International Journal of Nuts and Related Sciences 2(3):35-40, 2011
ISSN 2008-9937

Effect of some of anti frost on morphology, anatomy and proline of selective almond cultivars flower buds

[1]*Osman Mahmodzadeh,* [2*]*Ali Imani*
[1]Department of Horticulture Science, Abhar branch, Islamic Azad University , Abhar , Iran
[2]Horticultural Department of Seed and Plant Improvement Institute (SPII), P. O. Box 31585-4119 Karaj, Iran.
[1]Young researchers club, Abhar branch, Islamic Azad University , Abhar, Iran

Abstract
In this experimental, effect of some of antifrost on morphology, anatomy content of selective almond cultivars flower buds of 3 late, medium and early flowering cultivars of almond in Pheranshahr region using factorial design base on complete block randomize with 3 replications was investigated. This region is considerate as cold temperate. Experimental trees were planted with 6×6 m in 2000. In this research, trees no irrigated. Antifrost treatments including: Thiofer, Crop aid and Fosnutren that trees were sprayed using Thiofer and Crop aid in 5 /1000 in 26 November 2010 and 19 March 2011. But Fosnutren was applied with 5 /1000 at 5 may 2011. Of course before applying of treatments, sampling from flower buds for proline determination and bud characteristics study was carried out. This work was repeated in three stage of flower bud development. Results of present study showed that proline rate was significantly decreased by development stage of flower buds in all cultivars for example proline rate in cultivars of Sanky, Azar and Shekofeh before applying of treatments was0.44, 0.52 and 0.66 micromol per fresh weight (g.). While proline rate in medium of winter in cultivars of Sanky , Azar and Shekofeh treated by Thiofer, and Crop aid was 1.25 and 0.88 , 0.1.25, and 0.82 ,0.99 and 0.90 and in end of winter 0.56 and 0.44,0.59 and 0.87 and 0.47 and 0.56 (μmol)per fresh weight (g.) respectively. Also, it was fund that treatments no effect significantly on morphology and anatomy of selective almond cultivars flower buds.

Key words: almond, antifrost, flower buds, proline

INTRODUCTION

Among horticultural crops, almond, nut crop, is one of the most important with production of 110,000 tons and 120,000 hectares (FAO, 2004). In addition, Iran one of the most important domestic and wild almond centers in the world. Most genotypes of almond are early flowering and so often of late spring cold damage (Imani *et al.,* 2006). Almond's cultivation has been in Iran and elsewhere, with constraints such as drought, salinity and cold late spring features (Kester *et al.,* 1990). Although almonds are resistant to cold temperatures in winter but low temperatures in spring and continue for reproductive organs in flowering period is very danger. In some years even cold-resistant cultivars are damaged (Micke, 1996). The cold risk periods of almond are since the beginning of flowering and advance of vegetative growth (small bounce and friute growing) (Rodrigo, 2000). The minimum temperature that can be in various stages of phenological almond varieties are tolerant to adapt to the specific areas that are agroecology.

Flower buds are damaged at temperatures which depend on their developmental stage. Flower buds during Deep rest stage have the greatest resistance, but when they are swelled, their cold sensitivity is increased (Ashworth and Wishiewski, 1991). The spring frost damage in temperate zones than in effecte cold winter injury is much more. Cold tolerance, flower buds may be caused by various factors, including structural, morphological and phenological attributes (Rodrigo, 2000).

It is reported that in late frost spring sensitivity or resistance to factors such as genotype, growth stage, the formation of ice crystals, humidity and nutritional status have a key role (Friesen and Stuhnoff, 1985; Rodrigo, 2000; Miranda *et al.,* 2005).) Plants are suffering from frostbite due to tissue damage. Almonds are also due to frostbite in the reproductive organs are affected differently and mainly depends on the severity of injury is extremely cold and the plant material (Burke *et al.,* 1976; Strang *et al*., 1980; Niobium, 1992; Lu and Rieger, 1993).

Corresponding Author: Ali Imani, Horticultural Department of Seed and Plant Improvement Institute (SPII), P. O. Box 31585-4119 Karaj, Iran.

The amount of damage from freezing among different organs, including tree roots, trunks, branches and buds are different. Also, other factors can affect the severity of frost damage They can be cited nutrient deficiency, disease and pests, yield of years ago, irrigation, tree vigor, kind of pruning, environmental temperature before cold winter initiation for storing carbohydrates, short-term temperature changes and when the frost of the season occurs.

Flower bud at different stages of its development show different reacts to cold. therefore than the cold Incidence of adverse weather conditions, especially winter frost and spring colds the most important parameters determining species distribution and of course the most important selection criteria are established fruit orchards (Proebsting and Mills, 1978). Morphological damages can be included frostbite in the bud tissue discoloration, browning due to oxidation and loss of reproductive organs inside of developing flower buds due to necrosis (Niobium, 1992; Rodrigo, 2000). Hardening period of one or more tissues normally accumulate synthesized material such as sugars, amino acids, proteins and nucleic acids in plant cells and is associated mainly vacuoles (Irigoyen, *et al.*, 1992). Direct relationship has been founded between the increase resistances of woody and herbaceous plants to cold and carbohydrate content, generally assumed that the increase in the amount of carbohydrate in the cells, will lower the freezing point of cell sap (Benko, 1968).

Increasing concentrations of proline and carbohydrates and a decrease the amount of water in the in the leave of citrus generally has been associated with increased cold tolerance (Syverten.and Smith, 1983).In decreasing temperature, generally Proline of tissue and cold resistance is increasing .This change resulted in increased resistance to cold due to membrane fluidity (Aitbarka.and Audran, 1997).

Different ways of measuring the cold resistance in temperate zone trees there is, including measuring ion leakage and proline in the leaves.

Vervaeke et al (2004). Cold resistant of different varieties of *Aechmea* to cool using measuring the ion leakage and observed that the amount of ion leakage in resistant cultivars is the result image. Gusta *et al* (2003) suggested methods for determining cold tolerance including ion leakage. Ion leakage and the amount of carbohydrate in relation to cold tolerance during the dormancy sesean by Ameglio *et al* (2005) Has been studied and found that the EC after the first frost will reduce the amount of 100%. Ameglio *et al* (2003) to determine the resistance of different varieties of Rose in the south of France, the electrical conductivity of the leakage of ions in this plant is used. Soleimani *et al* (2003) for selection some olive varieties resistant to cold used ion leakage method.

The effect of low temperature in the poplar clone (Populus) through soluble sugars were examined and observed that increased levels of soluble sugars (sucrose, fructose and glucose) are associated with cold tolerance mechanisms. The metabolic changes in experimental Sorbus (*Sorbus domestica*) in the in vitro under cold stress was examined and founded the higher levels of fructose, glycerol, antioxidant and hydrogen peroxidase with the process of adaptation and resistance have association(Hausman *et al.*, 2003).

As noted, different reactions to frost ability in the plant genotype, tissue type and time of frostbite have been led to study the frost ability mechanism (Anderson and Seeley, 1993; Stushnoff, 1972). Cultivation techniques such as irrigation, reducing the consumption of nitrogen fertilizers, soil conservation System, garden heaters to prevent frost ability is often are used in commercial orchards, but recently, materials have been introduced under the anti frost that have been effective to prevent frost ability. The subject of this study was effect of some of anti frost on morphoantomy and prolin of selective almond cultivars flower buds.

Materials and methods

This experiment was carried out in the village Hava-Rah Beid which is located 14 kilmeters Piranshahr with the geographical latitude 45○and 36○ with height from the sea level 1670 m. in 2010 and 2011. The area of cold climate regions of the country's course in the warm summer weather without rain is almost and silty clay soil texture and average annual rainfall of 500-600mm and average local meteorological station recorded during April to late June, 150 -200mm (www.irimo.ir).

In this experimental, effect of some of antifrost on morphoantomy and proline content of selective almond cultivars flower buds of 3 late(cv Shekofeh), medium(cv Azar) and early(cv.Sanky) flowering cultivars of almond in Peranshahr region using factorial design base on complete block randomize with 3 replications was investigated. This region is considerate as cold temperate. Experimental trees were planted with 6×6 m in 2000. In this research, trees no irrigated. Antifrost treatments including: Thiofer, Crop aid and Fosnutren that trees were sprayed using Thiofer and Crop aid in 5 /1000 in 26 November 2010 and 19 March 2011. But Fosnutren was applied with 5 /1000 at 5 may 2011. Of course before applying of treatments, sampling from flower buds for proline determination and bud characteristics study was carried out. This investigation was repeated in three stage of flower bud development.

The before of applying treatments, was taken samples of desired varieties buds to determine the concentration proline and morphology and anatomical traits. The flower buds and morphological and anatomical imaging, and noted the figures given by binocular and scale of the cuts were necessary . To determine the concentration proline in each step and each sample approximately 0.5g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman # 2 filter paper. Two ml of filtrate was reacted with 2 ml acldninhdrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

[(µg proline/ml × ml toluene) / 115.5 µg/µmole]/[(g sample)/5] -= µmoles proline/g of fresh weight material(Bates et al., 1973).After obtaining the data using SAS software for data analysis and comparison according to Duncan's test was performed. Duncan was doing.

Results and discussion

Results of the notes taken from the morphological and anatomical details about the effects of different treatments on selective almond cultivars flower buds has been present in the table 1.

Table 1 .effects of different treatments on selective almond cultivars flower buds

Cultivar / treatment	Quantitative traits			Quality traits						
	bud weight	length bud	Wide bud	shape scales	Color scales	shape bud	Local of cork distrib ution	Center	Ovary color	Anther color
Snaky/ Cropaid	2.3 a	a 5.7	a 2.8	Tow loop appeared from behind the tip	Light Brown	Oval sharp	Margin s and the scales tip	Margin near the tip	Bright green with good growth	Completely yellow garlic
Snaky/ Thiofer	a 2.6	6.3 a	a 3.2	Large half-circle with the tip protruding	Light Brown	Oval sharp	litele	Near the margin s	Green	Full yellow
Azar/ Cropaid	a 2.5	a 5.5	a 3.3	Tow loop without tip or short	Mediu m Brown	Oval sharp	Low margin s, scales	Near the tip	Bright Green	Completely yellow with short to medium pistil
Azar/ Thiofer	a 2.6	5 a	a 3.6	Tow loop without tip or short	Light Brown	Oval sharp	Margin near the tip scales	Near the tip	Bright green growth and narrow	The full course yellow
Shkofeh/ cropaid	a 1.5	b 3.4	b 2.3	Dome-shaped with a long tip	Mediu m Brown	Oval sharp	Margin	Margin s in all scals	Bright green and very short and narrow growth	Bright yellow with little development
Shkofeh/ Thiofer	1.6 a	b 4.4	b 2.6	The dome in the crater with a sinking tip up	Mediu m Brown	Oval sharp	Margin	Margin s in all scals	Bright green and very short and narrow growth	Full growth with little yellow

Means with the same letters are not significantly using Duncan test at 5% level As is shown in Tables 1, between cultivars of almonds, there are significant differences in quantitative traits. The most bud weight (2. 6 g) in variety of Snaky in the Thiofer treatment was and the lowest bud weight (1.5 g) in cultivar of Shkofeh treated whit Cropaid was observed. However, the weight of bud in a cultivar using different treatments was not significant .Overall, bud weight difference between the cultivar was not in the relate with applying anti-Frost , but depended the cultivar and type of flowering time .

Cultivars with small flowers such as Shkofeh have bud weight lower than large flower cultivars (Azar and Sangy).This may be bud developing that in late flowering almond was observed.

The morphology and anatomical details about the flower buds of the desired cultivars by applying different treatments was not much difference between treatments observed. Differences in this relationship are more related to genotype or cultivar For example, as can be seen in Table 1. Effect of different treatments on ovarian growth and color of same cultivar was not significant. However, the only difference of ovarian growth and color between cultivars was founded; for example, ovarian growth and color in Sangy and Shkofeh cultivars were the light green with good growth and light green with very short using treatments of the Crop have been thiofer respectively.

The study showed that there were differences between varieties in different stages of phenological and how these differences are related to the percentage of cold injury should be considered, as a consequence of reports on the cold tolerance of flower buds of various factors, including structural and phenological and morfological (Ashworth and Wisniewski, 1991).

Phenological stage seems to be important in relation to cold damage as a result of almond trees in the flowering stage and petal fall were impressed (Micke, 1996).

Reports showed that species of Prunus including almond resistance to cold before flowering (flower buds rest stage), but the full flowering stage and the later stages of flowering are sensitive to cold (Miranda *et al.,* 2005). In this study, the differences of morphology and incremental growth of three different varieties of almonds (Shkofeh, Sangy and Azar) in a similar situation, regardless of treatment anti frost was observed (Table 1). These results may explain the fact that many factors may interfere with the resistance or the development of flower buds that are still unknown or difficult to control.

The results of some of the antifrost on flower bud proline of selective almond cultivars are presented in Table 2.

Table 2. The concentration (micro mol g fresh weight) of selected almond cultivars at various stages of germination and growth of various treatments Anti Frost

Cultivar(treatment)	Proline amount		
	Before applying of treatments	Medium of winter	End of winter
Snaky(Control)	* .44c		
Azar(Control)	.52 b		
Shkofeh(Control)	.66a		
Snaky(Thiofer)		1.25a	0.56
Snaky(Crop aid)		0.88 bc	0.44
Azar(Thiofer)		1.25a	0.59
Azar(Crop aid)		0.82 c	0.87
Shkofeh(Thiofer)		0.99 b	0.47
shkofeh(Crop aid)		0.90 bc	0.56

* Average of 5% level using Duncan test with the same letters are not significantly

Results of present study in Table 2 showed that proline rate was significantly decreased by flower bud developmental progress in all cultivars for example proline rate in cultivars of Sanky, Azar and Shekofeh before applying of treatments was0.44, 0.52 and 0.66 micromol per fresh weight (gr.). While proline rate in medium of winter in cultivars of Sanky , Azar and Shekofeh treated by Thiofer, and Crop aid was 1.25 and 0.88 , 0.1.25, and 0.82 ,0.99 and 0.90 and in end of winter 0.56 and 0.44,0.59 and 0.87 and 0.47 and 0.56 micromol per fresh weight (gr.) respectively.

There are reports that proline due to osmotic effects on plants under stress plays a useful role (Syverten and Smith, 1983). The genotypes that are under stress, proline levels increased, the amount of relative water decreases. Thus the increased effects of proline in reducing the effects of stress involved because it has a protective role in osmoregulation or Proline increase is associated with increased stress or the protection. There are significant differences between the different cultivars of almonds base on proline and cold stress. As, Azar cultivars that are treated with Cropaid had the highest levels of proline and highest cold tolerance ranked (results not presented). The reasons for these differences are consistent with results obtained by different researchers.

However, comprehensive data about the relationship between proline accumulation and resistance to stress and there has been conflicting report. Proline accumulation as a indicator for selection of stress resistant cultivars has been introduced. So that research on citrus, unlike sucrose, the exponential rate of accumulation of proline and its relationship with good cold tolerance have shown Until this matter as an indicator of the degree of cold resistance (Bates *et al*, 1973). So the relationship between proline accumulation and cold resistance, the only test of any specific plant, even as a cultivar and its relationship may be and still can not definitively is said about its role (Irigoyen *et al*, 1992).

In conclusion, considering that the morphological differences between cultivars, there certainly are differences in the amount of proline, which was evident as well in our tests .Mode to measure the overall results quantitative and quality traits and praline by applying deferent treatments of antifrost revealed that proline produced had significant differences between varieties at different growth stages. The changes increase with increasing proline levels of stress can be seen in cultivar. The highest proline levels in cultivars were observed that had the highest rates of resistance (unpublished results). Test results showed no significant differences within treatments on morphological and anatomical traits of the flower buds of the cultivars and differences in this relationship was more related to genotype or cultivar.

REFERENCES

Aitbarka E, Audran JC (1997).Response of changes of shoot and bud proline concentrations in response to low temperatures and correlations with freezing tolerance.J.Hort.Sci.72(4):577-582

Ameglio T, Alves, G, Decourteix M, Poirier MM, Bonhome M,Guilliot A, Sakr S, Brunel N, Petel G, Rageau R, Cochard H, Julien JLJ, Lacointe A (2005).Winter biology in walnut tree: Freezing tolerance by coil acclimation and embolism repair. Acta Hort. 705: 241-249

Ameglio T, Pigeon D, Archilla O, Frizot N, Saint joanis B, Reynoird JP,Guilliot A (2003). Adaptation to cold temperature and response to freezing in roses. Acta Hort. 618: 515-520

Anderson JL, Seeley SD (1993). Bloom delay in deciduous fruits. Hort. Rev. 15, 97-144.

Ashworth EN, Wishiewski ME (1991). Response of fruit tree tissues to freezing temperatures. HortScience 26, 501-504.

Barranco D, Ruiz N, Gomes M(2005). Frost tolerance of eight olive cultivars. HortScience 40(3) 558-560.

Bates LS, Walderen RD , Taere ID (1973).Rapid determination of free proline for water stress studies.Plant soil.39:205-207

Benko B (1968).The content of some amino acids in young apple shoots in relation to frost resistance .Bilo.plant(Prague).11:334-337.

Burke, M. J., Gusta, L.V., Quamme, H.A., Weiser, C.J., Li, P.H., 1976. Freezing and injury in plants. Ann. Rev. Plant Physiol. 27, 507-528.

Friesen LJ, Stushnoff C(1985). Spring frost injury relative to phenophase bud development in Saskatoon berry. HortScience 20, 744-746.

Gusta LV, Wisniewski M,Nesbitt NT, Tanino KT (2003). Factors to consider in artificial freeze tests. Acta Hort. 618 :493-507

Hausman JF, Reisen D,Evers D (2003).Chilling stress and physiological changes in Sorbus domestica grown in vitro: Anti oxidative systems and carbohydrate adjustment. Acta Hort.618:245-252

Imani A, Hassani D, Rahemi A (2006). Almond production and uses in Iran. Acta Horticulturae155-163.

Irigoyen JJ,Emerich DW,sanchez-Diaz M(1992).water stress induce changes in concentration of prolin and total soluble suger in nodulated alfa alfa(*Medicgo sativa*) plants.Plants.Physiologia plantarum,84:55-60.

Kester D E, Gradziel TM , Grasselly C (1990). Almond. In: Genetic resources of temperate fruit and nut crop. Moore, J. N. and Ballington, J. R., Jr. (eds). Acta Horticulturae, 290: 699-758

Lu S,Rieger M (1993). Effect of temperature precondition on ovary freezing tolerance of fully opened peach flowers. J. Hort. Sci. 68, 343-347.

Micke WC (Ed) (1996). Almond production manual. Univ. Calif Dir. Agr. Natural Resour. Agr. Sci. Pub1. 3364

Miranda, C., Santesteban, L.G., Royo, J.B., 2005. Variability in the relationship between frost temperature and injury level for some cultivated prunus species HortScience 4(2) : 357-361.

Niobium H (1992). Freeze damage to flower buds of some apple cultivars. J. Hort. Sci. 67, 171-177.

Proebsting EL, Mills HH (1978). Low temperature resistance of developing flower buds of six deciduous fruit species. J. Amer.Soc. Hort. Sci. 103: 192-198.

Rodrigo J (2000).Spring frost in deciduous fruit trees Morphological damage and flower hardiness. Scientia Hort. 85: 155-173.

Statistical yearbook of agriculture Iran,Tehran ,Iran.2004

Soleimani A, Lessani H, Talaie A (2003). Relationship between stomatal density and ionic leakage as indicators of cold hardiness in olive (*Olea europaea* L.). Acta Hort.618:521-525

Strang JG, Lombard PB, Westwood PB, Westwood, MN (1980a). Effect of simulates frost injury on fruit development in three pear cultivars. J. Am. Soc. Hort. Sci. 105, 63-65.

Stushnoff C (1972). Breeding and selection methods for cold hardiness in deciduous fruit crops. HortScience 7,10-13

Syverten JP, Smith ML (1983).Environment stress and seasonal changes in prolin concentration of citrus tree tissues and juice.J.Amer.Soc.Hort.Sci.108(5):861-866.

Vervaeke I , Londers E, Ceusters J, Godts C, Proft MPD,Deroose R (2004). Consequences of chilling and cold stress on the cultivation of *bromeliads*. Acta Hort.659:755-762