Journal of Stress Physiology & Biochemistry, Vol. 9 No. 3 2013, pp. 199-206 ISSN 1997-0838

ORIGINAL ARTICLE

Exogenous application of ascorbic acid alleviates chilling injury in apricot (*Prunus armeniaca* L. cv. Shahroudi) flowers

Hassan Bayat[1]*, Morteza Alirezaie Noghondar[1], Hossein Neamati[1], Ahmad Nezami[2]

[1] *Department of Horticultural science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.*
[2] *Department of Agronomy and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.*

*E-Mail: ***hassanbayat55@gmail.com***

Received March 19, 2013

One of the most important limiting factors in spread of apricot in Iran is late spring frost, which damages flower bud and decrease total yield of crop. It has been found that ascorbic acid (AA) plays a beneficial role during plant response to chilling and freezing stresses. To evaluate the effects of AA on alleviating of cold stress, the flower buds of *Prunus armeniaca* L. cv. Shahroudi were sprayed at pink cluster stage with AS at 4 levels (0, 100, 200 and 300 mg. L^{-1}) and were then exposed to artificial cold stress (4 h at – 4 °C) or without cold stress (+ 25°C). Experimental attributes including electrolyte leakage (EL) of flower buds and percentage of damage of pistil, anthers and petals to temperature treatments were determined. The results showed that at - 4°C the lowest and highest percentage of damage and EL of flower buds were observed in application of 200 and 0 mg.L^{-1} AA, respectively. The highest and lowest percentage of damage of flower organs and EL were obtained in application of 300 and 200 mg. L^{-1} AA, respectively at + 25 °C. Based on the results of this experiment, AA alleviates the negative effect of cold stress on EL and flower organ damages in apricot cv. Shahroudi, depending on the concentrations of AA used.

Key words: electrolyte leakage, foliar spray, pink cluster, stress

ORIGINAL ARTICLE

Exogenous application of ascorbic acid alleviates chilling injury in apricot (*Prunus armeniaca* L. cv. Shahroudi) flowers

Hassan Bayat[1]*, Morteza Alirezaie Noghondar[1], Hossein Neamati[1], Ahmad Nezami[2]

[1] *Department of Horticultural science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.*
[2] *Department of Agronomy and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.*

*E-Mail: ***hassanbayat55@gmail.com***

Received March 19, 2013

One of the most important limiting factors in spread of apricot in Iran is late spring frost, which damages flower bud and decrease total yield of crop. It has been found that ascorbic acid (AA) plays a beneficial role during plant response to chilling and freezing stresses. To evaluate the effects of AA on alleviating of cold stress, the flower buds of *Prunus armeniaca* L. cv. Shahroudi were sprayed at pink cluster stage with AS at 4 levels (0, 100, 200 and 300 mg. L^{-1}) and were then exposed to artificial cold stress (4 h at – 4 °C) or without cold stress (+ 25°C). Experimental attributes including electrolyte leakage (EL) of flower buds and percentage of damage of pistil, anthers and petals to temperature treatments were determined. The results showed that at - 4°C the lowest and highest percentage of damage and EL of flower buds were observed in application of 200 and 0 mg. L^{-1} AA, respectively. The highest and lowest percentage of damage of flower organs and EL were obtained in application of 300 and 200 mg. L^{-1} AA, respectively at + 25 °C. Based on the results of this experiment, AA alleviates the negative effect of cold stress on EL and flower organ damages in apricot cv. Shahroudi, depending on the concentrations of AA used.

Key words: electrolyte leakage, foliar spray, pink cluster, stress

The *Prunus armeniaca* L., belonging to the family Rosaceae, genus *Prunus* L., the subgenus *Prunophora* Focke, and the section *Armeniaca*, is one of the most cultivated stone fruits in the world (Rehder, 1967; Hurtado *et al.,* 2002; Vilanova *et al.,* 2003; Ercisli, 2009). It has been domesticated in the wide area covering, Iran, Turkistan, Afghanistan, Middle Asia and Western China, over 5,000 years ago (Faust *et al.,* 1998). Total production of fresh apricot in the world is between 2.2 and 2.7 million

tons per year. Because of its suitable climatic conditions, Iran is one of the major centers of apricot production ranking second in the world, and accounting for 12.7 percent of world apricot production (Ercisli, 2009).

Apricot tree is known as an early blooming and sensitive to frost. One of the main problems of apricot production in Iran, especially in Mashhad is the irregular and fluctuating production rates. For many years the apricot production of Iran has been unregulated because of late spring frost. This is the result of early flowering of native genotypes and coincidence of their flowering times with a late cold spring. Late flowering is considered important to avoid disastrous spring frost damage (Tsonev, 1995). In breeding programs, one of the objectives is to produce varieties which flower so late that all dangers practically to the blossoms from late frost are past. Another contributory factor to the crop loss due to late frost is the inherent susceptibility of the flowers to injury (Tsonev, 1995; Lin and Pliszka, 2001; Hodun *et al.,* 2002).

Growth regulators and chemical treatments sometimes cause higher resistance to cold of different parts of plants. Potassium nitrate in apricot has been reported to be effective in reducing the adverse effects of cold stress (Ozturk *et al.,* 2006). Abscisic acid (ABA) treatment (1 - 4 mol) caused increase in the cold resistance of *Cornus stolontifera* about 2 degrees (Fuchigami *et al.,* 1971). Also, control of flowering time in apricot and other stone fruits by application of growth regulators such as ethephon and gibberellic acid have been studied by other researchers (Soni and Yousif, 1978; Durner and Gianfagna, 1988; Gianfagna, 1988; Murdoc and Ferguson, 1990; Ganji Moghadam and Mokhtarian, 2006).

Ascorbic acid (AA) is the most abundant antioxidant in plants and serves as the major contributor to the cell redox state (Smirnoff, 2000). It is primarily known for its antioxidant properties, but it also acts as a cofactor for various enzymes and further contributes to the regulation of cell division and expansion (Smirnoff and Wheeler, 2000). It is essential for plant growth (Alhagdow et al., 2007; Dowdle et al., 2007) and seems to control flowering time and the start of senescence (Davey et al., 2000). In addition, AA can act as signaling agents (Fotopoulos et al., 2008) participating in the interaction with the environment. It is the most effective compound which increases the tolerance of the plants to oxidative stresses. A recent increase of evidences suggests that it may play a role in protection of plant against several environmental stresses such as heavy metal action (Vwioko et al., 2008), salinity (Shalata and Neumann, 2001), water loss (Fotopoulos et al., 2008), ozone ((Sanmartin et al., 2003), UV-B and pathogenesis (Fotopoulos et al.,2006).

The apricot trees, under Noghondar climatic conditions, because of early blooming in spring, are often injured by late frost, and losing the yield lost by frost at blooming period is 30 - 40 % almost every year, or sometimes there will be no production at all. This problem has not been solved effectively. Therefore, the aim of this study was to evaluate whether apricot flowers (cv. Shahroudi) would response favorably to AA under artificial cold stress.

MATERIALS AND METHODS

This experiment was conducted at agricultural college of Ferdowsi university of Mashhad, Iran in 2012. In this study, the apricot, suitable for fresh market, which is widely cultivated in Noghondar

region around Mashhad -North east of Iran- (36'22" latitude and 59'17" longitude), was selected. Three trees were selected and live flower buds at pink cluster stage were randomly collected from all quadrants of the trees. Each experimental unit consists of four branches (15 – 20 cm long) of one-year-old fruiting shoots. Each one contains ten buds, i.e. 40 buds for each treatment in four AA concentration treatments (0, 100, 200 and 300 mg. L^{-1} as spray application) and two thermal levels, including artificial cold stress (- 4 °C) or without cold stress (+ 25 °C) was studied. The all above branches were pretreated by spraying flower buds with AA 1 day before artificial cold stress treatment. The artificial cold stress treatment was performed on half of branches, which were kept in a freezing chamber and exposed at a temperature of -4 °C and 75% relative humidity in the dark (for 3 h) achieved by a continuous chilling decrease (2 °C h^{-1}). Then, they were evaluated carefully for observed chilling injuries. The other half of the branches, which were pre-treated by AA, were kept at a temperature of 25 °C and 75% relative humidity in the dark. The experiment was factorial based on a completely randomized design with 40 buds per replication. To assess specific symptoms of flower abnormalities such as the browning of tissues affecting pistils, stamens or petals, samples of buds (40, arranged as four replications) were bisected longitudinally and observed under a stereo-microscope (Nikon HFX-II). Data expressed as percentages.

Electrolyte leakage (EL) was evaluated by Barranco *et al.* (2005) method. 0.5 g fresh weight of the flowers were excised and placed in Erlenmeyer flask containing 20 mL distilled H_2O and incubated for 24 h in a shaker at 23 °C under continuous light. The initial electrolyte conductivity (EC_1) of each sample was measured to obtain an indirect indication of the amount of ion released at each treatment. Then, the samples were placed in an autoclave at 121 °C for 20 min and a second reading (EC_2) was recorded after cooling the solution to room temperature. The EL was calculated as EC_1/EC_2 and expressed as percent.

The statistical analysis was performed using JMP 8 software and means were compared using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, at -4 °C and no AA application (0 $mg.L^{-1}$) the highest damage of flower organs was observed (48.6, 59.2 and 88.3 percent for petals, anthers and pistils, respectively) ($P \leq 0.05$). The lowest damage symptoms was observed under -4 °C in application of 200 $mg.L^{-1}$AA (2.8, 3.9 and 6.1 percent for petals, anthers and pistils, respectively), but increasing AA concentrations from 200 to 300 mg. L^{-1} caused slight increase in damage. At 25 °C, the highest and lowest symptoms of damage to flower organs were obtained in application of 300 and 200 $mg.L^{-1}$ AA, respectively ($P \leq 0.05$). Among different flower organs, the highest and lowest symptoms of damage were observed in pistils and petals in all treatments, respectively. Damaged organs ranged from brown to yellow-brown which are distinctive morphological sign of chilling injury. Figures 1 show damaged and intact pistils in 0 and 200 $mg.L^{-1}$ AA pretreatment under artificial cold stress treatment (- 4 °C).

At -4 °C, the highest and lowest EL of flower buds were obtained in application of 0 and 200 mg. L^{-1} of AA, respectively (Fig. 2). Under 25 °C, the highest and lowest EL of flower buds were observed at 300 and 200 mg. L^{-1} of AA, respectively. With increasing of AA concentration from 200 to 300 mg. L^{-1}, EL was increased at both temperature

treatments (Fig. 2).

Percentage of damage and electrolyte leakage of flower organs is not only influenced by temperature but also is influenced by AA concentration. Under -4 °C and without AA application (0 mg.L^{-1}), the highest damage symptoms and % EL were observed. Our results coincide with Rouhani Nia *et al.* (2011), in apricot flowers. They studied the effect of cold stress on flower organs of some apricot cultivars and reported that 1, +2 °C didn't damage any flower organs, but decreasing temperature from +2 °C to 0°C and -2 °C cause gradually increased damage. From -2 °C to -4 °C the highest damage was observed (Rouhani Nia *et al.,* 2011). Among different flower organs, pistil was more sensitive to cold stress. Our results were consistent with results of Rouhani Nia *et al.* (2011), who reported pistil is more sensitive to cold stress than other apricot flower organs. There is often a good correlation between ion leakage and freezing tolerance (Levitt, 1980). Also, Electrolyte leakage is often used as a parameter for determining tissue damage as the loss of membrane's selective permeability (Bartoli *et al.,* 1995). In the present study, the lowest of damage symptoms and EL under cold stress (-4 °C) and 25 °C were observed in 200 mg.L^{-1} AA treatment and with increasing concentration to 300 mg.L^{-1}, damage symptoms and EL were increased. The effect of exogenous AA on the stress tolerance of plants is not always obvious. It depends not only on the applied concentration and the mode of application, but also on the overall state of the plant; developmental stage, oxidative balance of the cells, and acclimation by previous biotic or abiotic stresses (Shalata and Neumann, 2001). Although no similar studies have been done with the work ahead on flower buds of stone fruits, but recent studies show that AA may alleviate chilling injury not only at the whole-plant level but also when only the fruits are treated. When banana fruits were treated with 1% ascorbic acid, the cold tolerance of the fruits increased (Shivashankar, 2000).

Table 1. Effect of exogenous AA pretreatment (0, 100, 200 and 300 mg. L^{-1}) on damaged flower organs percentage in apricot flower buds under different temperature treatments (- 4 and + 25 °C).

Treatments		Flower organ damages (%)		
Temperatures	AA Concentrations (mg. L^{-1})	Petal	Anther	Pistil
- 4 °C	0	48.6 a	59.2 a	88.3 a
	100	24.4 bc	29.7 bc	44.4 bc
	200	28.2 b	34.4 b	51.4 b
	300	16.0 c	19.5 c	29.1 c
+ 25 °C	0	3.8 d	4.9 d	7.8 d
	100	3.5 d	4.7 d	7.4 d
	200	2.8 d	3.9 d	6.1 d
	300	5.7 d	7.0 d	12.3 d
Temperature		**	**	**
AA		**	**	**
Temperature × AA		**	**	**

Means within each column followed by the same letter (s) are not significantly different at 0.05 probability level according to Duncan multiple range test (DMRT).
*, ** and ns indicate significance at $P < 0.05$, $P < 0.01$ levels and non-significance, respectively.

Figure 1. Comparison of damaged (left) and intact pistil (right) pistils treated with 0 and 200 mg. L-1 AA, respectively under artificial cold stress (–4 °C)

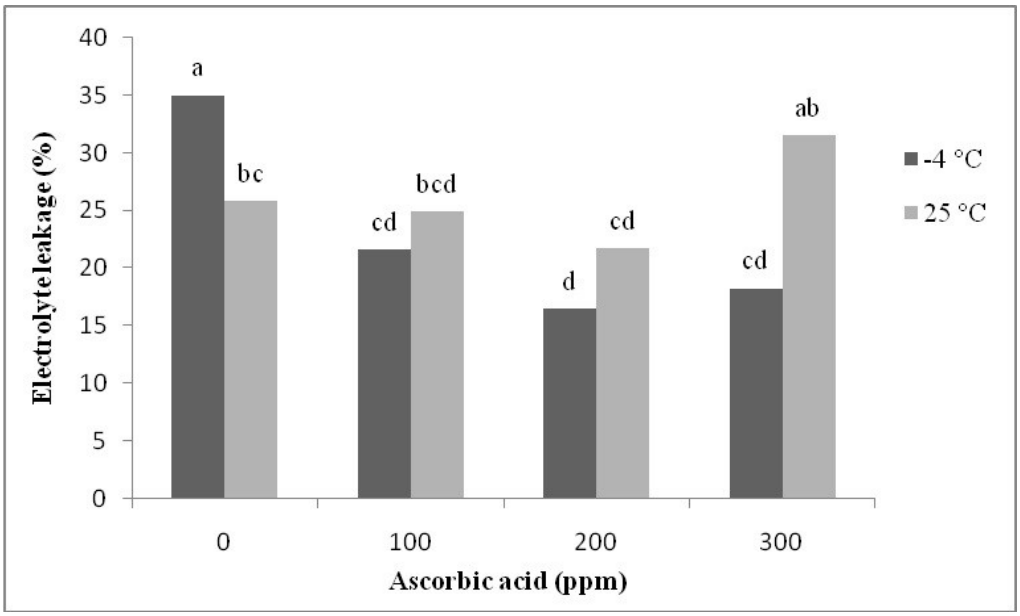

Figure 2. Effect of exogenous AA pretreatment (0, 100, 200 and 300 mg. L^{-1}) on relative electrolyte leakage in apricot flower buds under different temperature treatments (- 4 and + 25 °C).

CONCLUSION

Based on the present results, AA alleviates the negative effect of cold stress on electrolyte leakage and flower organ damages in apricot CV. Shahroudi, depending on the concentration of AA used. Maximum alleviation of cold stress was found with 200 mg.L^{-1} AA application.

REFERENCES

Alhagdow, M., Mounet, F. and Gilbert, L. (2007) Silencing of the mitochondrial ascorbate synthesizing enzyme L-galactono-1, 4-lactone dehydrogenase affects plant and fruit

development in tomato. *Plant Physiology,* **145**, 1408–1422.

Barranco, D., Ruiz, N. and Gomes, M. (2005) Frost tolerance of eight olive cultivars. *HortScience* **40,** 558-560.

Bartoli, C.G., Simomtacchi, M., Guiamet, J.J., Montaldi, E. and Puntarulo, S. (1995) Antioxidant enzymes and lipid peroxidation during aging of *Chrysanthemum morifolium* RAM petals. *Plant Sci.,* **104**, 161-168.

Davey, M.D., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A.K., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D. and Fletcher, J. (2000) Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, **80,** 825–860.

Dowdle, J., Ishikawa, T., Gatzek, S., Rolinski, S. and Smirnoff, N. (2007) Two genes in Arabidopsis thaliana encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. *The Plant Journal,* **52**, 673–689.

Durner, E.F. and Gianfagna, T.J. (1988) Fall ethephon application increases peach flower bud resistance to low-temperature stress. *J. Am. Soc. Hortic. Sci.,* **113,** 404-406.

Ercisli, S. (2009) Apricot culture in Turkey. *Sci. Res. Essays.,* **4,** 715-719.

Faust M, Suranyi D, Nyujto F (1998). Origin and Dissemination of Apricot. *Hort Rev.,* **22,** 225-266.

Fotopoulos, V., Sanmartin, M. and Kanellis, A.K. (2006). Effect of ascorbate oxidase over-expression on ascorbate recycling gene expression in response to agents imposing oxidative stress. *Journal of Experimental Botany,* **57,** 3933–3943.

Fotopoulos, V., De Tullio, M.C., Barnes, J. and Kanellis, A.K. (2008) Altered stomatal dynamics in ascorbate oxidase over-expressing tobacco plants suggest a role for dehydroascorbate signaling. *Journal of Experimental Botany,* **59,** 729–737.

Fuchigami, L.H., Evert, R.D. and Weiser, J.C. (1971) A translocatable cold hardiness promoter. *Plant Physiol*., **47,** 164-167.

Ganji Moghadam, E. and Mokhtarian, A. (2006) Delaying apricot (CV. Shahroudi) flower induction by growth regulators application. *J. Applied. Sci.*, **6,** 266-269.

Gianfagna, T.J. (1988) Chemical control with ethephon of bud dormancy, cold hardiness and time of bloom in peach trees. *Plant Growth Reg. Soc. Am. Quart.,* **17,** 39-47.

Hodun, G., Hodun, M., Swiecicki, W., Naganowska, B. and Wolko, B. (2002) Preliminary evaluation of sour cherry fruitlet susceptibility to late spring frosts. Broad Variation and Precise Characterization Limitation for the Future Proceedings of the XVI. EUCARPIA Genetic Resources Section Workshop, Poznan Poland, 16-20 May 2001, 335-337.

Hurtado, M.A., Romero, C., Vilanova, S., Abbott, G., Llácer, G., and Badenes, L. (2002) Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.) and mapping of PPV (Sharka) resistance. *Theor. Appl. Genet.,* **105,** 182-191.

Levitt, J. (1980) Responses of Plants to Environmental Stress. Academic Press. London.

Lin, W. and Pliszka, K. (2001) Blueberry flower spring injury in central Poland. *Small Fruits*

Rev., **1,** 43-49.

Murdoc, B.A. and Ferguson, N.H. (1990) Effects of fall ethephon and Gibberellic acid applications on bloom delay, flowering and fruiting of plum. *Hortic. Sci.,* **25,** 1110-1118.

Ozturk, K., Olmez, H., Colak, S. and Celik, B. (2006) Effects of potassium nitrate on cold resistance of Cataloglu apricot variety. *Acta Hortic.,* **701,** 713-718.

Rehder, A. (1967) Manual of Cultivated Trees and Shrubs. Macmillan. New York.

Rouhani Nia, M., Motallebi-Azar, A. and Davati-Kazemnia, H. (2011) Effects of cold stress on some Apricot (*Prunus armeniaca* L.) cultivars in different phonological stages. *AAB Bioflux,* **3,** 178-183.

Sanmartin, M., Drogoudi, P.D., Lyons, T., Pateraki, I., Barnes, J. and Kanellis, A.K. (2003) Over-expression of ascorbate oxidase in the apoplast of transgenic tobacco results in altered ascorbate and glutathione redox states and increased sensitivity to ozone. *Planta,* **216,** 918–928.

Shalata, A., and Neumann, P.M. (2001) Exogenous ascorbic acid (vitamin C) increases resistance of salt stress and reduces lipid peroxidation. *Journal of Experimental Botany,* **52,** 2207-2211.

Shivashankar, S. (2000) Effect of ascorbic acid and calcium chloride on chilling injury of banana. *Journal of Tropical Agriculture*, **38,** 94-96.

Smirnoff, N. (2000) Ascorbic acid: metabolism and functions of a multi-faceted molecule. Curr. Opin. Plant Biol., **3,** 229–235.

Smirnoff, N. and Wheeler, G.L. (2000) Ascorbic acid in plants: biosynthesis and function. *Critical Reviews in Biochemistry and Molecular Biology*, **35,** 291–314.

Soni, S.L. and Yousif, Y.H. (1978) Inducing delay in flowering of apricot with growth-regulators. *Indian J. Agric. Sci.,* **48,** 197-200.

Tsonev, R. (1995) Selection of parental forms of apricot for cold resistance. *Rasteniev'dni Nauki* **32,** 183-185.

Vilanova, S., Romero, C., Abbott, A.G., Llacer, G. and Badanes, M.L. (2003) An apricot (*Prunus armeniaca* L.) F2 progeny linkage map based on SSR and AFLP markers, mapping Plum Pox Virus resistance and self-incompatibility traits. *Theor. Appl. Genet.,* **107,** 239-247.

Vwioko, E.D., Osawaru, M.E. and Eruogun, O.L. (2008) Evaluation of okro (*Abelmoschus esculentus* L. Moench.) exposed to paint waste contaminated soil for growth, ascorbic acid and metal concentration. *African Journal of General Agriculture,* **4,** 39-48.