RESEARCH PAPER

Transcription factor TCP20 regulates peach bud endodormancy by inhibiting DAM5/DAM6 and interacting with ABF2

Qingjie Wang1,2,3, Gongxun Xu1,2,3, Xuehui Zhao1,2,3, Zejie Zhang1,2,3, Xuxu Wang1,2,3, Xiao Liu1,2,3, Wei Xiao1,2,3, Xiling Fu1,2,3, Xiude Chen1,2,3, Dongsheng Gao1,2,3,4, Dongmei Li1,2,3,4,5 and Ling Li1,2,3,*

1 College of Horticulture Science and Engineering, Shandong Agricultural University, Tai’an, Shandong, 271018, China
2 State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai’an, Shandong, 271018, China
3 Shandong Collaborative Innovation Center for Fruit & Vegetable Production with High Quality and Efficiency, Tai’an, Shandong, 271018, China

* Correspondence: dmli2002@sdau.edu.cn or liling217@sdau.edu.cn

Received 15 October 2019; Editorial decision 5 November 2019; Accepted 27 November 2019

Editor: Gerhard Leubner, Royal Holloway, University of London, UK

Abstract

The dormancy-associated MADS-box (DAM) genes PpDAM5 and PpDAM6 have been shown to play important roles in bud endodormancy; however, their molecular regulatory mechanism in peach is unclear. In this study, by use of yeast one-hybrid screening, we isolated a TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR transcription factor, PpTCP20, in the peach cultivar ‘Zhongyou 4’ (Prunus persica var. nectarina). The protein was localized in the nucleus and was capable of forming a homodimer. Electrophoretic mobility shift assays demonstrated that PpTCP20 binds to a GCCCR element in the promoters of PpDAM5 and PpDAM6, and transient dual luciferase experiments showed that PpTCP20 inhibited the expression of PpDAM5 and PpDAM6 as the period of the release of flower bud endodormancy approached. In addition, PpTCP20 interacted with PpABF2 to form heterodimers to regulate bud endodormancy, and the content of abscisic acid decreased with the release of endodormancy. PpTCP20 also inhibited expression of PpABF2 to regulate endodormancy. Taken together, our results suggest that PpTCP20 regulates peach flower bud endodormancy by negatively regulating the expression of PpDAM5 and PpDAM6, and by interacting with PpABF2, thus revealing a novel regulatory mechanism in a perennial deciduous tree.

Keywords: ABF2, bud endodormancy, dormancy-associated MADS-box, DAM5, DAM6, peach, TCP20, Prunus persica, teosinte branched1/cycloidea/proliferating cell factor.

Introduction

Perennial deciduous fruit trees in temperate and boreal zones have seasonal growth, and bud endodormancy is an adaptive way for plants to avoid damage in winter (Busov, 2019) in a process that is regulated by internal physiological factors (Lang, 1987; Tuan et al., 2017). Endodormancy is a complex mechanism that is essential for plant development and productivity (Chuiine and Beaubien, 2001), and it requires a sufficient period of chilling to be completed. For example, the peach cultivar ‘Zhongyou 4’ (Prunus persica var. nectarina) requires ~650–700 h of chilling accumulation (hours below
7.2 °C (Wang et al., 2015a). As a result of climate change, insufficient chilling accumulation in winter is becoming a major challenge for deciduous fruit production (Campoy et al., 2011; Kuroki et al., 2013). It is therefore crucial to understand the molecular mechanisms that regulate bud dormancy in order to maintain fruit yields.

The peach DORMANCY ASSOCIATED MADS-box (DAM) genes DAM5 and DAM6 belong to the MIKC-type MADS-box transcription factor family, which includes SHORT VEGETATIVE PHASE (SVP) and AGAMOUS-LIKE 24 (AGL24) in Arabidopsis (Yamane et al., 2011b). Bielenberg et al. (2008) found that there are six tandem genes (DAM1–6) in the peach mutant evergrowing (evg) that lacks dormant behavior. Subsequent studies have found that DAM5 and DAM6 are induced by short days (Li et al., 2009), and that the DAM genes are essential for the growth and development of peach (Jiménez et al., 2009). Yamane et al. (2011a) determined that the expression patterns of DAMs were associated with the endodormancy status. DAM-like genes have been studied in many perennial species in relation to bud dormancy, including leafy spurge (Horvath et al., 2010), apricot (Sasaki et al., 2011), pear (Liu et al., 2012; Niu et al., 2016), and apple (Mimida et al., 2015), which suggests that DAMs control bud dormancy in a similar manner across perennial plants.

Abscisic acid (ABA) is known to enhance the induction of dormancy and to regulate its maintenance in grape (Zheng et al., 2018). The ABA content decreases with chilling accumulation in grape (Zheng et al., 2015) and in peach (Wang et al., 2015a), and it is the major inhibitor that releases corm dormancy in gladiolus (Wu et al., 2019). It has been hypothesized that ABA can inhibit cell proliferation and shoot growth, and that dormancy can be induced by ABA biosynthesis, catabolism, and signaling (Wang et al., 2015a; Zheng et al., 2015). ABA signaling genes belong to the basic leucine zipper (bZIP) transcription factor family (Jakoby et al., 2002). ABA response elements (ABREs), such as the ABRE-binding factors AFB1, AFB2, AFB3, and AFB5, belong to the bZIP family and have important roles in ABA-dependent stress signaling (Zhang et al., 2015). The AREB1/ABF2 gene is induced by the ABA content in Arabidopsis (Fujita et al., 2005). In poplar, AREB/ABF is up-regulated during bud dormancy by increasing ABA content (Ruttink et al., 2007), and AREB1 binds to the DAM1 promoter region and negatively regulates its activity in pear (Tuan et al., 2017). These results suggest that the ABF transcription factor plays an essential role in bud dormancy.

Teosinte branched1/Cycloidea/Proliferating cell factors (TPCs) are a class of plant-specific transcriptional regulators that play an important role in development, including cell proliferation and growth (Nicolas and Cubas, 2016). The TPC transcription factor family has a highly conserved TPC domain (a non-canonical basic helix–loop–helix motif) that is involved in DNA binding and dimerization (Cubas et al., 1999). Based on the characteristics of this domain, TPC family members are divided into class I (also known as PCF or TCP-P) and class II (also known as TCP-C). The class-I TPC consensus binding site II motif is GGNCCCAAC, of which the core elements are GCCCR (R = A or G), and class-II TPCs bind DNA motifs of the sequence GTGGNCCC (Li et al., 2005). In Arabidopsis, class-II BRC1 (or TCP12) binds to, and is positively regulated, by HB21, HB40, and HB53. These four proteins enhance the expression of NCED3, leading to the accumulation of ABA and triggering a hormonal response that promotes axillary bud dormancy (González-Grandío et al., 2013, 2017). The class-I TCP14 has the ability to regulate embryo growth potential in seeds, and the lower ABA1 expression in tcp14 mutants delays germination (Tatematsu et al., 2008; Rueda-Romero et al., 2012). In addition, all class-ITCPs can interact with the REPRESSOR of GA1-3 protein (Davière et al., 2014), and TCP14 and TCP15 interact with DELLA proteins to regulate seed germination in Arabidopsis (Resentini et al., 2015). In addition, TCP19 modulates corm dormancy release by repressing NCED expression and increasing cytokinin levels in gladiolus (Wu et al., 2019). Recently, all TCP transcription factors from the peach genome have been identified and analysed in relation to fruit ripening (Guo et al., 2018); however, their relationships with bud endodormancy are still poorly understood.

In this study, we show that the TCP transcription factor PpTCP20 acts as a transcriptional repressor to inhibit the expression of PpDAM5 and PpDAM6 during the period of release of bud endodormancy in peach (the transition stage). In addition, PpTCP20 can interact with PpABF2 to synergistically regulate the release of endodormancy. Taken together, our findings reveal new molecular mechanisms for peach bud endodormancy.

Materials and methods

Plant material and identification of dormancy stages of flower buds

Peach trees (Prunus persica var.nectarina cv. Zhongyou 4) were grown in the Shandong Institute of Pomology in Tai’an, Shandong Province, China. To examine the dormancy stages of the flower buds, annual shoots were collected approximately every 15 d from 15 October 2017 to 30 January 2018, as described previously (Wang et al., 2015a). At each sampling time, 20 of the shoots were placed in tap water under 200 μmol m−2 s−1 light for 16 h at 25 °C, with a humidity of 75%. After 25 d, the percentage of flower buds that had broken dormancy was determined. If the bud break was less than 50%, the flower buds were considered to be in the endodormancy stage (Lang, 1987).

Morphological analysis of flower buds

Microscopic observation of flower bud morphology was performed by paraffin sectioning, as described previously (Ren et al., 2016). A series of flower buds (30 October 2017 to 15 January 2018) was fixed in 70% FAA (formaldehyde, ethanol, acetic acid) overnight at 4 °C. The paraffin sections were then eluted with xylene and absolute ethanol, stained with a red dye solution, and decolorized with absolute ethanol. The sections were then stained with a solid green dye solution and dehydrated with absolute ethanol. Finally, the sections were placed in xylene, sealed with a neutral gum, and observed under an optical microscope (Nikon Eclipse E100).

RNA extraction and quantitative PCR

Flower buds were sampled from 15 October 2017 to 30 January 2018. Total RNA was isolated from 0.5 g of bud tissue using a RNAprep Pure Plant Kit (Tian Gen, Beijing, China) according to the manufacture’s instructions. First-strand cDNA was generated using HiScript Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China) according to the manufacturer’s instructions. Quantitative PCR (qPCR) was performed using SYBR Premix Ex Taq (Takara) on a CFX96 real-time PCR detection system (Bio-Rad). Three biological replicates were
used for each analysis. The relative expression levels were calculated using the 2^−ΔΔCt method (Livak and Schmittgen, 2001) using the PpUBQ gene as the internal control. The data were analysed using SPSS Statistics v. 20. The qPCR primers are listed in Supplementary Table S1 at JXB online.

Measurement of ABA
The ABA content of flower buds collected from 15 October 2017 to 30 January 2018 was determined by HPLC/ electrospray ionization tandem MS (HPLC/ESI-MS/MS), as described previously by Zhao et al. (2019) with slight modifications. Accurately weighed samples of 0.5 g of flower buds were pulverized in liquid nitrogen, and 10 ml of isopropanol/hydrochloric acid extraction buffer was added followed by shaking at 4 °C for 30 min. Then, 20 ml of dichloromethane was added, and the mixture was shaken at 4 °C for 30 min. Following centrifugation at 13 000 rpm for 5 min at 4 °C, the lower organic phase was collected, dried under nitrogen gas, and redissolved in 400 ml of methanol (0.1% formic acid). HPLC/ESI-MS/MS was then used to measure the ABA content after passing through a 0.22-μm filter, using a 1290 HPLC system (Agilent) with a 6500 Qtrap MS/MS (AB SCIEX company).

Cloning and bioinformatic analysis of PpTCP20
The full-length open-reading frame (ORF) of PpTCP20 was amplified using the flower bud cDNA (sampled on 15 October 2017) and inserted into the expression vector, using the primer sequences listed in Supplementary Table S1. The sequences of proteins homologous to PpTCP20 in different species were obtained from PlantTFDB (Jin et al., 2013). The neighbor-joining method in MEGA6 was used to analyse the phylogenetic tree (Tamura et al., 2013).

Subcellular localization of PpTCP20 and transgenic plant material
The ORF sequence of PpTCP20 was amplified with the stop codon removed, and ligated into the PRI-GFP (35S::GFP) vector for detection of subcellular localization, as described previously (Hu et al., 2016). The primers are listed in Supplementary Table S1. PpTCP20-GFP and the control GFP construct were used to infect onion epidermal cells with Agrobacterium tumefaciens, respectively. TCP20-nLuc and ABF2-cLuc were transformed into A. tumefaciens strain GV3101. N. benthamiana leaves were infected with the mixed Agrobacterium strain. Detection of fluorescence was performed using an in vivo imaging system (IVIS Lumina II, Xenogen, Alameda, CA, USA). The primers are listed in Supplementary Table S1.

Yeast two-hybrid assays
The yeast two-hybrid (Y2H) interaction was verified using the Matchmaker Yeast Two-Hybrid System (Clontech) according to the manufacturer’s manual. The ORF of PpTCP20 was recombinated into the pGBK17 vector for verification of self-activation, and the ORFs of PpTCP20 and PpABF2 were cloned into the pGADT7 vector using the primers listed in Supplementary Table S1. The two recombinant plasmids were then co-transformed into yeast strain Y2H Gold and cultured on selective medium (SD/–Trp/–Leu) at 30 °C for ~3 d. After the yeast cells had grown, the putative transformants were transferred to selective medium (SD/–Leu/–Trp/–His/–Ade) with X-a-gal.

Electrophoretic mobility shift assays
The ORF of PpTCP20 was cloned into the pGE4T-1 vector to generate the PpTCP20-GST vector. PpTCP20-GST was transfected to Escherichia coli Transetta (DE3) for expression of the PpTCP20-GST fusion protein. The recombinant protein PpTCP20-GST was obtained and purified using a Pierce™ GST Spin Purification Kit (ThermoFisher Scientific) according to the manufacturer’s instructions.

Dual luciferase assays
Dual luciferase (dual-LUC) assays were performed as previous described (An et al., 2018). The full-length PpTCP20 cDNA was inserted into the pGreenII 0029 62-SK vector to generate the effector construct. The PpDAM5, PpDAM6, and PpABF2 promoter fragments were individually cloned into pGreenII 0800-LUC vectors to generate the reporter constructs. All recombinant constructs were individually transformed into A. tumefaciens strain GV3101. Niotiana benthamiana leaves were infected with the mixed Agrobacterium strain. Detection of fluorescence was performed using an in vivo imaging system (IVIS Lumina II, Xenogen, Alameda, CA, USA). The primers are listed in Supplementary Table S1.

Firefly luciferase complementation assays
Agrobacterium-mediated firefly luciferase complementation (LCl) assays were performed as previously described by Wang et al. (2013). The coding sequences of PpTCP20 and PpABF2 were fused to 35S::nLuc and 35S::cLuc, respectively. PpTCP20-nLuc and ABF2-cLuc were transformed into A. tumefaciens strain GV3101 and injected into N. benthamiana leaves. The leaves were then grown for 2 d in a humid environment, after which they were sprayed with 100 μM luciferin. After 6 min in the dark, the luciferase activity was monitored using a live imaging system (IVIS Lumina II, Xenogen, Alameda, CA, USA). Each assay was performed with three biological replicates.

Results
Identification of the dormancy stage and morphological changes in the flower bud
In order examine the molecular regulatory mechanism of PpDAM6, the dormancy stages of peach flower buds were first identified. From 15 October to 15 November, no bud-break was observed on the shoots. Thereafter, the buds started to break, and on 30 December the bud-break was
59% (Fig. 1A), at which time there was 1057 h of chilling accumulation (Supplementary Table S3). Zhongyou 4 requires ~700 h of chilling, and 725 h had been accumulated by 15 December. Therefore, we determined that the flower buds were in the endodormancy stage from 15 October to 15 December and in the endodormancy release period (transition stage) from 15 November to 15 December. In addition, the period 15 December 2017 to 30 January 2018 was considered as ecodormancy during which bud-burst was inhibited by the unfavorable environmental conditions (winter cold).

We examined the morphological characteristics of the flower buds using paraffin sections. As shown in Fig. 1B, from 30 October to 15 November, the stigma grew quickly, and there was no obvious change in internal morphology during the whole endodormancy stage. The expression of *PpDAM6* was determined every ~15 d from 15 October 2017 to 30 January 2018. The transcript levels began to increase on 15 October, peaked around 15 November during the endodormancy period, and then decreased markedly in the transition stage (Fig. 1C).

**PpTCP20 binds to the *PpDAM6* promoter**

Bioinformatic analysis revealed that the site-II motifs to which the TCP transcription factor can bind (–801 to –772 relative to the translational start site) were found in the promoter of *PpDAM6* (Supplementary Fig. S1). The bait fragment sequences of the *PpDAM6*-pAbAi vectors were used to search the TCP transcription factors in peach (Fig. 2A; Supplementary Fig. S2).

The Y1H Gold (*PpDAM6*-pAbAi) culture was grown on SD/-Ura with different concentrations of AbA and indicated that 200 ng ml⁻¹ was an appropriate concentration for Y1H screening (Fig. 2B). *Prupe.3G308700* and three other genes were thus identified. After comparison with the TCP transcription factor in Arabidopsis, we designated *Prupe.3G308700* as *PpTCP20* (Supplementary Fig. S3). Next, the ORF of *PpTCP20* was ligated into pGADT7 to construct AD-*PpTCP20* and this was verified with the bait protein in Y1H Gold (*PpDAM6*-pAbAi). As shown in Fig. 2C, the combination of Y1H Gold (*PpDAM6*-pAbAi/AD-*PpTCP20*) was able to grow on SD/-Leu/AbA 200, which indicated that *PpTCP20* bound to *PpDAM6* in yeast cells.

*PpTCP20* encodes a TCP protein and is located in the nucleus

*PpTCP20* was 4358 bp in length, and the clone of the coding sequence (CDS) was 933 bp (Supplementary Fig. S4A). The clone of the *PpTCP20* CDS from the flower bud of Zhongyou...
PpTCP20 regulates peach bud endodormancy

4 was three bases longer than the sequence, it encoded 311 amino acids (Supplementary Fig. S4B), and belonged to the class I family (Supplementary Fig. S3). The homologous sequences showed that PpTCP20 contained a conserved TCP domain–basic helix–loop–helix (bHLH) motif (Fig. 3A), which allows protein–protein interactions and DNA binding (Martín-Trillo and Cubas, 2010). The phylogenetic tree showed that PpTCP20 is closely related to Prunus mume and has the most distant relationship with Arabidopsis (Supplementary Fig. S5), with only 57.54% identity between PpTCP20 and AtTCP20.

To identify the subcellular localization of PpTCP20, the PpTCP20-GFP fusion protein was transiently expressed in onion epidermal cells. Confocal microscopy revealed that PpTCP20-GFP was only localized in the nucleus, while the empty control vector GFP was expressed throughout the cells (Fig. 3B). This indicated that PpTCP20 is a nuclear protein, consistent with its function as a transcription factor, which is typically in the cell nucleus. The TCP protein is capable of forming homodimers or heterodimers to regulate downstream gene expression (Parapunova et al., 2014). Our Y2H assays indicated that PpTCP20 had no autoactivation activity and could form homodimers (Fig. 3C).

**PpTCP20 binds to the site-II motifs and inhibits expression of PpDAM6**

To further demonstrate that PpTCP20 could bind to the site-II motifs, the PpTCP20-GST fusion protein was used for EMSAs. The results showed a clearly shifted band when PpTCP20-GST was incubated with labeled probes containing site-II motifs (Fig. 4B). In addition, the core sequence of the site-II motifs, GGGCCC, was replaced with AAATTT (Fig. 4A) and the shifted band disappeared, indicating that GGGCCC is essential for the binding of PpTCP20 to the site-II motifs. Because GCCCR is known to be a binding target of the PpTCP20 protein, we examined other GCCCR motifs in the promoter of PpDAM6 to which PpTCP20 might bind (Supplementary Table S2). EMSAs showed that PpTCP20 could not bind to the other three site-II motifs (Supplementary Fig. S6), indicating that PpTCP20 directly binds only to the motifs of the PpDAM6 promoter.

To determine whether PpTCP20 could regulate the expression of PpDAM6, dual-LUC assays were performed in tobacco leaves. Compared with the control, PpTCP20-SK co-expressed with Luc-PpDAM6pro showed significantly reduced luminescence intensity. In contrast, 35S::PpTCP20 failed to induce the Luc expression activity of Luc-PpDAM6pro (Fig. 4C, D). These results indicated that the PpTCP20 protein may inhibit the expression of PpDAM6.

**PpTCP20 binds to GCCR motifs and inhibits PpDAM6 expression**

PpDAM5 and PpDAM6 have been identified as candidate genes for bud endodormancy (Yamane et al., 2011a), and the expression of PpDAM5 was similar to that of PpDAM6. The PpDAM5 transcript levels also decreased rapidly in the transition stage (Fig. 5A). Three GCCCR motifs were found in the promoter of PpDAM5 (Supplementary Fig. S7) and EMSAs showed that PpTCP20 could bind to the promoter of PpDAM5 (Supplementary Fig. S6). When the core CCC sequence of the GCCCR motif was replaced with TTT, a clearly shifted band disappeared, indicating that PpTCP20 can also bind to PpDAM5 (Fig. 5B). Dual-LUC assays also showed that the PpTCP20 protein may inhibit the expression of PpDAM5 (Fig. 5C).
Fig. 4. PpTCP20 inhibits expression of PpDAM6. (A) The probes of the nucleotide sequence to which PpTCP20 may be bound. The predicted site-II motif is underlined (CACGTG), and the mutation site (Mut) is that for which the 5´-GGGCCC-3´ motif is replaced by 5´-AAATTT-3´. (B) Electrophoretic mobility shift assay (EMSA) showing that the PpTCP20-GST fusion protein binds to the site-II motifs of the PpDAM6 promoter. The PpTCP20 fusion protein was incubated with a labeled or a mutated probe DNA fragment. The unlabeled probe fragment was used as a competitor. The free and bound probes were separated on an acrylamide gel: –, absent; +, present. (C) Effector and reporter vector construction diagrams for the dual luciferase assays. (D) PpTCP20 inhibited the expression of PpDAM6 in a transient expression assay. In PpDAM6pro(Mut)-Luc the 5´-GGGCCC-3´ motif was replaced by 5´-AAATTT-3´. The graph shows a quantitative analysis of luminescence intensity, and the value for PpTCP20-62SK + PpDAM6pro-Luc was set to 1. Data are means (±SD) of the three biological replicates. Different letters indicate significant differences between means as determined by ANOVA followed by Duncan’s multiple range test (P<0.05). (This figure is available in colour at JXB online.)
PpTCP20 is involved in peach bud endodormancy

The PpTCP20 transcript levels were lowest on 15 November and then increased up to 15 December during the transition stage (Fig. 6A), which was the opposite to what was observed for PpDAM6 (Fig. 1C). This indicated that PpTCP20 may be involved in regulating peach bud endodormancy release.

Due to the difficulty in obtaining transgenic peach plants, the 35S::PpTCP20 fusion plasmid was heterologously transformed into tobacco. Three positive transgenic lines (PpTCP20-L1, PpTCP20-L2, and PpTCP20-L4) were identified (Fig. 6B). These PpTCP20-overexpressing lines exhibited enhanced germination compared with the wild-type (Fig. 6C; Supplementary Fig. S8A). In particular, the overexpressing lines showed an early flowering phenotype (Fig. 6C; Supplementary Fig. S8B).

Changes in ABA content and PpABF2 expression during bud dormancy

ABA metabolism has been reported as being involved in dormancy release (Zheng et al., 2015). The ABA content of peach flower buds was highest on 15 October, gradually decreased as endodormancy was released, and then remained at a low, constant level in the ecodormancy period (Supplementary Fig. S9). Previous studies have shown that the AREB1/ABF2 gene together with the ABA signal play an important role in the dormancy release process of grape and pear (Zheng et al., 2015; Tuan et al., 2017). PpABF2 was found to have the highest similarity to the ARB1/ABF2 protein in Arabidopsis (Supplementary Fig. S10), and germination is known to be significantly inhibited by ABF2 overexpression in Arabidopsis. PpABF2 has high expression during the endodormancy period in peach (Sun et al., 2016) and this is recognized as key regulator for bud dormancy. The expression level of PpABF2 was the lowest on 15 November (Fig. 7A). The expression peaked on 15 December as chilling accumulation increased, indicating that PpABF2 has an essential role in bud endodormancy release. Moreover, PpABF2 had an expression pattern similar to that of PpTCP20 (Fig. 6A), indicating that they may interact with each other.

PpTCP20 interacts with PpABF2 and directly binds to the PpABF2 promoter

To test whether PpTCP20 can interact with PpABF2, PpTCP20 and PpABF2 were fused to pGBK7T and pGADT7 to generate BD-PpTCP20 and AD-PpABF2, respectively, for Y2H assays. The results indicated that PpTCP20 was able to interact with the PpABF2 protein (Fig. 7B). A LCI assay was used to further verify the interaction, with the ORFs of PpTCP20 and PpABF2 being fused to pCAMBIA1300-nLUC and pCAMBIA1300-cLUC, respectively. PpTCP20-nLUC and PpABF2-cLUC co-expression resulted in strong luciferase activity compared to the control (Fig. 7C).

![Fig. 5](image-url)
Fig. 6. PpTCP20 is involved in peach bud endodormancy. (A) Relative expression of PpTCP20 during the peach flower bud dormancy. Data are means (±SD) of the three biological replicates, and expression is relative to that of PpUBQ. Different letters indicate significant differences between means as determined by ANOVA followed by Duncan’s multiple range test (P<0.05). (B) RT-PCR and qRT-PCR analyses of three PpTCP20-overexpressing transgenic lines of tobacco. NtACTIN is the reference gene. WT, wild-type. Data are means (±SD) of three biological replicates. (C) Comparison of seed germination for WT and the three transgenic lines after 5 d on MS medium. (D) Phenotypes of the WT and transgenic lines after 65 d. The circles indicate the positions of the flowers. (This figure is available in colour at JXB online.)

Fig. 7. Interaction between PpTCP20 and PpABF2. (A) Relative expression of PpABF2 in peach flower buds during different dormancy stages. Data are means (±SD) of the three biological replicates, and expression is relative to that of PpUBQ. Different letters indicate significant differences between means as determined by ANOVA followed by Duncan’s multiple range test (P<0.05). (B) PpTCP20 interacts with PpABF2 in yeast two-hybrid (Y2H) assays. PpTCP20 and PpABF2 were fused to pGBK7T and pGADT7, respectively. The Y2H-Gold yeast strain was co-transformed with the bait and prey to SD/-Leu/-Trp medium (DDO). The growth of yeast cells on SD/-Leu/-Trp/-His/-Ade/X-α-Gal medium (QDO/X) indicated an interaction. Yeast cells transformed with AD + BD-TCP20 were included as negative controls. (C) In vivo interaction of PpTCP20 and PpABF2 in tobacco leaves. The coding regions of PpTCP20 and PpABF2 were fused to pCAMBIA1300-nLUC and pCAMBIA1300-cLUC, respectively, and used to infect the leaves. Agrobacterium strains expressing nLuc and cLuc were used as negative controls. (This figure is available in colour at JXB online.)
The TCP-binding site (TBS) motif (GGTCCCCAC, −800 to −792 relative to the translational start site) to which AtTCP20 can bind was also found in the promoter of PpABF2 (Supplementary Fig. S11; Wu et al., 2016). EMSAs verified that PpTCP20 could bind to the GCCCR motif of PpABF2 promoter (Supplementary Fig. S12A). In addition, dual-LUC assays showed PpTCP20 inhibited the expression of PpABF2. Thus, as a result of the expression of PpTCP20 and PpABF2 (Figs 6A, 7A), PpTCP20 may inhibit the expression of PpABF2 during the deep bud endodormancy period in peach.

**Discussion**

**PpDAM5 and PpDAM6 are negative regulators of bud endodormancy in peach**

In previous studies, PpDAM5 and PpDAM6 have been found to be closely related to the required degree of cooling during bud dormancy and to flowering time in peach (Fan et al., 2010; Yamane et al., 2011a), and the expression levels of PpDAM5 and PpDAM6 are negatively correlated with terminal bud-break (Jiménez et al., 2010; Leida et al., 2010). Some studies have found that down-regulation of DAM genes is accompanied by release of endodormancy in peach (Leida et al., 2012), Japanese apricot (Prunus mume; Sasaki et al., 2011), and pear (Niu et al., 2016). Sasaki et al. (2011) found that ectopic overexpression of apricot PmDAM6 in poplar resulted in a phenotype with growth inhibition under favorable conditions. In this study, we found that PpDAM5 and PpDAM6 were down-regulated in the peach variety ‘Zhongyou 4’ in response to low temperature during the flower-bud endodormancy release period (transition stage) (Figs 1C, 5A), which was consistent with previous studies showing that these genes have essential roles in flower bud endodormancy in peach (Li et al., 2009; Yamane et al., 2011b; Leida et al., 2012). The molecular mechanisms by which DAM genes regulate dormancy have been previously reported. For example, the DAM-like gene SVP causes inhibition of FT expression and affects flowering in Arabidopsis (Lee et al., 2007). DAM1 in pear can bind to NCED3 to regulate bud dormancy (Tuan et al., 2017), and the poplar transcription factor SVL is closely related to Arabidopsis SVP and can also bind to NCED3 (Singh et al., 2018). Similarly, DAM can inhibit the expression of FT in leafy spurge (Hao et al., 2015) and pear (Niu et al., 2016) to regulate bud dormancy. Thus, we consider PpDAM5 and PpDAM6 to be negative regulators in the control of bud dormancy release in peach.

The CCCAC motif sequence is essential for PpTCP20 binding

TCPs have been identified as the DNA–binding proteins that can recognize specific motifs (Martín-Trillo and Cubas, 2010). There are 24 TCP transcription factors in Arabidopsis, divided into classes I and II (Danisman et al., 2012). Arabidopsis TCP20 belongs to the class-I subfamily and can bind to site-II motifs, the core elements of which are GCCCR (R = A or G) sequences (Li et al., 2005; Hervé et al., 2009). Using sequence analysis, we identified the site-II motif element GGGCCCAA in the peach PpDAM6 promoter (Supplementary Fig. S1), which was similar to the Arabidopsis GGNCCCA sequence to which AtTCP20 is targeted (Danisman et al., 2012). PpTCP20 could bind to the site-II motifs of PpDAM6 (Figs 2, 4B), and EMSAs also showed that it could bind to the GCCCR motifs in the promoter of PpDAM5 (Fig. 5B). However, there were other GCCCR sequences in the promoters of PpDAM5 and PpDAM6 that had no binding affinity with PpTCP20 (Supplementary Fig. S6). A previous study has suggested that TCP20 can bind to the TBS (GGTCCCCAC) motif sequence (Wu et al., 2016), and we found that PpTCP20 could bind to this sequence in the promoter of PpABF2 (Supplementary Fig. S12A). Our results clearly cannot fully explain the target sequence of PpTCP20 in peach, but they suggest that the CCCAC motif is the core sequence element (Supplementary Fig. S13).

**PpTCP20 regulates the release of flower bud endodormancy**

Previous studies have reported that TCP20 regulates plant growth (Li et al., 2005; Hervé et al., 2009), nitrate foraging by roots (Guan et al., 2014), and petal elongation (Wang et al., 2019). It has also been shown to regulate bud activation potential and flowering in Arabidopsis (Wu et al., 2016). Other studies have also found that TCP transcription factors play important roles in dormancy. TCP19 in gladiolus promotes corm dormancy release by influencing the ABA content (Wu et al., 2019). TCP18/BR-C1 negatively regulates axillary bud growth in Arabidopsis (Aguilar-Martínez et al., 2007; Niwa et al., 2013; Seale et al., 2017). In addition, poplar TCP18 is a direct target gene of the SVL protein and a negative regulator of bud-break (Singh et al., 2018). These studies indicate that TCP transcription factors have a function in dormancy. In our study, we found that PpTCP20-overexpressing lines exhibited enhanced germination and showed an early flowering phenotype in tobacco compared to the wild-type (Fig. 6C, D), and a previous study had indicated that seed germination involves the breaking of dormancy (Koo et al., 2015). Similarly, Arabidopsis TCP20 is involved in growth and cell division and contributes to the control of cell expansion (Hervé et al., 2009). In particular, expression of PpTCP20 increased during the transition stage in peach (Fig. 6A). These results confirmed that PpTCP20 may play a key role in peach bud endodormancy.

TCP proteins can act as transcriptional activators or repressors in plants, depending on the promoter sequence and the regulatory partner (Hervé et al., 2009). In Arabidopsis, TCP20 binds to the LOX2 promoter, thereby inhibiting its expression (Danisman et al., 2012). TCPs can also regulate the expression of the MADS-domain transcription factors SEPALLATA3 and APETALA1 (Danisman et al., 2012). Here, dual luciferase assays indicated that PpTCP20 inhibited the expression of PpDAM5 and PpDAM6 (Figs 4D, 5C). Previous studies have suggested that CBFs play important roles in regulating dormancy. The ectopic expression of peach CBF (PpCBF1) in apples alters the expression of DAMs and other dormancy-related genes and inhibits bud-break (Wisniewski et al., 2015), and the CBF transcription factor is the upstream gene that activates the
transcription of DAM1 in pear by binding the C-repeat/DRE element motif (Saito et al., 2013; Niu et al., 2016). CBF can bind to DAM in poplar, pear, and Chinese pear to regulate bud dormancy (Horvath, 2009; Saito et al., 2013; Niu et al., 2016). Our results indicate another mechanism for PpTCP20 to regulate PpDAM genes. Similar to the bHLH protein, the TCP transcription factor can bind to DNA to form homodimers (Manassero et al., 2013), and we confirmed that PpTCP20 is capable of forming homodimers (Fig. 4C), thus inhibiting the expression of PpDAM5 and PpDAM6 to regulate flower bud endodormancy.

**ABA and the ABA-signaling gene PpABF2 are involved in bud endodormancy**

ABA is generally considered to be a key hormone in regulating bud dormancy (Cooke et al., 2012; Wang et al., 2015a). It accumulates during seed development and the content decreases with the release of dormancy (Kermode, 2005). In grape, the ABA concentration decreases during the release of bud endodormancy, demonstrating that it inhibits bud-break (Zheng et al., 2015). In hybrid aspen under low temperature conditions, ABA levels decrease and inhibit expression of SVL, thereby promoting bud-break (Singh et al., 2018). In our study, the ABA content gradually decreased during the process of peach flower bud endodormancy (15 November to 15 December), and reached a minimum level during the ecodormancy stage (Supplementary Fig. S9), which was similar to the findings of a previous study in peach (Wang et al., 2015a). These results provide evidence that the ABA content plays a key role in the release of endodormancy in peach.

ABA signaling genes, such as ABFs, are known to play a role in bud endodormancy (Sun et al., 2016). In pear, AREB1/ABF2 binds to DAM1 and negatively regulates its activity during bud endodormancy (Tuan et al., 2017). We found that ABF2 showed high expression during the transition stage (Fig. 7), which indicated that ABF2 played an important role in bud endodormancy. The ABF protein can form a complex with other transcription factors and regulate the expression of downstream genes. For example, SnRK2s can phosphorylate AREB/ABFs to participate in ABA signaling in plants (Wang et al., 2015b), and the up-regulation of ABF2 that we observed was consistent with endodormancy release in pear (Tuan et al., 2017). The formation of a complex of AREB1(ABF2) and NAC2 in peanut regulates the downstream gene NCED1 (Liu et al., 2016). AREB1 in pear may also directly or indirectly regulate DAM1 expression in combination with the NAC transcription factor (Tuan et al., 2017). Similarly, we found that ABF2 could interact with the TCP20 protein (Fig. 7B, C), which indicated that PpABF2 and PpTCP20 synergetic regulated bud endodormancy in peach. The conformation of transcription factor binding sites has been shown to be affected by combinatorial regulation in yeast (Bilu and Barkai, 2005). Similarly, there is a need to understand the diversity of TCP binding sites and the combined regulation of TCP proteins and their chaperones (Hervé et al., 2009). However, as we were unable to obtain an PpABF2 fusion protein in peach, further studies are needed in order to determine whether the heterodimer between PpTCP20 and PpABF2 regulates the expression of PpDAM5 and PpDAM6. The PpTCP20 transcription factor inhibited the transcription of PpABF2 by binding the TBS

---

**Fig. 8.** A proposed model of the role of PpTCP20 in peach bud endodormancy. Long-term chilling accumulation in winter directly activates the accumulation of PpTCP20 and PpABF2. PpTCP20 inhibits expression of PpDAM5 and PpDAM6 to release endodormancy. In addition, PpTCP20 interacts with PpABF2 to form heterodimers to jointly regulate the release of bud endodormancy. During the deep endodormancy period, PpTCP20 inhibits PpABF2 expression, which might enhance the endodormancy. Induction of targets is represented by solid arrows and inhibition is represented by blocked lines. The dashed arrow represents potential induction. (This figure is available in colour at JXB online.)
motif (Supplementary Fig. S12). As a result of the expression of PpTCP20 and PpABF2 (Figs 6A, 7A), we propose that PpTCP20 inhibited PpABF2 in peach during the deep endodormancy stage (from 15 October to 30 October). Our results show that PpTCP20 may have different functions during different stages of peach endodormancy.

In summary, our study demonstrates that PpTCP20 plays an important role in flower bud endodormancy in peach by inhibiting the expression of PpDAM5 and PpDAM6. Specifically, PpTCP20 can also interact with PpABF2 to synergistically regulate the release of bud endodormancy (Fig. 8). Our findings characterize the molecular mechanisms relating to PpTCP20 in peach and provide new insights into bud dormancy in perennial deciduous fruit trees.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. The promoter sequence of PpDAM6.

Fig. S2. The PpDAM6-pAbAi bait sequence for the Y1H assay.

Fig. S3. Phylogenetic analyses of PpTCP20 and Arabidopsis TCP proteins.

Fig. S4. The sequence and gene structure of PpTCP20.

Fig. S5. Phylogenetic analysis of TCP20 proteins from different species.

Fig. S6. EMSA showing that PpTCP20 can bind to the PpDAM5 promoter.

Fig. S7. The promoter sequence of PpDAM5.

Fig. S8. Phenotypes of transgenic Nicotiana benthamiana expressing PpTCP20.

Fig. S9. The ABA content of peach flower buds during dormancy.

Fig. S10. Phylogenetic analyses of PpABF2 and AtAREB1.

Fig. S11. The promoter sequence of PpABF2.

Fig. S12. EMSA and transient expression assays showing that PpTCP20 inhibits expression of PpABF2.

Fig. S13. PpTCP20 may bind to the CCCAC motif sequence in peach.

Table S1. Primers used for amplification.

Table S2. Sequences of labeled probes used for EMSAs.

Table S3. Daily temperature data from 15 October to 30 December 2017.

Acknowledgments

This study was funded by the National Key Research and Developmental Program of China (2018YFD1000104), the National Natural Science Foundation of China (31872041 and 31601706), and the Shandong Province Modern Agricultural Technology System Fruit Innovation Team. We thank Prof. Yujin Hao (Shandong Agricultural University, China) for providing the dual luciferase plasmids (pGreenII 62-SK and pGreenII LUC). We thank Prof. Yuxin Yao (Shandong Agricultural University, China) for guidance during the writing of the paper. We thank Dr Jianping An and Xinglong Ji (Shandong Agricultural University, China) for technical assistance provided during this study. The authors declare that they have no conflicts of interest.

Author contributions

QW, DG, DL, and LL designed the study; QW, GX, XZ, ZZ, XW, and XL performed the experiments; QW, WX, XF, and XC analysed the data; QW wrote the paper.

References

Aguilera-Martínez JA, Poza-Carrión C, Cubas P. 2007. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. The Plant Cell 19, 458–472.

An JP, Yao JF, Xu RR, You CX, Wang XF, Hao YJ. 2018. Apple bZIP transcription factor MdzbZIP44 regulates abscisic acid-promoted anthocyanin accumulation. Plant, Cell & Environment 41, 2678–2692.

Bielenberg DG, Wang Y, Li Z, Zhabentayeva T, Fan S, Reighard GL, Scorzra R, Abbott AG. 2008. Sequencing and annotation of the evergrowing locus in peach [Prunus persica (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Trees Genetics & Genomes 4, 495–507.

Bilu Y, Barkai N. 2005. The design of transcription-factor binding sites is affected by combinatorial regulation. Genome Biology 6, R103.

Busov VB. 2019. Plant development: dual roles of poplar SLV in vegetative bud dormancy. Current Biology 29, R68–R70.

Campoy J, Ruiz D, Egea J. 2011. Dormancy in temperate fruit trees in a global warming context: a review. Scientia Horticulturae 130, 357–372.

Cao Y, Xie L, Ma Y, Ren C, Xing M, Fu Z, Wu X, Yin X, Xu C, Li X. 2019. PpMYB15 and PpMYBF1 transcription factors are involved in regulating flavonol biosynthesis in peach fruit. Journal of Agricultural and Food Chemistry 67, 644–652.

Chen M, Liu X, Jiang S, et al. 2018. Transcriptomic and functional analyses reveal that PpGLK1 regulates chloroplast development in peach (Prunus persica). Frontiers in Plant Science 9, 34.

Chuine I, Beaubien EG. 2001. Phenology is a major determinant of tree species range. Ecology Letters 4, 500–510.

Cooke JE, Eriksson ME, Junttila O. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant, Cell & Environment 35, 1707–1728.

Cubas P, Lauter N, Doebley J, Coen E. 1999. The TCP domain: a motif found in proteins regulating plant growth and development. The Plant Journal 18, 215–222.

Danisman S, van der Wal F, Dhondt S, et al. 2012. Arabidopsis class I and class II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically. Plant Physiology 159, 1511–1523.

Davière JM, Wild M, Regnaut T, Baumberger N, Eisler H, Genschik P, Achard P. 2014. Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height. Current Biology 24, 1923–1928.

Fan S, Bielenberg DG, Zhabentayeva TN, Reighard GL, Okie WR, Holland D, Abbott AG. 2010. Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (Prunus persica). The New Phytologist 185, 917–930.

Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K. 2005. AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. The Plant Cell 17, 3470–3488.

González-Grandío E, Pajaro A, Franco-Zorrilla JM, Tarancón C, Immink RG, Cubas P. 2017. Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. Proceedings of the National Academy of Sciences, USA 114, E245–E254.

González-Grandío E, Poza-Carrión C, Sorzano CO, Cubas P. 2013. BRANCHED1 promotes axillary bud dormancy in response to shade in Arabidopsis. The Plant Cell 25, 834–850.

Guan R, Wang R, Nacey P, Breton G, Kay SA, Pruneda-Paz JL, Davani A, Crawford NM. 2014. Nitrate foraging by Arabidopsis roots is mediated by the transcription factor TCP20 through the systemic signaling pathway. Proceedings of the National Academy of Sciences, USA 111, 15267–15272.

Guo Z-H, Shu W-S, Cheng H-Y, Wang G-M, Qi K-J, Zhang S-L, Gu C. 2018. Expression analysis of TCP genes in peach reveals an involvement
of PpTCPA2 in ethylene biosynthesis during fruit ripening. Plant Molecular Biology Reporter 36, 588–595.

Hao X, Chao W, Yang Y, Horvath D. 2015. Coordinated expression of FLOWERING LOCUS T and DORMANCY ASSOCIATED MADS-BOX-like genes in leafy spurge. Plant Molecular Biology 73, 169–179.

Hervé C, Dabos P, Bardet C, Jaunee A, Auriac MC, Ramboer A, Lacout F, Tremouysegue D. 2009. In vivo interference with ATTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development. Plant Physiology 149, 1462–1477.

Horvath D. 2009. Common mechanisms regulate flowering and dormancy. Plant Science 177, 523–531.

Horvath DP, Sung S, Kim D, Chao W, Anderson J. 2010. Characterization, expression and function of DORMANCY ASSOCIATED MADS-BOX genes from leafy spurge. Plant Molecular Biology 73, 169–179.

Hu DG, Sun CH, Ma QJ, You CX, Cheng L, Hao YJ. 2016. MdMYB1 regulates anthocyanin and malate accumulation by directly facilitating their transport into vacuoles in apples. Plant Physiology 170, 1315–1330.

Jakoby M, Weisshaar B, Drège-Laser W, Vicente-Carbajosa J, Tiedemann J, Kraj T, Parcy F. 2002. bZIP transcription factors in Arabidopsis. Trends in Plant Science 7, 106–111.

Jiménez S, Lawton-Rauh AL, Reighard GL, Abbott AG, Bielenberg DG. 2009. Phylogenetic analysis and molecular evolution of the dormancy associated MADS-box genes from peach. BMC Plant Biology 9, 81.

Jiménez S, Reighard GL, Bielenberg DG. 2010. Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Molecular Biology 73, 157–167.

Jin J, Tian F, Yang D-C, Meng Y-Q, Kong L, Luo J, Gao G. 2016. PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants. Nucleic Acids Research 45, D1040–D1045.

Kermode AR. 2005. Role of abscisic acid in seed dormancy. Journal of Plant Growth Regulation 24, 319–344.

Koo HJ, Park SM, Kim KP, Suh MC, Lee MO, Lee SK, Xini X, Hong CB. 2015. Small heat shock proteins can release light dependence of tobacco seed during germination. Plant Physiology 167, 1030–1038.

Kuroki K, Takemura Y, Matsumoto K, Takeda M, Tomiyama M, Kermode AR. 2005. Role of abscisic acid in seed dormancy. Journal of Plant Physiology 162, 1315–1330.

Lang G. 1987. Endo-, para- and ecodormancy: physiological terminology and classification for dormancy research. HorticScience 22, 271–277.

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH. 2007. Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes & Development 21, 397–402.

Leida C, Conesa A, Llácer G, Badenes ML, Rios G. 2012. Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. The New Phytologist 195, 67–90.

Leida C, Terol J, Martí G, Agustí M, Llácer G, Badenes ML, Ríos G. 2013. Effect of hydrogen cyanamide on breaking flower bud endodormancy and flowering period of major Japanese pear cultivars. Horticultural Research (Japan) 12, 179–185.

Liang G. 1997. Expression of ABA metabolism-related genes suggests similarities and differences between seed dormancy and bud dormancy of peach (Prunus persica) and peach (Prunus persica) expression and function of DORMANCY ASSOCIATED MADS-BOX genes. The Plant Journal 8, 482–485.

Rueda-Romero P, Barrero-Sicilia C, Gómez-Cadenas A, Carbonero P, Oñate-Sánchez L. 2012. Arabidopsis thaliana Dof6 negatively affects germination in non-after-ripened seeds and interacts with TCP4. Journal of Experimental Botany 63, 1937–1949.

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Blaberao RP, Boerjan W, Rohde A. 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. The Plant Cell 19, 2370–2380.

Saito T, Bai S, Ito A, Sakamoto D, Saito T, Ube BE, Imai T, Moriguchi T. 2013. Expression and genomic structure of the dormancy-associated MADS box genes MADS13 in Japanese pears (Pyrus pyrifolia Nakai) that differ in their chilling requirement for endodormancy release. Tree Physiology 30, 654–656.

Singh RK, Maurya JP, Azeez A, Miskolczi P, Tylewicz S, Stojković K, Dattamritra N, Busov V, Blaberao RP. 2018. A genetic network mediating the control of bud break in hybrid aspen. Nature Communications 9, 4173.

Sun MY, Fu XL, Tan QP, Liu L, Chen M, Zhu CY, Li L, Chen XD, Gao DS. 2016. Analysis of basic leucine zipper genes and their expression during bud dormancy in peach (Prunus persica). Plant Physiology and Biochemistry 104, 54–70.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0, Molecular Biology and Evolution 30, 2725–2729.

Tatematsu K, Nakabayashi K, Kuniya Y, Nambara E. 2008. Transcription factor ATTCP14 regulates embryonic growth potential during seed germination in Arabidopsis thaliana. The Plant Journal 53, 42–52.

Tuan PA, Bai S, Saito T, Ito A, Moriguchi T. 2017. Dormancy-associated MADS-box (DAM) and the abscisic acid pathway regulate flower bud outgrowth by a feedback mechanism. Plant & Cell Physiology 58, 1378–1390.

Wang D, Gao Z, Du P, Xiao W, Tan Q, Chen X, Li L, Gao D. 2015a. Expression of ABA metabolism-related genes suggests similarities and differences between seed dormancy and bud dormancy of peach (Prunus persica). Frontiers in Plant Science 6, 1248.

Wang J, Guan Y, Ding L, Li P, Zhao W, Jiang J, Chen S, Chen F. 2019. The CmTCP20 gene regulates petal elongation growth in Chrysanthemum morifolium. Plant Science 280, 248–257.
Wang J, Hu J, Qian Q, Xue HW. 2013. LC2 and OsVIL2 promote rice flowering by photoperiod-induced epigenetic silencing of OsLF. Molecular Plant 6, 514–527.

Wang L, Hu W, Sun J, et al. 2015b. Genome-wide analysis of SnRK gene family in Brachypodium distachyon and functional characterization of BdSnRK2.9, Plant Science 237, 33–45.

Wisniewski M, Norelli J, Artlip T. 2015. Overexpression of a peach CBF gene in apple: a model for understanding the integration of growth, dormancy, and cold hardiness in woody plants. Frontiers in Plant Science 6, 85.

Wu J, Wu W, Liang J, Jin Y, Gazzarrini S, He J, Yi M. 2019. GhTCP19 transcription factor regulates corn dormancy release by repressing GhNCED expression in gladiolus. Plant & Cell Physiology 60, 52–62.

Wu JF, Tsai HL, Joanito I, et al. 2016. LWD–TCP complex activates the morning gene CCA1 in Arabidopsis. Nature Communications 7, 13181.

Yamane H, Ooka T, Jotatsu H, Sasaki R, Tao R. 2011a. Expressional regulation of PpDAM5 and PpDAM6, peach (Prunus persica) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. Journal of Experimental Botany 62, 3481–3488.

Yamane H, Ooka T, Jotatsu H, Sasaki R, Tao R. 2011b. Expression analysis of PpDAM5 and PpDAM6 during flower bud development in peach (Prunus persica). Scientia Horticulorum 129, 844–848.

Zhang F, Fu X, Lv Z, et al. 2015. A basic leucine zipper transcription factor, AabZIP1, connects abscisic acid signaling with artemisinin biosynthesis in Artemisia annua. Molecular Plant 8, 163–175.

Zhao YF, Peng T, Sun HZ, et al. 2019. miR1432-OsACOT (Acyl-CoA thioesterase) module determines grain yield via enhancing grain filling rate in rice. Plant Biotechnology Journal 17, 712–723.

Zheng C, Halaly T, Acheampong AK, Takebayashi Y, Jikumaru Y, Kamiya Y, Or E. 2015. Abscisic acid (ABA) regulates grape bud dormancy, and dormancy release stimuli may act through modification of ABA metabolism. Journal of Experimental Botany 66, 1527–1542.

Zheng C, Kwame Acheampong A, Shi Z, Halaly T, Kamiya Y, Ophir R, Galbraith DW, Or E. 2018. Distinct gibberellin functions during and after grapevine bud dormancy release. Journal of Experimental Botany 69, 1635–1648.