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Effect of Salicylic Acid on Alleviating of Electrolyte Leakage and Flower Organ Damage in Apricot (*Prunus armeniaca* L. cv. 'Shahroudi') under Artificial Cold Stress

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Abstract

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 salicylic acid (SA) plays a beneficial role during plant response to chilling and freezing stresses. To evaluate the effects of salicylic acid on alleviating of cold stress, the flower buds (FBs) of *Prunus armeniaca* L. cv. 'Shahroudi' were sprayed at pink cluster stage with SA at 4 levels (0, 0.5, 1 and 2 mM) and were then exposed to artificial cold stress (4 h at -4°C) or without cold stress (+ 25). Experimental attributes including electrolyte leakage of FBs and percentage of damage (PD) of pistil, anthers and petals to temperature treatments were determined. The results showed that at -4°C the lowest and highest PD and EL of FBs were observed in application of 0.5 and 0 mM SA, respectively. The highest and lowest PD of flower organ and EL were obtained in application of 0 and 2 mM SA, respectively at +25°C. Based on the results of this experiment, SA alleviates the negative effect of cold stress on electrolyte leakage and flower organ damages in apricot cv. 'Shahroudi', depending on the concentrations of SA used.

Keywords: electrolyte leakage, pink cluster, salicylic acid, stress

Introduction

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 (Ercisli, 2009; Hurtado *et al.,* 2002; Rehder, 1967; Vilanova *et al.,* 2003). 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 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 (Hodun *et al.,* 2002; Lin and Pliszka, 2001; Tsonev, 1995).

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 (Durner and Gianfagna, 1988; Ganji Moghadam and Mokhtarian, 2006; Gianfagna, 1988; Murdoc and Ferguson, 1990; Soni and Yousif, 1978).

Salicylic acid (SA), a natural plant hormone, has various effects on the tolerance to abiotic stress as well as the regulation of plant growth and development (Bergmann *et al.,* 1994; Raskin, 1992; Van Breusegem *et al.,* 2001). During recent years, SA has received particular attention because of its role in modulating plant response to several abiotic stresses, such as chilling, heat, drought, salt and ultraviolet radiation. Studies show that SA increases chilling tolerance of wheat (Tasgin *et al.,* 2003), maize (Janda *et al.,* 1999; Kang and Saltveit, 2002), tomato (Ding *et al.,* 2002; Senaratna *et al.,* 2000), peach (Cao *et al.,* 2010; Wang *et al.,* 2006), pomegranate (Sayyari *et al.,* 2009), apricot (Guo *et al.,* 2007), anthurium (Promyoua *et al.,*

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2012), banana (Kang *et al.,* 2003), bean (Senaratna *et al.,* 2000), rice and cucumber (Kang and Saltveit, 2002).

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 SA under artificial cold stress.

Material 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 SA concentration treatments (0, 0.5, 1 and 2 mM. 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 SA (0, 0.5, 1 and 2 mM. L^{-1}) 1 d 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 pretreated by SA, 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.

In the second stage, the Electrolyte leakage was evaluated (EL) 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 same 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 Microsoft Excel (2007) and MSTAT C software and means were compared using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

Results

As it's shown in Tab. 1, at -4°C and no SA application (0 mM) the highest damage of flower organs was observed (27.2, 33.57 and 66.88 percent for petals, anthers and pistils, respectively) ($p \leq 0.05$). The lowest damage symptoms was observed under -4°C in application of 0.5 mM SA (7.11, 8.65 and 10.83 percent for petals, anthers and pistils, respectively), but increasing SA concentrations from 0.5 to 2 mM gradually caused slight increase in damage. No significant difference was observed between 0.5 and 1 mM concentrations. At 25°C, the highest and lowest symptoms of damage to flower organs were obtained in application of 0 and 2 mM SA, 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. Fig. 1 and 2 show intact and damaged pistils in 0 and 0.5 mM SA pretreatment under artificial cold stress treatment (-4°C).

At -4°C, the highest and lowest EL of flower buds (FBs) were obtained in application of 0 and 0.5 mM of SA, respectively (Fig. 3). Under 25°C, the highest and lowest EL

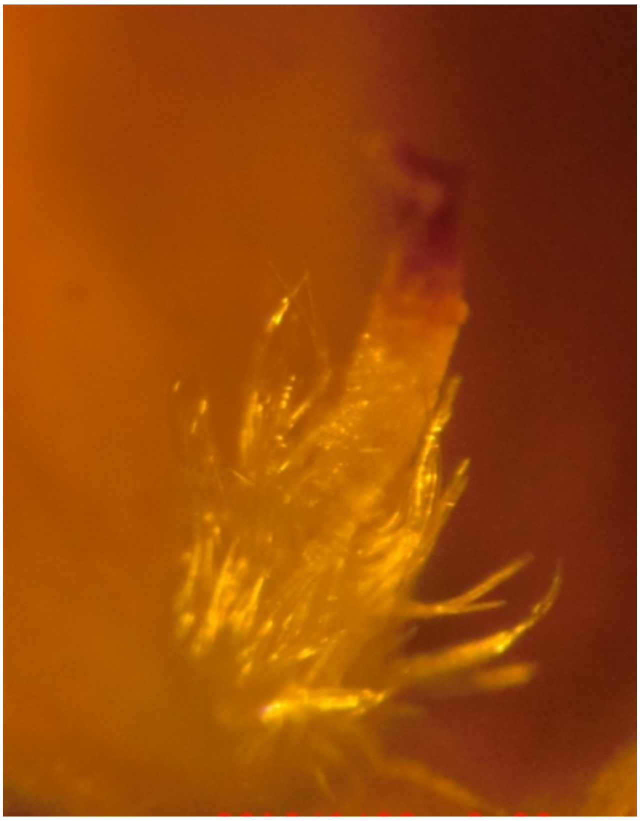

Fig. 1. Damaged pistil (0 mM SA and -4°C artificial cold stress)

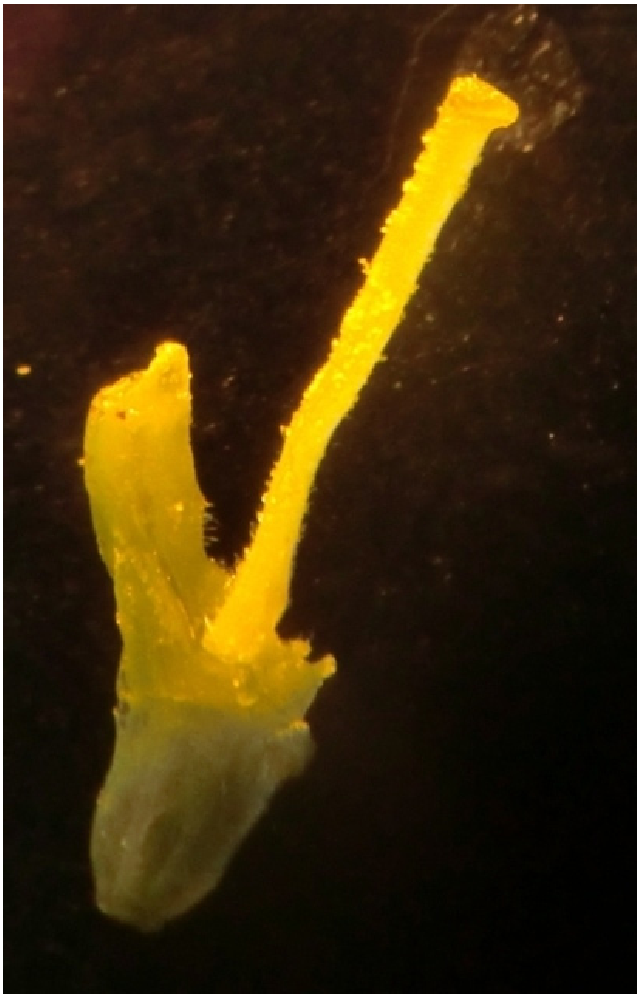

Fig. 2. Intact pistil (0.5 mM SA and -4°C artificial cold stress)

of FBs were observed at 0 and 2 mM of SA, respectively. With increasing of SA concentration from 0.5 to 2 mM, EL was increased at both temperature treatments (Fig. 3).

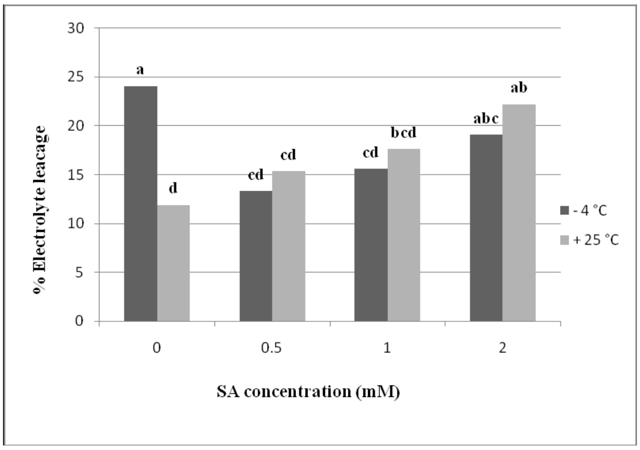

Fig. 3. Effect of exogenous SA pretreatment (0, 0.5, 1 and 2 mM) on relative electrolyte leakage in apricot flower buds under different temperature treatments (-4 and +25°C)

Percentage of damage and electrolyte leakage of flower organs is not only influenced by temperature but also is influenced by SA concentration. Under -4°C and without SA application (0 mM), 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 perme-

Tab. 1. Effect of exogenous SA pretreatment (0, 0.5, 1 and 2 mM) on damaged flower organs percentage in apricot flower buds under different temperature treatments (-4 and +25°C)

Treatments				
Temperature	SA concentration	Petals	Anthers	Pistil
-4°C	0	27.20a†	33.57a	66.88a
	0.5	7.11de	8.65e	10.83cd
	1	8.14de	9.37de	9.95cd
	2	13.46c	15.74c	19.44b
+25°C	0	2.81f	3.62f	5.80d
	0.5	5.85e	7.09e	9.03cd
	1	9.71d	12.13d	16.66bc
	2	19.34b	21.94b	24.08b
Analysis of variance				
Replication		10.66 *††	19.64*	10.66*
Treatment		194.65 **	282.06**	194.65**

Note: † 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

ability (Bartoli *et al.,* 1995). In our experiments, the lowest of damage symptoms and EL under cold stress (-4°C) were observed in 0.5 mM SA treatment and with increasing concentration from 0.5 to 2, damage symptoms and EL were increased. At 25°C, the lowest of damage symptoms and EL were obtained in application of 0 mM of SA and with increasing concentration from 0 to 2, these traits were increased. The effect of exogenous SA 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 (Horvath *et al.,* 2007). Although no similar studies have been done with the work ahead on flower buds of stone fruits, but recent studies show that SA may alleviate chilling injury not only at the whole-plant level but also when only the fruits are treated. When tomato plants were treated with 0.01 mM methyl salicylate and methyl jasmonate, the cold tolerance of the fruits increased (Ding *et al.,* 2002). Higher concentrations (0.1 and 0.5 mM), however, tended to decrease cold tolerance. Similar results were shown for peaches treated with SA and stored at low temperatures (Wang *et al.,* 2006).

Conclusion

The beneficial effect of SA pretreatment on protecting apricot flower buds from chilling injury was not observed at any concentration in neither temperature treatment. Based on the present results, SA alleviates the negative effect of cold stress on electrolyte leakage and flower organ damages in apricot cv. 'Shahroudi', depending on the concentration of SA used. Maximum alleviation of cold stress was found with 0.5 mM SA application.

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