J. Appl. Environ. Biol. Sci., 3(8)81-87, 2013

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Compare the grouping of winter wheat lines using SDS-PAGE electrophoresis data of seed storage proteins and quantitative traits data

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ABSTRACT

Plant modifier can have a great chance of success in him breeding programs with choosing the appropriate materials and the variety exist for him. Good understanding of the genetic diversity and classification of germplasm is necessary to select suitable parents for breeding purposes. In this study, the pattern of storage protein electrophoresis of winter wheat lines and data obtained from quantitative traits measurement were used to investigate the genetic diversity and classification of a set of promising wheat lines and control cultivars (Shahriar). For this purpose, the number of 9 promising lines with 1 control cultivar (Shahriar) was studied in a randomized complete block design with two replications. The experiments were done in the Biotechnology Laboratory, Islamic Azad University of Ardabil. Cluster analysis respectively placed genotypes into three groups by Ward's method for farm data and by UPGMA method for electrophoresis data based on the jacquard similarity matrix. Compare the grouping obtained from cluster analysis results of the electrophoresis data with results of the cluster analysis of morphological data showed that 10 genotypes were placed in the similar groups, in other words, grouping based on morphological characteristics and protein bands was consistent 35% together. The data from electrophoresis can somewhat have ability to complement the morphological data. Moreover, repeating the same experiments is recommended to achieve accurate results with a greater number of characters.

KEYWORDS: winter wheat, genetic diversity, electrophoresis, cluster analysis

INTRODUCTION

Variation is the key for any change, so the key for genetic change is genetic variation. The high chance of success in a breeding program follows the availability of a wide variety of appropriate materials for the breeder to choose from. Selecting appropriate parents for breeding purpose requires a good understanding on genetic variation and on classification of germplasms.

Wheat is important due to gluten features. The sticky part is the endosperm strict proteins and it causes the stretching or expansion of fermented dough, the term is peeling. When peeling is heated to bread production, this part of protein has been linked and it holds together. Only wheat and lesser extent of rye and triticale hold the property (Arzani, 2001).

As a rule, variation is the basis for all selections including genotypic selection. Naturally, the higher the genetic variation in a society, the wider the selection domain becomes (Abd Mishanei and Shah Nejat Boushehri, 1998). Genetic variation of cultivated plants is not randomly distributed throughout the world. In 1920s, Vovilov managed to identify regions where the highest possibly genetic variation for main crop species existed (Farshadfar, 1991).

Harlan demonstrated that plants have two different origins namely main center and secondary centers of genetic variation (Farshadfar, 1991). Plant breeding has been built upon the basis of variation and selection, while genetic variation broadens the range of activity and selection of the breeder for selection and other breeding operations. Currently, studies on genetic variation of the organisms are conducted in two ways: investigating gene expression (morphological and biochemical markers) and identifying sequences in DNA (DNA markers).

Use of genetic markers goes as far back as dozens of years before the discovery of DNA as a genetic substance. Morphology of DNA markers made possible by mutations visible within the morphology of live systems, has been in use ever since early twentieth century, while the application of biochemical markers, i.e. Isozymes and proteins, has been common for the past 40 years (Garayazi, 1996).

Glutenin subunits with high molecular weight can be used to identify cultivars as a supplementary indicator. Subunits can also are applied as an index for breeding projects to select parents and results with stronger gluten properties (Payne et al, 1982). In recent years, Polyacrylamide gel electrophoresis (PAGE) grains storage proteins has been valid method for assessing the chemical diversity of the wheat. This method may be used in order to collect germplasm where have considerable genetic diversity or in areas where there is expected a large variability

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(Dominici and Grottanelli, 1984). In the quality modification programs, choose the lines is permissible based on glutenin components with high molecular weight that are controlled by different locus. It is performed with crosses the parents and collecting allele in the offspring and their selection based on sedimentation test SDS and SDS-PAGE. Improvement is dramatically obtained in the quality of the protein (Miller, 1987). Encoder genes high-molecular-weight glutenin subunits place on the long arm of chromosomes 1A, 1B and 1D. The locus are shown as Glu-1; each gene contains two very close together Glu-1-1 and Glu-1-2 that respectively encode subunits of type x and y respectively in terms of molecular weight. Type y gene in the locus Glu-A1 usually is not present and it turned off. Sometimes both types of genes y and x in the locus Glu-A1 is not present in which case it will have a neutral allele. Both genes are often expressed in the Glu-B1, but both genes are expressed in the Glu-D1 forever. So in the Hexaploid wheat, 3-5 components (called bands) are seen in the glutenin with high molecular weight (Bushak and Rasper, 1996). Seed storage proteins are considered the most important effective factors in the making a difference in the quality of wheat (Rodriguez-Quijano et al, 2001). In fact, these proteins are responsible for the unique properties of adhesion and the elasticity of wheat flour dough (Branlard et al, 2001). Gupta and Macritche (1994) in the study of allelic diversity of high and low molecular weight glutenin subunits wheat and incremental effects and their interactions on the properties of the dough mentioned that some of the units have significant effects on dough strength (elastic and strength). The effects have been due to the environment that contains different levels of wheat protein. Therefore, the flour dough strength can be increased without increasing the protein which has negative relationship on the yield. Gupta et al (1995) showed that dough from the genotypes of subunits 5+10 and 17+18 have more resistant to genotypes subunit is 2 +12 and 14 +15. Also, the dough from the genotypes of subunits 7+9 have less resistant than genotypes subunit is 7 +8. Lafinard et al (1993) the preparation of the recombinant inbred of firolu varieties showed that there is no significant difference between the sub-units 2 +12 and 5 +12 in terms of SDS sedimentation. While, there was significant differences between sub-units 5+10, 5+12. Apparently the main reason for this difference is subunits 10. Lee et al (1983) reported that superiority of the subunit 5+10 than 2+12 is due to Position of the cysteine amino acid that make up subunits10 have a greater ability for intermolecular disulfide bond formation, share value Glu-D1 locus in SDS sedimentation is more than Glu-A1 locus and it is further from the Glu-B1. They also focused on the negative and significant effects of epitasis of this locus on the sedimentation rate (Carrillo et al, 1990). The purpose of this has been the study of genetic diversity of wheat lines and their grouping based on morphological data and wheat storage protein electrophoresis. With the hope that in addition identifying the cultivars and genotypes that have greater genetic distance and generally their crosses will have more than Heterosis, the grouping and identification of the genetic variation was done using the Electrophoresis and pattern of protein bands in a shorter time and these patterns can be used as genetic information per varieties.

MATERIALS AND METHODS

This study was conducted on a random sample of winter wheat in laboratory and farm. The experiment was conducted in a randomized complete block design with two replications in the Agricultural and Natural Resources Research Station, located 12 km south of Ardabil (Khalkhal Road). Seeds of 9 promising wheat lines and Shahriar cultivars (control) have been in table 1. Consumption has been 452 seeds per square meter and fertilizer consumption rate based on soil test results has been 92 kg nitrogen and phosphate and 62 kg potassium. Thus, total phosphorus and potash fertilizer was used in the autumn and nitrogen fertilizer was used at three stages tillering and heading. Broadleaf weed control was performed using 2, 4-D herbicide in the tillering stage. During the growth period characteristics such as time to 52% flowering and physiological maturity were recorded. Half-foot three lines were harvested when crop handling and the average of three samples, unfertilized spike dry matter per unit area and harvest index were determined. Plant height and number of grains per Spikes was measured the average of 12 spikes randomly harvested. Yield per unit of harvested crop were measured from each plot. Seed weight was measured after final harvest of the plots. In this study traits such as total number of tillers, the number of fertile tiller, the number of infertile tiller, length of spike, grain weight per spike, plant height, biomass, peduncle weight and length ,days to flowering, days to maturity, harvest index and grain yield were evaluated. Glutenin heavy subunits were performed using SDS-PAGE method on 10% gels according to the Laymly method (1970) that modified by Fullington et al (1983). Since the genotypes of the subunit 2 +12 with 10% acrylamide gel, the separation of subunits 2 and 2* is not done, therefore gel 7.5% acrylamide gel was used in the absence of urea. For this, after the prepare the requirement solutions, first, a healthy and medium grain were selected and it was milled after removing the fetus, the flour was poured in to a plastic tube (Apandrof) and on each of the samples was added 600 micro liters of protein extraction solution (12 ml twice distilled water, 5.1 ml extraction buffer and 0.90 ml 2 – mercapto ethanol) (0.1 ml solution to 8 mg sample weight). Eppendorf tube was immediately placed under vigorous stirring to mix the tube contents. During 2 hours proteins extraction, above procedure was repeated 3 - 4 times to extraction

protein done well. Centrifugation was carried out after 2 h for 10 min at 10000 g with 4 ° C. After centrifugation, the solids which were completely precipitated, 300 micro liter of clear solution Apandrof (supernatant) were gathered and was transferred to new Apandrof tube with preserving the profile (Maintenance of extracted protein was performed at -20 ° C). After polymerization the top gel, shoulders (Shane) were pulled out slowly and directly, 9 micro liter of extracted protein was injected into each well and current severity was adjusted on 30 mA for two gels. When gels colored lines were close to the end, it ended. It took 7-8 hours to reach the lines to the end. After power outages, gels were placed in trays on Shaker for painting. After staining gels were evaluated and recorded in terms of presence or absence of each band respectively the numbers 1 and 0. In this study, data obtained from the farm and banding pattern of wheat storage proteins, a separate cluster analysis was performed using SPSS software. For quantitative traits data, at first the distance ratio between individuals was calculated through Squared Euclidean distance method and after comparing the efficiency of different methods of cluster analysis, by correlation coefficient Kufentick (Rabie, 1996 and Romesburg, 1990), the dendrogram obtained from WARD with the correlation coefficient Kufentick 0.85% was determined as the best method. Simple matching coefficient method was used in order to grouping cultivars in terms of data obtained from protein-banding pattern and to calculate the similarity between each pair of genotypes (Rabie, 1996 and Valizadeh et al, 1994) and drawing was performed by UPGMA method.

Table 1 – List of study genotypes in this investigation

C-86-1	**Shahryar**
C-86-2	C-80-4
C-86-3	Bloudan/3/Bb/7C*2//Y50E/3*Kal/4/MV 17
C-86-4	Yan 7578. 128/4/Chil/24*Star
C-86-5	Yan 7578. 128/5/Chil/2*Star
C-86-6	Yan 7578. 128/6/Chil/2*Star
C-86-7	ID800994W/Vee//F900K/3/Pony/Opata
C-86-8	Bhr*5/Aga//Sni/3/Trk13/4/Drc
C-86-9	Bilinmiyen 96. 40
C-86-10	LC 909 Mima

RESULTS AND DISCUSSION

Cluster analysis (cluster) of the figures based on agronomic traits

By cutting the dendrogram obtained from the WARD distance of 6.40 unit (Figure 1) wheat lines were grouped in three clusters. The first and largest cluster included 6 genotypes, the second cluster, 2 genotypes and the third cluster included 2 genotypes. In this study, in order to investigate the contribution of 14 studied characters to create the clusters the mean of each cluster and total average of each cluster was calculated for all traits (Table 2). The first group included genotypes (1, 4, 5, 6, 7 and 9) that had less value in terms of yield and the most properties. The second group included genotypes 2 and 3 that had high value in terms of all properties with the exception of peduncle weight. They were genotypes with high tiller and with less fertile tiller and they had high yield than other genotypes. The third group included genotypes 8 and 10 that had moderate value in terms of all studied properties with the exception of biological yield and the number of infertile tiller. In conclusion, it can be stated that among these groups, the second group with genotypes C-80-4 (Number 2) and Bloudan/3/Bb/7C*2//Y50E/3*Kal/4/MV 17 (number 3) can be introduced as a superior group. Since genotypes in each cluster are of higher genetic proximity with each other than with genotypes from other clusters, thus phenomena such as Heterosis and transgressive segregation can be put into better use whenever hybridization of cultivars from different clusters is need.

Table 2. Average, deviation from the total mean in three clusters from cluster analysis for all traits

Cluster	Number	total number of tillers (m^2)	the number of fertile tiller (m^2)	the number of infertile tiller (m^2)	length of spike (m^2)	grain weight per spike (gr)	plant height (cm)	Biological yield
1	6	178.70	172.5	6.34	98.99	2.18	94.99	217.7
2	2	189.10	185.2	3.92	102.7	3.2	99.95	219.2
3	2	168.1	159.3	9	95.41	1.8	88.47	242.2
Total Average		178.6	172.40	6.38	99.01	2.31	94.68	**222.9**

Table2. Continue

Cluster	Number	Peduncle length (mm)	Peduncle weight (gr)	1000 grain weight (gr)	Harvest index (%)	Days to flowering	Days to maturity	Grain yield (ton/ha)
1	6	36.41	0.26	44.09	46.96	136	175.50	2.67
2	2	37.57	0.26	53.32	54.9	139.3	177	3.09
3	2	33.83	0.3	42.62	42.18	133.6	173.3	1.96
Total Average		36.12	0.28	45.64	47.59	136.2	175.4	**2.61**

Cluster analysis based on the pattern of protein bands

After ending the Electrophoresis, the carrier bands gels was photographed then numbers one and zero were respectively recorded based on the presence or absence of each band (Strip). Maximum number of bands was observed in genotype 2, 4, 5, 7, 8, 9 and 10 and the lowest number was in genotypes 1, 3 and 6. Among the banding most of the presence number was related to the bands 1, 8*, 10+2 and 5 + 7 and the lowest was related to the bands 2 + 7, 12 + 9, 6 + 8 and null (Table 3).

Table 3 - Total banding of protein electrophoresis and their presence number

Gene loci	Allele	Subunit	Number	Frequency %
Glu-A1	A	1	5	38
	B	2*	4	31
	C	Null	4	31
Glu-B1	B	7+8	2	16
	D	7+9	10	76
	H	6+8	1	8
Glu-D1	A	2+12	4	31
	D	5+10	9	69

The dendrogram cutting (Figure 2) in the distance of 10.21 unit placed cultivars based on the presence or absence of the protein bands from electrophoresis SDS-PAGE in the three groups (Table 4). To select the classification method, Kufentick coefficients were calculated using NTSYSc 2.02e software, the highest value based on Jacquard's similarity matrix (Jacquard, 1908) was (R=0.85) using UPGMA method (Figure 2). According to the results, 10 genotypes were divided into three groups (Table 4). So that, the first group included three genotypes 5, 7 and 9 that had moderate value in terms of yield and all studied properties. The second group included four genotypes which were high yielding, precocious, short legs, high tiller and the high spike length and in other words it was located the high level in terms of evaluated features. The third group included six genotypes 1, 2, 3, 6, 8 and 10 had most members, it had less value in terms of studied features (Table 5). In addition to high polymorphism they have, the storage proteins are also highly stable, while the electrophoretic protein patterns of ripen seeds have proven highly efficient criterion, either in solo or in combination with other markers, for identifying various plant realms and cultivars (Abd Mishanei and Shah Nejat Boushehri, 1998). Germplasms of crops originated in Pakistan have been researched with respect to diversity of grain storage proteins for pea (Ghaffour and Arshad, 2008), wheat (Seltana et al., 2007), blackgram (Ghaffour and Arshad, 2005), peanut (Javaeid et al., 2004) and cowpea (Iqbal et al., 2003).

Table 4 - Grouping of cultivars based on the pattern of protein bands

Cluster	Genotypes
1	G5 (Yan 7578. 128/5/Chil/2*Star), G7 (ID800994W/Vee//F900K/3/Pony/Opata) and G9 (Bilinmiyen 96. 40)
2	G4 (Yan 7578. 128/4/Chil/24*Star)
3	G1 (Shahryar), G2 (C-80-4), G3 (Bloudan/3/Bb/7C*2//Y50E/3*Kal/4/MV 17), G6 (Yan 7578. 128/6/Chil/2*Star), G8 (Bhr*5/Aga//Sni/3/Trk13/4/Drc) and G10 (LC 909 Mima)

Table 5 - Average of evaluated characteristics with separate the groups of Cluster analysis

Cluster	Number	total number of tillers (m^2)	the number of fertile tiller (m^2)	the number of infertile tiller (m^2)	length of spike (m^2)	grain weight per spike (gr)	plant height (cm)	Biological yield
1	3	171.55	165.88	5.95	93.10	2.26	97.41	191.25
2	1	181.33	176.15	5.17	107.56	2.42	92.93	206.85
3	6	181.72	175.01	6.18	100.56	2.31	93.60	241.41
Total Average		178.6	172.40	6.38	99.01	2.31	94.68	**222.9**

Table 5 continued –

Cluster	Number	Peduncle length (mm)	Peduncle weight (gr)	1000 grain weight (gr)	Harvest index (%)	Days to flowering	Days to maturity	Grain yield (ton/ha)
1	3	35.58	0.25	45.02	47.97	136.17	176	2.62
2	1	37.47	0.25	46.07	49.96	134.83	174.5	3.11
3	6	36.17	0.27	45.88	47.01	136.36	175.17	2.52
Total Average		36.12	0.28	45.64	47.59	136.2136.15	175.4	**2.61**

Comparison of morphological data grouping with protein electrophoresis data

In the next step, the grouping of cultivars was measured based on 14 morphological traits and banding protein pattern of the electrophoresis SDS-PAGE were compared. Comparing two clusters (Fig. 1 and 2) were observed that several cultivars located in both methods in one cluster (Table 4), that their close can be confirmed (Table 4). Grouping comparing from cluster analysis the electrophoretic data with grouping from cluster analysis the morphologic data showed that10 genotypes were placed in the similar groups. In other words grouping based on morphological characteristics and protein bands were compatible 35%.

In fact, data from electrophoresis were highly capable of complementing data obtained from field quantitative traits, whereas in vitro and in situ experiments can prove complementary to each other. It should not be overlooked that morphological data are based on traits measured on field, thus on one hand the number of accurate and unambiguous criteria they could potentially provide is limited, on the other hand study on them are both uneconomical and vulnerable against environmental conditions as they are related to plant growth conditions. For this end, in order for morphological data to be reliable, the evaluation of cultivars as well as their traits should be conducted over several years and locations. Moreover, proteins may also be influenced by environmental conditions, tissue as well as plant growth period. It may follow that classification based on data for protein electrophoresis may be limited, thus fail to provide a flawless criterion for distinguishing cultivars that are close, particularly, related to each other. In this case, more powerful markers such as those based on DNA may be used to distinguish between local, improved as well as foreign cultivars more efficiently by identifying trivial differences between the related cultivars. Thus, with breeding purpose in mind and based on information from this research, the superior parents may be selected from various clusters and hybridized to produce the superior progenies.

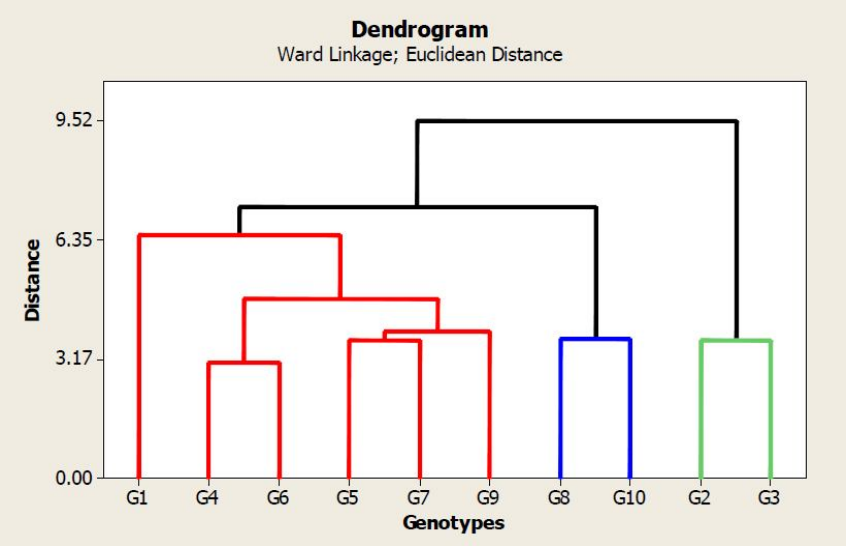

Figure 1 - Dendrogram obtained from cluster analysis of the minimum variance method (Ward) on all traits

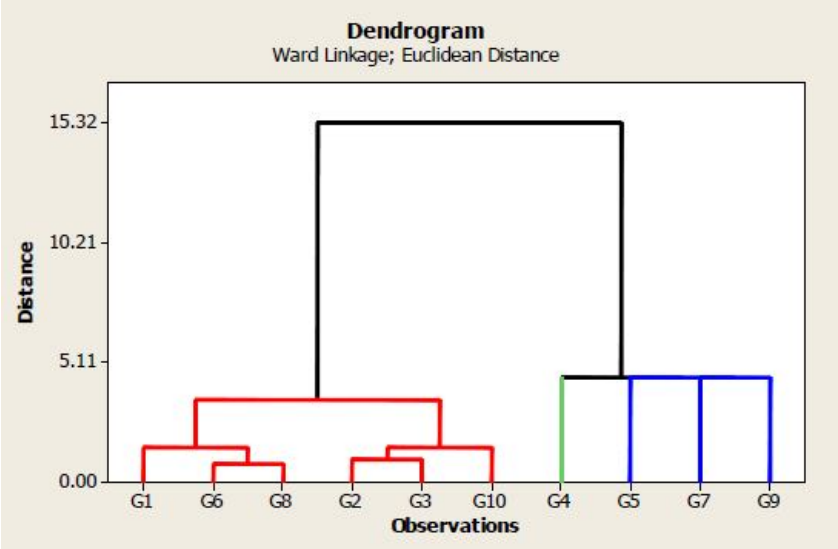

Figure 6 - Dendrogram obtained from cluster analysis the UPGMA method in winter wheat promising lines based on electrophoresis' banding patterns

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