Effect of Exogenous Salicylic Acid on the Physiological and Biochemical Processes of *Ligustrum lucidum* during Natural Cold Acclimation

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Abstract. The evergreen *Ligustrum lucidum* (glossy privet) suffers from freezing injury in northern China, where there are short growing seasons and early fall frost events. To investigate the influence of exogenous salicylic acid (SA) application on the natural cold acclimation of glossy privet, physiological and biochemical changes in glossy privet seedlings subjected to SA treatments at four concentrations (0, 150, 250, and 350 mg·L⁻¹) were evaluated from Sept. to Dec. 2016. The optimum application concentrations were between 250 and 350 mg·L⁻¹, which led to better freezing tolerance during natural cold acclimation. The improved freezing tolerance under exogenous SA application was associated with the accumulation of chlorophyll, proline, soluble protein, and soluble sugar, and the regulations of gibberellic acid (GA) and abscisic acid (ABA). Salicylic acid treatments started a cascade of steps for advancing the cold acclimation process of glossy privet. We suggest that exogenous SA application may be used on glossy privet grown in northern China.

Glossy privet, an evergreen horticultural plant, is increasingly popular for urban greening in northern China, where broad-leaved evergreen tree species are scarce. However, freezing injury has been a major constraining factor to the sustainability and profitability of glossy privet seedling production and has caused huge economic losses, which may affect the growth, productivity, and geographical distribution of horticultural plants. The susceptibility of plants to freezing injury may be due not only to insufficient freezing tolerance, but also to the timing and rate of cold acclimation (Suojala and Lindén, 1997).

Cold acclimation, the process by which plants transit from a cold-sensitive to a cold-hardy state, is essential for the survival of woody plants growing in temperate regions (Teets et al., 1989). Cold acclimation usually develops in two stages (Arora et al., 1992). In the first stage, short days induce growth cessation, periderm formation, and certain development of freezing tolerance (Palva et al., 2002). The second stage is an increase in freezing tolerance to a maximum level in response to decreasing air temperatures (Artlip and Wisniewski, 1997). In addition to environmental cues, there is a series of physiological and biochemical responses during cold acclimation, including the modification of membrane lipid composition, synthesis of protective proteins, increase of compatible compounds, and regulation of phytohormones (Guy, 1990; Thomashow, 1999; Welling and Palva, 2008).

Salicylic acid is a growth regulator in plants and is involved in many physiological processes (Hayat et al., 2010; Miura and Tada, 2010), and plays an important role in both biotic and abiotic stress (Ananieva et al., 2004; Cao et al., 2010; Kim et al., 2013). Suitable concentrations of SA in plants can increase resistance to freezing injuries or even prevent such injuries (Hashempour et al., 2014; Shin et al., 2018; Taggin et al., 2006). Mutlu et al. (2013) reported that exogenously applied SA results in cold tolerance by enhancing antioxidant enzymes, ice nucleation activity, and the patterns of apoplastic proteins.

Hence, as a cultural practice for improving the freezing tolerance of glossy privet, this study begins to shed light on the effect of SA, which would be beneficial to the horticulture industry. Physiological and biochemical responses of field-grown glossy privet seedlings to exogenous SA treatments during cold acclimation were investigated. Our hypothesis stated that SA would advance the cold acclimation process in glossy privet seedlings via physiological and biochemical regulations that mimicked environmental cues including short day, low temperature, or both. The following questions were addressed: 1) What is the optimum SA application concentration for advancing the cold acclimation process and ultimately improving the freezing tolerance of glossy privet? 2) Does freezing tolerance correlate with the accumulation of compatible compounds in glossy privet? 3) Does freezing tolerance correlate with the regulation of endogenous hormones in glossy privet?

Materials and Methods

Plant materials and experimental procedures. In early Apr. 2016, 1-year-old glossy privet seedlings were planted in our study site, the Dongdadi Experimental Base of Beijing University of Agriculture in Beijing, China (39°48′ N, 116°28′ E). The seedlings were randomly planted at 12 plots in our study site. Each 10 m × 3 m plot contained 20 plants. The plants were placed on 0.5 m × 0.5 m centers in each plot. The soil type of our study site was cinnamon soil. From April to August, the seedlings were kept well-irrigated and protected from bacterial pathogens and weed competition. Irrigation was stopped after 31 Aug. In a preliminary experiment, early September was shown to be the optimum time for SA application, and SA concentrations more than 400 mg·L⁻¹ were observed to cause leaf damage (data not shown). Thus, the seedlings were subjected to four concentrations of SA, respectively, consisting of 0, 150, 250, and 350 mg·L⁻¹.
between 1600 and 1800 HR on 1, 11, and 21 Sept. Each treatment involved three plots (three replications). The average height of the seedlings was 63.49 cm and the average ground diameter of them was 9.62 mm. Whole seedlings were sprayed with SA solutions to runoff with a 5-L handheld sprayer, averaging a spray volume of 0.5 L/seedling. The air temperature conditions in the site during our experimental period (from Sept. to Dec. 2016) were obtained from the Dongdadi Experimental Base (Fig. 1).

**Determination of leaf freezing tolerance.** Freezing tolerance of leaves, assessed as the low temperature where 50% injury occurred (LT50), was determined monthly from 30 Sept. to 30 Dec. 2016. Ten representative seedlings under each treatment were selected and one healthy upper crown leaf on each representative seedling was selected for LT50 measurement. There were seven designated temperatures, 0, –5, –10, –15, –20, –25, and –30 °C, in our freezing test. The leaves were cooled at a rate of 5 °C·h⁻¹ until the target temperatures were reached and were maintained for 2 h at each target temperature. The LT50 was measured according to the method of Li (2000).

**Determination of biochemical parameters in the leaves.** Biochemical parameters in the leaves were determined monthly from 30 Sept. to 30 Dec. 2016. Ten representative seedlings under each treatment were selected and one healthy upper crown leaf on each representative seedling was selected for biochemical measurements. The chlorophyll content was measured according to the method of Bates et al. (1973). The soluble protein content was measured according to the method of Bradford (1976). The soluble sugar content was measured according to the method of Li (2000). The endogenous hormone GA and ABA contents were measured according to the method of Li (2000).

**Statistical analysis.** All data were analyzed using SPSS Statistics 18.0 (SPSS Inc., Chicago, IL), including a one-way analysis of variance for main effects of different treatments and a correlation analysis within cold-hardiness and biochemical parameters. All tables and figures were produced using Microsoft Word 2007 and Microsoft Excel 2007 (Microsoft Inc., Redmond, WA), respectively.

**Results**

**Freezing tolerance in the leaves.** The LT50 values of the leaves under each treatment continued to decrease during cold acclimation (Fig. 2). However, the LT50 values under 250 and 350 mg·L⁻¹ were lower than other treatments during cold acclimation. Compared with the values without SA application, the LT50 values under these two treatments were reduced by 51% to 63%, 32% to 34%, 31% to 38%, and 21% to 22% in September, October, November, and December, respectively. In December, the leaves reached their top freezing tolerance (LT50 of –18.0, –19.8, –21.9, and –21.7 °C under 0, 150, 250, and 350 mg·L⁻¹, respectively).

**Chlorophyll content in the leaves.** The chlorophyll content in the leaves under each treatment kept decreasing consistently during cold acclimation (Fig. 3A). However, the decrease rate was significantly reduced by SA application, especially under 250 and 350 mg·L⁻¹. Compared with the values without SA application, the chlorophyll contents under these two treatments were higher, respectively, by 12% to 15% in September; 42% to 46% in October; 37% to 44% in November; and 173% to 188% in December. In December, the leaves reached their minimum chlorophyll content levels (0.3, 0.7, 0.9, and 1.0 mg·g⁻¹ under 0, 150, 250, and 350 mg·L⁻¹, respectively).

**Proline content in the leaves.** The proline content in the leaves under each treatment increased and reached its maximum level in November, and then decreased (Fig. 3B). However, the proline contents under 250 and 350 mg·L⁻¹ were higher than other treatments during cold acclimation. Compared with the values under 0 mg·L⁻¹, the proline contents under these two treatments increased by 7% to 9%, 17% to 18%, 8% to 12%, and 8% to 9% in September, October, November, and December, respectively. In November, the
leaves reached their maximum proline content levels [2.6, 2.9, and 3.0 mg·g⁻¹ dry weight (DW) under 0, 250, and 350 mg·L⁻¹, respectively].

**Soluble protein content in the leaves.** The soluble protein content in the leaves under each treatment increased consistently during cold acclimation (Fig. 3C). However, the values under 250 and 350 mg·L⁻¹ were higher than other treatments in each month. Compared with the values under 0 mg·L⁻¹, the soluble protein contents under these two treatments increased by 15% to 18%, 9% to 10%, 16% to 18%, and 11% to 15% in September, October, November, and December, respectively. In December, the leaves contained their maximum soluble protein levels (294, 340, and 326 mg·g⁻¹ DW under 0, 250, and 350 mg·L⁻¹, respectively).

**Soluble sugar content in the leaves.** The soluble sugar content in the leaves under each treatment increased consistently during cold acclimation (Fig. 3D). However, the values under 250 and 350 mg·L⁻¹ were higher than other treatments after October. Compared with the values under 0 mg·L⁻¹, the soluble sugar contents under these two treatments increased by 15% to 17% and 14% to 20% in November and December, respectively. In December, the leaves contained their maximum soluble sugar levels (46.0, 48.9, 55.4, and 52.6 mg·g⁻¹ DW under 0, 150, 250, and 350 mg·L⁻¹, respectively).

**GA content in the leaves.** Under each treatment, the GA content sharply decreased consistently during cold acclimation (Fig. 4A). However, the values under 250 and 350 mg·L⁻¹ were lower than other treatments in each month. Compared with the values under 0 mg·L⁻¹, the GA contents under these two treatments decreased by 35% to 37%, 37% to 45%, 47% to 48%, and 56% to 58% in

**Fig. 3.** Chlorophyll content (A), proline content (B), soluble protein content (C), and soluble sugar content (D) in the leaves of *Ligustrum lucidum* under SA treatments. T0–T3 indicate SA application at 0, 150, 250, and 350 mg·L⁻¹, respectively. Data are presented as means ± so (n = 10). SA = salicylic acid.

**Fig. 4A.** GA content in the leaves under each treatment. The GA content sharply decreased consistently during cold acclimation. However, the values under 250 and 350 mg·L⁻¹ were lower than other treatments in each month. Compared with the values under 0 mg·L⁻¹, the GA contents under these two treatments decreased by 35% to 37%, 37% to 45%, 47% to 48%, and 56% to 58% in
Fig. 4. GA (A) and ABA (B) contents in the leaves of *Ligustrum lucidum* under SA treatments. T0–T3 indicate SA application at 0, 150, 250, and 350 mg·L−1, respectively. Different lowercase letters indicate significant differences ($P < 0.05$) among treatments. Different capital letters indicate significant differences ($P < 0.05$) among months. Data are presented as means ± sd (n = 10). GA = gibberellic acid; ABA = abscisic acid; SA = salicylic acid.
September, October, November, and December, respectively. In December, the leaves reached their minimum GA levels (2.9, 2.3, 1.3, and 1.2 ng·g⁻¹ under 0, 150, 250, and 350 mg·L⁻¹, respectively).

**ABA content in the leaves.** Under each treatment, the ABA content sharply increased consistently during cold acclimation (Fig. 4B). However, the values under 250 and 350 mg·L⁻¹ were higher than other treatments in each month. Compared with the values under T0, the ABA contents under these two treatments increased by 44% to 59%, 45% to 55%, 85% to 91%, and 53% to 59% in September, October, November, and December, respectively. In December, the leaves reached their maximum ABA levels (113, 150, 174, and 180 ng·g⁻¹ under 0, 150, 250, and 350 mg·L⁻¹, respectively).

Correlation between freezing tolerance and chlorophyll, proline, soluble protein, soluble sugar, GA, ABA, and GA/ABA. A significant correlation was detected between freezing tolerance and the chlorophyll, proline, soluble protein, soluble sugar, GA, and ABA contents in the leaves of glossy privet seedlings under each treatment (Table 1). The LT50 value positively correlated with the GA content and negatively correlated with chlorophyll, proline, soluble protein, soluble sugar, and ABA contents.

**Discussion**

Similarly to other woody perennials, the freezing tolerance of glossy privet was strengthened during cold acclimation (Lim and Arora, 1998; Yang et al., 2015a, 2015b, 2015c, 2016). However, the seedlings treated with SA application showed more cold-hardiness during cold acclimation (Fig. 2). SA application has been reported to benefit many plant species in increasing freezing tolerance (Janda et al., 1999; Horváth et al., 2002; Siboza et al., 2014). In addition, more leaves of the seedlings without SA application were damaged in late December (visual observation), indicating the effectiveness of SA on increasing the freezing tolerance of glossy privet as well.

**Table 1. Correlation coefficients between freezing tolerance (estimated as LT50) and the chlorophyll, proline, soluble protein, soluble sugar, giberellinic acid (GA), and abscisic acid (ABA) contents in the leaves of *Ligustrum lucidum* under salicylic acid (SA) treatments.**

| Variable      | T0  | T1  | T2  | T3  | Correlation coefficient (R) |
|---------------|-----|-----|-----|-----|-----------------------------|
| Chlorophyll   | -0.91**| -0.88**| -0.89**| -0.86**|                            |
| Proline       | -0.80*| -0.76*| -0.83*| -0.81*|                            |
| Soluble protein | -0.79*| -0.75*| -0.81*| -0.78*|                            |
| Soluble sugar | -0.89**| -0.86**| -0.89**| -0.89**|                            |
| GA            | 0.84*| 0.89**| 0.88**| 0.92**|                            |
| ABA           | -0.78*| -0.75*| -0.80*| -0.79*|                            |

* and ** indicate significance at P < 0.05 and P < 0.01, respectively.

SA can increase chlorophyll contents of plants under abiotic stress (Li et al., 2014; Noriega et al., 2012), which was exhibited in our study. We detected that there was a significant relationship between chlorophyll content and freezing tolerance (Table 1) in glossy privet seedlings. Although the chlorophyll content under each treatment kept decreasing consistently during cold acclimation, the chlorophyll values of the seedlings applied with SA were higher (Fig 3A). It has been reported that SA improves chlorophyll levels in maize under low-temperature stress (Janda et al., 1999). On the other hand, higher accumulation of chlorophyll in leaves could help glossy privet look greener, which is especially estimable for a city lacking evergreen tree species in winter.

Our study also confirmed a significant correlation between freezing tolerance and proline content in glossy privet seedlings (Fig. 3B). As an amphiphilic molecule, proline can bind to hydrophobic surfaces using its hydrophobic moieties, thus converting them to hydrophilic ones. Such conversions enable the cell to preserve the structural integrity of cytoplasmic proteins under the dehydration conditions that develop under drought, salinity, and frost stresses (Papageorgiou and Murata, 1995). Hence, the higher proline content under SA applications of 250 and 350 mg·L⁻¹ (Fig. 3B) also accounts for increased freezing tolerance. It has been reported that SA could increase the proline content of plants under abiotic stress (Khan et al., 2013; Li et al., 2014; Shin et al., 2018).

Proline, however, was not the only biochemical factor responsible for freezing tolerance in glossy privet, as a significant correlation was observed between freezing tolerance and soluble protein content (Table 1), indicating a higher accumulation of soluble protein (Fig. 3C) also contributed to a higher level of freezing tolerance under SA applications of 250 or 350 mg·L⁻¹. Certain proteins can be conserved in higher plants (Palva et al., 2002) and accumulate during cold acclimation, freezing stress, or both in plants such as flowering peach (Arora et al., 1992) and rhododendron (Marián et al., 2003). Moreover, promoted accumulation of relative protein by SA has been detected in soybean under stress (Noriega et al., 2012). Soluble sugar can influence freezing tolerance via facilitating the deep supercooling of plant tissues (Kasuga et al., 2007), decreasing the freezing point of intracellular water (Morin et al., 2007), and preventing membrane and macromolecule injuries from freeze-induced dehydroxylation (Krasenskyy and Jonak, 2012). Shao et al., 2006). A significant correlation between freezing tolerance and soluble sugar content was observed in glossy privet (Table 1), suggesting that the improved freezing tolerance in the glossy privet seedlings treated with SA application of 250 and 350 mg·L⁻¹ was connected with soluble sugar accumulation (Fig. 3D). We were interested in the role of growth regulators on the accumulation of a variety of carbohydrates during cold acclimation in woody plants. However, the reports about the role of SA in promoting the sugar accumulation for developing cold acclimation were much fewer than those about ABA. Additional research studies should be designed to determine which kind of soluble sugars (e.g., glucose, fructose, sucrose, raffinose, and some others) are increased under SA application for promoting cold acclimation of glossy privet in the future.

Many factors are involved in the cold acclimation process of plants. SA can regulate various aspects of plant responses under both stressful and optimal environments through signaling cross-talks with other phytohormones (Horváth et al., 2007). The interaction between SA and phytohormones such as GA (Alonso-Ramírez et al., 2009) and ABA (Szepesi et al., 2009) has been established under both normal and stressed conditions. In our study, GA was positively correlated with LT50 values, whereas ABA was negatively correlated with LT50 values (Table 1), indicating a putative role for these two phytohormones in the freezing tolerance of woody plants. Lower GA content and higher ABA content in the leaves of glossy privet under SA application of 250 and 350 mg·L⁻¹ (Fig. 4) were evidences, proving the regulating role of SA on cold acclimation by phytohormones, as both ABA and the interactions between ABA and other plant hormones play an important role in inducing cold acclimation, which has been reported in many plant species (Churchill et al., 1998; Dallaire et al., 1994; Hansen and Grossmann, 2000; Hoffmann-Benning and Kende, 1992; Mora-Herrera and Lopez-Delgado, 2007; Vysotskaya et al., 2009). On the other hand, the accumulation of ABA triggered by SA in stressed plants in turn helped in the osmotic adaptation and improved photosynthetic pigments (Szepesi et al., 2009), which was also well exhibited in our study, by proline, soluble protein, soluble sugar, and chlorophyll contents (Fig. 3). In conclusion, an optimum application concentration of SA between 250 and 350 mg·L⁻¹ started a cascade of steps for advancing the cold acclimation process of glossy privet. An improved freezing tolerance under SA application was associated with the accumulation of chlorophyll, proline, soluble protein, and soluble sugar and the regulation of endogenous hormones GA and ABA. We suggest that exogenous SA application may be used on glossy privet grown in northern China where there are short growing seasons and early fall frost events.

**Literature Cited**

Alonso-Ramírez, A., D. Rodríguez, D. Reyes, J.A. Jiménez, G. Nicolás, M. López-Climent, A. Gómez-Cadenas, and C. Nicolás. 2009. Cross-talk between gibberellins and salicylic acid in early stress responses in *Arabidopsis thaliana* seeds. Plant Signal. Behav. 4:750–751.

Ananieva, E.A., K.N. Christov, and L.P. Popova. 2004. Exogenous treatment with salicylic acid
leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. J. Plant Physiol. 161:319–328.

Arora, R., M.E. Wisniewski, and R. Scorza. 1992. Cold acclimation in genetically related (sib-

ling) deciduous and evergreen peach (Prunus persica [L.] Batsch). I. Seasonal changes in cold hardness and polyphenols of bark and xylem tissues. Plant Physiol. 99:1562–1568.

Artlip, T. and M.E. Wisniewski. 1997. Tissue specific expression of a dehydrin gene in one-

year-old 'Rio Oso Gem' peach trees. J. Amer. Soc. Hort. Sci. 122:784–787.

Bates, L.S., R.P. Waldren, and L.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39:205–207.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Cao, S., Z. Hu, Y. Zheng, and B. Lu. 2010. Auxin- specific expression of a dehydrin gene in one-

year-old 'Rio Oso Gem' peach trees. J. Amer. Soc. Hort. Sci. 122:784–787.

Cao, S., Z. Hu, Y. Zheng, and B. Lu. 2010. Synergistic effect of heat treatment and salicylic acid on alleviating internal browning in cold-stored peach fruit. Postharvest Biol. Technol. 53:73–79.

Churchill, G.C., M.J.T. Reaney, S.R. Abrams, and L.V. Gusta. 1998. Effects of abscisic acid and abscisic acid analogs on the induction of freezing tolerance of winter rye (Secale cereale L.) seedlings. Plant Physiol. 116:1241–1249.

Dallaire, S., M. Houde, Y. Gagne, H.S. Saini, S. Boileau, N. Chevrier, and F. Sarhan. 1994. ABA and low-temperature induce freezing tolerance via distinct regulatory pathways in wheat. Plant Cell Physiol. 35:1–9.

Guy, C.L. 1990. Cold acclimation and freezing stress tolerance: Role of protein metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41:187–223.

Hansen, H. and K. Grossmann. 2000. Auxin-induced ethylene triggers abscisic acid bio-

synthesis and growth inhibition. Plant Physiol. 124:1437–1448.

Hashempour, A., M. Ghasemnezhad, R.F. Ghashvini, and M.M. Sohani. 2014. The physiological and biochemical responses to freezing stress of olive plants treated with salicylic acid. Russ. J. Plant Physiol. 61:443–450.

Hayat, Q., S. Hayat, M. Irfan, and A. Ahmad. 2010. Effect of exogenous salicylic acid under chang-

ing environment: A review. Environ. Exploit. Bot. 68:14–25.

Hoffmann-Benning, S. and H. Kende. 1992. On the role of abscisic-acid and gibberellins in the regulation of growth in rice. Plant Physiol. 99:1156–1161.

Horváth, E., T. Janda, G. Szalai, and E. Páldi. 2002. In vitro salicylic acid inhibition of catalase activity in maize: Differences between the isoenzymes and a possible role in the induction of chilling tolerance. Plant Sci. 163:1129–1135.

Horváth, E., M. Pál, G. Szalai, E. Páldi, and T. Janda. 2007. Exogenous 4-hydroxybenzoic acid and salicylic acid modulates the effect of short-term drought and freezing stress on wheat plants. Biol. Plant. 51:480–487.

Janda, T., G. Szalai, I. Tari, and E. Páldi. 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (Zea mays L.) plants. Plants 208:175–180.

Kasuga, J., K. Arakawa, and S. Fujikawa. 2007. High accumulation of soluble sugars in deep supercooling Japanese white birch xylem para-

thyma cells. New Phytol. 174:569–579.

Khan, M.I.R., N. Iqbal, A. Masood, T.S. Per, and N.A. Khan. 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signal. Behav. 8: E26374.

Kim, Y., S. Park, S.J. Gilmour, and M.F. Thomashow. 2013. Roles of CAMTA transcription factors and salicylic acid in configuring the low-
temperature transcriptome and freezing tolerance of Arabidopsis. Plant J. 73:564–376.

Krasensky, J. and C. Jonak. 2012. Drought, salt and temperature stress-induced metabolic rear-

rangements and regulatory networks. J. Expt. Bot. 63:1–16.

Li, H.S. 2000. Principles and techniques of plant physiological biochemical experiment. Higher Educ. Press, Beijing, China.

Li, T., Y. Hu, X. Du, H. Tang, C. Shen, and J. Wu. 2010. Salicylic acid alleviates the adverse effects of salt stress in Torreyra grandis cv. merivilli seedlings by activating photosynthesis and enhancing antioxidant systems. PLoS One 9:E10942.

Lim, C.C. and R. Arora. 1998. Comparing Gomp-

ertz and Richards functions to estimate freezing injury in Rhododendron using electrolyte leakage. J. Amer. Soc. Hort. Sci. 123:246–252.

Marjan, C.O., A. Eis, S.L. Krebs, and R. Arora. 2003. Environmental regulation of a 25 kDa dehydrin in relation to rhododendron cold acclimation. J. Amer. Soc. Hort. Sci. 129:354–359.

Miura, K. and Y. Tada. 2010. Regulation of water, salinity, and cold stress responses by salicylic acid. Front. Plant Sci. 5:1–12.

Mora-Herrera, M.E. and H.A. Lopez-Delgado. 2007. Freezing tolerance and antioxidant ac-
tivity in potato microplants induced by abscisic acid treatment. Amer. J. Potato Res. 84:467–475.

Morin, X., T. Améglio, R. Ahas, C. Kurz-Besson, V. Lanta, F. Lebourgeois, F. Miglietta, and I. Chuiine. 2007. Variation in cold hardiness and carbohydrate concentration from dormancy in-duction to bud burst among provenances of three European oak species. Tree Physiol. 27:817–825.

Mutlu, S., Ö. Karadaglioğlu, Ö. Atici, and B. Nalbantoglu. 2013. Protective role of salicylic acid applied before cold stress on antioxidative system and protein patterns in barley apoplast. Biol. Plant. 57:507–513.

Noriega, G., E. Caggiano, M.L. Lecube, D.S. Cruz, A. Battle, M. Tomaro, and K. B. Balestrasse. 2012. The role of salicylic acid in the pre-

vention of oxidative stress elicited by cadmium in soybean plants. Biometals 25:1155–1165.

Palva, E.T., S. Thtihariju, I. Tamminen, T. Puhakdinen, V. Lanta, F. Lebourgeois, F. Miglietta, and I. Chuiine. 2007. Freezing tolerance and antioxidant ac-
tivity of Ginkgo biloba during cold acclimation. J. Hort. Sci. Bio-
technol. 90:704–710.

Sang, and L.Y. Ma. 2015c. Physiological and biochemical pro-
tocols during acclimation to bud burst among provenances of three European oak species. Tree Physiol. 27:817–825.

Yang, Y., Z.K. Jia, F.J. Chen, Z.Y. Sang, J. Duan, and L.Y. Ma. 2015a. Natural cold acclimatisa-
tion and de-acclimatisation of Magnolia wufeng-
gensis in response to alternative methods of application of abscisic acid. J. Hort. Sci. Bio-
technol. 90:704–710.

Yang, Y., Z.K. Jia, F.J. Chen, Z.Y. Sang, and L.Y. Ma. 2015b. Comparative analysis of natural cold acclimation and de-acclimation of two Magnolia species with different winter hardi-

ness. Acta Physiol. Plant. 37:129.

Yang, Y., Z.K. Jia, F.J. Chen, Z.Y. Sang, and L.Y. Ma. 2015c. Physiological and biochemical pro-
cesses of Magnolia wufengensis in response to foliar abscisic acid application during natural cold acclimation. HortScience 50:387–394.

Yang, Y., N. Yao, Z.K. Jia, J. Duan, F.J. Chen, Z.Y. Sang, and L.Y. Ma. 2016. Effect of exogenous abscisic acid on cold acclimation in two Magnoli-

a species. Biol. Plant. 60:555–562.