

ORIGINAL ARTICLE

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

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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. Shahrودي 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. Shahrودي, depending on the concentrations of AA used.

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

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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 (Alhaghdow *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 H₂O and incubated for 24 h in a shaker at 23 °C under continuous light. The initial electrolyte conductivity (EC₁) 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 (EC₂) was recorded after cooling the solution to room temperature. The EL was calculated as EC₁/EC₂ 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⁻¹AA (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⁻¹AA, 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)

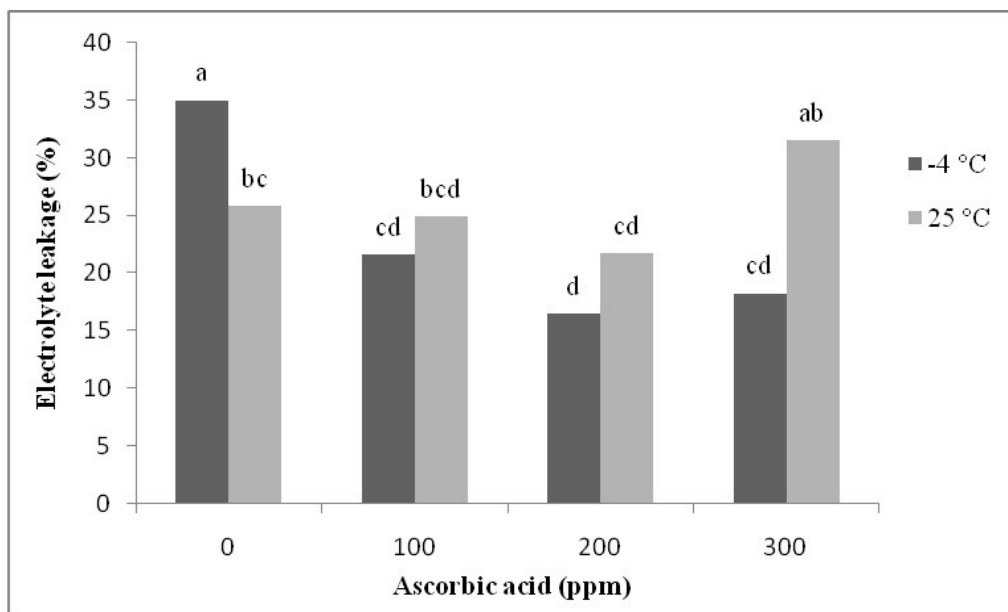


Figure 2. Effect of exogenous AA pretreatment (0, 100, 200 and 300 mg. L⁻¹) on relative electrolyte leakage in apricot flower buds under different temperature treatments (-4 and +25 °C).

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.

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