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An Investigation of Cold Tolerance on Chemical Properties (Proline, Protein, and Sugar) of the Flower Buds in Four Commercial Cultivars of Damghan Local Pistachio

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Abstract: Temperature reduction in early spring, especially corporate with buds swelling, sometimes causes severe damages. Most of the fruit trees in tropical and semi-tropical regions, especially in more critical conditions, moderate, at early spring are subjected to the frostbite and winter cold injuries. This study aimed to evaluate the amount of proline, total protein, and soluble sugars during the phonological period of buds under the late spring cold. So the research as factorial in completely randomized design with two factors, first factor was sampling time (1-mid- *February* 2- swelling of inflorescence buds 3-flowering 4-fruit set) and the second factor was four local pistachio cultivars of Damghan city (Shahpasand, Khanjari, Abasali, and Akbari cultivar) was established in three replications in the Damghan's regions. The results indicated that the highest amount of proline under cold stress in flowering stage was reported in Khanjari cultivar and the lowest amount in Shahpasand and Akbari cultivar. Also the result indicated that the highest amount of protein in flowering was observed in Khanjari cultivar and lowest amount in Akbari and Shahpasand varieties. Due to temperature reduction the total amount of proteins during the dormancy and swelling were decreased but during the flowering and fruit setting the total amount of protein was increased. During the winter, sugars were accumulated but their level decreased in early spring.

Keywords: Pistachio, Cold tolerance, Proline, Sugar, Protein, Inflorescence bud

INTRODUCTION

Pistacia vera L. is a deciduous tree which belongs to *Anacardiaceae*. There are 11 different species in this genus [1]. During the past years, Iranian pistachio growers were not immune against frost damage and in some years due to the cold stress the most parts of their yields have been lost. Despite the lack of scientific studies carried out on the extent of frost damage on pistachio, the observational experiences and received reports from different regions are focused on this point that the maximum damages are caused by early spring frost than the temperature lower than freezing in winter [2]. Pistachio is a deciduous tree and the emergence of its leaf occurs simultaneously with flowering stage in which the plant is very susceptible against spring cold. During the flowering stage, pistachio will experience the frostbite. This stage occurs between late March and early April, so this crop experiences severe damage. Frostbite occurs whenever air temperature, depending on the type of product, reaches below the threshold level which is equal or less than-4°C [3]. In 1997, in Semnan and Kerman due to spring frostbite half of the product was destroyed which was equal to 250 million dollar, followed by the 50

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to 70 percent reduction in export rate. In 2004 and 2005, in some regions of Kerman province due to spring cold about 60% of products were destroyed. Although, based on the early flowering, pistachio is not the same as almond, apricot, and peach but emerging frost and climate changes in recent years indicated that Iranian's pistachio is one of the products which are sensitive to spring cold. So the risk of frostbite in the next few years due to climate change will not be impossible. Therefore, given the importance of exchange of pistachio for our own country economical issues and more than thousands of people who are involved in this field in Semnan province, it is necessary to implement plans to prevent or decrease spring frost damages [4]. Dormancy beginning, dormancy, and breaking dormancy down periods accompanies with some changes in growth regulators and the metabolism of plant materials that make them ready for winter and spring different temperatures [5]. These changes in protein, fats, tissues water, resistance-inducing materials, carbohydrates, organic acids, free and combined amine acids, and nucleic acids have been seen during dormancy period [6]. Current researches on different cultivars indicated that buds dormancy and their cold tolerance are directly related to the soluble sugar, proline, and inter-tissues water level [7]. In this study, the level of available proline, sugar, and protein within the buds, flowers, and fruits of four cultivars under cold were measured and then evaluated.

MATERIALS AND METHODS

Sampling method

This study was carried out in one of the local pistachio cultivation orchards located in Damghan. Four different cultivars from 26 to 28 years-old-trees with almond base have been used for sampling. The irrigation treatments with 30 and 40 days intervals were applied, and texture of the soil was loam sandy. Sampling was conducted in four replications including: buds dormancy (11 February), buds swelling, (29 March), flowering (16 April) and fruit setting (30 April). In the above mentioned times, 20 buds from 10 trees of each cultivar were harvested and immediately transferred to the aqueous nitrogen tank at -176℃ and then, maintained at -20℃ in the refrigerator (Table 1).

Table 1. Weather conditions during sampling

Sampling time	month	Temperature
Bud recession (mid February)	11 February	(-4) ℃
Buds swelling	29 March	(-2) ℃
flowering	16 April	(-1) ℃
Fruit set	30 April	(+3) ℃

Methods for measuring proline, sugar, and total protein

First, for determination of proline (C2H3NO2) and sugar (C6H12O6) level, 0/5 gr of fresh buds were weighed and then squashed by Chinese mortar, after that 5cc of ethanol (C2H5OH) 95% was added to the mixture. The supernatant was then poured into the test tube. Adding 5cc of ethanol (C2H5OH) 95% to the mixture, the impurities were completely crushed. Finally, they were added again to the content of test tube. Using 15 min centrifuge in 1500 rpm, the impurities were exactly separated. In this case, the extract was used as basis to measure the amount of proline and sugar [8].

Determination of amount of proline

The standards of proline (0-0/1 mmol per ml) were prepared, and finally the absorption rate of standard solution and samples at 515 nm wavelength were measured by a spectrophotometer [8].

Determination of total soluble sugars

To draw a standard curve, pure glucose was used. Different concentrations of 0, 20, 40, 60, 80, 100, 120mg/l were prepared and they were used as original samples to performed intended experiment.

Their absorption at 625nm wavelength was red by spectrophotometer [8].

Protein measurement

For determination of buds protein percentage, the total measured nitrogen percentage was multiple by protein coefficient which is 5/3 in pistachio [9].

Protein factor × nitrogen percentage = Protein Percentage

This was a factorial research in the form of complete randomized blocks design with two factors, the first factor was sampling time (1-mid February 2-growth start and swelling of inflorescence bud 3-blooming time 4-fruit set) and the second factor was four cultivars of pistachio (Shah pasand, Khanjari, Abasali, and Akbari) that were performed in three replications in Damghan's regions. The data were subjected to analyses using the (SAS) software and the comparison among their related means was based on the Duncan test in level 1%.

RESULTS AND DISCUSSION

Changes in proline content during phonological stage of plant growth

The results of variance analysis of carried out experiments have shown that there is a significant relationship between variations in proline content among different cultivars and different sampling time in level of 1%. The results also have shown that there is a significant relationship between sampling time and cultivars in level 5%. The comparison of means using Duncan test showed that the highest amount of proline during dormancy was observed in Abasali and Khanjari as (7/086 mmol/g wet weight-bud) and (6/163mmol/g bud fresh weight), respectively. During the buds swelling the maximum proline content was observed in Khanjari (7/016 mmol/g buds fresh weight). The highest proline content during bud swelling was reported in Khanjari cultivar. During the fruit setting the level of proline in Khanjari was similar to Shahpasand and there was no statistically significant difference between them. The lowest proline content in four different stages of sampling was observed in Akbari cultivar. It seems that in Khanjari cultivar, under cold stress by increasing the amino acid, protein has tried to maintain the potential of its own water-tissues. So it can be said that it was more resistance to the cold, but small amount of this amino acid was found in Akbari which was probably less resistance (Fig 1). Hosseini (1379) stated that the pistachio fruit and leaves of different pistachio cultivars which are subjected to cold stress had more protein content than control groups which confirmed the results of the present study[10]. Taheri (1378), Schwabe and Lionakis(1996) stated that there was no comprehensive information about the relationship between the accumulation of proline and tension resistance but opposite reports have been stated. Some researchers believe that the accumulation of proline is as an index to select the drought-resistance varieties [11,12]. Blum (1996) and Heuer (1993) reported that While the accumulation of proline is an indicator of drought damage and it should be noted that it is not an appropriate index to make any conclusion about the degree of plant resistance [13,14]. Barca and Adran measured the proline level in the grap buds and shoots and calculated its coloration with the freezing resistance. It was revealed that the more the level of proline in tissues during different phonological stages increases, the better will be plant survival ability [15]. Proline measurement in this study show that the amount of proline in buds during dormancy will increase. Research on apricot and peach confirmed the results of the present study because this pattern is also seen in their proline level [16,17]. While the starch concentration decreases during the dormancy, the amount of proline increases which is in accordance with their results [18]. In germination stage, regarding the

water deficiency, plants are more stable than the breeding stage [19].

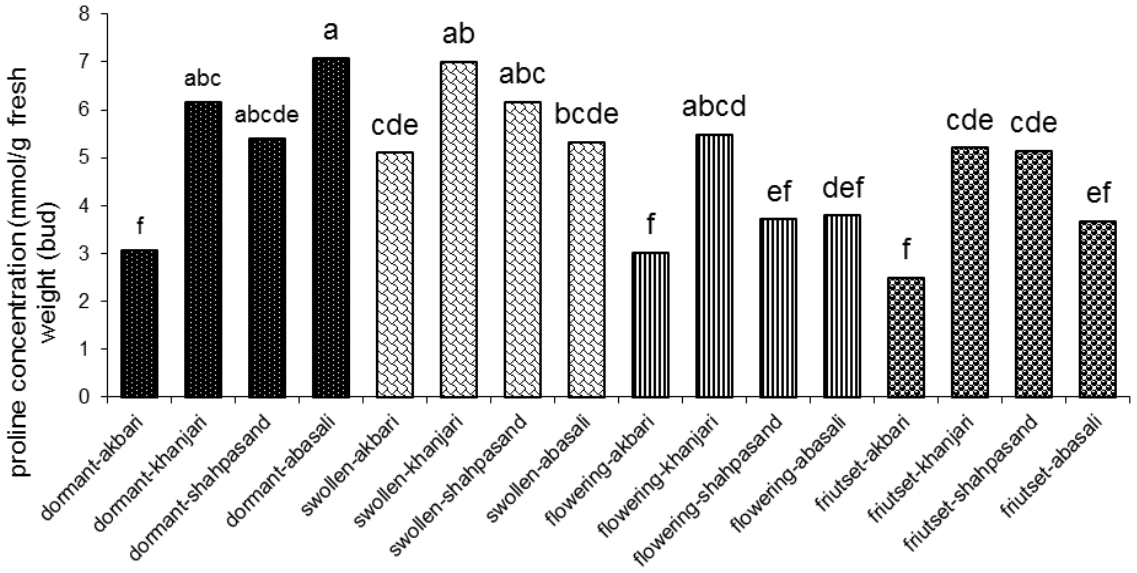

Fig 1. proline variation during phonological stages of plant growth

Changes in total protein content during phonological stages of plant growth

The results of variance analysis obtained from carried out experiments indicated that changes in protein content in different cultivars and different times (stages) and also their interactions in level 1% were significant. The comparison of obtained means using Duncan test indicated that the highest protein content during fruit setting was observed in Khanjari (53/980%) and the lowest in both Akbari and Shahpasand cultivars with no statistically significant difference. Also, it was reported that the highest amount of protein during bud bursting was observed in Khanjari cultivar (51/826%) and the lowest amount in Akbari with no statistically significant difference (24/125%). In dormancy, the highest amount of protein in buds was obtained in Khanjari cultivar (19/515%) and the lowest amount in Akbari cultivar (16/827%). In general, it can be concluded that in all four sampling stages, the highest amount of protein was in Khanjari cultivar and the lowest amount in Akbari cultivar (Fig 2). It can be stated that due to late flowering of Akbari cultivar, its vegetative stage occurred after the spring frost. Hosseini (2000) stated that due to the reserved compounds in their cells (especially proteins), the plant will not loss their protective ability and resistance under cold stress [10].

Adel Sio-Se-Marde et al. (2009) stated that accumulation of protein in wheat leaves like the amount of sugar are considered as adaptive reaction against the cold stress and therefore has significant role to reduce the impact of cold damage and frostbite on plant tissues[20]. kerepesi et al,(2004) stated that the accumulation of protein in leaf was as result of temperature reduction which is in accordance with our results obtained from pistachio buds and flowers[21]. During the winter, proteins act as nitrogen resource and also have an effective role in dehydration, cold tolerance and the protection of tree against the temperature reduction [22]. In dormancy, the degree of cold tolerance depended on the presence of anti ice-nuclei reagents such as protein, polysaccharide, amino acids and lipids [23]. Reducing the amount of

protein under tension might be related to the increased activity of the protein kinase [24].

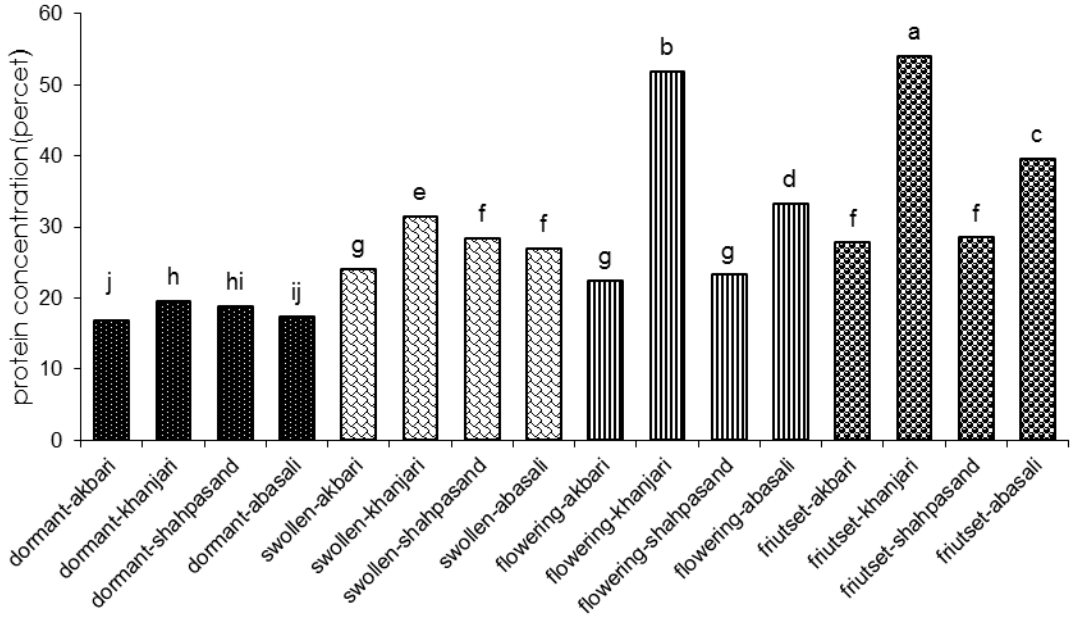

Fig 2. changes in protein level during phonological stages of plant growth

Changes in sugar content during phonological stages of plant growth

Results obtained from variance analysis indicated that there was no significant difference between changes in sugar content among different cultivars. Also, the results of variance analysis related to the sampling time and interaction between sampling time and cultivars showed a significant relationship between variables in level 1%. During the dormancy, the highest sugar content was observed in Abasali cultivar (64/817 mg/g fresh weight) and the lowest level in Khanjari cultivar (35/027mg/g fresh weight). During the buds swelling, the highest sugar content was reported in Khanjari (17/260 mg/g fresh weight) and Shahpasand (114/010 mg/g fresh weight) and the lowest level in Abasali and Akbari, respectively. During the flowering, the highest sugar content was observed in Akbari, Khanjari and Abasali with no statistically significant difference. The amount of glucose in these cultivars was similar and the lowest content was detected in Shahpasand cultivar (27/060 mg/g fresh weight). During the fruit stage, the highest amount of sugar was observed in Akbari (67/250mg/g fresh weight) and the lowest amount in Abasali cultivar (27/060 mg/g fresh weight) (Fig 3). Using the artificial cold stress on some cultivars, Afshari (2006) indicated that the accumulation of soluble sugar in -4°C compared with +2°C was decreased. Hence, regarding the temperature reduction in dormancy, soluble sugar accumulation was decreased . His theory was in accordance with previous researches and also he showed that due to the temperature reduction sugar accumulation in new flowers was reduced [25]. Levitt (1980) stated that There was a relationship between cold tolerance improvement and the content of soluble carbohydrate. Carbohydrate such as sucrose, sorbitol, and raffinose are subunits of plant protective units [26]. Rosa and Rallo (2000) reported that Sugar accumulation in buds is a result of their translocation from shoots and bark to the buds. Therefore, sugar reduction in shoots is related to the improved capacity of buds against cold stress

[27]. Atici et al. (2003) stated that Sugars have a significant role in reducing the freezing temperature of cell's water, providing accessible energy, and protecting the protein's structure and action against cold stress[28]. During the flowering, the amount of sugar in Khanjari, Akbari, and Abasali cultivars was not significantly different. Maximum Cold tolerance of the Khanjari cultivar was as result of the sugar existence during buds swelling stage. Sugar accumulation under low temperature is an important factor, so that superficial reduction in sugar content as a result of spring breathing caused reduction in cold tolerance [29].

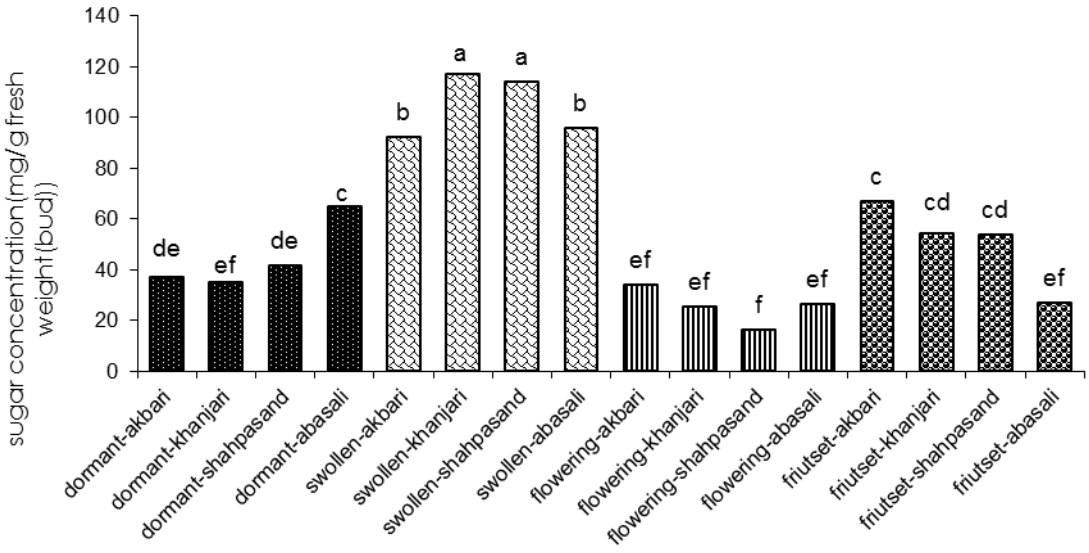

Fig 3. changes in sugar content during phonological stages of plant growth

Table 2. The results of variance analysis of cold stress on considered characteristics of pistachio

Source	DF	Mean Square		
		Proline	sugar	Protein
Block	2	0/113^{ns}	145/59^{ns}	13/56**
Cultivar	3	13/549**	50/29^{ns}	645/25**
Stage	3	10/697**	13837/87**	820/80**
Cultivar Stage	9	2/0043*	665/85**	133/48**
Error	30	0/843	97/651	1/088
C. V.		18/8%	17/4%	3/5%

ns,*,** non - significant, respectively. In level of 5% and 1%

CONCLUSION

The beginning of autumn and due to cold weather the amount of soluble sugars was increased, so that the highest amount of recovering sugar in coldest month of the year (Day) was accumulated within the buds and gradually the amount of sugars was decreased. The results of this study indicated that regarding the seasonal changes in the amount of soluble sugars, sugar accumulation in buds was going on during the autumns. The accumulation of sugar in autumn, especially during the resistance period appeared to be as a defensive mechanism against the cold damage. By losing the amount of water during this period, the plant was able to increase the concentration of materials. By this way, through the enhancement of the sugar content,

ice formation was decreased and ice-induced dehydration was prevented. Increase of proline and protein in Khanjari cultivars at flowering and appear to be as an index of cold tolerance of this cultivar during the flowering.

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