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GENETIC DIVERSITY IN WHEAT LANDRACES FROM PAKISTAN BASED ON POLYMORPHISM FOR HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS (HMW-GS)

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Abstract

A total of 77 wheat (*Triticum aestivum* L.) landraces collected from different areas of Pakistan were assessed for genetic diversity in terms of HMW-Glutenin sub-units. Considerable amount of variation was observed in this set of germplasm ranging from monomorphism to polymorphism as revealed by SDS-Polyacrylamide gel electrophoresis (SDS~PAGE). Of the 8 alleles detected at Glu-1 loci, three belong to each Glu-A1 and Glu-B1 and 2 belong to Glu-D1 locus. The most frequent subunits were 1 and 2* at Glu-A1 locus, 7+8 at Glu-B1 locus and 2+12 at Glu-D1 locus. Maximum variation was observed in Diamir followed by Ghizer. A higher gene diversity between the populations (Dst = 0.29%) was observed as compared to the gene diversity within the populations (Hs =10%). High value for gene diversity between population relative to total gene diversity (Gst = 0.7376) indicated a substantial amount of gene differentiation among the populations. The information generated could be exploited by the plant breeders to develop new cultivars and by the gene bank managers to properly document and maintain the germplasm collections.

Introduction

The knowledge about the level of genetic diversity in crop germplasm collection is necessary to improve the efficiency of breeding and genetic conservation programs. Traditionally, the data on agronomic, morphological and physiological plant traits are generally used to estimate the genetic variation. However, such data may not provide an accurate indication of genetic diversity because of the strong environmental influences upon the expression of these traits or difficulty in scoring due to the presence of multiple genes or alleles. Moreover field evaluation is a time consuming and laborious task when large number of accessions are to be analyzed. Considering these difficulties biochemical and molecular markers such as SDS-PAGE, isozymes, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have received much attention in recent years for genetic diversity studies in germplasm collections.

The analysis of storage protein variation in wheat have proved to be a useful tool not only for diversity studies but also to optimize the variation in germplasm collections (Ciaffi *et al*., 1993; Damania *et al*., 1983). Glutenins and gliadins are two major groups of seed storage proteins in hexaploid wheat. The high molecular weight subunits of glutenin constitute only about 10% of the storage proteins in the wheat endosperm but they have a major influence on the bread making properties of flour.

The genetics and biochemistry of high molecular weight glutenin sub~units in wheat is well known (Shewry *et al*., 1992). In wheat (*Triticum aestivum* L.) high molecular

weight glutenin sub-units are coded by genes at three loci designated Glu-A1, Glu-B1 and Glu-D1 and are close to the centromere on the long arms of Chromosome 1A, 1B and 1D, respectively (Payne *et al*., 1981b, 1984a). The allelic variations at the three loci have been used independantly or in combination with other genetic markers to estimate the genetic variability in different wheat species (Nevo & Payne, 1987; Ciaffi *et al*., 1993; and Marigotta *et al*., 1988). Wheat landraces collected in the primary and secondary center of diversity from various countries have been evaluated for variation in seed storage proteins (Lafiandra *et al*., 1987a; Lagudah *et al*., 1987; Marigotta *et al*., 1988).

Wheat is one of the most important food crops in the West Asian and North African (WANA) region. In Pakistan the crop accounts for three times the area and twice the value added share of cotton and rice. The enrichment of the gene pool of this important crop is necessary to overcome the effect of genetic erosion caused by large scale cultivation of commercial varieties.

Plant Genetic Resources Institute (PGRI), at National Agriculture Research Center, Islamabad holds a good collection of wheat germplasm from all over the country. To make the best use of this germplasm resource for breeding, it is necessary to obtain information on genetic variability present within the germplasm collection. This paper reports the level of genetic diversity present in these landrace genotypes based on the patterns of allele distribution of HMW glutenin subunits.

Material and Methods

Seeds from 77 landrace genotypes of wheat (*Triticum aestivum* L.) collected from Baluchistan and Northern Areas of Pakistan (Chitral, Diamer, Dir, Ghizer, Ghanchi, Gilgit, Kalat, Mastung, Noshki, Quetta and Skardo) were taken from the gene bank of PGRI. These 11 districts will hereafter be referred as R1, R2, R3, R4, R5, R6.R7, R8, R9, R10, R11, respectively. Chinese spring, Pavon and Pak 17951 with known banding pattern were used as standard varieties. The detail of wheat germplasm collection along with their altitudes is given in Table 1.

Total seed protein was extracted from 8 single seeds of each accession (616 samples) using 300µl protein extraction buffer. To maximize extraction, samples were mixed thoroughly by vortexing and centrifuged at 15,000 rpm for 5 minutes at room temperature. Seed protein was analyzed through slab type SDS-PAGE using 7.5% polyacrylamide gel following the method of Laemmli (1970).

A total volume of 8µl protein extract solution was loaded into each well with the help of a micro syringe. The electrophoresis was run at 100 V for the first 30 minutes until a blue line of BPB solution passes through the stacking gel. Thereafter, the electrophoresis was run at 150 V till blue line passes through the bottom of gel plate. After electrophoresis, the gels were stained in a staining solution with 0.2% (w/v) coomassie brilliant blue for one hour and destained until the colour of the background disappeared and electrophoretic bands were clearly visible. After destaining, the gels were dried using Gel Drying Processor and the HMW glutenin sub-units were identified following the numbering system suggested by Payne & Lawrence, (1983) and compared to the reference varieties of known composition such as Chinese spring (2+12, 7+8), Pavon (2*, 17+18, 5+10), and Pak 17951 (1, 13+16, 5+10).

Table 1. The detail of the wheat germplasm collection alongwith their altitude.

District	Region	Altitude in meters		
		Max.	Min.	Mean
Chitral	R1	2830	1665	2247.5
Diamir	R2	1885	1595	1740
Dir	R3	1440	1255	1347.5
Ghizer	R4	3000	1965	2482.5
Ghanchi	R5	3040	2505	2772.5
Gilgit	R6	2835	1650	2242.5
Kalat	R7	2160	1885	2022.5
Mastung	R8	1880	1760	1820
Noshki	R9	1725	1530	1627.5
Quetta	R10	1950	1875	1912.5
Skardo	R11	2500	2405	2452.5

Table 2. Allelic frequencies of HMW glutenin sub units at Glu-1 loci in wheat.

No. of allele	Samples	Proportion	Frequency (%)
Glu-A1			
1	422	0.6851	68.5
1*	9	0.0146	1.5
2*	185	0.3003	30.0
Total	616		
Glu-B1			
7+8	472	0.7662	76.6
13+16	29	0.0471	4.7
17+18	115	0.1867	18.7
Total	616		
Glu-D1			
2+12	468	0.7597	76.0
5+10	148	0.2403	24.0
Total	**616**		

*Allelic frequency revealed in 616 samples at three Glu-1 loci

Statistical analysis: The frequency of alleles at three Glu-1 loci (Glu-A1, Glu-B1, Glu-D1) were calculated across the entire 77 accessions. Expected heterozygosity or genetic diversity (He) at each locus; average heterozygosity (H) over the loci; the proportion of polymorphic loci (P); the gene diversity in total population (Ht); and the relative magnitude of gene differentiation among populations (Gst) were computed according to the method of Nei & Chesser (1983). All statistical analysis were carried out using a computer programme written in BASIC (GSTAT) and STATISTICA (ver 5).

Result

Allelic variation at the Glu-1 loci: Allelic frequency of HMW-GS at Glu-1 loci in 616 samples analyzed from 77 accessions is presented in Table 2. At the locus Glu-A1 the

allele controlling the expression of sub-unit 1 was most frequent as represented in 422 (68.5%) out of 616 samples. The allele for sub-unit 1* was found only in 9 samples, while allele controlling sub-unit 2* was observed in 30% of the samples analyzed. The Null allele which does not code for any protein at Glu-A1 locus was absent in this set of land race genotypes. In case of Glu-B1 locus the most frequent allele was 7+8 which was found in 472 (76.6%) samples. Allele controlling the sub-unit 13+16 was found in 29 whereas sub-unit 17+18 was found in 115 samples (18.7%).

At the Glu-D1 locus only two alleles controlling sub-units 2+12 and 5+10 were distinguished in these landrace genotypes. Majority of the genotypes had sub-unit 2+12 which was found in 76 % of the samples. Allele for sub-unit 5+10 was found only in 148 samples. The gel depicting the separation of HMW-GS is presented in Fig. 1.

Pattern of genetic variation: Eight seeds from each accession were subjected to SDS-PAGE analysis to determine the allelic variation. There was an appreciable amount of variation in the accessions ranging from monomorphism to polymorphism (Table 3). The maximium variation exists at Glu-A1 locus whereas 32 samples out of 77 were polymorphic. Maximum Genetic diversity 0.5667 for Glu-A1 was found in Pak 17285 from the region R10 (Quetta). At the locus Glu-B1 and Glu-D1 the variation was also observed where 27 and 18 samples, respectively were found polymorphic. Altogether, 41 samples were found monomorphic at all the three loci considered. Overall the highest average gene diversity (0.2389) and the proportion of polymorphic loci (P = 0.667) were obtained from the R2 (Diamir) indicating the higher level of polymorphism in the accessions collected from this region.

The analysis of glutenin gene diversity (Nei & Chessar, 1983) in 77 populations indicated a higher gene diversity between populations (29%) as compared to within population (0.10). A higher value for gene diversity between populations relative to total gene diversity (GST = 73.76) was also observed in this population (Table 4).

Regional distribution of alleles: The distribution of allelic patterns at the Glu-1 locus in 11 regions is presented in Figs. 2, 3 and 4. The subunit 1 at Glu-A1 locus was predominant in all the regions except R2, R3 and R5 where 2* was predominantly observed. Generally all three subunits were common in R7, R10 and R11. Sub-unit composition 1 has highest frequency of 0.89 in region R4 and sub-unit 2* was most frequent in region R3 (0.62). The “null” allele was absent in accessions from all the regions.

The allelic sub-unit 7+8 encoded at Glu-B1 locus was predominant in the accessions from all the regions with the exception of R2. The maximum frequency of 1 showed the pre-dominance of sub-unit composition 7+8 in the region R10. In region R2 its frequency 0.375 is very low as compared to the other regions. The sub-unit 17+18 was also found in most of the regions with a maximum frequency of 0.6 in R2, however, it was not present in R9 and R10. Sub-unit 13+16 was found only in the accessions from five regions with maximum frequency of 0.14 in region R8.

The allelic sub-unit pair 2+12 encoded at Glu-D1 was predominant in accessions from all the regions with the maximum frequency of 1 in region R9 and R10. A sub~unit pair 5+10 was also detected in most of the regions but was absent in regions R9 and R10. In region R2 the frequency 0.625 of the sub-unit 5+10 was maximum.

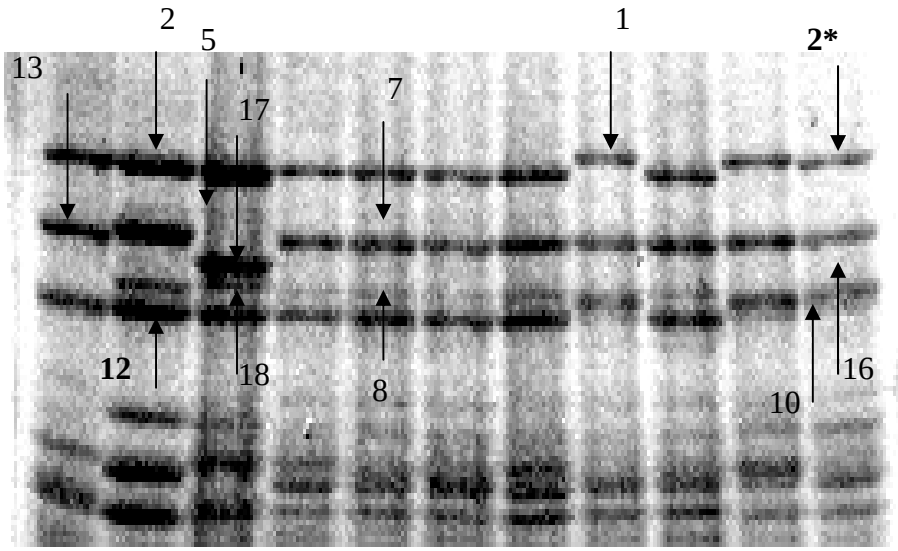

Fig. 1. SDS-PAGE separation of HMW-Glutenin subunit variation in wheat landrace genotypes.

Discussion

The characterization of cultivars based on biochemical and molecular genetic markers are now regarded as an important requirement for the modern seed industry. A substantial number of high yielding varieties of wheat have emerged from our national wheat breeding programs. These varieties have been studied extensively for various agronomic traits without paying much attention to quality characteristics. One of the solutions to resolve this issues is to discriminate the wheat land races based on allelic variation at the Glu-1 loci since the results are reproducible and are not influenced by the environmental factors. We studied 77 accessions of hexaploid wheat germplasm collected from Balochistan and Northern Areas of Pakistan. A reasonable amount of genetic diversity was observed in landrace genotypes of wheat on the basis of HMW glutenin subunit compositions. The level of polymorphism detected at Glu-1 loci is almost comparable to the previous studies of this nature (Cross and Guo, 1993; Lagudah *et al*., 1987; Marigotta *et al*., 1988; Ciaffi *et al*., 1993; Nevo and Payne, 1987; Tahir *et al*., 1995).

The "null" allele at the Glu-A1 locus has been predominantly observed in hexaploid wheat cultivars (Payne & Lawrence, 1983) and the germplasm accessions (Cross & Guo, 1993; Lagudah *et al*., 1987). However, none of the 77 accessions were found to possess the "null" allele at the Glu-A1 locus in this study. The significant proportion of landrace genotypes were found with allele 2* which impart better quality to the wheat flour. In an attempt to find worldwide distribution of Glu 1 alleles in bread wheats, Morgunov *et al*., (1993) analysed 9 varieties from Pakistan for MHW glutenin subunits. There results are in agreement with those obtained in this study. Similar results were obtained by Tahir *et al*., 1995 while evaluating wheat cultivars from Pakistan, where none of the 50 wheat varieties analyzed for HMW glutenin subunits was found to possess the null allele at the Glu~A1 locus. It appears that wheat breeders of Pakistan were selecting for allele 2* which imparts the better quality to the wheat flour.

Table 3. Gene diversity parameters for the 77 accessions of wheat pooled across the 11 regions.

Region	Gene diversity at three Glu-1 loci (He)			Average gene diversity over the loci	Proportion of polymorphic loci
	Glu-A1	Glu-B1	Glu-D1	H	P
R1					
Mean	0.0888	0.0407	0.0407	0.0567	0.1666
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.5000	0.5000	0.5000	0.5000	1.0000
R2					
Mean	0.3166	0.2000	0.2000	0.2389	0.6666
Minimum	0.2333	0.0000	0.0000	0.0778	0.3333
Maximum	0.4000	0.4000	0.4000	0.4000	1.0000
R3					
Mean	0.0777	0.0000	0.0000	0.0259	0.1111
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.2333	00000	0.0000	0.0778	0.3333
R4					
Mean	0.1619	0.1761	0.1761	0.1714	0.5238
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.5000	0.5333	0.5333	0.5222	1.0000
R5					
Mean	0.1888	0.1055	0.1055	0.1333	0.3888
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.5000	0.4000	0.4000	0.4000	1.0000
R6					
Mean	0.0666	0.0388	0.0388	0.0481	0.1666
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.4000	0.2333	0.2333	0.1556	0.6667
R7					
Mean	0.1737	0.0357	0.0905	0.1000	0.2619
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.4333	0.5000	0.5333	0.5000	1.0000
R8					
Mean	0.1458	0.2125	0.2041	0.1875	0.4583
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.5333	0.5667	0.5000	0.4889	1.0000
R9					
Mean	0.2111	0.0777	0.0000	0.0963	0.3333
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.4000	0.2333	0.0000	0.1556	0.6667
R10					
Mean	0.1933	0.0000	0.0000	0.0644	0.1333
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.5667	0.0000	0.0000	0.1889	0.3333
R11					
Mean	0.1799	0.0866	0.0800	0.1155	0.3333
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.4333	0.4333	0.4000	0.3556	1.0000

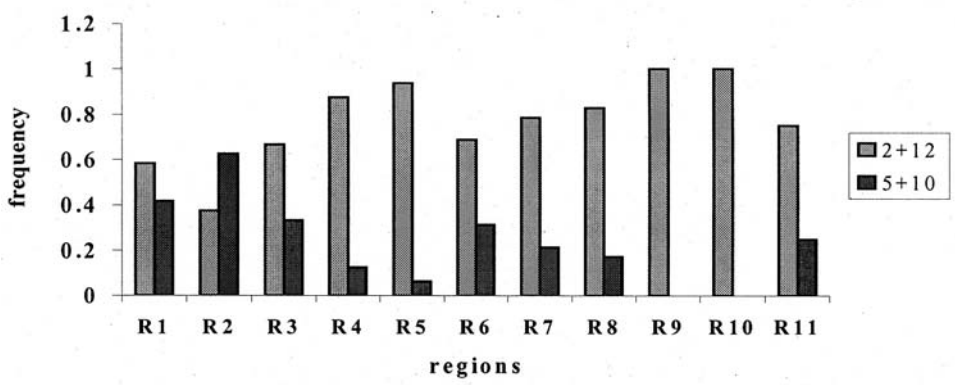

Fig. 2. Frequency distribution of Glu-A1 allele in the accessions from eleven regions

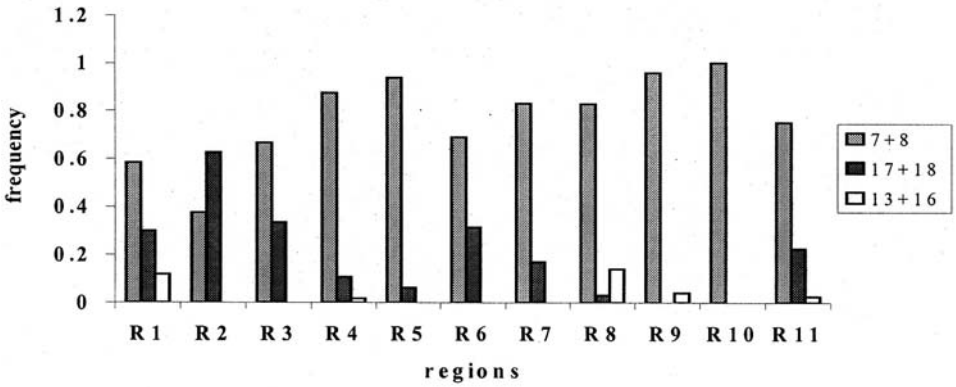

Fig. 3. Frequency distribution of Glu-B1 allele in the accessions from eleven regions

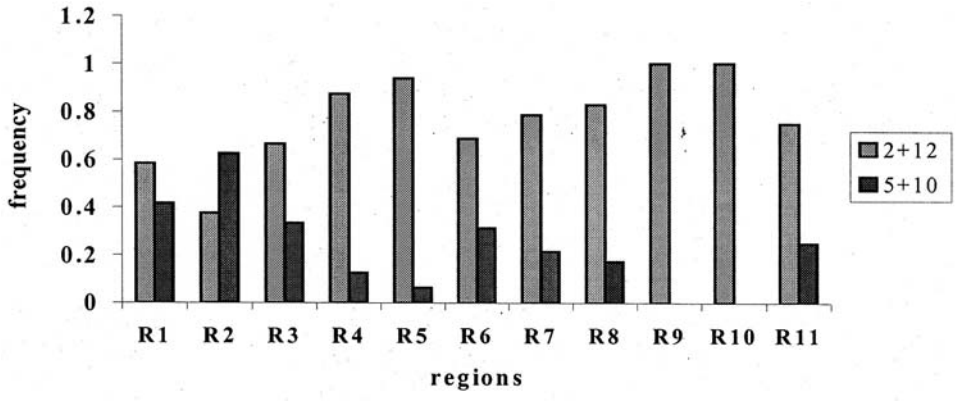

Fig. 4. Frequency distribution of Glu-D1 allele in the accessions from eleven regions

Table 4. Gene diversity statistics in landrace genotypes of wheat.

Locus	Ht	Hs	Dst	Gst
Glu-A1	0.4404	0.1459	0.2945	0.6687
Glu-B1	0.3759	0.0792	0.2967	0.7892
Glu-D1	0.3651	0.0848	0.2803	0.7676
Mean	0.3938	0.1033	0.2905	0.7376

Ht = Total gene diversity
Hs = Average gene diversity within populations
Dst = Average diversity between populations
Gst = Gene diversity between populations relative to Ht.

Two Glu-D1 sub-units 5+10 and 2+12 were commonly observed in hexaploid wheat (Payne & Lawrence, 1983). Both of these sub-units were detected in this set of local germplasm with a frequency of 76 and 24%, respectively. Since there is a significant association between certain HMW glutenin subunits and bread making quality (Payne *et al*., 1981), the variation at these loci is essential for the breeders to develop cultivars with improved bread making quality. The subunit 5 +10 which imparts better quality to wheat flour was predominantly found in district Diamer and Ghizer. These areas deserve more investigation for HMW-Glutenin subunits and needs to be focused while planning wheat expeditions in future. Some wheat breeding programs such as those at the former Plant Breeding Institute, Cambridge (PBI) and the International Maize and Wheat Improvement center (CIMMYT) has already used HMW glutenin subunit composition as a criterion of selecting parents for improving bread making quality. In order to improve bread making quality of Pakistani wheat in future cultivars, it should be advantageous to select for good quality subunits like 5+10, 17+18 and 2*

The information generated from this study could be utilized to devise an efficient breeding strategy aimed at improving bread making quality and to broaden the genetic base of this important food crop of Pakistan. Since the information obtained reflects the potential usefulness of the wheat germplasm collections therefore efforts are being made to expand the data base by characterizing the remaining germplasm.

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