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ORIGINAL PAPER

The Role of Glycogen Synthase Kinase-3 in Gibberellic Acid-Induced Chilling Tolerance and Defense Response in Postharvest Peach Fruit

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Abstract

The induction of chilling tolerance and defense response by glycogen synthase kinase-3 (GSK-3) under gibberellic acid (GA_3) treatment in peach fruit was explored at 4 °C up to 28 days. The fruits were treated with exogenous GA_3 and bikinin (GSK-3 inhibitor). Results showed that exogenous GA_3 alleviated chilling injury, and upregulated endogenous GA_3 content in peach fruit. Furthermore, *GSK-3* expression was activated by exogenous GA_3 treatment. GA_3 also upregulated gene expression of superoxide dismutase, peroxidase, catalase, glutathione reductase, glutathione S-transferase, and ascorbate peroxidase. Additionally, GA_3 enhanced gene and protein expression of small ubiquitin-like modifier and gene expression of methionine sulfoxide reductase, and weakened gene expression of lipoxygenase and phospholipase D. These above impacts stimulated by exogenous GA_3 were blocked by the addition of bikinin. Overall, GSK-3 was involved in stimulation of chilling tolerance and defense response under GA_3 treatment in postharvest peach fruit.

Keywords GA_3 · GSK-3 · Antioxidant system · Small ubiquitin-like modifier and methionine sulfoxide reductase · Lipoxygenase and phospholipase D · Peach fruit

Introduction

Refrigeration is a commonly used storage technology to extend the storage life and to inhibit decay of fruits. Peach fruit is recommended to be stored at − 1 to 0 °C (depending on the variety). Nevertheless, it has been widely reported that the temperature range of 2–7 °C induce chilling injury in peach fruit (Crisosto and Mitchell 2002; Lurie and Crisosto 2005). The most common visual symptoms of CI of harvested peach fruit are internal browning and flesh mealiness, thereby shortening postharvest life and reducing consumer acceptance (Lurie and Crisosto 2005). These chilling disorders resulted in the reduction of consumer acceptance and increase in postharvest losses of the fruit. Therefore, potential approaches to alleviate chilling injury are in need for its storage and transportation.

The phytohormones regulate many physiological processes of plant growth and development (Ding et al. 2013). Accordingly, gibberellic acid (GA_3) treatment effectively enhanced chilling tolerance in tomato (Zhu et al. 2016) and peach (Dagar et al. 2012; Gang et al. 2015; Pegoraro et al. 2015; Weksler et al. 2012) fruit. However, activation of endogenous signal compounds has been shown to be one of the mechanisms involved in chilling tolerance in postharvest fruit (Ruan et al. 2015). It is necessary to explore the downstream messengers involved in GA_3-alleviated CI in postharvest fruit. It has reported that glycogen synthase kinase-3 (GSK-3), a critical signal compound, mediated isoflavone biosynthesis in soybean sprouts in response to UV-B exposure (Jiao et al. 2017). Nevertheless, little is available in the existing reports concerning the mediation of GA_3-alleviated CI by GSK-3 in peach fruit.

Highlights
- GA_3 alleviated CI in peach fruit.
- GA_3 induced GSK-3 pathway.
- GSK-3 was involved in GA_3-enhanced antioxidant system.
- GSK-3 was involved in GA_3-enhanced *SUMO* and *MSR* expression.
- GSK-3 was involved in GA_3-weakened *LOX* and *PLD* expression.

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Oxidative damage has been considered as an early response in sensitive plant tissues to CI (Ding et al. 2016). Plants elevate levels of both antioxidant enzymes and non-enzymatic antioxidants to detoxification of free radical (Santa-Cruz et al. 2014). It has been reported that GSK-3 regulated NF-E2-related factor (Nrf2) antioxidant response in JFH1 HCV-infected Huh7.5.1 hepatocytes (Jiang et al. 2015). This suggested that GSK-3 may be involved in antioxidant system in animals. Nevertheless, the role of GSK-3 in antioxidant system in peach fruit under GA_3 treatment is not available in the literature. In addition, accordingly, small ubiquitin-like protein modifiers (SUMO) proteases regulated ROS production in Arabidopsis (Conti et al. 2007). A conserved functional SUMO interacting motif (SIM) in the gibberellin receptor has been identified in cereal crops (Nelis et al. 2015). Little attention is received about the mediation of the induction of *SUMO* by GSK-3 under GA_3 treatment in peach fruit.

Furthermore, the ROS generation during plant growth could result in lipid peroxidation, which may ultimately affect cell membrane permeability. Lipoxygenase (LOX) and phospholipase D (PLD) are shown to be the vital enzymes leading to membrane lipid degradation in plants (Yao et al. 2018). Apart from lipid peroxidation, excess ROS generation also resulted in the conversion of methionine (Met) residue to Met sulfoxide (MetO). The methionine sulfoxide reductase (MSR) could revert S- and R-diastereoisomers of MetO into Met (the reduced form), thereby protecting proteins against oxidative stress-caused damage. The *MSR* could be induced constantly by abscisic acid in tomato (Dai and Wang 2010). Nevertheless, little is available in regard to the effects of GSK-3 on LOX, PLD and MSR under GA_3 treatment in peach fruit.

Based on the above reports, little is available concerning the mediation by endogenous signal transduction of GA_3-alleviated CI in peach fruit. Therefore, the aims of the research were to investigate the regulation of the CI index and gene expression for enzymes participated in antioxidant system, *SUMO*, *LOX*, *PLD*, and *MSR* by GSK-3 under GA_3 treatment, and to explore the signal transduction mechanism in postharvest peach fruit.

Materials and Methods

Fruit Materials and Postharvest Treatments

Peach fruits (*P. persica* Batsch cv. Jinqiuhongmi) were harvested at commercial maturity from a local orchard in Beijing, China. The peaches were chosen for uniformity without any damage and randomly divided into three groups:

(1) Control group (CK): The fruit were immersed in sterile deionized water.
(2) GA_3: The fruit were immersed in 0.1 mM GA_3.
(3) GA_3+ bikinin (GSK-3 inhibitor): The fruit were immersed in 0.1 mM GA_3 plus 10 μM bikinin.

The concentration of GA_3 and bikinin was acquired according to our preliminary experiments (Supplemental Fig. 1). The fruit of each group were immersed for 10 min, and were dried in air for 40 min at room temperature afterwards. Subsequently, all three groups were stored at 4 °C and 80% relative humidity for 28 days. During storage, twenty fruit from each treatment were taken at 7 day intervals for determinations. Each assay was conducted three times. The fruit material were kept at − 80 °C for further analysis.

CI Index

During cold storage, CI index was determined after 0, 7, 14, 21, and 28 d, respectively. CI intensity was scored by the extent of internal browning symptom: 0 = no damage, 1 = superficial damage (damage < 5%), 2 = moderate damage (damage 6%–25%), 3 = severe damage (damage 26% to − 50%), 4 = very severe damage (damage > 50%). The CI index was calculated according to the following formula: CI index = Σ [(CI scale) × number of fruits at that CI)] / (5 × total number of fruit in each sample) × 100%.

Determination of GA_3 Content

A mass of 2.0 g fresh sample was extracted by 10 mL of 0.1% acetic acid in acetonitrile, which was shaken for 1 min. Subsequently, 4 g of anhydrous magnesium sulfate was added, followed by being shaken for 1 min, and was then centrifuged at 8000 rpm for 5 min. The GA_3 content in postharvest peach fruit was analyzed using high-performance liquid chromatography (HPLC) described by the method of Zhu et al. (2016). A Waters XBridge™ C18 reversed-phase column (2.1 × 150 mm, with a 5.0-μm particle size) was used, and the column temperature was maintained at 30 °C. The injection volume was 5 μL. The mobile phases consisted of 0.5 % (v/v) acetic acid (A) and methanol (B). The column was developed with stepwise linear gradient elution according to the following schedule: 0–2 min, 20% B; 3 min, 90% B; and 6.5–8 min, 20% B at a flow rate of 5 μL s^{-1}. The results were expressed as nanograms of GA_3 per gram of FW.

Gene Expression

Total RNA from 3 batches of peach fruit was obtained by an E.Z.N.A.™ Plant RNA Kit (Omega, Norcross, GA, USA; R6827-01) as described by instructions. The first-strand cDNA was obtained according to Zhu et al. (2016). cDNA sequences of the enzymes were obtained from NCBI database, and the primers designed and employed

in our research are exhibited in Table 1. Quantitative real-time PCR (qRT-PCR) assay was conducted using the SYBR®Premix Ex Taq™ (TAKARA: RR420A) in the ABI 7500 sequence detection system as described by instructions (Applied Biosystems, Foster City, CA, USA). The PCR conditions were 1 cycle of 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s, and then 63 °C for 25 s. Each assay was conducted with 3 biological replicates.

Western Blot

The peach samples at the end of storage (28 d) were ground with RIPA lysis buffer containing cocktail (a protease inhibitor). The mixture was then centrifuged to collect the tissue lysates. Western blot assay was conducted according to Jiao et al. (2017).

Statistical Analyses

All the data were shown as the mean ± standard deviation (SD). The assay of significant difference was conducted using the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) via the standard ANOVA and Duncan test.

Results

Effects of Exogenous GA_3 on CI in Peach Fruit at 4 °C up to 28 Days

CI symptoms in peach fruit appeared after 7 days, which elevated during the whole cold storage at 4 °C (Fig. 1). The CI index was effectively reduced by exogenous GA_3. At the end of cold storage, compared with the control, the CI index in peach fruit under GA_3 treatment decreased by 26%. However, the CI index significantly increased by bikinin (GSK-3 inhibitor) treatment under GA_3 treatment. At the end of storage, compared with GA_3-treated peach fruit, the CI index in bikinin-treated peach fruit increased by 15%.

Effects of Exogenous GA_3 on Endogenous GA_3 Content in Peach Fruit at 4 °C up to 28 Days

During the whole cold storage at 4 °C, endogenous GA_3 content gradually decreased and increased in control and exogenous GA_3-treated peach fruit, respectively (Fig. 2). Compared with the control, exogenous GA_3 upregulated endogenous GA_3 content. At the end of cold storage, the endogenous GA_3 content in peach fruit under GA_3 treatment was 1.7 times higher than that of the control.

Table 1 The primers in qRT-PCR assay

Gene	Accession number	Primer name	Primer sequences (5′ → 3′)
PpSOD	XM_007218235.2	Sense	CCAGAAGCACCACCAGAC
		Ant-sense	GACCTCCGCCATTGAACT
PpPOD	AJ583529	Sense	CCGACAACACTGAATACCGC
		Ant-sense	TTTGGAAGCCTGGACCCTG
PpCAT	AJ496419.1	Sense	TTGACTTCTTCTCCCACC
		Ant-sense	TTTCCCTGCCTTACTGAT
PpAPX	XM_007205713.2	Sense	GGGAAGGTGCCACAAGGA
		Ant-sense	AAGAGGGCGGAAGACAGG
PpGR	XM_007199710.2	Sense	GAATTGCAACGCCTCACTGG
		Ant-sense	TTCAGGAATGAAGGGTCGCC
PpGST	XM_007223887.2	Sense	AAGACCAGGGAGTGATAAGG
		Ant-sense	TGGGCAAGTGGGACAGAT
PpSUMO	XM_007207123.1	Sense	CAGCAACACTCAGCCACA
		Ant-sense	CTCCATTCCCAAATCAGC
PpLOX	EF568783.1	Sense	ACATGTTGCAAGCCTTTGAGG
		Ant-sense	CGGTCCAACTCGGTTCTTCA
PpPLD	EU925810.1	Sense	AACCCACGATGAAGAAAC
		Ant-sense	AGACCACCAACGAAACTC
PpMSR	XM_007209566.2	Sense	GGTTCGGGTCCAGTTTGA
		Ant-sense	TCCACTTGCTTTGCCTCC
β-actin	XM_007211382.2	Sense	GTTATTCTTCATCGGCGTCTTCG
		Ant-sense	CTTCACCATTCCAGTTCCAT TGTC

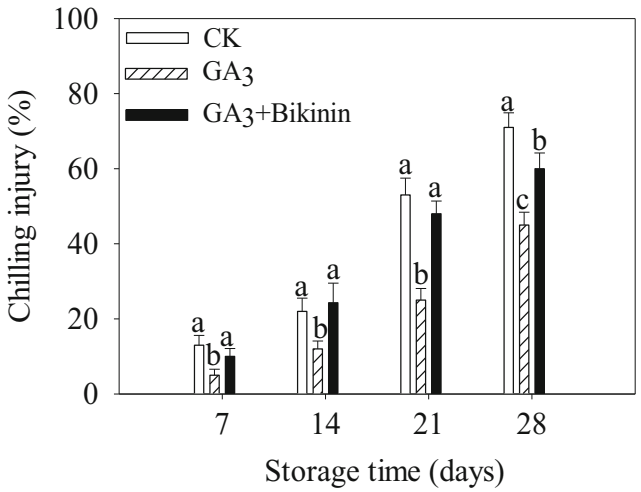

Fig. 1 Effects of gibberellic acid (GA_3) on chilling injury in peach fruit at 4 °C up to 28 days. Values are the mean ± SD (standard deviation). Values not sharing the same letter are significantly different at $p < 0.05$

Effects of Exogenous GA_3 on Gene Expression of *GSK-3* at 4 °C up to 28 Days

In general, during cold storage at 4 °C, the gene expression of *GSK-3* was enhanced at the early stages, and was weakened afterwards (Fig. 3). During 14 d of storage, compared with the control, exogenous GA_3 upregulated *GSK-3* expression. The highest *GSK-3* expression in GA_3-treated peach fruit was obtained on day 7, which was 2 times higher than that of the control. At the end of storage, there was no statistical difference in the *GSK-3* expression between control and GA_3 treatments. However, during the whole cold storage, except on day 21, bikinin inhibited the *GSK-3* expression under GA_3 treatment. At the end of storage, compared with GA_3-treated peach fruit, the *GSK-3* expression in bikinin-treated peach fruit decreased by 37%.

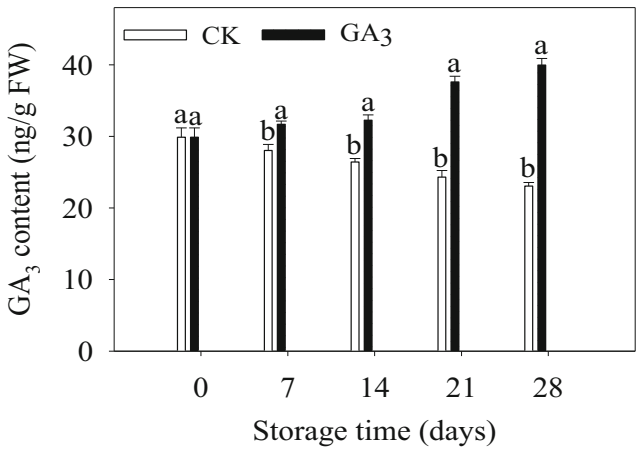

Fig. 2 Effects of GA_3 on endogenous GA_3 content in peach fruit at 4 °C up to 28 days. Values are the mean ± SD (standard deviation). Values not sharing the same letter are significantly different at $p < 0.05$

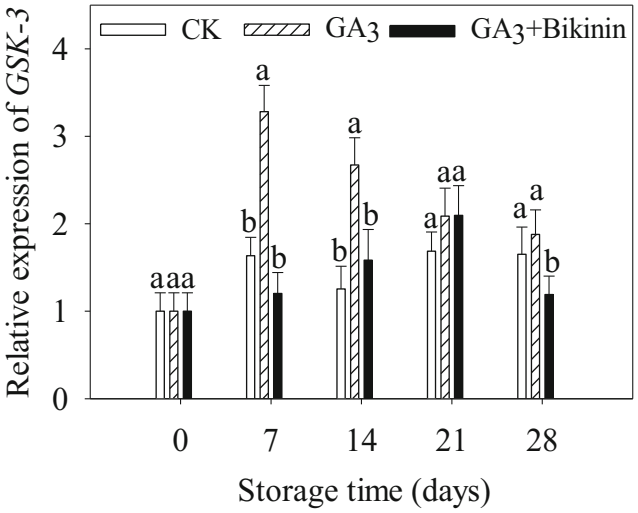

Fig. 3 Effects of GA_3 on gene expression of glycogen synthase kinase-3 in peach fruit at 4 °C up to 28 days. Values are the mean ± SD (standard deviation). Values not sharing the same letter are significantly different at $p < 0.05$

Effects of Exogenous GA_3 on Gene Expression of *SOD*, *POD*, *CAT*, *APX*, *GR*, and *GST* at 4 °C up to 28 Days

During the whole cold storage at 4 °C, the gene expression of *SOD*, *POD*, *CAT*, *APX*, *GR*, and *GST* enzymes showed the similar trends as the *GSK-3* expression (Fig. 4). Compared with the control, exogenous GA_3 upregulated gene expression of *SOD*, *POD*, *CAT*, *APX*, *GR*, and *GST*. The highest gene expression of *SOD*, *POD*, and *GR* under GA_3 treatment was obtained on day 14, which was 2.6, 1.4, and 2.1 times higher than that of the control, respectively. The highest gene expression of *CAT*, *APX*, and *GST* under GA_3 treatment was obtained on day 21, which was 1.4, 1.6, and 5.2 times higher than that of the control, respectively. At the end of storage, the gene expression of *SOD*, *POD*, *CAT*, *APX*, *GR*, and *GST* in GA_3-treated peach fruit was 1.9, 3.1, 4.0, 1.6, 1.3, and 1.2 times higher than that of the control, respectively. However, during the whole storage, the gene expression of *SOD*, *POD*, *CAT*, *APX*, *GR*, and *GST* under GA_3 treatment was weakened by bikinin. At the end of storage, compared with GA_3-treated peach fruit, the gene expression of *POD*, *CAT*, *GR*, and *GST* in bikinin-treated peach fruit decreased by 30%, 81%, 68%, 23%, 68% and 27%, respectively, and there were no statistical differences in the gene expression of *SOD* and *APX* between GA_3 and GA_3 plus bikinin treatments.

Effects of Exogenous GA_3 on Gene and Protein Expression of *SUMO* at 4 °C up to 28 Days

During the whole storage at 4 °C, the gene expression of *SUMO* in GA_3-treated peach fruit showed the similar trends as the *GSK-3* expression, while except on day 21, the gene expression of *SUMO* in control continuously increased (Fig. 5). Compared

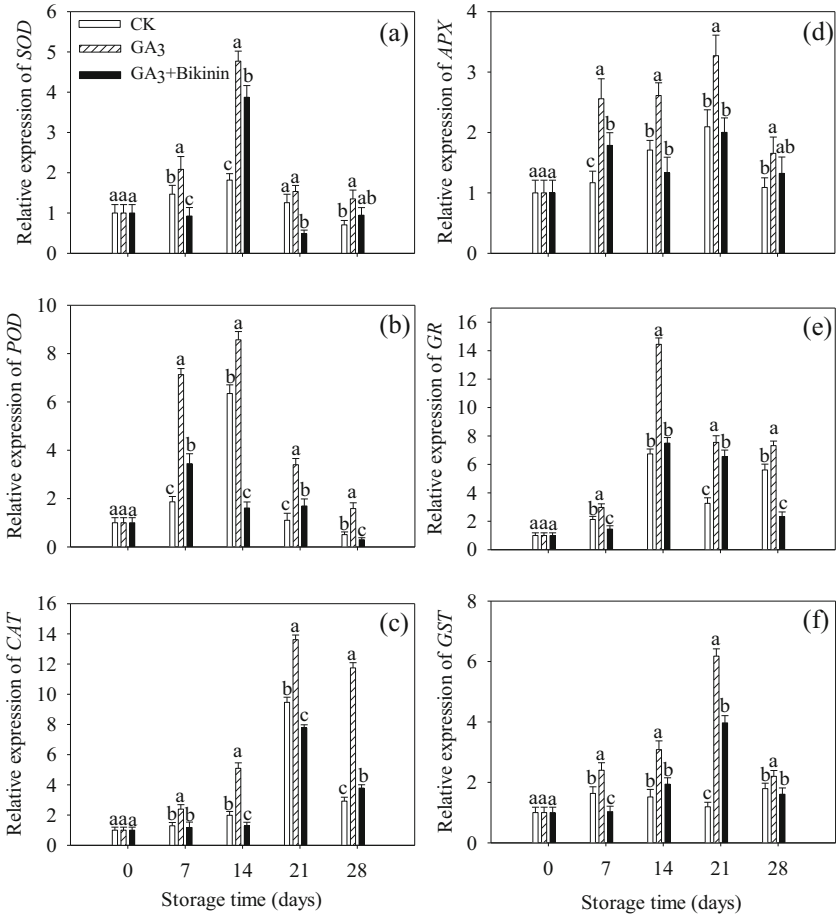

Fig. 4 Effects of bikinin on gene expression of superoxide dismutase (A), peroxidase (B), catalase (C), ascorbate peroxidase (D), glutathione S-transferase (E) and glutathione reductase (F) under GA_3 treatment in peach fruit at 4 °C up to 28 days. Values are the mean ± SD (standard deviation). Values not sharing the same letter are significantly different at $p < 0.05$

with the control, exogenous GA_3 enhanced the gene expression of *SUMO*. The highest gene expression of *SUMO* in GA_3-treated peach fruit was obtained on day 14, which was 3.1 times higher than that of the control. At the end of cold storage, the gene expression of *SUMO* in peach fruit under GA_3 treatment was 1.1 times higher than that of the control. However, during the whole storage, bikinin weakened the *SUMO* expression under GA_3 treatment. At the end of storage, compared with GA_3-treated peach fruit, the *SUMO* expression in bikinin--treated peach fruit decreased by 25% (Fig. 5A).

Also, the protein expression of SUMO was upregulated by exogenous GA_3. At the end of storage, the protein expression of SUMO in GA_3-treated peach fruit was 1.7 times of the control. However, the protein expression of SUMO under GA_3 treatment was weakened by bikinin. At the end of storage, compared with GA_3-treated peach fruit, the protein expression of SUMO decreased by 35% in bikinin-treated peach fruit (Fig. 5B and C).

Effects of Exogenous GA_3 on Gene Expression of *LOX* and *PLD* at 4 °C up to 28 Days

During the whole cold storage at 4 °C, the gene expression of *LOX* and *PLD* exhibited the similar trends as the *GSK-3* expression (Fig. 6). Compared with the control, exogenous GA_3 weakened the gene expression of *LOX* and *PLD*. The highest gene expression of *LOX* and *PLD* in GA_3-treated peach fruit was obtained on days 21 and 14, which decreased by 20% and 75%, respectively compared with the control. At the end of cold storage, the gene expression of *LOX* and *PLD* in peach fruit under GA_3 treatment decreased by 35% and 57%, respectively compared with the control (Fig. 6A and B). However, during the whole storage, bikinin blocked the GA_3 treatment-inhibited gene expression of *LOX* and *PLD*. At the end of storage, compared with GA_3-treated peach fruit, the gene expression of *LOX* and *PLD* in bikinin-treated peach fruit increased by 36% and 75%, respectively (Fig. 6A and B).

Effects of Exogenous GA_3 on Gene Expression of *MSR* at 4 °C up to 28 Days

During the whole storage at 4 °C, the gene expression of *MSR* both in control and GA_3-treated peach fruit continuously increased (Fig. 6C). Compared with the control, exogenous GA_3 enhanced the gene expression of *MSR*. At the end of cold storage, the gene expression of *MSR* in peach fruit under GA_3 treatment was 1.4 times higher than that of the control. However, during the whole storage, bikinin weakened the

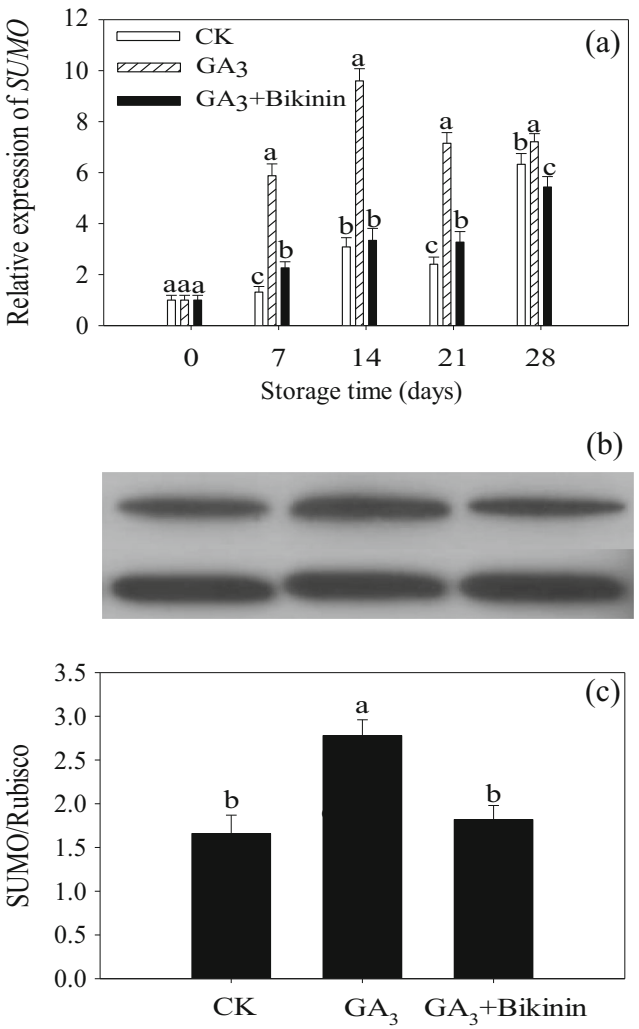

Fig. 5 Effects of bikinin on gene (A) and protein (B and C) expression of small ubiquitin-like modifier under GA_3 treatment in peach fruit at 4 °C up to 28 days. Panels show representative bands (B). Histograms represent relative protein levels of small ubiquitin-like modifier (C) normalized to the corresponding rubisco. Values are the mean ± SD (standard deviation). Values not sharing the same letter are significantly different at $p < 0.05$

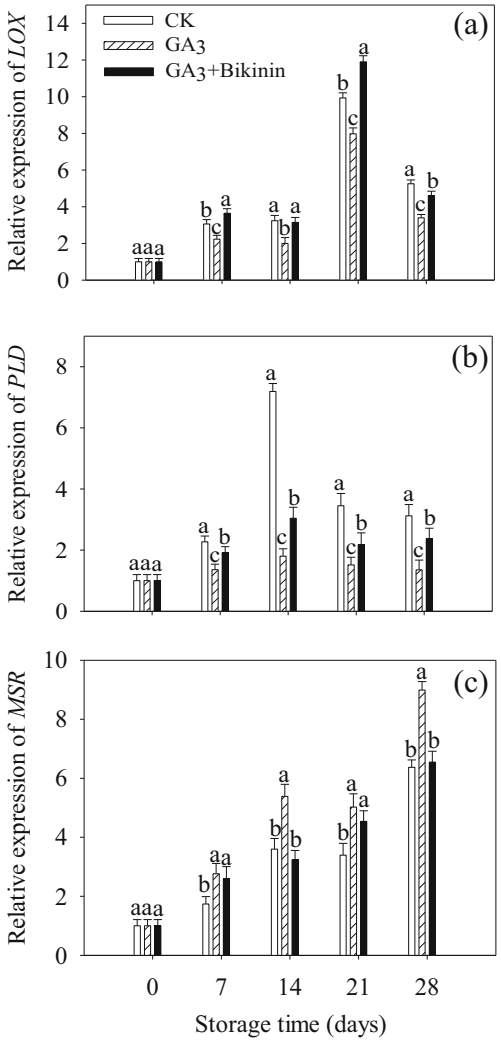

Fig. 6 Effects of bikinin on gene expression of lipoxygenase (A), phospholipase D (B) and methionine sulfoxide reductase (C) under GA_3 treatment in peach fruit at 4 °C up to 28 days. Values are the mean ± SD (standard deviation). Values not sharing the same letter are significantly different at $p < 0.05$

MSR expression under GA_3 treatment. At the end of storage, compared with GA_3-treated peach fruit, the *MSR* expression in bikinin-treated peach fruit decreased by 27% (Fig. 6C).

Discussion

Our results demonstrated that during storage, GA_3 treatment significantly reduced CI (Fig. 1), and upregulated endogenous GA_3 content (Fig. 2) in peach fruit. It suggested that GA_3 treatment could be used as a possible method that alleviated CI in peach fruit.

The ROS production in plants can interfere cellular redox equilibrium, hence leading to oxidative damage (Shi et al. 2007). Redox homeostasis is important for alleviating the damage caused by oxidative stress in plants (Chaves and Oliveira 2004). To counterbalance ROS generation, the ROS scavenging systems were activated in plants. It turned out that during cold storage, GA_3 treatment up regulated the gene expression of *SOD* in peach fruit (Fig. 4A). SOD has been identified as the first line of defense against ROS, which generates H_2O_2 and oxygen by scavenging superoxide radicals (Apel and Hirt 2004). In addition, the gene expression of *POD* and *CAT* also increased by GA_3 treatment (Fig. 4B and C). The joint effects of SOD, POD and CAT are necessary for alleviating oxidative stress-caused damage as a result of their complementary roles in cell metabolism (Benavides et al. 2005). To regulate the levels of ROS, an ascorbate-glutathione pathway also exists in plants. GA_3 treatment also activated *APX* expression (Fig. 4D). APX is the primary enzyme that participates in ROS detoxification in chloroplasts and the cytosol of

plant cells. Further, the *GR* expression was stimulated by GA_3 (Fig. 4E). GR, as an enzyme in the glutathione pathway, could reduce oxidized glutathione (GSSG) to reduced glutathione (GSH). GA_3 also induced the *GST* expression (Fig. 4F), another enzyme in the glutathione cycle and metabolism. Additionally, the increase in gene expression of *SOD*, *POD*, *CAT*, *APX*, *GR*, and *GST* triggered by GA_3 was weakened by bikinin (GSK-3 inhibitor) in different degrees (Fig. 4). These data demonstrated that GSK-3 was involved in GA_3 treatment-induced antioxidant system enhancement in peach fruit. GA_3 treatment, an external signal, could not directly induce defense responses in plants, instead of triggering its downstream effectors like GSK-3 (Fig. 3). Then GSK-3 transduced the GA_3 signal to defense reactions (Eilert 1987) like antioxidant system enhancement through a modulation of transcripts for enzymes involved in antioxidant system (Fig. 4). Ca^{2+} possibly mediated the antioxidant system enhancement by GSK-3. Accordingly, *AtCBL1* and *AtCP1*, as two kinds of the Ca^{2+} sensor family called AtCBLs, could be activated upon the overexpression of *AtGSK1* in transgenic Arabidopsis. AtCBL1 was considered to be a Ca^{2+} sensor involved in stress signal transmission (Kudla et al. 1999), and AtCP1 was identified as a small Ca^{2+}-binding protein with EF hands (Jang et al. 1998). Both of the proteins interact with CBL-interacting protein kinases (CIPKs) (Shi et al. 1999). Hence, GSK-3 acts as an effective mediator of Ca^{2+} mobilization in plant. Ca^{2+} could elevate total antioxidant capacity and DPPH* scavenging capacity (Yang et al. 2016). Thus, mediation of antioxidant system enhancement by GSK-3 might be via a Ca^{2+}-mediated signal pathway in peach fruit under GA_3 treatment. In addition, the gene and protein expression of SUMO also increased by GA_3 treatment (Fig. 5). Moreover, it turned out that GA_3-regulated expression of *SUMO* was mediated by GSK-3 (Fig. 5). SUMO, a covalent protein modification, could be attached to key protein targets to regulate their activity, thus playing an important role in cell signal transduction (Conti et al. 2007). Accordingly, SUMO proteases regulated ROS production in Arabidopsis (Conti et al. 2007). SUMO conjugation caused inhibition of NADPH oxidase, thereby decreasing in intracellular ROS production in HEK293 and HeLa cells (Kim et al. 2011). In addition, SUMO modification facilitated human SOD1 stability and aggregation (Fei et al. 2006). Hence, SUMO may be involved in GSK-3-induced antioxidant system enhancement under GA_3 treatment.

ROS generated in plants may lead to lipid peroxidation, which damages membrane integrity. LOX and PLD have been considered to be critical enzymes participated in membrane lipid degradation in plants. LOX degrades polyunsaturated fatty acids, destroying the bilayer of phospholipids. PLD leads to the peroxidation of membrane phospholipids. GA_3 treatment could weaken the gene expression of *LOX* and *PLD* (Fig. 6A and B), elevating unsaturated fatty acids level and maintaining membrane integrity and consequently alleviating CI in peach fruit. Our results further showed that GA_3-regulated gene expression of *LOX* and *PLD* was mediated by GSK-3 (Fig. 6A and B). Ca^{2+} possibly modulates LOX (Shi et al. 2002) and PLD association with membranes (Zheng et al. 2000) in plants. Hence, Ca^{2+} may mediate GSK-3-modulated LOX and PLD. Apart from membrane lipid degradation and peroxidation, ROS production also resulted in the oxidation of Met residue to MetO, damaging the structure and function of some proteins. GA_3 treatment induced the *MSR* expression in peach fruit (Fig. 6C). MSR could catalyze the reduction of MetO to Met, consequently protecting proteins against oxidative stress-caused damage. Moreover, our results showed that GA_3-regulated gene expression of *MSR* was mediated by GSK-3 (Fig. 6C). Excess ROS could result in transient oxidation of CaM, downregulating energy metabolism and the further ROS generation through respiratory control mechanisms, which allows for rapid restoration of protein function through repair of oxidized Met by MSR (Bigelow and Squier 2005). Therefore, oxidation of CaM may be a vital regulator for GSK-3-activated MSR under GA_3 treatment.

Conclusions

Exogenous GA_3 treatment protected peach fruit against CI, and elevated endogenous GA_3 content. The *GSK-3* expression was enhanced by GA_3 treatment. GA_3 also stimulated *SOD*, *POD*, *CAT*, *GR*, *GST*, and *APX* expression. Additionally, the elevation of gene and protein expression of *SUMO* and gene expression of *MSR* and the inhibition of gene expression of *LOX* and *PLD* were in response to GA_3 treatment. However, bikinin, a GSK-3 inhibitor, weakened the above GA_3-triggered impacts. Overall, GSK-3 was involved in stimulation of chilling tolerance and defense response under GA_3 treatment in postharvest peach fruit. However, further work is still required to reveal the complex molecular networks regulated by GSK-3 under GA_3 treatment in response to chilling stress.

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