Journal of Nuts
Journal homepage: ijnrs.damghaniau.ac.ir

Evaluation of Freezing Damage in some Pistachio Seedling Rootstocks

Maryam Afrousheh*[1], Hossein Hokmabadi[2], Hassan Arab[1], Ali Tajabadipour[1]

[1]*Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran*

[2] *Damghan Pistachio Station, Agricultural and Natural Resources Research Center of Semnan Province (Shahrood), AREEO, Iran*

A R T I C L E I N F O

Keywords:

Freezing tolerance,
Ionic leakage,
pH,
Pistachio rootstocks

A B S T R A C T

A greenhouse experiment was conducted to evaluate the freezing damage in some pistachio rootstocks by ion leakage and pH changes of leaked solution. A factorial experiment was carried out in randomized block designs (RBD) with three factors: Temperature (A) including 4˚C, 0˚C, -2˚C, -4˚C, -6˚C, Time (B) including 3, 12, 24h , and Rootstock (C) including *P. vera* cv 'Badami Zarand' (V13) and 'Sarakhs' (S5), *P. mutica* (M1)and *P. atlantica* (A7). For this, one-year-old seedlings were kept at these five temperatures in incubator for 2 hours. Then in the first 24 hours in three hour intervals and during four days, EC and pH in leaked solution were measured daily. After four days the seedling samples were autoclaved at temperatures 105°C for 4 minutes to destroy all cell membrane. EC and pH of remaining solution were measured again and the percentage of ionic leakage was calculated. The results showed that the best time to evaluate the pH and ionic leakage was 24 hours after incubation of samples. Based on the results, ionic leakage dramatically increased with decreasing temperatures from 0°C to -6°C, while pH of leaked solution had no significant difference in 0°C and 4°C temperature treatments. When temperature reduced from 0°C to -6°C, like ionic leakage, pH greatly reduced. So the pH of the leaked solution could be an appropriate tool to study the freezing damage of pistachio rootstocks. Based on the results of pH and ionic leakage, *P. mutica* and *P. atlantica* were the most frost tolerant and sensitive rootstocks of this experiment, respectively.

Introduction

Low temperature stress is one of the most important problems that restrict agricultural productivity of plants (Xin and Browse, 2000). The most important problem facing the economic crop production in pistachio orchards are spring frost. This stage usually occurs in late March to early April and causes damages to flower buds of pistachio. During spring frost, temperature drops to -6°C in some parts of the Kerman pistachio growing areas causing heavy damage to pistachio production in the region which ultimately results in reducing export by 50 to 70% (Afshari *et al*., 2008). Frost damage occurs when ice forms inside the plant tissue and injures the plant cells and may lead to cell death (Chen and Li, 2002). The evaluation of frost damage through ionic leakage, first time used by Dexter *et al*., (1932). Cold stress affects the structure and permeability of cell membrane and increases the leakage of ions from cells. Therefore increasing membrane permeability and ionic leakage associated with tissue sensitivity to frost (Flinn and Ashworth, 1995; Leinonen, 1996; Flint *et al*., 1967; Mikal, 2002). Bartolozzi and Fontanazza (1999)

***Corresponding author**: Email: afrousheh@pri.ir
Received: 22 February 2017; Received in revised form: 2 October 2017; Accepted: 14 May 2018

studied 12 cultivars of olives native to various regions of Italy with ionic leakage, their results showed that ionic leakage clearly showed the difference in tolerant and susceptible cultivars. Electrical conductivity (EC) determines the amount of water-soluble ionic. Therefore, electrical conductivity increases with high levels of water-soluble ionic (Murata and Tatsumi, 1979). So far, this method studied in freezing tolerance in plants such as Passiflora species (Patterson *et al*., 1976), avocado leaves (McKellar *et al*., 1992), peach (Ashworth, 1982), plum (Cary, 1985), clover (Nunes and smith, 2003), olive (Soleimani *et al*., 2003) and pistachio (Afshari *et al*., 2010). Results showed that ionic leakage is proportional to the severity of injury to the plant cells. Mavali (2011) studied on ionic leakage of pistachio that they showed tolerance to the spring frost in 2008. Results showed that four rootstocks of five selected rootstocks as tolerant that exhibited lowest ionic leakage. Measurement of ion leakage can help to understand how much damage caused to cells and their membranes. By measuring the amount of ionic leakage of cells, we can determine the different varieties of plants in terms of tolerance to freezing stress (Mirmohamadi Meibodi and Tarkeshe Esfahani, 2004). Hokmabadi *et al*., (2011) studied the tolerance of three commercial cultivars of pistachio including,'Kalleh Ghoochi', 'Ahmad Aghaei' and 'Ouhadi' to spring frost damage. Results showed that the flowering stage of 'Ahmad Aghaei' had less leakage than the other two cultivars. In addition, in following fruit set stage,'Kalleh Ghoochi' in comparison to 'Ahmad Aghaei' and 'Ouhadi' was more sensitive to the spring frost damage. The effect of temperature on the rate of ionic leakage showed that with decreasing temperature from 0°C to -6°C, ionic leakage dramatically increases. The results established that pH of leaked solution, same as percentage of ion leakage, can be an appropriate indicator for studying the frost damage in pistachio. The results of interaction effects of temperature and cultivar on the rate of pH of leaked solution indicated that cultivar of 'Ouhadi' in different temperature was more susceptible to spring frost damage than the other cultivars and with decreasing temperature from 4°C to -6°C, pH levels in this cultivar was less than the others. Freezing stage is when the intra-cellular water freezes. This causes the death of the cells. Damage of cold killing temperatures is caused through drying plant tissues directly by freezing the cellular. Ice crystals may form inside (intracellular) or outside (intercellular) cells. Their sharp edges can puncture the cell walls and cause cellular contents to leak away. Killing temperatures for leaves were determined based on the electrolyte leakage test (Sukumaran and Weiser, 1972) and pH (Hokmabadi *et al*., 2011; Yoshida *et al*., 1999). Choosing the rootstock is one of the most important decisions in orchard establishment. The aim of this study was to evaluate the freezing damage in four pistachio rootstocks by ionic leakage and pH changes.

Materials and Methods

Rootstock selection is one of the most important decisions in orchard management. In Iran specially in Kerman province , pistachio growing areas, pistachio cultivars are grown on pistachio seedling rootstocks of different cultivars such as 'Badami Zarand' and 'Ghazvini'. Native *P. vera* forests are located in north eastern part of Iran particularly in Sarakhs region. This native *P. vera* is the origin of cultivated pistachio trees in Iran. Wild pistachio species, *P. atlantica* , *P. Khinjuk* Stock and *P. mutica* F and M 'Beneh', are grown in Iran. *P. mutica* is a wild species indigenous to Iran, growing with almond, oak and other forest trees common to most Alpine regions. *P. atlantica* is a species of pistachio tree that adapted to drought and the Mediterranean climate. Literatures showed that pistachio rootstocks differ in their ability (Tavallali and Rahemi, 2007). This experiment was carried out to evaluate the tolerance of four pistachio seedling rootstocks- including *P. vera* cv. 'Badami Zarand' (V13) and 'Sarakhs' (S5) as domesticated species and *P.mutica* (M1) and *P. atlantica* (A7) as wild species-to the freezing damage by ionic leakage and pH changes of leaked solution. A greenhouse experiment conducted as factorial with three factors: A (temperature), B (time) and C (seedling rootstock) and three replications. For evaluation of physiological characteristics, one year old seedlings of rootstocks were used. Three seedlings from each rootstock were kept in five different temperatures (4°C, 0°C, -2°C, -4°C, and -6°C) for 2 hours in refrigerated incubator. After incubation(applying of cold treatments), 2g samples of old leaves were removed from the seedlings immediately and added to 30 ml deionized water and shaken on the shaker. EC and pH in leaked solution were measured in 3, 12 and 24 h daily and for four days. In fourth day, the leaf samples were autoclaved at temperatures 105°C for 4 minutes in order to destroy all cell membrane. EC and pH values in remaining solution were measured again and the percentage of ionic leakage calculated based on the following formula:

$$\text{Percentage of ionic leakage} = \frac{Ec_1}{Ec_2} \times 100$$

In the formula, EC_1 and EC_2 are electrolytic conductivity before and after autoclaving, respectively. It is assumed that the conductivity after autoclaving represents complete (100%) ionic leakage (Arrora *et al*., 1992).

The aim of this research was evaluate the seedling behavior in freezing damage among native Iranian pistachio species can be used as rootstock.

Statistical analysis

Data were analyzed using MSTAT-C software. Mean comparisons were grouped by Duncan's multiple range tests at the probability level of 5 percent.

Results

The results of analysis of variance indicated that the interaction effect of temperature, time and rootstock was statistically significant on ionic leakage and pH in leaked solution (Table1).

Table 1. Analysis of Variance (ANOVA) of the effect of frost temperature on pH and ionic leakage in four pistachio seedling rootstocks

Source of variation	df	Mean Square	
		Ionic leakage	pH
Factor A (Temperature)	4	3503.513**	5.691**
Factor B (Time)	2	21126.734**	3.364**
Factor C (Rootstock)	3	6336.082**	3.172**
A * B	8	1143.629**	1.298**
A * C	12	1476.291**	1.297**
B * C	6	200.159**	0.579*
A * B * C	24	356.499 ^{ns}	0.156^{ns}
Error	120	129.719	0.219
CV%	180	22.19	15.12

Significant F-test at **P < 0.01, at *P <0.05 and non-significant (NS)

The interaction effect of temperature and seedling rootstocks on pH in leaked solution

The comparison of means showed that pH of the leaked solution under frost temperature in *P. mutica* and *P. vera* cv. 'Sarakhs' were significantly higher than the other seedling rootstocks. The lowest pH after frost damage was observed in *P. atlantica* (Fig. 1). These results indicated that *P. mutica* and *P. vera* cv.'Sarakhs' were the most tolerant rootstocks whereas *P. atlantica* was sensitive.

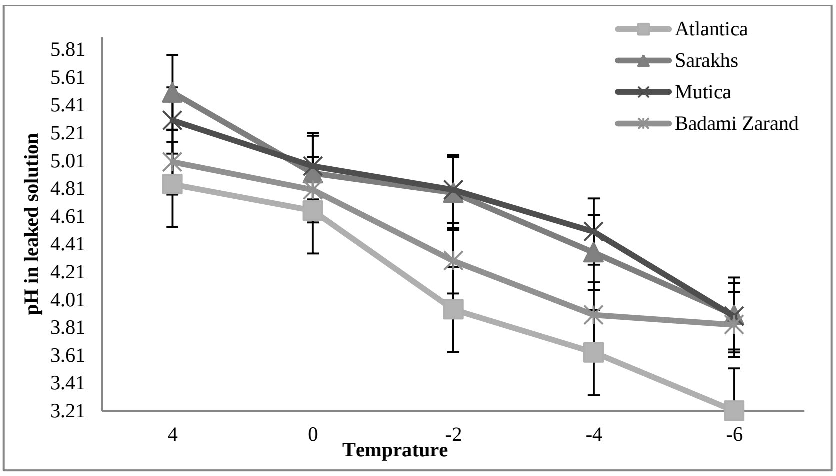

Fig. 1. Interaction effect of temperature and seedling rootstock on pH in leaked solution
(Means followed by the same letter are not significantly different at 5% probability using Duncan's test).

The interaction effect of temperature and time on pH of leaked solution

The effect of temperature and time on pH of leaked solution indicated that the leaking solution pH until the temperature of 0°C was not significant compared to control (4°C). By reducing the temperature of 0°C to -6°C, pH of leaked solution was significantly reduced (Fig. 2).

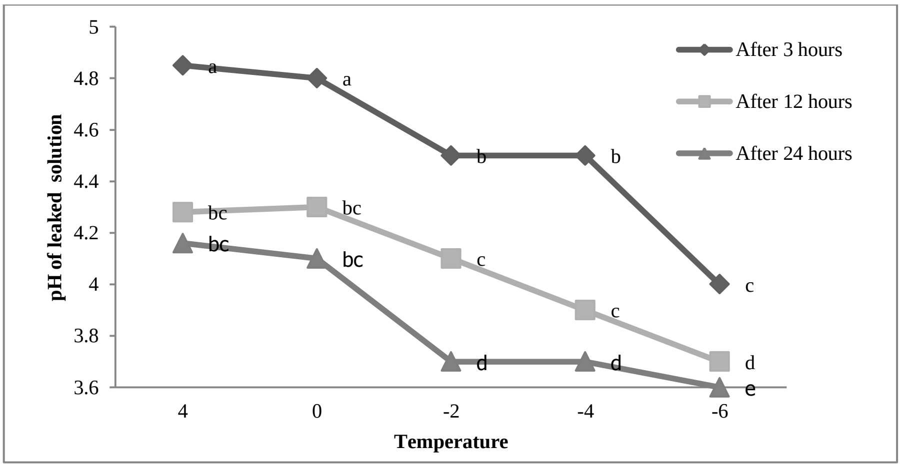

Fig. 2. Interaction effect of temperature and time on pH of leaked solution
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The effect of frost damage on ionic leakage

The comparison of means by the Duncan's method showed that best time to evaluate the solution of ionic leakage was 24 hours after samples were incubated. Ionic leakage fixed at 24 hours after the treatments and then increased exponentially. Increased ionic leakage after 24 hours of frost treatments could occur due to cell death. The maximum time for measuring of ionic leakage was between 20 to 24 hours after samples were incubated. Similar observations were also reported by Emmert and Howlett (1953); Flinn and Ashworth (1995); Flint *et al*., (1967); Burke *et al*., (1976) and Leinonen (1996). The results revealed that the *P. mutica* seedling rootstock had less leakage than the others. In contrast, *P. atlantica* was the most sensitive and had the highest ionic leakage than the others.

The interaction effect of temperature and seedling rootstocks on ionic leakage

Results showed that the highest percentage of ionic leakage was in *P. atlantica* than other seedling rootstocks. The lowest rate of ionic leakage related to *P. mutica* and *P. vera* cv.'Sarakhs' (Fig. 3).

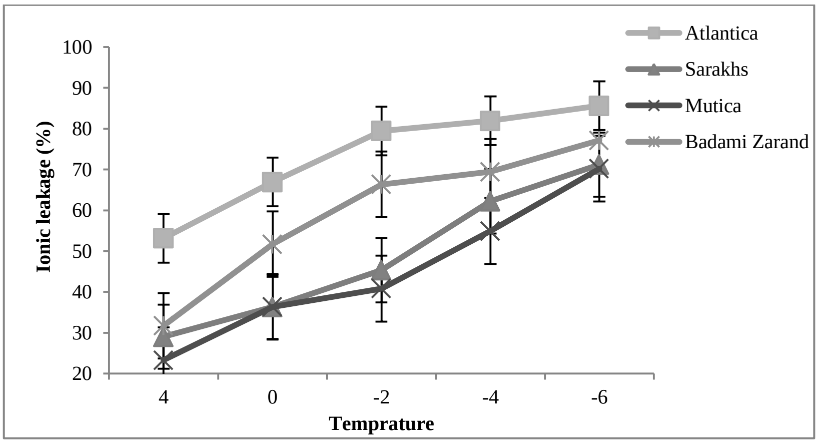

Fig. 3. Interaction effect of temperature and seedling rootstock on ionic leakage
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The effect of different temperatures and time on ionic leakage

The results regarding the effects of different temperatures on the percentage of ionic leakage showed that ionic leakage at 4°C was lower than the other treatments. By reducing the temperature of 0˚C to -6˚C, the amount of ionic leakage increased and -6˚C reached its highest level (Fig. 4).

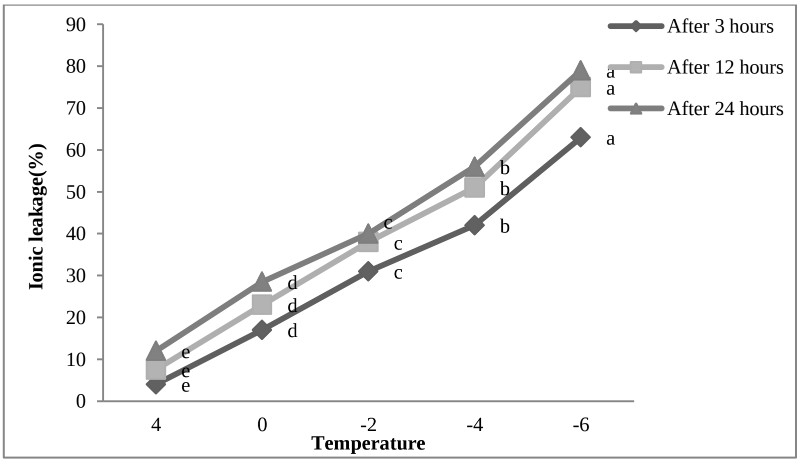

Fig. 4. Interaction effect of temperature and time on ionic leakage
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Discussion

The results indicated that pH of leaked solution was gradually reduced during 3 to 24 hours after treatments. It remained constant between 21 to 24 hours after treatments and was severely reduced after 24 hours. Hokmabadi *et al*., (2011) established that after 24 hours of cold treatments due to cell damage and cell contents leak into the space, cellular pH levels decreased. After 24 hours of frost stress, acidity was decreased due to cellular damage and cell contents were leaked into the space between them. Therefore, the best time to evaluate pH is 24 hours after the temperature treatments. The result of this research showed that when temperature decreased from 0°C to -6°C, ionic leakage increased and pH of the solution reduced. The results of the effect of temperature and pH of the solution at different temperatures showed that *P. atlantica* is more sensitive than the other seedling rootstocks to frost stress. The measured values of pH in *P. atlantica* were lower than other seedling rootstocks with reducing the temperature from 4°C to 6°C. The results also showed that the *P. mutica* is more tolerant to-4 °C temperatures than the other seedling rootstocks. *P. vera* cv.'Sarakhs' was tolerant at higher temperatures after *P .mutica*. In general, based on the results of ionic leakage and pH changes, tolerant seedling rootstocks were *P .mutica*> *P. vera* cv.'Sarakhs'> *P. vera* cv.'BadamiZarand'>*P .atlantica*, respectively. Similar results were obtained by Hokmabadi *et al.* (2011). Hokmabadi *et al*. (2011) studied the tolerance of three commercial cultivars of pistachio including, 'Kalleh Goochi', 'AhmadAghaei' and 'Ouhadi' to spring frost damage. The results indicated that the pH and ionic leakage were useful tools to study the pistachio rootstock in frost stress.

Conclusions

Pistachio is one of the products that is sensitive to spring frost. Temperature fluctuations has become this a major challenge, especially in Kerman province. Therefore, considering the importance of pistachio products in our country in terms of economics and valuation, the necessity of evaluation of rootstocks to reduce the damage caused by spring cold is necessary. The existence of unique pistachio species in Iran and variety in the genus could help us to select of suitable species for cultivation, under various environmental stresses including spring frostbite.

The present results identified that pH of leaked solution same as percentage of ion leakage can be regarded as an appropriate tool for studying the freezing and frost damage in pistachio. The results revealed that the *P. mutica* had less ionic leakage than the other seedling rootstocks. The results interaction effects of temperature and rootstock on the rate of pH of leaked solution indicated that *P. atlantica* was more susceptible to frost damage in different temperature than the other seedling rootstocks and with decreasing temperature from 4°C to -6°C pH levels in this seedling rootstock was less than the others. Generally, the results of percentage of ion leakage and the solution pH showed that *P. mutica* is the most resistant and *P. atlantica* is the most susceptible rootstock. Results also showed that ionic leakage and pH showed the difference in tolerant and susceptible cultivars.

Acknowledgements

Special thanks to Pistachio Research Center in Rafsanjan for financial support of this research.

References

Afshari H, Hokmabadi H, Ebadi A, Arab H, Ghorbanian A (2008) Investigation of frost damage tolerance of pistachio commercial cultivar of Damghan area. Journal of plants and ecosystems (Quarterly).18, 60-76. [In Persian].

Ashworth EN (1982) Properties of peach flower buds which facilitate super cooling. Journal of Plant Physiology. 70, 1475- 1479.

Arrora R, Wisniewski ME, Scorza R (1992) Cold acclimation in genetically related (sibling) deciduous and ever green peach (*Prunus persica* L. Batsch). Plant Physiology. 99, 1562-1568.

Bartolozzi F, Fontanazza G (1999) Assessment of frost tolerance in olive (*Olea europaea* L.). ScientiaHorticulturea. 81, 309–319.

Burke MJ, Gusta LV, Quamme HA, Weiser CJ, Li PH (1976) Freezing and injuring plants. Annual Reviews in Plant Physiology. 27, 507-528.

Cary JW (1985) Freeze survival in peach and prune flowers. Plant Science Letters. 37, 265-274.

Chen PW, Li PH (2002) Membrane stabilization by abscisic acid under cold aids proline in alleviating chilling injury in maize. Plant Cell and Environment. 25, 955-962.

Dexter ST, Tottingham WE, Graber LF (1932) Investigations of the hardiness of plants by measurement of electrical conductivity. Journal of Plant Physiology. 7, 63-78.

Emmert FH, Howlett FS (1953) Electrolytic determinations of the tolerance of fifty-five apple varieties to low temperatures. Proceedings of the American Society for Horticultural Science. 62, 311-318.

Flinn CL, Ashworth EN (1995) The relationship between carbohydrates and flower bud hardiness among three Forsythia taxa. Journal of the American Society for Horticultural Science. 120, 607-613.

Flint HL, Boyce BR, Beattie DJ (1967).Index of injury – A useful expression of freezing injury to plant tissues as determined by the electrolytic method. Canadian Journal of Plant Science. 47, 229-230.

Hokmabadi h, Alipoor H, Sohrabi N, Dargahi R. Askari R, Ismaili Ranjbar A (2011) Investigation of frost damage tolerance of three Iranian commercial pistachios by rates of ionic leakage, proline, soluble sugar and nutrition element content. The final report of the research project: Pistachio Research Institute in Iran. [In Persian].

Leinonen I (1996) A simulation model for the annual frost hardiness and freeze damage of Scots pine. Annals of Botany. 78, 687-693.

Mavali M (2011) Pistachio rootstocks evaluation which is tolerance to cold. MS thesis, Islamic Azad University of Jiroft.

McKellar M, Buchanan A, Ingram DL, Campbell CW (1992) Freezing tolerance of Avocado leaves. HortScience. 27, 341-343.

Mikal ES (2002) The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. Postharvest Biology and Technology. 26, 295-304.

Mirmohamadi Meibodi A, Tarkeshe Esfahani S (2004) Aspects of physiology and breeding for cold and freezing in crops. Golbon Publication, Isfahan, Iran. pp. 223. [In Persian].

Murata T, Tatsumi Y (1979) Ion leakage in chilled plant tissues. In: Low temperature stress in crop plants, the role of the membrane. Academic Press, New York.

Nunes M ES, Smith GR (2003) Ionic leakage assay capable of quantifying freezing tolerance in Rose Clover. Crop Science. 43, 1349-1357.

Patterson B, Murata D, Urata T, Graham D (1976) Electrolyte leakage induced by chilling in Passiflora species tolerant to different climates. Australian Journal of Plant Physiology. 3, 435-42.

Soleimani A, Lessan H, Talaie A (2003) Relationship between density and ionic leakage as indicators of cold hardiness in Olive (*Olea europea* L.). Acta Horticulturae. 618, 521-525.

Sukumaran NP, Weiser CJ (1972) An excised leaflet test for evaluating potato frost tolerance. HortScience. 7, 467-468.

Tavallali V and Rahemi M (2007) Effects of Rootstock on Nutrient Acquisition by Leaf, Kernel and Quality of Pistachio (*Pistacia vera* L.).American-Eurasian Journal of Agricultural and Environmental Science. 2 (3), 240-246.

Xin Z, Browse J (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. Journal of Plant Cell. 23, 893-902.

Yoshida S, Hotsubo K, Kawamura Y, Murai M, Arakawa K, Takezawa D (1999) Alterations of intracellular pH in Response to Low Temperature Stresses. Journal of Plant Research. 112, 225-236.