Study the effect of cold treatments on some physiological parameters of 3 cold resistance Almond cultivars

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Abstract: One of the main problems of almond producers in Iran is the irregular and fluctuating production rates. This is the result of early flowering of native genotypes and coincidence of their flowering times with a cold spring. An experiment was done on three major commercial of Monagha, Sh-12 and , Sh-18 through a double-factor factorial in the form of random blocks(cultivar, cold and phenological phase at 3, 6 and 3l levels respectively) with three replications with ages around 15 years. The results showed that the highest rate of proline was found at -6° C in Sh-18 (3.43µmol/g). While the highest rate of carbohydrates at -6 °C were found in cultivar Monagha (0.305mg/g). The rate of calcium, magnesium, potassium and sodium at different stages of bud development of the experimental cultivars under cold stress was diverse significantly. According to this research results, Sh- 12 of almond was observed more resistant than other cultivars.
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Introduction

One of the main problems of almond producers in Iran is the irregular and fluctuating production rates. This is the result of early flowering of native genotypes and coincidence of their flowering times with spring cold. Under such conditions, chilling or lack of suitable pollination and insemination in the absence of pollinator insects at the time of flowering and the existence of incompatibility phenomenon and the lack of coincidence of flowering of late flower varieties were reduced the total product and producers suffered large losses. This type of damage has been estimated at 60 to 100 percent in some years (Soleimani et al., 2003; Rahemi,2002; Browicz, 1969). Nowadays these problems to some extent have been diminished in the world by applying appropriate management particularly introducing late flower and resistant varieties to cold (Rodrigo, 2000). Therefore, performing research projects in line with almond breeding programs, especially to achieve resistant to cold and high-yielding varieties, early bearing, easy to hull, easy to harvest and marketing is the most important almond breeding objectives(Duncan and Widholm, 1987; Bonhomme et al., 2005). Mechanisms of cold resistance and environmental compatibility are one of topics in plant biology researches, including agriculture, horticulture and forestry. So estimating the degree of cold tolerance in plants has significant importance in applied studies (Wilson, 1996). The role of leaf is important not only in the production of photosynthetic materials that are essential for cold resistance but also in the production of substances that cause resistance to cold. The Results of different researches have shown that leafy branches that in the vicinity to light did not have photosynthetic capacity toward the branches that were in dark adjusted to cold better (Quamme, 1974).

Abscisic acid reduces lipid per oxidation of cell membranes and causes membrane stability and maintains within a cell proline and cell survival at the end. In samples treated with Abscisic acid,

proline rate was 2-3 times higher than that of control levels. According to early flowering of almond tree in areas such as Semnan where is capable of the spring frost, some parts of product disappear annual. Thus, to identify cultivars with higher cold resistance and less injury, three commercial cultivars named Monagha, variety 12 and variety 18 which are planted in the area were evaluated.

Materials and Methods

Plant material

The experiment was run on three major commercial almond cultivars, namely Monagha, variety 12 and variety 18. Native trees of the region with ages around 15 years were used for the experiment. Sampling was done in the collection orchard belonging to Semnan Province Agricultural Research Center (Shahroud County). Branches with equal length and diameter were harvested from 3 almond cultivars in three different phenological stages (bud swell, blossoming and Fruit formation) were placed in a special containers and carried immediately to the laboratory. Branches were taken from all position on the tree.

Statistical analysis

Selective Test Procedure: Double factor factorial in the form of random blocks with three replications (each replication with one tree). The first factor of the experiment was almond cultivar at three levels (including Monagha, varieties 12 and 18) and the second factor was frost treatment at 6 levels. The cold factor at 6 levels including the following treatments applied with incubators capable of regulating temperature at the required level:

1. Control group: 4 ºC in a dark room
2. Temperature: 2 ºC
3. Temperature: 0 ºC
4. Temperature: -2 ºC
5. Temperature: -4 ºC
6. Temperature: -6 ºC

Treatments were applied to three phenological phases of almond cultivars, including the following:

Time 1 –swell bud: winter 2009 (March)
Time 2 - Flower Opening: March 26, 2010
Time 3 - Fruit formation: April 18

Data analysis was performed in two trials using SAS statistical software and comparison of means using the Duncan test with probability level of 5 percent.

Procedures

Test freezing

For test freezing, branch 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 -20 to +30 °C range with 0.3 °C precision. 5 thermo par 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 5° C for 50 min and then programmed to decline by 2 °C per hour until the desired frost temperature was reached. The frost temperature was maintained for 30 min and was then increased up to 7° C by 2 h^{-1}. Frost damage rate was evaluated 24 h after frost treatment. Flower buds, blossom and fruit let were regarded as frost damaged when they showed brownish.

determining the proline

For determining the proline rate, the plant material was crushed in a mortar with 10 ml sulfo salicylic 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 1 h 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).

determining the total carbohydrates

To determine the total rate of soluble carbohydrates solution 100 micro liters of leakage solution taken and 3 ml Known Antron freshly prepared (150 mg of net Antron + 100 ml of sulfuric acid 72 percent) was added. Then put it in boiling water bath for 10 minutes and after cooling of samples, their absorption in wavelength 625 nm was read with a spectrophotometer. Pure glucose was used for drawing standard curve. Concentrations of 0, 20, 40, 60, 80, 100 and 120 mg l (ppm) were prepared. Like main samples, possible operation

was performed on them (Xavier Morin, 2007; Annette et al, 2010).

determining the nutritious elements

Atomic absorption device (Perkin-Elmer, model HGA 700) was used to measure calcium and magnesium rate in leakage solution and Flame photometer device (Corning, UK, Model 405) was used to measure potassium and sodium (Soleimani et al., 2003).

Results

Proline Rate in Leakage Solution

Results indicated that the highest proline rate was found at -6° C in solution leakage because proline rate in leakage solution was significantly higher than the other temperatures. The lowest proline rate in leakage related to the temperature of 2 and 4 ° C. at These temperatures the rate of was around zero, which means at the temperatures above zero plant in terms of less stress will not release any proline (Table 1). proline rate in leakage solution shows sharp increase by reducing temperature from 4 to -6°C. This occurrence is more tangible especially after decreasing temperature from -4 to -6°C.

The comparison of means with the reaction of different cultivars on proline rate in leakage solution indicated that most proline rate in leakage solution related to variety 12 (3.43µmol/g) and the lowest rate was allocated to Monagha (2.70µmol /g). In relation to variety 18, the results showed that proline rate in leakage solution was between two other varieties (Table 3).

The lowest proline rate in leakage solution was in the stage of swell bud (1.47µmol/g) and the highest proline in the fruit stage (4.70µmol/g), means that among the studied phonological stages, newly formed fruit stage was the most sensitive phase and swell bud stage was the strongest phase (Table 2).

The results of interaction effects of cultivar in phenological stage on proline rate in leakage solution indicated that the lowest proline rate in leakage solution in Monahga variety, variety 12 and variety 18 was seen in swell bud stage. And the highest proline rate was observed in flowering and newly formed fruit stages in all three cultivars (Table 4).

The results of interaction effects of cultivar in phonological stage on the rate of proline in leakage solution indicated that the lowest rate of proline was seen in swell bud stage even at -6°C. The highest proline rate in leakage solution occurred in newly formed fruit stage compared with swell bud and flowering stages (Table 4).

The results in relation to effect of cultivar on the rate of proline in leakage solution indicated that variety 12 at first stage and variety 18 at second stage had the highest rate of proline in leakage solution and Monagha variety regarding to two other varieties had the lower rate of proline in leakage solution (Table 4).

The Rate of Carbohydrates in Leakage Solution

The results in relation with the effect of different temperatures on the rate of carbohydrates in solution leakage showed that the highest rate was observed at -6°C in leakage solution (0.305mg/g), because the rate of carbohydrates in leakage solution was significantly more than other temperatures. The lowest rate of leak also related to 2 and 0°C and at these temperatures the rate of carbohydrates was around zero which means that plant degree at above zero temperatures did not release any carbohydrates because of less stress (Table1). The rate of carbohydrates in leakage solution showed intense increase by reducing temperature from 4 to -6°C, this occurrence was more tangible after decreasing temperature from -4 to -6°C.

The comparison of means in relation to different cultivars' interaction on the rate of carbohydrates in leakage solution indicated that the highest rate of carbohydrates in leakage solution related to Monagha variety (0.15 mg/g) and the lowest rate related to variety 12 (0.02 mg/g). Results in relation to variety 18 indicated that the rate of carbohydrates in leakage solution was between two other varieties (Table 3).

The comparison of means showed that the lowest rate of carbohydrates in leakage solution was in plant swell bud stage (0.042mg/g) and the highest rate was in flowering stage (0.168mg/g), this means that among the studied phenological stages, flowering stage was the most sensitive stage and swell bud stage was the most resistant stage (Table2).

The results of interaction effects of cultivar in phenological stage on the rate of carbohydrates in leakage solution indicated that the highest rate of carbohydrates in leakage solution was seen in Monagha variety and variety 18 in flowering stage, and the lowest rate was observed in variety 12 in swell bud stage (Table 4).

The results in relation with the interaction effect of cultivar and temperature on the rate of carbohydrates in leakage solution indicated that variety 12 at first stage and variety 18 at second stage had the highest rate of carbohydrates in leakage solution, and Monagha variety in all temperatures regarding to two other varieties had less rate of carbohydrates in leakage solution (Table 4).

Nutritious Content in Leakage Solution

Calcium Rate in Leakage Solution

The results in relation with the effect of different temperatures on calcium rate in leakage solution indicated that the highest rate of calcium was observed at -6°C in leakage solution because calcium rate in leakage solution was significantly more than other temperatures (Table 1).

The comparison of means in relation with the effect of different cultivars on the rate of calcium in leakage solution indicated that the highest rate of calcium in leakage solution related to variety 18 (7.2mg/100g) and the lowest rate related to Monagha variety (6.2mg/100), in relation with variety 12, the results showed that the rate of calcium in leakage solution was between two other varieties (Table 3).

The mean comparison indicated that the lowest calcium rate in leakage solution was in swell bud stage of bud (6.18mg/100g), and the highest rate of calcium was observed in newly formed fruit stage (6.90mg/100g) that means among studied phenological stages, fruit stage was the most sensitive stage and swell bud stage of buds was the most resistant stage (Table 2).

The results of interaction effects of temperature in phenological stage on calcium rate in leakage solution showed that the lowest calcium rate was seen in dormancy stage even at -6°C (Table 3).

The results in relation with interaction effect of cultivar and temperature on calcium rate in leakage solution showed that variety 12 at first stage and variety 18 at second stage had the highest calcium rate in leakage solution and Monagha variety had less calcium rate at all temperatures in comparison with two other varieties (Table 4).

Potassium Rate in Leakage Solution

The results in relation with the effect of different temperatures on potassium rate in leakage solution indicated that the highest potassium rate was seen at -6°C in leakage solution (48mg/100g). Because potassium rate in leakage solution was significantly more that other temperatures (Table 1). Potassium rate in leakage solution showed severe increase by reducing temperature from 4 to -6°C. This occurrence was more tangible especially after reducing temperature from -4 to -6°C.

The lowest potassium rate in leakage solution was seen in swell bud stage of plant (15mg/100g) and the highest rate was seen in flowering stage (20mg/100g), means that among studied phenological stages, flowering stage was the most sensitive stage and swell bud stage of buds was the most resistant stage (Table 2).

Interaction effects of cultivar and phenological stage on potassium rate in leakage solution indicated that there wasn't a significant difference among varieties regarding potassium rate in leakage solution (Table 4).

The results of interaction effects of temperature in phenological stage on potassium rate in leakage solution indicated that the lowest potassium rate in leakage solution was seen in dormancy stage even at -6°C. The highest potassium rate in leakage solution occurred in flowering stage in comparison with dormancy and fruit stages (Table 5).

Magnesium Rate in Leakage Solution

The results in relation with the effects of different temperatures on magnesium rate in leakage solution showed that the highest rate was observed in leakage solution at -6°C because magnesium in leakage solution was significantly more than other temperatures (Table 1).

The comparison of means in relation with reaction of different cultivars on magnesium rate in leakage solution demonstrated that the highest magnesium rate in leakage solution related to

Monagha variety (16.40mg/100g) and the lowest rate was allocated to variety 18 (14.90mg/100g), the results in relation with variety 12 illustrated that magnesium in leakage solution was between two other varieties (Table 3).

Mean comparison showed that the lowest magnesium rate in leakage solution was seen in swell bud stage of plant (13.5mg/100g) and the highest magnesium rate was observed in flowering stage (17mg/100g), thus among examined phenological stages, flowering stage was the most sensitive stage and swell bud stage of buds was the most resistant stage (Table 2).

The results of interaction effects of cultivar in phenological stage on magnesium rate in leakage solution demonstrated that there wasn't a significant difference among varieties of magnesium rate in leakage solution (Table 4).

The results of interaction effects of temperature in phenological stage on magnesium rate in leakage solution demonstrated that the lowest magnesium rate in leakage solution was observed in swell bud stage even at -6°C. The highest magnesium rate in leakage solution took place in flowering stage in comparison with fruit and swell bud stages (Table 3).

The results in relation with interaction effects cultivar and temperature on magnesium rate in leakage solution indicated that variety 12 at first stage and Monagha variety at second stage had the highest magnesium rate in leakage solution (Table 4).

Sodium Rate in Leakage Solution

The results in relation with the effects of different temperatures on sodium rate in leakage solution showed that the highest sodium rate was observed at -6°C in leakage solution because sodium rate in leakage solution was significantly more than other temperatures. Sodium rate in leakage solution demonstrated intense increase by reducing temperature from 4 to -6°C, this event was more sensible especially after reducing temperature from -4 to -6°C (Table 1).

The comparison of means in relation with reaction of different cultivars on sodium rate in leakage solution indicated that the highest sodium rate in leakage solution related to variety 18 (0.0240mg/100g) and the lowest was allocated to Monagha variety (0.0032mg/100g). (Table 3)

The lowest and the highest sodium rates in leakage solution were seen in swell bud stage (0.30mg/100g) and flowering stage (0.0260mg/100g), respectively (Table 2).

Interaction effects of cultivar in phenological stage on sodium rate in leakage solution showed that there wasn't a significant difference among varieties of sodium rate in leakage solution (Table 4).

The results of interaction effects of temperature in phenological stage on sodium rate in leakage solution indicated that the lowest sodium rate was seen in dormancy stage even at -6°C. The highest sodium rate in leakage solution occurred in flowering stage in comparison with dormancy and fruit stages. The results in relation with interaction effect of temperature and cultivar on sodium rate in leakage solution demonstrated that variety 12 at first stage and Monagha variety at second stage had the highest sodium rate in leakage (Table 5).

Discussion

Each plant species has its unique set of temperature requirements, which are optimum for proper growth and development. Low temperature is one of the abiotic stresses that are principal cause of crop failure world wide, dipping average yields for most major crops (Bray et al.,2000). Cold stress induced an accumulation in soluble protein content in Medicago sativa (Mohapatr et al., 1987). Synthesis of specific

proteins is an important mechanism involved in increasing freezing tolerance during cold acclimation(Antikainen et al., 1996). Low temperature can result in the synthesis of proteins (Hughes and Dunn, 1996). Proline accumulates in higher plants in response to serious abiotic and biotic stresses such as water stress and chilling stress. It has been reported that praline content was increased in potato hybrids when plants subjected to cold treatment (Rhodes et al, 1999). In present research proline accumulated when the plants were transferred to chilling temperatures (4°C)(Yadegari et al., 2007). In cold acclimated plants proline content was higher than non-acclimated plants and cold acclimated plants recovered faster than non-acclimated ones. In this work, we conclude that

proline accumulation, typical plant cold stress response.

Our results demonstrate that proline content increased in swell bud and flower of Almonds is activated by cold stress. We found a significant correlation between freezing tolerance and an increase of proline concentration in bud and flower of Almonds after exposure to low temperatures. In this study, low temperature significantly ($p < 0.01$) increased proline accumulation in pepper upon chilling.

The Comparison of Different Physiological Stages

Injury rate is influenced not only by temperature but also by developmental stage that means the buds had the highest cold resistance in dormancy stage which is related to increase growth inhibitors in buds, reduce water of buds, and form scale on the buds at this time. Also in invigorated buds a lot of ice is formed inside the bud scales and bud axis and no ice formed within the flower organs, but in not resistant bud ice kernels are formed within developing flower organs and in bud scales and axis (Rada et al, 2001). Sensitivity to environmental factors increases by progress of bud developmental so that there is more sensitivity in flowering stage, and more injury is seen. Among the various components of flower, pistil is more sensitive to stress that means cold causes more damage to pistil. Also in pistil studying the sensitivity of the ovary is more than that the stigma and cream and shows more damage that the injury is in the form of thickening of cell wall, reducing meristem activity and damaging vascular system(Rada et al, 2001).

Another critical stage is after the falling of petals, so that compared with other stages shows greater damage that causes are particular situation of cell at this stage and not fully formed cell wall.

Proline rate is high in the stage after the petal drop and cell specific location causes the more damage at this stage. This means that cell wall did not form completely has caused more sensitive than stress. proline rate increases after cold treatment. Resistant cultivars often have more proline than susceptible cultivars. However there are exceptions like this case has been observed in citrus, so increase rate of proline in the leaves of trifoliate orange as a known cold-resistant plant was about four times lower than that in some Rafelmun leaves was observed after cold treatment (Yelenosky, 1979).

The Study of Relationship between Proline Rate and Cold Resistance

Proline production increases in higher plants under stress conditions take place using two Glutamate(nitrogen deficiency) and Urnetin (nitrogen is high in the cell) cycles (Chen and Li, 2002)

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 (Sakai and Larcher, 1987). proline production prevents of excessive acid of cell. Proline is analyzed after stress that helps to phosphorylation Mitochondrial oxidative and helps to improve the damage due to stress ATP production (Hore and Cress, 1977) in this regard proline rate of samples was measured after cold treatment. So that it is observed the highest proline rate was in fruit stage and the lowest rate was in swell bud stage. Although proline rate is high at fruit production stage but most of damage can be attributed to special situation of cells at this stage, which means lack of form a complete cell wall has caused more sensitivity of these cells to stress. The results are compatible with Rohaninia and colleagues' observations about apricot. (Rohaninia et al, 2008).

Most resistant cultivars have more proline than susceptible cultivars. However there are exceptions. For example, winter wheat cultivar w1-121 6023 that is a moderately resistant cultivar shows more proline than cultivar 2-93 AF that is a resistant variety (Pecta and Terbea, 1995). Like this case has been observed in citrus fruits so that proline increase in the leaves of trifoliate orange known as a cold-resistant plant was about four times lower than that in leaves of Rafelmun observed after cold treatment (Yelenosky, 1979).

Among studying cultivars although Shahrood cultivar 12 showed greater resistance in ion leakage test, but Shahrood cultivar 18 had more proline from part of proline production. Early flowering and sensitive Monagha cultivar also

produced the lowest proline. As Yelenosky concluded in citrus that there is not linear relationship between increase of cold resistance and proline increase (Yelenosky, 1979), in studied cultivars, similar results were obtained with respect to ionic leakage of cultivars and proline rate.

According to our results and the studies cited previously, one role of carbohydrates is in the osmotic adjustment that contributes to the prevention of intracellular freezing. When the risk of freezing injury is low (Above zero temperatures), total carbohydrate concentration is not necessarily related to cold hardiness because soluble carbohydrates are either allocated to processes such as cell growth or converted to starch(Table 1)(Xavier Morin et al, 2007).

Correlation between Carbohydrate Rate and Cold Resistance

Correlation between carbohydrate rate and cold resistance has been reported in some woody species. There are also reports that carbohydrate rate grown in the organs of some plants in the winter and carbohydrate rate in root was higher than that in leaves, stems and buds in the winter half. Unlike the obtained results of measured ion leakage rate, the results of measuring carbohydrate rate in different cultivars show that although Monagha indicates high ion leakage and less resistance. But the rate of soluble carbohydrates in this cultivar is higher than other cultivars, with regarding to the fact that Monagha variety is an early flowering, high soluble carbohydrate rate in this cultivar can be known as a stimulus to begin metabolic activities in growth season start. As it is also evident in the table which is related to comparison of soluble carbohydrates in various developmental stages, the rate of soluble carbohydrates is more in flowering time when metabolic activities are more and more active.

The results from the idea that high levels of soluble carbohydrates or osmotic active can not be considered as cold resistance mechanisms, but rather it is suitable because of increased metabolic activities at the time of approaching to hot growth season.

This is compatible with the observations of Rada and colleagues (Rada et al., 2001) about Polylepis Tarapacana plant and also species of the Afroalpine including Lobelia, Senecio and Alchemilla.

Nutrients and Cold Resistance

Temperature reduction causes changing of concentration or activity of enzymes that are regulators of biochemical processes within the cell. Reduction of enzyme activity of cell membrane causes disorder in transferring of ions and substances. Activity change of intracellular enzymes (decrease or increase) causes different compounds' production. Some of these compounds are toxic, but some are useful and effective on cold resistance (Quame, 1974).

Presence of calcium ion in the intercellular space pectin compounds causes tissue strength. Calcium effect has observed on the inner membrane stability during numerous experiments. membrane strength has direct effect on semi-permeability characteristic of membrane and therefore calcium deficiency around the cell (due to lack of transfer or lack of absorption) caused loss of semi permeability or permeability property of intracellular materials out and penetration of harmful substances into the cell and ultimately "leads to cell death ". So the presence of ion concentration of 1 to 5 mmol has been known necessary surrounding cells for maintaining cell membrane. Environmental stresses such as water deficit, salinity, aluminum toxicity and low PH impair calcium absorption and transmission. The reason of concentration reduction of ion and loss cell membrane strength and consequently, at these conditions permeable of intracellular substances such as potassium are observed. So there are reports that prevent potassium removal in calcium ion stress conditions and cause the cell resistance. Some of calcium is isolated from cell membrane in cold conditions. Calcium ion can play an important role in cold conditions to protect cell membrane. But in this study was observed that all the elements measured because of temperatures of -6 ° C in leakage solution had more concentration that is sign of cell wall destruction and leak of all elements (Hore and Cress, 1997; Rada et al., 2001; Soleimani et al., 2003).

Most parts of different elements needed for buds are provided from stored resources in branches and roots in the spring and concurrently with

buds' turgidity when the roots' activity is still the lowest size. Also the concentration of nutrient elements, including magnesium, manganese, boron and chlorine slightly increases at time of flower burgeoning. But if there is enough nutrients in soil, sodium amount increases steady. Calcium amount as mentioned previously is always started with a low dose and significantly grows.

According to our results, the effect of different temperatures on the rate of proline in leakage indicated that the highest rate of proline was found at -6° C in cultivar 18 (mol/gµ3.43)-while the highest rate of carbohydrates at -6 ° C were found in cultivar Monagha (0.305mg/g).

The rate of calcium, magnesium, potassium and sodium at different stages of bud development of the experimental cultivars under cold stress was various significantly.

According to this research results, cultivar 12 of almond was observed more resistant than other cultivars.

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Table 1. the effect of different temperatures on the content of proline, carbohydrate, and leakage elements

				Traits			
Treat		Proline	Sugar	Ca	K	Mg	Na
	-6	11.90a	0.305a	23a	48a	39a	0.655a
	-4	4b	0.120b	17b	38a	35b	0.33b
Temperature	-2	1.70c	0.75c	0c	24c	15.5c	0c
	0	0.20d	0.23d	0c	0d	0d	0c
	2	0e	0e	0c	0d	0d	0c
	4	0e	0e	0c	0d	0d	0c

Letters represent data significantly ($p< 0.005$) different form other data with different letters

Table 2. The effect of different sampling stages on the content of proline, carbohydrate, and leakage elements

				Traits			
Treat		Proline	Sugar	Ca	K	Mg	Na
	T	1.47c	0.042c	6.18c	15c	13.5c	0.030c
stage	F	4.70a	0.070b	6.90a	18b	15.3b	0.044b
	B	2.65b	0.168a	6.70b	20a	17a	0.260a

Letters represent data significantly ($p< 0.005$) different form other data with different letters. T: Turgidity, B: Bloom, F: Fruit

Table 3. The effect of different temperatures on the content of proline, carbohydrate, and leakage elements

				Traits			
Treat		Proline	Sugar	Ca	K	Mg	Na
	M	2.70c	0.15a	6.2c	18.40a	16.40a	0.0032c
Cultivars	Var12	3.43a	0.02c	6.5b	13.20c	15.10b	0.0051b
	Var18	3.21b	0.09b	7.2a	17.10b	14.90c	0.0240a

Letters represent data significantly ($p< 0.005$) different form other data with different letters. M:Monagha, Var12:Variety12, Var18:Variety 18.

Table 4: Interaction effects of cultivars and phenological stage on measured parameters

Cultivar*stage	proline	sugar	Ca	k	mg	Na
M-T	1.333 b	0.07000 bc	6.000 a	15.000 a	13.167 a	0.00333 b
M-B	2.500 ab	0.24000 a	6.500 a	21.667 a	17.500 a	0.00500 b
M-F	4.167 ab	0.15667 ab	6.333 a	15.500 a	15.500 a	0.00167 b
Var18 –T	1.417b	0.03833 bc	7.667 a	19.833 a	17.333 a	0.05000 ab
Var18 –B	4.333 ab	0.22000 a	7.500 a	19.500 a	16.500 a	0.00333 b
Var18 –F	4.333 ab	0.02500 c	6.667 a	14.833 a	12.500 a	0.00333 b
Var12 –T	1.883 b	0.01500 c	5.333 a	14.833 a	13.833 a	0.00500 b
Var12 –B	5.500 a	0.03500 bc	6.500 a	20.333 a	18.333 a	0.00333 b
Var12 –F	2.500 ab	0.02333 c	8.000 a	17.500 a	15.667 a	0.00333 b

Letters represent data significantly ($p < 0.005$) different form other data with different letters, T: Swell bud, B: Flower opening, F: Fruit formation

Table 5: interaction effects of temperature in phonological stage on measured parameters

Stage*temperature	proline	sugar	Ca	K	Mg	Na
T/-6	1.1000 e	0.00000 c	0.00000 d	31.333 d	25.667 d	0.00000 b
T/-4	2.6667 d	0.05000 c	2.6667 c	4.5888 fg	34.333 c	0.00667 b
T/-2	0.5000 e	0.00667 c	16.3333 b	20.333 e	18.333 e	0.00000 b
T/0	0.0000 e	0.00000 c	0.00000 d	0.000 g	0.000 g	0.00000 b
T/2	0.0000 e	0.00000 c	0.00000 d	0.000 g	0.000 g	0.00000 b
T/4	0.0000 e	0.00000 c	0.00000 d	0.000 g	0.000 g	0.00000 b
B/-6	11.6667 b	0.51000 a	0.00000 d	49.667 a	42.667 a	0.15000 a
B/-4	3.6667d	0.22000 b	16.3333 b	42.000 bc	36.333 bc	0.00000 b
B/-2	7.3333 c	0.02667 c	0.0000 d	2.255 fg	41.000 ab	0.00000 b
B/0	0.0000 e	0.19000 b	0.0000 d	0.000 g	0.000 g	0.00000 b
B/2	0.0000 e	0.00000 c	0.0000 d	0.000 g	0.000 g	0.00000 b
B/4	0.0000 e	0.23333 b	0.0000 d	0.000 g	0.000 g	0.00000 b
F/-6	17.0000 a	0.27000 b	23.6667 a	44.667 ab	40.000 ab	0.01667 b
F/-4	7.0000 c	0.12667 bc	18.3333 b	37.333 c	33.667 c	0.00000 b
F/-2	3.6667 d	0.01333 c	0.00000 d	8.667 f	8.667 f	0.00000 b
F/0	0.3333 e	0.00000 c	0.00000 d	0.000 g	0.000 g	0.00000 b
F/2	0.0000 e	0.00000 c	0.00000 d	0.000 g	0.000 g	0.00000 b
F/4	0.0000 e	0.00000 c	24.6667 a	0.000 g	0.000 g	0.00000 b

Letters represent data significantly ($p < 0.005$) different form other data with different letters, T: Turgidity, B: Bloom, F: Fruit

Table 6: interaction effects of cultivars and temperature on measured parameters

cultivar*temperature	proline	sugar	Ca	K	Mg	Na
M/-6	10.0000 b	0.03667 cd	17.0000 d	50.000 a	45.000 a	00667 ab
M/-4	3.3333 cd	0.20000 b	15.6667 e	35.667 cd	32.000 c	0.00000 b
M/-2	2.3333 de	0.03333 cd	0.0000 f	31.000 d	26.333 d	0.00000 b
M/0	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
M/2	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
M/4	0.0000 e	0.23333 b	0.0000 f	0.000 g	0.000 g	0.02000 b
Var12/-6	13.3333 a	0.10333 bcd	22.6667 b	42.667 b	39.000 b	0.14667 a
Var12 /-4	5.0000 c	0.46667 a	22.0000 b	39.667 bc	0.000 g	0.00000 b
Var12 /-2	1.0000 e	0.00000 d	0.0000 f	8.333 f	7.000 f	0.00000 b
Var12 /0	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
Var12 /2	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
Var12 /4	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
Var18-6	12.1667 ab	0.40000 a	25.3333 a	47.667 a	39.667 b	0.04000 b
Var18 /-4	5.0000 c	0.16000 bc	18.3333 c	42.000 b	33.667 c	0.00000 b
Var18 /-2	1.9333 de	0.00667 d	0.0000 f	0.000 g	19.333 e	0.02333 b
Var18/0	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
Var18 /2	0.0000 e	0.00000 d	0.0000 f	0.000 g	0.000 g	0.00000 b
Var18 /4	0.0000 e	0.00667 d	0.0000 f	21.000 e	38.667 b	0.00000 b

Letters represent data significantly ($p < 0.005$) different form other data with different letters