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ORIGINAL ARTICLE

Evaluation the Resistance of Almond Cultivars to Spring Frost at different Phenological stages

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ABSTRACT
Almond cultivation was restricted to regions with low risk of spring frosts. Spring frost is a limiting factor for yield and its stability and for almond production and very little is known about frost susceptibility of almond cultivars. Therefore Shoots with flowers in three phenological stages (popcorn, flowering and young fruit) were harvested from three almond cultivars then frost treatment were applied in each stage separately. Frost damage of ovary, proline, ion leakage, peroxidase and catalase measurements were evaluated. The results showed that the most resistant cultivar to spring frost was 6-8 cultivar. Among all cultivars and different phenological stages the highest proline amount was in 6-8 cultivar and the lowest rate was in Shahroud 12 cultivar. The most peroxidase activity was observed in Shahroud 12 cultivar in popcorn stage at -0.5˚ C. Among different cultivars the highest catalase(CAT) activity was in flowering stage while in Shahroud 12 cultivar in popcorn stage CAT activity was the highest. The highest amount of ion leakage in Shahroud 12 cultivar in popcorn stage at -5 and -7˚ C was occurred. As a general result, among all of cultivars, 6-8 was the most resistant cultivar to spring frost damage.
Keywords: *spring frost, phenological stage, proline, ion leakage, catalase, peroxidase.*

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INTRODUCTION

Almond (*Prunus amygdalus*) is an early blooming species, thus susceptible to spring frosts [18]. Although, the almond is resistant to low temperature in the winter, but certain degree and duration of low temperature in the spring frost is lethal to the most reproductive organs at the blooming period [15]. Almond cultivation was restricted to regions with low risk of spring frosts [18]. Not only almond cultivation in Iran but also in world is characterized by high degree of risk due to a range of adverse climatic factors such as drought, soil salinity and particular spring frost [31].Low temperatures during and after bud break may limit the distribution of deciduous fruit trees and may also seriously impair fruit production caused by freezing injuries in areas where the particular crop is already well established [31]. The periods of major frost risk are in the beginning of the an thesis and towards to active growth [30].
In *Prunus*, fruit damage is detectable by observing ovule browning, ice formation [33] and browning of the petals and pistils [18]. Some factors such as genotype, developmental stage, the formation of ice, moisture content, nutritive status of the pistil and even environmental factors have been mentioned in the resistance of flower buds to spring frosts[12]. In addition, destructive temperatures vary on not only the phonological stage, but also vary among species, cultivars, orchards [30] and even within the trees [50].Floral buds of fruit trees are resistant to low temperature impact while in dormancy, but later in spring, the period of dormancy ends, plant begins to grow and resistance to cold gradually decreases [14].

Bud development is influenced by plant age and healthiness, bud positioning and density in the shoot, climatic conditions, geographical area, agrotechnical methods, usage of pesticides [41].Two main causes are behind fruit set failure after flowering pollination problems [32] and spring frost damage [12].In some cases, the accumulation of organic osmolite like proline, glycine, betaine can be considered, that their amounts increase in stress conditions [9]. Proline seems to have various roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species [5, 46]. Frost affects cell membranes, which become less permeable and even break, giving rise to the leakage of solute from damaged cells. There is often a good correlation between ion leakage and freezing tolerance [21].

BACKGROUND RESEARCHES

Kodad and Socias(2004) reported that frost damage in almond is highly dependent on the phonological stage of the bud/ flower/ fruit, the early developing fruit being the most susceptible stage [18]. Imani et al. (2012) found that the severity of frost damage in almond was influenced by genotype. Genotypes that had more resistant to frost damage had higher amount proline content [15]. Stepulaitiene et al.(2013) found that frost resistance is associated with development of *Prunus cerasus* generative buds [37]. The hardiness of flower buds of apricot at three different developmental stages was tested at -4̊C for 1 hour and 3 hours [12]. Survival rates of pistils of seven apple varieties at various stages from tight cluster to full bloom on trees were determined after frost [3].The objective of the present study was to assess the late spring frost damage in the flower bud phenological stage and determine cultivar or genotype resistant to spring frost.

MATERIALS AND METHODS

The study was conducted at Meshkinshahr Collection Orchard, Seed and Plant Improvement Institute (SPII), Karaj, Iran in 2013. Nonpareil, 6-8 and Shahroud12 cultivars were used in this experiment. 6 years old Experimental trees were planted 5×5 m apart. This experiment was based on randomly complete design with three replications. Each plot consisted of three trees.

Shoots with flowers in three phenological stages (popcorn, flowering and young fruit) with equal length (10-12 cm) and diameter (1.5-2cm)were harvest from three almond cultivars in early morning then were placed in a container include water and carried quickly to the laboratory. Shoots were taken are represented of all positions on the trees. For frost treatment, shoots were spread in to a chamber(432 L;ASLAparatos Cientificos,Madrid Spain).This programmable chamber model is equipped with a heat–cold unit working in the – 20˚ C to 30˚ C. Five thermopar probes connected to a data logger (LI-100; LI-COR, Inc., Lincoln, Neb) were placed near the samples. Air temperature in the chamber was maintained at 7˚ C for 50 min and then programmed to decline by 2˚ C.h^{-1} until the desired frost temperature was reached. The frost temperature was maintained for 30 min. then transferred to refrigerator at 4˚ C for 6 hand Frost damages rate was evaluated 24 h after Frost treatment in 21˚ C.Then flowers detached from theshoots, placed for dissection into a petri dish and observed for frost damages onmicroscope. The pistil is the most frost sensitive of organ in the flower.In almond, the first tissue affected can be the pistils, the petals, or both. For this reason, flowers were considered frost damaged when pistils in them were brownish, because of pistil is the effective organ for developing into nut. Frost was applied in the popcorn stage three temperature (-3, -5 and -7˚C) and also in the flower stage three temperature (-1.5, -3 and –4.5˚ C) and in young fruit stage three temperature (-0.5, -1.5 and -2.5˚ C)[16].

Determining the proline

For determining the proline rate, the plant material was crushed in a mortar with 10 ml sulfosalicylic acid and centrifuged at 4000 rpm for 20 min. The 2ml of the supernatant was mixed with 2ml ninhydrin and 2ml acetic acid, and incubated for 1h at 100°C. The 4ml toluene was added, and absorbance was determined by a

spectrophotometer at 520nm.Proline content was derived from a standard curve obtained with pure proline (Merck KGaA,Darmstadt, Germany), according to Bates et al. (1973) [5]. The statistical analysis was performed using Microsoft Excel(2007) and SAS software and means were compared using Duncan's Multiple Range Test (DMRT).

Ion Leakage

EC was measured according to Barranco et al. (2005) [4]. 0.5 g fresh weight of the flowers excised and washed in deionized water. Afterward samples were placed in an Erlenmeyer flask containing 15 mL of deionized water. The flasks were then shaken for 24h using a conductance meter (Consortmodel C831, Turnhout, Belgium) at 120 rpm in light conditions and temperature of 20 to 22°C. The initial electrolytic conductivity of each solution (initial EC, in µS.cmG1) was measured, to obtain an indirect indication of the

amount of ion released at each freezing temperature. Sample tubes were then autoclaved (1h, 100˚ C, 1 atm) to kill the tissues completely. After 2 h shaking at 200 rpm in light conditions, electrical conductivity was measured again,to obtain a reference value for total ions. EC was calculated as:

$EC= ((EC_2-EC_1)/EC_2)\times100$

Peroxidase assay:

PO activitywas assayed spectrophotometrically with guaiacol by measuring an increase in absorbance at 470 nm(ε= 26.6 mM-1cm-1) according to Maehly and Chance (1954) [24]. The mixture of 0.5 cm3 of the enzyme extract, 0.5 cm3 of 50 mM acetate buffer (pH 5.6), 0.5 cm3 of 20 Mm guaiacol and 0.5 cm3 of 60 mM $H_2O_2$was used. The enzyme activity was expressed in units (mmol tetraguaiacol min-1) per g fresh weight.

Catalase assay:

CA activity was determined at 25 ˚C according to Aebi [1]. The reaction mixture contained 40 mM phosphate buffer pH 7.0 and 0.1 ml pure enzyme in a total volume of 3ml. CAT activity was estimated by decreased in absorbance of H2O2 at 240nm.

RESULTS AND DISCUSSION

Frost damage

Results showed that the severity of frost damage was influenced by variety; frost damage rate was significantly affected by morphological and phenological stage of flower buds. Shahroud 12 cultivar suffering the greatest frost damage rate and the least frost damage was observed in 6-8 cultivar. The most frost damage in Nonpareil and 6-8 cultivars was occurred at flowering stage at -4.5˚ C and small fruit stage at -1.5 and -2.5˚ C. while Shahroud 12 cultivar suffering the greatest frost damage rate at flowering stage at -4 and -3˚ C, at small fruit stage at -1, -2, and -5˚ C and at popcorn (balloon) stage at -5 and -7˚ C (Fig.1).In all above cultivars the amount of frost damage was 100%. The 6-8 cultivar at popcorn stage had the maximum resistant to frost [15].

Phenological stage seems to be important regarding the degree of frost damage, as trees were more affected at full bloom than at the popcorn stage [16]. The risk of frost injury of the reproductive organs may increase with phenological development stage and growth and low temperature [15].

Almond has demonstrated hardiness at pre bloom and less hardly at post bloom [25]. Miranda et al. (2005) concluded that *Prunus* species such as almond resists to frost without major damage before the pre bloom phase, but is susceptible to frost during and after anthesis [26]. Almond is most susceptible to frost from the first swell bud stage on ward, and much less susceptible in fully dormant [15].

Proline

Proline of almond cultivars concern to variety type was different. The highest proline rate was in popcorn stage at -7˚C and after that flowering stage at -3˚ C in 6-8 cultivar and the lowest rate was in popcorn stage at -7˚ C in Shahroud 12 cultivar. Among all cultivars and different phenological stages the highest proline amount was in 6-8 cultivar and the lowest rate was in Shahroud 12 cultivar(Fig.2).

Proline production increases in higher plants under stress conditions take place using two glutamate (nitrogen deficiency) and uretin (nitrogen is high in the cell) cycles [15].

In sensitive plants to cold, cell proline increase is not sufficient to cause cold resistance increase, unless high amounts of proline are added before the stress. Of course cell proline increase always doesn't cause an increase in cold resistance [9]. The highest proline rate was in an thesis stage and the lowest rate was in popcorn stage of sprout [15].

The results are similar with Rodrigo [31] reports that emphasized the important attention on structural, phenilogical, morphological and physiological feature of almond cultivars when selection plant material for new varieties, as this is a mechanism of frost escape. Proline increased in the leaves of citrus species after cold treatment [20] and the content of amino acids in leaves of maize increased during chilling period [40].

Peroxidase

Among all cultivars and different developmental stages under cold stress the highest peroxidase activity was in Shahroud 12 and Nonpareil cultivars and the lowest peroxidase activity was in 6-8 cultivar. The most peroxidase activity was observed in Shahroud 12 cultivar in popcorn stage at -0.5˚ C (Fig. 3). Peroxidases are a large group of isoenzymes with an extreme range of isoelectric points, serving a multitude of functions [44]. Each group is thought to have a different function in the cell.Acidic peroxidases are the isoenzymes most likely involved in lignin formation and wall-associated, whereas function of basic isoenzymes has been suggested that they might provide H_2O_2 for other peroxidases [48]. Cansev et al. [7] reported expression of acidic peroxidase bands with different band intensities which are responsible for tolerance to freezing stress of 15 olive cultivars. Gulen et al., [11] indicated a correlation

between peroxidase activities and cellular damage provoked by low temperature treatment. The considerable increase of peroxidase activities could not stop the deleterious effects of low temperature, but reduced stress severity thus showing a reduction in the percentage of injury. The highest level of APX activity in citrus unshiu unripe fruits was at 3˚ C, and 0˚ C for ripened fruits[2].

Environmental stresses increase the formation of ROS that then oxidize photosynthetic pigments, membrane lipids, proteins and nucleic acid [10, 35]. Several lines of evidence suggest that low temperatures alter cellular homeostasis by increasing the level of ROS at the transcript protein and activity levels [39].

In addition, the induction of oxidative stress by low temperatures is likely to be the main factor contributing to frost injury in different fruits [36]. Sala(1998) observed that chilling injuries were reduced in cold [36]. Tolerant mandarin cultivars that had a more efficient antioxidant system [2]. Plants with high levels of either constitutive or induced antioxidants were reported to have greater resistance to oxidative damage [38].

Differences in stress tolerance among plant species and intraspecific genotypes are intrinsically associated with the development of antioxidant systems under stress [27]. Peroxidase (POD) activity plays on important role in the oxidative degradation of phenolic compounds, which can lead to the production of brown polymers [43]. POD activity in fruit peel may be linked to the higher cell damage as a response to stress [22]. POD enzyme activity in the flavedo tissue of unripe citrus lemon and citrus unshiu fruit significantly increased during temperature reduction [2]. Peroxidase activity increased steadily in zucchini squash during storage at 5˚ C [49]. Chilling temperatures also enhanced peroxidase activity in mango fruit [51] and avocado fruit under restricted ventilation [45].

Catalase

Catalase activity was lowest in Shahroud 12 cultivar in small fruit stage at -0.5˚ C and the highest amount of catalase activity was in Nonpareil cultivar in flowering stage -3˚ C. Among different cultivars the highest catalase activity was in flowering stage while in Shahroud 12 cultivar in popcorn stage CAT activity was the highest(Fig.4).Several active free-radical scavenging enzyme systems exist in plant tissues as defenses against free-radical attack [49]. Catalase uses hydrogen peroxide both as a donor of hydrogen and as a substrate in the catalytic decomposition of hydrogen peroxide to form oxygen and water [6].

Catalase activity was found to decrease in zucchini squash tissue during chilling [49]. Decrease in catalase activity during chilling has also been found in avocado fruit [34], cucumber leaves [29], and maize leaves [42].Decreasing catalase activity and increasing peroxidase activity could lead to the slow removal of H_2O_2 may aggravate oxidative damage, such as the oxidation of the sulfhydryl group, and intensify chilling damage in tissues [49]. Process of stress tolerance in plants may be accompanied by an increased activity of one or more antioxidative enzymes [8].

Cansev et al. (2011) was found that CAT and APX activities displayed seasonal alternations in both leaf and bark tissues of olive. Certain changes occur in plant antioxidative enzyme activities during adaptation to cold [47]. Kuk et al. (2003) reported that cucumber leaves that are tolerant to cold exhibited higher CAT and APX activities compared with the leaves of those that are non-tolerant indicating an association of low temperature tolerance with enhanced antioxidative enzyme activities [19]. Chen et al., 2006 explained that CAT and APX activities were increased during cold adaptation in ever green Sabina plant [8].

Ion leakage

The highest amount of ion leakage in Shahroud 12 cultivar in popcorn stage at -5 and -7˚ C was occurred. And the lowest amount of ion leakage was in small fruit stage of 6-8 and Nonpareil cultivar at -0.5˚ C (Fig. 5).Imani et al., 2011 reported Sh16 as the late blooming had the maximum resistant to frost with at least ion leakage and Nonpareil cultivar 87.5% damage in same temperature with 88.67% ion leakage [17].According to investigations of Murata and Tatsumi, (1979), Hardwick and Anderews, 1980 and Lindon, 2002, the level of cold tolerance among cultivars of species and the amount of ion leakage in response to stress had been the different [28, 13, 23]. They also concluded that electrolyte leakage, a public property for all species is not sensitive to freeze. While Imani et al., 2011 was found that the electrolyte leakage of almond cultivars flowers in response to freeze stress increased. There was relation between frost damage and ion leakage in almond cultivars that had the more resistant to frost damage had less ion leakage [17].

Cell membrane damage is the first indication of irreversible injury following stress in plants. Determination of this damage by ion leakage method bears principal significance in identification of stress tolerance in a given plant and has been utilized to characterize plant tolerance against many types of stress, including temperature stress. Researchers investigated the degree of cell membrane damage by

ion leakage method in order to determine the response of olive leaves and barks to various low temperatures (-4, -5, -10 and -20˚ C) [17].

The electrolyte leakage test is based on the principal that the damage to cell membranes results in an enhanced leakage of electrolytes (mainly K^+) from the cell. Recording the amount of leakage will thus provide an estimate of tissue damage [17, 23].

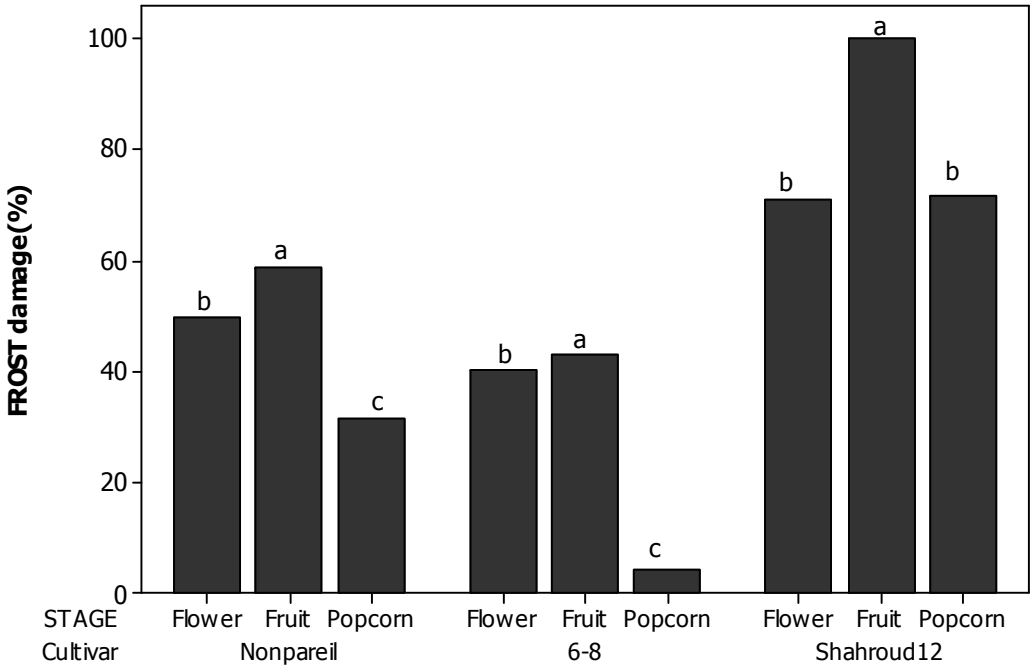

Figure 1 Frost damage in 3 cultivars and different flower bud developmental stage.

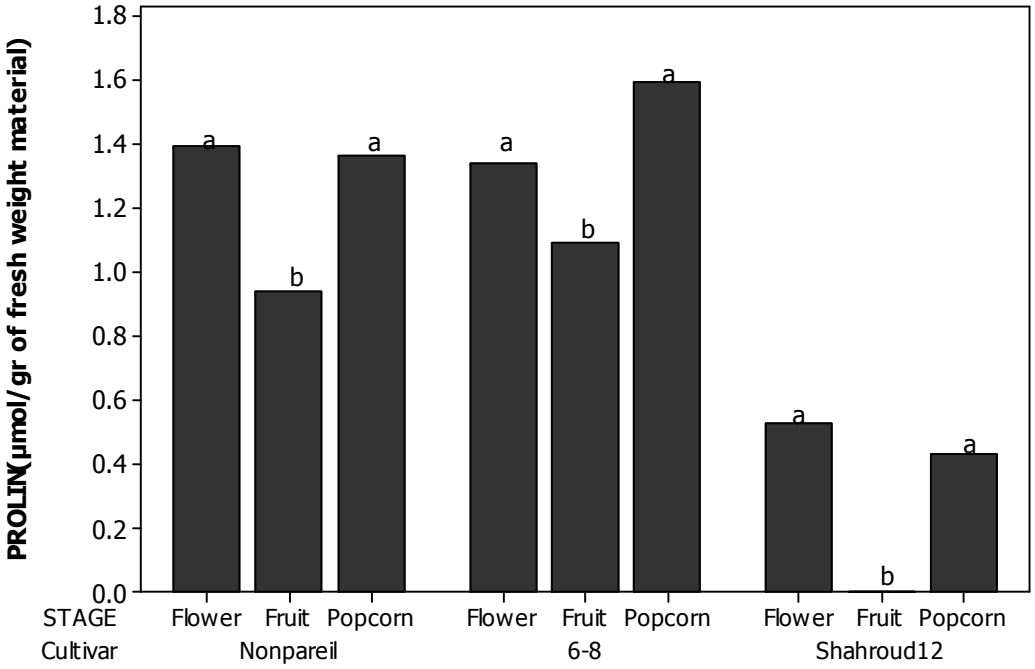

Figure 2Proline amount in 3 cultivars and different flower bud developmental stage.

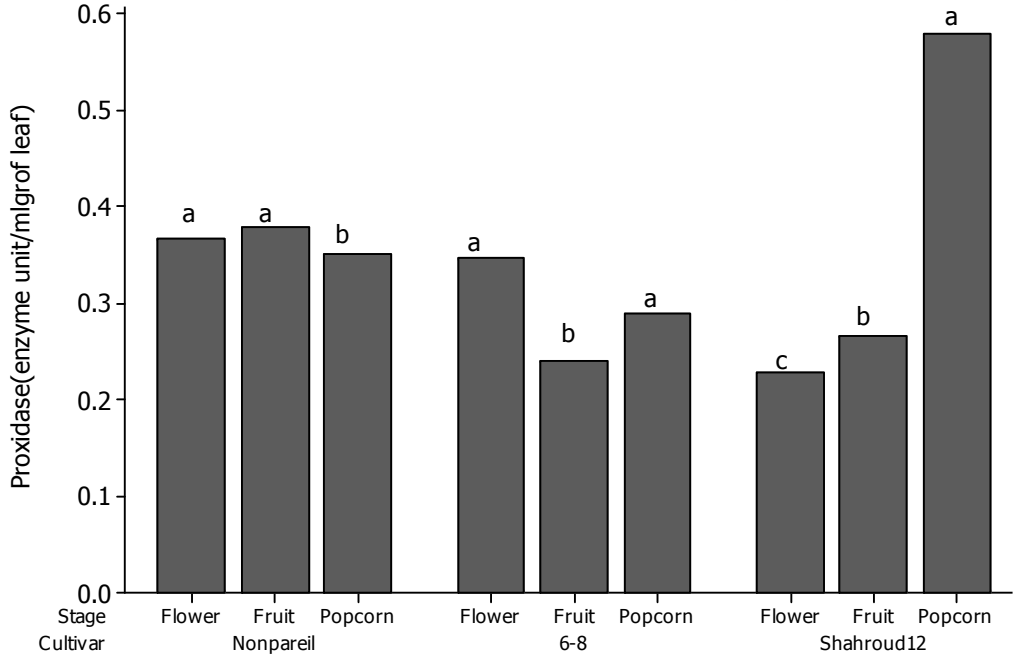

Figure 3Peroxidase activity in 3 cultivars and different flower bud developmental stage.

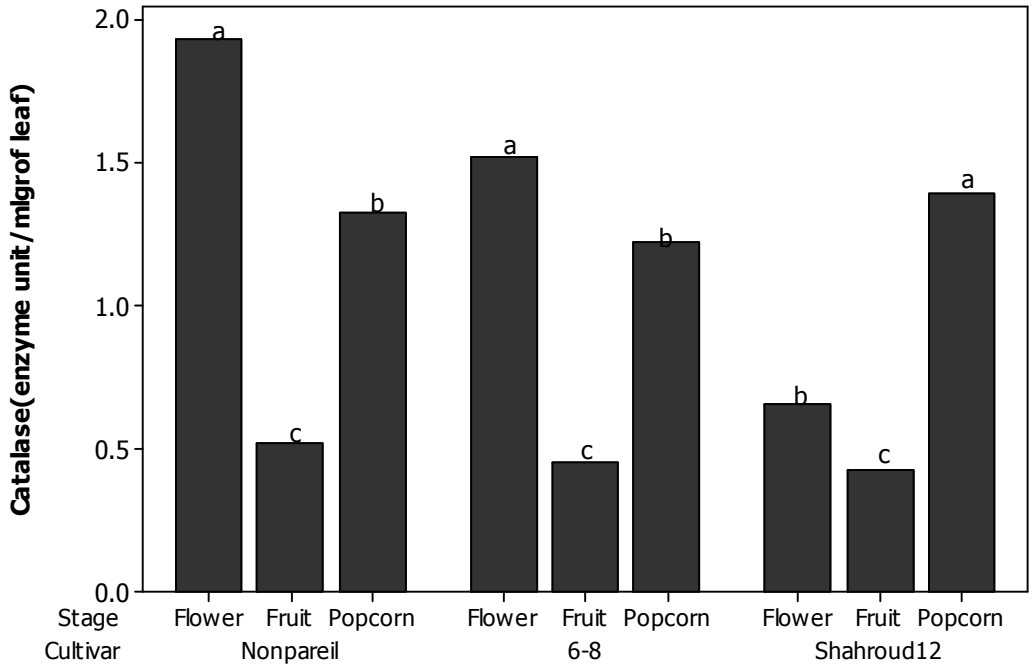

Fig.4. Catalase activity in 3 cultivars and different flower bud developmental stage.

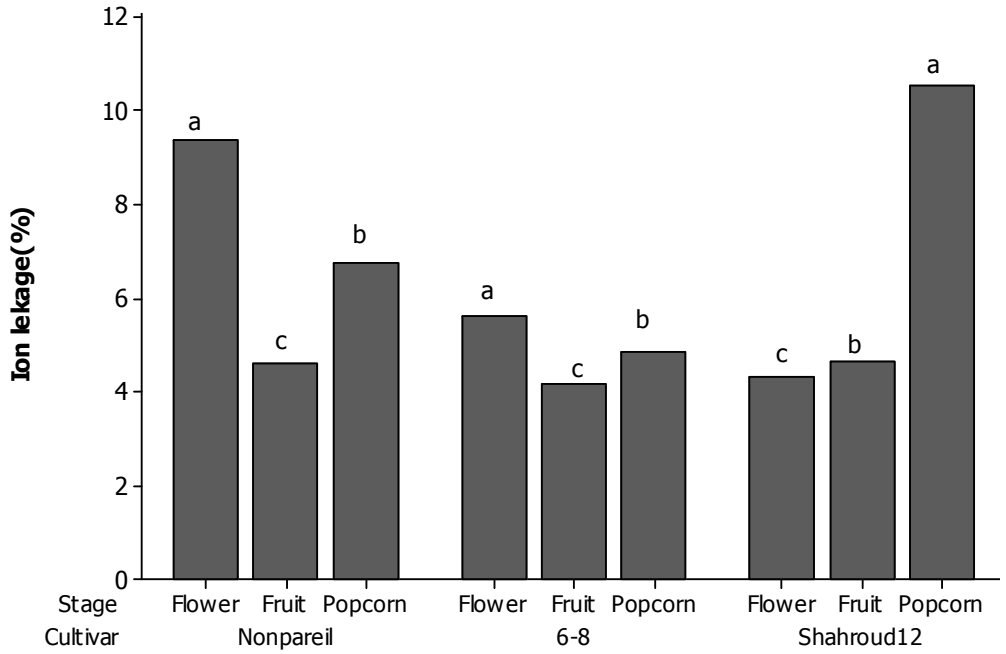

Fig.5. Ion leakage in 3 cultivars and different flower bud developmental stage.

REFERENCES

1. Aebi, H. (1984). Catalase. In: L. Packer (Ed), methods in enzymology, Academic Pres, Orland, 105: 121-126.
2. Afshar Mohammadian, M., Khosravi Larijani, Z. and Sajedi, R.H. (2012). Quantitative and qualitative comparison of antioxidant activity in the flavedo tissue of three cultivars of Citrus fruit under cold stress. Australian J. of Crop Sci., 6(3): 402-406.
3. Aygun, A. and San, B. (2005). The late spring frost hardiness of some apple varieties at various stages of flower buds. Tarim Bilimleri Dergisi., 11(3): 283-285.
4. Barranco, D., Ruiz, N. and Gomes, M. (2005). Frost tolerance of eight olive cultivars. Hortsci., 40(3): 558-560.
5. Bates, L.S., walderen, R.D. and Taere, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil., 39: 205-207.
6. Burris, R.H. (1960). Hydroperoxidase (peroxidases and catalases). In: W. Ruhland (Editor), Encyclopedia of plant physiology, Vol.12. Springer Verlag. Berlin., 365-400.
7. Cansev, A., Koksal, N., Gulen, H., Ipek, A. and Eris, A. (2005). Dusuk sicaklik stresi altindaki bazi zeytin cesitlerinin peroksidaz aktivitesine gore gruplanmasi. XIV. Biyoteknoloji Kongresi, Bildiriler Kitabi., 313-317.
8. Chen, Y., Zhang, M., Chen, T., Zhang, Y. and An, T. (2006). The relationship between seasonal changes in anti-oxidative system and freezing tolerance in the leaves of evergreen woody plants of Sabina. S. Afr. J. Bot., 72: 272-279.
9. Duncan, D.R. and Jack, M. (1987). Proline accumulation and its implication in cold tolerance of regenerable maize callus. Plant Physiol., 83(3): 703-708.
10. Egert, M. and Tevini, M. (2002). Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives. Environ. Exp. Bot., 48: 43-49.
11. Gulen, H., Cetinkaya, C., Kadioglu, M., Kesici, M., Cansev, A. and Eris, A. (2008). Peroxidase activity and lipid peroxidation in strawberry plants under low temperature. J. Biol. Environ. Sci., 2(6): 95-100.
12. Gunes, N.T. (2006). Frost hardiness of some Turkish apricot cultivars during the bloom period. Hort. Sci., 41: 310-312.
13. Hardwick, R.C. and Anderews, D.J. (1980). A method of measuring differences between varieties in tolerance to suboptimal temperatures. Ann. Appl. Biol., 95: 235-246.
14. Iezzani, A.F. (1985). Genetic differences for spring floral bud development among sour cherry cultivars. Acta Hort., 169: 123-126.
15. Imani, A., Ezaddost, M., Asgari, F., Masoumi, S.H. and Raesi, I. (2012). Evaluation the resistance of almond to frost in controlled and field conditions. Inter. J. of Nut. and Rel. Sci., 3(1): 29-36.
16. Imani, A. and Mahamad Khani, Y. (2011). Characteristics of almond selections in relation to late frost spring. Inter. J. of Nut. and Rel. Sci., 2(2): 77-80(a).
17. Imani, A., Barzegar, K. and Pirireivatlou, S. (2011). Relationship between frost injury and ion leakage as an indicator of cold hardiness in 60 almond selections. Inter. J. of Nut. And Relat. Sci., 2(1): 22-26(b).
18. Kodad, O. and Socias, R. (2004). Differential flower and fruit damages by spring frosts in almond. I company unidad de fruticultura CITA, DGA Apartado 727.

19. Kuk, Y.L., Shin, J.S., Burgos, N.R., Hwang, T.E., Han, O.H., Cho, B.H., Jung, S. and Guh, J.O. (2003). Antioxidative enzymes offer protection from chilling damage in rice plants. Crop Sci., 43: 2109-2117.
20. Kushad, M.M. and Yelenosky, G. (1987). Evaluation of polyamine and proline levels during low temperature acclimation of *Citrus*. Plant Physiol., 84: 692-695.
21. Levitt, J. (1980). Responses of plants to environmental stress. Vol2. 2nd. Academic Press. London.
22. Li, M.H. (2003). Peroxidase and superoxide dismutase activities in fig leaves in response to ambient air pollution in a subtropical city. Arch. Environ. Contam. Toxicol., 45: 168-176.
23. Lindon, L. (2002). Measuring cold hardiness in woody plants. PhD. Thesis, Helsinki Univ. Pub.
24. Maehly, A.C. and Chance, B. (1954). The assay of catalases and peroxidases. In: Glick D. (Ed). Methods of biochemical Analysis. John Willey and Sons, Inc., New York. P, 357-425.
25. Micke, W.C. (1996). Almond production manual. Univ. Calif. Dir. Agr. Natural Resour. Agr. Sci. Pub1. 3364.
26. Miranda, C., Santesteban, L.G. and Royo, J.B. (2005). Variability in the relationship between frost temperature and injury level for some cultivated *Prunus* species. Hortsci., 4(2): 357-361.
27. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.
28. Murata, T. and Tatsumi, Y. (1979). Ion leakage in chilled plant tissues. In: low temperature stress in crop plants, the role of the membrane. Academic Press, New York.
29. Omran, R.G. (1980). Peroxidase levels and the activities of catalase, peroxidase, and indoleacetic acid oxidase during and after chilling cucumber seedlings. Plant Physiol., 65:407-408.
30. Probsting, E.L. and Mills, H.H. (1978). Low temperature resistance of developing flower buds of six deciduous fruit species. J. Amer. Soc. Hort. Sci., 103: 192-198.
31. Rodrigo, J. (2000). Spring frost in deciduous fruit trees morphological damage and flower hardiness. Scientia Hort., 35: 155-173.
32. Rodrigo, J. and Herrero, M. (1996). Evaluation of pollination as the cause of erratic fruit set in apricot "Moniqui". J. Hortic. Sci., 71: 801-805.
33. Saunier, R. (1960). La lute contre les gelees printanieres chez les arbres fruitiers. Poml. Fr., 2: 5-12.
34. Sharon, O. and Kahn, V. (1979). Browning potential, PPO, catalase and acid phosphatase activities during ripening of non-chilled and chilled avocado. J. Sci. Food Agric., 30: 634-638.
35. Smirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 125: 27-58.
36. Sala, J.M. (1998). Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. Postharv. Biol. Technol., 13: 255-261
37. Stepulaitiene, I., Zebrauskiene, A. and Stanys, V. (2013). Frost resistance is associated with development of sour cherry generative buds. Zemdirbyste-Agric., 100(2): 175-178.
38. Sudhakar, C., Lakshmi, A. and Giridarakumar, S. (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry under NaCl salinity. Plant Sci., 161: 613-619.
39. Suzuki, N. and Mittler, R. (2006). Reactive oxygen species and temperature stresse: a delicate balance between signaling and destruction. Plant Physiol., 126: 45-51.
40. Szalai, G., Janda, T., Barok, T. and Pald, E. (1997). Role of light in changes in free amino acid and polyamine contents at chilling temperature in maize. Physiol. Plant., 101: 434-438.
41. Szalay, L. (2006). Comparison of flower bud development in almond, apricot and peach genotypes. Inter. J. of Hort. Sci., 12(2): 93-98.
42. Taylor, A.O., Stack, C.R. and Mcpherson, H.G. (1974). Plant under climatic stress. IV. Chilling and light effects on photosynthetic enzymes of sorghum and maize. Plant Physiol., 54: 696-701.
43. Tomas, B.F.A. and Espin, J.C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric., 81: 853-876.
44. Van Huystee, R.B. (1987). Some moleculare aspects of plant peroxidases biosynthetic studies. Ann. Rev. Plant Physiol., 38: 205-219.
45. Van Lelyveld, L.J. and Bower, J.P. (1984). Enzyme reaction leading to avocado fruit mesocarp discoloration. J. Hortic. Sci., 59: 257-263.
46. Vanrensburg, L., Kruger, G.H.J. and Kruger, R.H. (1993). Proline accumulations drought tolerance selection criterion: Its relationship to membrane integrity and choloroplast ultra structure in *Nicotiana tabacum*. J. Plant Physiol., 141: 188-194.
47. Walker, W.A. and Mckerise, B.D. (1993). Role of ascorbate glutathione antioxidant system in chilling resistance of tomato. J. Plant Physiol., 141: 234-239.
48. Walter, M.H. (1992). Regulation of lignification in defense. In: Plant gene research-genes involved in plant defense, (Ed: T. Boller and F. Meins). Springer-Verlag, 329-352.
49. Wang, C.Y. (19950. Effect of temperature preconditioning on catalase, peroxidase, and superoxide dismutase in chilled zucchini squash. Postharv. Biol. Technol., 5: 67-76.
50. Westwood, M.N. (1993). Temperate-zone pomology: physiology and culture. Timber Press, Portland, Ore.
51. Zauberman, G., Fuchs, Y., Rot, I. and Wexter, A. (1988). Chilling injury, peroxidase, and cellulose activities in the peel of mango fruit at low temperature. Hortsci., 23: 732-733.