Summary

Endodormancy is a crucial physiological state for survive under low temperature in winter for deciduous woody plants including Japanese pear (*Pyrus pyrifolia* Nakai). Induction of endodormancy in Japanese pear has been known to be triggered off by low temperature rather short photoperiod in autumn. To complete endodormancy, adequate amount of chilling accumulation (generally less than 7.2 °C) is required depending on the genotypes. However, the regulatory mechanism of endodormancy has not yet been fully understood so far. In this study, in order to provide insight into the molecular mechanisms underlying endodormancy phase transition in Japanese pear, the focus is given on *dormancy-associated MADS-box* (*DAM*) and the experiments were designed as follow: i) expression analysis of *DAM* during endodormancy phase transition and ii) comparison of the genomic structures including the 5′-upstream region of *DAM* from Japanese pear 'Kosui' and Taiwanese pear TP-85-119 ('Hengshanli') in which chilling requirement for endodormancy release is about 1,000-1,200 chill unit (CU) for 'Kosui' and less than 200 CU for 'Hengshanli', respectively and iii) elucidation of regulatory mechanism of *DAM* expression mediated by epigenetic regulation including histone modification and iv) clarification of signaling cascade of DAM during endodormancy.

We isolated three *DAM* genes (*PpMADS13-1, PpMADS13-2,* and *PpMADS13-3*) and showed regulated expression concomitant with endodormancy establishment and release in the leaf buds of Japanese pear 'Kosui'. Application of hydrogen cyanamide accelerated endodormancy

release with a reduction in *PpMADS13* expression, whereas heat treatment (25 °C) in autumn inhibited endodormancy establishment without induction of *PpMADS13* expression, thus confirming tight relationships between the *PpMADS13* expression patterns and endodormancy phase transition. Comparative study between 'Kosui' and ‘Hengshanli’ revealed that the reduction of ‘Kosui’ *PpMADS13-1* and ‘Hengshanli’ *PpMADS13-1tw* expression level in *PpMADS13-1tw* occurred earlier than in *PpMADS13-1* toward endodormancy release, suggesting the *PpMADS13-1* homolog expression might work as a dose-dependent growth inhibitor and might reflect the differences in the amount of chilling requirement in the cultivars. Genomic structures of ‘Kosui’ *PpMADS13-1* and ‘Hengshanli’ *PpMADS13-1tw* revealed a 2,317 bp insertion in the 1st intron of ‘Hengshanli’ that might be ascribed to the earlier reduction of ‘Hengshanli’ *PpMADS13-1tw* expression; however, the insertion was also found in the high chilling requiring pear genotypes for endodormancy release.

To gain insight into the reduction of a Japanese pear *DAM* homolog (*PpMADS13-1*) expression toward endodormancy release, we first investigated the methylation status of the 5’-upstream region of *PpMADS13-1*, but there were no obvious changes in methylation status toward endodormancy release. Then, the histone H3 tail and the occupancy of histone variant H2A.Z (H2A.Z) in *PpMADS13-1* locus via chromatin immunoprecipitation quantitative PCR (ChIP-qPCR) were investigated. The results indicated that not induction of inactive histone mark, trimethylation of the histone H3 tail at lysine 27 but reduction of active histone mark, trimethylation of the histone H3 tail at lysine 4

contributed to the reduction of *PpMADS13-1* expression toward endodormancy release. The loss of H2A.Z also coincided with the down-regulation of *PpMADS13-1*. It has been supposed that C-repeated binding factor (CBF) could regulate DAM expression via the binding of CBF protein to the promoter of *DAM*. Indeed, the results suggested that PpCBF2, a pear C-repeated binding factor protein interacted with the 5′-upstream region of *PpMADS13-1* by ChIP-qPCR and transient reporter assay; thus, this complex could accelerate *PpMADS13-1* expression. Furthermore, transient reporter assay confirmed no interaction of the PpMADS13-1 protein and *PpFT1a*, a pear *FLOWERING LOCUS T*.

The results in this study showed that reduction of *PpMADS13* expression was correlated with endodormancy release; thereby, *PpMADS13* expression could use as expression marker to determine the dormancy status. Moreover, it was also demonstrated that the *PpMADS13* regulated by histone modification, suggesting the endodormancy of Japanese pear may control artificially using activator and/or inhibitor for histone modifying enzyme. Taken together, the results obtained in this study have led to a deeper understanding of the molecular mechanisms underlying endodormancy phase transition in Japanese pear, which could be greatly beneficial in future efforts for the stable and economical production of fruits even under global warming conditions.