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STUDY OF THE EFFECT OF LOW TEMPERATURES AND CALCIUM CHLORIDE TREATMENT ON THE GERMINATION OF IRANIAN AND EUROPEAN BARLEY CULTIVARS

ABSTRACT

Low temperature stress is one of the limiting factors of seed germination. In order to investigate the effect of low temperatures on germination of barley cultivars, identification of traits related to low temperature stress at germination stage and the effect of calcium chloride on these traits, 44 Iranian and European barley cultivars were evaluated in a factorial experiment within completely randomized design with 3 replications in the Laboratory of Plant Physiology, Agronomy and Plant Breeding department, Razi University. The first factor was 44 Iranian and European barley cultivars, the second factor included four temperature (0, 5, 10 and 20°C), and the third factor was the use of calcium chloride (10 mM) and its non-use (distilled water). Analysis of variance showed that there was a significant difference between cultivars for all traits except root length and seed vigor. Applying calcium chloride treatment at a concentration of 10 mM did not significantly affect the traits under the studied temperatures. Reducing temperature from 20°C to 10°C and 5°C reduced root length, shoot length, coleoptile length, root number, coefficient of velocity of germination, seed vigor and promptness index. The results of correlation analysis showed that there was a significant positive correlation between promptness index with average velocity of germination, coefficient of velocity of germination and seed vigor, germination percentage and root number in all studied temperatures. There was little differentiation between Iranian and European cultivars by both cluster and discriminant analysis.

Key words: barley, correlation, germination, low temperature stress

INTRODUCTION

Barley as an agronomic plant compatible with drought stress and tolerant to adverse environmental conditions and possessing characteristics such as green grazing in the tillering, grain extraction and its use in food industry, has a special place in the agricultural systems of the arid regions of the world, including Iran (Rezaikalu *et al.*, 2012). Barley is cultivated in many parts of the world due to its high resistance to environ-

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mental stresses and less need for moisture and adaptation to the environment (Behnia, 1996). Barley is planted in an area of 1.8 million hectares in Iran, of which 60% is devoted to rainfed areas. Most of the rainfed lands are located in cold and humid areas. In cold regions, in addition to cold and drought stresses, most of the years, due to delays in precipitation in early autumn, seedling emergence due to cold occurrence is difficult causes decreasing the growth period and ultimately decreasing yield (Abdolrahmani *et al.*, 2011). Low temperature stress is one of the limiting factors for plants germination. Germination plays an important role in grain quality and malt quality (Chloupek *et al.*, 2003). Germination is a trait that varies greatly among populations (Baskin and Baskin, 1998).Cultivars with fast germination properties are more likely able to absorb more water and are more suitable for rainfed conditions due to resistance to winter cold (Rastegar, 1992). Calcium is an essential nutrient, and it plays an important role in the activation of metabolic activities, such as membrane stabilization, signal transduction through the second transducer, membrane preservation, and control of the transfer of ion particles and of the activity of enzymes in counteracting the conditions of environmental stresses (Arshi *et al.*, 2006). When the plant is exposed to environmental stresses, including low temperature stress, calcium can withstand stress by regulate many physiological and cellular reactions (Hirschi, 2004). In a study by Perine *et al.* (2008), in order to increase the rate of germination and increase hormonal activity, instead of using hormones, NaOH, $Mg(OH)_2$, $Ca(OH)_2$ and $NaHCO_3$ was used. The results showed that calcium hydroxide was effective in increasing germination by 60-66%. Sedaghathoor *et al.*, 2015, to study the germination rate of seeds of three species of grass (*Lolium perenne*, *Poa pratensis*, *Cynodon dactylon*) used calcium chloride (2%). Calcium chloride treatment alone has had covert not a significant effect on the traits, but the effects of the type of grass and calcium chloride on the average daily germination had the most effect.

The aims of this study were to investigate the effect of low temperatures stress on germination of Iranian and European barley cultivars, to identify the traits related to tolerance to low temperatures in germination stage and to investigate the possible effect of calcium chloride on germination acceleration and other growth parameters of seedlings under low temperatures stress.

MATERIALS AND METHODS

In order to investigate the effect of low temperatures on germination of barley cultivars, identification of traits related to low temperature stress at germination stage and the effect of calcium chloride on these traits, 44 Iranian and European barley cultivars were evaluated in a factorial experiment within completely randomized design with 3 replications in the Laboratory of Plant Physiology, Agronomy and Plant Breeding department, Razi University. The first factor was 44 Iranian and European barley cultivars, the second factor included four temperature (0, 5, 10 and 20°C), and the third factor was the use of calcium chloride (10 mM) and its non-use (distilled water). Iranian cultivars recieved from Kermanshah Agricultural and Natural Resources Research Center and European cultivars seeds recieved from the Genomics and Post Genomics Institute (CRA-GPG) in Fiorenzola, Italy. Table 1 shows the name, source and some of the characteristics of the studied cultivars. Cultivars are named from 1 to 44.

Table 1

Proprties of studied barley cultivars

Code	Cultivar name	Pedigree	Origin
1	ALIMINI	FIOR 2551 x Federal	European
2	RODORZ	Baraka x Gotic	European
3	SFERA	((Katy x HJ54/30) x Igri x Arda) x (Tipper x Sonja)) x Amillis	European
4	ALFEO	Tipper x Igri	European
5	SIRIO	FIOR 2136 x Arco	European
6	ARDA	Igri x HJ 51-15-3	European
7	PONENTE	(Vetulio x Arma) x Express	European
8	ALDEBARAN	Rebelle x Jaidor	European
9	TREBBIA	selection from Fior Synt 3	European
10	ZACINTO	IABO 329 x Arda	European
11	ALISEO	(Plaisant x Gerbel) x Express	European
12	ALCE	(Tipper x Igri3) x [(Tipper x Alpha)x(Sonja x Wb117/18)]	European
13	PARIGLIA	Airone x Arco	European
14	AQVIRONE	FIOR 5186 x Naturel	European
15	ASTARTIS	(IABO x Arda3) x Amillis	European
16	AIACE	FO 1078 x FO 1638	European
17	COMETA	PO202.169 x FO 3358	European
18	NURE	(FIOR 40 x Alpha2) x Baraka	European
19	AIRONE	Gitane x FIOR 763	European
20	SCIROCCO	FIOR 1000 x Express	European
21	MARTINO	FIOR 3007 x Federal	European
22	EXPLORA	[(Onice\Arma\\Onice\Mirco\\\Jaidor) x (Plaisant\Jaidor\Express)] x Gotic	European
23	VEGA	Rebelle x FIOR 1341	European
24	PANAKA	Amillis x Diadem	European
25	Sahra	L. B. LRAN/ Una8271// Giorias"s" Com	Iranian
26	Yusef	Lignee527/chn-01//Gustoe/4/Rhn-08/3/Deir Alla 106//DI71/strain 205	Iranian
27	Denmarck	Denmark55	Iranian
28	Zarjoo	1-28-9963	Iranian
29	Makoie	Star	Iranian
30	Karoon	Strain- 205	Iranian
31	Mahoor	Wi2291/Wi2269//Er/Amp	Iranian
32	Fajr30	Lignee131/ Gerbet//Alger- Ceres/ jonoob	Iranian
33	Sararood	Chicm/An57//Albert	Iranian
34	Gorgan4	Herta	Iranian
35	Jonoob	Gloria" s"/ Copal"s"	Iranian
36	Reihani	Rihane-03/4Alanda/lLignee527/Arar/3/Centinela/2*	Iranian
37	Nimrooz	Trompillo, CMB74A-432-25B-1Y-IB-IY-OB	Iranian
38	Nosrat	Karoon/Kavir	Iranian
39	Afzal	Chahafzal	Iranian
40	Aras	Arumir	Iranian
41	Ansar	Not Clear	Iranian
42	Nader	Not Clear	Iranian
43	Local	Not Clear	Iranian
44	Sararood1	Not Clear	Iranian

From each barley, for each experimental unit, 20 healthy seeds were selected and disinfected according to the following steps: First, the seeds were washed with distilled water and then disinfected with 70% alcohol for 1 minute and 3% hypochlorite for 3 minutes. Then, three times washed with distilled water for 1 minute, 3 minutes and 5 minutes. Seeds were then dressed with mancozeb fungicide (at a rate of 2 g a.i/kg) and cultured in Petri dishes under sterile conditions. The germinated seeds were counted daily for 10 days. The traits were measured based on the average of 10 seedlings including root length (cm), number of root, shoot length (cm), coleoptile length (cm) and the following traits:

$$GP = \frac{Ni}{S} \times 100$$

where
GP – Germination percentage
Ni – Number of germinated seeds
S – Total number of seeds

$$AVG = \frac{\sum Nt}{\sum t}$$

where
AVG – Average Velocity of Germination in day / number:
ΣNt – Total number of germinated seeds at time
Σt – Total time (day), (Salehzade *et al.*, 2009)

$$CVG = \frac{N_1 + N_2 + \cdots + N_x}{N_1 \times T_1 + \cdots + N_x \times T_x} \times 100$$

where
CVG – Coefficient of Velocity of Germination:
N_1 to N_X – the number of seeds germinated from the first day to the end of the test.
T_1 to T_x are the time of counting
This index is a characteristic of the seed germination rate (in day)., calculated from the following equation; (Scotte *et al.*, 1984)

$$PI = nd_2(1.0) + nd_4(0.8) + nd_6(0.6) + nd_8(0.4) + nd_{10}(0.2)$$

where
PI – Promptness Index:
nd2, nd4, nd6, nd8 and *nd10* – the number of germinated seeds on the second, fourth, sixth, eighth and tenth day (Bouslama and Schapaugh, 1984).

$$SV = (SL + RL) \times GP$$

where
SV – Seed Vigor:
RL: Root length,
SL: Shoot length,
GP – Germination percentage; (Hamidi *et al.*, 2009)

$$PCT = \frac{X_n - X_s}{X_n}$$

where
PCT– percentage change of traits
Xn – the mean of trait in control conditions
Xs – the mean of traits in the stress conditions

Statistical analysis

Data were analyzed based on based on a 44 × 2 × 4 factorial experiment within completely randomized design. Mean comparisons were determined with Least Significant Difference (LSD) test by the SAS software ver.9.2. Pearson's correlation coefficients between measured traits evaluated in all tempratures level and cluster analysis based on the Euclidean distance square using Ward's method were done by SPSS software (Ver. 16.0.1, SPSS Inc).

RESULTS

Analysis of variance

None of the studied cultivars germinated at 0°C temperature in all three replications, so the temperature level of 0°C was eliminated from the statistical analysis. Analysis of variance of germination traits in 44 barley cultivars showed that there was a significant difference between cultivars for all traits except root length and seed vigor index (Table 2). The mean comparisons of 44 barley cultivars for the studied traits were done by using the least significant difference test (LSD). Considering the significance of the two and three way interactions for the studied traits, except for root length and seed vigor index, LSD test was performed only on these interactions, some of which are mentioned. Comparison of the significant interaction effect of calcium chloride and temperature for measured traits (Table 3) showed that at 5° C, except for the coleoptile length, other traits in the calcium chloride treatment decreased compared to distilled water. At 10°C, no significant difference was observed in the measured traits between calcium chloride and distilled water treatments. Only a significant decrease for coefficient of velocity of germination in distilled water treatment compared to calcium chloride was observed at 20°C (Table 3). The comparison of the mean of temperature effect (Table 4) for root length and seed vigor index indicated significant differences in these traits at 20°C compared to 10°C and 5°C.

Table 2

Analysis of variance for germination related traits in 44 barley cultivars

Mean squares						
Source of variations	DF	CL [cm]	SHL[cm]	RL [cm]	GP	RN
Cultivar	43	2.763**	15.129**	1541.6965ns	4461.55**	4.771**
$CaCl_2$	1	2.371**	7.99216ns	2481.009ns	575.28**	0.0960ns
Temperature	2	1197.3**	5918.56**	9622.65**	50475.66**	187.40**
$CaCl_2$ × Cultivar	43	0.8073ns	5.428ns	1490.4792ns	212.751**	0.260ns
Cultivar × Temperature	86	2.76**	16.61**	1565.1858ns	2509.57**	3.11**
$CaCl_2$ × Temperature	2	2.371**	2.266ns	2095.3263ns	654.64**	0.004ns
Cultivar ×Temperature × $CaCl_2$	86	0.807**	4.44ns	1492.3237ns	180.47ns	0.337ns
Error	528	0.57	4.23	1439.31	122.28	0.27
Source of variations	DF	PI	SV	CVG	VG	
Cultivar	43	133.543**	14207020ns	0.0198**	2.753**	
$CaCl_2$	1	15.4448*	24573048ns	0.001ns	0.3140*	
Temperature	2	1457.13**	141361556**	2.184**	31.400**	
$CaCl_2$ × Cultivar	43	4.913**	12353878ns	0.0020ns	0.1429**	
Cultivar × Temperature	86	77.649**	14355716ns	0.0205**	1.552**	
$CaCl_2$ × Temperature	2	17.904**	15985379ns	0.0127**	0.420**	
Cultivar ×Temperature × $CaCl_2$	86	3.852*	12366553ns	0.0025ns	0.109*	
Error	528	3.02	11657824	0.0024	0.077	

Table 3

Results of mean comparison of interaction effect of temperature and $CaCl_2$ or significant traits in barley cultivars

Variant	Temperature [°C]	Coleoptile length [cm]	Average germination velocity	Germination velocity coefficient	Promptness Index	Germination percentage [%]
Distilled water	5	0.109 d	1.451 c	0.18 cd	7.365 c	57.917 c
$CaCl_2$	5	0.047 d	1.322 d	0.17 d	6.486 d	52.765 d
Distilled water	10	1.616 c	2.029 a	0.188 c	10.759 b	81.061 a
$CaCl_2$	10	1.580 c	2.055 a	0.188 c	10.821 ab	82.083 a
Distilled water	20	3.853 a	1.536 b	0.33 b	11.183 a	61.364 b
$CaCl_2$	20	3.524 b	1.519 b	0.347 a	11.162 ab	60.379 bc

Values followed by the same letter in the same column are not significantly different

Table 4

Results of mean comparison for main effect of temperature on root length and seed vigor

Temperature [°C]	Root length [cm]	Seed Vigor
20	12.290 a	1471.5 a
10	3.44 b	461.9 b
5	0.748 c	41.2c

Values followed by the same letter in the same column are not significantly different

Percentage changes in traits at different temperatures compared to 20°C.

Reducing the temperature from 20°C to 10°C and 5°C resulted in a significant decrease in coleoptile length (Table 5). The roots number decreased by about 36.71 % compared to 20°C by reducing the temperature to 5°C. At the temperature of 10°C, the germination percentage and the average velocity of germination increased compared to the temperature of 20°C, but the reduction of temperature to 5°C reduced these traits. Coefficient of velocity of germination and promptness index decreased at 10°C and 5°C than 20°C.

Table 5

Variation percentage of traits related to germination in 44 barley cultivars in different temperatures compare to 20°C

RL: root length ‹SHL: shoot length ‹CL: coleoptile length ‹RN: root number ‹GP: germination percentage,

PI [%]	SV	CVG [%]	AVG	GP [%]	RN	CL [cm]	SHL [cm]	RL [cm]	Temperatures [°C]
3.424	68.610	44.490	-33.649	-34.008	4.496	81.618	81.618	58.070	10
38.011	96.656	48.339	9.252	9.085	36.717	99.129	99.129	90.901	5

AVG : average velocity of germination ‹CVG: coefficient of velocity of germination ‹SV: seed vigor ‹ PI: promptness index

Correlation analysis

Pearson correlation analysis for all three temperatures are presented in Table 6. Correlation analysis of traits showed that there is a positive and significant correlation between root length and all traits measured at 5°C. Root number at 5°C had a positive and significant correlation with all traits except shoot and coleoptile length. There was a positive and significant correlation between shoot length and coefficient of velocity of germination at 5 and 10°C. Celoplite length showed a positive and significant correlation with root length, shoot length and seed vigor at 5°C. At 10°C, coleoptile length had significant positive correlation with coefficient of velocity of germination, shoot length and seed vigor and at 20°C with all traits except root length and seed vigor. Germination percentage showed positive and high correlation with seed vigor at all three temperatures. There was a positive and significant correlation between promptness index with coefficient of velocity of germination, average velocity of germination, seed vigor index, germination percentage and root number in all three temperatures.

Table 6

Correlation matrix between measured traits related to germination in 44 barley cultivars under different temperatures

Traits	Temp .	RL	SHL	CL	RN	GP	AVG	CVG	SV	PI
	5°C									
RL	10°C									
	20°C									
	5 °C	.431**								
SHL	10°C	.074								
	20°C	.101								
	5°C	.431**	1.000**							
CL	10°C	.074	1.000**							
	20°C	.164	.684**							
	5°C	.552**	.231	.231						
RN	10°C	.509**	.277	.277						
	20°C	.110	.776**	.799**						
	5 °C	.428**	.046	.046	.662**					
GP	10°C	.120.	.117	.117	.267					
	20°C	.205	.325*	.506**	.607**					
	5 °C	.428**	.046	.046	.662**	1.000**				
AVG	10°C	.120	.116	.116	.267	1.000**				
	20°C	.204	.313*	.496*	.596**	1.000**				
	5 °C	.626**	.230	.230	.430**	.461**	.460**			
CVG	10°C	.267	.326*	.326*	.546**	.325*	.325*			
	20°C	.065	.776**	.754**	.905**	.617**	.605**			
	5°C	.867**	.569**	.703**	.665**	.710**	.709**	.617**		
SV	10°C	.105	.703**	.703**	.244	.301*	.302*	.292		
	20°C	.982**	.119	.195	.166	.369*	.369*	.129		
	5°C	.509**	.091	.230	.670**	.983**	.983**	.587**	.770**	
PI	10°C	.203	.230	.230	.436**	.918**	.917**	.668**	.306*	
	20°C	.191	.324*	.493**	.603**	.997**	.997**	.632**	.356*	

Cluster analysis

The cluster analysis for the data obtained from the germination test was performed using the Ward method based on the Euclidean distance square (Fig. 1). The results of the discriminant analysis divided the dendrogram into two groups and did not differentiate between Iranian and European cultivars (Table 7). The mean of measured traits in each cluster is shown in Table 8. The first cluster consists of 8 Iranian cultivars and 15 European cultivars and the second cluster consists of 12 Iranian varieties and 9 European cultivars. The first cluster had the highest mean for all studied traits (Table 8).

Fig 1. Cluster analysis of 44 barley cultivars based on traits related to germination using Ward method and square Euclidean distance

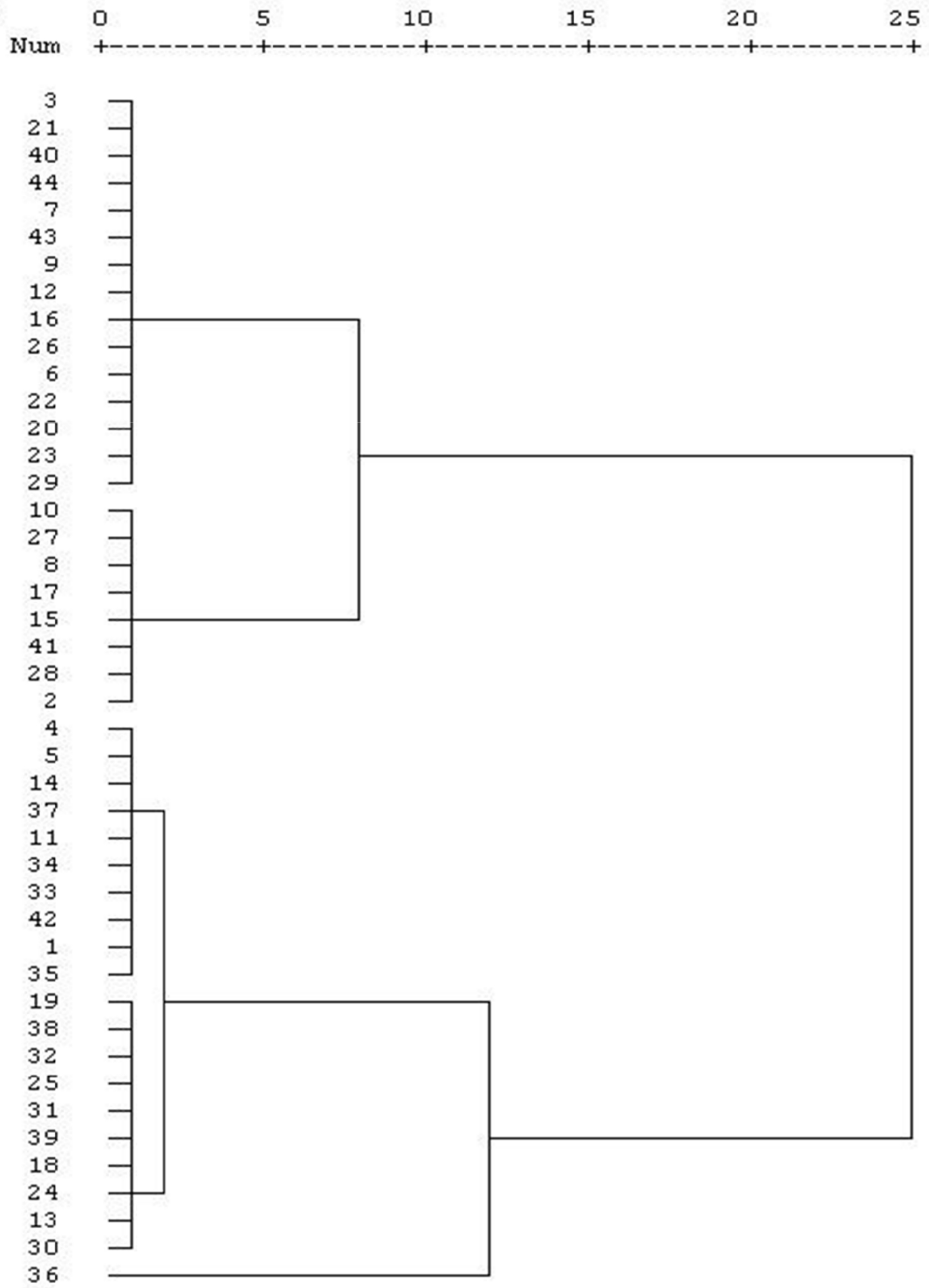

Table 7

Discriminant analysis for grouping 44 barley cultivars based on traits related to germination

Predicted groups			Groups result from cluster analysis	Percentage
Total	2	1		
21	0	21	1	100
23	23	0	2	
100	0	100	1	100
100	100	0	2	

Table 8

Mean of measured traits of 44 barley cultivars in two clusters

Standard deviation ± Mean					Number of cultivars	Cluster
PI	SV	CVG [%]	AVG	GP [%]		
1.27 ± 11.92	12.26 ± 623.30	0.08 ± 0.24	0.15 ± 1.98	6.23 ± 79.36	23	1
1.84± 7.53	179.52 ± 405.56	0.03 ± 0.22	0.26 ±1.34	10.93± 53.65	21	2
Standard deviation ± Mean					Number of cultivars	Cluster
RN	CL [cm]	SHL [cm]	RL [cm]			
0.29 ± 3.88	0.24 ± 1.39	1.87 ± 3.97	1.33 ± 7.12		23	1
0.56 ± 3.39	0.44 ± 1.08	1.14± 3.47	1.21 ± 4.01		21	2

DISCUSSION

The effect of low temperature stress on reducing plant growth is one of the clearest response of plants. Analysis of variance showed that there was a significant difference between cultivars for all traits except root length and seed vigor. Applying calcium chloride treatment at a concentration of 10 mM did not significantly affect the traits under the studied temperatures. Askarian (2004) investigated the effect of CaCl2 on germination of two rangelands species namely Kochia prostrate and Elymus junceus, reported that with increasing CaCl2, germination decreases and even reaches zero. The results showed that the root and shoot length under low temperature stress are accompanied by a decrease, which is consistent with the results of Ghorbani *et al.*, 2009. Abbasal-Ani and Hay, 1983 reported that the growth rate of root and shoot in barley, oat, rye and wheat at low temperature (5 ° C) was low and at high temperature (15 and 25 ° C) is fast. It has been reported that at lower temperatures, the rooting of plants and roots grow decreases (Akbaraghdami *et al.*, 2013). Macduff and Wild (1986) reported that the length and number of roots in germinated barley increased by 27 times, with increasing temperature from 3 to 25°C, after 20 days. The sensitivity of germination percentage and average velocity of germination were lower than other traits, so that at the temperature of 10°C, even the germination percentage and the average velocity of germination increased compared to the temperature of 20°C , but the reduction of temperature to 5°C reduced these traits. Mei and Song, 2010 reported the optimum temperature for the germination percentage in barley is 5-20°C. In the study conducted by Dinari and Meighani, 2014, the effect of cold stress on seed germination and growth of *Hordeum spontaneum* L (root and shoot length and weight) were studied. Reducing the temperature reduced seedling growth, but seed germination was more tolerant to cold stress than seedling growth. Klos and Brummer (2000) stated that the temperature of the environment determines the success of germination and seedling growth, and affects the capacity and velocity of germination. Particularly temperatures below the optimum can cause poor seed germination. Cultivars with fast velocity of germination are more likely to able to absorb water and adapt to snvironment and, due to their winter resistance, are more suitable for rainfed conditions (Rastegar, 1992).

One of the most sensitive traits to low temperatures was seed vigor, which decreased by about 96.65% at 5°C. Due to changes induced by low temperatures, root capacity decreases for water absorption and ultimately plant growth reduces (Akbaraghdami *et al.*, 2013). Root length showed significant positive correlation with all the traits measured at 5° C. Root length can be an important indicator for predicting the emergence of seedling in the field and it is also considered as the primary index of growth and development of seedlings and its changes as an indicator of seedling vigor are analyzed (Bagheri *et al.*, 2012).

Root number at 5°C showed positive correlation with most of traits. Most cold-resistant plants, including barley, when exposed to low temperatures, show signs of water stress (low water potential and leaf inflammation), which is known as drought stress due to frostbite (Ghorbani *et al.*, 2009. Creating a deep and widespread root system as a result of an increase in root number and length with fast growth rate resulted resistance to stress (Kafi, 1997). Tikonov (1973) studied the roots number in the germination stage in 40 wheat cultivars, and observed that the varieties with the highest roots number at germination time had the highest yield under rainfed conditions. Positive and significant correlation between shoot length and coefficient of velocity of germination at 5 and 10°C observed. Cultivars with high coefficient of velocity of germination and shoot length when exposed to cold stress, have better tolerance and better growth (Akbaraghdami *et al.*, 2013).

Celoplite length showed a positive and significant correlation with most of traits at all tempratures. The importance of the coleoptile length in rapid emergence, early deployment and plant diameter, which protects the plant from environmental damage, such as cold and drought, has been reported (Shakeri *et al.*, 2013). Positive and high correlation of germination percentage with seed vigor at all three temperatures indicated seeds that have better seed germination under stress conditions have stronger seedlings (Jajarmi, 2012). Promptness index showed correlation with coefficient of velocity of germination, average velocity of germination, seed vigor index, germination percentage and root number in all three temperatures. In plants such as barley, which are planted early in autumn, less germination time can result in faster seedling growth, and consequently rosseting and resistance to cold weather (Jajarmi, 2012).

Cluster analysis classified cultivars with more desirable germination characteristics under all studied temperatures into separate group. The cultivars in this cluster can be considered as resistant to low temprreratures during germination and seedling growth stages. The varieties grouped in the first cluster can be used in breeding programs for improvement of parameters related to germination in low temperatures. However, in order to be more reliable, the test should be repeated in a range of low temperatures and in field conditions.

CONCLUSION

- In general, the temperature decrease reduced the characteristics related to germination in the barley cultivars. The cultivars with desirable root number and length, shoot and coleoptiles,
- Length showed better germination characteristics under low temperature conditions. Application of calcium chloride treatment with a concentration of 10 mM did not significantly influence the traits under the studied temperatures. It would seem that other concentrations should be considered. Cluster analysis classified cultivars with more desirable germination characteristics under all studied temperatures into separate group. Considering the importance of the ability of cultivars to face low temperature stress at the germination stage, it is recommended that the cultivars in this group be used for further studies and test confirmation.

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