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The Effect of Low Temperatures on Domestic and Wild Pistachio Rootstocks and Interspecific Hybrids Based on Physio-biochemical Indices

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A R T I C L E I N F O

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A B S T R A C T

In recent decades, spring frost considered a serious threat in pistachio production (*Pistacia vera*). Considering that, Iran is one of the most important centers of pistachio production and has the highest variety of pistachios in the world, the identification of domestic and wild different rootstocks and their crosses to reach cold-tolerant rootstocks could be one of the effective solutions to face the challenge of spring frost. In this study, cold tolerance was assessed in fourteen rootstocks of pistachio domestic species (Badami Zarand, Sarakhs and Qazvini), interspecific hybrids (Qazvini×Khinjuk, Qazvini× Mutica, Qazvini × Baneh Baghi, Qazvini × Atlantica, Qazvini× Integerrima), non-domesticated species (Atlantica (*P. atlantica)*, Integerrima, Khinjuk, Sarakhs, Mutica (*P. atlantica* sub. mutica) and Baneh Baghi (*P. mutica* × *P. vera*)) and UCB1 hybrid rootstock at low temperatures (4, 0 and -4 °C) for 2 hours. This experiment was conducted in a factorial completely randomized design with three replications on one-year-old seedlings in greenhouse conditions. The results showed that the rate of ion leakage, malondialdehyde and injury index percentage was significant ($p<0.01$) and their values were decreased in the cold-tolerant rootstocks than the cold-sensitive rootstocks. The interaction between treatments showed that the temperature of - 4 °C caused a significant reduction in ion leakage and malondialdehyde in the cold-tolerant rootstocks. Proline was increased in the cold-tolerant rootstocks compare to cold-sensitive rootstocks. The results of cation leakage percentage were significant in temperature and rootstocks ($p<0.01$). The results showed that the highest percentage of cation leakage related to potassium and there was a positive and significant correlation between calcium and magnesium and cell membrane stability. According to the results, the most sensitive rootstock was UCB1, Integerrima, Khinjuk and Baneh Baghi, respectively. Hybrids Qazvini , Baneh Baghi, Khinjuk and Integerrima rootstocks were significantly reduced under low-temperature stress. Mutica, Qazvini×Mutica, Sarakhs, Qazvini, Qazvini×Atlantica rootstocks, improved physiological and biochemical traits and maintained cell membrane integrity and finally showed greater tolerance to frost and freezing temperatures, which can be considered in the breeding program.

Introduction

Pistachio (*Pistacia vera* L.) is one of the most important nut crops (Agricultural Statistics, 2019 & 2020; Norozi et al., 2019; Sharifkhah et al., 2020). Although pistachio is known as a plant adapted to adverse environmental conditions, however, several reports are available in regard of sensitivity of pistachio to environmental stresses (Alipour, 2018; Shamshiri and hasani, 2018), among which spring frost is very important. So that, more frost injury due to climate change is expected in the next few years (Afshari *et al*., 2009; Kodra *et al*., 2011). Therefore, the identification of resistant or tolerant rootstocks is

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one of the best ways to overcome this challenge. Physiologically, the cold decreased cytoplasmic flow, turgor pressure and general organ dysfunction (Aslamarz *et al.*, 2010). Exposure of plant tissue to low temperatures also causes ion leakage of calcium to the cytoplasm, which is related to variation in enzyme activity because of the break of cell walls (Sayyari *et al*., 2009; Habibie *et al.*, 2019). Thus, electrolyte leakage occurs when the membrane is damaged (Hanna and Bischofa, 2004; Esenta *et al*., 2003; Aslamarz and Vahdati, 2010). The frost damage depends on the temperature, the rate of reduction, and the compatibility of the plant species. Plants that have previously adapted to this phenomenon are somewhat tolerant (Guy, 2003; Gusta *et al*., 2003; Aslamarz *et al.*, 2011). In cold-tolerant cultivars, the cell membrane preserves in the liquid phase.

Many plants respond to environmental stresses, such as cold stress, by increasing free proline to improve osmotic adjustment which stabilizes the membrane during stress (Nasibi et al., 2020; Aslamarz *et al.*, 2011; Rahemi *et al.*, 2016). In this regard, the literature showed that cold resistance is directly related to increased proline (Ghasemi Soluklu et al., 2014; Chen *et al*., 2005; Ait Barka *et al*., 2006). The mechanism of the increase in cold resistance by proline underlies osmotic regulation and reduction of water potential, oxygen neutralization, and cell membrane stability (Matysik *et al*., 2002). Afshari *et al*. (2010) reported that the highest amount of soluble sugars and proline were intolerant cultivars, and the lowest was related to susceptible cultivars in three commercial cultivars of pistachio in Damghan. Similar observations were reported by Tajabadipour *et al*. (2018). Evaluation of plant physiological parameters such as ion leakage, malondialdehyde (MDA), proline content, and activity of antioxidant enzymes reflects the physiological response of plant in response to cold stress (Imahori *et al*., 2008).

Afrousheh *et al*. (2018) studied cold resistance in four pistachio rootstocks to spring frost. Their results showed that the maximum time to evaluate the rate of ion leakage and pH of leaked solution occurred after 24 hours in sample incubation. Based on their results the rate of ionic leakage increased with decreasing temperature from 0 ° C to -6 ° C. The present results showed that the percentage of ion leakage could be a suitable tool for studying the cold and freezing stress. Hokmabadi *et al* (2016) evaluated cold resistance in Damghan three commercial cultivars. They reported that Shahpasand and Abbas Ali cultivars had the highest potassium leakage, while Khanjari cultivar had the lowest leakage of potassium. They reported the cold injury in rootstocks were observed at -4 °C. In this temperature, Badami and Sarakhs rootstocks were sensitive and Qazvini and Atlanta rootstocks recognized as tolerant. At temperatures of -4 and -6 °C, Qazvini rootstock was more resistant than other rootstocks.

Tajabadipour *et al*. (2018) classified several cold-resistant trees in pistachio orchards of Sirjan city (spring frost in 2008). Temperatures of -4 °C caused a significant increase in ion leakage, proline, hydrogen peroxide, and malondialdehyde compared to -2 °C, especially in cold-sensitive rootstocks. Sajadian *et al.* (2019) studied the physio-biochemical responses of rootstocks and hybrids of pistachio species to low temperature. According to their results, the highest ion leakage, malondialdehyde, soluble sugars, proline, antioxidant capacity, and protease activity were in the temporal treatment -4°C. The hybrid of Integerrima×Badami Zarand was the most sensitive rootstock to cold. The aim of the present study was to investigate the frost and freezing temperatures on domestic and wild species of pistachio and their interspecific hybrids base on physiological and biochemical traits

Materials and Methods

Plant material

In this experiment, the seeds were prepared from the Pistachio Research center (PRC), Rafsanjan, Iran.

They were soaked in distilled water for 24 hours. After germination, three seeds were planted in each pot. In this study, 14 rootstocks (domestic, non-domestic, hybrids between pistachio species and UCB1) were evaluated in frost and freezing temperature (4, 0, and -4 °C for two hours). Rootstocks included *P. vera* (Badami Zarand, Sarakhs and Qazvini), interspecific hybrids (Qazvini×Khinjuk, Qazvini× Mutica, Qazvini × Baneh Baghi, Qazvini × Atlantica, Qazvini× Integerrima), non-domesticated species including Atlantica (*P. atlantica)*, Integerrima, Khinjuk, Sarakhs, Mutica (*P. atlantica* sub. mutica) and Baneh Baghi (*P. mutica* × *P. vera*) and UCB1 (tissue culture-generated plant). This research was carried out as factorial in a completely randomized design (CRD) with three replications on one-year-old seedlings. Three pots were selected and transferred to the laboratory in late April. The incubator (freezing room) was used to apply the low-temperature treatments. The seedlings were placed in a refrigerated incubator. The incubator temperature reached 4 °C within 5 hours, then cold treatments of 4, 0, and -4 °C were applied for two hours. After treatments, physiological and biochemical traits were measured. The studied traits were measured as follow.

Electrolyte leakage measurement

Following the application of the temperature treatments, the cell death index was measured by Sairam (1994) method. According to this method, 0.1 g of leaves were placed in 10 ml of double distilled water. Then it placed in a water bath of 40 °C for 30 minutes and its electrical conductivity (EC) was read with the EC meter (C1), then the sample was placed in a bath of Ben Marie for 15 minutes at temperature 100 °C and for the second time its electrical conductivity was read (C2) and the indices were calculated based on the following equations.

$$EL = \frac{C1}{C2}$$

$$MSI = (1 - \frac{C1}{C2}) \times 100$$

$$I\% = 1 - (\frac{T-D}{T-C}) \times 100$$

$$CMS = (\frac{1-\frac{t1}{t2}}{1-\frac{c1}{c2}}) \times 100$$

In the I% formula (Injury Index), the D, C, and T represent the ion leakage in control, stress, and total states (C + D), respectively. In the CMS formula (Cellular Membrane Stability), the c and t represent the electrical conductivity under control and stress conditions, respectively. Indices 1 and 2 show the initial and final electrical conductivity, respectively.

Proline

The concentration of proline was measured by Bates et al. (1973) method. First, the leaf sample (0.5 g) was crushed in liquid nitrogen, and homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid. The homogenized mixture was filtered through a Whatman filter paper. Then, 2 mL ninhydrin and 2 mL glacial acetic acid were added to 2 mL of filtered extract. The extract was incubated in a boiling water bath for 1 hour, and the reaction was finished with the ice. 4 ml of toluene was added to the mixture, and the organic phase was extracted. Absorbance was measured at 520 nm by the spectrophotometer, while toluene was used as blank.

Lipid peroxidation

Lipid peroxidation was measured base on MDA concentration, according to Heath and Packer's method (1968). In this method, 0.25 g of the leaf sample was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 6000 rpm for 5 min. At the next step, 250 µl of the supernatant was added with 1 ml of malondialdehyde solution (containing 20% trichloroacetic acid and 0.5% thiobarbituric acid). The resulting mixture was heated for 30 minutes at 95 °C in a Ben Marie bath then, immediately placed in ice, and centrifuged again for 10 minutes. The Absorbance was measured at 532 nm by the spectrophotometer.

Measurement of ion leakage of cations (sodium, potassium, magnesium and calcium)

Concentrations of cations including sodium, potassium, magnesium and calcium were measured in the leaked solution (Hokmabadi *et al*., 2016, Ryyppö *et al*., 1998).

Cation leakage (%)= Cation concentration before autoclave / Cation concentration after autoclave×100

Statistical analysis

In this study, statistical analysis of data performed using SPSS software and comparison of data means with Duncan's method at 5% level. Graphs were drawn using Excel.

Results

Measurement of indicators of cell death

The results showed that the indices of electrolyte leakage (EL), membrane stability index (MSI), injury index (I), and leaf cell membrane stability (CMS) under low-temperature treatments were significant at the level of 1% probability (Table 1). In this study, ion leakage increased with decreas in temperature. The highest rate of ion leakage was significantly related to -4 °C. The cell death damage results showed that these indices were significant at -4 °C in all studied rootstocks (Table 2). Based on the results, with temperature reduction to -4 °C, ion leakage (EL) and injury index (I%) increased 22.7% and 27.39%, respectively. Membrane stability index and cell membrane stability decreased 15.3% and 20.4%, respectively (Table 2). The interaction effect indicated that the highest amount of ion leakage was related to UCB1 and Khinjuk rootstocks (Fig. 1). Based on the results, the lowest amount of membrane stability was observed in these two rootstocks (Fig. 2). The results of the variance analysis showed that the malondialdehyde content in temperature and rootstock treatments had a significant difference at the probability level of 1 and 5%, respectively (Table 1). Overall, malondialdehyde content was increased with decreasing temperature and significantly increased at -4 °C. According to the results, the highest content of malondialdehyde was observed in UCB1 (0.52 μg g^{-1} fresh weight) and Khinjuk (Table 2). The results of the interaction effects also indicated that the content of malondialdehyde in all rootstocks significantly increased with decreasing temperature. The highest was observed in UCB1 (0.70 μg g^{-1} fresh weight) and Khinjuk and Baneh Baghi, Integerrima, hybrids Qazvini × Integerrima, and Qazvini× Baneh Baghi, (Fig. 3). There was a significant difference between the rootstocks in proline content (Table 1). The proline level at -4 °C was significantly higher than 0 and +4 °C (Table 2). The highest was observed in Mutica, Qazvini× Mutica, and Sarakhs rootstocks (0.411, 0.339, and 0.313 mg g^{-1} fresh weight) and the lowest were observed in the hybrid rootstock of UCB1 (0.181 mg g^{-1} fresh weight), Integerrima, Khinjuk, Baneh Baghi and hybrid Qazvini× Integerrima (0.21, 0.205, 0.22 and 0.238 mg g^{-1} fresh weight) (Table 2).

Table 1. Analysis of variance of the effect of cold treatments on some physiological and biochemical traits of different pistachio rootstocks

Source of variation	df	Mean Square					
		Proline ($\mu molg^{-1}$ FW)	MDA ($\mu molg^{-1}$ FW)	EL (%)	MSI (%)	I (%)	CMS (%)
Temperature (T)	2	0.209^{**}	1.123^{**}	25332.3^{**}	25332.6^{**}	8048.7^{**}	4980.1^{**}
Rootstock (R)	13	0.034^{**}	0.066^{**}	416.45^{**}	416.6^{**}	768.1^{**}	664.5^{**}
T× R	26	0.004^{**}	0.032^{*}	264.02^{**}	265.4^{**}	512.7^{**}	528.6^{**}
Error	84	0.0001	0.0029	24.43	24.18	34.4	67.9
CV%	-	5.72	6.92	4.3	3.2	2.1	5.1

ns, * and **: non-significant, significant at 5% and 1%, respectively

Table 2. Mean Comparison of the effect of cold treatments on some physiological and biochemical traits of different pistachio rootstocks

	Treatment	Proline ($\mu molg^{-1}$ FW)	MDA ($\mu molg^{-1}$ FW)	EL (%)	MSI (%)	I (%)	CMS (%)
Cold	4 °C	0.194c	0.145c	29.1c	70.5a	5.01c	98.9a
	0 °C	0.263b	0.278b	35.9b	64.8b	10.22b	92.2b
	-4 °C	0.335a	0.457a	44.8a	55.2c	27.39a	78.5c
Rootstock	Badami Zarand	0.240f	0.308c	34.9e	65.1cde	11.9de	89.5cd
	Qazvini	0.3d	0.238d	29.5g	72.5a	7.5g	95.6ab
	Sarakhs	0.313c	0.221d	28.19gh	71.8a	4.8h	94.7ab
	Mutica	0.411a	0.146e	26.9h	73.1a	5.1gh	97.8a
	Baneh Baghi	0.220g	0.361c	40.5cd	58.5g	19.1bc	83.8e
	Khinjuk	0.205h	0.467b	41.25c	53.7g	21.7b	79.1f
	Atlantica	0.269e	0.292cd	33.63ef	66.3bcd	10.4ef	89.7cd
	Integerrima	0.210gh	0. 328c	46.3b	55.78g	23.22b	76.2e
	UCB1	0.181i	0.525a	50.7a	49.3h	34.5a	66.1g
	Qazvini× Mutica	0.339b	0.199e	28.13gh	71.8a	4.8h	94.7ab
	Qazvini × Baneh Baghi	0.243f	0.320c	37.9de	60.7ef	9.3ef	90.1c
	Qazvini×Khinjuk	0.237f	0.346c	39.8d	62.1def	10.5ef	91.9c
	Qazvini × Atlantica	0.293d	0.247d	28.3gh	69.6b	7.9g	95.7ab
	Qazvini× Integerrima	0.238f	0.350e	39.8d	61.1ef	17.4c	82.9e

(Means followed by the same letter are not significantly different at 5% probability using Duncan's test)

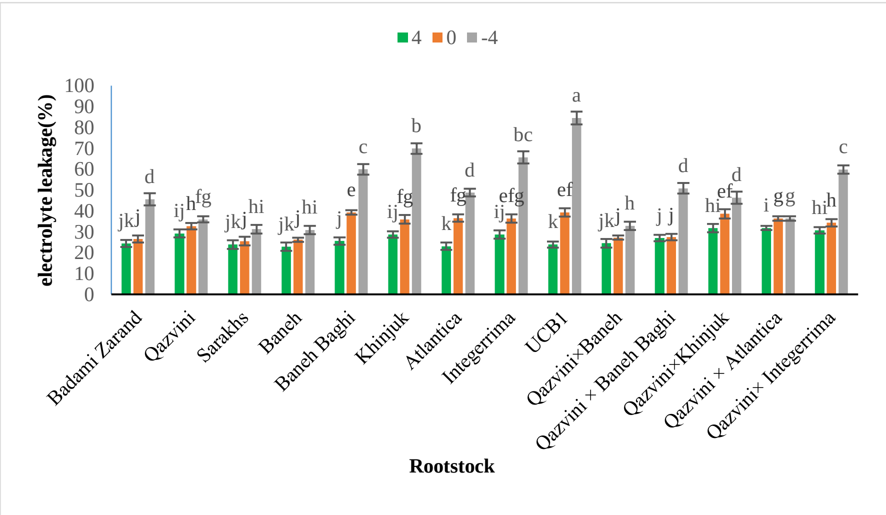

Fig.1. Evaluation of electrolyte leakage in the studied rootstocks under low-temperature treatments. (Means followed by the same letter are not significantly different at 5% probability using Duncan's test)

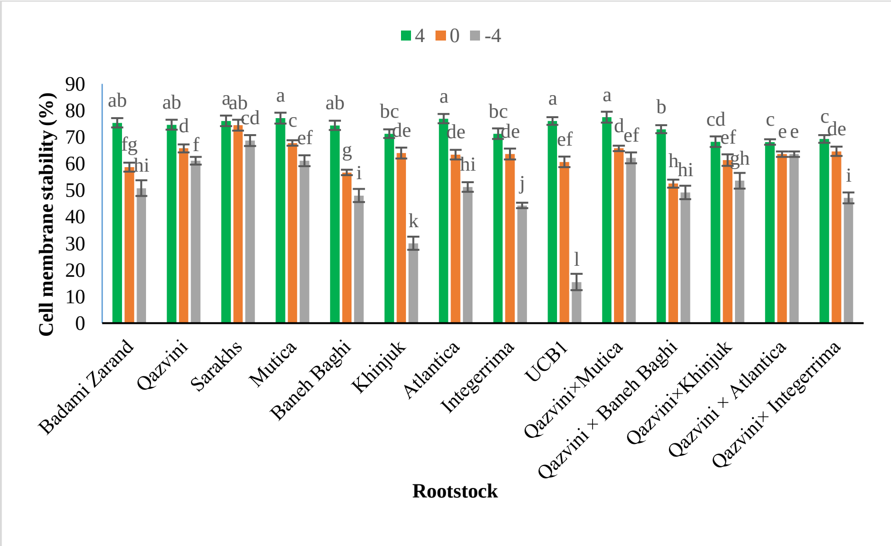

Fig. 2. Evaluation of leaf cell membrane stability in the studied rootstocks under low-temperature treatments. (Means followed by the same letter are not significantly different at 5% probability using Duncan's test)

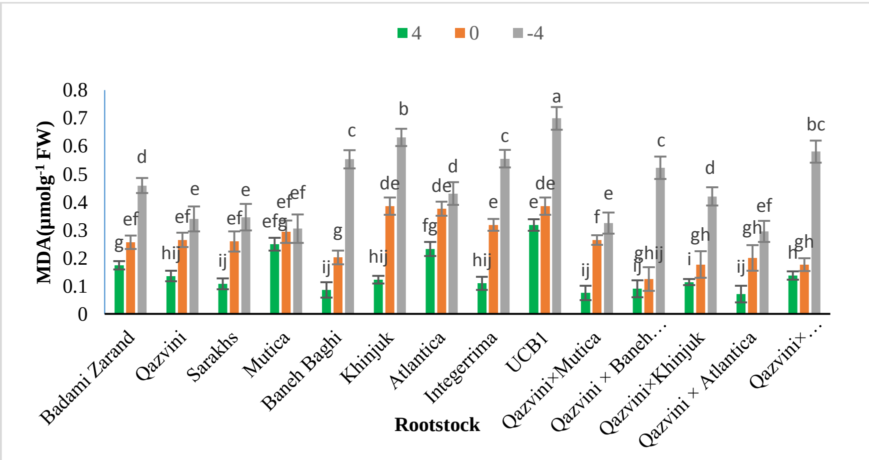

Fig. 3. Evaluation of leaf cell membrane stability in the studied rootstocks under low-temperature treatments. (Means followed by the same letter are not significantly different at 5% probability using Duncan's test)

Measurement of ion leakage percentage of cations

The results of the variance analysis indicated that the percentage of ion leakage was significant at the level of 1% probability in rootstock and temperature treatments. But the interaction effects were not significant (Table 3). Overall, the percentage of cation leakage significantly increased at -4 °C (Table 4). The mean comparison results showed that the percentage of cation leakage was significant in the rootstocks (Table 4). According to the results, the highest of sodium leakage was in UCB1, Integerrima, Khinjuk, and Qazvini × Integerrima rootstocks. The highest leakage of potassium was in UCB1, Integerrima, hybrid Qazvini × Khinjuk, and hybrid Qazvini × Integerrima. The highest leakage of calcium was in UCB1 and Integerrima, and the highest of magnesium leakage was in UCB1.

Table 3. Analysis of variance of the effect of cold treatments on the percentage of cation leakage of different pistachio rootstocks

Source of variation	df	Mean Square			
		Leakage Sodium (%)	Leakage Potassium (%)	Leakage Calcium (%)	Leakage Magnesium (%)
Temperature (T)	2	1243.22**	2064.22**	226.46**	398.67**
Rootstock (R)	13	390.52**	1493.52**	131.09**	168.15**
T× R	26	50.8 ns	210.2ns	27.02 ns	37.01ns
Error	84	69.15	314.8	79.53	92.6
CV%	-	8.93	6.7	5.05	10.05

ns, * and **: non-significant, significant at 5% and 1%, respectively

Table 4. Mean Comparison of the effect of cold treatments the percentage of cation leakage of different pistachio rootstocks

	Treatment	Leakage Sodium (%)	Leakage Potassium (%)	Leakage Calcium (%)	Leakage Magnesium (%)
Cold	4 °C	18.40c	22.44b	4.46c	3.85c
	0 °C	22.91b	26.05b	7.01b	5.22b
	-4 °C	29.23a	35.98a	15.84a	16.16a
Rootstock	Badami Zarand	18.34b	13.8c	10.19bc	18.59de
	Qazvini	22.82b	24.27bc	8.36cd	10.70f
	Sarakhs	24.78b	25.88bc	10.08bc	18.93de
	Mutica	20.28b	23.69bc	4.25g	1.70g
	Baneh Baghi	16.62b	13.54c	7.5def	27.93bc
	Khinjuk	30.67a	14.8c	6.3efg	22.27cd
	Atlantica	20.93b	15.03c	10.16bc	12.91ef
	Integerrima	34.52a	49.83a	19.85a	15.2def
	UCB1	35.63a	50.43a	20.91a	44.46a
	Qazvini× Mutica	19.69b	26.25bc	8.87cde	2.25g
	Qazvini × Baneh Baghi	18.10b	26.75bc	5.62fg	32.75b
	Qazvini×Khinjuk	20.47b	38.26ab	9.01bcd	16.01def
	Qazvini × Atlantica	21.29b	26.87bc	8.85bc	1.57g
	Qazvini× Integerrima	34.03a	44.71a	8.87bc	29.03bc

(Means followed by the same letter are not significantly different at 5% probability using Duncan's test)

Discussion

Cold damage first causes metabolic and cellular changes (electrolyte leakage), and then damage symptoms in the plant under stress appears (Mirmohammadi Meybodi and Tarkesh Isfahani, 2004). The most important indicators are the change in cell membrane fluidity by cold damage, which results in functional disorders such as increased permeability, loss of intracellular water solids, inactivation of transport channels, and finally, electrolyte leakage occurs (Dionne *et al*., 2001; Larcher, 2004; Buchanan *et al*., 2015). Based on the results of this study, the rate of electrolyte leakage increased under low-temperature stress. It was consistent with the previous literature (Rahemi et al., 2016). Our results showed that in freezing temperature -4 °C, the ion leakage and injury index percentage increased 22.7% and 27.39%, respectively, and membrane stability index and cell membrane stability decreased by 15.3% and 20.4%, respectively. The highest percentage was in UCB1 and Khinjuk rootstocks under cold stress. The lowest cell membrane stability was in these two rootstocks. The results obtained are consistent with the results of the percentage of damage. According to the damage results, the UCB1 rootstock had 33.33% seedling drying damage and 10% leaf wilting. Cold-tolerant species (Mutica, Sarakhs, hybrid Qazvini × Mutica) had a more stable cytoplasmic membrane and less electrolyte leakage than susceptible genotypes. Our results were consistent with other researchers (Azzarello *et al*., 2009; Ahmad and Prasad, 2012). Mavali (2011) investigated electrolyte leakage on five pistachio cultivars under cold stress. Their results showed that the resistant rootstocks had the least electrolyte leakage in spring frost damage. Malondialdehyde (MDA) is one of the terminal products of lipid peroxidation (LPO). Therefore, it is an important indicator of structural membrane damages and cellular metabolic status exposed to low-temperatures (Zeinali Yadegari *et al*., 2009; Sofo *et al*., 2004; Fahimirad *et al*., 2013). The results of this study explained that rootstocks Mutica, Sarakhs, Qazvini, and hybrid Qazvini × Mutica had the minimum amount of malondialdehyde. This indicator states that the cell membrane was more resistant to cold stress in the tolerance-rootstocks. Our result was in agreement with the previous studies (Posmyk *et al*., 2004; Guo *et al*., 2012; Guo *et al*., 2016). Also, the Pearson correlation effects indicated that the malondialdehyde had a positive correlation with ion leakage (0.902 **) and the percentage of injury index (0.804 **) and showed a negative and significant correlation with the membrane stability (-0.855 **) and cell membrane stability (-0.843 **) (Table 5).

The proline amount in the studied rootstocks at -4 °C was significantly higher than that of 0 and +4 °C. The highest was in the rootstocks of Mutica, hybrid Qazvini× Mutica, and Sarakhs, and the lowest amount was in the rootstock of UCB1 hybrid (0.181 mg g^{-1} fresh weight) and then in Integerrima, Khinjuk and Baneh Baghi (0.21, 0.21 and 0.22 mg g^{-1} fresh weight). In the other studies, an increase in proline synthesis has been reported in many tolerance species under cold stress (Mansour, 2000; Ruelland *et al*., 2002; Couée *et al*., 2006; Wahid *et al*., 2007; Buchanan *et al*., 2015; Wisniewski *et al*., 2017). Proline is a storage source for carbon, nitrogen, and a free radical scavenger (Chen and Murata, 2000). Proline stabilizes extracellular structures (including membranes and proteins) and eliminates the stress-induced cell redox potential. This compound protects cell membrane phospholipids against degradation and acts as a hydroxyl radical scavenger, resulting in membrane stability and less water loss through cell membranes (Ashraf and Foolad, 2006; Szabados and Savoure, 2009; Verslues and Sharma, 2010; Shang *et al*., 2011). Based on the results, the accumulation of proline increased in the tolerance rootstocks to low-temperature stress, which was consistent with the results of Nikoogoftar Sedghi *et al.* (2021) on cold tolerance of 15 pistachio cultivars. Their results showed that proline concentration could be an indicator in the selection of cold-tolerant species. The Pearson correlation effects indicated that the amount of proline exhibited a negative and significant correlation with malondialdehyde (-0.494 **), ion leakage (-0.889**), and injury index percentage (-0.692**) (Table 5).

In this research, the percentage of cation leakage at -4 °C significantly increased. Base on the results, the highest leakage of sodium was in UCB1, Integerrima, Khinjuk, and hybrid Qazvini ×Integerrima. The highest leakage of potassium was observed in UCB1, Integerrima, hybrid Qazvini × Khinjuk, and hybrid Qazvini × Integerrima. The most leakage of calcium was in UCB1 and Integerrima, and the highest leakage of magnesium was in UCB1.

It has been reported that when leaves are exposed to cold stress, the concentration of calcium in cold-sensitive plants transiently increases and acts as a second messenger. Potassium and magnesium concentrations play an important role in plant response to low temperature stress (Czek-Kwinta *et al*., 2007; Song *et al*., 2008). The high percentage of potassium in cell content is related to osmotic regulation, cell sap potential and the reduction of electrolyte leakage due to low-temperature (Singer *et al*., 1996; Beringer and Troldenier, 1980). The results of this study indicated that among the studied cations, the high leakage of cations was related to potassium, sodium, magnesium, and calcium cation, respectively, and sensitive species had higher cationic leakage compared to resistant species. The lowest leakage of cations was observed in Mutica (tolerant-rootstock) and the highest in UCB1 (sensitive-rootstock). Mir Mohammadi Meybodi (2004) stated that the highest percentage of damages under cold stress occurred on ion leakage. They have also determined that the most released element in damaged cells was potassium, which is consistent with our results of this study. Hosseini (2000) indicated that in cold-sensitive pistachio cultivars, the leakage of calcium, sodium, and potassium ions was higher than resistant cultivars, which is consistent with the results obtained from this study. The results of the Pearson correlation showed that the percentage of calcium cation leakage had a positive relationship with ion leakage (0.670 **) and injury index percentage (0.679 **) and had a significant negative correlation with membrane stability (-0.557**) and cell membrane (-0. 696 **). The percentage of magnesium leakage had negatively correlated with proline (-0.707**), membrane stability index (-0.690**) and cell membrane (-0.575*) and had a positive and significant correlation with malondialdehyde (0.760**), ion leakage (0.702**), and percentage of injury index (0.536*) (Table 5). The results also indicated that sensitive rootstocks have more electrolyte leakage and less proline than tolerant rootstocks. Therefore, it can be concluded that preventing damage of the cell membrane is a mechanism to increase cold stress tolerance in the tolerant rootstocks.

Table 5. Correlation analysis of some physiological Characteristics in low-temperature pistachio rootstocks

Characteristics	Leakage Sodium	Leakage Potassium	Leakage Calcium	Leakage Magnesium	Proline	MDA	EL	MSI	I	CMS
Leakage Sodium	1									
Leakage Potassium	0/715**	1								
Leakage Calcium	0/660*	0/689**	1							
Leakage Magnesium	0/424	0/323	0/371	1						
Proline	-0/388	-0/208	-0/529	-0/707**	1					
Malondialdehyde	0/489	0/235	0/481	0/760**	-0/89**	1				
Electrolyte Leakage	0/515	0/433	0/670**	0/702**	-0/89**	0/902**	1			
Membrane Stability Index	-0/357	-0/268	-0/557*	-0/690**	0/864**	-0/85**	-0/95**	1		
Injury Index	0/445	0/423	0/679**	0/536*	-0/69**	0/804**	0/896**	-0/888**	1	
Membrane Stability	-0/502	-0/356	-0/696**	-0/575*	0/685**	-0/84**	-0/83**	0/812**	-0/935**	1

* and **; significant at the 5% and 1%, respectively.

Conclusions

The measurement of physiological and biochemical traits was studied on the fourteen rootstocks under low-temperature stress (temperatures of +4, 0 and -4 °C). Base on the results, the studied rootstocks had different physicochemical reactions in cold stress tolerance, which are related to the genetic diversity of domestic and wild species of pistachios. The results showed that tolerant-rootstocks including Mutica (*P. atlantica* sub. mutica), hybrid Qazvini × Mutica, hybrid Qazvini × Atlantica, Sarakhs, Qazvini. Semi-sensitive rootstocks included Badami Zarand, Atlantica, hybrid Qazvini × Baneh Baghi, Qazvini × Khinjuk. Sensitive rootstocks were related to Khinjuk, Baneh Baghi and Qazvini × Integerrima, Integerrima, UCB1, respectively. Interspecific hybrid Qazvini with Baneh Baghi, Khinjuk and Integerrima rootstocks were able to significantly reduce the stress caused by low temperatures.

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