Molecular and genetic basis of bud dormancy regulation in Japanese apricot (*Prunus mume*)

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Introduction

Bud dormancy is an important adaptive process for woody perennials to survive under extreme cold in winter and to ensure robust plant development in spring. Many deciduous fruit tree species, including Japanese apricot (*Prunus mume* Sieb. et Zucc), require exposure to a genetically determined chilling period, known as chilling requirement (CR) fulfillment, to ensure bud dormancy break. Exposure to a subsequent warm period, known as heat requirement (HR) fulfillment, leads to bud break and blooming. Global climate change affects dormancy release and CR/HR fulfilment. Understanding their regulation mechanisms, therefore, is important to ensure stable fruit production. Previous studies showed that the levels of plant hormones and the expression of *DORMANCY-ASSOCIATED MADS-box* (*DAM*) genes correlate with bud dormancy process in Japanese apricot, but the underlying molecular mechanisms remain largely unknown. Moreover, little information is available about the genetic factors controlling bud dormancy. This study explored the molecular mechanisms of bud dormancy regulation involving putative key dormancy regulators, abscisic acid (ABA) and *PmDAM6*. Furthermore, a genome-wide association study (GWAS) was conducted to determine genomic regions and genetic factors controlling bud dormancy.

Chapter 1. Seasonal changes of ABA level and transcript levels of the genes for ABA metabolism/signaling in dormant buds

Seasonal changes in the endogenous ABA level were found to correlate with dormancy release in both dormant vegetative and flower buds of Japanese apricot. The ABA level was higher in high-CR individuals than low-CR ones of an F_1 segregating population derived from the cross between high-CR ‘Nanko’ and low-CR ‘SC’. This result strongly suggested that ABA is involved in bud dormancy

release and genetic regulation of CR in Japanese apricot. Furthermore, mRNA-seq analysis revealed that changes in the transcript levels of *9-cis-EPOXYCAROTENOID DIOXYGENASE* (*NCED*), a key ABA biosynthesis gene, and *ABA RESPONSIVE ELEMENTS-BINDING FACTOR 2,3* (*ABF2,3*) genes involved in ABA signaling, were higher in 'Nanko' than 'SC' dormant buds, corresponding well with the ABA levels in these cultivars. The transcript levels of *CYP707A*s encoding ABA 8'-hydroxylases involved in ABA catabolism, were increased during dormancy release and CR fulfillment, while the levels of ABA catabolites (PA and DPA) were not well-connected to dormancy process. Furthermore, the results obtained from the exogenous applications of ABA and fluridone (an ABA biosynthesis inhibitor), suggested that ABA controlled *PmDAM6* transcription in vegetative buds. In summary, this chapter confirmed that the expressions of *NCED* and *ABF2,3* genes were higher in high-CR 'Nanko' dormant buds than in low-CR 'SC', and the expression patterns correspond well to ABA accumulations in both vegetative and flower buds. Furthermore, the exogenous hormone application tests suggested that ABA controls *PmDAM6* expression in vegetative buds. Overall, the regulation of NCED-ABA-ABF-DAM may play a central component in regulating bud dormancy and CR in Japanese apricot cultivars.

Chapter 2. Primary functions of Japanese apricot PmDAM6 in vegetative bud dormancy

Previous results showed that PmDAM6-overexpressing transgenic apples exhibit inhibited growth, repressed bud break competency of dormant buds, and delayed bud dormancy release in apple (*Malus* × *domestica*) plants. Furthermore, mRNA-seq analysis was conducted using dormant buds of *35S:PmDAM6* transgenic apple and two Japanese apricot cultivars with contrasting CR to explore the molecular function of PmDAM6 in dormant buds. Analyses of phytohormone metabolism genes showed that the transcript levels of *CYTOKININ DEHYDROGENASE* encoding a cytokinin (CK) catabolic enzyme and *GA 2-OXIDASE* encoding a gibberellin (GA) catabolic enzyme were higher, in *35S:PmDAM6* than in wild type. These changes in gene transcript levels may have resulted in lower

GA and CK levels in *35S:PmDAM6* apple and high-CR Japanese apricot cultivars. To clarify the role of PmDAM6 in cellular metabolism, the cell structure of the dormant vegetative meristem was observed by transmission electron microscopy (TEM). Compared with wild-type plants, the *35S:PmDAM6* transgenic plants showed higher lipid body accumulation and lower cell density in the dormant meristem. The same tendency was observed in Japanese apricot high-CR 'Nanko' as compared with low-CR 'Ellching'. In addition, gene expression analysis suggested that PmDAM6 may down-regulate *GDSL ESTERASE/LIPASEs*, encoding lipid catabolic enzymes, and *CYCLINS*, encoding cell cycle proteins, during dormancy. These findings suggest that PmDAM6 represses cell division during dormancy, accompanied by low accumulation of active CK and GA and high accumulation of lipid bodies.

Chapter 3. Genome-wide association study provides insights into the genetic basis of bud dormancy in Japanese apricot

Based on previous genomic and evolutionary studies, Japanese apricot germplasms were classified into the 'Japanese group', 'Taiwanese group', and 'Chinese group'. However, GWAS analysis using accessions from Japanese cultivars in bud dormancy study has yet to be conducted, and the previous studies mainly focused on blooming using Chinese ornamental cultivars. In this Chapter, a GWAS for six bud dormancy-related traits (blooming date, leafing date, and the CR and HR of vegetative and flower buds) was conducted using 117 Japanese apricot accessions consisting of a "Japanese group" and a "Taiwanese group". Phenotypic data was recorded in at least two different seasons. Three loci associated with CR of vegetative buds were consistently identified on chromosomes 1, 2, and 7 across seasons. Furthermore, several candidate genes were identified near lead SNPs of CR of vegetative buds, such as *CYCLINs*. Although genomic loci associated with other traits were not consistently detected across seasons, *DAM*s were identified on and near the genetic loci associated with HR of vegetative buds and CR of flower buds, respectively. Furthermore, ABA transporter (i.e.,

ABC TRANSPORTER 31) and orthologs of *Arabidopsis* flowering-related genes (i.e., *AP1* and *14-3-3 PROTEIN 7*) were located on the loci associated with CR and HR of flower buds. In summary, these results provided further evidence for the involvement of *DAM*s as genetic components in controlling bud dormancy in Japanese apricots.

Conclusion

This study clarifies the molecular mechanisms underlying bud dormancy regulatory pathway involving ABA and DAMs in Japanese apricot. This study first identified genomic regions associated with bud dormancy, which may provide valuable information for future Japanese apricot breeding strategy.