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Original Contribution

ASSESMNT OF GENETIC DIVERSITY IN HIGH-MOLECULAR-WEIGHT GLUTENIN SUBUNITS AND RELATIONSHIP TO BREED-MAKING QUALITY IN COMMON WHEAT

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ABSTRACT

Quantitatively, HMW-Gs are minor components of the wheat storage proteins but they are key factors in the process of bread-making. In this research SDS-PAGE electrophoresis was used to study of genetic variation and protein banding patterns in twelve common wheat varieties. Laemmli protocol (1970) was used for protein extraction, with some modification. The average of similarity coefficient was 0.525 and this indicated high variation between varieties. Cophenetics coefficients indicated that Jaccard coefficient and UPGMA algorithm (r_{coph} = 0.94178) were appropriate methods. Derndrogram was clustered 12 varieties to 4 groups. Also four discriminant function were calculated to classify cultivars and this method confirmed the cluster analysis. Each variety contained a range of two to five subunits and 14 different glutenin subunit patterns were observed in hexaploid wheats. A high frequency of the null allele at the *Glu-A1* locus and a low frequency of subunits 5+10 at the *Glu-D1* locus were observed. According to Payne scoring system, Kav and Bol 1, as a good quality cultivars holding alleles encoding subunit 5+10 and no subunits 2+12, 3+12 and 4+12 and other cultivars were recognized as poor cultivars in baking and dough quality. We suggested hybridization between Bol and Kal or Str and Kal . This hybridization will produce the highest F_2 segregation from breed-making quality point of view.

Key words: Wheat (*Triticum aestivum* L.), genetic diversity, HMW and LMW subunits, Payne scoring, cluster and discriminant analysis.

INTRODUCTION

Common wheat (2n=6x=42; AABBDD; *Triticum aestivum* L.) is an allohexapolid derived from hybridization between a domesticated form of tetraploid, wild emmer, *Triticum turgidum* spp. *dicocoides* (genomic constitution; AABB), and the diploid *Aegilops tauschii* (genomic constitution; DD), (1-4). Of all the cereal grains, wheat is unique, because it is the major and most important agricultural crop (5), and was widely cultivated by human in the past (6), as it was a key factor enabling the emergence of city-based societies at the start of civilization. The various forms of wheat represent almost 30% of the world´s grain production, and it is estimated that by 2020, the global wheat requirement will double the current production level (7).

The last decade has witnessed the emergence of wide spread concern to solve the major problem of the erosion of genetic resources caused by the global extension of modern crop plants (8). Moreover, Genetic characterization and evaluation of crop populations and cultivars diversity are very important step in plant improvement programs (9). For this reason it is necessary to broaden the genetic base of wheat, and its germplasm accessions most distinct from modern cultivars are predicted to contain the highest number of unexploited potentially useful alleles (7). The study of genetic diversity is the process by which, variation among individuals or groups of individuals or population is analyzed by specific method or a combination of methods (9).

Wheat endosperm proteins were the first proteins to be were studied when Beccari in 1745 reported the isolation of gluten (5). Wheat seed storage proteins have been studied extensively for their pivotal role in determining nutritional and bread-making quality of flour (10, 11). Some researchers have focused on wheat protein components with respect their structure and linkage map (12, 13), content, variation, and role

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of them on bread-making quality (6, 14). These proteins (Glutens) are composed of two major components: gliadins and glutenins (15). Gliadins are generally considered to contribute to the viscosity and extensibility of gluten. Although some authors have associated specific gliadin alleles with bread-making quality, it is now accepted that these proteins may not have a direct effect on wheat (5). These molecules are monomeric prolamins, controlled by the *Gli-1* and *Gli-2* loci, located on the short arm of chromosome of the homoeologous group 1 and 6, respectively (16, 17). Instead, glutenins are devided in two groups, high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (5, 15). The HMW and LMW glutenin subunits are encoded by the *Glu-1* loci situated on the long arm of group 1 homoeologous chromosomes and *Glu-3* that located on the short arm of these same chromosomes, respectively (12). Also, these subunits are minor components quantitatively, but they are key factors in the processes of bread-making because they are major determination of gluten elasticity (18). Osborn classified cereal-seed proteins based on their sequential extraction and differential solubility into four different groups, albumins (soluble in water and dilute buffers), globulins (soluble in saline solutions), prolamins (soluble in 70-90% ethanol), and glutenins (soluble in dilute acid or alkali) (5).

The objective of this study is to estimate the genetic diversity of 12 hexaploid common wheat varieties and fingerprinting them by SDS-PAGE protein banding pattern and the relative quality of HMW subunits in relation to wheat flour quality as well.

MATERIAL AND METHODS

Twelve common wheat cultivars were used in this research **(Table 1)**. These materials were obtained from Sistan Agricultural Research Centre.

Storage proteins were extracted from wholemeal flour, using Laemmli protocol with some modification. Electrophoresis of storage proteins was accomplished using one dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis method. Extraction buffer was consisted of 1.5 M Tris-HCl (pH=6.8), 12% (w/v) SDS, 60% (v/v) glycerol, 0.05% (w/v) commasie blue R-250, and 23.5% (v/v) mercaptoethanol (should be added fresh). After vortex at 6 times in 850 rpm, the extracts were heated at 94°C for 5 min and centrifuge for 5 min at 7000 rpm. The supernatant was transferred to new tubes for loading in gel. Acrylamide stock solution (30%) was prepared in 29% (w/v) acrylamide and 1% (w/v) N,N´-methylenbisacrylamide ratio. The stacking and resolving gels were provided 4% (pH=8.8) and 10% (pH=6.8), respectively. We loaded 10 µl protein´s sample in each well and also 30 µl unstained protein molecular weight marker (Fermentase #SM0431) in first well. Electrophoresis buffer consisted of 25 mM (w/v) Tris, 25 mM (w/v) glycine, and 0.1% SDS. Electrophoresis was performed at a constant current of 18 mA at room temperature for the time required for the tracking marker dye in extraction buffer to migrate off the gel. Gels were stained overnight with 6% (w/v) TCA (trichloroacetic acid) solution containing 5% (v/v) ethanol and 0.038% (w/v) commasie blue R-250. De-staining only was carried out with 10% (w/v) TCA.

The bands of HMW-GSs on SDS-PAGE gels were read using HMW-GS methodology and the nomenclature described by Payne and Lawrence, 1983. Evaluation of variation in the endosperm proteins was performed by the calculation of the individual band frequency for each cultivar. Polymorphism was scored for presence (1) or absence (0) of bands. The clustering of cultivars was based on Jacquard´s coefficient by the Un-weighted Pair Group Method with Arithmetic Average (UPGMA) using the software package program NTSYS-PC, ver 2.0 (19), in combination with Microsoft Excel 2007. Molecular weights of bands were calculated using semi-logarithmic regression model. Discriminant function analysis was performed by SPSS statistical software (20).

RESULTS AND DISSCUTION

One sample of SDS-PAGE profile of different cultivars is presented in **Fig.1.** Average bands weight was 69.56 KD (range from 44.8 KD to 129 KD) and the number of bands decrease as the size and weight increased. The highest banding number related to Kal and the lowest number related to Bol 1, Mah, and Kav. The average of similarity coefficient was 0.525 and this indicated normal distribution of diversity between varieties. This parameter ranged from 1 for closely related cultivars (for example Bol 3 and Alv) to zero for Mah and Ham pair of cultivars. In the present study only fourteen alleles at the *Glu-1* loci were detected, reflecting a suitable genetic base for this material. Caballero et al., (6) reported that genetic diversity estimates based on Low-Molecular-Weight glutenin subunits in spelt wheat were extensive. So we suggested hybridization between Bol and Kal or Str and Kal. These hybridization will produce the highest F_2 segregation from breed-making

quality Point of view.

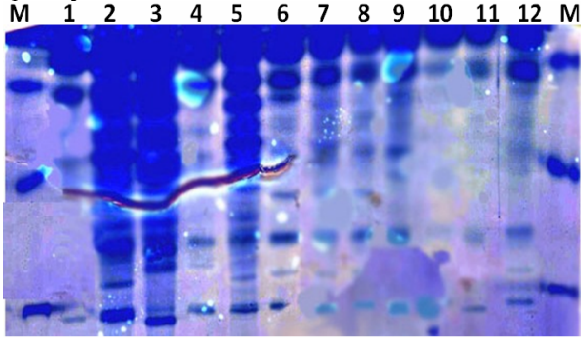

Fig 1. A sample of electrophoresis separation profile of cultivars. Numbers upside of the stained gel (M=Molecular Weight Marker) indicate the cultivars so recommended in **table 1**.

Genetic diversity differentiated primarily by ecological factors such as soil mineral content, moisture stress, and microclimatic condition (21). Genetic variation observed between cultivars in our study could also may effect and be the result of different factors such as soil texture, heat stress, and/or salinity stress (genotype environment interaction).

The HMW-GS compositions of cultivars are shown in Table 1. As mentioned above, fourteen alleles were found for HMW-GS; two, five, and seven alleles in *Glu-A1*, *Glu-B1*, and *Glu-D1* respectively. Payne and Lawrence, (22) could determine 21 different subunits in his studies on wheat cultivars. From three alleles should be found at *Glu-A1* locus, the subunit 1 wasn´t found in any cultivar. In this locus, the most frequent allele was the Null (83.33%), which generally ranks poorly for wheat quality parameters. These results are quite different to those observed in Argentinean cultivars, where allele Null is present in 1.1% of the cultivars (23). At *Glu-B1*, except 7 subunit, 91.7% were accounted for by 6+8, 6, 7+8, and 13+16. Larger allelic variation was observed at the *Glu-B1* locus, although this variability was lower than that previously reported by Moragues et al., (24), Lerner et al., (25). In this locus, both Bol 1 and Kal dont show any subunit. At *Glu-D1* maximum frequency corresponded to 2+12 subunit (Table 2). Also these cultivars have almost low frequency of subunites 2* (related with extensibility) and 5+10 (associated with good quality). Nakamura, (26) reported that frequency of 5+10 subunit in Chinese wheat varieties is relatively low. Regarding to HMW-GS, analyze of these alleles show that two cultivar (Bol 1 and Kav) were corresponded to good bread-making quality and other cultivars were recognized as poor cultivars in baking and dough quality. Three possible factors could affect the distribution of alleles at each *Glu-1* locus: (a) linkage to genes of adaptive value, resulting in the selection of particular alleles for specific areas; (b) the use of a particular parental gene pool; (c) *Glu-1* alleles might be influenced by selection pressure towards specific quality properties such as bread-making quality (23). It appears that the third option is likely to be the cause of the distribution of the alleles at the *Glu-1* loci in these cultivars.

Table 1. *Cultivars of common wheat used in the study and HMW-Glutenin subunits composition*

No	Cultivar Name	Code	HMW- Glutenin subunits		
			A1	B1	C1
1	vees/3/bows/vees/keF/h/Bolani	Bol 1	Null	---	2+10,5+10
2	Kalakafghan	Kal	Null	---	2+10
3	Mahdavi	Mah	Null	7	2+10,3
4	Bolani	Bol 2	Null	6	2+10,3
5	Pethncer-2123/Bolani	Bol 3	Null	6+8	2+12,3+12
6	Alvand	Alv	Null	6+8	2+12,4+12
7	Hamoon	Ham	Null	6+8	2+12,3+12
8	Star	Str	Null	6	2+12,5
9	Hirmand	Hir 1	2*	13+16;7+8	2+12,5
10	Kavir	Kav	2*	13+16;7+8	2+10,5+10
11	V.8187.Arvand-1	Arv	Null	6	2+12,5
12	pethncer-2123/Hirmand	Hir 2	Null	13+16;7	2+12,3+12

Comparing our results with those obtained in Iranian wheat cultivars by Najafian et al., (27) notable similarities were observed; for example, both Kav and Hir1 cultivars show 2* and 2+10 subunits, however their subunits in *Glu-B1* locus wasn't similar. To improve the bread-making quality of Iranian wheat, Masoudinejad et al., (28) suggested that, substitution allele 5+10 instead of 2+12 through back cross or single seed descent procedure. Solouki and Emamjomeh., (29) were studied storage protein of 29 wheat

substitution lines and reported that only Kapla 3A was known as strong cultivar in bread-making quality and other substitution lines were recognized as poor cultivars in bread-making quality except Shayen 7A and Shayen 4B.

Table 2. *Allelic frequency observed in common wheat cultivars for the three glutenin loci under study*

Locus	Allele	Number of cultivars	Frequency (%)
Glu-A1	Null	10	83.33
	2	2	16.66
Glu-B1	6+8	3	25.00
	6	3	25.00
	7+8	2	16.60
	7	1	8.33
	13+16	3	25.00
Glu-D1	2+10	5	41.66
	5+10	2	16.00
	3	2	16.00
	2+12	7	58.33
	3+12	2	25.00
	4+12	1	8.33
	5	4	33.33

Cophenetic´s correlation coefficient (which measures the correspondence between pair of methods) were calculated (r_{coph}=0.94178) for various methods and algorithms, then we selected Jaccards coefficient and algorithm of UPGMA. This method has been used very widely for the analysis of data on the base of biochemical items (30, 31, 25). The dendrogram on the basis of SDS-PSGE profile endosperm proteins revealed the clustering of the cultivars into four groups (Fig. 2). Cluster 1 included Bol 1, Bol 2, Bol 3, Alv, Ham and Str. As shown in Table 1, cluster 1 include cultivars with HMW-GS compositions mainly including Null, 6+8 and 2+12 alleles in *Glu-A1*, *Glu-B1*, and *Glu-D1* respectively. Mostly these alleles are associated with poor quality bread-making parameters. Within this cluster two different subgroups were noticed. These results are in agreement with those of obtained previously by Masoudinejad et al., (28). In this cluster Bol 3, Alv and Ham showed 100% identity in endosperm protein profile. Cluster 2 and cluster 4 contain only one cultivar, Mah and Kal, respectively. Cluster 3 contains the rest of varieties. Both Hir 1 and Kav, containing 2* subunit in *Glu A1* locus, situated in cluster 3. Also Hir 1 and Hir 2 were grouped together with similarity value 65%. It seems that this cluster is more variable from other clusters. Rout and Chrungoo., (31) clustered 23 different Himalayan buckwheats using different marker approaches such as morphological traits, seed storage proteins and RAPD data marker. Cluster analysis of the endosperm protein profiles of the selected accessions revealed three broad clusters. Genetic diversity of 270 European spelt, 15 Iranian spelt and 25 bread wheat cultivars was evaluated by constructing the dendrogram for HMW and LMW glutenin subunit bands (14). Based on cluster analyze, they suggested that spelt and common wheat form distinct groups.

Also discriminant function analysis was calculated to classified cultivars. This method is concerned with the problem of how well it is possible to separate two or more groups of individuals on the basis of the available measurements (32). Four discriminant functions were calculated using data of these cultivars (data not shown). As it was expected and mentioned above, this analysis was correspondence with cluster analysis and confirmed it. Hailu et al., (33) evaluated variability of 121 tetraploid wheat accessions using multivariate methods such as cluster, discriminant function and principal component analysis. Cluster analysis grouped the accessions into 15 clusters and discriminant analysis showed that 83% of the accessions were correctly placed in their respective altitudinal class. Discriminant analysis in Italian emmer wheat accessions showed that two functions were sufficient to explain all the variability, the first function accounting for 91% of eigenvalues (34).

Frequency of Null and 2+12 alleles is high in this cultivar collection, and so we know, they are associated with poor characters of bread-making quality. Nonetheless, this effect can be partly ameliorated by combining its use with certain HMW-GS and LMW-GS alleles, which implies that further information is required about the effects on quality and end-use of the LMW-GS alleles (25).

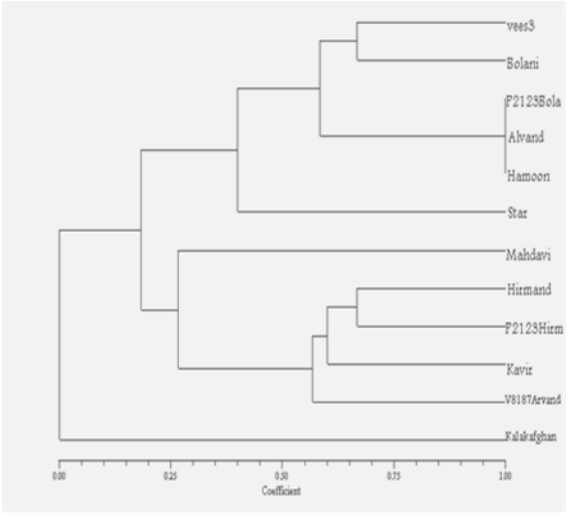

Fig. 2 Dendrogram of all cultivars obtained by UPGMA algorithm using Jaccard's similarity coefficient

Although other methods such as PCR based markers provide interesting tools for the type of study reported here, the used of 1D SDS-PAGE continues to be a valuable, efficient, and economic method (15, 25), if high definition gels can be obtained. Prediction of wheat quality by electrophoresis studies is currently useful in directing breeding strategies (10). It is therefore concluded that seed storage protein profiles could be useful markers in cultivar identification and registration of new varieties (35), and in the study of genetic diversity and classification of crop and cultivar evolution (26, 36).

It is also be interesting to studying of other components of proteins effective on bread-making quality (such as *Glu-A3*, *Glu-B3*, and *Glu-D3*), SDS-PAGE in combination with densitometry, RP-HPLC (Reverse Phase-High Performance Liquid Chromatography), molecular markers and/or gene sequencing. Through these studies we can better understand biochemistry and genetics base of common wheat, our most important food crop.

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REFERENCES

1. Dudnikov, A. JU., Allozyme variation in transcaucasian population of *Aegilops squarrosa*. *Heredity*, 80: 248-258, 1998.
2. Moghaddam, M., Ehdaie, B., and Waines, J. G., Genetic diversity in populations of wild diploid wheat *Triticum urartu* Tum. Ex. Gandil. revealed by isozyme markers. *Genetic Resources and Crop Evolution*, 47: 323–334, 2000.
3. Peterson, G., Seberg, O., Yde, M., and Berthelsen, K., Phylogenetic relation of *Triticum* and *Aegilops* evidence for the origin of the A, B and D genomes of common wheat (*Triticum aestivum*). *Molecular Phylogenetic and Evolution*, 39: 70-82, 2006.
4. Schneider, A., Molnar, I., and Molnar-Lang, M., Utilization of Aegilops (goatgrass) species to widen the genetic diversity of cultivated wheat. *Euphytica*, 163: 1–19, 2008.
5. Gianibelli, M. C., Larroque, O. R., Macritchie, F., and Wrigley, C. W., Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. *Cereal Chem*, 78: 635–646, 2001.
6. Caballero, L., Martin, L. M., and Alvarez, J. B., Genetic variability of the low-molecular-weight glutenin subunits in spelt wheat (*Triticum aestivum* ssp. spelta L. em Thell.). *Theor Appl Genet*, 108: 914–919, 2004.
7. Hegde, S. G., Valkon, J., and Waines, J.G., Genetic diversity in wild and weedy *Aegilops*, *Amblyopyrum*, and *Secale* species - A Preliminary survey. *Crop Sci*., 42: 608-614, 2002.
8. Brown, A. H. D., Isozymes, Plant Population Genetic Structure and Genetic Conservation. *Theor Appl Genet*, 52: 145-157, 1978.
9. Mohammadi, S. A., and Prasanna, B. M., Analysis of Genetic Diversity in Crop Plants-Salient Statistical Tools and Considerations. *Crop Sci*., 43: 1235–1248, 2003.
10. Bietz, J. A., Genetic and biochemical studies of nonenzymatic endosperm proteins. In: Heyne, E.G. (Ed.), Wheat and Wheat Improvement, pp. 215-241. American Society of Agronomy, Madison, WI, USA, 1987.
11. Ranhorta, G. S., Gelroth, J. A., Glaser, B. K., and Lorenz, K. J., Nutrient composition of spelt wheat. *J. Food Comp. Anal.* 9: 81-84, 1996.
12. Singh, N. K., and Shepherd, K. W., Linkage mapping of genes controlling endosperm proteins in wheat. 1. Genes on the short arms of group-1 chromosomes. *Theor Appl Genet*, 75:628–641, 1988.
13. Nelson, J. C., Andreescu, C., Breseghello, F., Finney, P. L., Gualberto, D,. G., Bergman, C. J., Pena, R. J., Perretant, M. R., Leroy, P., Qualset, C. O., and Sorrells, M. E., Quantitative trait locus analysis of wheat

quality traits. *Euphytica*, 149: 145–159, 2006.
14. Xueli, A., Qiaoyun, L., Yueming, Y., Yinghua, X., Hsam, S. L. K., and Zeller, F. J., Genetic diversity of European spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.) Revealed by glutenin subunit variations at the *Glu-1* and *Glu-3* loci. *Euphytica*, 3: 193-201, 2005.
15. Bradova, J., and Matejova, E., Comparison of the results of SDS PAGE and chip electrophoresis of wheat storage proteins. *Chromatographia*, 67: 83-88, 2008.
16. Payne, P. I., Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annu Rev Plant Physiol*, 38:141–153, 1987.
17. Payne, P. I., Holt, L. M., Lawrence, G. J., and Law, C. N., The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. *Plant Foods Hum Nutr*, 31:229-241, 1982.
18. Tatham, A. S., Miflin, B. J., and Shewry, P. R., The beta-turn conformation in wheat gluten proteins: Relationship to gluten elasticity. *Cereal Chem*, 62: 405-442, 1985.
19. Rohlf, F. J., NTSYS-PC. Numerical taxonomy and multivariate analysis system, version 2.00. Exeter Software, Setauket, NY. 1998.
20. SPSS Inc. SPSS 11.0 for Windows, USA, 2001. (http://www.spss.com),
21. Bonfil, D., Czosnek, H., and Kafkafi, U., Changes in wheat seed storage protein fingerprint due to soil mineral content. *Euphytica*, 95: 209–219, 1997.
22. Payne, P. I., and Lawrence, G. J., Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, and *Glu-D1* which code for the highmolecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun*., 11:29-35, 1983.
23. Gianibelli, M. C., Echaide, M., Larroque, O. R., Carrillo, J. M., and Dubcovsky, J., Biochemical and molecular characterisation of *Glu-1* loci in Argentinea wheat cultivars. *Euphytica*, 128: 61–73, 2002.
24. Moragues, M., Zarco-Hernandez, J., Moralejo, M. A., and Royo, C., Genetic diversity of glutenin protein subunits composition in durum wheat landraces [*Triticum turgidum* ssp. turgidum convar. durum (Desf.) MacKey] from the Mediterranean basin. *Genetic Resources and Crop Evolution*, 53: 993–1002, 2006.
25. Lerner, S. E., Kolman, M. A., and Rogers, W. J., Quality and endosperm storage protein variation in Argentinean grown bread wheat. I. Allelic diversity and discrimination between cultivars. *Journal of Cereal Science*, 49: 337–345, 2009.
26. Nakamura, N., Genetic diversity of high-molecular-weight glutenin subunit compositions in landraces of hexaploid wheat from Japan. *Euphytica*, 120: 227–234, 2001.
27. Najafian, G., Bahraee, S., Baghaee, N., Morteza-gholi, M., and Babaee-Goli, E., Bread-making quality attributes of Iranian trade cultivars of wheat and their HMW glutenin subunits composition. 11th International Wheat Genetics Symposium. Sydney, Australia. 2008.
28. Masoudinejad, A., Yazdisamadi, B., Sarblouki, M. N., and Abdmishani, S., Genetic Classification of Iranian Wheats by Ectrophoresis and Cluster Analysis. *Journal of Agricultural Science*. 28: 97-107, 1997.
29. Solouki, M., and Emamjomeh, A., Determination of variations of protein banding patterns of high molecular weight-glutenin's subunits in wheat after chromosomal substitution. *International Journal of Biology and Biomedical Engineering*, 3:53-56, 2007.
30. Mirali, N., Elkhouri, S., and Rizq, F., Genetic diversity and relationships in some *Vicia* species as determined by SDS-PAGE of seed proteins. *Biologia Plantarum*, 4: 660-666, 2007.
31. Rout, A., and Chrungoo, N. K., Genetic variation and species relationships in Himalayan buckwheats as revealed by SDS-PAGE of endosperm proteins extracted from single seeds and RAPD based DNA fingerprints. *Genet Resour Crop Evol*, 54:767-777, 2007.
32. Manly, B. F. J., Multivariate statistical methods a primer. Chapman and Hall, London, 1994.
33. Hailu, F., Merker, A., Singh, H., Belay, G., and Johansson, E., Multivariate analysis of diversity of tetraploid wheat germplasm from Ethiopia. Genet Resour Crop Evol, 53: 1089–1098, 2006.
34. Pagnotta, M. A., Laghetti, G., Mondini, L., Codianni, P., and Fares, C., Agronomical, quality, and molecular characterization of twenty Italian emmer wheat (*Triticum dicoccon*) accessions. *Genet Resour Crop Evol*, 56:299–310, 2009.
35. Cooke.,R.J.,The characterisation and identification of crop cultivars by electrophoresis.*Electrophoresis*,5:52-79,2005
36. Degaonkar, A. M., Tamhankar, S. A., and Rao,V.S.,An assessment of cultivated emmer germplasm for gluten proteins. *Euphytica*, 145: 49–55, 2005.