IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 5, Issue 6 (Nov. – Dec. 2019), PP 18-25
www.iosrjournals.org

Molecular Mechanism of MADS-Box Genes as Candidate Genes for Regulation of Terminal Bud Dormancy in Peach

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Abstract:*Peach (Prunus persica L.), a member of the Rosaceae family, remains one of the most important fruit tree crops in temperate regions. The timing of flowering in peach is one of the most fundamental and complex developmental processes in the temperate areas. MADS-box family genes are essential transcription factors involved in plant development including growth terminal, bud formation, and endodormancy regulation in peach. Current understanding of the regulatory network(s) involved in dormancy induction is complicated. To understand the role of MADS-box genes orthologs in peach, we isolated and characterized nine transcription factors "TFs" including three DAM genes and six other "TFs" involved in dormancy regulation. The expression profiles of these genes were assayed during different flowering stages development and monitored their expression in apical buds throughout the full growing season using quantitative real-time polymerase chain reaction analyses (qRT-PCR). Among them, the gene expression profiles of PpeDAM6, PpeDAM62, PpeDAM79 and Ppe1148 are may possible responsible for dormancy regulation in different peach cultivars, while some ABA, GA, Ppe1851 and Ppe286 genes were identified as candidates genes in bud formation and ecdodormancy mechanisms. These results indicated that the molecular mechanisms of controlling dormancy are supposed associated with the dynamic interaction between gene expression profiles and cultivar-specific chilling requirements. These study is distinctive strategy to reveals the intersection between environmental signaling and the dormancy cycle in Peach.*

Keywords: *Prunus persica L.; MADS-box; RT-PCR; dormancy; bud formation.*

Date of Submission:04-11-2019 Date of Acceptance:20-11-2019

I. Introduction

The life cycle control of deciduous trees species is complex, especially the dormancy phenomena, as it is one of the most fundamental and complex developmental processes in plants. Dormancy has piqued additional attention from the perspective of global climate change, given that organization of the plant's activity-dormancy cycle with local climatic conditions is critical for the survival of tree species in the warm region and ensures simultaneous blooming in the orchard and upon the following season's fruit production [1]. Lang [2] classified the dormancy in three types states as being; eco-dormancy, para-dormancy, and endo-dormancy.

Now, it is well known that the genetic factors are controlling the regulating between the perception of dormancy-inducing signals and growth arrest, bud formation, and entry into end-dormancy are underlying. The time required between the entrance of the endodormancy and the flowering and bud break vary between cultivars, and those with the lower chilling requirement in mild winter regions, allow the harvest of fruits in shorter supply times [3]. This is particularly true for chilling requirement varies from species to species and also within a species based on their adapted location and the genetic control [4]. The current understanding of the regulatory genetic network(s) involved in bud set and endodormancy establishment will enable the targeted adaptation of superior genotypes to alternative winter climate regions [5]. As with plant dormancy regulation, phytohormones such as gibberellic acid (GA), abscisic acid (ABA) and ethylene are involved in the regulatory network(s) connecting signal and response, as demonstrated by reverse genetic approaches and seem to integrate in bud dormancy regulation [1].

Gene expression analysis is an increasingly key strategy towards developing our awareness of the complicated signaling and metabolic pathways underlying developmental and cellular and biological processes in peach. Real-time quantitative PCR (RT-PCR) has relatively gained much importance and wider applicability for the quantification of gene expression, owing to their inherent advantages of speed, high throughput, and automation potential. Hence, among the golden rules of the qRT-PCR technique, transcript level normalization with the ideal reference gene(s) is a critical factor in the analysis of gene expression [6].

In plants, genetic and molecular approaches have led to the identification of putative genes regulating bud endodormancy, among which *MADS*-boxgenes are best identified as master regulators of floweringtime and

floral organ development. In peach, a set of six dormancy associated *MADS*-box1-6 genes (*DAM*1-6), have been discovered partially deleted in the ever-growing (*evg*) mutant showing nondormant behavior[7]. In wild peach, the expression of *DAM* genes is decreased toward endo-dormancy breaking compare to the lost *DAM* genes in the ever-growing peach mutant [8].The expression of *DAM* genes is extremely dependent on the establishment, maintenance and release of bud dormancy. Recent studies have also demonstrated that *DAM* genes are differentially expressed either up-regulated towards dormancy establishment or down-regulated towards dormancy release in response to seasonal dormancy transitions in other plant systems[9].This *DAM* genes were identified as strong candidates genes for the regulation of growth cessation and terminal bud formation in peach [10].

In the current study, we sought to shed light on the behavior of *DAM* with a phytohormone key genes ABAand GA using qRT-PCR. In detail, we discuss our results in terms of changes in expression profiling of peach *DAM* genes family during the key phenological events, including various stages of dormancy, dormancy release and flower stages within the four peach cultivars.

II. Materials and Methods

Plant Material

Peach (*P. persica*) specimens used in this study contained four cultivar Malwina (MW), Disertred (DR), Nanshang (NH) and Jinxiang (JX). These four specimens are maintained at Chinese Academy of Sciences, China. All material were collected in four stage, dormancybud, balloon, full- balloon stages and young leaves in juvenile stage that had just unfolded, respectively. All the samples were immediately frozen in liquid nitrogen and then stored at 80_C until use.

RNA Extraction and qRT-PCR Analyses

Total RNA was extracted from 100 mg using a rapid plant RNA extraction kit (Aidlab, Beijing, China) according to the manufacturer's instructions. The single-stranded cDNAs were synthesized from 1 µg of RNA using a cDNA synthesis kit according to the manufacturer instructions (Takara Biotechnology, Dalian, China). qRT-PCR was performed with a gene specific primer pair and *TEF2* actin primer pair as an internal control using Step OnePlus Real-Time polymerase chain reaction (PCR) system. Reactions were achieved on a CFX96 real-time PCR detection system with SYBR Premix Ex Taq (Takara).

Gene-specific primers for *TEF2* actin as a reference gene and with 13 genes named (ABA1, ABA2, ABA3, GA, *PpeDAM* 6, *PpeDAM 62*, *PpeDAM*79, Home Box like, *Ppe*1148, *Ppe*3082, *Ppe*286, *Ppe*393 and *Ppe*1851) were involved in the biosynthesis of dormancy and break dormancy in peach. These genes were designed using the primer designing tools of IDTdna (http://www.idtdna.com), as listed in Table 1. The thermo cycling parameters were as follows: 10 min at 95° C, followed by 40 cycles of 15 s at 95° C for denaturation and 1 min at 60° C for annealing and extension. Results presented are the average of three independent biological replicates repeated three times. The quantified data were analysed using the Bio-Rad IQTM5 Multi-color Real-Time Manager software. The ΔΔCt was calculated by subtracting the ΔCt of each stage from the ΔCt of the plant tissue. The formula 2^-(ΔΔCt) was used to determine the relative fold change between the flowering development stages, while the mean and SE values of relative transcript abundance were determined.

III. Results

It is well-know that the flowering time is a complex stage affected by climatic conditions and environmental factors; this is varies depending on cultivar-specific chilling and heat requirements for dormancy releases. The specific response to climatic and environmental influences involving dormancybud, balloon, full-balloon stage and juvenile stage were studied in four peach cultivars. To verify whether the overall gene expression was trained by cultivar-specific, the qRT-PCR was used to examine the changes of *P. persicaDAM* genes family members during the four stages. The *DAM* genes really presented seasonal expression patterns throughout the four stages of the year. However, these expression profiles were somehow differently within the four species. Here, the *DAM* transcript levels during the four stages have revealed that their mRNA expression profiles associated with various dormancy cycle levels. A summary of the expression analysis that identified three *DAM*, six *DAM* like genes and four phytohormone key genes (ABA1, ABA2, ABA3 and GA2) were differentially expressed during the regulation of bud dormancy cycle as presented in Figure 1,2,3 and 4.In detail, our results showed that, in the dormancystage these genes(*PpeDAM*62, *PpeDAM*79 and *Ppe*3082), were significantly up-regulated, a similar trend was observed for abscisic acid hormone genes of ABA2 and ABA3, respectively. However, the expression of *PpeDAM*6, *PpeDAM*62, *PpeDAM*79, *Ppe*1148, and *Ppe*3082 genes (Figure 1) began to increase with the highest expression levels in the balloon stage. These genes were correlated positively with significantly up-regulated with ABA2 and ABA3 genes (Figure 2). From our results, we found three genes named *Ppe*286,*Ppe*393 and *Ppe*1851(Figure 1) showed the highest expression levels in the full-

balloon stage. Additionally, an increase was noted in the phytohormone genes expression of ABA1, ABA2 and GA3, respectively (Figure 2). Conversely, the expression pattern of the *PpeDAM*6, *PpeDAM*62, *PpeDAM*79 and home box-like genes were down-regulated during the full-balloon stage.

The expression profiles of four phytohormone key genes were further examined in the juvenile stage. The results indicated that ABA1,ABA2, ABA3 and GA2 gene expression were elevated (Figure 2). The same trend in expression level with slightly elevated was observed in home box-like, *Ppe*286, *Ppe*393, *Ppe*851and *Ppe*1148genes (Figure 1), while *PpeDAM*6 was downregulated in juvenile stage. Collectively, the up-regulated expression of these genes may facilitate flowering metabolism during the juvenile stage.

To verify whether the overall gene expression was conditioned by cultivar-specific factors, four different species of peach were compared their expression at a fixed date across the four-stage of dormancy (Figure 3 and 4). Despite different expression levels of each gene between the tested peach genotypes, it could be noticed that the level of *PpeDAM*6, *PpeDAM*62 and *PpeDAM*79 gene expressions (Figure 3), were higher in the first two collection stages compared to the level of expression in the remaining stages. The cultivar Nanshang was primarily distinguished by a high level of expression in *PpeDAM*6, *PpeDAM*62 and *PpeDAM*79 genes, which sharply increased within the dormancy and balloon stages, and were followed by Disertred and Malvina cultivars.

Conversely, the expression levels of these genes were decreased in all cultivars tested during the full-balloon stage and juvenile stage. Interestingly, highly significant up-regulated in the Disertred cultivar was noticed in the relative expression levels of *Ppe*1148, *Ppe*286, *Ppe*393 and *Ppe*1851 genes during the full-balloon stage (Figure 3). The same trend with the intensity elevated of the gene expression level was observed in the ABA1 and ABA2 phytohormone genes, respectively (Figure 4). As a result, during the juvenile stage, there was a widespread down and up-regulated are noted in the expression of several genes tested. Notably, in the Malwina cultivar, there was slightly increased in the expression level of several genes, e.g., *Ppe*1148, *Ppe*286, *Ppe*393, *Ppe*1851, ABA1 and ABA3, these genes were preferentially expressed in the juvenile stage (Figure 3 and 4). It is noteworthy, *DAM* home box gene showed a sharply increased in the expression level within Jinxiang and Nanshang cultivars through the juvenile stage (Figure 3). Consequently, these genes seem to be correlated positively with bud break ability following the dormancy cycle in the cultivar-specific and showed a wide up-regulated distribution of gene expression in Nanshang, Disertred and Malvina cultivars, respectively.

IV. Discussion

Crosstalk among peach bud dormancy and plant hormones along with the bud break is a serious dilemma for peach flowering and production; this controlled by a complicated regulatory network involving several expression genes family members including several *"TFs "*and plants phytohormone key genes [11]. In the present study, the quantification of the *DAM* transcript levels throughout the whole season has revealed that their mRNA expression profiles connect to various dormancy cycle periods; these performances were somehow reliable even between diff erent species. In Rosaceous evidence suggested that the *DAM* genes created a group subdivided into two clades; one comprising Prunus *DAM* genes and the other clade formed Malus and Pyrus. This emphasizes the evolutionary parallels of these Rosaceous species, but simultaneously the variance among genus [12]. In view of the performance of our results, the vast majority of *DAM* and our*"TFs "* genes exhibiting their highest expression levels during endodormancy and bud break, as recommended by the previous finding of the *DAM* gene family in peach and other Rosaceous species [13]. In the early spring, the *DAM* genes are regularly displaying the highest expression pattern before bud break. Our data infer similar features as reported previously in peach, apple and kiwifruit genes [14]. Recently, Falavigna [12] proposed the hypothesized that each of the *DAM* and *DAM* like genes could assimilate various environmental signals to let a subtle modifying regulation of the diff erent stages of tree dormancy cycle. Another striking characteristic, involving *DAM* genes sensitivity to biological signals, was recognized after dormancy demonstration when the branch presented to increase cold temperatures in controlled conditions. This approach led to the down-regulation of *DAM* genes in a cultivar-dependent behavior, as reported in our results of the cultivar-specific approach in each of Malwina and Disertred.

During dormancy, a lot of changes in buds in the regulatory network, mostly in the endogenous hormone contents such as the two phytohormone key genes (ABA and GA) which plays an important role in regulating dormancy and the developmental pathways bud break.Evidence mentioned that the overexpression of ABA biosynthetic enzymes enhances dormancy, suggests that the roles of ABA as the initiation and development of the dormancy phase and that ABA phytohormone key genes seem to be involved in mediating environmental signals [15]. While the regulation of GA phytohormone key genes during dormancy is a complex model with variance effects dependent on accumulation and developmental stages. GA seems to be stimulated the metabolic pathways due to dormancy release. Recent hypotheses proposed that the major role for GAs in preserve growth before the induction of dormancy and stimulating growth through ecodormancy [16]. Accordingly, GA processing has a complex effect on dormancy and bud break, as presented in our study and many perennial

genera since GA pathways may substitute for chilling [17]. Indeed, our findings agree with the several earlier report in peach and cherry supports this opinion as most of GA expression profiles genes are up-regulated during the ecodormancywith a significant increase in bud break [4,15]. It is worth noting that within dormancy, a cross-talks occur between ABA–GA phytohormone key genes, this of course, due to the interaction overlapped correlated with the ABA/GA percentage affects differ depth of dormancy and flower initiation response to environmental cues [18]. This concept has gained much acceptance and support through our findings and other previous studied [15,19]. Another striking point, the connection balance of *DAM* transcription factors and phytohormone key genes, especially ABA and GA seem to be allowed hormones and other internal signals correlated with the transition among several phases of bud dormancy. This concept was also recommended in our results and other reported species as Japanese pear and peach [20].

Recently, insufficient chilling accumulation in the winter season has become a big challenge due to global warming, leading to the inability to complete endodormancy, therefore, the chilling requirements differ from species to species and inside a species, proposing a broad genetic control of this process, as observed in the regulation of seasonal growth cessation and terminal bud formation in Malwina and Disertred cultivars [3,17,21].

V. Conclusion

The timing of flowering in peach is one of the most fundamental and complex developmental processes in the temperate areas. In the present investigation the studied phytohormone key genes are most likely involve in the regulation of bud dormancy cycle. However, GA pathways have various functions and could differentially control dormancy. Following the notes that the ABA levels are clearly associated with dormancy depth, and its biosynthetic pathway was shown to be operated by *DAM* and candidate *"TFs* "genes in recent study during the transition from endo to eco-dormancy. These genes highlight the presence of specific putative transcription factors during bud dormancy progression and release, offer a reasonable process for the regulation of these genes, which is significant in dormancy processes. However, these genes were proposed to have a role in the regulation of seasonal growth of peach cultivars and terminal dormancy, dormancy release and flower stages.

Acknowledgements

The author is thankful to Dr. Mohamed Ewas, Researcher, Department of Genetics, Desert Research Center, Egypt, for helping the analysis of data and revised.

Reference

[1]. Cooke, J. K., Eriksson, M. E. and Junttila, O. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ, 35: 1707–1728.
[2]. Lang, G.A. 1987. Dormancy: a new terminology. Hort Science, 22: 817–820.
[3]. Alves, G., Gelain, J., Vidal, G.S., Nesi, C.N., Mio, L.L.M.D. and Biasi, L.A. 2018. Flowering period and fruit quality of peach trees selections and cultivars in the metropolitan region of Curitiba. RevistaBrasileira de Fruticultura, 40:(3).
[4]. Yamane, H. 2014. Regulation of bud dormancy and bud break in Japanese apricot (*Prunus mume* Siebold &Zucc.) and peach [*Prunus persica* (L.) Batsch]: a summary of recent studies. Journal of the Japanese Society for Horticultural Science, CH-Rev4.
[5]. Rohde, A., Howe, G.T., Olsen, J.E., Moritz, T., Van Montagu, M., Junttila, O. and Boerjan, W. 2000. Molecular aspects of bud dormancy in trees. In Molecular biology of woody plants, Springer Dordrecht, 89-134.
[6]. Imai, T., Ubi, B.E., Saito, T. and Moriguchi, T. 2014. Evaluation of reference genes for accurate normalization of gene expression for real time-quantitative PCR in *Pyrus pyrifolia* using different tissue samples and seasonal conditions. PLoS One, 9(1): p.e86492.
[7]. Bielenberg, D.G., Wang, Y.E., Li, Z., Zhebentyayeva, T., Fan, S., Reighard, G.L., Scorza, R. and Abbott, A.G. 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. Tree Genetics & Genomes, 4(3): 495-507.
[8]. Ubi, B.E., Sakamoto, D., Ban, Y., Shimada, T., Ito, A., Nakajima, I., Takemura, Y., Tamura, F., Saito, T. and Moriguchi, T. 2010. Molecular cloning of dormancy-associated MADS-box gene homologs and their characterization during seasonal endodormancy transitional phases of *Japanese pear*. Journal of the American Society for Horticultural Science, 135(2):174-182.
[9]. Wells, C.E., Vendramin, E., Tarodo, S.J., Verde, I. and Bielenberg, D.G. 2015. A genome-wide analysis of MADS-box genes in peach [*Prunus persica* (L.) Batsch]. BMC plant biology, 15(1):41.
[10]. Jiménez, S., Lawton-Rauh, A.L., Reighard, G.L., Abbott, A.G. and Bielenberg, D.G. 2009. Phylogenetic analysis and molecular evolution of the dormancy associated MADS-box genes from peach. BMC Plant Biology, 9(1):81.

[11]. Khalil-Ur-Rehman, M., Wang, W., Xu, Y.S., Haider, M.S., Li, C.X. and Tao, J.M. 2017. Comparative study on reagents involved in grape bud break and their effects on different metabolites and related gene expression during winter. Frontiers in Plant Science, 8:1340.
[12]. Falavigna, V., Guitton, B., Costes, E., and Andrés, F. 2019. I Want to (Bud) Break Free: The Potential Role of *DAM* and *SVP*-Like Genes in Regulating Dormancy Cycle in Temperate Fruit Trees. Frontiers in plant science, doi:10.3389/fpls.2018.01990.
[13]. Sasaki, R., Yamane, H., Ooka, T., Jotatsu, H., Kitamura, Y., Akagi, T. and Tao, R. 2011. Functional and expressional analyses of *PmDAM* genes associated with endodormancy in *Japanese apricot*. Plant physiology, 157(1):485-497.
[14]. Xiang, Y., Huang, C.H., Hu, Y., Wen, J., Li, S., Yi, T., Chen, H., Xiang, J. and Ma, H. 2016. Evolution of Rosaceae fruit types based on nuclear phylogeny in the context of geological times and genome duplication. Molecular biology and evolution, 34(2):262-281.
[15]. Liu, J. and Sherif, S.M. 2019. Hormonal Orchestration of Bud Dormancy Cycle in Deciduous Woody Perennials. Frontiers in plant science, 10.
[16]. Zhang, Z., Zhuo, X., Zhao, K., Zheng, T., Han, Y., Yuan, C. and Zhang, Q. 2018. Transcriptome Profiles Reveal the Crucial Roles of Hormone and Sugar in the Bud Dormancy of *Prunus mume*. Sci. Rep, 8:1–15.
[17]. Vimont, N., Schwarzenberg, A., Domijan, M., Beauvieux, R., Arkoun, M., Yvin, J.C., Cortijo, S., Wigge, P.A., Dirlewanger, E. and Wenden, B. 2018. Hormonal balance finely tunes dormancy status in sweet cherry flower buds. bioRxiv, 423871.
[18]. Duan, C., Li, X., Gao, D., Liu, H., and Li, M. 2004. Studies on regulations of endogenous ABA and GA3 in sweet cherry flower buds on dormancy. Acta Hortic. Sinica, 31:149–154.
[19]. Wen, L. H., Zhong, W. J., Huo, X. M., Zhuang, W. B., Ni, Z. J., and Gao, Z. H. 2016. Expression analysis of ABA- and GA-related genes during four stages of bud dormancy in Japanese apricot (*Prunus mume*Sieb. et Zucc). J. Hortic. Sci. Biotechnol., 91:362–369.
[20]. Wang, D., Gao, Z., Du, P., Xiao, W., Tan, Q., Chen, X., Li, L. and Gao, D. 2016. 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.
[21]. Tuan, P.A., Bai, S., Saito, T., Ito, A. and Moriguchi, T. 2017. Dormancy-associated MADS-Box (DAM) and the abscisic acid pathway regulate pear endodormancy through a feedback mechanism. Plant and Cell Physiology, 58(8):1378-1390.

Table (1). Primers and their sequences used for real-time PCR analysis in this study.

	Oligonucleotide sequence pairs used for amplification (5–3)	
Gene name	F	R
*Ppe*DAM6	TACTGGACCTGCGTTTGTGGAGCC	TGTTGCAGCTGGTGGAGGTGGCAATT
*Ppe*DAM62	AGCTGGTTGTTGTTATGC	TGGGCGAGTCAATGAGTTCTAT
*Ppe*DAM79	GGCACAACAGCAGCAAACAA	CGCCTCCAAGATTACAGGGA
Ppe Home box Like MAD	GCTGAGCTGGTAGAAGCCAA	GCCGTCTTCTTCTCCTCCAC
*Ppe*286	GCCTTGCCTAATCATTCG	TCCATAACTTCCTTCACTCT
*Ppe*1148	CGATGATGATGGTGATGATG	ACGACGAAGAGATTGAAGA
*Ppe*393	CTCTGATACACGCCTGAT	TGTGGTTATGTTCTCCTTCA
*Ppe*3082	CCTTGCTCCTCTTCTACAT	TGGTTTCCTGCTGATGAT
*Ppe*1851	GGCTATTCTCCCACATTTG	GAGTTGGTCACTGAAGTTG
ABA 1	ACAGACCAACAGATTGCTGACAAC	CCTTCATCATCACCCTCCTCTTCTT
ABA 2	TCGGCAACAATAGGGACTTTGAAATG	CCATCACTCTTCCTTCTCTTCGCTAT
ABA 3	TCACCAAGGAGACTACCACAATAGC	CAAGGAAGCCAACATCAAAGGAGAAC
GA2	CTTTCAGCCTTGTGCTAGAA	CGTACAGAGCCAGTAGAATG
TEF2	GGTGTGACGATGAAGAGTGATG	TGAAGGAGAGGGAAGGTGAAAG

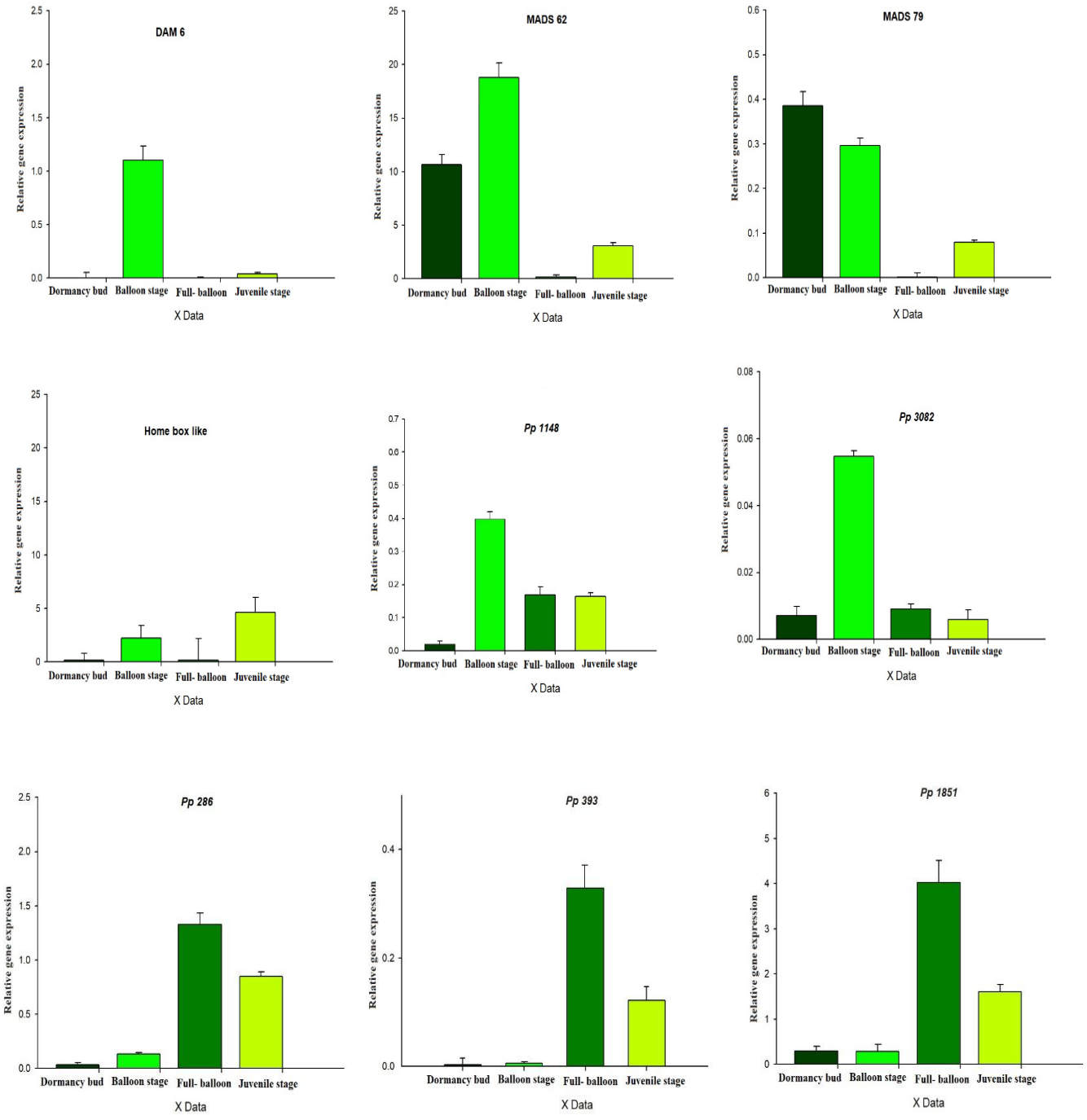


Figure (1). Expression profiles of *PpeDAM*6, *PpeDAM*62, *PpeDAM*79, Home Box like, *Ppe*1148, *Ppe*3082, *Ppe*286,*Ppe*393and *Ppe*1851 genes in different stage of dormancy.

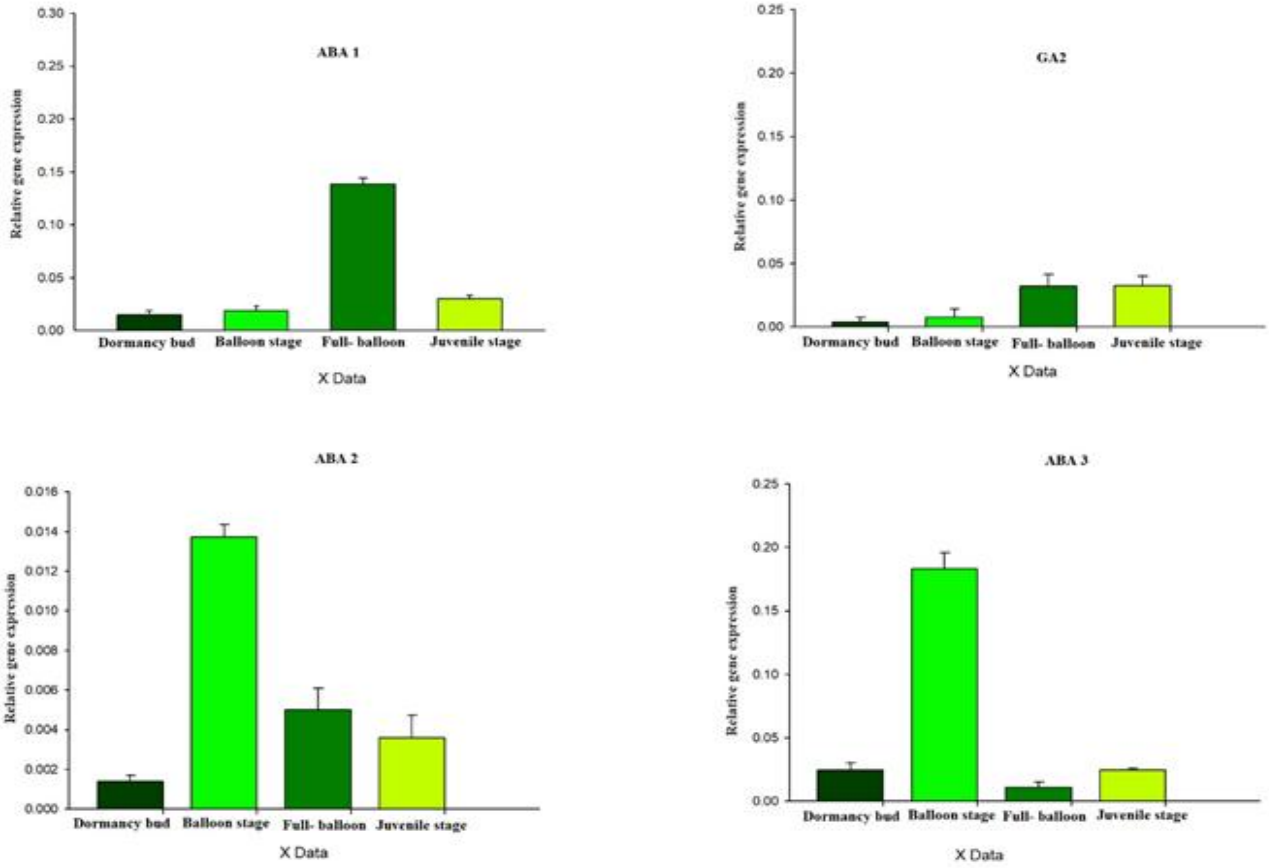
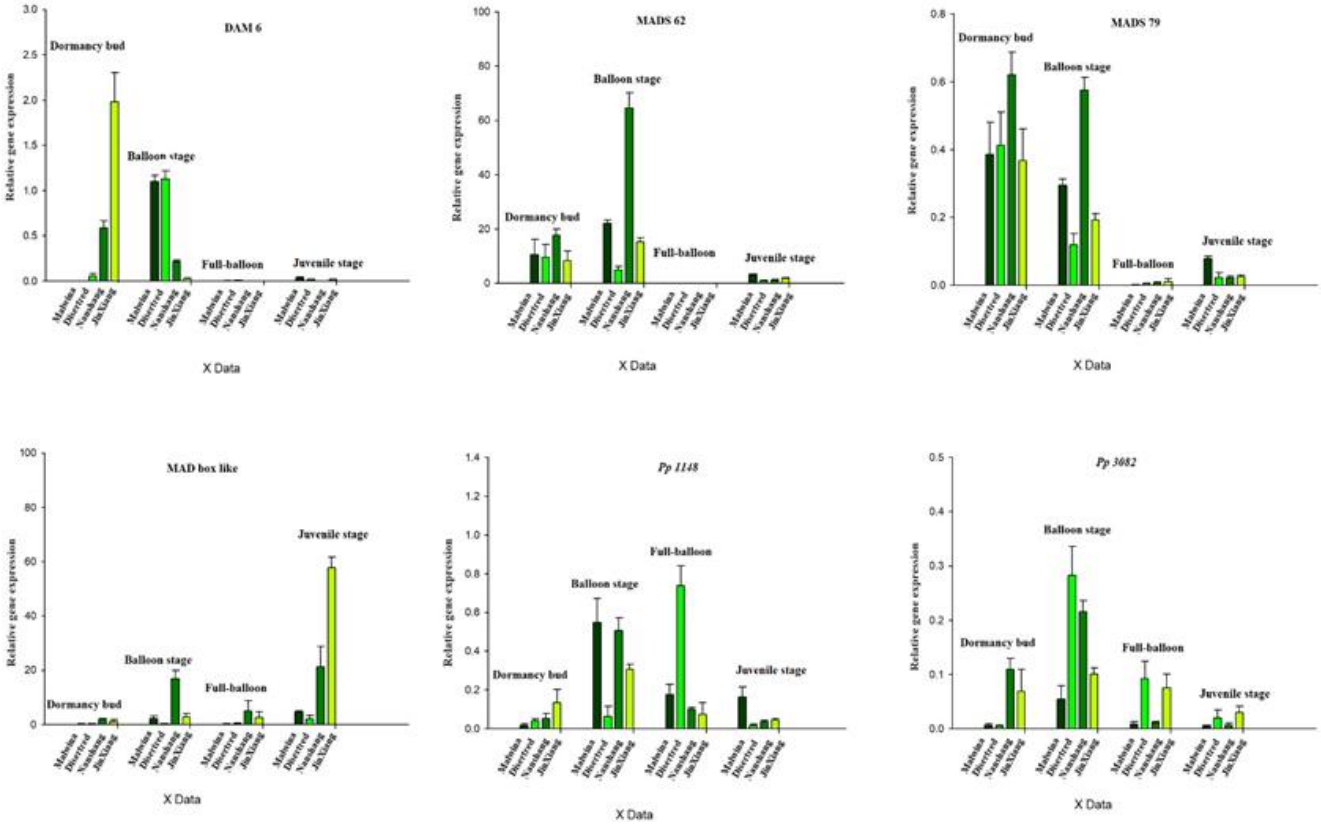


Figure (2). Expression profiles of four phytohormone genes ABA1,ABA2,ABA3and GA2 genes in different stageof dormancy.



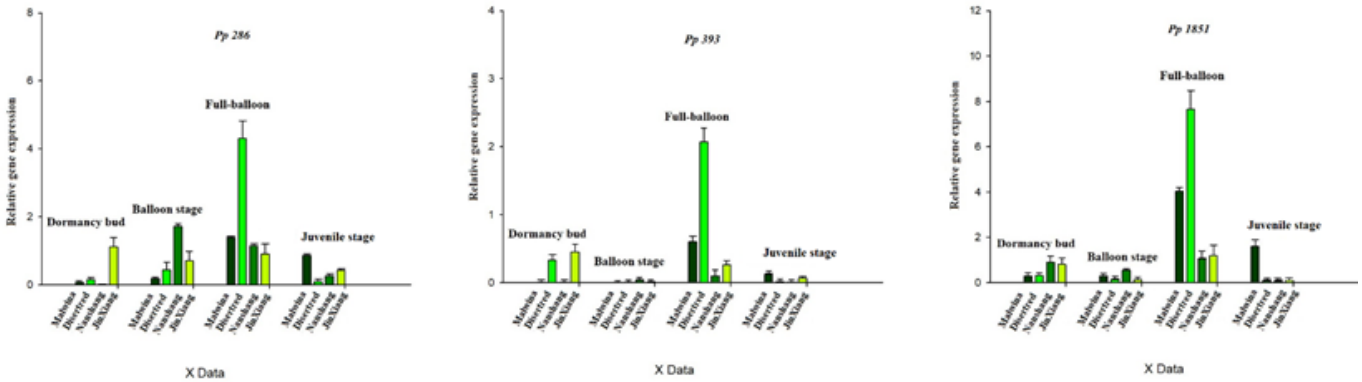


Figure (3). Expression profiles of *PpeDAM* 6, *PpeDAM 62*, *Ppe DAM*79, Home Box like, *Ppe*1148, *Ppe*3082, *Ppe*286, *Ppe*393and *Ppe*1851 genes in four species of *Prunus persica* L. within the four stages of dormancy.

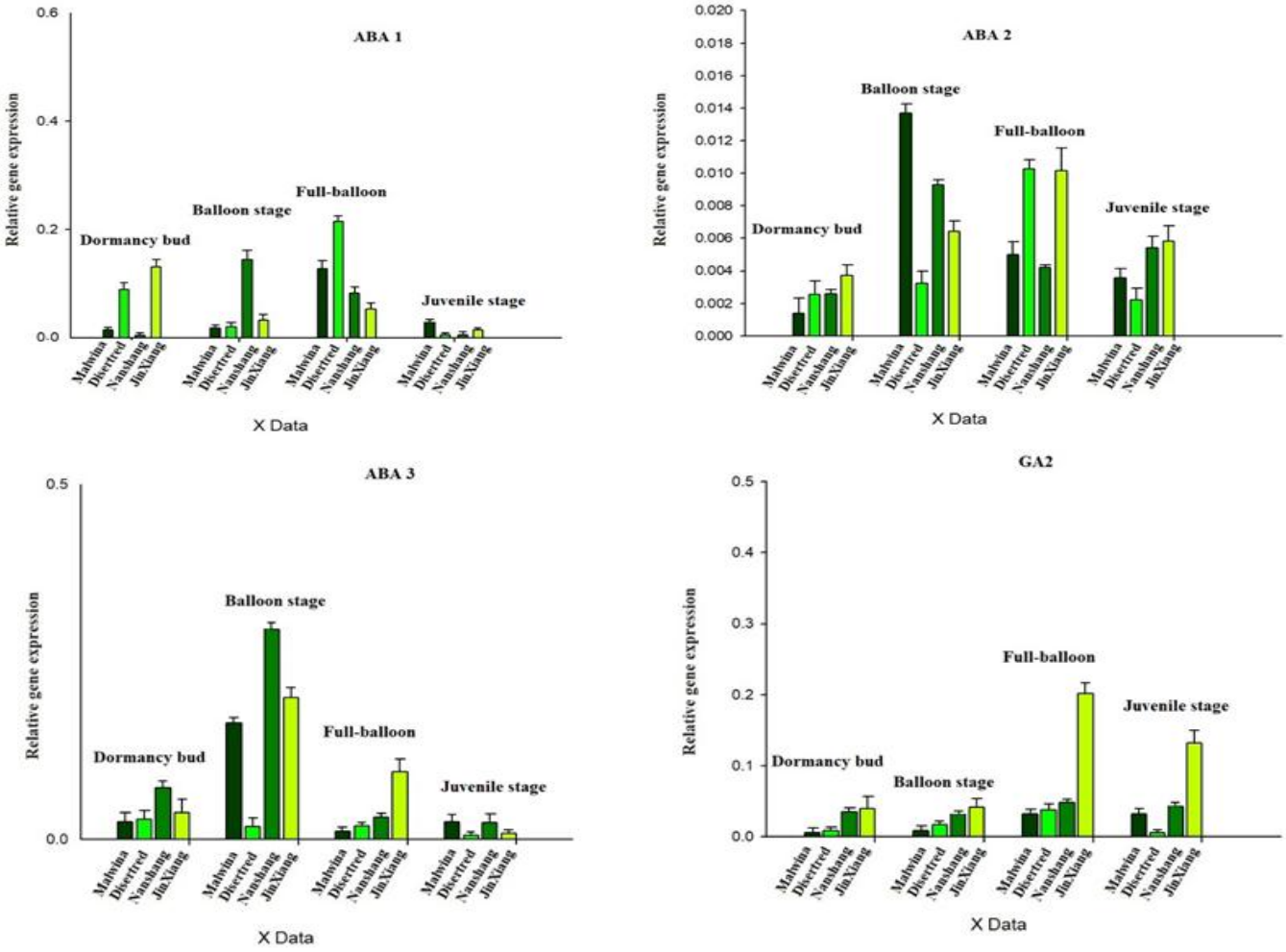


Figure (4). Expression profiles of four phytohormone genes ABA1,ABA2,ABA3andGA2 genes in four species of *Prunus persica* L. within the four stages of dormancy.