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Genetic Evaluation of Crown Freezing Tolerance and Some Physiological Traits in Barley (*Hordeum vulgare* L.) Lines

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Abstract

In order to investigate cold tolerance in 20 barley genotypes based on crown survival percentage, a greenhouse experiment was conducted as split plots with three replicates, with temperatures (-8, -10, -12, -14 and -16°C) as main plots and barley genotypes constituting subplots. Randomized complete block design was performed to analyze physiological traits measured after acclimation and before applying chilling temperatures. Crown survival percentage was measured zero at -16°C. Error was not significant for main factor in split plots, therefore, data analysis for -8, -10, -12 and -14°C was executed as factorial. Results indicated that temperature, genotype and their interactions had significant influence on the crown survival percentage. Also, the genotypes were significantly different in terms of LT50, the glycine betaine content and leaf relative water content before and after adaptation to cold. Comparison of the means, based on LT50 and crown survival percentage, suggested the genotype number 15 (with K-096M3 pedigree) as the most tolerant to crown freezing, and genotypes 36 (Schulyer), 15 (K-096M3) and 14 (GK Omega) as possessing the most desirable physiological traits, with genotypes 15 and 36 possessed the lowest difference before and after leaf relative water content, and the maximum quantity of glycine betaine after adaptation to cold. Cluster analysis of the genotypes, based on the aforementioned traits, divided them into three distinct tolerant, semi- tolerant and sensitive groups.

Keywords

Barley; Crown survival percentage; Freezing tolerance; Glycine betaine; LT50; Relative water content

Introduction

Cold stress is an abiotic stress that limits the distribution, growth, and productivity of crop plants [1] in 42 percent of the surface of the earth [2] where lands experience temperatures below -20°C. Plants exhibit different degrees of cold tolerance, and some can increase their tolerance through a process known as cold acclimation, adaption to low temperatures. Cold acclimation involves a series of physical and biochemical mechanisms, which occur at low temperatures, or above the freezing point [3,4], including processes such as stability of cell permeability, change in the composition of membrane lipids and increase in antioxidants [5]. After cold adaptation, plant may withstand the following freezing conditions, in which case it is called cold tolerant [6].

In order to assess tolerance in cereals after acclimation, they are grown in natural environment or controlled conditions (growth chamber or fridge), which is known as direct evaluation. In indirect methods, on the other hand, molecular markers are employed to assess physiological and biochemical modifications during cold acclimation [7]. One direct method is fast, controllable and repeatable; uses controlled freezing tests to measure LT50 in cold acclimatized cultivars, and commonly used as a winter survival signifier of tolerant plants.LT50 is a temperature stage that causes 50 percent death to plants grown in a freezing chamber during monitored freezing tests [8,9] In some studies, there has been a significant linear correlation between LT50 and survival at -50°C [10]. In winter wheat, for example, physiological traits such as LT50, crown water content (CWC) and leaf relative water content (RWC) correlate with cold survival [11].

Most temperate plants, through evolution, have acquired varied abilities to develop cold tolerance in response to acclimating conditions. Accumulation of certain molecules with a cryoprotective role, for example, is a mechanism adapted by plants in response to low temperature conditions [12]. Glycine betaine (GB) is one such cold tolerance associated osmolyte [13], which plays many roles, including preserving the quaternary structure of enzymes and proteins [14], stabilizing membranes [15] and photosynthetic apparatus [16,17], under cold and freezing temperatures. It also reduces the peroxidation of membrane [18]. In some species, cold acclimation induces glycine betaine accumulation proportional to the degree of cold tolerance [19,20]. There is also evidence that GB concentrations in leaf correlates with leaf relative water content [21].

Losses of water and tissue water content are other attributes of cold tolerance. According to Fowler et al. [22], tissue water content measurement, as one important laboratory indicator, possesses in it all the desired characteristics of cold tolerance. It has also been established that leaf water content has had a major correlation with viability of plants) [11], and tissue water content declined in response to cold acclimation, which in turn leads to increase in cold tolerance [23].

Among autumn cereals, barley is the third sub tolerant to cold stress [24]. In terms of global production, it comes forth in the rank, after wheat, rice and corn. Barley autumn cultivars have higher yield than spring ones. Since they spend a part of their vegetative growth exposed to cold conditions, to avoid late heat and droughts, developing cold tolerate barley varieties is an important goal of breeding programs worldwide [25]. World have suffered significant economic losses due to injuries imposed by freezing temperatures to crop and horticultural industries [26]. In carrying out this study, the objectives have been to identify barley genotypes tolerant of freezing based on the crown freezing test, as well as determining the relationship between freezing tolerance and some physiological and biochemical characteristics.

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Materials and Methods

Preparation of plant materials

In this experiment, plant materials, including 20 Barley (*Hordeum vulgare* L.) genotypes obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran (Table 1). Evaluation of the Barley genotypes was carried out using a split plot experiment with three replicates, within greenhouse and growth chamber in the Faculty of Agriculture of University of Tabriz (from November 2013 to the middle of February 2014).

Seeds, after have being sterilized in Mancozeb 2 ppt, were planted the rows in rectangular 50 × 40 cm plastic pots containing agronomy soil. Each pot involved four rows. 25 seeds were planted along each row, sown two cm down the soil. Irrigation performed when necessary. The greenhouse temperature was kept at 21°C and 18°C during the days and nights, respectively. After reaching three, four- leaf stage, the seedlings were transferred to a growth chamber for four weeks, with 4°C daytime and 2°C during nights, under a 12-h day length at 250 µmol m-2s-1 photo synthetically active radiations, in order to get acclimatized to low temperatures.

Relative water content (RWC) measurement

In order to execute RWC measurements prior and post acclimation, the third developed leaves were sampled from each genotype, fresh weights were determined immediately after wards. To determine saturated weight, leaf specimens were submerged in 100 ml water at room temperature for four h. The same samples were, then, wrapped in aluminum foil, put inside an Avon set at 75°C to dry out, and, finally, weighed to measure their dry mass. The RWC was determined using the following equation [27].

$$RWC = [(\text{fresh weight- dry weight})/(\text{saturated weight- dry weight})] \times 100.$$

Glycine betaine (GB) measurement

For glycine betaine measurement, sampling was carried out before and after cold acclimation from the third developed leaves. GB content was measured according to Grieve and Grattan [28]. After stirring leaf samples in distilled water for 48 h at 25°C and filtering, the solution was diluted using 2NH2SO4. Cold KI-I2 was added to the diluted liquid, and after centrifugation, the supernatant was mixed with 1, 2- dichloroethane. Absorption was recorded at 365 nm.

Crown survival percentage (CSP) assessment

CSP investigated after plants have been adapted to cold with the roots and leaves were cut two cm below and one cm above the crown respectively, so plants could recover by developing new roots and leaves. Ten crowns belonging to the same genotype were banded together. Samples were placed in aluminum cans filled with wet sand and transferred to a programmable freezer where they were, first, kept at -2°C. After 12 hours, the temperature plummeted gradually. From -8°C onwards, materials of the respective temperatures were taken out at two-hour intervals, and the crowns were put in a regular fridge to thaw at 4°C. The next day, the crown of any given temperature were planted in pots, then, grown in the greenhouse at 23°C for a 21-day period. The records of surviving and dead plants, as well as the CSP were calculated as followings [29].

$$CSP = (\text{the number of seedlings after freezing} / \text{the number of seedling before freezing}) \times 100.$$

LT50 measurements

LT50 in genotypes studied was calculated using data related to survival percentage for all temperatures and transformation of the probits [30]; variance analysis was conducted as randomized complete blocks. Comparison of the means was carried out with Duncan's test. Prior to analysis, data was suitably transformed in cases where some assumptions of the variance analysis were not true.

Statistical analysis

Before performing analyses, the assumption of variance homogeneity and error normality was examined. Most data relative to survival percentage scored zero at -16°C, and brought in homogeneity and abnormality to the variance, therefore, the pertaining data was excluded from the analysis. Since the amount of biochemical variables were measured prior and post acclimation, a complete randomized block design with three replicates was implemented to analysis the data. Data was analyzed in SPSS19 and MSTATC computer software.

Results

Data was, first, analyzed in the split plot, due to the nature of the experiments. However, as a result of main plot error being non-significant, a factorial design was used to analyze the variables.

The results of variance analysis pertaining to CSP of 20 barley genotypes at -8, -10, -12 and -14°C showed that the F for temperature, genotype and the interaction of temperature and genotype was significant at 1%. Interaction being significant indicates that genotypes

Table 1: Code/name and pedigree of barley genotypes us ed in evaluation of cold s tres s

Genotype No	Genotype Code/ Cultivar Name	Pedigree
1	EC79-10	Walfajre/Miraj 1
4	EC80-7	YEA389.3/ YEA475.4
5	EC80-11	ALGER/(CI10117/ CHOYO
9	EC82-5	Alger/(CI10117/ Choyo
11	EC82-11	Np106/Minn14133-Gva xduois //Gi10143
14	EC83-10	GkOmega
15	EC83-12	K-096M3
16	EC83-15	SCHUYLER//(M.RNB89.80/ NB1905//L.527)
18	A1C84-7	Star/Dundy
20	A1C84-12	Kozir/330
21	A1C84-14	As trix(C)/3/Mal/OWB753328-5H//Perga/ Boyer
22	A1C84-15	Monolit/Plais ant
28	A2C84-14	Cyclone/Arar
29	A2C84-18	Mal/OWB753328-5H//11840-76/3/ Radical
31	Makouee	Makouee
33	Rihane	Rihane
34	Kavir	Kavir
35	73M4-C	73M4-30
36	Schulyer	Schulyer
38	Aths	Aths

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Table 2: Mean of s urvival percentage at -8, -10, -12, -14°C in barley genotypes

			-12°C		-14°C		The average mean temperatures
Genotype No.	-8°C	-10°C	The original data	The converted data	The original data	The converted data	
1	85.93	33.33	0	0.7080	0	0.0708	27.32
4	96.67	53.33	10	0.2902	0	0.0708	40
5	100	63.33	33.33	0.6151	0	0.0708	49.17
9	100	70	10	0.3300	3.33	0.1572	45.84
11	100	66.67	6.67	0.2436	3.33	0.1572	44.17
14	100	73.33	50	0.7904	6.67	0.2436	57.5
15	96.97	92.13	55	0.8424	10	0.3300	63.49
16	100	36.67	0	0.0708	0	0.0708	34.17
18	93.33	68.9	30	0.5851	0	0.0708	48.05
20	96.67	83.33	33.33	0.6151	0	0.0708	53.34
21	100	53.33	0	0.0708	0	0.0708	38.34
22	90	60	16.67	0.4233	3.33	0.1572	42.5
28	90	76.67	10	0.3300	3.33	0.1572	45
29	100	50	10	0.3300	3.33	0.1572	40.83
31	100	43.33	6.66	0.2038	0	0.0708	37.5
33	86.30	26.67	0	0.0708	0	0.0708	28.25
34	47.50	20	0	0.0708	0	0.0708	16.88
35	63.50	33.33	0	0.0708	0	0.0708	24.21
36	100	82.50	30	0.5816	10	0.3300	55.63
38	43.33	13.33	0	0.0708	0	0.0708	14.17
LSD5% LSD1%	0.1383 0.1852	0.1568 0.2100		0.1568 0.2100		0.0527 0.0700	

did not change equally at different temperatures. Therefore, analysis of variance and comparison of the means of genotypes for CSP for each individual temperature was conducted using a randomized complete block design, as genotypes performed differently at different temperatures, (Table 2).

There was a significant difference between genotypes for -8, -10, -12°C at level of 1% and for -14°C at level of 5%. At -8°C, all genotypes, except for 34, 35 and 38, scored above 80 percent survival, which also displayed a significant contrast to other genotypes at 1%. At -10°C, genotypes 15, 20 and 36 showed the maximum percentage of survival; the lowest percentage was obtained by genotype 38 as 13.33. As temperature declined to -14°C, some genotypes were killed; genotypes 15 and 14 obtained the maximum scores, respectively, with 55 and 50 percent survival. This was significant at 1%, compared to other genotypes.

At -14°C, most genotypes were destroyed. Genotypes 15 and 36 with 10 percent survival were significantly different from others at 1%, hence, designated as tolerant genotypes. Genotype 38 had the lowest average of survival across average temperatures; genotypes 15, 14, 36, 20 and 5 had survival percentage of at least 50 across mean temperatures (Table 2 and 3). The results of variance analysis for LT50 in genotypes studied revealed a significant difference at the level of 1% (Table 4). The coefficient of variation (C.V.) for this trait was 6.77, indicating a low experiment error. LT50 was higher in genotypes 15, 36, 14, 5, 20 and 9 and lower in genotypes 38, 34 and 35 than the rest. Genotypes with smaller LT50 had higher tolerance than those with bigger LT50 (Table 4).

Genotypes 15, 14, 36, 20 and 5 also scored a higher mark for CSP during the freezing test. The negative, significant correlation between LT50 and CSP indicated that the more the CSP, the less the number of dead plants.

Analysis of variance relative to the amount of GB and RWC were conducted as a factorial experiment involving temperature (in two levels) and genotype (in 20 levels) based on a complete randomized block design. Results showed that there was a significant difference between the two temperature conditions- prior and post acclimation to cold – in regard to aforementioned traits ($p \leq 0.01$). Likewise, a significant difference was observed between barley genotypes concerning RWC and GB, implying a variation in barley genotypes for these traits ($p \leq 0.01$). The interaction between genotype and temperature for GB showed significant difference at 1%, which, by comparing the means of this effect, made clear that the discrepancy was rooted from variation in GB content as developing adaptation to cold. In other words, there was no significant difference between genotypes before adaptation.

Investigating the means of GB and RWC between the genotypes under two temperature conditions showed that leaf RWC dropped significantly after adaptation to cold ($p \leq 0.01$) (Figure 1), and the amount of GB increased significantly post adaptation to cold ($p<0.01$) (Figure 2).

To better understand the contrasts between the genotypes, analysis of variance for two variables-GB content and RWC, before and after adaptation to cold and changes in the value of these traits-was conducted as a complete randomized block design in two conditions (Table 4). Analysis of variance showed that, in contrast to before adaptation to cold, which showed no significant difference between the genotypes, the amount of GB in genotypes had experienced a change from 1125 m mol per gram fresh weight in Sensitive genotypes to 2472 m mol per gram fresh weight in resistant ones, conferring a significant difference at 1%. Likewise, the change in the content of GB before and after adaptation brought about a significant difference among the genotypes at 1%, with the highest and lowest changes belonged to the genotypes 36 and 34, respectively (Figure 3). Accordingly, genotypes 38 and 34 are sensitive to cold and genotypes 5, 15 and cultivar 36 (Schulyer) are cold tolerant. Which means more GB carries with it more tolerance to cold. The same results have been reported on the accumulation of GB inducing tolerance in other plants undergoing drought and salinity stress [31].

Furthermore, there was no significant difference between the barley genotypes before cold acclimation for RWC, contrary to significant decline in RWC at 1% after the genotypes having been acclimatized. Variations of RWC before and after adaptation to cold were not significant between genotypes. However, these changes showed that, among all 20 genotypes, 38 and 34 possessed the maximum and 14, 15 and 36 had the least chaining of RWC in two conditions (Figure 4). Which means sensitive genotypes displayed a bigger fluctuation in RWC in response to cold adaptation. In other words, cold condition causes more loss of water in cold sensitive genotypes.

A negative, significant correlation existed between LT50 and GB content after acclimation to cold, and the difference between to temperature conditions. Which indicates that more tolerate genotypes has produced more GB. There was a significant, negative correlation between GB content after cold adaptation and RWC, before, after and the difference between the two cold treatments. Genotypes with lower RWC had greater GB content. Likewise, a significant, positive correlation was found between LT50 and RWC, before, after and the difference between the two cold treatments, indicating that sensitive genotypes possessed greater RWC in leaves (Table 5).

Table 3: Mean of LT50 in barley genotypes

Genotype NO	Genotype code or cultivar name	Mean
51		-12.39
63	EC83-12	-11.785
51	Schulyer	-11.274
1	EC83-10	-11.125
02	EC83-11	-11.068
9	A1C84-12	-11.048
51	EC82-5	-10.997
09	A1C84-7	-10.872
00	A2C84-18	-10.864
65	A1C84-15	-10.819
01	Makouee	-10.806
55	A2C84-14	-10.703
1	EC82-11	-10.547
05	EC80-7	-10.052
53	A1C84-14	-9.987
5	EC83-15	-9.52
66	EC79-10	-8.389
61	Rihane	-8.731
61	73M4-C Kavir Aths	-7.322
61		-6.7
1%LSD		0.7500
5%LSD		1.005

Table 4: Analys is of variance of GB and RWC content in barley genotypes leaves

M.S.							
G.B.					**R.W.C.**		
S.O.V	D.F.	Control	Acclimation	Changes between control and acclimation	Control	Acclimation	Changes between control and acclimation
	2	129.398ns	34243.438ns	335370.09ns	52.557ns	12.840ns	1114.439ns
Replication Genotype Error	19	6001.014ns	288364.093**	310132.916**	180.818**	69.680**	37.741ns
Non-additive	38	2556.659	30036.324	30746.398	23.617	11.688	47.746
Res idual	1	1.874**	2477714.5**	303005.948**	3.013ns	0.468*	1.160*
	37	2625.707	24151.590	23388.02	24.173	11.991	49.005
C.V		36.01	11.21	19.22	6.30	4.90	95.3

ns : not s ignificant, * s ignificant at 0.05% and ** s ignificant at 0.01%

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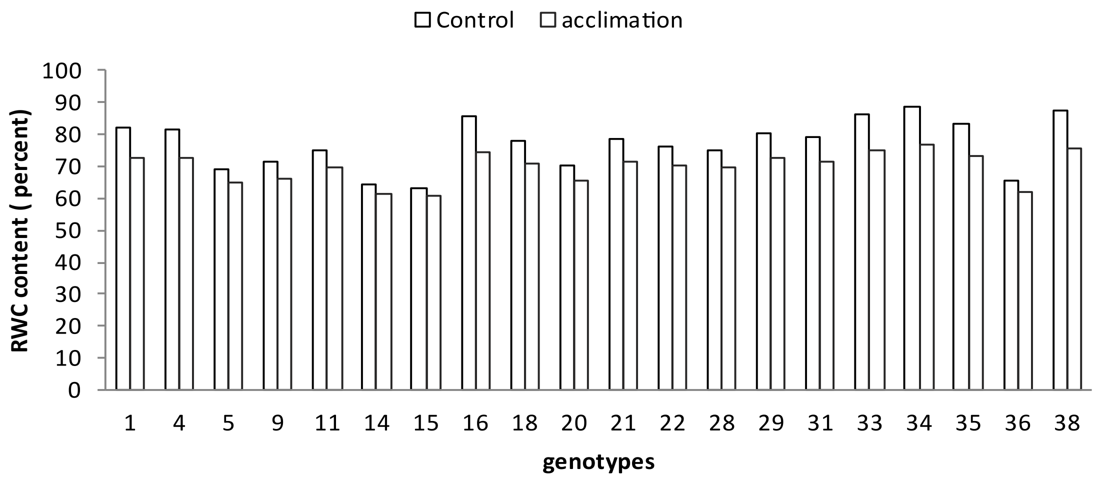

Figure 1: Content of RWC in the leaves of barley genotypes under control and cold conditions.

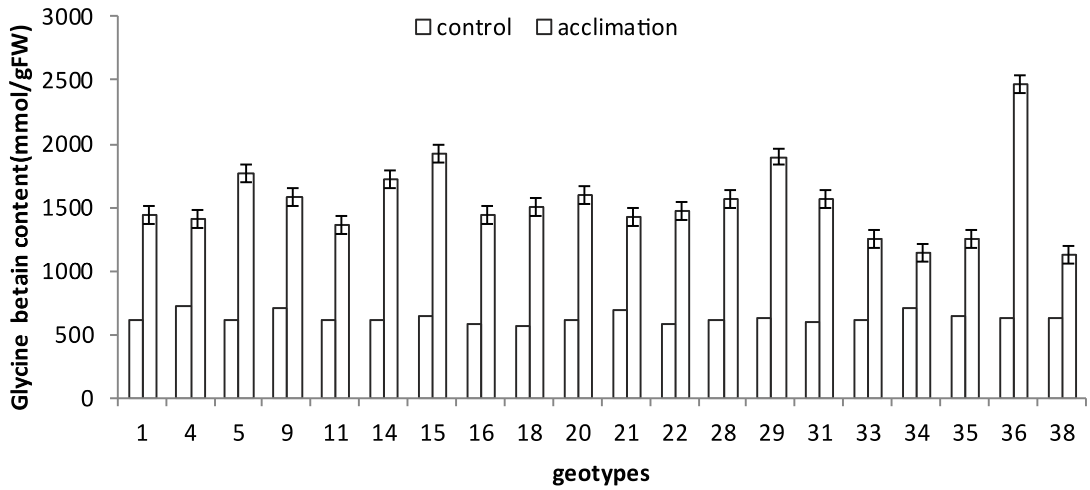

Figure 2: Changes in the content of GB in the leaves of barley genotypes under control and cold conditions.

Discussion

In this experiment, barley genotypes possessed different survival percentage and LT50 after cold adaptation at different temperatures. Autumn cultivars 36 and 15, due to being regularly cultivated in cold and moderate regions, displayed bigger survival percentage than spring's cultivar 38 did. Spring cultivars do not need adaptation to cold; they enter reproductive stage after a short time. Therefore, they are very sensitive to late spring as well as early fall cold weather. Transition from vegetative to reproductive stage is a vitally important phenomenon which keeps genes associated with cold tolerance under suppression, and raises the temperature in the crown [32]. In most areas, injury to the crown accounts for the main cause of death in plants. Therefore, soil temperature around the crown during the cold adaptation process is very important, and the crown will spoil if the soil temperature is lower than that of the crown [33]. In spring cultivars in which the temperature of the crown is warmer than the surrounding soil, this tissue spoils. Besides the plant's vegetative habit, genetics potential of the plants will also count for adaptation to cold [32] Autumn's genotypes varied in LT50 and survival percentage. And although the genetic potential varies in response to cold stress, the general pattern of response to cold weather during winter is the same for genotypes either inside or between cereal species. As a result, genetic variations related to cold tolerance can be determined using genetic coefficients of LT50 [34].

The results of this experiment and others [8,9] confirm the validation and reliability of LT50 as an indication of cold tolerance in barley. A high inheritability has been reported for LT50 [11] Here, the maximum LT50 scored by autumn genotype 15 as -12.39°C, and the minimum obtained by spring genotype 38 as -6.7°C. LT50 for wheat cultivar Nourstar has been determined in myriad of experiments around -23°C [35,36]. Therefore, it would be wise to use Nourstar as the landmark in identifying the precise LT50 for other genotypes, and setting them against Nourstars' LT50, as a means to reflex their potential capabilities [33]. Some management styles can additionally influence cold tolerance, which include cultivation date, plant age, depth of plantation and so forth [37]. In the current experiment, the effort was made to make sure every seed was planted in the same depth; the seedlings were acclimatized to cold stress in the same stage- a 3 to 4 leaf stage- to make sure the management errors have been averted, and LT50 was the only indicator of genetic potential.

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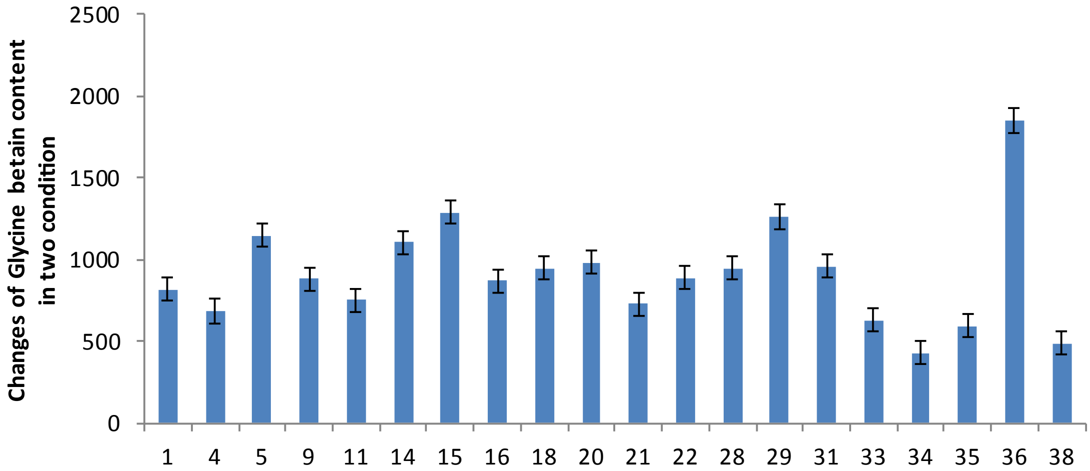

Figure 3:Changes of Glycine betaine content in the leaves of barley genotypes in two conditions.

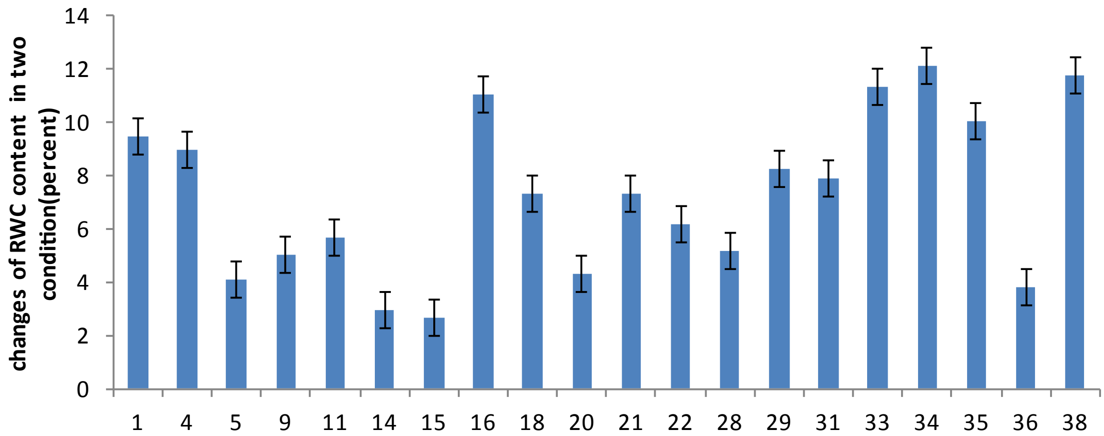

Figure 4. Changes of RWC content in the leaves of barley genotypes in two conditions.

Previous studies have confirmed a reduction in leaf RWC after acclimation to cold stress. Cold injury starts from the cell membrane, where low temperatures change the status of the membrane and leads to its damage [38]. The less cell membrane is damaged by freezing, the less amount of water is lost, and the greater the rate of survival will be [39].

Huner et al. [40] also reported that the leaves adapted to cold have 23% less water than plants without cold adaptation. Another research has also shown that plants tolerant to cold stress have higher competency to absorb and retain water during cold stress, hence experiencing fewer drops in RWC in leaves [41]. In the current study, the significant, positive correlation between leaf RWC and LT50, under normal conditions, signifies that tolerant genotypes with lower LT50 had a lower Leaf RWC than sensitive ones. Mirzaie-Asl et al. [42] also reported that tissues with less RWC in wheat were more tolerant to cold stress than those with bigger RWC. Ice formation is very damaging. Since ice crystals cannot exert a hydrophobic force necessary for preserving the bipolar status of lipids in cell membrane, they cause the disruption of cell membrane in contact [43]. Less cell membrane disruption in tolerate cultivars is due to less leaf RWC, less formation of ice inside the cell, and less production of H_2O_2.

In the current study, a significant, negative correlation existed between LT50 and GB content after cold adaptation as well as the difference between the two cold treatments, meaning GB content has increased with the reduction of LT50 (in more tolerate genotypes).

GB is one of the more common osmolytes, whose accumulation in surviving organisms is said to be used as a strategy for combating environmental stresses [44]. Although they are put in different groups, osmolytes assume similar functions in protecting plants against stress. However, the exact function of such solutes, including GB, under abiotic stresses, is not fully understood. There are two main functions attributed to these osmolytes: osmosis regulation and cell adaptation. Osmosis is regulated by the influence of forces related to concentration on osmosis pressure, which absorbs more water from the surrounding environment. In the cell adaptation process, these osmolytes substitute water in biochemical reactions, keeping the metabolism moving under stress conditions [45]. GB can substitute the lost water in tolerant genotypes, helping the plants survive by preserving the metabolism against cold stress. Contribution of GB accumulation to plants' tolerance to drought and salinity has been also reported in another study [31]. In the current study, GB content had increased in both tolerant and sensitive genotypes, but a bigger increase was seen in the former.

Table 5: Correlation between LT50, RWC, GB and Crown s urvival percent.

	LT50	**Rwc before cold**	**Rwc after cold**	**Rwc difference between before and after cold**	**Gb before cold**	**Gb after cold**	**Gb difference between before and after cold**	**Crown s urvival percent at -8°C**	**Crown s urvival percent at -10°C**	**Crown s urvival percent at -12°C**	Crown s urvival percent at -14°C
Rwc before cold	.819**	1									
Rwc after cold	.784**	.992**	1								
Rwc difference between before and after cold	.847**	.980**	.948**	1							
Gb before cold	.288	.154	.132	.185	1						
Gb after cold	-.759**	-.759**	-.775**	-.711**	-.190	1					
Gb difference between before and after cold	-.772**	-.754**	-.765**	-.711**	-.322	.991**	1				
Crown s urvival percent at -8°C	-.898**	-.597**	-.556*	-.643**	-.264	.598**	.613**	1			
Crown s urvival percent at -10°C	-.892**	-.912**	-.873**	-.944**	-.189	.704**	.705**	.688**	1		
Crown s urvival percent at -12°C	-.689**	-.866**	-.881**	-.814**	-.256	.643**	.655**	.412	.780**	1	
Crown s urvival percent at -14°C	-.591**	-.745**	-.770**	-.683**	-.073	.761**	.744**	.326	.661**	.649**	1

** Significant at 1%

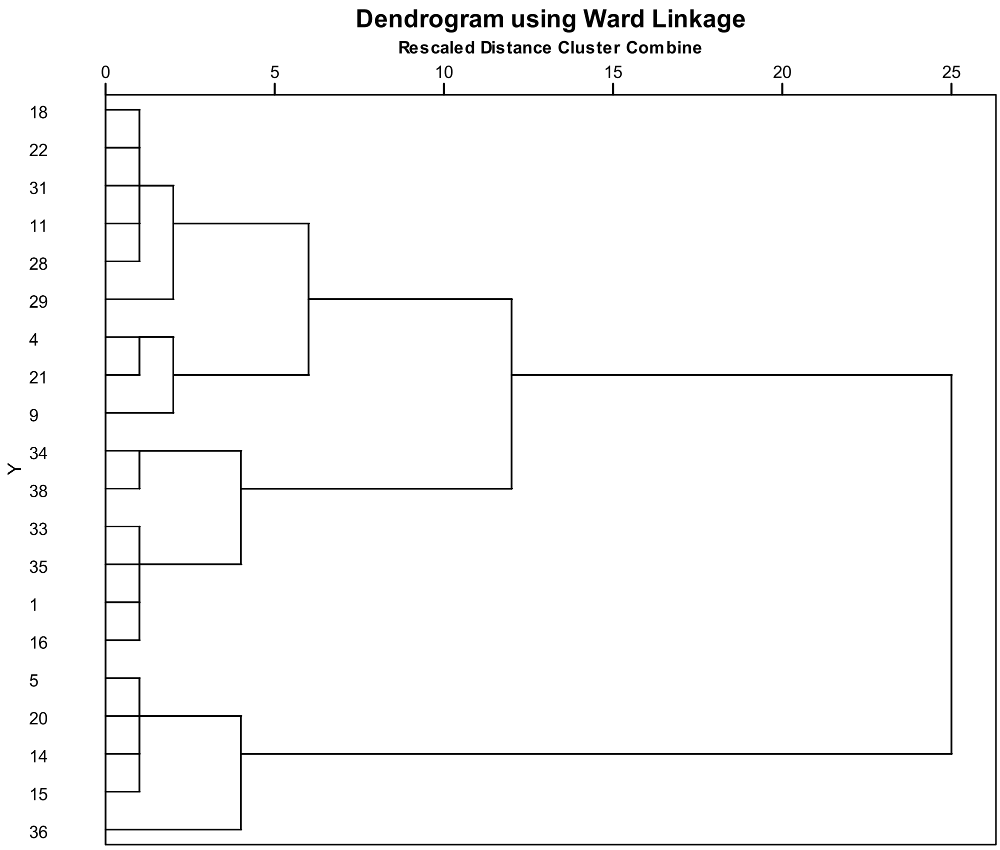

Figure 5: Cluster analysis for GB and RWC traits using the Ward method.

Cluster analysis

Cluster analysis was carried out using Ward method according to the squared Euclidean distance on standardized data (Figure 5). At a cut off 10 the dendrogram revealed three clusters. Group one includes 9 genotypes (18, 22, 31, 11, 28, 29, 4, 21,9) with negative deviation of mean (-16.38) for LT50, changing of GB and amount of RWC in two conditions, but deviation of mean percent is not a lot for GB and RWC. The group can considered as semi- tolerant genotypes based on the investigated characteristics under cold stress. Second group includes 34, 38, 33, 35, 1, 16 has positive deviation of mean (62.47) for LT50, negative deviation for changing of GB and RWC in two conditions. This group considered as sensitive to cold stress. Group three includes 5 genotypes (5, 20, 14, 15, 36) with highest negative deviation (-45.48) for LT50, positive changing of GB and RWC in two conditions, ranked as tolerant genotypes to cold stress. For LT50, more negative deviation from the mean and more positive deviation from the mean for GB and RWC, is a desirable features.

Conclusion

To sum up, LT50 is a suitable indication of tolerance to crown freezing but this method requires much time and cost and needs special systems for freezing test, thus by studying physiological traits and LT50 in seedling stage, a robust correlation can be made between these traits and tolerance to cold stress, hence differentiating tolerant genotypes from sensitive one at a lower cost and time. Glycine betaine and relative water content have a significant correlation with LT50, so that GB is more increased after cold in tolerant genotypes but RWC is more decrease in sensitive genotypes in response to stress condition. In conclusion, according to the results we suggested that in absence of freezing test systems, by measuring glycine betaine before and after cold stress, could be detected barley genotypes tolerance to cold conditions.

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