Asian Journal of Chemistry Vol. 22, No. 3 (2010), 2356-2366

Measurement of Chemical and Non-Chemical Parameters of Three Native Pistachio Cultivars of Damghan Region (Iran) for Studying Spring Frost

HOSSEIN AFSHARI*, HOSSEIN HOKMABADI†, ABDOLGHAPHAR EBADI‡ and GHANBAR LAEE
Department of Horticulture, Islamic Azad University, Damghan Branch, Damghan, Iran
E-mail: h_afshari@ymail.com; afshari2000ir@yahoo.com

Pistachio (*Pistacia Vera* L) is one of the most strategic products of Iran. Frost injury in recent years has exposed this product to serious risks. Determining the critical temperatures causing such damages in early spring, which are sometimes quite huge, has greatly contributed to developing and using timely prevention methods and measures aimed at reducing damages and may still be valuable in estimating inflicted losses caused by spring frost. A research project was undertaken at Islamic Azad University, Damghan Branch, in years 2007 and 2008. The experiment was conducted as factorial in a completely randomized design with two factors: cultivar type at three levels (Abbasali, Khanjari, Shahpasand) and temperature at five levels (+2, 0, -2, -4 and -6 °C) in three phases: growth of reproductive organs (swollen buds, opened flowers, newly fertilized fruits in late winter and early spring. Samples were places inside an incubator and temperature was reduced at a rate of 2 °C/h. At each temperature level some samples were taken out for studying the rate of damages. Also to determine the accumulation of proline and soluble sugars the samples were first treated and then measured in terms of proline and soluble sugar contents. Results indicated that the highest sensitivity to early spring cold was in the fully-opened flower stage and maximum damages were inflicted at -6 °C. Results also demonstrated that Abbasali and Shahpasand were the most and least resistant cultivars to spring frost, while Khanjari cultivar was recognized as semi-resistant.

Key Words: Abbasali, Frost resistance, Khanjari, Pistachio, Shahpasand.

INTRODUCTION

Pistachio is a winter dormant tree and its leaves nearly appear at the same time as its blossoms when the plant is totally sensitive and vulnerable to spring frost. Pistachio is cold stricken in the flowering stage which normally occurs from the second half of March to the second half of April. In this stage the product is quite sensitive and will suffer considerable damage in face of spring cold. Cold strike comes when temperature falls to the plant's resistance threshold that of course depends on its cultivar type. For pistachio this temperature is 4 °C and lowers[1].

†Pistachio Research Institute, Rafsanjan, Iran.
‡Department of Biological Sciences, Islamic Azad University, Jouybar Branch, Jouybar, Iran.

Pistachio trees end their dormant state late in winter and quickly loose their resistance to low temperatures, this is when their buds appear and begin their hasty growth period. In such a time temperatures near zero are more than enough to damage the young flowers or blossoms of pistachio[1]. In 1997 about 50 % of pistachio crops in Kerman and Semnan provinces were lost due to spring frost. Such a rate makes a loss of about 2500 billion rials (Iranian currency) and reduced pistachio exports about 50-70 %. In 2004 and 2005, some pistachio farms of Kerman province lost up to 60 % of their crops due to spring cold. Though pistachio is not an early-flowerer like almond, apricot and peach yet frost and climatic changes in recent years have shown that Iranian pistachio is a very sensitive plant to spring frost and it is not impossible to witness frost stricken products in coming years given the state of climatic changes. Therefore, due to the strategic significance of pistachio for the country in terms of economy and producing foreign currency, particularly in Semnan province with more than 10 thousand pistachio farmers, there is urgent need for research and development of plans aimed at preventing or reducing damages caused by spring frost[2]. Frostbite damages depend much on the temperature and duration of exposure. The less the temperature the quicker the damages approach rates such as 50 or 100 %[3]. In 2005 Gholipour studied the resistance of Qazvini and Ohadi cultivars and found that the critical temperature causing damages to these two was -4 °C for the buds, -2 for opening bud and +2 for flowers. It was also shown that decline of temperature to 2 °C below the critical threshold would expose the plant to serious irreversible damages (tissue browning). In this experiment the two cultivars of Qazvini and Ohadi demonstrated no significant difference in terms of critical temperature levels. Also opened flowers and unopened buds were the most sensitive and most resistant organs of the plants in face of temperature reduction[4]. Decline of temperature to critical levels at each stage will result in the reduction of natural bud opening rate, opening of the flowers, pollination and most importantly reduction of pollen tube growth and fertilization; these in turn cause fruit hollowness and decline of final performance of the tree[3]. Arpasi *et al.*[5] (2002) exposed the reproducing buds of several domestic pistachio cultivars of Turkey to low tempruture. Results indicated that exposure of the buds to a temperature of -3 °C for 2 h would cause 85 % damage, exposure to -1 °C for 2 h killed more than 60 % of the buds and the same temperature killed 40 % of the Buds in 1 h.

The present study works on Shahpasand, Khanjari and Abbasali cultivars which are particularly important in Damghan region and studies them in terms of resistance and critical temperature levels.

EXPERIMENTAL

The present study was conduced in 2007 and 2008 in order to study frostbite in three pistachio cultivars Khanjari, Shahpasand and Abbasali and determine their damage threshold. The site of sampling was a pistachio orchard in Damghan region. Samples were taken from trees 25-30 years old. The orchards were irrigated in 55-60 day cycles. The water was salty and the soil was sandy loam texture. The parcels of land being used for the experiment were 1 hectare each and the distance between rows of trees was 4 m. The experiment was in the form of completely randomized statistical factorial design with 2 factors in three replications. Two factors assessed that included, factor 1: Cultivar, three pistachio cultivars (Khanjari, Shahpasand, Abbasali) and factor 2: Temperature, in five levels (+2, 0, -2, -4 and -6 °C).

The experiment was done in three phonological stages including swollen bud, newly-opened flowers and newly-fertilized fruit.

Sampling was done in this manner: 5 trees were randomly chosen for each cultivar and from each tree 25 one-year branches having flowers or buds were taken and immediately transferred to laboratory. The branch ends were put in distilled water inside incubator and treated with +2 to -6 °C (temperature falling 2 °C/h).

At the end of each period (2 h) each time 60 buds or flowers (20 in three replications) were taken out of the incubator and their branch ends were put inside water container. After 24 h (time to return to ambient temperature and demonstration of damage symptoms) the flowers and buds were studied in terms of morphology, tissue and organ pathology (with unequipped eye and binoculars). Results of observations were recorded and photos were made. The number of flowers or buds showing any level of damage from natural to necrosis were counted and produced data (based on 20 sample branches taken out in each iteration) were statistically analyzed and their averages were compared through Duncan range test (probability level 1 %).

A binocular was used for microscopic studies and the damage patterns in tissues and reproductive organs of the flowers were thoroughly examined and photos were made when necessary. The goal of such studies is to examine and record the macroscopic effects of frost at organ and tissue levels. Damage indices in this level include change of tissue colors (being a sign of primary and possible reversible damages) and browning (serious irreversible damages to cells and tissues) and necrosis (total destruction of tissues).

Measuring proline and soluble sugars: In order to study the accumulation of proline and soluble sugars in frost organs some samples were chosen after treatment and the following procedure was follows to measure their proline and soluble sugar content *i.e.*, 0.5 g of flower buds were prepared and weighed, crushed in a china container and added with 5 mL of 95 % ethanol. The upper part of the solution (the floating content) was separated and added to 5 mL of 70 % ethanol and this was repeated on the remaining residues. The extract was centrifuged for 15 min at 4500 rpm and then kept in freezer until the time of measuring proline and soluble sugar content at -20 °C. Then proline was measured through conventional methods[6].

To determine the total soluble sugar content 100 µL of the prepared extract or standards were taken and were added with 3 mL freshly-made anteron identifier (15 mg pure anteron + 100 mL sulfuric acid 72 %). They were then placed inside boiling water bath for 10 min. Once the samples cooled down their absorption at 625 nm wavelength was read with spectrophotometer. Curves were drawn by standard of pure glucose[6].

Since studies focused on three organs of swollen bud, opened flower and newly-fertilized fruit, each stage and organ is discussed separately.

Resistance (tissue color change or reversible damages) in swollen bud stage: Differences of cultivars in terms of resistance to frost were studied and it was shown that Abbasali cultivar was more resistant to lower temperature in comparison to the other two, followed by Khanjari and Shahpasand cultivars (Figs. 1-3). According to present results Abbasali cultivar was more resistant to lower temperatures in comparison to Shahpasand and Khanjari cultivars with proportions of 11 and 5 %, respectively, in the swollen bud stage. Therefore Abbasali pistachio is more resistant to frost damage in swollen buds stage.

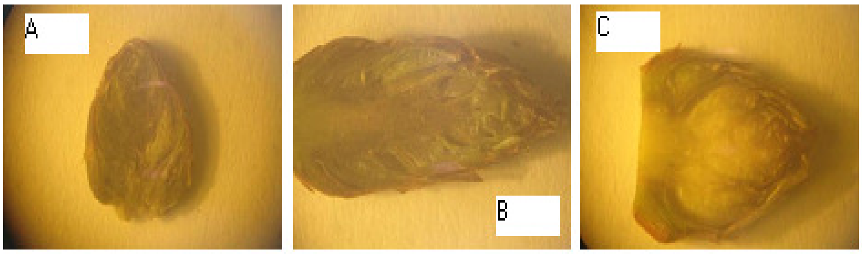

Fig. 1. Swollen bud of Shahpasand (A), Khangari (B) Abbasali cultivar at -6 °C

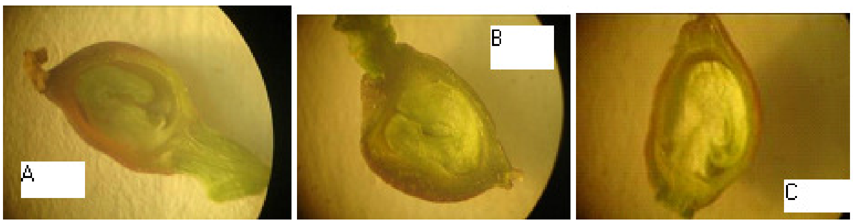

Fig. 2. Fruit of of Shahpasand (A), Khangari (B) Abbasali cultivar

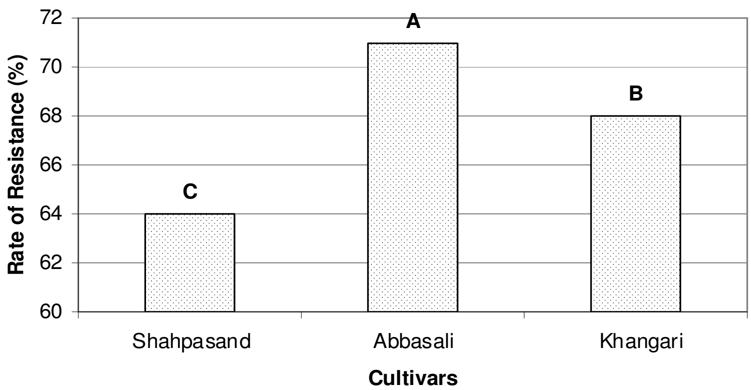

Fig. 3. Rate of resistance (reversible damage) to spring frosts among all cultivars at swollen bud stage

In connection with the effect of temperature comparison of means showed that probability of resistance and survival of swollen buds was 40 % at -6 °C, 50 % at -4 °C, 65 % at -2 °C, 85 % at 0 °C and near 100 % at +2 °C (Table-1). It was expected that the swollen bud stage is the most resistant. The results of present study proved that swollen buds are sensitive to low temperature tensions and there is good chance of damage in this stage as well. In many cases initialized and swollen buds stop growing and never open due to frost effects of nature, which is a good sign of the damages they receive from low temperatures.

Interactions of cultivar type and temperature levels are shown in Table-1. As can been seen here, at -6 °C resistance of Abbasali pistachio was 25 % more than those of Shahpasand and Khanjari cultivars. However, at -4 °C this difference was not significant. At -2° and 0 °C Abbasali cultivar had better survival and resistance scores than the other two.

Resistance rate (tissue color change or reversible damages) in opened flower stage: Results of comparisons showed that resistance to low temperatures and frost damage was highest in Abbasali followed by Khanjari and then Shahpasand cultivars (Table-1). Thus in the opened flower stage Abbasali and Khanjari cultivars showed 30 and 17 % more resistance as compared to Shahpasand.

In connection with the effect of temperature and according to Table-1 results of present study demonstrated that resistance was 100 % at +2 °C and possibility of damage was 0 %. However, decline of temperature to zero and subzero degrees makes resistance rates to fall rapidly. At 0 °C resistance falls 35 % in comparison to +2 °C and the lowest resistance was observed at -4° and -6 °C, *i.e.* about 40 %.

In connection with the effects of temperature and cultivar types, the results of Table-1 point to resistance of Abbasali cultivar at 0, -2 and -4 °C in comparison to the other two cultivars. However, when temperature falls to -6 °C all three cultivars show the same rate of resistance. Khanjari cultivar was more resistant than Shahpasand, yet less resistant than Abbasali. In case of subzero temperatures resistance of Khanjari will be more than Shahpasand.

Resistance (color change or reversible damages) in newly-fertilized fruits: Comparison of all cultivars showed that Khanjari was the most resistant and Shahpasand was the most sensitive type in such a way that the first was 15 % more resistant than the second. Also, Shahpasand cultivar was 12 % more resistant in comparison to Shahpasand (Table-1).

In connection with the effects of temperature, given the fact that newly-fertilized fruits were of female gender comparison of means demonstrated that their resistance was above 90 % at +2 and 0 °C. However, reducing the temperature to -2 °C resulted in a resistance decline to 65 % and a -4 °C temperature produced a resistant rate of 58 % and finally -6 °C faced a resistance of 50 % (Table-1).

Effects of temperature and cultivar type in light of the feminine nature of newly fertilized fruits indicated that Khanjari and Abbasali cultivars were more resistant than Shahpasand at +2 °C. At 0 °C Abbasali was most and Shahpasand was least resistant; while at -2 °C Khanjari was most resistant and at -4° and -6 °C Khanjari was most resistant too (Table-1).

Proline accumulation

Proline accumulation in swollen bud: Variance analysis showed that the effects of cultivar type on the accumulation of proline in swollen buds stage were insignificant. Yet effects of temperature were significant at a 1 % level and mutual effects of temperature and cultivar type were insignificant (Table-2). Given the mean comparison test by duncan method in term of temperature effects, it was shown that reducing the temperature to below zero degree caused a reduction in proline accumulation. Thus at -6 °C it fell 24 % in comparison to +2 °C.

Proline accumulation in opened flower: In this stage the variance analysis showed no significant effect for the cultivar type. Effects of temperature were significant at 1 % level but effects of cultivar and temperature were not significant (Table-2).

In connection with the effects of temperature on proline accumulation in opened flowers stage, it was shown that reduction of temperature from 0° to 6 °C will cause a decline in proline accumulation in such a way that at -6 °C proline accumulation dropped 14 % in comparison to +2 °C. Of course the values obtained for the rate of proline accumulation showed that this accumulation in swollen buds was 50 % less than opened flowers.

Proline accumulation in newly-fertilized fruit: Like swollen bud and opened flower, this organ did not show any significant effect of cultivar and temperature on proline accumulation, yet differences were significant for effects of temperature. Proline accumulation dropped in line with reduction of temperature in newly fertilized fruits so that at -6 °C it showed a 15 % decline. It was obvious of course that proline accumulation in newly-fertilized fruits was 1.5 times that of the opened flower and 3 times that of swollen bud (Table-2).

Accumulation of Soluble Sugars

Soluble sugar accumulation in buds: Given the results of variance analysis the number of effects of cultivar type and mutual effects of cultivar and temperature on the accumulation of soluble sugars in swollen buds was not significant (Table-3). But the effects of temperature were significant at 5 % level and mean comparison demonstrated that accumulation of soluble sugars decreased with the reduction of temperature. Thus at -4 and -6 °C this accumulation dropped 5 % in comparison to +2 °C.

Accumulation of soluble sugars in opened flowers: Variance analysis showed that mutual effects of cultivar and temperature on accumulation of soluble sugars were insignificant for opened flowers (Table-3). Yet the effects of temperature on accumulation of soluble sugars in opened flowers was significant at 1 % level the reduction of temperature decreases accumulation of soluble sugars in opened flowers.

TABLE-1

EFFECTS OF TEMPERATURE AND CULTIVAR TYPE ON RESISTANCE TO SPRING FROSTS AT DIFFERENT STAGES OF OPENING OF REPRODUCING BUDS

	-6 °C			-4 °C			-2 °C			0 °C			2 °C		
Bud stage	K	A	S	K	A	S	K	A	S	K	A	S	K	A	S
Swollen Bud	38.6^{j}	50.3^{i}	38.6^{j}	50.3^{i}	51.9^{i}	50.3^{i}	63.6^{g}	63.6^{g}	68.6^{f}	90.3^{d}	91.9^{c}	71.9^{e}	96.9^{a}	96.9^{a}	93.6^{b}
Open Flower (testa)	11.1^{k}	11.1^{k}	27.7^{i}	11.1^{k}	41.1^{g}	27.7^{i}	29.4^{h}	44.4^{f}	26.1^{j}	46.1^{e}	41.1^{g}	59.4^{d}	84.4^{a}	82.7^{b}	79.4^{c}
Open Flower (Ovary)	12.3^{l}	12.3^{l}	13.9^{k}	12.3^{l}	42.3^{g}	13.9^{k}	33.5^{j}	45.6^{f}	35.6^{i}	37.3^{h}	50.6^{e}	68.9^{d}	98.8^{a}	95.6^{b}	88.9^{c}
Open Flower (stigma)	48.9^{h}	48.9^{h}	48.9^{h}	40.6^{i}	67.3^{d}	30.6^{j}	60.6^{f}	67.3^{d}	55.6^{g}	65.6^{d}	72.3^{c}	60.6^{f}	100^{a}	100^{a}	97.3^{b}
fertilized Fruit	61.9^{i}	61.9^{i}	53.6^{j}	70.3^{g}	61.9^{i}	61.9^{i}	83.6^{c}	75.3^{e}	73.6^{f}	96.9^{b}	100^{a}	76.9^{d}	100^{a}	100^{a}	100^{a}

Values with the same letters within a row are not significantly different according to DMRT ($p \leq 0.05$). K: Khanjari, A: Abbasali, S: Shahpasand.

TABLE-2

EFFECTS OF TEMPERATURE AND CULTIVAR TYPE ON ACCUMULATION OF PROLINE IN DIFFERENT STAGES OF OPENING OF REPRODUCING BUDS

	-6 °C			-4 °C			-2 °C			0 °C			2 °C		
Bud stage	K	A	S	K	A	S	K	A	S	K	A	S	K	A	S
Swollen Bud	4.9^{h}	5.0^{e}	4.9^{h}	5.5^{cd}	5.3^{f}	5.1^{g}	5.4^{de}	5.5^{cd}	5.7^{bc}	5.9^{bc}	5.8^{bc}	5.9^{bc}	61^{abc}	6.2^{a}	61^{ab}
Opened Flower	11.0^{ef}	10.9^{f}	11.3^{def}	11.4^{de}	11.4^{de}	11.6^{cd}	11.6^{cd}	11.8^{bcd}	11.9^{bc}	11.7^{bcd}	11.9^{bc}	12.2^{abc}	12.2^{abc}	12.8^{a}	12.4^{ab}
fertilized Fruit	14.5^{e}	14.9^{de}	14.7^{de}	15.0^{cde}	15.1^{cd}	15.0^{cde}	15.6^{bc}	15.6^{bc}	15.1^{cd}	15.9^{b}	16^{b}	15.9^{b}	16.5^{a}	16.6^{a}	16.7^{a}

Values with the same letters within a row are not significantly different according to DMRT ($p \leq 0.05$). K: Khanjari, A: Abbasali, S: Shahpasand.

TABLE-3

EFFECTS OF TEMPERATURE AND CULTIVAR TYPE ON ACCUMULATION OF SOLUBLE SUGARS IN DIFFERENT STAGES OF OPENING OF REPRODUCING BUDS

	-6 °C			-4 °C			-2 °C			0 °C			2 °C		
Bud stage	K	A	S	K	A	S	K	A	S	K	A	S	K	A	S
Swollen Bud	295^{a}	296^{a}	294^{a}	295^{a}	296^{a}	296^{a}	299^{a}	302^{a}	293^{a}	301^{a}	301^{a}	301^{a}	306^{a}	205^{a}	304^{a}
Opened Flower	363^{e}	364^{de}	364^{de}	368^{d}	269^{d}	371^{cd}	369^{d}	375^{c}	377^{c}	378^{c}	381^{bc}	382^{bc}	385^{abc}	393^{a}	387^{ab}
fertilized Fruit	410^{h}	420^{fg}	417^{fgh}	414^{gh}	425^{de}	422^{ef}	419^{fg}	431^{bc}	427^{cde}	424^{de}	435^{ab}	431^{bc}	430^{bcde}	445^{a}	433^{bc}

Values with the same letters within a row are not significantly different according to DMRT ($p \leq 0.05$). K: Khanjari, A: Abbasali, S: Shahpasand.

Accumulation of soluble sugars in newly-fertilized fruits: Effects of temperature on accumulation of soluble sugars in newly-fertilized fruits were significant at 1 % level. Yet the effects of cultivar type and temperature were insignificant. In connection with the effects of temperature on accumulation of soluble sugars, mean comparison proved that reduction of temperature decreased accumulation of soluble sugars so that at -6 °C this accumulation falls 5 % as compared to +2 °C. The other result related to accumulation of soluble sugars in different organs was maximum accumulation was observed in newly-fertilized fruits and its minimum was seen in swollen buds, leaving the intermediate level for opened flowers (Table-3).

RESULTS AND DISCUSSION

Present results showed that the late spring frost damage of pistachio was variable according to stages of flower bud development and genotypes. Proebsting and Mills[7] indicated that during blossom bud development from dormancy to post bloom T_{50} rose to near -3 ºC for 'Delicious' apple, 'Bartlett' pear, 'Bing' cherry, 'Elberta' peach, 'Early Italian' prune and some apricots. In controlled freezing tests, -6 ºC was determined as the critical temperature for 50 % (T_{50}) kill of all pistachio cultivars at swollen bud stages. But 'Abbasali' pistachio have been more resistant than the average laboratory value on several occasions.

Stage of bloom development was significant in response to frost damage. At full bloom, the first symptom observed after thawing in pistachio flowers is a brown discoloration at the base of the style and damage may extend both to the style and to ovary, resulting in death of the placenta abortion of ovules, or formation of large breaks in the cortical tissue. Factors affecting spring frost hardness are environmental, pruning, crop load, nutrition, rootstock, chemical and genotype. Spring frost hardiness in deciduous fruit is influenced primarily by genotype[8].

Critical temperatures were known in which the stage of opening of the buds was accompanied by evolving female organs facing severe problems and there was possibility of disorders in their growth and opening of their flowers. While in opened flowers the primary damages were mostly inflicted to stigmas, which might pronounced problems in the reception of pollens or growth of pollen tubes. Obviously, exposure of trees in shorter periods of time results in less damages, while longer exposure times will bring deep damages. Decline of temperature at a rate of 2 °C in each stage may result in the browning of reproducing organs in any of the three stages discussed above, which is called loss of physiologic life; and lower temperatures may result in total destruction of the organ which is called necrosis[9,10]. Though it is said that this level stress damage is reversible to large extents, more accurate observations have proved that decline of temperature to critical levels will result in reduced natural opening of buds, opening of flowers, pollination and above all reduction of pollen tube growth and fertilization. These result in increased rates of blank fruits and decline in the final yield of the tree[3].

Lower temperatures result in more severe cellular oxidation activities, destruction of cellular structure and eventually browning of tissue which is final irreversible damage of the stress. The first signs of browning may be observed in stigmas. This damage results in inhibition of pollen tube growth which in turn results in no fruit produced[6,11].

Studies have shown that frost damages happen much sooner than what we see on the trees at macroscopic level. In fact major parts of damages may be tracked at the level of reproducing organs, which must be taken into account in related studies and in estimation of damages. Though parts of these damages are reversible, they eventually result in reduction of production rates due to reduced yield of pollination and fertilization.

If we can foresee temperature falls we may be able to take some agronomic or other measures to delay the opening of the buds so that damages caused by temperature loss are controlled. For example, a two-year study of pistachio orchards demonstrated that the time of beginning agronomic operations such ploughing and irrigation have a direct positive relationship with the time of opening of buds and flowers. Thus delaying such operations may be a good preventive measure[12].

On the other hand, results of these tests demonstrated how temperature changes of one or two degrees may convert chilling damages from minor reversible to major irreversible losses finally leading to browning of the tissues. This proves that just a couple of degrees increase in temperature through various methods like chemical and/or agronomic- and reducing the lower end of super cooling (temperature at which tissues begin to freeze) through methods such as spraying frost protective substances, fighting bacteria producing ice cores, *etc*. may be quite effective in preventing damages inflicted in low temperatures. This field obviously requires more study[13]. In cold acclimated cultivar (Abbasali) proline content was higher than nonacclimated plants and cold acclimated cultivars recovered faster than non-acclimated ones. So, because of prolin,s protective role in plants in stress conditions, we can say, cold acclimated cultivars could tolerate chilling temperatures better than non-acclimated cultivars. In leaf samples, amount of praline increased in acclimation specially in cold acclimated cultivar (Abbasali), but it decreased in chilling temperatures and again increased in recovery phase[14]. Proline synthesis is implicated as a mechanism of alleviating cytoplasmic acidosis and may maintain $NADP^+$/NADPH ratios at values compatible with metabolism. Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress-induced damage[15,16].

In several reports, it has been suggested that amino acids, especially proline, may protect higher plants against frost injury[17-20]. In potato, frost tolerance appeared positively correlated with leaf proline content and additionally, exogenous application of proline resulted in an increase in frost tolerance in the leaves[21]. Therefore, high proline levels may serve as selection criterium in screening for frost tolerance in potato. Also soluble sugars, such as sucrose, may serve as indicators of frost tolerance in quinoa breeding material[22]. Bravo *et al*.[23] mentioned that in barley, after 11 days of cold acclimatization, the sugar content increased 8 times, whereas in quinoa a 10 times increase was seen. As soluble sugars act as osmo regulators, a higher content of dissolved solutes in the intracellular liquid reduces the freezing point, increasing tolerance to cellular freezing. The significant difference between sugar content and both freezing temperature and LT_{50} means that soluble sugar content may be utilized as an indicator of frost tolerance.

Findings of the present study may be summarized as follows: (i) At the swollen bud stage it was seen that Abbasali cultivar was most resistant and Shahpasand cultivar was the lease resistant to low temperatures, leaving the medium level of resistant to Khanjari cultivar. (ii) At the swollen bud stage a temperature of -2 °C was recognized as the critical threshold and temperatures below -2 °C increases the probability of damage to buds up to 50 %. (iii) Unlike most people think that the swollen bud stage is quite resistant, these results indicate that the swollen bud stage is sensitive to low temperatures as well and there is an obvious probability of damage in this stage like other stages. In many cases failure of initialized and swollen buds in opening is observed in face of cold weather in nature, which is probably due to chilling damages they take. (iv) In the opened flower stage too Abbasali and Khanjari cultivars were 30 and 17 % more resistant than Shahpasand. (v) At the opened flower stage a temperature of -2 °C was the critical threshold and when it fell to below -2 the possibility of serious damages and total destruction of the crops was increased by 50 %. As for newly-fertilized fruits, Shahpasand cultivar was the most sensitive and Abbasali was the most resistant. (vi) At the newly-fertilized fruit stage a temperature of -4 °C was critical and below that there was an increased 50 % possibility of damage. (vii) Among studied organs, the opened flower was the most sensitive followed by swollen bud and newly-fertilized fruit. Of course this is against the results obtained for other fruits for in most of them newly-fertilized fruits were more sensitive than opened flowers[9,12,24].

Stage of bloom development was significant in response to frost damage. At full bloom, the first symptom observed after thawing in pistachio flowers is a brown discoloration at the base of the style and damage may extend both to the style and to ovary, resulting in death of the placenta abortion of ovules, or formation of large breaks in the cortical tissue. Factors affecting spring frost hardiness are environmental, pruning, crop load, nutrition, rootstock, chemical and genotype. Spring frost hardiness in deciduous fruit is influenced primarily by genotype[8].

REFERENCES

1. Z. Jahangiry, G.H. Kamali, K. Nouhi and M. Ahmadi, First Gathering of Apply and Scientific Method of Frost Control (In Persian) (2005).
2. H. Hokmabadi, K. Arzani and P.F. Grierson, *Aust. J. Agric. Res.,* **56**, 135 (2005).
3. H.A. Quamme, *J. Am. Soc. Hort. Sci*., **103**, 5761 (1978).
4. Y. Gholipour, *Acta Hort*., **726**, 121 (2005).

5. S. Arpasi, H.S. Atli, H. Tekin and M. Burak, *Acta Hort*., **726**, 82 (2005).
6. Y.L.W. Chien, *Hort. Sci.*, **29**, 98687 (1994).
7. E.L.J. Proebsting and H.H. Mills, *J. Am. Soc. Hort. Sci*., **103**, 192 (1978).
8. A. Aygun and S. Bekir, *Tarim Bilimleri Dergisi*, **11**, 283 (2005) (In Turkish).
9. D.H. Byrne, *Hort. Sci.*, **15**, 1235 (1986).
10. M. Kaneda, H. Yoshiaki, S. Takashi and S. Takuo, *J. Jap. Soc. Hort. Sci*., **70**, 516 (2001).
11. Z. Szabo, J. Nyeki and M. Soltesz, *Acta Hort*., **410**, 23 (2000).
12. J. Rodrigo, *Sci. Hort.*, **85**, 155 (2000).
13. X. Hao, G. Marlyn, L.P. Cheryl and R. Bernard, *Can. J. Microbiol*., **44**, 64 (2000).
14. L. Zeinali, R. Heidari and J. Carapetian, *Res. J. Biol. Sci*., **3**, 74 (2008).
15. P.D. Hare and W.A. Cress, *Plant Growth Regul*., **21**, 79 (1997).
16. P.D. Hare, W.A. Cress and J. Van Staden, *Plant Cell Envir*., **21**, 535 (1998).
17. S.F. Boggess and C.R. Stewart, *Plant Sci. Lett*., **17**, 245 (1980).
18. A.J. Delauney and D.P.S. Verma, *Plant J*., **4**, 215 (1993).
19. T.E. Elthon and C.R. Stewart, *Plant Physiol*., **70**, 567 (1982).
20. S.F. Boggess, D. Aspinall and L.G. Paleg, *Aust. J. Plant Physiol*., **3**, 513 (1976).
21. A.C. Van sawaij, H. Nijdam, E. Jacobsen and W.J. Feenstra, *Euphytica*, **36**, 369 (1987).
22. S.E. Jacobsen, C. Monteros, L.J. Corcuera, L.A. Bravo, J.L. Christiansen and A. Ujica, *Eur. J. Agron.*, **26**, 471 (2007).
23. L.A. Bravo, G.E. Zuniga, M. Alberdi and L.J. Corcuera, *Physiol. Plant*, **103**, 17 (1998).
24. M.N. Westwood, W.H. Freeman and Company, San Francisco, p. 303 (1996).

(*Received*: 14 August 2009; *Accepted*: 2 December 2009) AJC-8130