

Estimation of Genetic Divergence Based on Different Quantitative Traits and Allelic Variation in Bread Wheat (*Triticum aestivum* L.) Germplasm Through SDS-PAGE Techniques

¹Muhammad Mohibullah, ²Malik Ashiq Rabbani, ¹Malik Munir,
¹Sarfraz Khan Marwat, ¹Shoaib Gangohi, ¹Muhammad Iqbal, ³Adnan Amin,
¹Abdul Aziz Khakwani, ¹Mamoon ur Rashid and ¹Irfan Ullah

¹Faculty of Agriculture, Gomal University, KP, Pakistan

²PGRI, National Agricultural Research Centre, Islamabad, Pakistan

³GCBB, Faculty of Agriculture, Gomal University, KP, Pakistan

Abstract: To study the variability and allelic variation of one hundred wheat (*Triticum aestivum* L.) entries, the experiment was conducted during the growing season 2004-2005 in augmented field design at research area of the Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan (NWFP). The germplasms were evaluated and characterized in two phases. In the 1st phase the phenotypic traits i.e. days to emergence, days to heading, days to maturity, number of tillers plant⁻¹ and Plant height (cm) were studied. Days to emergence varied from 7.10 to 20.10 days having 24.03% coefficient of variation. Days to heading ranged from 79.15 [PARC/NIAR 00203 (05)] to 130.25 [PARC/MAFF 004271 (01)] days with coefficient of variation 9.35 %. The minimum days to maturity (136) and the maximum days to maturity (193) were taken by PARC/NIAR 00203 (05) and PARC/MAFF 004271 (01), respectively. The entry PARC/NIAR 002809 (01) produced maximum number of tillers plant⁻¹. The entries PARC/NIAR 00203 (05) and PARC/MAFF 004270 (03) have the maximum (125.6 cm) and the shortest plant heights (53.2 cm) respectively. Days to heading have a significant and positive correlation with days to maturity and plant height has a significant correlation with number of tillers plant⁻¹. In the 2nd phase all the accessions were analyzed for allelic variation through SDS-PAGE. The frequency distribution of various alleles at three Glu-1 loci (Glu-A1, Glu-B1 and Glu-D1) contains the subunit "null" with 53%, subunit 2* (31%), subunit 2 (7%) and subunit 1 (09%) at the Glu-A1 locus. The most frequent HMW glutenin subunits at Glu-B1 locus were 7+8 with 58%. Thus variation in HMW glutenin Sub-units of wheat are correlated with bread making quality. Therefore, the accessions PARC/MAFF 4355 (02), 4280 (03), 4269 (02), 4279 (01), 4277 (01), 4277 (02), 4358 (03), 4292 (01), 4354 (02), 4354 (01), 4264 (03) and PARC/JICA 3835 (05) depicted the sub units for good bread making quality. Hence, all the above noted accessions provides best opportunity for further wheat breeding programme.

Key words: Wheat • Allelic • Germplasm • Glutenin • Crop

INTRODUCTION

Wheat is the most important food crop of the world. The largest cropped area is devoted to wheat and the quantity produced is more than that of any other crop. In 2011-12, the leading wheat-producing countries were China, the former USSR, India, the United States, France and Canada [1]. Wheat is the leading cereal grain crop in terms of production, consumption, nutritive value, storage qualities, adaptation and trade and has been intimately

linked with the development of both agriculture and civilization over a long period of time. Because of its wide usage in the form of human food (65%), feed for livestock (21%), seed (8%) and industrial and other products (6%), wheat has played a significant role in establishing permanent settlements, fostering the development of human civilization through cultural/religious development and in continuous population growth all over the world. Since this is the center of its wild progenitor's geographical distribution [2]. Its great popularity as

human food is due to its mild, acceptable flavor and to the unique ability of its principal proteins to form gluten when mixed with water. Due to rapid growth of population, increased production must come not only from land already available but also from new lands brought under cultivation and by increasing cropping intensity.

The genetic diversity is the key component of any agricultural production system. The history of gene pool assemblage on a systematic way goes back when Vavilov [3] undertook first successful expedition to Northern Iran and the Pamirs. The material from diverse geographical origin of the crop species can help to ensure conservation of co-adapted gene complexes because, genetically heterogeneous populations produce more and stable yield than genetically homogeneous lines [4]. Therefore, the present research project was carried out to evaluate genetic variability determined by morphological traits and biochemical markers (SDS-PAGE) to produce new wheat varieties with qualitative and quantitative improvements.

MATERIALS AND METHODS

One hundred entries of wheat (*Triticum aestivum* L.) of N.W.F.P reserved in Institute of Agricultural Biotechnology & Genetic Resources (IABGR) at National Agricultural Research Center (NARC), Islamabad were grown during the growing season 2004-2005 in augmented field design at the research area of the Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan. Recommended cultural practices and fertilizers were thoroughly applied. Parameters for days to emergence, days to heading, days to maturity, Plant height (cm) and number of tillers plant⁻¹ were taken by selecting maximum ten plants at random of each entry. For allelic variation through (SDS-PAGE) analysis a single spike was harvested separately from each of 100 wheat accessions and ground into fine powder. 10 mg of seed flour was added with 400µl of protein extraction buffer i.e. @ “0.5M Tris-HCl (pH 6.8), 2.5 % SDS, 10 % glycerol and 5 % 2-mercaptoethanol” for 5 minutes, centrifuged at 15,000 rpm for 10 minutes and stored at -20.0°C. The crude protein extract was run through slab type SDS-PAGE using 12.25 % Polyacrylamide Gel according to Laemmli [5] with 70 voltage until the bromophenol blue marker reached the bottom of the gel. The gels were stained with a staining solution i.e. Methanol (440ml), Acetic acid (60ml), Distilled water (500ml), CBB* (Coomassie Brilliant Blue) RG250 (2.25gm) and were dried for about two hours.

Analysis of Data: The basic statistics i.e. mean, range, standard deviation, coefficient of variation and correlation were analyzed by the method used by Steel & Torrie [6] and Sneath & Sokal [7]. While, for allelic variation quality score were recorded on the basis of Payne and Lawrence catalogue [8].

RESULTS

Morphological Traits

Days to Emergence: A distant variability was observed for days to emergence. It ranged from 7.10 to 20.10 with mean value of 13.5±3.24 and coefficient of variation 24.03 % (Table 1). The frequency distribution in (Fig. 1) ranged from 7 to 20 days for emergence. Three entries [PARC/NIAR 002303 (05)], [PARC/JICA 003841(02)] and [PARC/MAFF 004295 (04)] took minimum (7 days) for emergence and one entry [PARC/NIAR 002771 (03)] emerged very late and took as large as 20 days to emergence.

Days to Heading: The frequency distribution of days to heading (Fig. 2) indicates that days to heading ranged from 79 days to 118 days. The maximum number of accessions (21) headed between 99 to 103 days followed by 20 entries, which headed between 104 days to 108 days. The accession PARC/NIAR 002303 (05) took minimum (79 days) to heading and the accession 188667 PARC/NIAR002450(01) took maximum duration 130 days for heading, which was very late. Among the parameters studied the maximum variation was observed among the germplasm for days to heading. It varied from 79.15 to 130.25 days for heading with a mean value of 104.59 ± 9.78 and coefficient of variation for this parameter was 9.35 % (Table 1).

Days to Maturity: The medium level of variation existed among the germplasm for days to maturity. It ranged from 136.31 to 193.16 days to maturity with a mean value of 155.49± 11.16 and coefficient of variation for this trait is 7.17% (Table 1). The frequency distribution for days to maturity (Fig. 3) presents that days to maturity ranged from 136 to 199. The maximum number of entries (37) matured between 152 to 159 days followed by 28 entries that matured between 144 to 151 days. The accession PARC/MAFF 004271 (01) took maximum duration (193 days) for maturity, which was very late and entry [PARC/NIAR 002303 (05)] took minimum duration (136 days) for maturity.

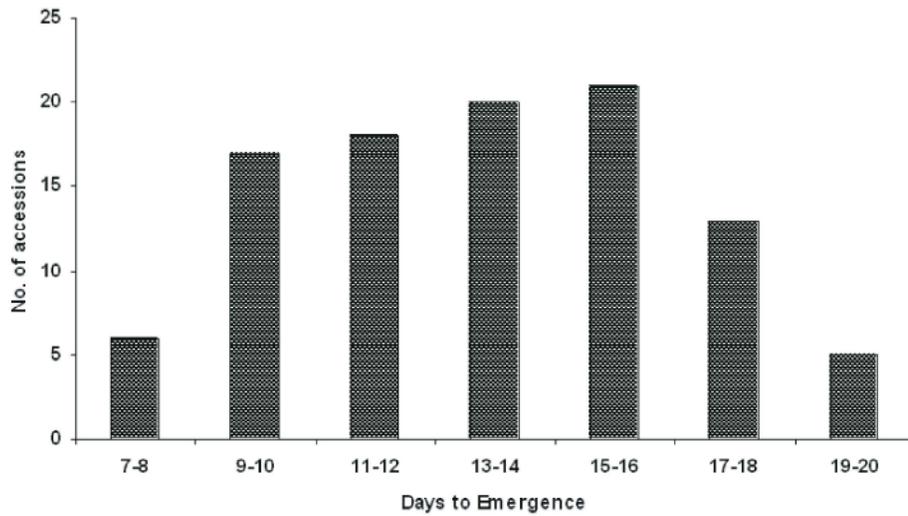


Fig. 1: Frequency distribution of days to emergence

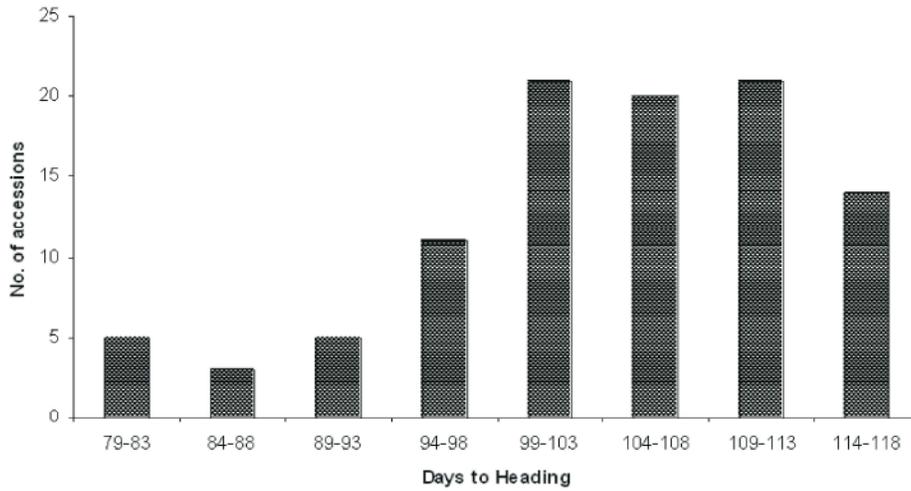


Fig. 2: Frequency distribution of days to heading

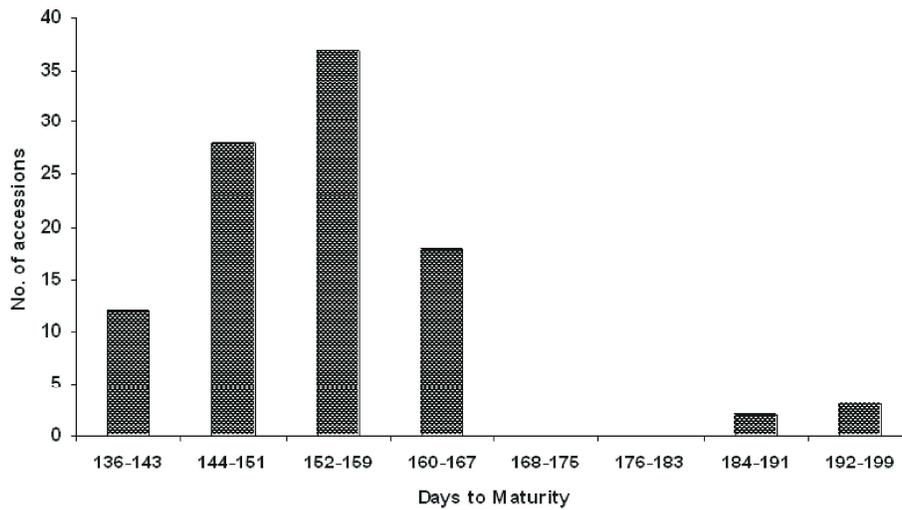


Fig. 3: Frequency distribution of days to maturity

Table 1: Basic statistics for comparison of wheat accessions

Trait	Mean	Minimum	Maximum	Variance	SD	CV (%)	SE
DE	13.5	7.10	20.10	10.47	3.24	24.03	0.32
DH	104.59	79.15	130.25	95.58	9.78	9.35	0.96
DM	155.49	136.31	193.16	124.46	11.16	7.17	1.10
Ph (cm)	82.68	53.20	125.60	187.31	13.69	16.55	1.35
Tillers plant ⁻¹	9.32	1.20	29.50	11.33	3.37	36.12	0.33

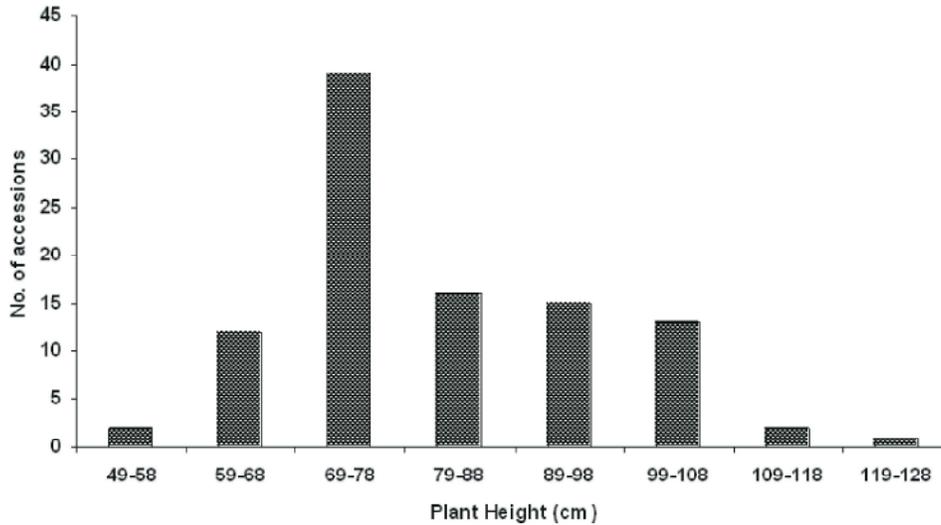


Fig. 4: Frequency distribution of plant height (cm)

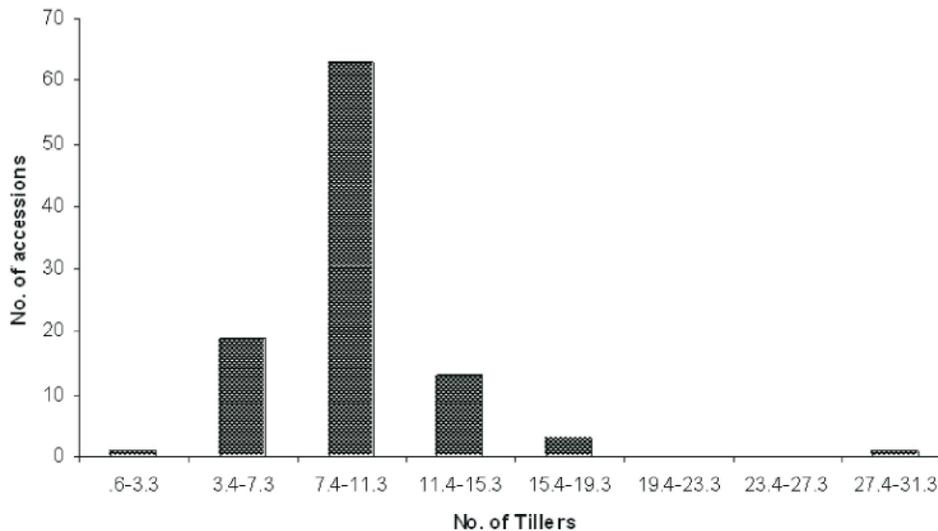


Fig. 5: Frequency distribution of number of tillers plant⁻¹

Plant Height (cm): The distribution for plant height among the germplasm studied varied from 49 to 128 cm (Fig. 4). The maximum number of entries (39) were in the range of 69 to 78 cm followed by 16 accessions in between 79 to 88 cm and 15 entries in the range of 89 to 98 cm. Entry [PARC/MAFF 004270 (03)] was the least in height (53.2 cm), while entry PARC/NIAR 002303 (05) had a maximum plant height of 125.6 cm. It varied from 53.20 to

125.60 cm with mean value of 82.68±13.69 (cm) and coefficient of variation for this parameter was 16.55 % (Table 1).

Tillers Plant⁻¹: Enormous variability was observed for the number of tillers plant⁻¹. It ranged from 1.20 to 29.50 with mean value of 9.32±3.37 and 36.12 % coefficient of variation (Table 1). In Fig. 5 the frequency distribution

varied from 0.6 to 31.3 number of tillers plant⁻¹ indicated that 63 accessions had 7.4 to 11.3 number of tillers plant⁻¹ followed by 19 entries with 3.4 to 7.3 and 13 with 11.4 to 15.3 number of tillers plant⁻¹. The line PARC/NIAR 002303 (05) had 9.1 number of tillers plant⁻¹. The entry [PARC/NIAR 002809 (01)] possessed the minimum number (1.2) tillers plant⁻¹, while entry [PARC/MAFF 004270 (01)] had a maximum number (29.5) tillers plant⁻¹.

Correlation Coefficients: The computation of relationships revealed that days to emergence had significant and positive correlation with number of tillers plant⁻¹ ($r = 0.20$), While this parameter had significant negative correlation with days to maturity ($r = -0.23$) and negative correlation with days to heading ($r = -0.06$), plant height ($r = -0.09$). Days to heading showed strong positive significant correlation with days to maturity ($r = 0.15$), while this parameter presents also a positive correlation with plant height ($r = 0.05$), number of tillers plant⁻¹ ($r = 0.04$). However, days to maturity had negative correlation with plant height ($r = -0.05$), number of tillers plant⁻¹ ($r = -0.03$). The plant height indicates significantly positive correlation with number of tillers plant⁻¹ ($r = 0.24$).

RESULTS

Allelic Variation Through Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): The results showed a broad and interesting range of variation for HMW glutenin sub-units genes in the landraces under investigation. In (Table 3) all the accessions were declined into 12 individual allelic variants for various HMW- glutenin subunit combinations. In which most frequent at Glu-1 loci the subunit composition were null, 7+8 and 2+12. In this group of accessions other subunits with various unique compositions were also found. The frequency of various alleles found in the entire set of germplasm at three Glu-1 loci (Glu-A1, Glu-B1 and Glu-D1) is depicted in (Table 3). At the locus Glu-A1 the subunit “null”, which does not code for any protein, was the most frequent and represents in 53% of the accessions. The remaining accessions were found to possess the subunit 2* (31%), subunit 2 (7%) and subunit 1 (09%) at the Glu-A1 locus. The most frequent HMW glutenin subunits at Glu-B1 locus were 7+8, which appeared in 58% accessions. The other subunit was 17+18 (25%), subunit 7 (5%) and subunit 7+9 (12%). It has been further noticed that in some of the accessions the Glu-B1 subunit 7 was found to be slightly faster as compared to others.

Table 2: Correlation coefficients for wheat accessions

Traits	DE	DH	DM	PH (cm)	Tillers plant ⁻¹
DE	1.00				
DH	-0.06	1.00			
DM	-0.23*	0.15*	1.00		
PH (cm)	-0.09	0.05	-0.05	1.00	
Tillers plant ⁻¹	0.20*	0.04	-0.03	0.24*	1.00

* = Significant at 0.05 and 0.01 probability level respectively

Table 3: Allele frequencies of HMW glutenin subunits in different Wheat accessions

	Allele	No. of Accessions	Proportion	Frequency
Glu-A1	1	09	0.09	9%
	2	07	0.07	7%
	2*	31	0.31	31%
	Null	53	0.53	53%
Glu-B1	7	02	0.02	2%
	8	03	0.03	3%
	7+8	58	0.58	58%
	7+9	12	0.12	12%
	17+18	25	0.25	25%
Glu-D1	2+12	81	0.81	81%
	5+10	17	0.17	17%
	4+12	02	0.02	2%

At the Glu-D1 only three pairs of subunits 2+12, 4+12 and 5+10 were found in most of accessions with frequency of 81%, 2% and 17%, respectively. The most frequent subunit 2+12 was depicted in 81% of the accessions.

DISCUSSION

A very high degree of variability was noted for days to emergence, days to heading, days to maturity, Plant height (cm) and number of tillers plant⁻¹. These evaluations are at par with the results notified by Moghaddam *et al.* [9] on southwestern Iranian bread wheat landraces and Tesemma *et al.* [10] in the Ethiopian highland wheat landraces. Significant genotypic differences were observed for all traits which are at par with the results observed for yield and quality traits in 40 wheat genotypes grown under normal and late sowing conditions [11]. Correlation coefficients observations also agree with the results of [12] as they concluded that grain yield was positively correlated with number of tillers plant⁻¹, number of grains spike⁻¹ and grain weight. Similar observations have been recorded by Mohibullah *et al.* [13] on wheat. Simultaneously more selection stress could be applied on minimum days to heading, maturity, maximum number of tillers plant⁻¹ and maximum plant height (cm) in segregating generations to increase grain yield. Our observations are in agreement with the results

stated by Mohibullah *et al.* [14], that genotypes of wheat had high magnitude of genetic variability. The results of Xu *et al.* [15] also showed that significant variation was observed for all the evaluated parameters.

For allelic variation the quality score noted in these accessions are quite at par with the scores obtained by the earlier researchers [16, 17] which were 8.3 in Indian wheat varieties and 8.7 in Canadian bread wheat varieties respectively. HMW glutenin sub-units provide useful information on genetic variation in evolution and domestication of wheat and enhance the genetic variability available to improve its industrial and commercial quality [18]. Glutenins and gliadins are two major groups of the seed storage proteins and valuable determinants of bread-making quality (BMQ) [19]. The inheritance of protein is well characterized and their high levels of polymorphism, has proved immense value in breeding programme [20]. A quality score assigned to individual or pair of HMW glutenin sub-units has made it possible to evaluate the potential BMQ of lines/ variety on the basis of glutenin pattern. The above results depicts that the pattern of HMW glutenin sub-units in Pakistani landraces are almost similar to those reported by different researchers [21, 22].

CONCLUSION

By observations and evaluation of experimental findings through (a) morphological traits it was concluded that germplasm [PARC/NIAR 002303 (05)], [PARC/JICA 003841(02)] and [PARC/MAFF 004295 (04)] having short duration with only 7 days for emergence, 79 days to heading and 136 days to maturity could be utilized for evolving short duration wheat cultivars. While, the entries [PARC/NIAR 002303 (05)], [PARC/JICA 003841(02)] and [PARC/MAFF 004295 (04)] having maximum plant height 125.6 (cm) and 9.1 number of tillers plant⁻¹ respectively. Allelic variation through SDS-PAGE a considerable variation in total 12 different HMW glutenin subunit compositions were noted. The frequency of 7+8 and 2+12 was the highest in the entire set of all germplasms. During the present investigation then germplasms PARC/MAFF 4355 (02), 4280 (03), 4269 (02), 4279 (01), 4277 (01), 4277 (02), 4358 (03), 4292 (01), 4354 (02), 4354 (01), 4264 (03) and PARC/JICA 3835 (05) possessed a known source of good bread making quality. Hence the said germplasms has the required characteristics and recommended for general cultivation and for further wheat breeding program to transfer a desirable characters under different agro climatic conditions in the country.

Abbreviations

DE	DH	DM	PH
Days to emergence	Days to heading	Days to maturity	Plant height (cm)

ACKNOWLEDGEMENT

This research work has been financially supported under the Indigenous Scholarship programme to Muhammad Mohibullah Khan from Higher Education Commission, Islamabad, which is highly acknowledged.

REFERENCES

1. Anonymous, 2012. Islamabad: Government of Pakistan, Ministry of food, Agriculture and Cooperatives, pp: 104.
2. Zohary, D., 1970. Wild Wheat's In: Genetic Resources in Plants – Their Exploration and Conservation, (ed.) O.H. Frankel and E. Bennett. Oxford: Blackwell Scientific Publication, pp: 239-247.
3. Vavilov, N.I., 194. Five Continents (eds.) L.E. Rodin, Semyon Reznik and Paul Stapleton, International Plant Genetic Resources Institute, Rome Italy, pp: 1-197.
4. Rasheed, A., A.S. Mumtaz and Z.K. Shinwari, 2012. Genetic characterization of novel Lr gene stack in spring wheat variety Chakwal86 and its effectiveness against leaf rust in rain fed areas of Pakistan. *Pak. J. Bot.*, 44(2): 507-510.
5. Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of the head of Bacteriophage T4. *Nature*, pp: 680-685.
6. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. 2nd Edition, McGraw-Hill Book Co. Inc., New York, USA, pp: 633.
7. Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.F. Freeman & Co., San Francisco, pp: 573.
8. Payne, P.I. and G.J. Lawrence, 1983. Catalogue of the alleles for the complex gene loci, Glu-A1, Glu-B1, Glu-D1 which code for high-molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.*, 11: 29-35.
9. Moghaddam, M.B., Ehdai and J.G. Waines, 1998. Genetic variation for interrelationships among agronomic traits in landraces of bread wheat from southwestern Iran. *J. Genet. & Breed.*, 52: 73-81.
10. Tesemma, T., S. Tsegaye, G. Belay, E. Bechere and D. Mitiku, 1998. Stability of performance of tetraploid wheat landraces in the Ethiopian highland. *Euphytica*, 102: 301-308.

11. Arya, V.D., I.S. Pawar and R.A.S. Lamba, 2005. Genetic variability, correlation and path analysis for yield and quality traits in bread wheat. *Haryana Agric. Uni. J. Res.*, 35(1): 59-63.
12. Belay, G., T. Tesemma, H.C. Becker and A. Merker, 1993. Variation and interrelationships of agronomic traits in Ethiopian tetraploid wheat landraces. *Euphytica*, 71: 181-188.
13. Mohibullah, M., M.A. Rabbani, Shah Jehan, Zakiullah, A. Amin and Ghazanfarullah, 2011. Genetic variability and correlation analysis of bread wheat (*Triticum aestivum* L.) accessions. *Pak. J. Bot.*, 43(6): 2717-2720.
14. Mohibullah, M., M.A. Rabbani, Irfanullah, M. Iqbal, Zakiullah, M.I. Khattak and O. Sayal, 2012. Correlation and Frequency distribution for different parameters among bread wheat (*Triticum aestivum* L.) Accessions. *Pak. J. Bot.*, 44(1): 255-259.
15. Xueli, S., X. Song, D. Qing, Z. Ni, B. Dou, Q. Sun and H. Pen. 2005. 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*, 146: 193-201.
16. Bhagwat, S.G. and C.R. Bhatia, 1988. Variation in high molecular weight glutenin subunits of Indian wheat varieties and their Glu-I quality scores. In: T. E. Miller and K.M. Koener (eds.), *Proc. Seventh Int. Wheat Genetics Symp.* Bath Press, Bath, pp: 933-936.
17. Lukow, O.M., P.I. Payne and R. Tkachuk, 1989. The high HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread making quality. *J. Cereal Sci.*, 15: 29-37.
18. Vallega, V. and J.G. Waines, 1987. High-molecular weight glutenin subunits in *Triticum turgidum* var *dicoccum*. *Theor. Appl. Genet.*, 74: 706-710.
19. Payne, P.I., K.G. Corfield, L.M. Holt and J.A. Blackman, 1981 a. Correlation between the inheritance of certain high molecular weight subunits of glutenin and bread making quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric.*, 32: 51-60.
20. Morgunov, A.I., W.J. Rogers, E.L. Sayers and Metakovsky, 1990. The high-molecular weight glutenin subunit composition of Soviet wheat varieties. *Euphytica*, 51: 41-52.
21. Naushad, A.T., M.A. Farhatullah, Rabbani and Z.K. Shinwari, 2012. Genetic diversity in the locally collected Brassica species of Pakistan based on microsatellite markers. *Pak. J. Bot.*, 44(3): 1029-1035.
22. Igrejas, G., H. Guedes-Pinto, V. Carnide and G. Branlard, 1999. The high and low molecular weight glutenin subunits and ω -gliadin composition of bread and durum wheats commonly grown in Portugal. *Plant Breeding*, 118: 297-302.