Tarım Bilimleri Araştırma Dergisi 8 (1): 35-39, 2015
ISSN: 1308-3945, E-ISSN: 1308-027X, www.nobel.gen.tr

Classification of Barley Cultivars Using Seed Storage Protein Markers Related to Some Physiological Traits

Omid SOFALIAN[1*] Ali ASGHARI[1] Rasool ASGHARI ZAKARIA[1] Behnam FIROOZI[1]
[1]Agronomy and Plant Breeding Department, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil

*Corresponding author
e-mail: sofalian@gmail.com

Received: March 01, 2015
Accepted: April 22, 2015

Abstract

In breeding programs, classification of genotypes and genetic information within or between species of crop plants were useful for any selection and hybridization perspectives. In the present study 17 barley lines and cultivars were used for studing seed storage protein by electrophoresis technique and some physiological traits. Based on ANOVA results, studding genotypes had significant differences at level of $P \leq 0.01$. The resulting dendrogram using WARD method indicated that the studding lines could be divided into three groups. First group lines had a high Fv/Fm value. Second group contained lines numbered 10, 11, 12 and 8, had a low chlorophyll content values. The results of electrophoresis profile showed 11 protein bands with maximum in number 2 and 17 lines (10 bands) and minimum in number 6 and 16 lines (1 band). Based on jaccard's similarity coefficient, There was the most similarity (or the least difference) among number 8 and 10 lines (1), 2 and 17 (1), 11 and 17 (0.9), 11 and 2 (0.9) and the least similarity (or highest difference) between number 1 and 3 lines (0), 3 and 6 (0), 3 and 8 (0), 3 and 10 (0), 3 and 16 (0), 4 and 6 (0), 6 and 8 (0), 6 and 10 (0) and 6 and 16 (0) respectively. Also principal coordinate analysis confirmed that 80.9 percent of total variance of barley storage proteins was explained by the first three components. Finally it is clear that genetic variance was existed between studding lines.

Key Words: Chlorophyll content, Electrophoresis, Proline, Soluble sugars content.

INTRODUCTION

Barely (*Hordeum vulgare*) is one of the first plants domesticated by humans and thus genetic variation occurred in its genome and it has been causing genetic diversity in its wild ancestors [1]. In terms of global production, Barley is the fourth cereal after wheat, rice and maize [2]. Barely has a wide range of adaptability and cultivates in areas where other crops doesn't grow well due to the low rainfall, salinity, heat and cold conditions [3]. This crop used as a fodder for animals, malt preparation and other human consumptions [4]. Based on FAO [5] statistics cultivation of barely are over 56 million hectares yielding to 137 million tons in the world and 1.7 million hectares those yielding to 2.9 million tons in Iran.

Protein electrophoresis that distinguish and identify plant populations by biochemical system, has found wide application over the other methods used in species, populations and samples. In this regard, a variety of enzymatic and non-enzymatic proteins (usually seed storage proteins) have been investigated. Storage proteins are very stable, while having a high polymorphism. Although environmental factors affect the amount of protein storage, but their impact is low on mature seeds. The important point about storage proteins is, if exact genetic studies did not carry out on control samples, it is difficult to interpret different samples genetically. Some studies have done about several major crops such as barley, wheat, corn, pea and bean in order to investigate the genetic controls of seed storage proteins. The problem about interpretation of seed storage proteins, are the polygenic nature of this type of proteins that observed in some species like as wheat, corn, pea and bean. Usually each protein group controls by group of genes and/or a group of DNA sequences. Heterogeneous nature of electrophoretic pattern of the bands is due to the different number of copies of the gene clusters, internal heterogeneous sequences of a group and different products of a protein.

An application of storage proteins in plant breeding is using the association between some of the alleles with desired traits in seed. In some species, special proteins have a better balance of amino acids. Some of them also made plants to resistant to diseases and pests [6]. Barely seeds contain eight to 15 percent protein, 60-70 percent starch, two-three percent minerals and 10 percent humidity. Prolamins (hordeins) are the major proteins in barley seed and constitute 50% of the total. Hordeins are multigene and their synthesis controlled by several genes, therefore they act so specific. Hordeins-produced multi genes created by duplication and differentiation of their ancestral genes and the nucleotide substitution. These two mutations cause different numbers of repetitive sequences produced [7, 8]. This group of multi-gene has been cause to polymorphism in storage proteins. It is believed that the high genetic diversity in hordein indicate that this group of proteins hada high resistance to mutation and natural selection [9].

Having knowledge about genetic distance between Individuals or populations and kinship relations of species in breeding programs may be organized germplasm and efficient sampling of genotypes. To determine the genetic diversity of plants several markers, such as morphological, biochemical and DNA marker may be used. Characterization of germplasm by means of DNA fingerprinting techniques provides a tool for precise germplasm identification and a quantitative estimate of genetic diversity [10].

McCausland and Wrigley [11] reported high polymorphism in Australian barley for the first time. Shewry et al. [12] studied storage proteins of 29 barely varieties using SDS-PAGE technique and indicated that only seven cultivars are similar to each other. Marchylo and

LaBerge (13] by electrophoresis technique compared the amount of seed hordeins in 62 Canadian barley cultivars and six American barley landraces and stated that only 25 cultivars are similar to each other. Hamza et al. [14] used 17 microsatellite markers to assessing the genetic diversity of 26 barley cultivars and the association between these markers and morphological traits. The 15 of 17 microsatellite markers were polymorphic.

Due to the role of genetic diversity in breeding programs, present study was conducted to investigate the probable variability of barely storage proteins and physiological and grouping them according to genetic diversity.

MATERIALS AND METHODS

The plant material used in this experiment consisted of 17 genotypes of barley cultivated in Iran. The name and origin of these genotypes are shown in Table 1. Seeds of these lines were obtained from the Agricultural Research Center of Moghan. These lines were cultivated in a randomized complete block design with three replications in 2013 in University of Mohaghegh Ardabili Research Field, Ardabil, Iran (latitude: 48°34′E, longitude: 38°31′Naltitude: 1350). This area has temperately cold climate.

Seed protein extracted based on Laemmli [15] method. At first a seed was chosen from each line and ground to fine powder using mortar and pestle. The 0.04g of flour sieved and was poured into a separate Eppendorf tube. The 1500 µl of extraction buffer (0.61g Tris, 2.93g NaCl, 30 ml of distilled water. pH=7.5 and the final volume was 100ml) and 100 µl 2-Mercaptoethanol were added and shaked. After 15 min incubation in the freezer, 15 min maintained at room temperature. Vortex and freezing procedure repeated three times. Finally, samples were centrifuged at 10,000 rpm for 10 min and the supernatant was transferred to a new Eppendorf tube. Protein staining solution (0.75 g Tris, 8 g of sucrose, 2 g of sodium dodecyl sulfate, 0.06g bromophenol blue, 30 ml of distilled water, pH=6.8, the final volume is 100 mL) and extracted proteins mixed with the ratio 1: 3 and 30 µl of the mixture loaded into the wells of SDS-PAGE gel. After electrophoresis ended the gel soaked into staining solution (Coomassie Brilliant Blue, methanol, acetic acid and double-distilled water concentration of 144 mg, 60 ml, 24 ml and 60 ml respectively) and then was taken to the destaining solution (a mixture of 60 ml of methanol, 20 ml acetic acid, 120 ml of double-distilled water). Next steps include fixing bands by using 7% acetic acid solution, gel scanning and scoring it as 0 and 1, respectively.

Proline concentration was measured by method of Bates and et al. [16].Using the standard solution and the regression equation between concentration and absorption at 520 nm, proline concentration of each sample was calculated as proline per fresh weight of leaf (µg/g). Chlorophyll content was measured according to Arnon [17] method. Fresh young leaves (0.1 g) were selected from plants, washed with deionized water and grind by acetone 80% in a porcelain mortar. Remained leftovers in the mortar were completely washed and final volume was reached to 25 ml by acetone 80%. After centrifugation at 400 rpm for 10 min, absorption of samples was read at wavelengths of 663 and 645 nm. The device was first calibrated by control sample of acetone 80%. The following formulas were used for estimation of total chlorophyll, chlorophyll a and chlorophyll b contents:

(1) Chl a (mgg-1 FW) = [12.7 (A 663) – 2.69 (A 645)] × V/W

(2) Chl b (mgg-1 FW) = [22.9 (A 645) – 4.68 (A 663)] × V/W

(3) Total Chl (mgg-1 FW) = [20.2 (A 645) + 8.02(A 663)] × V/W

Whereas, V: Volume of extract (ml), W: Fresh weight of the sample (g), and A: Absorbance at specific wave length.

Chlorophyll fluorescence values were obtained by placing leaves in darkness for 30 min by attaching light-exclusion clips to the leaf surface; chlorophyll fluorescence was measured using a osI30 portable device (ADCBioscietificCompany). Fluorescence parameters recorded were as follows:

1. Minimal fluorescence, or F0, as a measure of the stability of the light-harvesting complex

2. The ratio of variable (Fv = Fm – F0) to maximal (Fm) fluorescence (Fv/Fm, which represents the maximum quantum yield of photosystem II), which in turn ishighly correlated with the quantum yield of net photosynthesis.

Table 1. Name and pedigree of barley lines used in this research

Lines No	Lines name	Lines pedigree
1	20 line	F-A1-1
2	21 line	F-A1-2
3	22 line	F-A2-11
4	27 line	F-GRB-84-11
5	30 line	F-GRB-85-5
6	3 hulless	PETUNIA1/CHINIA(a)
7	7 hulless	CONGONA/BORR
8	8 hulless	STIPA/PETUNIA1//KOLLA/BBSC(b)
9	9 hulless	PETUNIA1/CHINA (c)
10	10 hulless	STIPA/PETUNIA1//KOLLA/BBSC (c)
11	11 hulless	GLORIA-BAR/COPAL//BEN.4D/3/S.P-B/4/DC-B/SEN/5/CONGONA
12	4 hulless	STIPA/PETUNIA1//KOLLA/BBSC (a)
13	14 hulless	CHAMICO/TOCTE//CONGONA (a)
14	15 hulless	PALLIDUM48//NORDIC/563.6.5/3/CEL-B…2/MZQ//CEL-B/5/LINO/6/CONGONA
15	19 hulless	CHAMICO/TOCTE//CONGONA (d)
16	Licivi	Licivi
17	Sahra	Sahra

Soluble sugars were measured as described by Irigoyen et al. [18]. Then the sample absorbance at the wavelength of 625 nm was measured using a spectrophotometer. Calculation of the total soluble sugar content was done by creating a standard curve using a standard glucose and was expressed in µg/g fresh weight (µg/g FW) [19].

Statistical software

Analysis of variance and mean comparison of physiological traits were done by SPSS ver. 16 software. Analysis of electerophoretic data (0 and 1) obtained from SDS-PAGE were performed using the NTSYS-pc2 software.

RESULT AND DISCUSSION

Based on ANOVA results (Table 2) there was significant difference among lines in all of measured physiological traits at 1%. The block effect was significant for all of traits except for sugar solution content. Signification of block effect on majority of agronomical traits indicated farm blocking was done in the proper direction. It seems signification of block effect on physiological traits is because of measuring and sampling them in different period of times. Coefficient of variation (CV) varied from 0.78% for chlorophyll fluorescence to 19.18% for soluble sugar content. Pectu and Terbea [20] reported that free proline content in plants maybe depend on their phonological condition and growth habit. Mateos and Hayes [21] found that the effect of salinity and drought stress lead to increase content of amino acids and the increase of proline content was more than others. However increasing of soluble carbohydrates concentration in plants lead to keep osmotic equilibrium in environmental stresses [22]; On the other hand, accumulation of sugars in the shoots causes impairment in photosynthesis and reduction in yield.

Prado et al. [23] suggested increasing the amount of carbohydrates is a strategy to reduce the effects of osmotic and ionic stress and the adaptation of plants with this condition. Comparison of the mean of Fv/Fm and chlorophyll content traits showed lines 1, 14 and 17; and lines 8, 10 and 16 had largest and lowest chlorophyll fluorescence respectively. The content of chlorophyll in lines 6 and 7 and cultivar 11 was largest and lowest respectively. Yong et al. [24] stated that the reduction in leaf chlorophyll content and quantum yield of photosystem II is the result of damage to the chloroplasts membrane during cold stress.Measurement of chlorophyll fluorescence, measures the efficiency of absorption of light related to leaves photosystem II complex. Chlorophyll fluorescence is a reliable and nondestructive strategy for monitoring photosynthetic event and judgment about physiological status of plant [25].

Rizza et al., [25] investigated the effect of duration of cold acclimation on the efficiency of the quantum of photosystem II in the spring and winter oat species. They found that placing the plants in cold hardening condition, decreased the efficiency of quantum photosystem II at first, but then increased and return to its early level. Ratio of Fv/Fm showed maximum performance of quantum photochemical reaction II and decrease of efficiency of quantum photosystem II due to low temperature effect, that display low temperature has negative effect on photosystem II and electron transport in photosynthesis. Therefore measuring of Fv/Fm may be a successful strategy in determining of cold tolerant varieties [26].

Table 2. Analysis of variance for physiological traits in studied barley lines

Sources of variance	df	Means square			
		Proline	Fv/Fm	Soluble sugar content	Chlorophyll content
Replication	2	0.005*	0.014*	0.11^{ns}	156.99**
Genotype	19	0.297*	0.011**	0.42**	25.72**
Error	38	0.001	0.004	0.05	3.45
CV	-	4.97	0.78	19.18	4.69

$^{ns, *, **}$ Non significant and significant, at P ≤ 0.05, 0.01 respectively

For reaching to maximum heterosis, researchers choose cultivars or genotypes that have more genetic distance from each other. This purpose can achieve by investigating genetic distance between genotypes based on morphological traits using cluster analysis method. Therefore cultivars or genotypes those are in far groups from each other selects as parents in crosses to create more genetic variation. In this study cluster analysis carried out based on physiological traits using Ward's method and divided lines into three groups (Fig. 1).

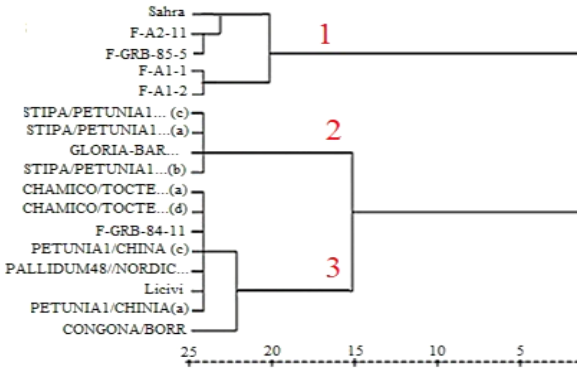

Figure 1. Cluster analysis of studied lines base on physiological traits

In the first group were 17,3,1,2 and 5 lines. This group had a Fv/Fm in high value. The second group consists of 10, 11, 12 and 8 lines that had low chlorophyll content. Finally third group consists of 13, 15, 4, 9, 14, 6, 16, and 7 lines. According to Fig. 1, it is observed that the genetic distance between groups is high. Thus, crosses between genotypes that are more distant groups can produce offspring with great variety and maximum heterosis. The most distant was between 1 and 2 cluster and cross between these two groups may obtain the maximum variety. Within clusters must be chosen lines that have more value in morphological or physiological traits. According to the information obtained from protein bands score, 11 protein bands observed for 17 lines that the 17 and 2 lines had maximum bands (10 bands) and 16 and 6 lines had minimum (1 band) band. Lines had common and specific bands. Protein band showed variation in the location of the protein bands on gels, molecular weight, density and intensity. Sofalian and Valizadeh [27] and Asghari et al. [28] used DNA and protein markers to assess the phylogeny of some species of wild wheat relatives and vetch (Vicia spp) respectively and found in the phylogenic

tree based on protein markers. Cluster analysis based on RAPD marker data showed these samples were placed in one group and other species were in its sub cluster. Drawing phylogenic tree using combined data's provided better result. In that study the clustering of samples using principal components analysis was partly compatible with the results of phylogenic analysis in each case. Similarity matrix calculated based on Jaccard's similarity coefficient. According to this matrix there were most similarity (minimal difference) between 8 and 10(1), 2 and 17 (1), 11 and 17 (0.9), 11 and 2 (0.9) lines and least similarity (maximum difference) between 1 and 3 (0), 3 and 6 (0), 3 and 8 (0), 3 and 10 (0), 3 and 16 (0), 4 and 6 (0), 6 and 8 (0), 6 and 10 (0), 6 and 16 (0) lines. By examining the tree diagram at a distance0.3, four groups obtained (Fig.2). The first group consists of four lines (1, 8, 10 and 16), the second group consisted of 11 lines (2, 17, 11, 12, 13, 14, 15, 4, 5, 7 and 9), and the third and fourth groups consist of three and six lines respectively. According to the Fig. 2, 14 and 15 lines and 12 and 13 lines had 78% and 87% similarity respectively.

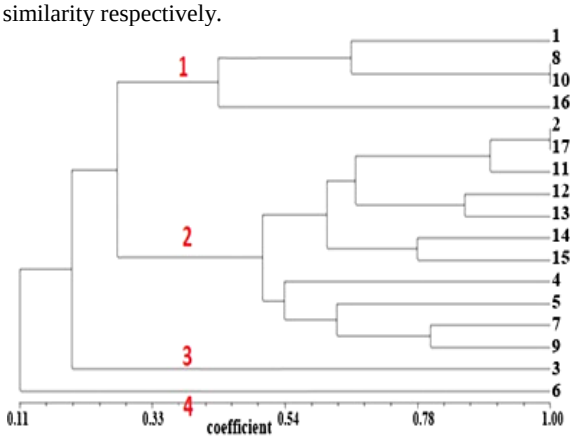

Figure 2. Cluster analysis based on storage protein electrophoresis in studied barley genotypes

Similarity between the 8 and 10 lines, 2 and 17 lines were 100% that could indicate the fact that there is no variation among them as studied proteins. Therefore this result may be because of the low number of protein markers studied here and their weaknesses in distinguishing lines. There is great interest to the content of genetic diversity in germplasm research today and relationship between genotypes is important for effective maintenance of germplasm resources [29; 30, 31].In plant breeding this information is valuable in the selection of crosses between parents and leads to maximizing the efficiency of the selection and maintenance of genetic diversity.

Mantel test carried out between coefficient and similarity matrices in order to ensure the appropriateness of the methods used in the cluster analysis and correlation equal to 89.85% obtained that indicated the method is suitable for determining the distance matrix. The principle coordinate analysis provides useful information about the distinction groups. Based on this analysis, the first three components explained 82% of total variation. The first, second and third component explained 63.18%, 22.12% and 6.6% of total variances respectively. PCoA 2-D graph drown for classification and study of the relationship between samples and were classified genotypes into four main groups (Fig. 3).This grouping was very similar to the results of the cluster analysis.

In the stepwise regression analysis protein markers entered the model as a dependent variable and molecular characteristics as fixed variables (Table 5). According to regression analysis, for all studied traits there was significant relation with some of the studied protein markers. Soluble sugar content had a most association (P1, P9 and P10) among other traits with protein markers. Also, this trait had the most coefficient of determination (R2) between measured traits. Metakovsky and branlard [32] reported that in the French wheat varieties, some of gliadin alleles were associated with resistance to cold. In the cold tolerant varieties compared with cold sensitive varieties, frequency of Gli-A2r, Gli-B1l, Gli-D2g alleles were significantly higher and Gli-D2g, Gli-A1a, Gli-B2c alleles were lower. In the study of Witkowski et al. [33], SDS-sedimentation value, resistance to leaf blotch and frost tolerance showed statistically significant associations with the status of the Glu-A1 locus. It appears that chromosome 1A with the null allele at Glu-A1 carries a closely linked locus responsible for frost tolerance.

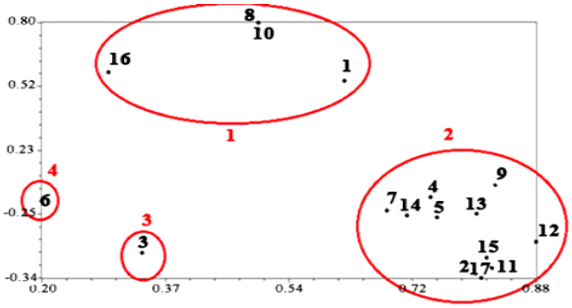

Figure 3. 2D plot of principle coordinate analysis

Table 3. Regression analysis for seed storage proteins and some physiological traits

Marker	Proline	Fv/Fm	Soluble sugar content	Chlorophyll content
Intercept	1.05	0.82	1.45	0.96
P1			0.42	
P2				
P3		- 0.49		
P4	- 0.48			- 0.48
P5				
P6				
P7				
P8				
P9			0.34	
P10			- 0.6	
R^2	0.23	0.24	0.69	0.23

CONCLUSIONS

The seed storage protein pattern is considered as the genotypic fingerprint. It is, therefore, used for several purposes such as plant variety protection, registration, certification, patents and as a breeding tool especially in flour quality breeding programs. At present study, lines to be used grouped based on seed storage proteins and physiological traits. In addition to these markers, DNA-based markers could be used in order to discovery of genetic diversity at high level. It seems that since each of these markers demonstrates different aspects of diversity in different populations, simultaneous application of those can present researchers a brighter view of diversity. But the assessment of genetic variation in large samples of plant genetic resources requires a high costing time and amount of consumables. Thus, SDS-page methods are reliable and economical techniques could be preferred, at least in the first screening of genetic variability.

REFERENCES

[1] Ghaderi, M. G., P.Vojdani, A.Gerami, 2003. Genetic diversity in the Iranian landraces of barley and its relation to geographical and climatic variation. Journal of Agricultural Science and Technology, 9(4):55-70.

[2] Zong-Yun, F., L. Xian-Jun, Z. Yi-Zhang, L. Hong-Qing, 2006b. Genetic diversity analysis of Tibetan wild barley using SSR marker. ActaGeneticaSinica, 33:917-923.

[3] Mer, R. K., D.H.Pandye, A. N.Pandye, 2000. Effect of salt on germination of seeds and growth of young plants of *Hordeumvulgar* L., *Triticumaestivum* L., *Cicerarietinum* L., and *Brassica juncea* L. Journal of Agronomy and Crop Science, 185:209-217.

[4] Masoudi, B., M. R.Bihamta, H.R.Babayi,S.A.Peighambari, 2009. Evaluation of Genetic Diversity for Agronomic, Morphological and Phenological Traits in Soybean. Journal of Plant and Seed, 24:413-427.

[5] Fao. 2006. http://faostat.fao.org.

[6] Abdmishani, S.,A.Booshehri, 1998.Avdvanced Plant Breeding, Vol I. Publication of Tehran University, 322 pp.

[7] Gepts, P.1990. Genetic diversity of seed storage proteins in plants. In: Brown AHD, Clegg MT, Kahler AL and Weir BS (eds.), Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, Massachusetts, 64-82.

[8] Shewry, P. R. 1995. Plant storage proteins. Biology Reviews 70:375-426.

[9] Nevo, E., D.Zohary, A.Beiles, D.Kaplan,N.Storch, 1986. Genetic diversity and environmental associations of wild barley (*Hordeumspontaneum*) in Turkey. Genetics, 68:203-213.

[10] Manifesto, M. M., A. R.Schlatter, H. E.Hopp, E. Y.Suarez, J.Dubcivsky, 2001. Quantitative evaluation of diversity in wheat germplasm using molecular markers. Crop Science, 41:682-690.

[11] McCausland, J., C. W.Wrigley, 1977. Identification of Australian barley cultivars by laboratory methods: gel electrophoresis and gel isoelectric focusing of the endosperm proteins. Australian Journal of Experimental Agriculture, 17:1020-1027.

[12] Shewry, P. R., J. M.Hill, H. M.Pratt, M. M.Leggatt,B. J.Miflin, 1987. An Evaluation of Techniques for the Extraction of Hordein and Glutelin from Barley Seed and a Comparison of the Protein Composition of. Journal of Experimental Botany, 29(3):677-692.

[13] Marchylo, B. A., D. E.LaBerge, 1981. Barley cultivar identification by electrophoretic analysis of hordein proteins Catalogue of electrophoregram for mulae for Canadian-grown barley cultivars. Canadian Journal of Plant Science, 61:859-870.

[14] Hamza, S., W. B.Hamida, A.Rebai, M.Harrabi, 2004. SSR-Based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica, 135:107-118.

[15] Laemmli, U. K. 1970. Cleavage of structural protein during assembly of the head of bacteriophage T4. Nature, 22: 680-685.

[16] Bates, L. S., R. P.Woldern, I.Dteave, 1973. Rapid determination of free Proline for water stress studies. Plant and Soil, 39:205-207.

[17] Arnon, A. N. 1967. Method of extraction of chlorophyll in the plants. Agronomy Journal, 23:112-121.

[18] Irigoyen, J. J., D. W.Emerich, M.Sanchez-Diaz, 1992. Water stress induced changes in concentration of praline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiologia Plantarum, 84:55-60.

[19] MovahhedyDehnavy, M., S. A. M.ModarresSanavy, A. Soroushzadeh, A. Jalai, 2004. Changes in proline, total soluble sugars, and chlorophyll fluorescence in winter safflower cultivars under drought stress and foliar application of zinc and manganese. Desert, 9(1):93-109.

[20] Petcu, E., M. Terbea, 1995. Proline content and the conductivity test as screening methods for frost tolerance of winter wheat. Bulgarian Journal of Plant Physiology, 21:3-11.

[21] Matus, I., P. M.Hayes, 2002. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome. 45:1095- 1106.

[22] Hanson, A. D. 2002. Drought and salt tolerance: Towardunderstanding and application. Trends in Biotechnology, 10:358-362.

[23] Prado, F. E., C.Boero, M.Gallardo, J. A.Gonzalez, 2000. Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* (Wild.) seeds. Botanical Bulletin of Academia Sinica, 41:27–34.

[24] Yong, I. J. S., R. Nilda, E.Tay, H.Oksoo, S.Baik,G.Ock, 2003. Antioxidative enzyme offer protection from chilling damage in rice plants. Crop Science, 43:2109-2117.

[25] Rizza, F., D.Pagani, A. M.Stance,L.Cattivelli, 2001. Use of chlorophyll fluorescence to evaluatethe cold acclimation and freezing tolerance of winter and spring oats. Plant Breeding, 120:389 -396.

[26] Zobayed, S., F.Afreen, T.Kozai, 2005. Temperature stress canalter the photosynthetic efficiencyand secondary metabolite concentrations. Plant Physiology and Biochemistry, 43:977- 984.

[27] Sofalian, O., M.Valizadeh, 2009. Investigation of seed storage proteins in some wild wheat progenitors using SDS-PAGE and Acid- PAGE. Not. Bot. Hort., 37: 179- 182.

[28] Asghari, A., Z.Bozorghi, M.Shokrpour, A. A.Jafari, 2012. Study of phylogenetic relationships of some *Vicia* species using storage proteins and RAPD markers. Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 20(1):39-54.

[29] Davila, J. A., M. P. S.Hoz, Y.Loarce, E.Ferrer, 1998. The use of random amplified microsatellite polymorphic DNA and coefficients of parentage to determine genetic relationships in barley. Genome, 41:477-486.

[30] Manjunatha, T., I. S.Bisht, K. V.Bhat, B. P.Singh, 2006. Genetin diversity in barley (*Hordeum vulgare* L.) landraces from Uttaranchal. Genetic Resources and Crop Evolution, 54:55-65.

[31] Russel, J. R., J. D.Fuller, M.Macaulay, B. G.Hatz, A.Jahoor, W.Powell, R.Waugh, 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLP, AFLPs, SSRs and RAPDs. Theoretical and Applied Genetics, 95:714-722.

[32] Metakovsky, E. V.,G.branlard, 1998. genetic diversity of French common wheat germplasm based on gliadin alleles. Theoretical and Applied Genetics, 96:209-218.

[33] Witkowski, E., J.Waga, K.Witkowska, M.Rapacz, M.Gut, A.Bielawska, H.Luber,A. J.Lukaszewski, 2008. Association between frost tolerance and the alleles of high molecular weight glutenin subunits present in Polish winter wheats. Euphytica, 159:377–384.