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Genetic Diversity of Hexaploid Wheat Based on Polymorphism in Quality Characteristics

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Abstract: High Molecular Weight Glutenin Subunits (HMW-GS) were used as markers to assess the genetic diversity among 52 local wheat genotypes and their yield producing capacity with the object of exploiting diversity in the commercial varieties and landraces grown in different regions of Pakistan. HMW-GS profiling of wheat genotypes was done through SDS-PAGE; polymorphism was revealed at HMW-GS encoding loci; *Glu-A1*, *Glu-B1* and *Glu-D1*; alleles 3, 6 and 4 were identified, respectively. The most common composition of HMW-GS in the population was 2*, 17+18, and 2+12. ANOVA revealed significant variation among the varieties for yield parameters and also there is correlation found between these parameters. The average intrapopulation heterozygosity for the three *Glu-1* loci was high in Punjab (60.36%) compared to the other populations that is Sindh (48.88%), Baluchistan (33.33%), Azad Jammu Kashmir (30.36%) and Khyber Pakhtunkhwa (55.98%). Among populations Punjab had maximum genetic similarity of 93.04% with Sindh thus a small genetic distance of 7.21% which showed that the two populations are more identical. Genetic distance was large between the population of Azad Jammu Kashmir and Khyber Pakhtunkhwa. Though the bread-making quality of wheat is good but the heterogeneity is low among the wheat varieties of Pakistan showing a need for improving the genetic pool of the local genotypes for the future breeding programs.

Key words: Bread-making, genetic diversity, genetic pool, heterogeneity, HMW-glutenins, quality, yield parameters

INTRODUCTION

In the past wheat research was more focused on improving yield of the crop, that plant breeders have ignored the importance of quality of wheat. By quality it means the presence of glutenin and gliadin proteins which determine the dough properties of wheat flour and the protein content which gives the quantitative estimation of protein level of the flour. These two traits together with the yield production capability of different wheat varieties actually define the overall quality of wheat whether good or poor. Grain yield in wheat is one of the most important and complex character affected directly or indirectly by gene present in plant (Bhutta *et al*., 2005) as well as interaction of environment. Knowledge of the genetic association between grain yield and its components can help the breeders to improve the efficiency of selection (Heidari *et al*., 2005). Wheat bread making potential is derived largely from the quantity and quality of its protein. Protein quantity is influenced by environmental factors, while the quality of protein is genetically determined. In wheat varieties that are grown under comparable environmental conditions, high quality wheat will produce good bread over a fairly broad range of protein levels. Poor quality wheat will yield relatively low quality wheat bread even at high protein contents (Hruskova and Famera, 2003). The ability of wheat flour to be processed into different foods is largely determined by the gluten proteins (Weegels *et al*., 1996). Gluten composition is the main factor that determines the quality characteristics of wheat cultivars (Du-Cros, 1987; Kovacs *et al*., 1993). Gluten is complex protein composed of glutenins and gliadins. Glutenins are further separated into high molecular weight (HMW-GS) and low molecular weight (LMW-GS) subunits. LMW-GS are associated with the omega-gliadins, thus difficult to study as bands are found clustered.

The protein content of the grain appears to be determined by a combination of genetic and environmental factors; with variation in nutrition (particularly availability of N) resulting in considerable variation in protein content within genetically determined limits (Shewry, 2006). Also there is a strong negative correlation between grain protein content and grain yield; cultivars with high grain protein content tend to be lower yield (Khan *et al*., 2000; Gonzalez-Hernandez *et al*., 2004). Grain yield and grain protein concentration through, its contribution to end-use quality, are the most important characters determining the economic value of a bread wheat crop (Oury and Godin, 2007). The allelic diversity of the HMW-GS found in bread wheat has been described by many scientists who routinely use these

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proteins as genetic markers for quality improvement in breeding programs. It has been claimed that plant breeding reduces genetic diversity in elite germplasm which could seriously jeopardize the continued ability to improve crops (Reif *et al.*, 2005). Diversity levels of plant populations may change rapidly with changing environment, with scio-economic factors such as the introduction of new varieties, the movement of people with their germplasm, and commercial marketing of both seed for planting and the grain produced, or with changing agronomic practice including cultivation methods and use of fertilizers or pesticides (Donini *et al*., 2000; Christiansen *et al*., 2002). Plant breeders are destroying the genetic base for new generation of varieties produced by the breeders showed narrow genetic diversity. To study whether this concept is hold true or not, diversity between the wheat genotypes locally grown was studied and also to understand the quality of our wheat that this research paper has focused on the HMW-Glutenin composition, protein content and yield and the diversity regarding the glutenin compositions found in different regions of Pakistan.

MATERIALS AND METHODS

Plant material: The seed material used for this study comprised of 48 local hexaploid cultivars and 4 landraces of Pakistan. This local wheat germplasm was obtained from National Agriculture Research Centre (NARC), Islamabad, Sindh Agriculture University, Tandojam and Agricultural University, Faisalabad, Pakistan. The seeds were sown in the years 2005-2006 and 2006-2007. Plantations were made in the screen house within the premises of Department of Genetics, University of Karachi, Pakistan. In this study the varieties were divided into five groups in accordance to their origin or place of release, with the objective to test significant differences if any in the occurrence of allelic frequencies at *Glu-1* loci among the wheat genotypes of Pakistan. These five regions were Sindh (S), Baluchistan (B), Azad Jammu Kashmir (AJK), Punjab (P), and Khyber Pakhtunkhwa (KP).

Cultural practice: Plots were prepared by mixing sandy loam soil and manure in a ratio of 1:1. Sowing of 50 seeds was done in Complete Randomized Design in plots keeping a distance of 50 cm between the rows. Normal cultural practice was observed throughout the season that included irrigation, according to the need and weather. Germination percentage was recorded after 15 days of sowing. After 15 days of germination thinning was done manually leaving 10 plants per row with a distance of 1.5 inches between each plant. A record of daily temperature and rain was maintained. After maturation crop was harvested in April.

Agronomic parameters: Observations were recorded from twenty representative plants, 10 for each row of each wheat variety. The data of number of tillers per plant, flag leaf area, plant height, number of spikes per plant, spike length, number of spikelet per spike, number of grains per plant, 1000 grain weight, biomass, harvest index and grain yield were recorded. The experiment was repeated twice for the years 2006 and 2007.

Quantitative estimation: Total proteins were extracted the homogenizing buffer containing 20 mM Tris-HCl (pH-7.5), 3 mM Na-EDTA, 0.25 mM Sucrose and 0.2 mM β-mercaptoethanol. Seeds were crushed in a pre-chilled pestle and mortar containing 2 mL of cold homogenizing buffer solution per gram fresh weight of material (1:2). Centrifuged at 14,000 rpm for 15 min and then, the resultant supernatant containing the soluble fraction was collected and stored for further use at -4°C. Quantitative estimation of total protein content was performed as described by Lowry *et al*. (1951).

Qualitative analysis: A single seed has been used of each wheat variety with four repeats for the protein quality analysis using SDS-PAGE as described by Lammeli (1970). Protein extraction was done using Singh *et al*. (1991) modification method. The HMW-GS were analyzed through SDS-PAGE using 10% running gel and 3% stacking gel. The presence of 2*was confirmed on 7% running gel.

Table 1: ANOVA of the agronomic traits of the hexaploid wheat genotypes

Trait	SoV	df	MS
No. of tillers	b/w Genotypes	50	5.974**
	within Genotypes	437	1.561
No. of spikes	b/w Genotypes	50	4.313**
	within Genotypes	437	1.289
Flag leaf area	b/w Genotypes	50	490.801**
	within Genotypes	437	36.231
Plant height	b/w Genotypes	50	634.328**
	within Genotypes	437	58.196
Spike length	b/w Genotypes	50	13.475**
	within Genotypes	437	1.238
No. of spike	b/w Genotypes	50	87.240**
lets/spike	within Genotypes	437	4.305
No. of seeds	b/w Genotypes	50	11074.944
/plant	within Genotypes	437	2340.970
Grain yield	b/w Genotypes	50	21.405**
	within Genotypes	437	3.195
1000 grain	b/w Genotypes	50	22242.931**
weight			
	within Genotypes	437	550.228
Biomass	b/w Genotypes	50	121.749**
	within Genotypes	437	15.582
Harvest index	b/w Genotypes	50	1131.175**
	within Genotypes	437	171.362

**: Significance at 0.01 level of probability ($p \leq 0.01$); SoV: Sources of Variation

Table 2: Correlation coefficient between the agronomic traits of hexaploid wheat genotypes

	T	S	FLA	PH	SL	SPKL	G	GY	GW	B	HI
T	1	0.938**	0.370**	0.385**	0.228	0.160	0.696**	0.773**	0.554**	0.835**	- 0.129
S		1	0.311*	0.331*	0.128	0.082	0.727**	0.789**	0.459**	0.814**	- 0.133
FLA			1	0.517**	0.583**	0.365**	0.402**	0.401**	0.522**	0.521**	- 0.203
PH				1	0.300*	0.196	0.172	0.246	0.461**	0.409**	- 0.372**
SL					1	0.725**	0.440**	0.415**	0.420**	0.430**	0.075
SPKL						1	0.537**	0.486**	0.364**	0.435**	0.223
G							1	0.931**	0.358**	0.770**	0.229
GY								1	0.500**	0.865**	0.196
GW									1	0.645**	- 0.221
B										1	- 0.258
HI											1

**: Indicates highly significant correlation; *: Indicates significant correlation; T = No. of tillers; S = No. of spikes; FLA = Flag leaf area; PH = Plant height; SL= Spike length; SPKL = No. of spikelets; G = No. of grains; GY = Grain yield; GW = 1000 grain weight; B = Biomass; HI = Harvest index

Table 3: Total protein contents in hexaploid wheat genotypes

Genotypes	Protein (mg/g f.wt)	%	Genotypes	Protein (mg/g f.wt)	%
Abadgar	131	13.1	Fasialabad -85	148	14.8
Anmol-91	120	12.0	GA-2002	81	8.1
Bhittai	131	13.1	Inqilaab-91	86	8.6
Khirman	60	6.0	Kohistan	106	10.6
Kiran	110	11.0	LU-26	136	13.6
Marvi-2000	66	6.6	Manthar	100	10.0
Mehran-89	154	15.4	Mexipak	136	13.6
Moomal-2002	164	16.4	Pak-81	126	12.6
Sarsabz	76	7.6	Pasban-90	76	7.6
TD1	148	14.8	Pavon	106	10.6
TJ-83	100	10.0	Punjab-81	48	4.8
Zarlashta-99	116	11.6	Punjab-96	100	1.0
Zamindar-80	148	14.8	Rawal-87	170	17.0
Zarghoon-79	71	7.1	SA-75	180	18.0
Sariab-92	142	14.2	SH-2003	71	7.1
Foder	142	14.2	Shehzor	296	29.6
Macs	232	23.2	Watan	90	9.0
Sagar	116	11.6	Bakhtawar-94	66	6.6
Arz	126	12.6	Fareed-06	156	15.6
Aqaab-2000	131	13.1	Haider-2000	66	6.6
AS-2003	81	8.1	Khyber-87	142	14.2
Barani-70	154	15.4	Margalla-99	100	10.0
Barani-83	146	14.6	Pirsabak-85	172	17.2
Chakwal-86	116	11.6	Pirsabak-91	170	17.0
Chenab-70	90	9.0	Saher-06	136	13.6
Chenab-2000	124	12.4	Tatara	131	13.1

Scoring and data analysis: Analysis of variance (ANOVA) was performed to estimate the differences between and within the varieties for different agronomic parameters. Correlation coefficient was also calculated to determine the association between various agronomic parameters. Statistical analysis of agronomic data was done by using computer software 'SPSS version-11.0 (2001)'. Banding pattern of each variety was studied and identified using the nomenclature of Payne and Lawrence (1983). *Glu-1* score was calculated according to Payne (1987) by adding the score of individual subunits. Allelic diversity was estimated according to Nei (1973).

RESULTS

The analysis of variance revealed significant differences among the wheat genotypes for all the traits (Table 1). The wheat cultivars have highly significant variation for all the yield parameters; spike length per plant, number of spikelets per spike, number of seeds per plant, grain yield, and 1000 grain weight. Cultivars under study showed highly significant differences regarding grain yield. The minimum grain yield was recorded in Foder (0.83 g) which is a landrace; where as the maximum yield was observed in Haider-2000 (8.50 g). The correlation between agronomic traits was also performed to find out association between the parameters (Table 2). The data showed significant correlations between the different agronomic traits. Grain yield had highly significant correlation with all the yield parameters which is important to decide the selection criteria. Grain yield showed a highly significant positive correlation with flag leaf area (0.401**) which is important as flag leaf area can be anindicator of grain yield in wheat.

The current research is an extension to earlier study by Afshan and Naqvi (2011), data not shown. The

Table 4: Intrapopulation homozygosity and heterozygosity for the hexaploid wheat genotypes

		Allelic frequencies				
Locus	Alleles	S	B	AJK	P	PK
A	1	0.27	0.75	1.00	0.28	0.22
	2*	0.45	0.00	0.00	0.56	0.22
	null	0.27	0.25	0.00	0.16	0.55
Locus homozygosity		0.3539	0.625	1.000	0.4176	0.4072
Locus heterozygosity		0.6461	0.375	0.000	0.5824	0.5928
B	6.1	0.27	0.00	0.00	0.08	0.00
	7	0.00	0.25	0.33	0.08	0.33
	7+8	0.00	0.00	0.00	0.08	0.00
	7+9	0.27	0.00	0.00	0.12	0.22
	13+16	0.00	0.25	0.00	0.20	0.22
	14+15	0.00	0.00	0.00	0.00	0.00
	17+18	0.72	0.50	0.66	0.52	0.22
Locus homozygosity		0.6763	0.375	0.5445	0.344	0.259
Locus heterozygosity		0.3237	0.625	0.4555	0.656	0.741
D	2+12	0.45	0.00	0.66	0.44	0.22
	5+10	0.54	1.00	0.33	0.48	0.77
	4+12	0.00	0.00	0.00	0.04	0.00
	4+12*	0.00	0.00	0.00	0.04	0.00
Locus homozygosity		0.5031	1.000	0.5445	0.4272	0.6542
Locus heterozygosity		0.4969	0.000	0.4555	0.5728	0.3458
Average heterozygosity	Hi	0.4888	0.3333	0.3036	0.6036	0.5598
Average homozygosity	Ji	0.5112	0.6667	0.6963	0.3964	0.4402

S = Sindh; B = Baluchistan; AJK = Azad Jammu Kashmir; P = Punjab; KP = Khyber Pakhtunkhwa

Table 5: Average interpopulation heterozygosity among the five sub-populations

S/B	S/AJK	S/P	S/KP
0.6100	0.5932	0.5812	0.6535
B/AJK	B/P	B/KP	
0.5025	0.6467	0.5600	
AJK/P	AJK/KP		
0.6339	0.7088		
P/KP			
0.6832			

published data reported the presence of alleles 1, 2* and null at *Glu-A1* locus. The most polymorphic locus of the three was *Glu-B1*, with the presence of 7, 7+8, 7+9, 13+16, 17+18, and 6.1 subunits. At the *Glu-D1* locus four different combinations were found, that of 5+10, 2+12, 4+12 and 4+12*. The reported *Glu-1* score ranges from 6-10 with an average of 8, indicating that the Pakistani wheat possesses strong gluten strength and have good bread-making quality. *Glu-1* score of 6.1 and 8 subunit was not known.Total crude protein contents measured represent about the amount of microgram protein/gram fresh weight in different wheat genotypes (Table 3).

The maximum amount of protein content was observed in Shehzor and Macs; 296 (29.6%) and 232 (23.2%) µg protein /g f.w.t., respectively. The lowest protein content was noted in Khirman having 66 µg protein/g f.wt, while Marvi-2000, Bakhtawar and Haider-2000 all have 66 µg protein/g f.wt of protein content which makes 6.6% of protein concentration. But most of the varieties showed protein content level ranging between 11-18% which is considered as a good level of protein quantity.

The correlation between the quality score and yield parameter was also performed and mostly non-significant results were obtained but the important association was found between quality score and flag leaf area which showed positive and significant (0.286*).

The whole sample population was divided into five sub-populations according to the origin of the wheat genotype and where the variety been released from. The five sub-populations are Sindh, Baluchistan, Azad Jammu Kashmir, Punjab and Khyber Pakhtunkhwa.The frequency distribution of glutenin subunits at the three *Glu-1* loci in five sub-populations is demonstrated in Fig. 1.

Locus heterozygosity: Locus heterozygosity was calculated for the individual subpopulation using Nei's formula (1973). Considerable heterozygosity was observed within the populations at the three *Glu-1* loci. In Sindh (S) at *Glu-A1* 64%, at *Glu-B1* 32% and at *Glu-D1* 49% gene diversity was obtained showing the dissimilarity that exists within the varieties from the same population (Table 4). Here more variation was found at the *Glu-A1*. Similarly from Baluchistan (B) *Glu-A1* locus gave a heterozygosity of 37%, *Glu-B1* of 62%. But at the *Glu-D1* locus no gene diversity was found as 100% of the population possessed subunit 5+10. In landraces from Azad Jammu Kashmir (AJK) there was similar intrapopulation diversity for *Glu-A1*, for *Glu-B1* and for *Glu-D1*; that showed same percent locus heterozygosity of 45%. Varieties from Punjab (P) exhibited more heterozygosity, *Glu-A1* had 58%, *Glu-B1* 65% and *Glu-D1* had 57% locus heterozygosity. In Khyber Pakhtunkhwa (KP) *Glu-A1* showed gene diversity of 59%, *Glu-B1* had 74% and *Glu-D1* had 34%, here *Glu-B1* locus was most polymorphic.

Average intrapopulation diversity: For Sindh it was 48.88%, Baluchistan 33.33%, AJK 30.36%, Punjab 60.36% and Khyber Pakhtunkhwa had 55.98%. The most polymorphic subpopulation was Punjab with 60.36% heterozygosity and the least polymorphic was AJK which possessed 30.36% gene diversity.InAzad Jammu Kashmir 69% average homozygosity exists within the population, which is higher compared to the other populations, indicating fixation of alleles in this population.

Interpopulation diversity: The interpopulation homozygosity and heterozygosity was also determined to understand the percentage of similarities and dissimilarities that existed among the five sub-populations

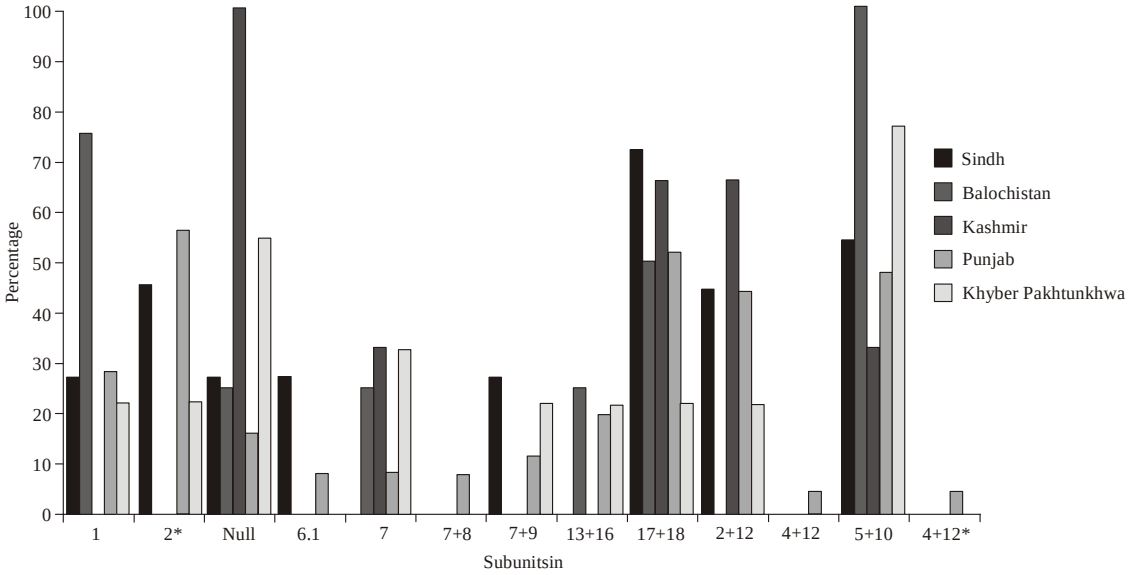

Fig. 1: Percent frequency distribution of GLU-1 subunitsin wheat genotypespooled a cross five regions of Pakistan

(Table 5). The maximum interpopulation heterozygosity was observed between the genotypes from regions AJK and KP, which had a value of 70.88%. The minimum heterozygosity was between Baluchistan and Azad Jammu Kashmir with a value of 50.25%.Sindh varieties with rest of the sub-populations had the diversity of 61% with Baluchistan varieties, 59.32% diversity with Azad Jammu Kashmir, 58.12% with Punjab and 65.35% with Khyber Pakhtunkhwa respectively. Baluchistan showed maximum gene diversity with Punjab of 65%. It had quite similar difference with AJK and KP with the 52% and 56% diversity, respectively. AJK showed minimum diversity with the wheat varieties from Baluchistan, 52%. With Punjab, Azad Jammu Kashmir had the heterozygosity of 63%. Khyber Pakhtunkhwa showed the difference of 71% with Punjab.

DISCUSSION

The current study revealed that grain yield which is a parameter of prime importance has significant variation among the wheat cultivars. The flag leaf contributes the most photosynthetic assimilates in wheat; therefore, it assumes the greatest importance in terms of grain yield (Lupton, 1973). A greater flag leaf area will eventually help to increase photosynthetic efficiency by increasing their weight. Therefore, flag leaf area has a direct relationship to grain yield (Riaz and Chowdhry, 2003). Another important correlation was found between flag leaf area and high molecular weight glutenins quality score, which showed significant and positive association (0.286*). This correlation of flag leaf area with grain yield and quality score indicates an indirect association or correlation of grain yield with quality score. But there is a strong negative correlation between grain protein content and grain yield, and also between grain protein content and quality score, however there are genotypes which possess high yield potential along with good protein quantity and quality as well.

The overall diversity calculated was minimal in the sample population, indicating that the varieties released by the plant breeders are some what similar to each other in their allelic compositions (Afshan and Naqvi, 2011). There are slight variations found like the presence of 6.1, 4+12* and 4+12. In order to improve the bread making quality of Pakistani bread wheat it was recommended to be advantageous to select for good quality subunits like 5+10, 17+18 and 2* (Masood *et al*., 2004) but in doing so the breeders are also decreasing the genetic variation in the common wheat as more and more alleles are getting fixed. In a highly self pollinated crop like wheat whose gene pool is already getting narrow such an approach is not suitable. This could be a problem for the future improvement of wheat crop regarding its quality and yield. Heterozygosity is the presence of allele at one or more loci on homologous chromosomes. Heterozygosity is the estimate of similarity and dissimilarity for individual locus in a population or between populations in terms of frequencies of alleles found in each population. The average heterozygosity and homozygosity was estimated among the five sub-populations, results revealed the differences in the presence of allelic combinations in wheat genotypes but the diversity calculated between the varieties was minimum that indicates that the varieties selected by the breeders to develop new varieties are quiet similar to each other in

their qualitative composition. This is probably due to the fact that most breeding programs in Pakistan were more focus on improving the yield of the crop that the researchers somehow neglected the quality improvement of the Pakistani wheat.In the current study the maximum difference was observed between the sub-population of Azad Jammu Kashmir and Khyber Pakhtunkhwa, this difference is needed to be confirmed by studying more samples from both the populations. The least population difference between the varieties from Sindh and Punjab has the probability of sharing somewhat same environment.Genetic distance on the other hand is the estimate of the genetic relatedness of two populations. It refers to the genetic divergence between two species or two populations within specie.

The genetic distance calculated showed that at the genetic level varieties share more common genes. Though the wheat varieties grown in Pakistan possess good bread making quality but carry a narrow gene pool. There is high genetic diversity present among the Pakistani wheat which has been reported previously (Tahir *et al*., 1996; Branlard, 2004; Ahmed, 2004; Afshan and Naqvi, 2011), and it is necessary to maintain this variation for the improvement of Pakistani wheat.

CONCLUSION

After the green revolution Pakistani farmers were successful in producing good quality wheat but fewer efforts were made for the broadening of the gene pool of our crop. Previous breeding programs have focused more on improving the wheat producing capacity of wheat but in doing so the genes responsible for the bread-making property became fixed. Though the quality of wheat grown is good but the new varieties being produced are some what similar thus indicating the narrowness of the gene pool. It is necessary to focus our research towards the wheat landraces to find out the variations and use it in improving the genetic diversity of Pakistani wheat.

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