JCHR (2022) 12(3), 335-347

Journal of Chemical Health Risks

www.jchr.org

**ORIGINAL ARTICLE**

# Study the Freezing Resistance of some Barley Varieties Using Rates of Electrolyte Leakage

Shahram Ashraf Rad*, Seyyed Mohammad Taghavi

*Department of Soil Science, Damghan Branch, Islamic Azad University, Damghan, Iran*

*(Received: 14 November 2014 Accepted: 5 May 2021)*

**KEYWORDS**

Freezing tolerance;
Barley;
Electrolyte leakage

**ABSTRACT:** In order to study the freezing resistance of some barley varieties using rates of electrolyte leakage this experiment was carried out on three barley variety, including Reyhan, kavir, and Nosrat. These varieties are the most important varieties that cultivated in Semnan province. For this experiment, barely seeds were sown in small containers. The current experiment was carried out as Factorial experimental design plan based on completely randomized design (CRD).The first factor in this study was barley variety in three levels. The second factor was temperature in 5 levels including +4°C, -4°C, -8°C, -12°C and -15°C. Seedlings in the stage of tillering, was sprayed by water and then were put in the incubator for two hours. Then two gram samples were selected and 20 mm deionized water was added and shakes for 24 hours. Then every day for a week EC in solution was measured. Analysis of variance (ANOVA) was used to determine significant ($p < 0.01$ and $p < 0.05$) differences between barley genotypes. The differences between means of barley varieties were inspected using Duncan test. The results showed that the percentage of ion leakage between the three cultivars had significant differences .The effect of temperature on the percentage of ion leakage in all days, had significant difference.The highest percentage of ion leakage measurements on all days was at the -15°C and the lowest percentage of leakage occurred at 4°C. Kavir variety in comparison with other varieties had lower cold tolerance. Interaction of temperature and genotype factors on ion leakage measurements showed that all varieties had significant differences.

## INTRODUCTION

Barley is one of the important products of Damghan. Winter barley should be planted in autumn and harvested in summer.The autumn-seeded barley take advantage of autumn rainfall and thereby yield significantly is much more than spring-seeded crops. The capability of autumn-seeded varieties to survive referred to winter hardiness. Low-temperature tolerance is a complex quantitative character that plants tolerates temperatures that leading to freezing. Cold stress lead to low pollen producing and results low fruit.Low temperatures change the shape of the flower and lead to sterilization of the flower and deformation of the fruit [1, 2]. Frostbite occurs when the temperature drops sharply and causes damage to the plant [3].

The sensitiveness of plant issues to the cold weather is different. The leaves have a little compatibility. The roots have less resistance than the stem in the cold weather [4]. This character is determined by a highly integrated system of structural and developmental genes that are regulated by environmentally responsive and complex pathways [5]. According to gene growth theory, the duration and intensity of gene expression controls the degree LT resistance [6].

However, the study of plants against cold resistance under controlled conditions can not be directly related to factors

*Corresponding author: shahramashraf35@gmail.com (Sh. Ashrafi Rad)
DOI: 10.22034/jchr.2021.577905.0

limiting plant survival in the field evaluation method [7]. There are many methods to evaluate frost test and identify frost tolerant of plant genotypes. Freezing tolerance of winter barley is one of the main factors of winter survival. To avoid winter kill it is very important to choose freezing tolerant of barley genotypes. Many studies have been conducted to find an effective and rapid method to evaluate the plants tolerance to freezing temperatures. One safe method is to measure the electrolyte leakage of the cytoplasmic membrane or the conductivity of compatible organs that the plant is damaged by cold stress [8].

Since the cytoplasm membrane is the first place that can be damaged, it is possible to determine the amount of injury through the electrolyte leakage of damaged tissues. It is predicted sensitive cells to suffer more damage than resistant cells and must have a more electrolyte leakage [9].

The electrolyte leakage (EL) method is easy and reliable and less expensive than other methods, so it is a good way to determine the plant's tolerance to cold. [10]

Some researchers [11, 12], worked on some cultivars of wheat, rye and winter barley, found a significant correlation between the EL% of plant leaves and crop survival from freezing. Similarly [13] studied 9, 10 and 12 cultivars of wheat , rye ,red clover , and found a significant correlation between crop survival and EL% of leaves ,though this relation was not significant for rye crops. Another evaluation method for freezing tolerance is to measure the growth characteristics and the plant regrowth after the recovery period which is followed by the freezing test in controlled conditions.

Fowler and Carlers [14] investigated on wheat; they found that there was a significant relationship between shoot dry weight of plants during the recovery period and temperature of 50 in field conditions.

In addition, a positive significant correlation was found between the chlorophyll amount (SPAD), plant height, leaf area and dry weight with LT50 under the recovery period [15]. Nezami et al, [16] found the dry weight of the plant at -12°C with resistant genotype is less than the dry weight of the control plant at temperature at 0°C. However the dry weight of sensitive genotypes was only 10% the control plant. The objective of this study was to evaluate various barley genotypes exposed to the freezing stress under controlled conditions using rates of electrolyte leakage.

## MATERIALS AND METHODS

This experiment was carried out on three barley varieties, including Reyhan, kavir, and Nosrat. These varieties are the most important varieties that cultivated in Semnan province. For this experiment, barely seed planted used in small container. The current experiment was carried out as Factorial experimental design plan based on completely randomized design (CRD). The first factor in this study was barley variety in three levels. The second factor was temperature in 5 levels including +4°C, -4°C, -8°C, -12°C and -15°C. When the pots reached the stage of tillering, were sprayed water and put inside the incubator for two hours. Then two gram samples obtained and 20 mm demonized water was added and shakes for 24 hours. Then until on week every day EC in solution was measured. Analysis of variance (ANOVA) was used to determine significant ($p < 0.01$ and $p < 0.05$) differences between barley genotypes. The differences between means of barley varieties were inspected using Duncan test.

## RESULTS

The effect of temperature on ion leakage measurements in the first day showed there was significant difference between the trearments at the 1% level. The highest percentage of ion leakage (53.52%) was at - 15°C .The lowest percentage was 18% at 4 ° C. Comparison of treatment means using the Duncan test showed that there were significant differences between the percentage of ion leakage values (82.23, 32 and 50.47) at -4, -8, -12°C. The effect of temperature ion leakage measurements showed that there was significant difference between the treatments at the 1% level (Figure 1). The rate of ion leakage at 4°C (24.84 %) was lowest rate of ion leakage. In temperatures between 4 - and 8 - , the percentage of ion leakage (36.78 and 35.72) had no significant difference.

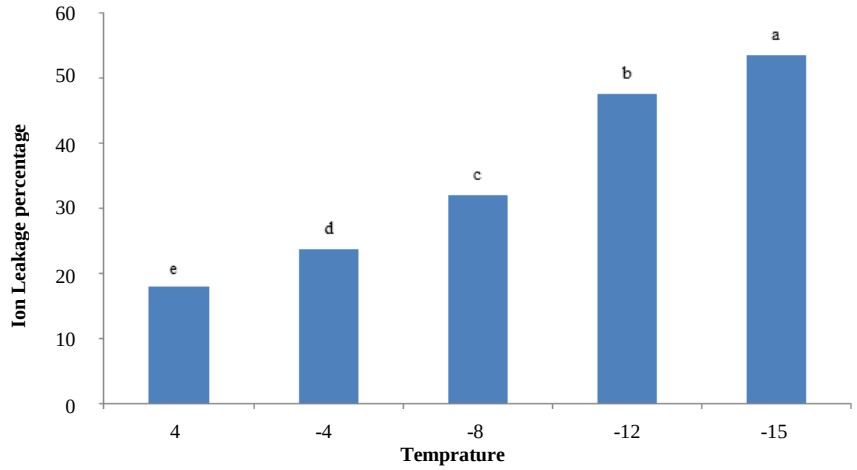


**Figure 1 .**The effect of temperature on the first day of ion leakage measurements.

The effect of temperature on the third day day of ion leakage measurement showed significant difference between the temperatures at the 1% level (Figure 2). The rate of ion leakage at 4°C with a 24.84 showed the lowest rate of ion leakage. In temperatures between -4 and -8 the percentage of ion leakage respectively 36.78 and 35.72 had no significant difference. Most of ionic liquids at temperature of -15 degrees were 68.60. Lowest percentage of ion leakage was 28.77 at 4ºC. Values 35.61, 40.46 and 53.49 had the percentage of ion leakage at -4, -8 and -12°C respectively. The rate of ion leakage were significantly different at the three temperatures were together.

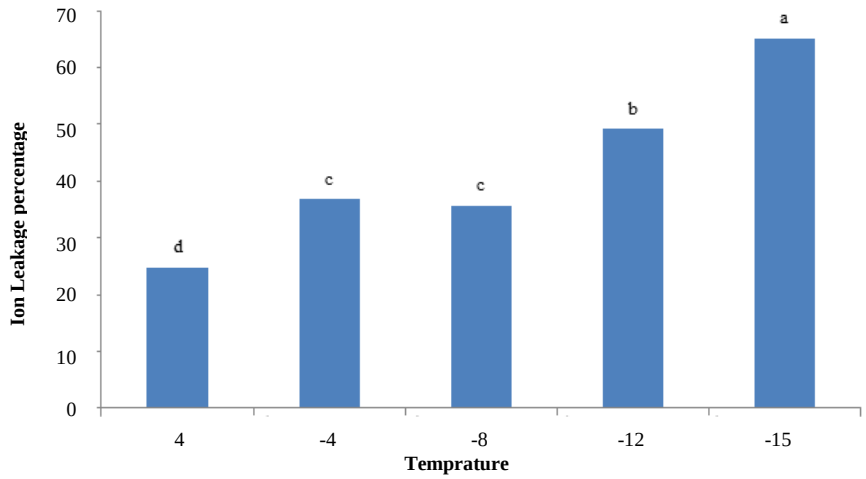


**Figure 2.** The effect of temperature on the second day day of ion leakage measurement.

Effect of temperature on the rate of ion leakage showed there was no significant difference between the temperatures at the 1% level. The rate of ion leakage at 4°C (26.33%) showed the lowest rate of ion leakage (Figure 3). Most ionic leakage (70.21%) was at temperature of -15°C. In temperatures between -12 and - 8 the percentage of ion leakage (52.07 and 52.83) had not any significant difference.

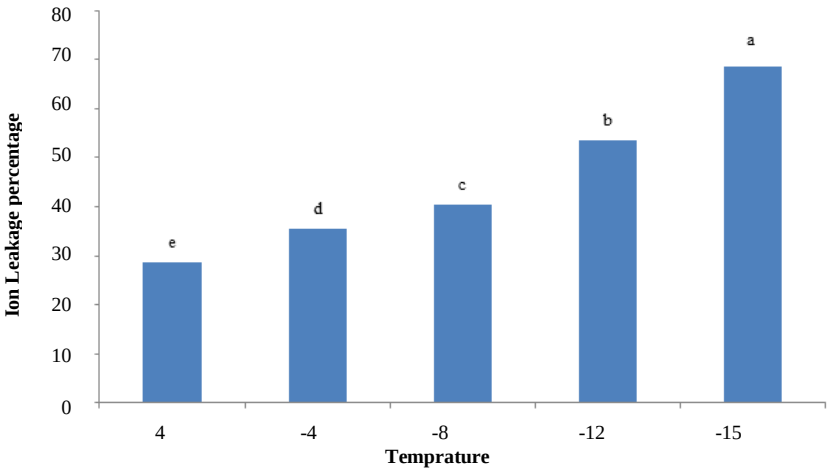


**Figure 3 .**The effect of temperature on the third day of ion leakage measurement.

The effect of temperature on of ion leakage measurements in fifth day (Figure 4) Showed there was significant difference between the temperatures at the 1% level. The lowest rate of ion leakage was at 4°C (31.44%). Most of ionic leakage deal (71.56%) was at temperature of - 15°C. In temperatures between -12 and -8°C, there was not any significant difference

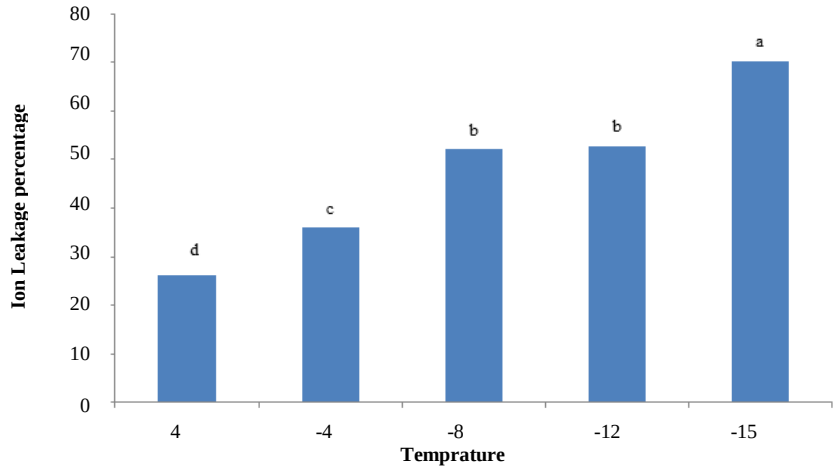


**Figure 4.** The effect of temperature on the forth day of ion leakage measurements.

The effect of temperature on the rate of ion leakage (Figure 5) showed that there was significant difference between the treatments at the 1% level.Most of ionic leakage (74.40) was at temperature of -15°C. The lowest rate of ion leakage (26.33) was at 4°C. In temperatures between -12, -4 and -8°C, the percentage of ion leakage had significant difference.

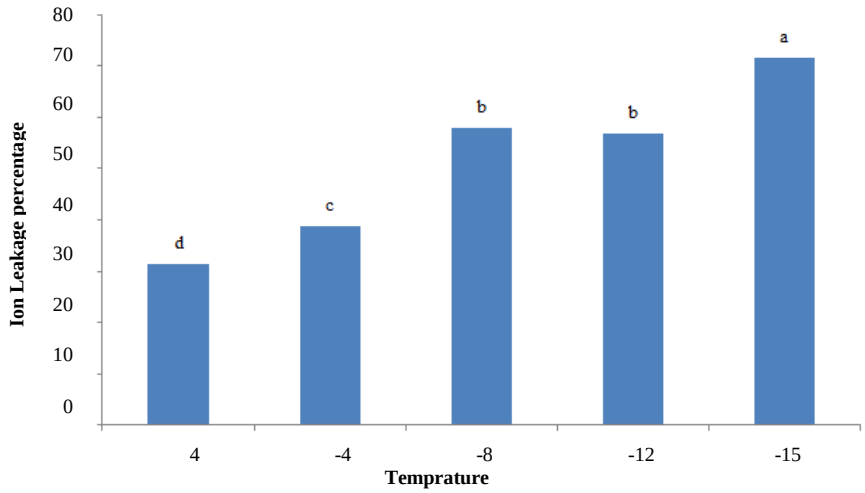


**Figure 5.** The effect of temperature on the fifth day of ion leakage measurements.

The effect of temperature on ion leakage measurements in seventh day showed (Figure 6) that there was significant difference between the treatments at the 1% level.Most of ionic leakage was at temperature of -15°C .The lowest rate of ion leakage was at 4°C. In temperatures between -12, -4 and -8°C, the percentage of ion leakage, had significant difference.

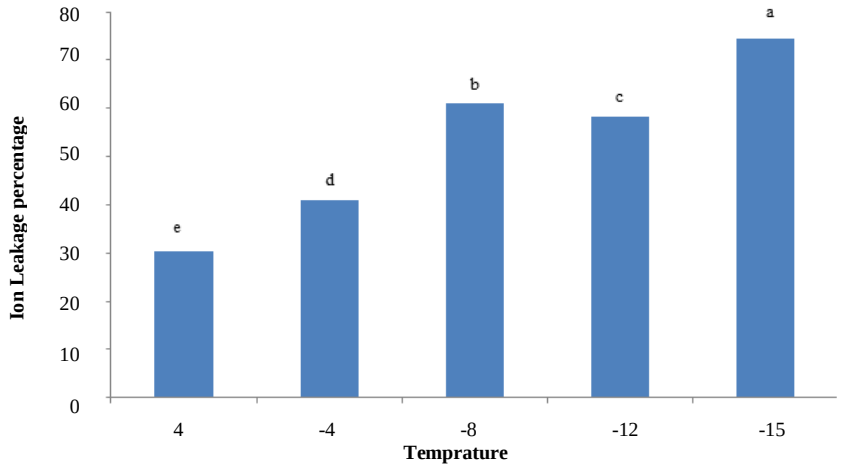


**Figure 6.** The effect of temperature on the sixth day of ion leakage measurements.

The effect of verity on ion leakage measurements in the first day showed (Figure7) there was significant difference between the temperatures at the 1% level.Most of ionic leakage was at Kavir verity on -15°C. In temperatures between -12, -4 and -8°C, the percentage of ion leakage had significant difference. Between Nosrat and Reyhan varieties, the percentage of ion leakage rate the difference was not significant.

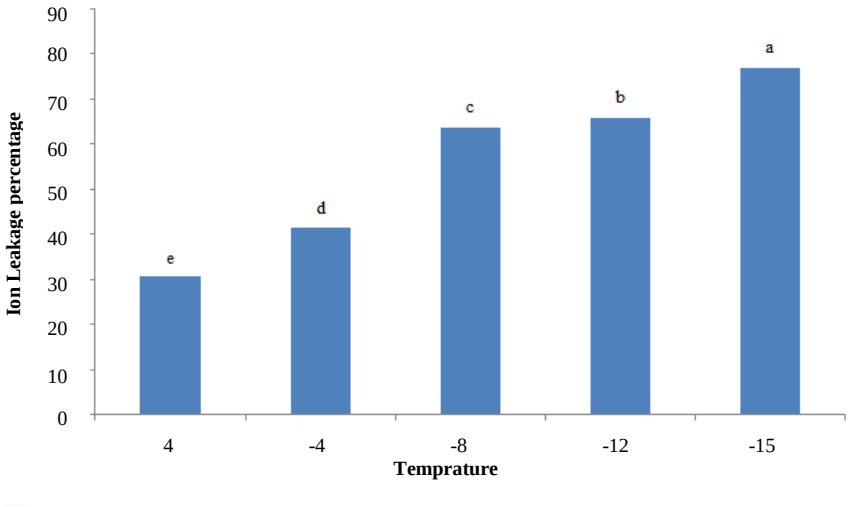


**Figure 7.** The effect of temperature on the of ion leakage in seventh day.

The effect of variety on ion leakage measurements in the second day showed (Figure 8) there was significant difference between the varieties at the 1% level. Most of ionic leakage was at Kavir variety. In Kavir and Nosrat varieties the percentage of ion leakage were 38.85 and 39.26 . Between Nosrat and Reyhan varieties, percentage of ion leakage rate had not significant difference.

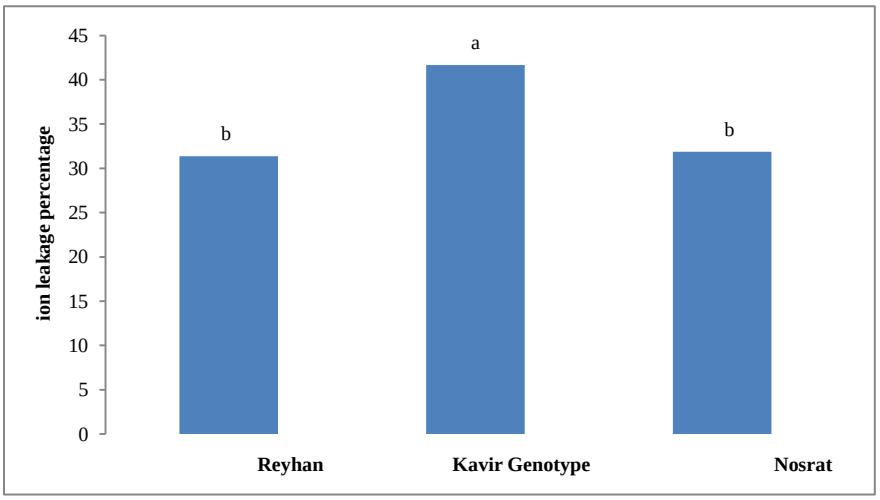


**Figure 8.** The effect of verity on ion leakage measurements in the first day

The effect of variety on ion leakage measurements in the thirth day showed (Figure 9) there was significant difference between the varieties. Most of ionic liquids were at Kavir variety .Percentage of ion leakage in Reyhan and Nosrat varieties were 43.85 and 40.81 %.

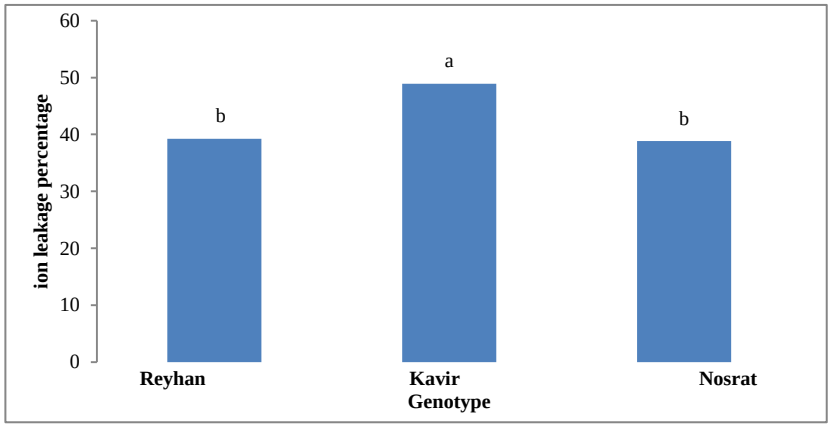


**Figure 9.** The effect of variety on ion leakage measurements in the second day.

The effect of variety on ion leakage measurements in the forth day showed (Figure 10) there was significant difference between the varieties.Most of ionic liquids was at Kavir variety. Lowest percentage of ion leakage were related to Nosrat variety

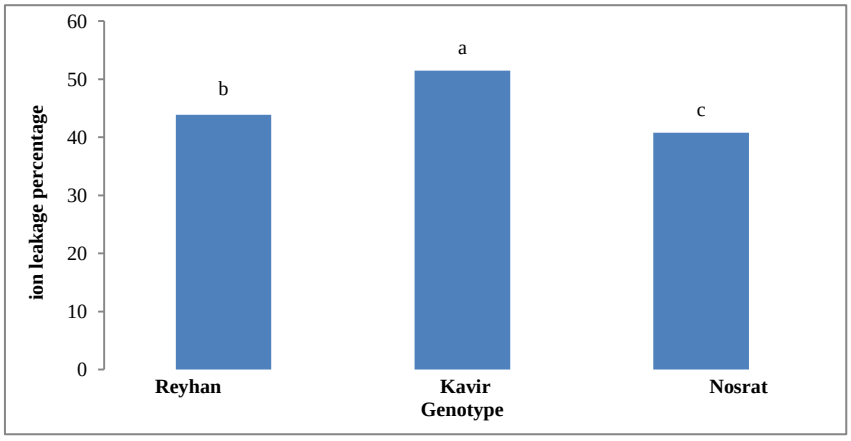


**Figure 10.** The effect of variety on ion leakage measurements in the thirth day.

The effect of variety on ion leakage measurements in the fifth day showed (Figure11) that there was significant difference between the varieties. Most of ionic liquids was at Kavir variety .Lowest percentage of ion leakage were related to Nosrat variety.

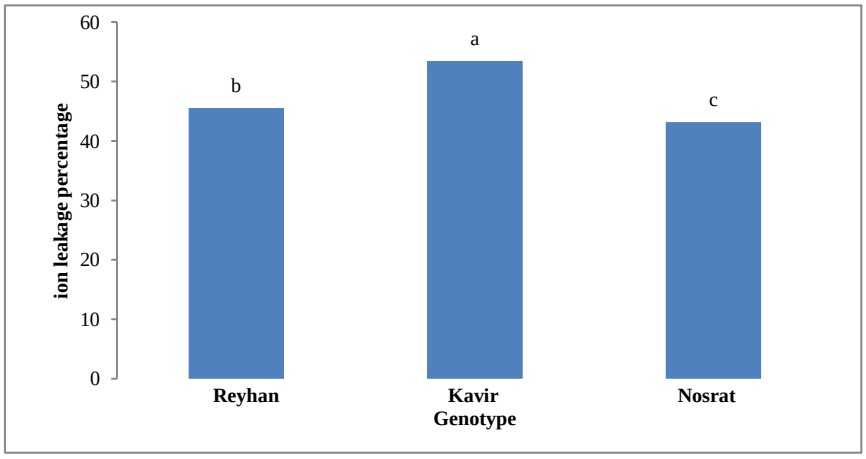


**Figure 11.** The effect of variety on ion leakage measurements in the forth day.

The effect of variety on ion leakage measurements in the sixth day showed that there was significant difference. Most of ionic liquids were at Kavir variety. Percentage of ion leakage in Reyhan and Nosrat varieties were 50.26 and 50.43 % that there were not significant difference in these varieties.

The effect of variety on ion leakage measurements in the sevevth day showed (Figure12) that there was significant difference. Most of ionic liquids were at Kavir variety. Percentage of ion leakage in Reyhan and Nosrat varieties were 52.17 and 53.91% that there were significant difference.

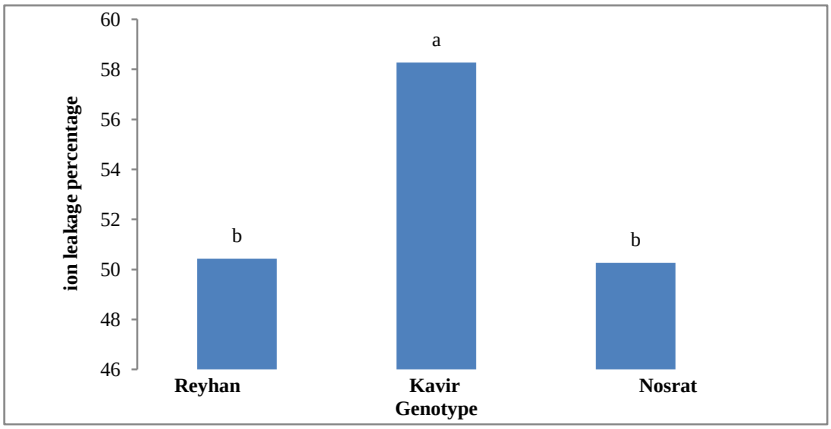


**Figure 12.** The effect of variety on ion leakage measurements in the sixth day.

Interaction of temperature and genotype on the rate of ion leakage measured in first day showed (Figure13) that there was a significant difference. Highest leakage was in Kavir variety at 15°C. Nosrat varety was the third at -12°C. Nosrat and Reyhan varieties at -8°C had the percentage of ion leakage of 31.47 and 31.07%, also were not significantly different.The lowest percentage of ion leakage measurements from the first day belonged to Reyhan and Nosrat varieties.

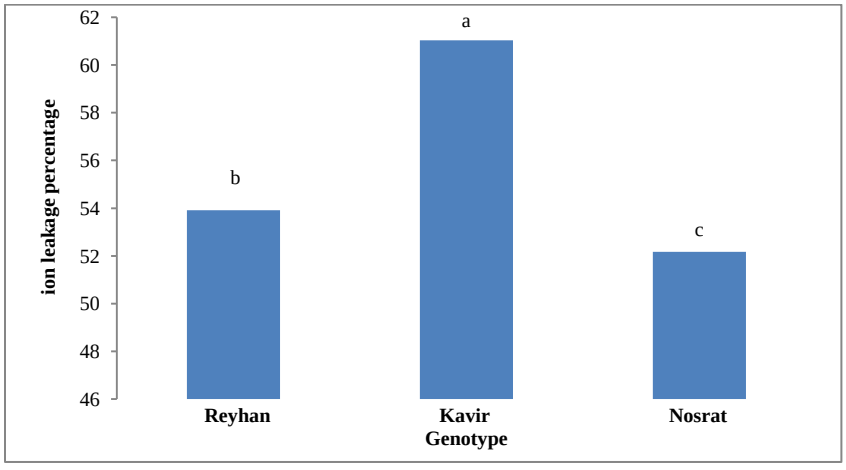


**Figure 13**. The effect of variety on ion leakage measurements in the sevevth day

Interaction of temperature and genotype on the rate of ion leakage measured in second dayshowed (Figure14) that there was a significant difference. Highest leakage was in Kavir variety at -15°C. Reyhan and Nosrat were the second at -12°C varieties, also were not significantly different. Nusrat variety at -12, -15°C temperature with Nosrat variety had not a significant difference. Lowest percentage of ion leakage at 4°C belonged to Nosrat variety.

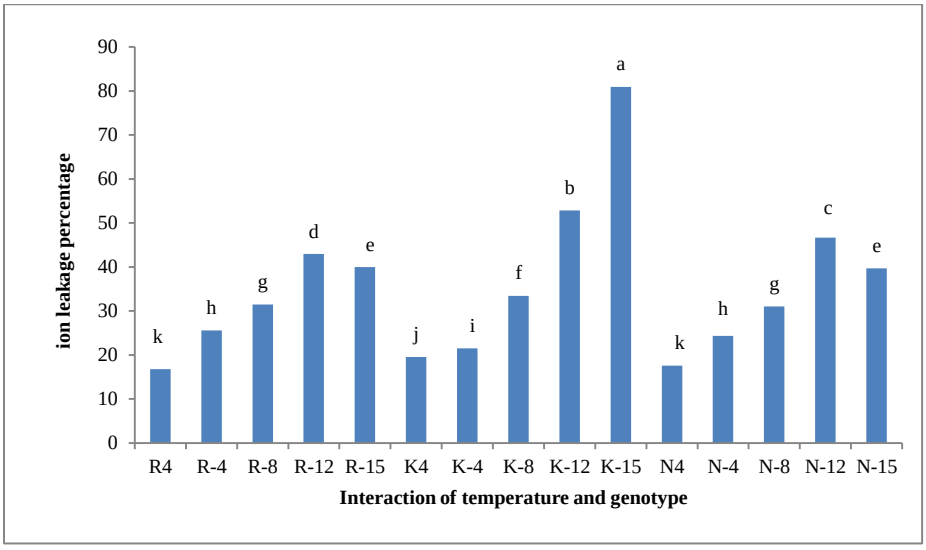


**Figure 14.** Interaction of temperature and genotype on the rate of ion leakage measured in first day.

Interaction of temperature and genotype on the rate of ion leakage measured in third day (Figure15) the highest percentage of ion leakage belonged to Kavir at -15°C. Per the second one was Kavir queries at a temperature of -12°C and Reyhan under the -15°C. Lowest percentage leaks to the two varieties of basil and assistance with treatment temperature was 4°C with no significant difference.

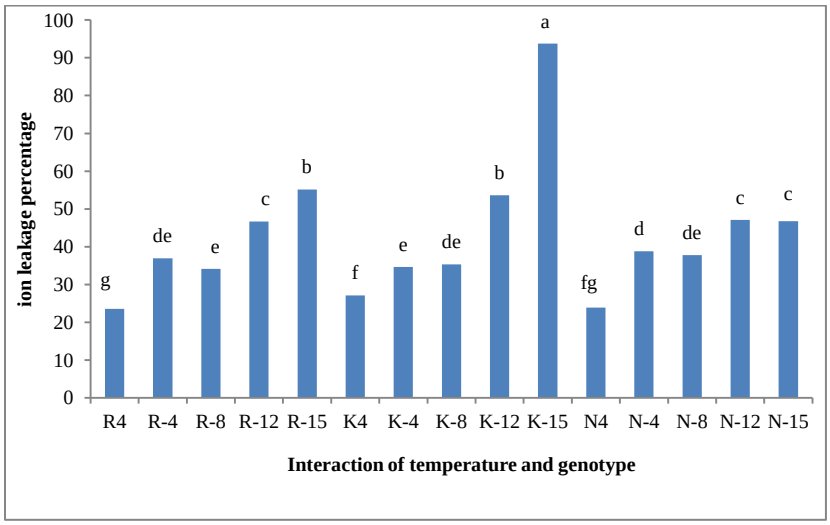


**Figure 15.** Interaction of temperature and genotype on the rate of ion leakage measured in second day

Interaction of temperature and genotype on the rate of ion leakage measured in forth day showed (Figure16) that highest percentage of ionic leakage was the Kavir varety at -15°C. The lowest percentage leakage rate at 4° belonged to Nosrat variety. Kavir at -12°C with Nosrat at the –15°C has not been significantly different. Kavir treatment at -4°C had ion leakage percent about 43/3%. The treatments had not significant difference.

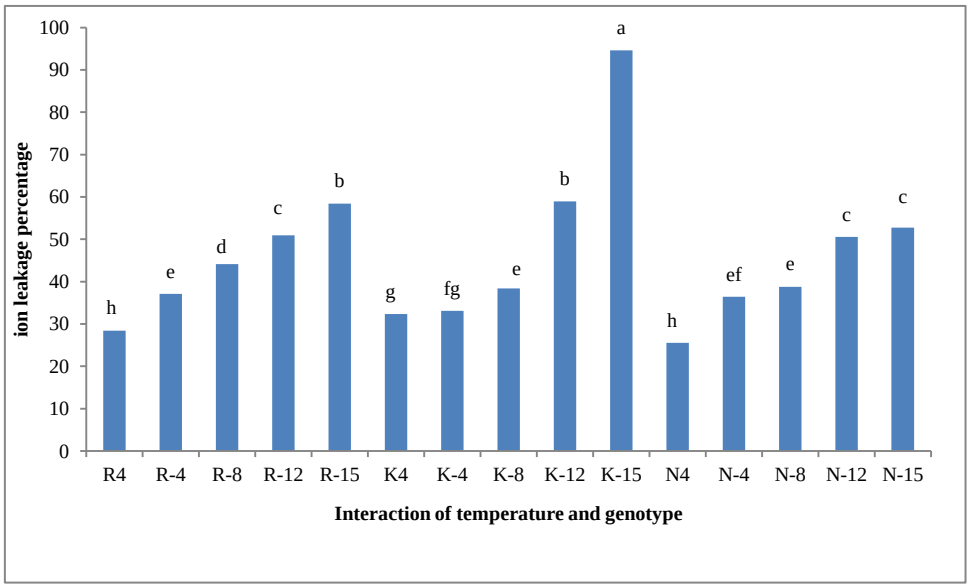


**Figure16.** Interaction of temperature and genotype on the rate of ion leakage measured in third day.

Interaction of temperature and genotype on the rate of ion leakage measured in fifth day showed **(**Figure17) that three varieties had significant difference. The highest percentage of ion leakage in Kavir temperature -15°C. Lowest percentage of ion leakage of three varieties at 4°C had not significantly differences.

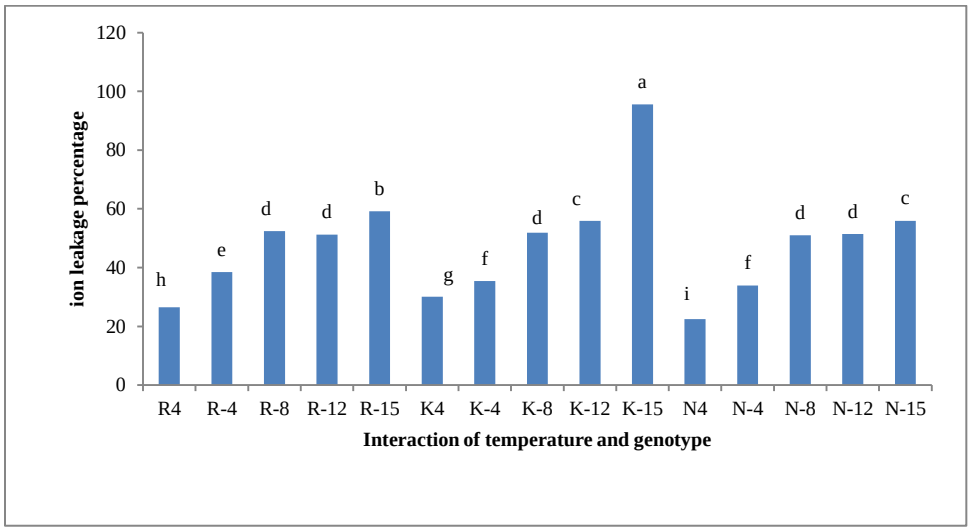


**Figure17.** Interaction of temperature and genotype on the rate of ion leakage measured in forth day.

Interaction of temperature and genotype on the rate of ion leakage measured in sixth day showed (Figure18) that highest percentage was belonged to Kavir at -15°C. Lowest percentage of ionic liquids in the three varieties at 4°C was also not significantly different.

Interaction of temperature and genotype on the rate of ion leakage measured in seventh day showed (Figures 19, 20) that Lowest percentage of leakage at 4 c belonged to Nosrat.The highest percentage of ion leakage was belonged to Kavir variety under –15°C .

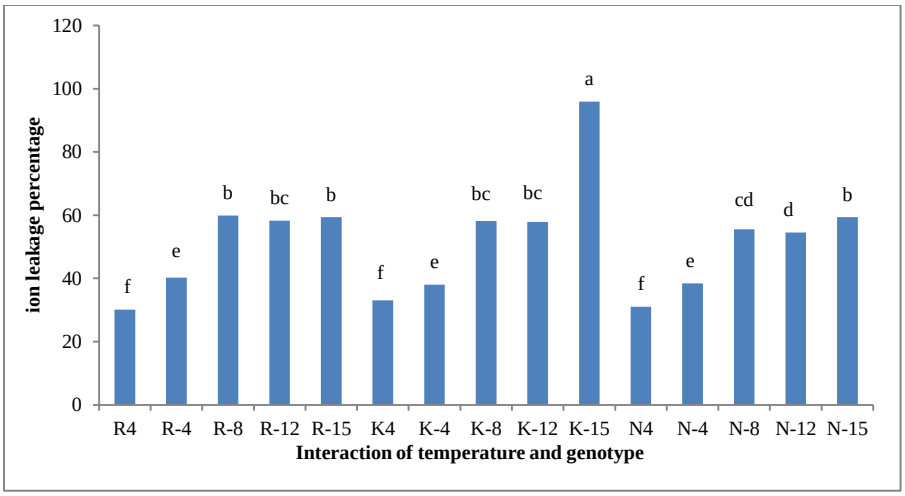


**Figure 18.** Interaction of temperature and genotype on the rate of ion leakage measured in fifth day.

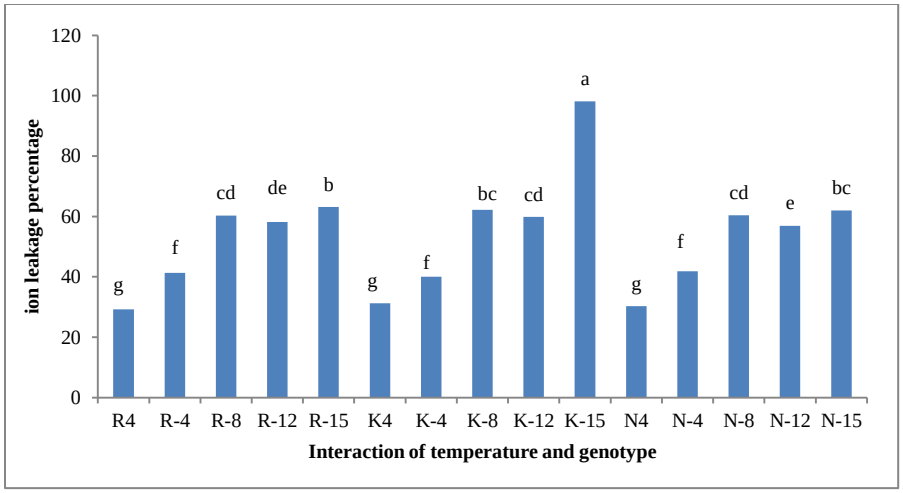


**Figure 19.** Interaction of temperature and genotype on the rate of ion leakage measured in sixth day

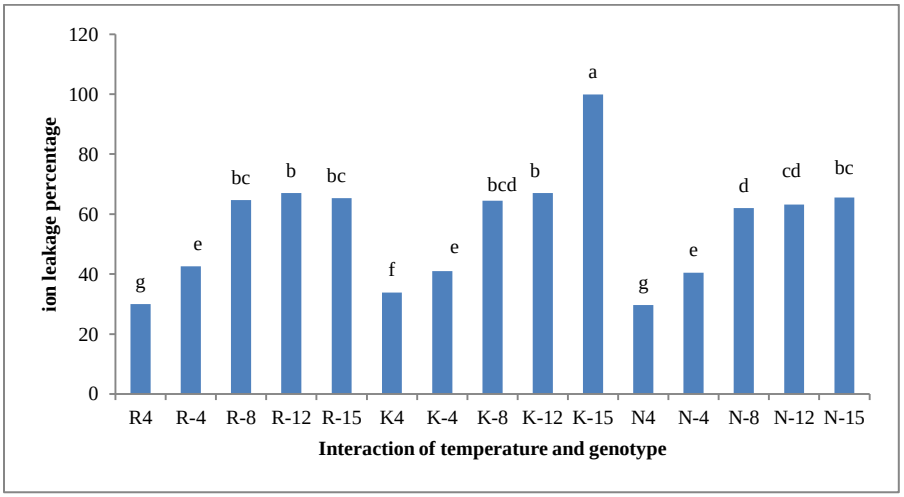


**Figure 20.** Interaction of temperature and genotype on the rate of ion leakage measured in seventh day.

## DISCUSSION

The results showed that the percentage of ion leakage between the three cultivars had significant differences .The effect of temperature on the percentage of ion leakage in all days had significant difference.The highest percentage of ion leakage measurements on all days was at the -15°C and the lowest percentage of leakage occurred at 4°C. Kavir variety in comparison with other varieties had lower cold tolerance .Interaction of temperature and genotype on ion leakage measurements showed that all varieties had significant differences. Plants ability to tolerate temperatures below zero can without causing significant damage is defined as an important indicator for assessing the potential of crop species and cultivars [16]. Each plant may have an internal rhythm; cold resistance that is independent of environmental factors. Time and adaptability to cold is usually genetic, but can be altered by environmental factors. So that within a species of cold tolerance among varieties can distinguish differences. But this is not always true. Measuring solute leakage from plant tissue is a long-standing method for estimating membrane permeability in relation to environmental stresses, growth and development, and genotypic variation. Expressing electrolyte leakage can be as good index for distinguishing plant stress especially temperature stress. It is important to identify rapid and accurate methods for assessing hardwood seedling quality and physiological status. Evaluation of electrolyte leakage (EL) from plant tissues is promising for this purpose. It has successfully predicted the physiological status of conifer seedlings and has been used experimentally on European hardwood species. Physiological methods of testing cold hardiness are also rapid [18] allowing for timely management decisions in nursery operations. Many methods have been employed for evaluating cold hardiness of plants [19]. The membranes are sensitive to environmental stresses such as chilling and freezing conditions. Cold temperatures reduce enzymatic activity, alter metabolism, and decrease the photosynthetic capacity of plant tissues [20].In plant membranes, these changes are often associated with increases in permeability and loss of integrity [21]. An estimation of cell damage and hardiness can be made by comparing the conductivity of the leaked contents from injured and uninjured tissues in water. The efficacy of EL in predicting hardiness has resulted in its use in operational practice at some nurseries, particularly for determining lifting windows and storability [22].For hardwoods, relatively little information is available [23].

## ACKNOWLEDGEMENTS

The authors would like to thank their colleagues Dr Hokmabadi for his contribution in conducting some of the experiments and support and help in completing the research.

### *Conflict of interests*

We have no conflict of interest to declare.

## REFERENCES

1. Liang W., Xie M., Dong D., 1994. Genetic improvement of hazelnut for cold hardiness and culture. Northern Nut Growers Association (U.S.). 85, 149-151.

2. Ito A., Hamaya H., Kashimura Y., 2002. Sugar metabolism in buds during flower bud formation: acomparison of two japanes Pear .

3. Wilson J.M., 1996. The mechanism of chill and drought hardiness. New Physiologist. 97, 257-270.

4. Weiser C.J., 1970. Achievments in plant chilling stress and injuries studies. Science. 169, 1269-1275.

5. Fowler D.B., Limin A.E., 2007. Progress in breeding wheat with tolerance to low temperature in different phenological developmental stages. Buck H., Wheat production in stressed environments. Dordrecht, the Netherlands. p. 301–314

7. Fowler D.B., Limin A.E., Ritchie J.T., 1999. Low-temperature tolerance in cereals: model and genetic interpretation. Crop Science. 39, 626–633

8. Rapacz M., Tyrka M., Kaczmarek W., Gut M., Wolanin B., Mikulski W., 2008. Photosynthetic acclimation to cold as a potential physiological marker of winter barley freezing

tolerance assessed under variable winter environment. Agronomy and Crop Science, 194 (1), 61–71

9. Mirzai-Asl, A., Yazdi-Samadi B., Zali A., Sadeghian-Motahhar Y., 2002. Measuring cold resistance in wheat by laboratory tests. J Sci and technol Agric and Natur Resour. 6, 177-186

10. Beirami zade E., Yazdi-Samadi B., Arshad Y., Bihamta M.R., 2006. A geneticanalysis of frost resistance in ten bread wheat through diallele method. J Agric Sci. 37, 45-59

11. Lyons J.M.M.R., 1973. Chilling injury in plants. Annu Rev of Plant Physiol and Plant Mol Biol. 24, 445–466

12. Hömmö L.M., 1994. Winterhardiness of winter cereal species in Finnish conditions, with special reference to their frost and snow mould resistance. In K. Dorffling, B. Brettschneider, H. Tantau & K. Pithan, eds. Crop Adaptation to Cool Climates, Workshop, Hamburg, Germany. pp. 65-73.

13. Fowler D.B., Gusta L.V., 1979. Selection for winter hardiness in wheat (*Triticum aestivum* L.). I. Identifcation of genotypic variability. Crop Science. 19 (6), 769–772

14. Azizi H., Nezami A., Khazaie H.R. Nassiri Mahallati M., 2008. Evaluation of cold tolerance in wheat (*Triticum aestivum*) cultivars under controlled conditions. Iranian Journal of Field Crops Research. 6(2), 343-352.

15. Armoniene R., Liatukas Z., Brazauskas G., 2013. Evaluation of freezing tolerance of winter wheat (*Triticum aestivum* L.) under controlled conditions and in the field. December. 100(100), 417-424

16. Nezami A., Bagheri A., Rahimian H., Kafi M., Nasiri Mahalati M., 2007. Evaluationof freezing tolerance of chickpea (*Cicer arietinum* L.) genotypes under controlled conditions. J Sciand Technol Agric and Natur Resour. 10, 257-269

17. Lyon B.G., Lyon C.E., 1990. Texture profile of broiler pecto-ralis major as influenced by post-mortem deboning time and heat method. Poult Sci. 69, 329–340

18. Xi-Man K., Qian Z., Xin Z., Bao-Dong W., Shu-Juan J., 2020. Transcription factor CaNaCl regulates low-temperature-induced phospholipid degradation in green bell pepper. Journal of Experimental Botany. 71(3), 23, 1078–1091,

19. Liu Q., Kasuga M., Sakuma Y., ABE H., Miura S., Yamaguchi-Shinozaki K., Shinozaki K.,1998. Two transcription factors, Drebi and Dreb2, with an Erebp/Ap2 DNA binding domain separate two cellular signal transduction pathways in drought and low-temperature-responsive gene expression, respectively, in Arabidopsis. The Plant Cell. 10(8), 1391-1406.

20. Dubey J.P., 1998. Toxoplasma gondii oocyst survival under defined temperatures. Journal of Parasitology. 84, 862-865.

21. Campos R.M.L., 2007. Fatty acid and volatile compounds from salami manufactured with yerba mate (Ilex paraguariensis) extract and pork back fat and meat from pigs fed on diets with partial replacement of maize with rice bran. Food Chemistry. 103, 1159-1167

22. McNabb K., Takahashi E., 2000. Freeze damage to loblolly pine seedlings as indicated by conductivity measurements and out planting survival. Research Report, Auburn University Southern Forest Nursery Management Cooperative, Auburn.

23. Tinus R., 1996. Root growth potential as an indicator of drought stress history. Tree Physiology. 16, 795-799.