Effect of Postharvest Brassinolide Treatment on Phenylpropanoid Pathway and Cell Wall Degradation in Peach Fruits

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Abstract: Peaches are subject to flesh softening during postharvest storage and transport, which affects the storage life of the fruit and causes huge economic losses. Previous research has demonstrated that postharvest brassinolide treatment can maintain flesh firmness, ascorbic acid and soluble solids contents, and enhance disease resistance in peach fruits. This study assessed the influence of postharvest brassinolide treatment on the expression of key genes involved in cell wall degradation and the phenylpropanoid pathway in peach fruits by real-time fluorescence quantitative polymerase chain reaction (qPCR). The results showed that brassinolide dipping inhibited the gene expression of pectate lyase 1, polygalacturonase 21 and pectin methylesterase 1, and significantly enhanced the gene expression of peroxidase, cinnamoyl-CoA reductase, phenylalanine ammonia lyase and caffeoyl-CoA-*O*-methyltransferase 5 in peach fruits. It also increased the gene expression levels of chaleone synthase, chaleone isomerase, dihydroflavonol-4-reductase and flavanone 3-hydroxylase at the early stage of storage. These findings imply that brassinolide can suppress the expression of key genes involved in cell wall degradation and enhance the expression of key genes involved in the phenylpropanoid pathway, thereby delaying peach fruit softening and enhancing disease resistance.

Keywords: brassinolide; peach fruit; phenylpropanoid pathway; cell wall degradation; softening

采后油菜素内酯处理对桃果实苯丙烷途径和细胞壁降解的影响

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摘 要：桃果实在采后物流过程中容易发生果肉软化，影响其贮藏期，造成巨大的经济损失。前期研究表明，采后油菜素内酯处理能够保持桃果肉的硬度及抗坏血酸、可溶性固形物含量，并能提高果实抗病性。本研究利用油菜素内酯处理桃果实，通过实时荧光定量聚合酶链式反应分析相关基因的变化，探讨油菜素内酯处理对桃果实细胞壁降解及苯丙烷途径关键基因表达的影响。结果表明：油菜素内酯浸泡处理能抑制果胶裂解酶1、多聚半乳糖醛酸酶21和果胶甲酯酶1基因的表达；并显著促进果实中过氧化物酶、肉桂酰辅酶A还原酶、苯丙氨酸解氨酶和咖啡酰辅酶A-*O*-甲基转移酶5基因的表达；同时，提高贮藏早期果实中查耳酮合成酶、查耳酮异构酶、二氢黄酮醇4-还原酶和黄烷酮3-羟化酶基因的表达量。综上，油菜素内酯可以抑制细胞壁降解关键基因的表达，促进苯丙烷途径关键基因的表达，从而延缓桃果实软化并提高其抗病性。

关键词：油菜素内酯；桃果实；苯丙烷途径；细胞壁降解；软化
DOI:10.7506/spkx1002-6630-20230203-031
中图分类号：TS255.36 文献标志码：A 文章编号：1002-6630（2023）21-0175-09
引文格式：
ZHANG Shuran, LI Canying, XU Hengping, et al. Effect of postharvest brassinolide treatment on phenylpropanoid pathway and cell wall degradation in peach fruits[J]. 食品科学, 2023, 44(21): 175-183. DOI:10.7506/spkx1002-6630-20230203-031. http://www.spkx.net.cn

收稿日期：2023-02-03
基金项目：国家自然科学基金青年科学基金项目（31801595）

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ZHANG Shuran, LI Canying, XU Hengping, et al. Effect of postharvest brassinolide treatment on phenylpropanoid pathway and cell wall degradation in peach fruits[J]. Food Science, 2023, 44(21): 175-183. (in English with Chinese abstract)
DOI:10.7506/spkx1002-6630-20230203-031. http://www.spkx.net.cn

Peach (*Prunus persica* L. Batsch) fruit is one of the important horticultural crops with large cultivated area in China[1]. It exhibits attractive appearance and delicious flavor as well as contains high levels of antioxidants and anticarcinogenic compounds, including vitamins, carotenoids and phenolic compounds[2-3]. As a typical climacteric fruit, peaches have strong respiratory metabolism and large ethylene release after harvest, therefore accelerating softening and decay[4-5]. Softening is one of the main factors of fruit quality deterioration, which not only affects the appearance, taste and disease resistance, but also directly affects the commercial value of fruit[6]. Moreover, softened fruit is more susceptible to fungal infections, which can result in huge economic losses and also produce harmful toxins in the tissues[7]. Therefore, developing eco-friendly measures to control postharvest decay and delay fruit softening is the major issue of peach industry. Study has shown that ultraviolet (UV)-C and UV-B treatments effectively reduced decay incidence and extended storage life of peaches[8]. Similarly, nitric oxide could delay respiratory peak and reduce respiratory rate and ethylene production, thus alleviating softening, decay and chilling of peaches[9]. Zhu Yongchao et al.[10] reported that changes in the DNA methylation in different genomic regions inhibited the expressions of a series of genes related to peach fruit softening, senescence and stress response. Mou Linyun et al.[7] reported that controlled atmosphere storage could alleviate flavor deterioration, inhibit softening and decay index of peaches. Additionally, 1-methylcyclopropene (1-MCP) treatment can significantly reduce the weight loss and decay rate, inhibit physiological and biochemical metabolic activities, and delay the softening of peaches during storage[11]. Glucose oxidase immobilized on ZnO nanoparticles treatment maintained the hardness and total soluble solids, inhibited decay and extended storage life of peaches[12]. It has been suggested that both methyl jasmonate treatment and combined 1-MCP and laser microporous plastic bag packaging enhanced ascorbate-glutathione cycle and antioxidant ability to enhance postharvest shelf life of peaches[13-14]. These findings suggest that physical and eco-friendly chemicals treatments could suppress softening and enhance disease resistance of peaches.

Brassinolide is a new and highly effective plant hormone, which plays an indispensable role in growth and development of plants as well as in response to biological and abiotic stresses[15]. It has been proven that brassinolide can extend shelf life of fruit and vegetable[16]. Postharvest application of brassinolide could enhance chilling tolerance in grapes, bananas and peppers by maintaining membrane integrity, enhancce the activities of anti-oxidative enzymes and decrease reactive oxygen species (ROS) accumulation[17-19]. Recent studies have also revealed that brassinolide increased the soluble solids and total soluble sugar contents to maintain quality of grapes[20]. A study showed that brassinolide increased the non-enzymatic and enzymatic antioxidant defense systems by inhibiting malondialdehyde and ROS production, inducing total phenols and proline accumulation, which significantly alleviated chilling injury and rib-edge darkening of carambola fruit[16]. In addition, brassinolide can control blue mold of peach fruit[21]. Taken together, brassinolide can induce disease tolerance and maintain storage quality of peaches.

Activation of phenylpropanoid pathway is one of the mechanisms involves in inducing resistance which plays pivotal roles in tolerance to biological and abiotic stress[22]. Phenylalanine is the precursor that can be catalyzed by phenylalanine ammonia lyase (PAL) to synthesize phenolic compounds, flavonoid and lignin, which has anti-fungal and anti-oxidative activities[23-26]. Additionally, lignin can strengthen the cell wall structure and act as a physical barrier at the early stage of pathogenic infestation[27]. Pectin substance is also one of the components of the cell wall, which can be broken down by various cell wall degrading enzymes, thus resulting in softening of the fruit and reducing storage ability[28]. Studies have reported that hydrogen sulfide or 1-MCP combined with laser microporous plastic bag packaging reduced cell wall degradation enzymes activities and inhibited the degradation of pectin, hemicellulose and cellulose to maintain the structural integrity of the cell wall and delay the softening and deterioration of peaches[13,29]. Ji Nana et al.[30] also reported that methyl jasmonate effectively promoted the accumulation of total phenol, flavonoid and lignin to enhance disease resistance by activating the phenylpropanoid pathway of harvested peaches. Similarly, hot water treatment enhanced the key gene expression in phenylpropanoid pathway, which by improving the phenolic

compounds accumulation to control chilling injury of peaches[31]. All these findings imply that elicitors can affect the production of secondary metabolites by regulating phenylpropanoid pathway to strengthen the cell wall and enhance resistance of fruit. Previous studies in our laboratory demonstrated that brassinolide treatment decreased respiratory rate, maintained higher flesh firmness, ascorbic acid and soluble solids contents, enhanced antioxidant capacity and disease resistance against blue mold through mediating the enzyme activity and secondary metabolites accumulation in peaches[5,21,32]. Therefore, we further investigated the effect of brassinolide on the key gene expression in phenylpropanoid pathway and cell wall degradation of peaches.

Studies have demonstrated that hydrogen sulfide had the ability of delaying senescence via inhibiting ethylene production and cell wall metabolism to suppress softening in peaches[6,29]. Additionally, methyl jasmonate could increase disease resistance and delay senescence by increasing phenylpropanoid pathway related enzyme activity and metabolite content of peaches[30]. However, little information is available regarding the relationship between fruit softening and phenylpropanoid pathway in peach fruit after application of brassinolide. Therefore, the goals of the current study were to assess the effect of brassinolide treatment on the expressions of genes encoded cell wall degrading enzymes and involved in phenylpropanoid pathway; to elucidate the relationship between them in peaches during storage.

1 Materials and Methods

1.1 Materials and reagents

Peach (*P. persica* L. Batsch) fruit were handpicked at commercial maturity in Jinzhou (China) and shipped to Fruit and Vegetable Physiology Laboratory, Bohai University (China). Brassinolide was purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. RNAprep Pure Plant Kit, FastQuant RT Kit, 2 × *Taq* Polymerase Chain Reaction (PCR) Master mix, and Super Real PreMix Plus (SYBR Green) Kit were purchased from Tiangen Biochemical Technology (Beijing) Co., Ltd. Biowest Agarose was purchased from Wuhan Kehaojia Biotechnology Co., Ltd. 4S Green Nucleic Acid Staining Agent was purchased from Beijing Baiao Leibo Technology Co., Ltd.

1.2 Instruments and equipment

NanoDrop™ One/OneC ultra-micro ultraviolet spectrophotometer was purchased from Thermo Fisher Scientific (China) Co., Ltd. ABI-2720 PCR amplification instrument was purchased from Nanjing Eruoda Instrument Equipment Co., Ltd. Uvitec multi-color fluorescence/chemiluminescence gel imaging analysis system was purchased from Beijing Shengke Xinde Technology Co., Ltd. LightCycler® 96 real-time PCR system was purchased from Keyu Xingye Technology Development Co., Ltd.

1.3 Methods

1.3.1 Fruit and treatment

Flesh firmness and soluble solids content of the peach fruit were (6.92 ± 0.14) kg/cm^2 and (12.68 ± 0.26)% at the harvest day, respectively. Fruit with similar surface color and size, and absent of visual defects were randomly divided into two batches (180 fruit per batch). The first batch was dipped in 5.0 μmol/L brassinolide for 10 min (the concentration of brassinolide was previously screened by Fruit and Vegetable Physiology Laboratory[21]). The second batch was dipped in deionized water (control) for 10 min. All treated fruit were drained for 2 h prior to keeping in a plastic bag at 20–22 ℃ and 75%–80% relative humidity.

1.3.2 Sample collection

Flesh tissue was taken from 3–10 mm below the exocarp around the equator of brassinolide-dipped and control fruit from 0 to 5 d after treatment. Each sample was individually packed with aluminum foil, then frozen in liquid nitrogen and kept at –80 ℃ until the biochemical determination following Ge Yonghong et al.[21] approach.

1.3.3 Extraction of total RNA

The RNAprep Pure Plant Kit was utilized to extract total RNA from flesh tissue (0.5 g). Then, 1.0 g/L agarose gel and ultra-micro spectrophotometer were used to monitor the integrity and purity of RNA. Synthesis of first-strand cDNA for real-time quantitative PCR (qPCR) was performed using the FastQuant RT Kit. As shown in Table 1, the primer sequences of the selected genes were designed and synthesized by Sangon Biotech (Shanghai, China).

Table 1 Primer sequences used for qPCR

Gene	Primer sequence (5′-3′)	GenBank ID
PpPL1	Forward: AGCACTACGGGTGGAGGACTAT Reverse: GCATCAACCAACCCATCTTT	AB264095.1
PpPG21	Forward: GCTCTAGACACTGGGTTTGCTAGAAAT Reverse: GGGGTACCAACAACTTGTAGGCTGAAC	ppa006839m
PpPME1	Forward: ATCAATGGCTACCAGGACACG Reverse: TGCTGTGATGACCGTGAACTG	AB231903.1
PpPAL	Forward: GCAACCCTGTCACCAACCAT Reverse: GCTCACCATTGAAGCCCACT	HM543574.1
PpCHI	Forward: GAGTCAAAAATCAAGGTGGAGAA Reverse: AAAGAGCCCAAAACCATACAAC	JQ717266.1

Table 1 continued

Gene	Primer sequence (5′-3′)	GenBank ID
PpCHS	Forward: TTAGCCCTGAAGCCTGAGAA Reverse: TGGTCCAAACCCAAACAGC	HM543568.1
PpCCR	Forward: CCATCAACGCCAGCATCAT Reverse: TTTCCACCACATCTCCACGA	AY012685.1
PpF3H	Forward: ATTGTGGAGGCTTGTGAGGA Reverse: ATGGCTGGAGACGATGAAAC	HM543570.1
PpDFR	Forward: TGTTCCATGTCGCCACTCCTAT Reverse: ATCGCTCCAGTCGGTTTCGT	HM543571.1
PpPOD	Forward: CTCCCTTGACCGCCTTATTT Reverse: CACTGAGCCTGCCCTATTGTA	JN791440.1
PpCCoAOMT5	Forward: GTCTTCACTGGTTACTCCCTCC Reverse: ACGACCCAAGTCATAGTTTTCC	XM_007201175.2
Ppactin	Forward: GCCATTCAGGCTGTTCTTTC Reverse: CGGACAATTTCCCGTTCA	AB046952.1

Notes: PL. pectate lyase; PG. polygalacturonase; PME. pectin methylesterase; CHI. chaleone isomerase; CHS. chaleone synthase; CCR. cinnamoyl-CoA reductase; F3H. flavanone 3-hydroxylase; DFR. dihydroflavonol-4-reductase; POD. peroxidase; CCoAOMT. caffeoyl-CoA *O*-methyltransferase.

1.3.4 qPCR analysis

qPCR analysis was done using LightCycle 96 referring to the approach of Zhu Jie et al.[33]. Easy Dilution Kit was utilized to dilute the synthesized cDNA for five times. Then, the reaction was done referring to the SuperReal PreMix Plus protocol (SYBR Green) using a three-step amplification. The procedure was as follows: denaturation at 95 ℃ for 7 s, annealing at anneal temperature for 30 s, and extension at 72 ℃ for 15 s with 40 cycles. Gene expression was figured out by utilizing $2^{-\Delta\Delta Ct}$ compared with the *Ppactin*. Each gene was analyzed using six biological replicates.

1.4 Statistical analysis

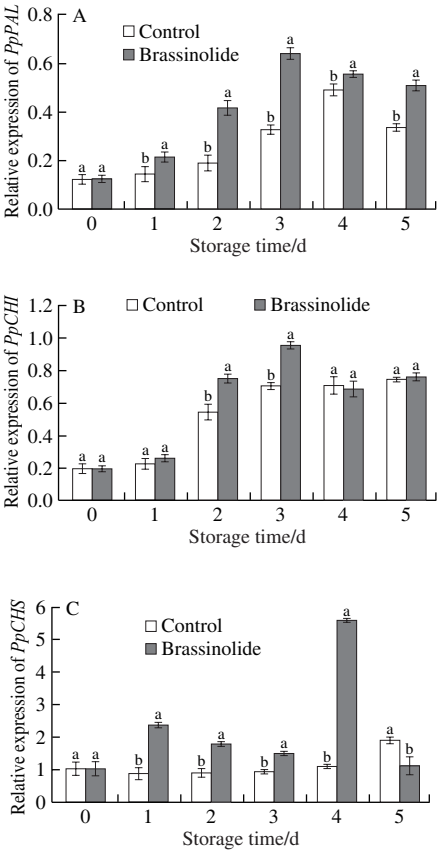
All statistical analyses were performed by SPSS 19.0 and Excel 2010 software. Triplicates data of the control and brassinolide-treated groups were subjected to a one-way analysis of variance (ANOVA) and mean separations were compared by the least significant difference (LSD) test at level $P < 0.05$. Correlation analysis was conducted by Origin 2021 software, and expressed as Pearson's correlation coefficient and presented as a heat map.

2 Results and Analysis

2.1 Expression of the gene involved in phenylpropanoid metabolism

2.1.1 *PpPAL*, *PpCHI* and *PpCHS*

Control fruit showed an increasing trend in *PpPAL* expression between days 0–4 but decreased afterwards (Fig. 1A). While, *PpPAL* expression in the brassinolide-dipped sample increased between days 0–3 but decreased between days 3–5. Moreover, brassinolide improved *PpPAL* expression along the storage time, and the maximum value was recorded on day 3 as 1.96-fold higher than that in untreated group. *PpCHI* expression in control presented a continuous increasing trend along the storage period (Fig. 1B). While, *PpCHI* expression increased between days 0–3 and days 4–5 with a decreasing trend between days 3–4 in the fruit treated with brassinolide. Additionally, the maximum level of *PpCHI* expression was measured on day 3 as 1.35-fold higher in brassinolide-dipped sample than that in control. *PpCHS* expression significantly increased in control fruit on the fifth day after storage (Fig. 1C). In contrast, *PpCHS* expression increased on days 0–1 and decreased in the following period except day 4 in brassinolide-dipped sample. In the meantime, the greatest difference was measured on day 4 as 5.08-fold higher in the fruit with brassinolide treatment than without one.



Each column value and bar represents mean and standard error. Different letters represent significant difference between control and brassinolide treatment based on LSD on the same day ($P < 0.05$). The peach fruit was storage at (21 ± 1) ℃ and (80 ± 5)% relative humidity. The same as Figs. 2–4.

Fig. 1 Expression of *PpPAL* (A), *PpCHI* (B) and *PpCHS* (C) in peach fruits after brassinolide treatment

2.1.2 *PpCCR*, *PpF3H* and *PpDFR*

Both control and brassinolide-dipped samples showed an increasing trend in *PpCCR* expression between days 0–3 and a slight decreasing trend between days 3–5 (Fig. 2A). Moreover, brassinolide improved *PpCCR* expression along the storage time. The maximum level of *PpCCR* expression was recorded on day 3 as 1.25-fold higher in the fruit with brassinolide treatment than without. *PpF3H* expression in brassinolide-dipped group presented an increasing trend between days 0–1 and days 3–5, but a decreasing trend between days 1–3. While in control it exhibited an irregular trend, especially presented a rapid increasing on day 5 (Fig. 2B). The greatest difference was determined on day 5 as 4.89-fold higher in control than that in brassinolide-dipped fruit. *PpDFR* expression slightly increased between days 0–1 and decreased between days 1–2, and increased again between days 3–5 in brassinolide-dipped group. While, *PpDFR* expression remained basically stable between days 0–4, followed by a dramatic increase in the following period (days 4–5) in control (Fig. 2C). Furthermore, the greatest difference was recorded on day 5, being 1.46-fold higher in control than that in brassinolide-dipped group.

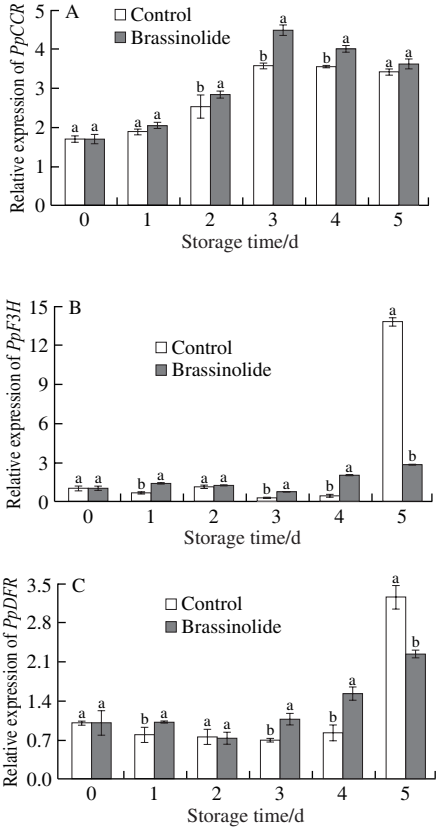


Fig. 2 Expression of *PpCCR* (A), *PpF3H* (B) and *PpDFR* (C) in peach fruits after brassinolide treatment

2.1.3 *PpPOD* and *PpCCoAOMT5*

PpPOD expression in brassinolide-dipped fruit increased between days 0–3, but declined afterwards; whereas in control it showed a slowly increasing trend along the storage period (Fig. 3A). Moreover, brassinolide significantly promoted *PpPOD* expression on days 1–4 and the maximum value was recorded on day 3 as 2.5-fold higher in the fruit with brassinolide treatment than without. *PpCCoAOMT5* expression in control decreased in the first 3 d of storage, and decreased thereafter; whereas the brassinolide-dipped fruit manifested an irregular trend on days 0–5 (Fig. 3B). Furthermore, brassinolide increased *PpCCoAOMT5* expression during the entire storage time. The maximum level of *PpCCoAOMT5* expression was measured on day 5, which was 1.69-fold higher in the fruit with brassinolide treatment than without.

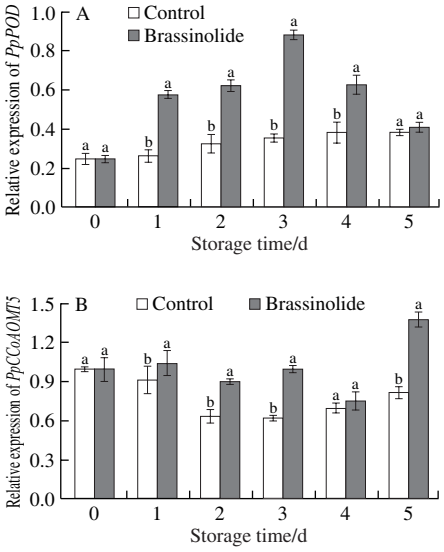


Fig. 3 Expression of *PpPOD* (A) and *PpCCoAOMT5* (B) in peach fruits after brassinolide treatment

2.2 Expression of the gene involved in cell wall degradation

PpPL1 expression increased between days 0–2, but declined between days 2–5 in both the fruit with brassinolide treatment and without (Fig. 4A). Brassinolide suppressed *PpPL1* expression along the storage time, and the maximum value was recorded on day 2 as 1.11-fold higher in the fruit without brassinolide treatment than with. However, the maximum difference between the fruit without brassinolide treatment and with was recorded on day 3, being 1.34-fold higher in control than that in brassinolide-dipped fruit. *PpPG21* expression showed an increasing trend between days 0–4 and a decreasing trend between days 4–5 in both groups (Fig. 4B). Moreover, brassinolide suppressed

PpPG21 expression in the fruit between days 1–5. On day 4 of storage, control fruit showed remarkably higher (1.18-fold) *PpPG21* expression than the brassinolide-dipped sample. *PpPME1* expression increased between days 0–2 and days 3–4, but dropped off between days 2–3 and days 4–5 in the fruit with and without brassinolide treatment (Fig. 4C). Moreover, brassinolide inhibited *PpPME1* expression along the storage period except day 5.

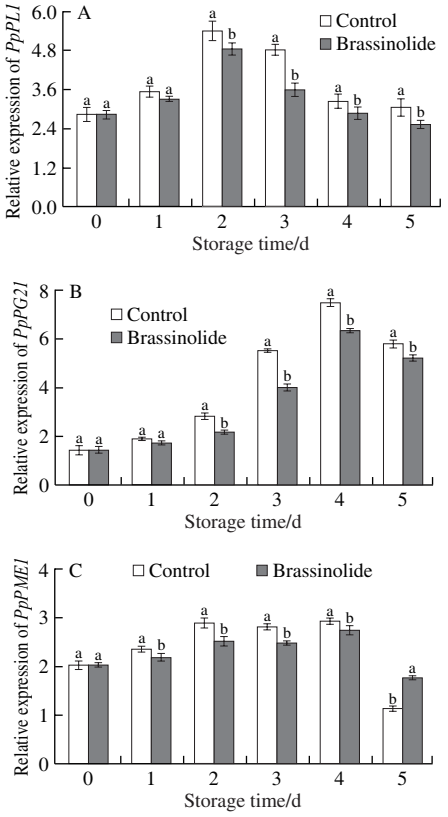
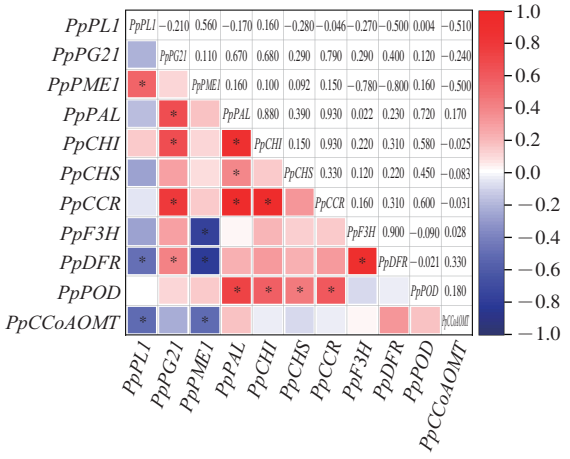


Fig. 4 Expression of *PpPL1* (A), *PpPG21* (B) and *PpPME1* (C) in peach fruits after brassinolide treatment

2.3 Correlation analysis

Correlation coefficients between all the selected parameters were calculated (Fig. 5). *PpPL1* expression has a significant positive correlation with *PpPME1*, whereas significant negatively correlated with *PpDFR* and *PpCCoAOMT5* in peaches. *PpPG21* expression was significantly and positively correlated with *PpPAL*, *PpCHI*, *PpCCR* and *PpDFR* in peaches. Similarly, *PpPME1* expression has a significant negative correlation with *PpF3H*, *PpDFR* and *PpCCoAOMT5* in peaches. Moreover, *PpPOD*, *PpPAL*, *PpCHI*, *PpCHS* and *PpCCR* expressions were significant positively correlated each other in peaches.



Each square in different color indicate the correlation coefficient. The red (+) and blue (−) colors represent positive and negative correlations, respectively. ∗. Significant correlation ($P < 0.05$).

Fig. 5 Pearson's correlation coefficients between cell wall degradation and phenylpropanoid metabolism in peach fruits after brassinolide treatment

3 Discussion

Softening, one of the main characteristics for fruit ripening and senescence, is largely determined by the disassembly of cell wall components, such as pectin substances, semi-cellulose and cellulose[34-35]. PL, PG and PME play crucial roles in the regulation of fruit ripening and postharvest softening[36-37]. Our previous studies have shown that brassionolide could inhibit the increase of weight loss and respiratory intensity and maintain flesh firmness, thereby inhibiting fruit softening and delaying senescence during storage[5,32]. This study demonstrated that brassinolide dipping treatment significantly suppressed the expressions of *PpPL1*, *PpPG21* and *PpPME1* in peaches. PL degrades pectin into a 4,5-unsaturated oligopoly galacturonic acid and a short-chain pectin molecule through *β*-elimination mechanism[38]. While, PG and PME are considered as the main hydrolysis enzymes acting on pectin in the fruit[37]. Moreover, PME catalyzes the demethylation of polygalacturonic acid, converts pectin methyl acid into pectin acid, and renders the cell wall susceptible to decomposition by PG[39]. PG catalyzes the rupture of the 1,4-2-*D*-galactoside bond of pectin acid, resulting in the cleavage of pectin to oligogalacturonic acid or galacturonic acid, and the disintegration of the cell wall[34]. Similarly, hydrogen sulfide treatment inhibited PG and PME activities and reduced the decrease of water insoluble pectin, soluble hemicellulose and cellulose, thus inhibiting cell wall degradation and maintaining fruit hardness of peaches[13].

Li Canying et al.[28] also reported that methyl jasmonate effectively delayed the increase of cell wall degrading enzyme activity, improved disease resistance and delayed fruit softening in peach fruit. Some studies have shown that the decrease of *PL* expression significantly delayed fruit softening in tomatoes and strawberries[40-41]. Additionally, *PpPG* expression is highly correlated with the softening of peaches during low temperature storage[42]. Similarly, temperature-induced the expressions of *PpPME* and *PpPG* to control the development of softening and quality deterioration in peaches[39]. Brassionolide also enhanced chilling tolerance and delay softening of banana fruit through mediating the activities of antioxidative enzymes and phenolic compounds contents[19]. Altogether, brassinolide inhibited the degradation of pectin substances and maintained the stability of the cell wall structure by mediating the key gene expression in cell wall metabolism.

Phenylpropanoid pathway is a major secondary metabolic pathway, which plays an important role in fruit and vegetable against stresses[30,43]. PAL is the rate-limiting enzyme in this pathway in which phenylalanine is transformed into 4-coumaroyl CoA to enter anthocyanin and lignin biosynthesis pathway[44]. In plants, CHS, CHI, F3H and DFR are crucial structural genes for the production of chalcone, naringenin and dihydrokaempferol, then catalyzed by DFR to synthesize anthocyanin[45-46]. We previously found that postharvest brassinolide treatment improved PAL and 4-coumarate/coenzyme A ligase (4CL) activities in peaches[21]. This study further demonstrated that brassinolide treatment up-regulated *PpPAL* expression, enhanced *PpCHI*, *PpCHS*, *PpF3H* and *PpDFR* expressions at the earlier storage, but suppressed their expressions at the end of storage in peaches. A recent study found that 1-MCP treatment up-regulated the anthocyanin biosynthesis genes expressions, and inhibited softening in postharvest peaches[11]. Similar research manifested that 1-MCP treatment affected the color change, stimulated the expressions of *PpPAL*, *PpCHS*, *PpF3H* and *PpDFR*, promoted the biosynthesis of flavonoids and stability of anthocyanin in postharvest peaches[3]. These evidences imply that brassinolide might regulate the expression of the anthocyanin biosynthesis gene to promote fruit coloration and scavenge excessive free radicals to delay senescence of fruit, thus maintaining storage ability. At the later stage of storage, fruit color was stable, and the expressions of the related genes with anthocyanin biosynthesis were down-regulated to inhibit browning and delay senescence of peaches.

Plants respond to biological and abiotic stresses and strengthen the cell wall structures by synthesizing and depositing lignin or lignin phenolic polymers on the cell walls[47]. CCR and CCoAOMT are the key enzymes in the synthesis of lignin monomers and play pivotal roles in the methylation step of the lignin monomer pathway[27,48]. In addition, POD plays a protective role in plant cells by regulating the synthesis of lignin and increasing the thickness of the secondary wall, which indirectly affects cell wall related degrading enzymes[49]. Previous study has demonstrated that brassinolide induced resistance to *Penicillium expansum* of peaches through increasing the accumulation of phenolic compounds, flavonoids and lignin[21]. This study further evaluated the transcriptional level of the genes involved in this pathway and found that brassinolide treatment significantly enhanced the transcription levels of *PpPOD*, *PpCCR* and *PpCCoAOMT5* in peaches, suggesting the pivotal role of the phenylpropanoid pathway in promoting the effective accumulation of lignin to maintain the cell wall structure. Ji Nana et al.[30] reported that methyl jasmonate activated the key enzyme activities and gene expressions of the phenylpropanoid pathway, resulting in lignin accumulation. Similarly, *P. membranaefaciens* enhanced the antioxidant capacity of peaches, stimulated flavonoids and lignin accumulation, and delayed the occurrence of fruit softening[50]. Moreover, based on the results of correlation analysis, it can be seen that overall cell wall degradation showed a negative correlation with phenylpropanoid pathway. This may be due to the fact that inhibition of cell wall degradation can maintain flesh firmness, whereas promotion of phenylpropanoid metabolism can produce secondary metabolites to strengthen the cell wall structure and improve resistance, thus delaying peach fruit softening. The findings prove our previous studies, which found that brassinolide increased antioxidant capacity, enhanced resistance and maintained peach fruit quality[5,21,32]. Overall, these results reveal that brassinolide could accumulate sufficient secondary metabolites for deposition in the cell wall by activating the phenylpropanoid metabolic pathway, whereas inhibiting the expression of genes related to cell wall degradation to suppress the breakdown of cell wall components, thereby delaying softening and increasing resistance of peaches.

4 Conclusion

Brassinolide treatment down-regulated *PpPL1*, *PpPG21* and *PpPME1* expressions to suppress cell wall degrading

of peaches. Moreover, brassinolide up-regulated the gene expressions of *PpPAL*, *PpCHI*, *PpCHS*, *PpF3H*, *PpDFR*, *PpPOD*, *PpCCR* and *PpCCoAOMT5* in phenylpropanoid pathway to enhance storage ability of peaches. Altogether, brassinolide treatment can be used as an effective and feasible technique to delay softening and induce resistance of peaches during storage.

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