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Effect of salicylic acid on freezing injury in peach floral organs and the expressions of *CBF* genes

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Abstract

We used flowering branches at the budding stage of two peach cultivars Xiahui 6 and Xiacui with different cold resistance to explore the effect of exogenous salicylic acid (SA) on the freezing injury of peach floral organs and the molecular mechanism. Using water application as the negative control, the effects of spraying with SA at concentrations of 20 or 100 mg dm^{-3} on stigma receptivity, frost damage characteristics of floral organs, and the expressions of *C-repeat-binding factor* (*PpCBF*) gene family members were investigated at 0 °C. No significant frost damage was observed on petals in all treatments. No frost damage was seen in the ovary and style under 20 mg dm^{-3} SA treatment, but damage was substantial at the other two treatments. Cultivar Xiahui 6 was more susceptible to freezing than cv. Xiacui. The expression peaks of *PpCBF*s in the SA-pretreated floral organs occurred at 3 or 6 h after low temperature treatment, and peak time was closely related to peach cultivar, organ, and SA concentration. This indicates that appropriate concentration of exogenous SA may alleviate freezing damage to floral organs and enhance cold resistance by the regulated expression pattern of *PpCBF*.

Additional key words: ovary, petals, *Prunus persica*, stigma, style.

Introduction

Low temperature is a key limiting factor affecting plant survival, distribution, growth, development, and production (Liu *et al*. 2012, 2016). In recent years, cold injury has often occurred during cold invasion in early spring in many countries (Chu *et al*. 2010, Hufkens *et al*. 2012). Plants growing in the field often face the danger of freezing damage, and this is a major cause of crop loss. The stresses of late spring and rapid temperature changes cause various types of injury directly and indirectly associated with the freezing of water in plant tissues (Burke *et al*. 1976). Plant physiological characteristics are strongly affected by cold stress (Huang and Guo 2005). When facing freezing stress, many plants show notable changes in membrane lipid composition (Uemura and Steponkus 1994), in accumulation of proline (Wanner and Junttila 1999) and soluble sugars (Carpenter *et al*. 1986), and in activities of antioxidant enzymes (Janda *et al*. 2003). Cold acclimation results in, and is governed by, a distinct set of changes in gene expression (Thomashow *et al*. 2001, Chinnusamy *et al*. 2006, Wisniewski *et al*. 2011).

Salicylic acid (SA) is a common signal molecule responsible for inducing resistance to a number of biotic and abiotic stresses (Métraux *et al*. 1990, Karlidag *et al*. 2009, Guo *et al*. 2013). It is involved in establishing the local and systemic disease resistance after pathogen attack (Kachroo *et al*. 2005). The synthesis of many pathogenesis-related proteins involved in systemic acquired resistance in plants is also induced by SA application (Ward *et al*. 1991, Wang *et al*. 2006). It has been reported that many defence responses are induced by SA (Kang and Saltveit 2002), *e.g.*, SA can prevent lipid peroxidation in membranes under cold stress (Senaratna *et al*. 2000, Promyou *et al*. 2012) as well as it

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Abbreviations: CBF - C-repeat-binding factor; COR - cold responsive; LT - low temperature; RT-qPCR - reverse transcriptase quantitative polymerase chain reaction; SA - salicylic acid.

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can induce an increase in hydrogen peroxide (H_2O_2) content in plant tissues (Janda *et al*. 2003).

In recent years, researchers have increasingly paid attention to the role of cold-regulated genes in cold tolerance. A number of studies have shown that the C-repeat-binding factor (CBF) cold response pathway is the best suited to understanding the cold tolerance induction (Fowler and Thomashow 2002, Van Buskirk and Thomashow 2006). The CBF transcription factors are a part of the Apetala2-ethylene responsive factor (AP2/ERF) domain family of DNA-binding proteins that recognize a C-repeat response *cis*-acting element that regulates a number of cold-responsive (*COR*) genes (Artlip *et al*. 2013). They are involved in the induction of many *COR* genes through the binding of specific elements known as C-repeat/dehydration-responsive elements (CRT/DREs) present in their promoters (Chinnusamy *et al*. 2007). The *CBFs* regulate expression of many genes involved in osmolyte biosynthesis, detoxification of reactive oxygen species, membrane transport, and hormone metabolism. Karimi *et al*. (2015) reported that *CBF* gene expression of two *Vitis vinifera* cultivars increases at the beginning of cold stress and then decreases. Yuasa *et al*. (2014) revealed that cold stress upregulates a tomato *CBF* homolog. In *Arabidopsis thaliana*, the SA-accumulating lines *siz1* and *acd6*, which have a dwarf phenotype, showed that sensitivity to cold stress is associated with increased endogenous SA accumulation and decreased expression of *DREB1A*/*CBF3* and its regulon genes (Miura and Ohta 2010). Transcription of peach *CBF*s during postharvest cold storage was studied by Liang *et al*. (2013).

Peach buds have low resistance to freezing conditions. Their floral organs, such as the petals, stigma, and ovary, are susceptible to freezing injury. There is a lack of knowledge on the relationships between *CBF* expression and cold tolerance of peach buds under low temperature. In addition, although the effect of SA on peach petals has been tested, other floral organs have been rarely investigated. Because the stigma and ovary are especially important for pollination and fertilization, the effect of SA on cold injury of all peach floral organs should be systematically studied. Therefore, in this study, the effect of SA on peach buds under low temperature was investigated to test the possible effect of SA on cold injury.

Materials and methods

Plants and experimental conditions: Eight-year-old peach trees (*Prunus persica* L. cv. Xiahui 6 with low cold tolerance and cv. Xiacui with relatively high cold tolerance), growing at the Experimental Orchard of the Jiangsu Academy of Agricultural Sciences, P.R. China, were used. The experiment was conducted during the peach blooming season in spring 2015. Solutions of salicylic acid (SA, *Sigma-Aldrich*, St Louis, MO, USA) at 0.0 (control), 20, and 100 mg dm^{-3} were sprayed on branches once the budding stage commenced. The spraying ceased as soon as the liquid commenced dripping off the trees. The SA solutions were allowed to be absorbed for 2 h. Then, the sprayed branches were removed from the tree. Peach branches of length of 50 - 60 cm were collected and inserted into the same SA solutions for another 2 h. Then, the branches were transferred to a phytotron set at 0 °C. After 0, 3, and 6 h, images of floral organs (petal, stigma, and ovary) were taken. Then, organs were sampled, dipped in liquid nitrogen and stored at -70 °C. Of each treatment, 60 buds were chosen and divided into three replications (20 buds for each replication).

Stigma receptivity observation: Stigma receptivity was tested at each defined time using the benzidine-H_2O_2 method (Dafni 1992). A fresh peach stigma was placed on a slide and submerged by a drop of benzidine-H_2O_2 solution [1 % (v/v) benzidine + 3 % (v/v) H_2O_2 + water; 4:11:22], and then examined under a stereomicroscope (*SZX7*, *Olympus*, Tokyo, Japan). Three fresh stigmas were used for stigma receptivity observations for each treatment, with three replicates.

Real-time reverse transcriptase quantitative polymerase chain reaction: Total RNA was isolated with a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Chang *et al*. 1993). The RNA (0.5 μg) was used for the synthesis of the first stand cDNA with a *Prime Script* reverse transcriptase (RT) reagent kit (*TaKaRa Bio*, Kyoto, Japan). The RT-qPCR analysis was performed using a *My-IQ 2* (*Bio-Rad*, Hercules, CA, USA) and *SYBR Premix Ex Taq*™ (*TaKaRa Bio*). The mixture (volume 20.0 mm^3) contained 2.0 mm^3 of diluted cDNA, 0.4 mm^3 of each primer, 10.0 mm^3 of *Master Mix*, and 7.2 mm^3 of double distilled water. Thermo-cycling conditions were: an initial polymerase activation step at 95 °C for 2 min , followed by 40 cycles at 95 °C for 15 s (template denaturation), at 60 °C for 15 s (annealing), and at 72 °C for 20 s (extension and fluorescence measurement). The primers *PpCBF1-PpCBF5* were designed by Liang *et al*. (2013), and *PpCBF6* by Wisniewski *et al*. (2011) (Table 1). The reference gene *β-actin* was the internal control (Table 1). The gene expression was normalized to the internal reference gene in three independent biological replicates, each with three technical replicates. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical analysis: The experiment was arranged as a completely randomized design with three replications. All samples were analyzed at least three times. Data were statistically analyzed by *ANOVA* using the *SPSS*-17 software (*SPSS Inc*., Chicago, IL, USA), and the data from the different treatments were compared using Duncan's Multiple Range Test at 5 % probability.

Results

Both peach cultivars exhibited the strongest stigma receptivity at the beginning of the experiment (0 h), with no significant difference between them (Table 2 and Fig. 1*A*,*B*). After 3 h of low temperature (LT) treatment, the strongest stigma receptivity in both cultivars was observed under 20 mg dm^{-3} SA, then followed by 100 mg dm^{-3} SA treatment and control. After 6 h of LT treatment, both cultivars showed strong stigma receptivity

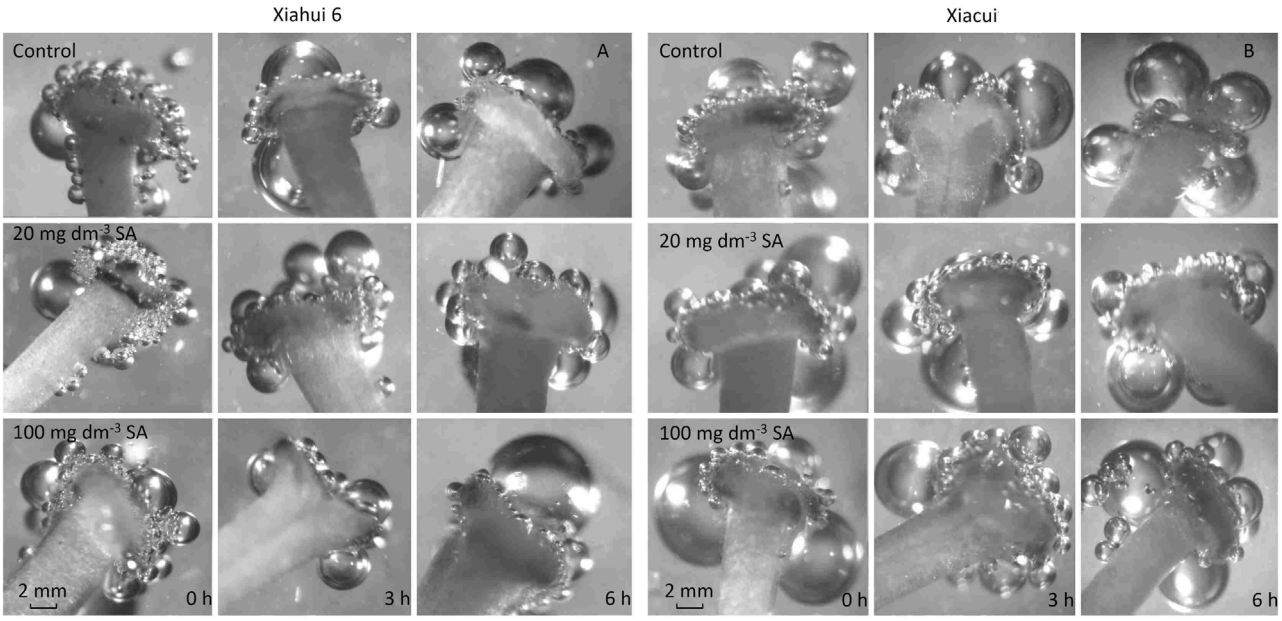

Fig. 1. The stigma receptivity observations of peach floral organs under pre-treatments with 0, 20, and 100 mg dm^{-3} salicylic acid (SA) and cold stress (0 °C) for 0, 3, and 6 h. *A* - cv. Xiahui 6, *B* - cv. Xiacui.

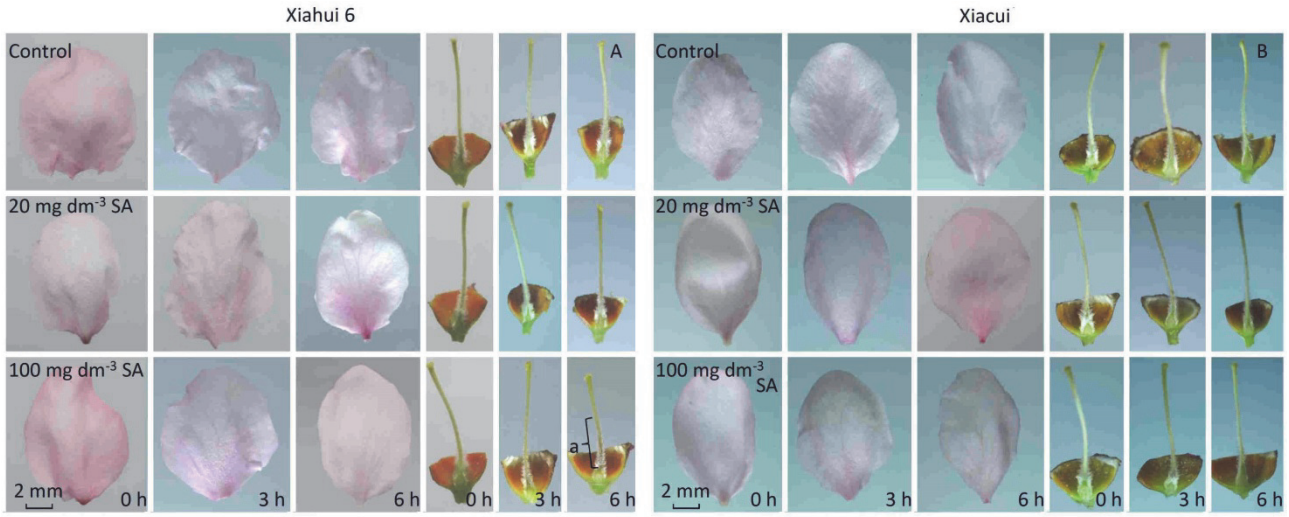

Fig. 2. Effect of 0, 20, and 100 mg dm^{-3} salicylic acid (SA) application on morphological changes in peach floral organs under cold stress (0 °C) for 0, 3, and 6 h. *A* - cv. Xiahui 6, *B* - cv. Xiacui (a shows the mid-bottom part of the style).

again at 20 mg dm^{-3} SA treatment, followed by 100 mg dm^{3} SA, but the control Xiahui 6 lost stigma receptivity, whereas the control Xiacui showed weak receptivity. This suggests that 20 mg dm^{-3} SA maintained the highest stigma receptivity at low temperature.

Low temperature did not significantly affect the petal appearance of Xiahui 6 under various treatments (Fig. 2*A*). The longitudinal section of the ovary showed mild symptoms of frost damage after 6 h of LT treatment. The mid-bottom part of the style (Fig. 2*A-a*) treated with 100 mg dm^{-3} SA turned dark brown after 3 h, and this was more pronounced after 6 h. No obvious symptoms of frost damage were observed in the ovary under 20 mg dm^{-3} SA treatment, and the style only exhibited mild damage shown by a darker color.

Similarly to Xiahui 6, the petals of Xiacui showed no obvious frost damage under low temperature (Fig. 2*B*). No substantial frost damage was observed in 20 mg dm^{-3}

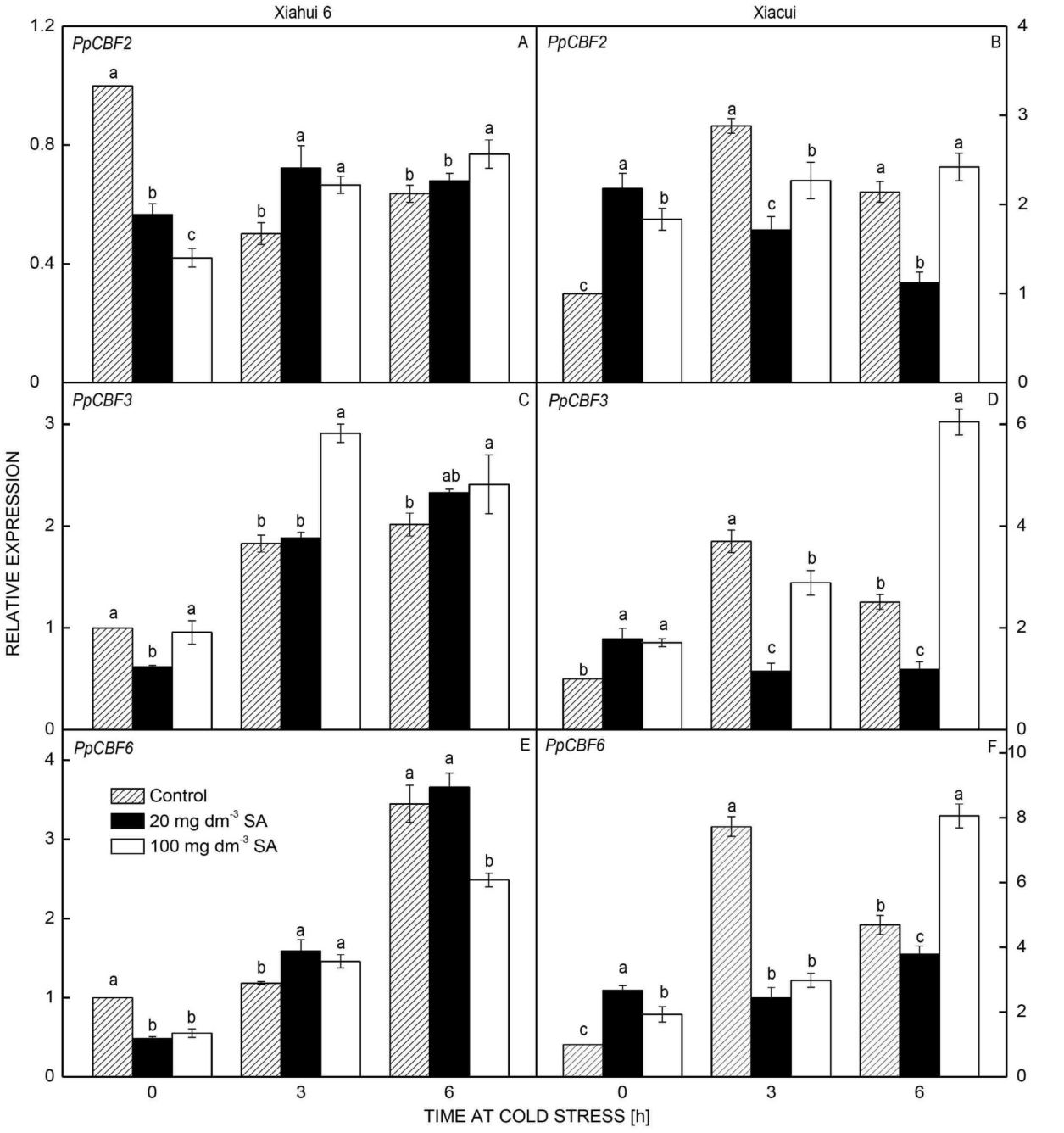

Fig. 3. Effect of SA application on relative expression of *CBFs* genes in peach petals under cold stress (0 °C) for 0, 3, and 6 h. *A*, *C,* and *E* - cv. Xiahui 6; *B*, *D,* and *F* - cv. Xiacui. Means ± SEs, $n = 3$. For each treatment time, columns with different letters indicate significantly different values at $P < 0.05$.

SA treated and control pistils, but the bottom of the style with 100 mg dm^{-3} SA treatment showed mild damage at 3 h after treatment, and the damage extended inward after 6 h as it was shown on the ovary section. Therefore, higher concentration of exogenous SA did not induce higher cold tolerance of peach floral organs.

Our study showed almost no expression of genes *PpCBF1*, *PpCBF4*, and *PpCBF5* in peach floral organs and, therefore, data for their relative expressions are not shown in this paper. The *PpCBF2* and *PpCBF6* expressions in the petals of Xiahui 6 were significantly reduced after SA spraying without exposure to LT stress. The expression of *PpCBF3* substantially decreased following treatment with 20 mg dm^{-3} SA, but no difference was observed after 100 mg dm^{-3} SA treatment, suggesting that pre-treatment of SA did not induce the *CBF* expression in Xiahui 6 petals. When the duration of the LT treatment increased, the expressions of the three

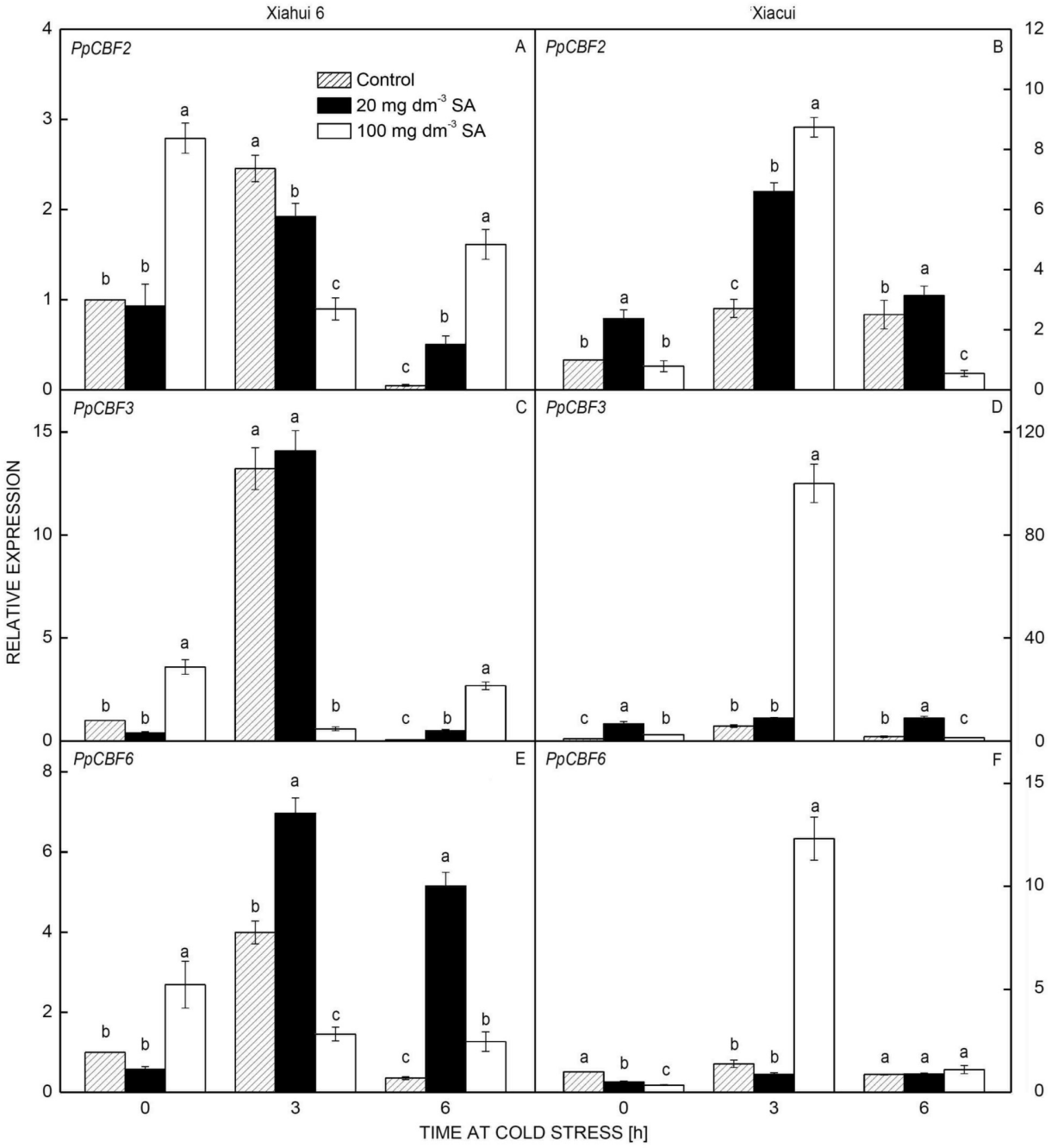

Fig. 4. Effect of SA application on relative expression of *CBFs* genes in peach stigma under cold stress (0 °C) for 0, 3, and 6 h. *A*, *C,* and *E* - cv. Xiahui 6; *B*, *D,* and *F* - cv. Xiacui. Means ± SEs, $n = 3$. For each treatment time, columns with different letters indicate significantly different values at $P < 0.05$.

CBF genes increased in all SA treatments, but *PpCBF2* expression initially decreased and then increased in plants treated with water. The *CBF* expressions showed up-regulation at most treatments, but expression of *PpCBF2* at 20 mg dm^{-3} SA and of *PpCBF3* at 100 mg dm^{-3} SA increased at first and then decreased (Fig. 3*A*,*C*,*E*).

The SA pre-treatment significantly induced *CBF* expressions in Xiacui, and the 20 mg dm^{-3} SA treatment showed the strongest induction (Fig. 3*B*,*D*,*F*). The LT treatment significantly enhanced *CBF* expression in control petals. However, in LT stress, *PpCBF2* and *PpCBF3* expressions were reduced at 20 mg dm^{-3} SA but that of *PpCBF6* increased, whereas all three genes showed an upward trend at 100 mg dm^{-3} SA. Differences in petal *CBF* expressions regulated by SA and low temperature were observed between the two cultivars (Fig. 3). For

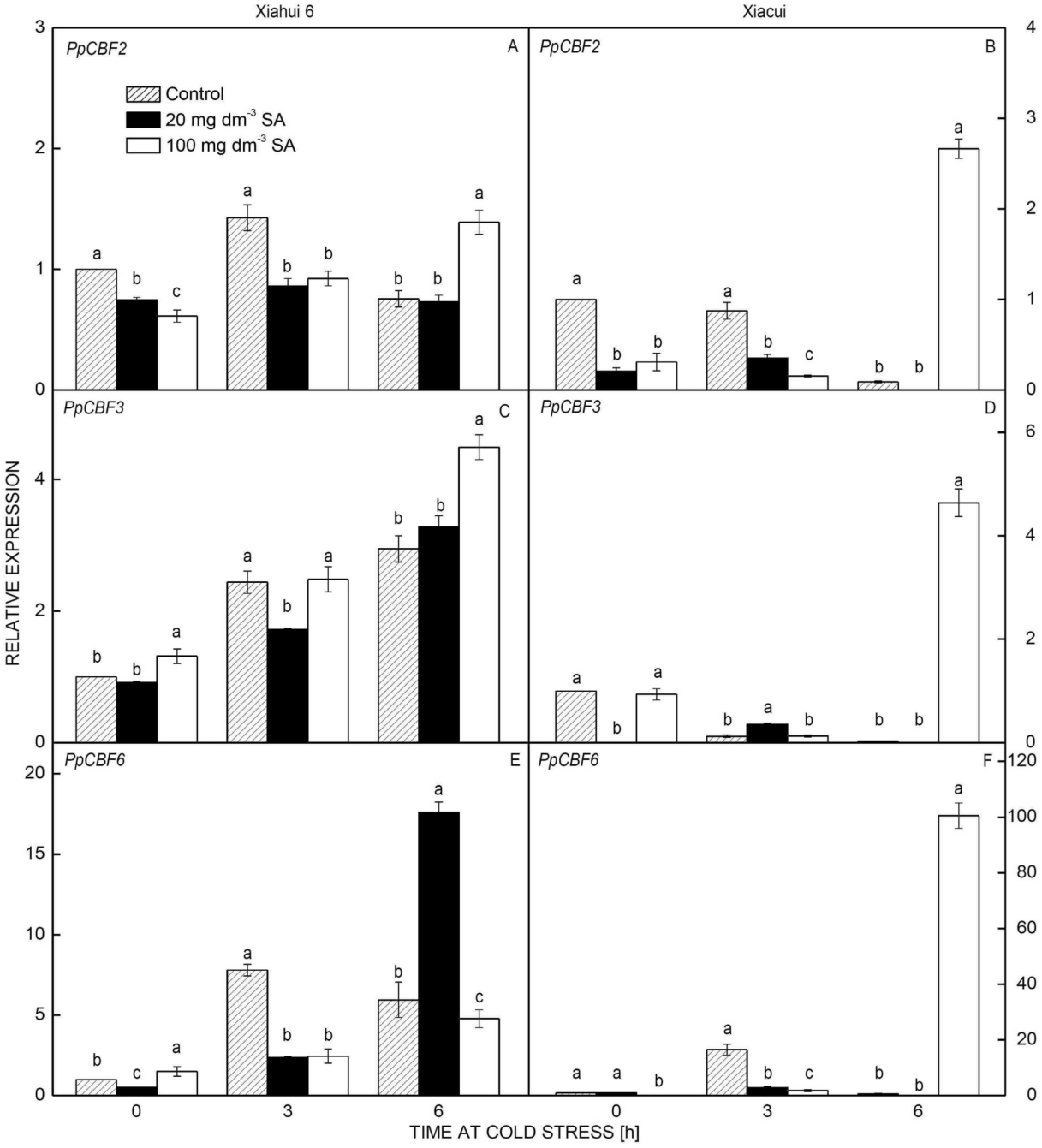

Fig. 5. Effect of SA application on relative expression of *CBFs* in peach ovary under cold stress (0 °C) for 0, 3, and 6 h. *A*, *C,* and *E* - cv. Xiahui 6; *B*, *D,* and *F* - cv. Xiacui. Means ± SEs, $n = 3$. For each treatment time, columns with different letters indicate significantly different values at $P < 0.05$.

Xiahui 6, the cold susceptible cultivar, there were no significant differences in the expressions of three *CBF* genes between control and 20 mg dm^{-3} SA treatment at 6 h after LT treatment, but expressions of *PpCBF2* and *PpCBF3* in 100 mg dm^{-3} SA treated samples were higher than the other two treatments. For Xiacui, the cold resistant cultivar, the expressions of the three *CBF* genes in 20 mg dm^{-3} SA treated samples were significantly lower than those of control, but they were higher at 100 mg dm^{-3}. The genes *PpCBF3* and *PpCBF6* in peach petal might be regulated by low temperature. When the floral organ was treated with SA and exposed to low temperature, *PpCBF6* was possibly the key gene regulated by SA.

High concentration of SA (100 mg dm^{-3}) significantly increased the expressions of three *CBF* genes in the style of Xiahui 6 at 0 h, but 20 mg dm^{-3} SA treatment showed no substantial difference from the control at this stage (Fig. 4*A*,*C,E*). The expressions of three *CBF* genes initially increased and then decreased in both 20 mg dm^{-3} SA treatment and the control. In 100 mg dm^{-3} SA treated samples, the expressions of *PpCBF2* and *PpCBF3* were reduced at first and then increased. The *PpCBF6* expression gradually decreased. At 6 h after LT stress, the maximum expressions of *PpCBF2* and *PpCBF3* were at 100 mg dm^{-3} SA, followed by 20 mg dm^{-3} SA and then the control ($P < 0.05$); however, the order for *PpCBF6* was 20 mg dm^{-3} SA > 100 mg dm^{-3} SA > control ($P < 0.05$).

The pre-treatment with 20 mg dm^{-3} SA significantly increased expressions of *PpCBF2* and *PpCBF3* in the style of Xiacui, and the highest expression of *PpCBF6* was in the control, followed by 20 and 100 mg dm^{-3} SA ($P < 0.05$) (Fig. 4*B*,*D,F*). The gene expressions were high in the 100 mg dm^{-3} SA treated samples at 3 h after LT stress.

In addition, the *PpCBF2* expression was significantly higher than that of the control. At 6 h after LT stress, the expression order of *PpCBF2* and *PpCBF3* among treatments was as follows: 20 mg dm^{-3} SA > control > 100 mg dm^{-3} SA ($P < 0.05$), while there was no substantial difference in *PpCBF6* expression among the three treatments. For all treatments, the *CBF* expression exhibited an initial increase followed by a decrease with longer duration of LT stress.

When the ovaries of the two peach cultivars were treated with SA at two different concentrations, differences in *CBF* expression were recorded. At 0 h, high SA concentration induced a significant elevation of *PpCBF3* and *PpCBF6* in the ovaries of Xiahui 6, but substantially reduced *PpCBF2* expression; 20 mg dm^{-3} SA treatment also lowered the expressions of *PpCBF2* and *PpCBF6* compared to the control (Fig. 5*A*,*C,E*). During LT treatment, *PpCBF2* and *PpCBF6* of the control and *PpCBF2* of the 20 mg dm^{-3} SA treated sample showed initial increases followed by decreases, but there was an overall upward trend for all other treatments. The highest expressions of *PpCBF2* and *PpCBF3* were found at 100 mg dm^{-3} SA treated sample and the expression of *PpCBF6* at 20 mg dm^{-3} SA treatment.

The *CBF* expression in the ovaries of Xiacui was not substantially enhanced after SA pre-treatment (Fig. 5*B*,*D,F*). During LT stress, the expressions of *PpCBF2* and *PpCBF3* were reduced, and *PpCBF6* showed an initial increase followed by a decrease in the control variant. The change trend for these three genes was similar to that for 20 mg dm^{-3} SA treatment, which initially increased, and then decreased. At 6 h after cold stress, the expressions of the three genes reached their peak at 100 mg dm^{-3} SA treatment.

Discussion

Many studies have demonstrated that appropriate concentration of exogenous SA can enhance cold tolerance of seedlings and fruits. The application of SA at concentrations of 0.5 and 1 mM increases cold tolerance of *Plukenetia volubilis* seedlings by reducing superoxide anion generation rate and H_2O_2 content and elevating the activity of antioxidant enzymes (Luo *et al*. 2014). Luo *et al*. (2011) substantiated that exogenous SA inhibits respiration and ethylene production during cold storage of plum and so fruit damage. Application of SA is commonly used in the field for preservation of fresh cut flowers (Alaey *et al*. 2011, Marandi *et al*. 2011, Promyou *et al*. 2012). Postharvest SA application prolongs vase-life in cut rose flowers by improving the reactive oxygen species scavenging capacity and by better regulation of the water balance (Alaey *et al*. 2011). In the present study, the stigmas treated with 20 mg dm^{-3} SA exhibited relatively strong receptivity under cold stress, which was maintained to some degree, even 6 h after the stress. Moreover, frost damage to the pistil was not substantial at this SA concentration, indicating strong cold tolerance. We also showed that floral damage was more severe in Xiahui 6 than Xiacui, with weak stigma receptivity of the pistil indicating that cold sensitivity of the floral organ was cultivar dependent, which is consistent with previous results (Promyou *et al*. 2012). In addition, the high concentration of SA (100 mg dm^{-3}) did not improve the cold resistance of peach floral organs, suggesting that an appropriate SA concentration was the key in relieving cold damage to plants.

Low temperature stress affects the normal transcription and translation by altering the secondary structure of RNA and DNA, resulting in physiological disfunction. The CBF regulon includes the *COR15a* gene and others that encode late embryogenesis abundant (LEA) or LEA-like hydrophilic polypeptides thought to

play roles in freezing tolerance. In *Arabidopsis thaliana*, low temperature generates a short-lived signal that induced the expression of *LEA*/*COR* and *LEA*/*COR-like* genes through a pathway independent of the CBF cold response pathway (Fowler and Thomashow 2002). In grapevine, low temperature elevates the expressions of *CBF1-CBF4* in leaves, but the increase amplitude and the timing of the highest expression vary in different cultivars. In addition, overexpression of four genes, *VvCBF2*, *VvCBF4*, *VvCBFL*, and *VvZFPL* in grapevine improves cold resistance of the fruit (Takuhara *et al*. 2011). Jaglo-Ottosen *et al*. (2001) showed that overexpression of *CBF* in *Arabidopsis* and rapeseed significantly increases tolerance to low temperature, drought, and high salinity. Constitutive overexpression of peach *CBF1* in apple results in a 4 - 6 °C increase in freezing tolerance in both the non-acclimated and acclimated states compared with untransformed apple trees (Wisniewski *et al*. 2011). For a comparison in the present study – except for *PpCBF2* in the petal of Xiahui 6 and *PpCBF2* and *PpCBF3* in ovary of Xiacui showing reductions at the early experimental stage – the expression of *CBF* genes exhibited a significant increase in all other treatments. Based on the cold damage to floral organs and the stigma receptivity (Table 2 and Figs. 1 and 2), the induced expression of *CBF* gene under cold stress only alleviated frost damage to some extent, and damage to peach floral organs was irreversible with long exposure to low temperature. In addition, relatively high expressions of *PpCBF2*, *PpCBF3* and *PpCBF6* might be the reason for the cold tolerance of the style in peach, with *PpCBF3* and *PpCBF6* responsible in the petal and *PpCBF6* in the ovary.

In some plants, no *CBF* expression is detected at room temperature, but it increases upon low temperature (Zhao *et al*. 2009). However, in the present study, *CBF* expression was detected in the petal, style, and ovary at 0 h in both peach cultivars and was enhanced under low temperature, suggesting different effects of temperature on *CBF* expression in different species.

SA and low temperature were capable of inducing *CBF* expression, but the time for the induced peak varied depending on the organ and SA concentration. In the present study, we showed the expression pattern of different *CBF* genes in peach floral organs treated with various SA concentrations upon cold treatment. For example, the expressions of *PpCBF3* and *PpCBF6* in the 20 mg dm^{-3} SA treated styles of Xiahui 6 exhibited an initial increase and then a decrease. Expressions of *PpCBF2*, *PpCBF3*, and *PpCBF6* showed an upward trend in 100 mg dm^{-3} SA treated petals of Xiacui. Previous studies indicated that there are three *CBF* homologs present in tomato, *LeCBF1*, *LeCBF2*, and *LeCBF3*, but only *LeCBF1* was induced by cold treatment (Zhang *et al*. 2004). Expression of *Arabidopsis* drought responsing *cis*-acting element 1A (*DREB1A*)/*CBF3* gene reaches its peak at 3 h after the onset of cold treatment, and gradually decreases thereafter (Miura and Ohta 2010). Zhang *et al*. (2014) confirmed that two types of *CBF* expression in apricot, constitutive and induced expression, and SA along with low temperature coordinately strengthen *CBF* induction. This is consistent with the results of the present study. This suggests that expression patterns of *CBF* genes not only differ among species but also among organs.

The present study reveals that enhancement of SA-induced freezing resistance in peach floral organs was associated with an up-regulation of *CBF* genes. The SA concentration of 20 mg dm^{-3} was the appropriate to alleviate freezing damage to peach floral organs. In addition, Xiacui had better freezing tolerance than Xiahui 6 in the budding stage.

References

Alaey, M., Babalar, M., Naderi, R., Kafi, M.: Effect of pre- and post-harvest salicylic acid treatment on physio-chemical attributes in relation to vase-life of rose cut flowers. - Postharvest Biol. Tech. **61**: 91-94, 2011.

Artlip, T.S., Wisniewski, M.E., Bassett, C.L., Norelli, J.L.: *CBF* gene expression in peach leaf and bark tissues is gated by a circadian clock. - Tree Physiol. **33**: 866-877, 2013.

Burke, M.J., Gusta, L.V., Quamme, H.A., Weiser, C.J., Li, P.H.: Freezing and injury in plants. - Annu. Rev. Plant Physiol. **27**: 507-528, 1976.

Carpenter, J.F., Hand, S.C., Crowe, L.M., Crowe, J.H.: Cryoprotection of phosphofructokinase with organic solutes: characterization of enhanced protection in the presence of divalent cations. - Arch. Biochem. Biophys. **250**: 505-512, 1986.

Chang, S., Puryear, J., Cairney, J.: A simple and efficient method for isolating RNA from pine trees. - Plant mol. Biol. Rep. **11**: 113-116, 1993.

Chinnusamy, V., Zhu, J., Zhu, J.K.: Gene regulation during cold acclimation in plants. - Physiol. Plant. **126**: 52-61, 2006.

Chinnusamy, V., Zhu, J., Zhu, J.K.: Cold stress regulation of gene expression in plants. - Trends Plant Sci. **12**: 444-451, 2007.

Chu, J.Z., Yao, X.Q., Zhang, Z.N.: Responses of wheat seedlings to exogenous selenium supply under cold stress. - Biol. Trace Element Res. **136**: 355-363, 2010.

Dafni, A.: Pollination Ecology. - Oxford University Press, New York 1992.

Fowler, S., Thomashow, M.F.: *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the *CBF* cold response pathway. - Plant Cell **14**: 1675-1690, 2002.

Guo, Q., Meng, L., Mao, P.C., Jia, Y.Q., Shi, Y.J.: Role of exogenous salicylic acid in alleviating cadmium-induced toxicity in Kentucky bluegrass. - Biochem. Syst. Ecol. **50**: 269-276, 2013.

Huang, M., Guo, Z.: Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity. -

Biol. Plant. **49**: 81-84, 2005.

Hufkens, K., Friedl, M.A., Keenan, T.F., Sonnentag, O., Bailey, A., O'Keefe, J., Richardson, A.D.: Ecological impacts of a widespread frost event following early spring leaf-out. - Global Change Biol. **18**: 2365-2377, 2012.

Jaglo-Ottosen, K.R., Kleff, S., Amundsen, K.L., Zhang, X., Haake, V., Zhang, J.Z., Deits, T., Thomashow, M.F.: Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. - Plant Physiol. **127**: 910-917, 2001.

Janda, T., Szalai, G., Rios-Gonzalez, K., Veisz, O., Páldi, E.: Comparative study of frost tolerance and antioxidant activity in cereals. - Plant Sci. **164**: 301-306, 2003.

Kachroo, P., Venugopal, S.C., Navarre, D.A., Lapchyk, L., Kachroo, A. Role of salicylic acid and fatty acid desaturation pathways in *ssi2*-mediated signaling. - Plant Physiol. **139**: 1717-1735, 2005.

Kang, H.M., Saltveit, M.E.: Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. - Physiol. Plant. **115**: 571-576, 2002.

Karimi, M., Ebadi, A., Mousavi, S.A., Salami, S.A., Zarei, A. Comparison of *CBF1*, *CBF2*, *CBF3* and *CBF4* expression in some grapevine cultivars and species under cold stress. - Sci. Hort. **197**: 521-526, 2015.

Karlidag, H., Yildirim, E., Turan, M.: Salicylic acid ameliorates the adverse effect of salt stress on strawberry. - Sci. Agr. **66**: 180-187, 2009.

Liang, L., Zhang, B., Yin, X.R., Xu, C.J., Sun, C.D., Chen, K.S.: Differential expression of the *CBF* gene family during postharvest cold storage and subsequent shelf-life of peach fruit. - Plant mol. Biol. Rep. **31**: 1358-1367, 2013.

Liu, H., Ouyang, B., Zhang, J.H., Wang, T.T., Li, H.X., Zhang, Y.Y., Yu, C.Y., Ye, Z.B.: Differential modulation of photosynthesis, signaling, and transcriptional regulation between tolerant and sensitive tomato genotypes under cold stress. - Plos ONE **7**: e50785, 2012.

Liu, J.Y., Zhu, Y., Huang, L.L., Xu, X., Li, G.Z., Hao, L.: Combined effect of ethylene- and salicylic acid-signaling insensitive mutation on *Arabidopsis* response to low temperature. - Biol. Plant. **60**: 523-531, 2016.

Livak, K.J., Schmittgen, T.D.: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. - Methods **25**: 402-408, 2001.

Luo, Y.L., Su, Z.L., Bi, T.J., Cui, X.L., Lan, Q.Y.: Salicylic acid improves chilling tolerance by affecting antioxidant enzymes and osmoregulators in sacha inchi (*Plukenetia volubilis*). - Braz. J. Bot. **37**: 357-363, 2014.

Luo, Z.S., Chen, C., Xie, J.: Effect of salicylic acid treatment on alleviating postharvest chilling injury of Qingnai plum fruit. - Postharvest Biol. Tech. **62**: 115-120, 2011.

Marandi, R.J., Hassani, A., Abdollahi, A., Hanafi, S.: Application of *Carum copticum* and *Satureja hortensis* essential oils and salicylic acid and silver thiosulfate in increasing the vase life of cut rose flower. - J. Med. Plant Res. **5**: 5034-5038, 2011.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyssbenz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., Inverardi, B.: Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. - Science **250**: 1004-1006, 1990.

Miura, K., Ohta, M.: *SIZ1*, a small ubiquitin-related modifier ligase, controls cold signaling through regulation of salicylic acid accumulation. - J. Plant Physiol. **167**: 555-560, 2010.

Promyou, S., Ketsa, S., Doorn, W.G.V.: Salicylic acid alleviates chilling injury in anthurium (*Anthurium andraeanum* L.) flowers. - Postharvest Biol. Tech. **64**: 104-110, 2012.

Senaratna, T., Touchell, D., Bunn, E., Dixon, K.: Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. - Plant Growth Regul. **30**: 157-161, 2000.

Takuhara, Y., Kobayashi, M., Suzuki, S.: Low-temperature-induced transcription factors in grapevine enhance cold tolerance in transgenic *Arabidopsis* plants. - J. Plant Physiol. **168**: 967-975, 2011.

Thomashow, M.F., Gilmour, S.J., Stockinger, E.J., Jaglo-Ottosen, K.R., Zarka, D.G.: Role of the *Arabidopsis CBF* transcriptional activators in cold acclimation. - Physiol. Plant. **112**: 171-175, 2001.

Uemura, M., Steponkus, P. L.: A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance. - Plant Physiol. **104**: 479-496, 1994.

Van Buskirk, H.A., Thomashow, M.F.: *Arabidopsis* transcription factors regulating cold acclimation. - Physiol. Plant. **126**: 72-80, 2006.

Wang, L., Chen, S., Kong, W., Li, S., Archbold, D.D.: Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. - Postharvest Biol. Tech. **41**: 244-251, 2006.

Wanner, L.A., Junttila, O.: Cold-induced freezing tolerance in *Arabidopsis*. - Plant Physiol. **120**: 391-400, 1999.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahlgoy, P., Metraux, J.P., Ryals, J.A.: Coordinate gene activity in response to agents that induce systemic acquired resistance. - Plant Cell **3**: 1085-1094, 1991.

Wisniewski, M., Norelli, J., Bassett, C., Artlip, T., Macarisin, D.: Ectopic expression of a novel peach (*Prunus persica*) *CBF* transcription factor in apple (*Malus* × *domestica*) results in short-day induced dormancy and increased cold hardiness. - Planta **233**: 971-983, 2011.

Yuasa, T., Nakamura, J., Ishibashi, Y., Iwayainoue, M.: Tomato inducer of *CBF* expression 1 (*SlICE1*) is involved in cold and salt stress signaling. - Amer. J. exp. Agr. **4**: 785-796, 2014.

Zhang, J.H., Wang, Y.Z., Sun, H.Y., Yang, L., Jiang, F.C.: Effects of exogenous salicylic acid on antioxidant enzymes and *CBF* transcription factor in apricot (*Prunus armeniaca* L.) flowers under chilling stress. - Plant Physiol. J. **50**: 171-177, 2014.

Zhang, X., Fowler, S.G., Cheng, H., Lou, Y., Rhee, S.Y., Stockinger, E.J., Thomashow, M.F.: Freezing-sensitive tomato has a functional *CBF* cold response pathway, but a *CBF* regulon that differs from that of freezing-tolerant *Arabidopsis*. - Plant J. **39**: 905-919, 2004.

Zhao, D.Y., Lin, S., Bei, F., Yu, M.M., Yang, Z., Lv, S.N., Sheng, J.P.: Ethylene and cold participate in the regulation of *LeCBF1* gene expression in postharvest tomato fruits. - FEBS Lett. **583**: 3329-3334, 2009.