant (Yamane et al., 2008). In fact, a second flushing of ‘Nanko’ trees under field conditions during summer suggested that they are not completely
dormant. However, bud burst has never been observed in the long branches collected in autumn under any of our forcing conditions (Sasaki et al., 2011; Yamane et al., 2008), suggesting that these buds are deep dormant. Until the cessation of shoot growth, axillary buds were unable to grow due to apical dominance; however, after cessation of growth until leaf abscission, axillary buds were dormant through correlative inhibition, internal inhibitory factors within the bud itself, or both. According to the review by Erez (2000), the inhibitory effects of bud break of axillary or lateral buds start from outside organs such as the leaves, and then gradually change to come from the bud itself, and this shift is believed to be modulated by low temperature (Crabbé, 1994; Faust et al., 1997) and short daylength (Nitsch, 1957).

The effects of chilling deprivation on dormancy release and bud break were tested using pot-grown trees that were defoliated and transferred to the greenhouse (20°C–28°C, natural daylength, Kyoto climate) in autumn. Bud outgrowth did not resume during the period of observation (Oct. to the following Aug.), and most buds aborted. These results suggested that the dormancy of Japanese apricot can be maintained if chilling exposure is avoided, and that long photoperiods and warm temperature cannot compensate for this completely. Again, chilling exposure seems to be one of the essential factors that lead dormant buds to be released. In the case of pot-grown peach trees, bud break was observed in a few vegetative buds in April following cold deprivation during winter, suggesting that a long photoperiod itself and/or other factors can partly break peach dormancy (H. Yamane, unpublished data).

After passing through autumn, bud burst in forcing conditions using single node cuttings was seen to occur shortly after leaf abscission in mid-December. When a whole branch cutting approach was used, bud burst was observed after January, suggesting that dormancy is released during late December to January, and that ‘Nanko’ trees are non-deep dormant during these months. However, the bud-burst percentage continues to increase and time to bud burst continues to decrease under forcing conditions from January onwards. Thus, February to

Fig. 3. The seasonal changes of axillary/lateral bud development and dormancy depth of Japanese apricot ‘Nanko’ grown in Kyoto, Japan. Dormancy depth was estimated by repetitive sampling over several years. As a reference, the information on annual daylength changes and annual average temperature changes in Kyoto (June 2012–March 2013) was downloaded from the National Astronomical Observatory of Japan (http://eco.mtk.nao.ac.jp/koyomi/dni/) and the Japan Meteorological Agency website (http://www.jma.go.jp/jma/index.html), respectively.
March are considered to be less dormant. The average air temperature is normally lower in February than January, and additional chilling exposure seems to raise the bud-break frequency after the fulfillment of CR. This hypothesis is supported by other reported chilling treatment experiments (Sasaki et al., 2011; Yamane et al., 2008). Indeed, other factors, such as increasing daylength from January onwards, also seem to induce bud break, as experimentally demonstrated in peach (Erez et al., 1966). Although dormancy is released during January, active vegetative growth in the field does not occur until April. This suggests that heat accumulation or a longer daylength after dormancy release is required for bud flushing in the field, as is observed with other temperate fruit tree species.

2. Genetic approaches for Prunus dormancy study

Among the different dormancy events, dormancy release and CR are the most important factors for fruit production; therefore, most genetic, biochemical, and molecular studies have focused on these dormancy events. It is well known that the CR differs among species and cultivars (genotypes) (Westwood, 1993). In peach, a systematic breeding program to create cultivars adapted to subtropical climates began in 1907 in the USA (Topp et al., 2008). This has led many low-chill cultivars with commercially acceptable fruit quality to be released. To date, peach cultivars with CRs ranging from less than 50 chill units (CU) to over 1000 CU have been developed and used for cultivation and breeding worldwide. For Japanese apricot, low-chill lines and evergreen-like lines have been found in southern China, Taiwan, and Southeast Asia.

Genetic studies have revealed that in Prunus spp., both CR for dormancy release and leafing and blooming time in the field are quantitative polygenic traits that are genetically determined (Arora et al., 2003; Tzonev and Erez, 2003). In Prunus, the CR rather than the heat requirement is the major factor determining leafing and blooming time (Egea et al., 2003; Fan et al., 2010; Ruiz et al., 2007; Sánchez-Pérez et al., 2012). Early genetic studies in apple and apricot have indicated that the low-chill characteristic is dominant and results from the involvement of at least one dominant gene (Hauagge and Cummins, 1991; Tzonev and Erez, 2003); recent reports however, have questioned this hypothesis (Campoy et al., 2011b; Fan et al., 2010) and further evidence is required to determine whether the low-chill characteristic is dominant in Prunus.

The first successful comprehensive study on flower bud CR quantitative trait locus (QTL) analysis in Prunus was published by Fan et al. (2010) using peach. They used an F2 population of 378 genotypes, developed from two genotypes with contrasting CR, for map construction and QTL detection. QTLs for CR were found in linkage groups of G1, G4, G5, G6, G7, and G8 in the Prunus (n = 8) genetic map. Among these, one major QTL (LOD > 18) in G1 was also detected as a QTL for heat requirement and blooming date, suggesting that there may be one unified temperature sensing system in this region that regulates CR and leads to blooming. In apricot, the use of F1 pseudo-testcross progenies identified QTLs associated with the CR of vegetative buds, in G1, G2, G3, G5, and G8 (Olukolu et al., 2009). In almond, a major QTL for CR of flower buds was located in G4, and minor QTLs were located in G1, G3, and G7 (Sánchez-Pérez et al., 2012). Among these, QTLs for blooming time overlapped with QTLs for CR in G1 and G4. Unfortunately, none of these identified QTLs in Prunus have yet been fine-mapped and efforts are continuing (Zhebentyayeva et al., 2014). Genetic and QTL studies on the blooming time of Prunus have been conducted by several researchers (Olukolu and Kole, 2012). Although this trait is expected to be more affected by environmental factors in comparison to CR, Dirlewanger et al. (2012) reported that several QTLs for the blooming time of three Prunus species (peach, apricot, and sweet cherry) were highly stable, suggesting that they were not affected by climate change. Recently developed techniques, such as genotyping-by-sequencing (GBS) using second-generation sequencing (SGS), such as Illumina sequencing, and the release of a well-assembled whole peach genome (The International Peach Genome Initiative, 2013) and other Prunus genome sequences, such as the Japanese apricot genome (Zhuang et al., 2012), will accelerate QTL studies of CR in Prunus (Bielenberg, 2013). Currently, QTL studies of Japanese apricot CR, blooming and leafing time are ongoing at the Kyoto University Experimental Farm, Takatsuki, Japan.

3. Biochemical and molecular biological approaches for Prunus dormancy study

Early biochemical studies on Prunus bud dormancy regulation have investigated seasonal carbohydrate concentration changes and carbohydrate absorption potentials (Marquat et al., 1999). During dormancy, the bud exhibited a low sugar absorption potential, while later during dormancy release absorption potentials increased. Soluble sugars accumulated during winter. The active sucrose absorption could be explained by increased activity of plasma membrane H+-ATPase (Aue et al., 1999; Gévaudant et al., 2001). Bonhomme et al. (2005) investigated the influence of cold deprivation during dormancy on the carbohydrate content of buds. Since sugar concentrations remained high during cold deprivation, bud necrosis caused by cold deprivation could be the consequence of an inability to use carbohydrate reserves. Another example of a biochemical study was the analysis of seasonal changes of the water status of peach flower buds by magnetic resonance imaging (Yooyongwech et al., 2008). Surprisingly, there have only been a few studies on the analysis of phytohormone contents during dormancy transition in Prunus. These include analyses by immunoassay (Ramina et al., 1995)
and gas chromatography (Luna et al., 1990); however, conclusive results have yet to be obtained. Several studies have investigated the effects of the external application of phytohormones such as GA (Reinoso et al., 2002) and cytokinin (Campoy et al., 2010) on bud burst in Prunus. As the crucial roles of ABA and GA in seed dormancy regulation become more evident (Finkelstein et al., 2008), it is becoming more important that the association of hormonal content with dormancy regulation is taken into account.

One of the early proteomic approaches was to analyze bud or bark protein changes associated with the seasonal changes of dormancy in peach (Arora et al., 1992). Dehydrins, such as late embryogenesis abundant (LEA) proteins were identified as being associated with dormancy transition (Arora et al., 1994, 1996). Yamane et al. (2006) found that seasonal patterns of a dehydrin protein and transcript accumulation differed between two Japanese apricot cultivars, with greater accumulation over a longer period in late flowering ‘Nanko’ than in early flowering ‘Ellching’. This supports the findings reported by Artrip et al. (1997) for peach dehydrin accumulation between evergreen and deciduous genotypes. Dehydrins are believed to protect plant cells against cellular dehydration and are therefore expected to accumulate in cold-hardened tissues. Therefore, dehydrins are thought to be more closely associated with cold and/or drought hardiness than with dormancy regulation (Rowland and Arora, 1997). However, Faust et al. (1997) proposed that dehydrins bind water, leading to freeze protection and a simultaneous deepening of dormancy. Yakovlev et al. (2008) also speculated that dehydrin expression was related to the timing of bud burst in Norway spruce (Betula pubescens Ehrh.). Recently, proteomics studies in Japanese apricot using matrix-assisted laser desorption/ionization time of flight/time of flight mass spectrometry (MALDI-TOF/TOF MS) identified 34 differentially expressed proteins during dormancy phase transition among more than 400 highly reproducible proteins (Zhuang et al., 2013b). In the future, developments in techniques such as metabolomics and hormonomics will provide us with a more comprehensive picture of the biochemical aspects of the regulation of bud dormancy release and of bud break in Prunus.

During the last decade, marked progress in the understanding of the molecular aspects of bud dormancy regulation has been made through transcriptomic approaches. The history of Prunus transcriptomic tool development is summarized by Trainotti et al. (2012). Initial attempts to discover genes related to bud dormancy release used strategies of relatively small-scale expression profiling at the genomic level. These included the cDNA-AFLP technique in apricot (Čechová et al., 2012) and the RNA subtraction technique in Japanese apricot (Yamane et al., 2008) and peach (Leida et al., 2010, 2012). Then, strategies moved to a more comprehensive genome-wide basis that used RNA sequencing with SGS (RNA-seq) (Habu et al., 2012; Zhong et al., 2013) or microarray analysis (Habu et al., 2014) in Japanese apricot. These new techniques were also applied to bud dormancy studies of temperate fruit trees other than Prunus, such as Japanese pear (Bai et al., 2013; Liu et al., 2012; Nishitani et al., 2012) and grapevine (Díaz-Riquelme et al., 2012). Recent findings using gene ontology analysis suggested that some characteristic gene networks, including the rhythmic process, reproductive process, stress response, and metabolic process, were possibly involved in dormancy release of these temperate fruit trees and ornamental trees (Gai et al., 2013) (Table 1). Currently, third-generation sequencing technology is being developed. This will allow direct sequencing of RNA molecules and therefore omit the cDNA preparation and amplification steps that are presently required for the SGS system, and which may bring bias into the results. In the future, this newly developed sequencing technique is expected to facilitate a better understanding of the molecular aspects of the regulation of bud dormancy release in Prunus.

1) Identification of DORMANCY-ASSOCIATED MADS-box genes in Prunus

As described above, functional genomics could promote the discovery of gene function and identify gene networks associated with bud dormancy regulation at the transcript level on a genome-wide basis. In addition, the use of functional genomics can be useful in breeding as functional genomics approaches can be used to generate robust molecular markers for bud dormancy traits. The next step will require the functional validation of candidate genes and gene networks and validation of the marker (allele)-trait relationship between the genotype and phenotype. In model plants, functional validation is often achieved by ectopic expression and gene silencing. Much effort has been made for the development of a transformation system for Prunus (Gao et al., 2010); however, the efficiency of transformation remains low. Nonetheless, transformation studies using a heterologous plant genetic system can occasionally be used as an alternative approach. The discussion now focuses on one such candidate gene that regulates bud dormancy release and bud break of Japanese apricot and peach, from its discovery to functional characterization by transgenic studies.

Yamane et al. (2008) performed RNA subtraction to identify genes expressed preferentially in deep-dormant buds, which are March buds from trees grown under cold deprivation from Oct. to March, compared to less-dormant buds, which are March buds from cold-exposed field-grown trees of Japanese apricot. The aim was to identify candidates for internal factors that maintain a bud dormant state and prevent it from dormancy release. This work identified a MADS-box gene with dormancy-associated expression. Seasonal expression analysis suggested that the gene was up-regulated during bud dormancy release. The gene was speculated to be related to bud break and dormancy release in Prunus. Therefore, the gene is expected to be a good marker for the regulation of bud break and dormancy release in Prunus.
Table 1. Selected GO terms of differentially expressed genes during dormancy release of horticultural woody crops identified by genome-wide transcriptomic analysis (as of Oct. 1st, 2013).

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Organ used for experiment</th>
<th>Method</th>
<th>Comparison (generally endo vs eco)</th>
<th>Selected GO (biological process) of differentially expressed genes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese apricot</td>
<td>Vegetative buds</td>
<td>Microarray (60K)</td>
<td>Nov. buds vs chilled buds</td>
<td>Up in chilled buds: response to chitin, oxylipin/jasmonic acid biosynthetic process, response to carbohydrate stimulus, response to wounding, oxylipin/jasmonic acid metabolic process, cell wall organization or biogenesis, response to other organism, response to biotic stimulus, response to external stimulus, defense response, plant-type cell wall modification, response to fungus, multi-organism process, cell surface receptor linked signaling pathway, small molecule metabolic process. Down in chilled buds: vegetative to reproductive phase transition of meristem, photoperiodism, hypersomotic salinity response, circadian rhythm, reproductive developmental process, reproduction, reproductive structre development, positive regulation of developmental process, post-embryonic development, positive regulation of biological process, RNA processing, cellular response to radiation, cellular response to light stimulus.</td>
<td>Habu et al. (2014)</td>
</tr>
<tr>
<td>Tree peony</td>
<td>Floral buds (mixed)</td>
<td>Microarray (15K)</td>
<td>Nov. buds vs chilled buds</td>
<td>Cellular process, metabolic process, response to stimulus, biological regulation, regulation of biological process, developmental process, multicellular organizational process, localization, establishment of localization, reproduction, reproductive process, multi-organism process, anatomical structure formation, immune system process, death, rhythmic process, biological adhesion.</td>
<td>Gai et al. (2013)</td>
</tr>
</tbody>
</table>

<sup>a</sup> When over-represented GOs were divided into up-regulated or down-regulated in references, the information is highlighted by underlining.
dormancy progression, and down-regulated during dormancy release. Full-length cDNA cloning of the MADS-box gene and phylogenetic analysis revealed that the gene was similar to the \textit{SiMADS11} clade MADS-box genes of \textit{Arabidopsis}, such as \textit{SHORT VEGETATIVE PHASE} \textit{(SVP)} and \textit{AGAMOUS-LIKE24} \textit{(AGL24)} (Yamane et al., 2008). Bielenberg et al. (2008) independently identified six \textit{SiMADS11} clade MADS-box genes as candidate genes associated with terminal bud formation in peach. Early studies had identified a mutant that failed to cease growth and to enter dormancy under dormancy-inducing conditions in peach; this is known as \textit{evergrowing} \textit{(evg)} (USDA PI442380) and was first identified in southern Mexico (Rodriguez et al., 1994). The \textit{evg} trait segregates as a single recessive nuclear gene (Rodriguez et al., 1994). Wang et al. (2002) generated an F2 mapping population for the segregating \textit{evg} trait and found that \textit{evg} was located in G1. Sequencing and expression analysis of the \textit{evg} locus identified six \textit{SiMADS11} \textit{(SVP/AGL24)} clade MADS-box genes as candidate genes associated with terminal bud formation in peach (Bielenberg et al., 2008). These were named \textbf{DORMANCY-ASSOCIATED MADS-box 1–6 (DAM1–6)} genes. The gene Yamane et al. (2008) found in Japanese apricot appears to be an ortholog of peach \textit{DAM6} and was named \textit{PmDAM6}. 

2) Expression analysis of \textbf{DAM} genes

In the Japanese apricot genome, six tandem arrayed \textit{PmDAM} genes (\textit{PmDAM1–PmDAM6}) have been identified (Sasaki et al., 2011; Zhang et al., 2012). Seasonal expression analysis using reverse transcription-quantitative PCR (RT-qPCR) analysis of \textit{PmDAM} genes (Sasaki et al., 2011), genome-wide transcriptomic analyses using the Japanese apricot EST dormant bud database (http://bioinf.mind.meiji.ac.jp/JADB/) (Habu et al., 2012) and 60K-microarray analysis (Habu et al., 2014) demonstrated that \textit{PmDAM} genes were preferentially expressed in dormant buds and down-regulated during the dormancy release of lateral vegetative buds (Fig. 4). Moreover, both RT-qPCR and microarray analysis revealed that all six \textit{PmDAM} genes were down-regulated following artificial prolonged cold exposure (Fig. 5). Among them, the expression levels of \textit{PmDAM1} to \textit{PmDAM3} decreased long before dormancy release, and at a similar rate in both high-chill (‘Nanko’) and low-chill (‘Elching’) genotypes. Interestingly, in the high-chill genotype, a short period of cold exposure led to a slight increase in \textit{PmDAM4} to \textit{PmDAM6} expression, whereas in the low-chill genotype, the same treatment repressed \textit{PmDAM4} to \textit{PmDAM6} expression. The results may indicate that the low-chill genotype reacts to cold temperature in Oct. as chilling but the high-chill genotype does not. Alternatively, a certain amount of chilling accumulation may be necessary for \textit{PmDAM4} to \textit{PmDAM6} downregulation in the high-chill genotype. The distinct changes in \textit{PmDAM4} to \textit{PmDAM6} expression may possibly contribute to the different amounts of chilling requirements for dormancy release of the genotypes. This suggests that there is an association of \textit{PmDAM5} with the genetic control of chilling requirement for dormancy release. Zhong et al. (2013) recently conducted RNA-seq using Japanese apricot flower buds of an early-flowering genotype and demonstrated that \textit{PmDAM3}, \textit{PmDAM5}, and \textit{PmDAM6} were abundantly expressed in endodormant (November and December) to ecodormant (January) buds; their expression was negatively correlated with bud-burst frequency (Zhong et al., 2013). In peach, six \textit{DAM} genes showed distinct seasonal expression changes in the shoot apex. Peach \textit{DAM1}, \textit{DAM2}, and \textit{DAM4} were most closely associated with terminal bud formation (Li et al., 2009), whereas peach \textit{DAM5} and \textit{DAM6} expression was negatively correlated with the time required for terminal bud break in peach (Jiménez et al., 2010). Negative correlation of peach \textit{PpDAM5} and \textit{PpDAM6} expression with the time required for bud break was also reported for lateral vegetative (Yamane et al., 2011a) and flower (Yamane et al., 2011b, c) buds. In other temperate fruit trees, down-regulation of the \textit{SVP-like} gene during dormancy release has been reported in raspberry (\textit{Rubus idaeus} L.) (Mazzitelli et al., 2007). In Japanese pear, the expression of the \textit{DAM}-like gene \textit{MADS13} was up-regulated towards dormancy establishment and down-regulated towards dormancy release (Saito et al., 2013). Wu et al. (2012) suggested that \textit{SVP-like} genes in kiwi-fruit (\textit{Actinidia spp.}) may have distinct roles in dormancy and flowering. In the perennial herbaceous species leafy spurge (\textit{Euphorbia esula}), the \textit{DAM} homologs \textit{DAM1} and \textit{DAM2} are associated with dormancy induction (Horvath et al., 2010).

3) Functional characterization of \textbf{DAM} genes

To elucidate the biological functions of \textit{PmDAM6}, hybrid poplar (\textit{P. tremula} × \textit{P. tremuloides}; clone T89) plants constitutively expressing \textit{PmDAM6} under the control of the cauliflower mosaic virus 35S promoter (35S:\textit{PmDAM6}) were generated, and phenotypes were compared with control plants that were either wild-type poplar or poplar transformed with an empty vector (Sasaki et al., 2011). When grown under long day (LD) conditions (16h light/8h dark), the shoot growth of 35S:\textit{PmDAM6} poplars was inhibited (Fig. 6A). In addition, 35S:\textit{PmDAM6} poplars set terminal buds earlier than control poplars (Fig. 6B). Shoot growth was inhibited and terminal bud set was observed earlier in 35S:\textit{PmDAM6} poplars relative to controls, even under greenhouse conditions (air cooling was set at 25°C, therefore kept under 25°C) with natural daylength in Kyoto, from April to Aug. 2012 and 2013 (Fig. 6C, D). However, the growth of suckers was stimulated more in 35S:\textit{PmDAM6} plants than in controls. After terminal bud set was observed even in control plants in the same greenhouse from April to Aug. 2012, two different experiments were conducted. In experiment 1, trees were defoliated and transferred to a greenhouse (approximately 25°C, natural daylength).
Lateral bud opening was observed after one to two months in some of the 35S:PmDAM6 and control poplars; however, some 35S:PmDAM6 poplars showed earlier lateral bud opening than the control. In experiment 2, trees were defoliated and terminal portions of shoots were removed from each plant (decapitated), then trees were transferred to a greenhouse (approximately 25°C, natural daylength). Bud burst was observed in the buds at the terminal position in the control trees, whereas first bud burst was observed in buds at the base position or from suckers, and the time to bud burst was later in some 35S:PmDAM6 trees compared to the control. Finally, buds in the upper position opened in some 35S:PmDAM6 plants. Collectively, these results suggested that overexpression of PmDAM6 in poplar inhibited apical growth during the active growing season but could not maintain all parts of the trees in deep dormancy.

Phenotypic observation of 35S:PmDAM6 poplar trees is ongoing, and dormancy release and bud break under chilling exposure and subsequent forcing conditions are still being assessed. The biological function of Japanese apricot DAMs during dormancy will be further clarified following these experiments. Hopefully, transgenic studies using Japanese apricot will be performed despite the

Fig. 4. Seasonal expression changes of PmDAM1–PmDAM6 genes in Japanese apricot ‘Nanko’. (A) Microarray results (Habu et al., 2014) (B) RT-qPCR results (Sasaki et al., 2011). In A, three DAM1, one DAM3, three DAM4, six DAM5, and seven DAM6-annotated probes are shown in each graph. No DAM2-annotated probe was loaded on the 60K microarray.
etative buds during dormancy, but does not appear to be the single determinant of bud dormancy regulation. Because the expression of all Japanese apricot DAMs decreased when most buds were ready to burst in the lack of acceptable transformation efficiency (Yamane et al., 2013). The current working hypothesis of the biological role of PmDAM6 in Japanese apricot is that it participates in the inhibition of tip growth of lateral vegetative buds during dormancy, but does not appear to be the single determinant of bud dormancy regulation. Because the expression of all Japanese apricot DAMs decreased when most buds were ready to burst in the
field (Habu et al., 2012, 2014; Sasaki et al., 2011; Zhong et al., 2013) in response to chilling exposure (Habu et al., 2014; Sasaki et al., 2011), DAMs are expected to take part in chilling-mediated dormancy release and resumption of growth. Additionally, it has been reported that in a peach genotype a major QTL for CR and bloom date overlapped the genomic regions where peach DAMs are located (Fan et al., 2010; Zhebentyayeva et al., 2014). However, questions remain as to whether DAMs play a central role in bud dormancy regulation of Japanese apricot and peach. Firstly, DAMs expression levels were not affected when low-chill peach dormancy was broken by dormancy breaking reagent, cyanamide, in October (Hosaka et al., 2012), although cyanamide could decrease DAM expression when high-chill peach dormancy was broken by cyanamide in December (Yamane et al., 2011a). Secondly, in the early flowering Japanese apricot cultivar ‘Taoxingmei’, digital expression of DAMs in flower buds was maintained at high levels in ecodormancy (Jan.) in comparison to that observed in endodormancy (Dec.), and decreased during February (Zhong et al., 2013). This is not consistent with the dormancy-associated expression patterns of PmDAMs in vegetative buds (Sasaki et al., 2011) and peach DAMs in flower buds (Jiménez et al., 2010; Yamane et al., 2011b, c). Recently, chromatin modifications in the peach DAM6 gene were investigated to characterize the repression mechanism of DAM6 expression during dormancy release (Leida et al., 2012). Further functional studies, including the identification and analyses of the target genes of DAM transcription factors, will be required to determine the molecular and biological function of DAMs.

4. Conclusion and future studies

Winter dormancy of woody perennials is a complex trait involving many genetic networks that are regulated under the influence of environmental factors. Although PmDAMs were identified as candidates for bud dormancy regulation in Japanese apricot, many other genes, proteins, and metabolites are likely to be involved in this trait. For example, the association of a SOC1-like MADS-box gene with chilling requirements was recently found in apricot (Trainin et al., 2013), and European plum (P. domestica) trees overexpressing popular FTI have a reduced chilling requirement for bud break (Srinivasan et al., 2012). There is accumulating evidence of the possible involvement of MADS-box genes and other flowering-related genes in dormancy regulation (Horvath, 2009). Furthermore, recent genome-wide transcriptomic studies have suggested significant roles for phytohormones in the dormancy phase transition of various plant species (Bai et al., 2013; Diaz-Riquelme et al., 2012; Gai et al., 2013; Habu et al., 2014; Liu et al., 2012; Zhong et al., 2013; Zhuang et al., 2013a). Although this review focused on bud dormancy regulation, dormancy is not only a trait of bud phenology but one of the seasonal growth-regulatory phases for entire trees; thus our final goal should be to uncover how entire trees are induced and maintained in the dormancy state and subsequently released from dormancy following chilling exposure.

For DAM genes, the roles of DAM homologs in fruit tree species other than Prunus should also be clarified since DAM (SVP) homologs seem to be related to the dormancy release of not only peach and Japanese apricot but also Japanese pear (Saito et al., 2013), raspberry (Matzitzelli et al., 2007), and kiwifruit (Wu et al., 2012). However, their role has been reported to be less or not significantly related to that of grape (Diaz-Riquelme et al., 2012) and leafy spurge (Horvath et al., 2010). Elucidation of the molecular basis of dormancy regulation in temperate fruit tree species could enable artificial control of dormancy through innovated cultural and chemical practices. It will also lead to the development of rapid breeding techniques such as marker-assisted seedling selection. The author’s group is seeking to advance research in this area with the aim of creating innovations that will elevate sustainable fruit production worldwide.

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