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Full Length Research Paper

Genetic diversity and disease response of rust in bread wheat collected from Waziristan Agency, Pakistan

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In the present study, thirty indigenous landraces exclusively collected from Waziristan Agency, Khyber Pakhtunkwa, Pakistan along with 14 cultivars were investigated for genetic diversity and resistance to fungal diseases. The germplasm was evaluated for morphological traits, response to rust and smut, and seed protein markers. A low level of allelic variation was observed for morphological trait, while the agronomic traits exhibited higher level of coefficient of variation. In the present study, six lines (*MIRALI (ECDOCK) NWA*, *MIRALI NWA-1*, *MIRALI NWA-2*, *BANNU PROPER*-1 and *FR-BANNU DOMEL*) were highly resistant to rust disease. The SDS-PAGE was carried out to assess the genetic diversity and selection of elite genotypes based on proteomic homology of high molecular weight (HMW) glutenin (*Glu-A*1) using Jaccard's similarity coefficient. Almost 16% genetic diversity (low level) was observed in HMW glutenin protein and the germplasm was grouped into five clusters. The cluster 5 sorted resistant lines, while the others were inter-spread, although few of these were grouped on the basis of collection sites. The genotype from Chakdara-2 of Cluster 1 was unique due to the presence of all the polypeptides of HMW glutenin protein. Based on HMW glutenin, the resistant line from *Bannu Proper*-1 and *FR-Bannu Domel* were similar to the improved line *RAJ* and *BAKHTAWAR-92*. On the basis of this initial investigation it is suggested to investigate this germplasm for their adaptability and for molecular markers to employ this unique germplasm in modern wheat cultivars.

Key words: Diversity in *Glu-A*1 loci, novel immune lines, Pakistani wheat, *Puccinia triticina*, SDS-PAGE,

INTRODUCTION

Wheat is a staple food for 35% of the world's population and is grown on 17% of the cultivated area in the world (Kronstad 1998). It occupies 70% of Rabbi (winter season) and 37% of total cropped area in Pakistan (Shah et al., 2003). Wheat is affected by leaf rust and smut, serious fungal diseases that is a continuous threat to its cultivation. Infection can cause up to 20% yield losses and resulting grain to shrivel. Pakistan is rich with unique germplasm variation in many crop species, especially wheat. Efficient genetic evaluation is the first step to any crop breeding program. Once the genetic nature of the germplasm is investigated, then one can develop a possible outcome for the future generation for food security that is experiencing a lot of risks at present.

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The knowledge of genetic diversity is a useful tool in gene-bank management and breeding experiments like tagging of germplasm, identification and/or elimination of duplicates in the gene stock and establishment of core collections. Due to vitality characterization, Dotlacil et al. (2002) studied 123 wheat lines; Gupta et al. (2002) and Pawar et al. (2003) reported genetic divergence among different wheat advanced cultivars to document the morphological and agro-economical data.

During recent years, biochemical and molecular genetic techniques have emerged as a complementary strategy in conjunction with traditional approaches in the management of plant genetic resources. Lot of work had been published for estimation of genetic diversity using SDS-PAGE for Low as well as high molecular weight glutenin subunits in wheat (Benmoussa et al., 2000; Popa et al., 2006; Sultana et al., 2007; Caballero et al., 2009). Wiser (2007) indicated that gluten proteins play a key role

in determining the unique baking quality of wheat by conferring water absorption capacity, cohesively, viscosity and elasticity on dough. Tahir et al. (1996) identified novel high molecular weight glutenin subunit in a hexaploid wheat landrace collected from Baluchistan that is known for wider genetic diversity for most of the cereal. The wheat endosperm storage proteins are widely associated with the bread making quality (Wrigley et al., 2006). There is no report on indigenous wheat genetic resources from Waziristan Agency, Pakistan that is highly vulnerable to biological as well as geo-political instability.

The work was devoted to explore the untouched agro-ecological zones of Pakistan for wheat collection and to document the genetic resources of wheat using morphological and seed protein markers for future utilization for wheat improvement program.

MATERIALS AND METHODS

Plant materials

In the present study, thirty indigenous landraces collected from Waziristan Agency, Khyber Pakhtunkhwa, Pakistan, along with 14 cultivars (Pakistani origin) was also included (Table 1). Different experiments were conducted to investigate the genetic diversity and resistance to fungal diseases. The genetic diversity was investigated through morphological characterization and biochemical markers.

Morphometrics study

The morphological characterization was split into qualitative and quantitative traits. The qualitative traits were scored through general visualization (phenotypic observation) on the genotype basis and 3 qualitative traits (plant color, growth habit and logging activity) were studied at the relevant stage of development in the available germplasm. Data on quantitative traits were recorded for plant height (cm), spike length (cm), Awn length (cm), 1-leaf length, 2-leaf length, 3-leaf length, 4-leaf length, biomass (g), 100-grain weight, grain yield and harvest index.

Disease status

The disease status was examined visually and the severity of disease was presented in percentage for rust disease. The genotypes showing resistance under natural infestation were artificially inoculated with *Puccinia triticina* spores by tapping heavily infected plant parts over the leaves. Severity of the disease was categorized into 0, 2.32, 2.85, 5.26, 5.88, 6.06, 6.66, 6.97, 10, 10.6, 14.28, 15.15, 17.39, 21.7, 28.99, 43.9, 48, 53.19, 54.83, 59.25, 61.1, 61.76, 70, 90.9, 91.3 and 100%, respectively. Effect on different phases as well as on different parameters was observed.

Biochemical assay

To explore the genetic diversity on the basis of protein, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. For SDS-PAGE analysis, grains of each genotype were crushed into a fine powder with mortar and pestle. Protein extraction buffer (400 µl) was added to 0.01 g of grain flour and mixed well with Automatic Lab-Mixer DH-10. The extraction buffer contained the following final concentrations: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol blue (BPB) was added to the protein extraction buffer as tracking dye to watch the movement of protein in the gel. The molecular weight of the dissociation polypeptides was determined by using molecular weight protein marker MW-SDS-70 Kit (Sigma).

The SDS-PAGE of total grain protein was carried out in the discontinuous buffer system according to the method of Laemmli (1970). Acrylamid gel concentration 12.5% and 6 µl of sample were used for analyzing germplasm. After staining and de-staining, the gel was dried using (Atto, Rapidery-Mini Japan) gel drier. In order to check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. The data was scored on the presence (1) or absence (0) of protein bands and high intensity of glow was considered as major bands and low intensity as minor bands.

Data analysis

Similarity indices of 43 genotypes were calculated for all possible pairs of protein types and used to construct dendrogram by the UPGMA (Sneath and Sokal, 1973) by computer software NTYSIS Version -2.0. To find out genetic similarity between the collected genotypes from North Waziristan Agency and obtained cultivars, Jaccard's similarity index (S) was calculated for possible pairs of protein types by S = W/ (A+B-W) (Sneath and Sokal, 1973). Whereas W = the number of bands of common mobility. A = the number of bands in protein type 'A' and B = The number of bands in protein type 'B'. Cluster analysis and principal components analysis was calculated using the un-weighed pair-group method arithmetic average (UPGMA) using NTYSIS vir-2.0.

RESULTS

Morphometrics study

The results regarding qualitative and quantitative traits are presented in Table 1 and 2, respectively and all the characters showed significant level of genetic divergence. Thirteen genotypes that is FR (Frontier Region)-Bannu Marghali Pirbakhel; FR- Bannu Domel-1; Mirali NWA-3; Miranshah NWA-1; Miranshah NWA-2; Bannu Proper-2; Mirali NWA-4; Mirali NWA-5; North Waziristan Agency-3; Waziristan NWA; Bannu-2, Mardan, Chakdara showed best performance in the present collection. The lodging ability of all the collected genotypes was also investigated. In the collected germplasm, the genotype of Mirali (ECDOCK) NWA, Bannu Plains Areas, FR-Bannu, FR-Bannu (Dry Area), FR-Bannu Domel, Miranshah NWA lodged after > 100 days (Table 1).

Plant height was positively and significantly correlated with 1st leaf length (r = 0.64), 2nd leaf length (r = 0.58), 3rd leaf length (r = 0.61), biomass (r = 0.71) and grains yield (r = 0.79). However it was negatively correlated with 100-grain weight. Spike length showed positive correlation with awn length, while it was negatively correlated with 100-grain weight. First leaf length showed highly positive correlation with 2nd leaf length, 3rd leaf length, and

Table 1**.** Genetic diversity based on qualitative traits in wheat germplasm.

R/No.	Genotypes	Plant color	Growth habit	Logging
1	Mirali (ECDOCK) North Waziristan Agency	Green	Weak/normal	Susceptible
2	Mirali North Waziristan Agency-1	Green	Normal	Resistant
3	North Waziristan Agency-1	Green	Weak/normal	Resistant
4	FR-Bannu Marghali Pirbakhel	Green	Vigorous	Resistant
5	FR-Bannu-1	Green	Weak	Resistant
6	Mirali North Waziristan Agency-2	Green	Weak/normal	Resistant
7	FR-Bannu-2	Dark green	Normal	Resistant
8	Bannu proper-1	Green	Weak/normal	Resistant
9	Bannu plain areas-1	Green	Weak	Resistant
10	Bannu plain areas-2	Pale Green	Weak	Susceptible
11	FR-Bannu-1	Green	Weak	Susceptible
12	FR-Bannu (dry area)	Green	Weak	Susceptible
13	FR-Bannu Domel-1	Green	Weak/normal	Resistant
14	FR-Bannu Domel-2	Green	Weak	Susceptible
15	FR-Bannu Marghali Pirbakhe-l	Green	Normal	Resistant
16	Mirali North Waziristan Agency-3	Dark green	Vigorous	Resistant
17	FR-Bannu-2	Dark green	Weak/normal	Resistant
18	FR Bannu Domel-3	Dark green	Normal	Resistant
19	Miranshah North Waziristan Agency-1	Dark green	Vigorous	Susceptible
20	Miranshah North Waziristan Agency-2	Dark green	Vigorous	Resistant
21	Bannu Proper-2	Dark green	Vigorous	Resistant
22	North Waziristan Agency-2	Dark green	Vigorous	Resistant
23	Mirali North Waziristan Agency-4	Dark green	Vigorous	Resistant
24	North Waziristan Agency-3	Dark green	Vigorous	Resistant
25	Mirali North Waziristan Agency-5	Dark green	Vigorous	Resistant
26	Bannu-1	Dark green	Normal	Resistant
27	Waziristan North Waziristan Agency	Dark green	Vigorous	Resistant
28	Bannu-2	Dark green	Vigorous	Resistant
29	Mardan-1	Dark green	Vigorous	Resistant
30	Mardan-2	Dark green	Vigorous	Resistant
31	Chakdara-1	Dark green	Vigorous	Resistant
32	Chakdara-2	Dark green	Vigorous	Resistant
33	Check 29	Dark green	Vigorous	Resistant
34	Check 30	Dark green	Weak	Resistant
35	Check 31	Dark green	Normal	Resistant
36	Check 32	Dark green	Weak	Resistant
37	Check 33	Dark green	Vigorous	Resistant
38	Check 34	Dark green	Vigorous	Resistant
39	Check 35	Dark green	Vigorous	Resistant
40	Check 36	Dark green	Vigorous	Resistant
41	Check 37	Dark green	Vigorous	Resistant
42	Check 38	Dark green	Weak	Resistant
43	Check 39	Dark green	Weak/normal	Resistant
44	Check 40	Dark green	Weak	Resistant

biomass and with grain yield, while it was negatively correlated with 100-grain weight and harvest index. Second leaf length had positive relationship with 3rd leaf length, 4th leaf length, biomass and grain yield, while it was inversely associated with 100-grain weight and harvest index. The 3rd leaf length showed high positive correlation with 4th leaf length, biomass and grain yield, while it was negatively correlated with 100-grain weight and harvest index. The fourth leaf length had positive correlation with biomass and grain yield, while it was

Table 2. Comparative descriptive statistics of eleven quantitative traits of Pakistan wheat genotypes and cultivars.

Traits	Mean ± St-dev		St-EE		Range		Min		Max		CV %	
	Genotype	Check	Genotype	Check	Genotype	Check	Genotype	Check	Genotype	Check	Genotype	Check
Plant height (cm)	58.7 ± 19.33	48.12 ± 6.90	3.47	2.08	89.42	24.48	27.6	36.66	117.02	61.14	*32.92***	14.35
Spike length (cm)	8.1 ± 4.47	8.19 ± 1.40	0.8	0.42	26.6	4.98	2	5.74	28.6	10.72	*54.62***	17.12
Awn length (cm)	5.9 ± 1.15	6.23 ± 0.80	0.21	0.24	5.9	2.42	1.2	5.06	7.1	7.48	19.42	12.84
1-leaf length	19.05 ± 3.6	22.82 ± 1.23	0.65	0.37	16.2	3.52	13.6	20.98	29.8	24.50	*18.91*	5.39
2-leaf length	23.28 ± 4.47	25.72 ± 1.63	0.8	0.49	21.18	6.20	15.54	23.32	36.72	29.52	*19.19*	6.32
3-leaf length	22.15 ± 4.27	22.77 ± 1.53	0.77	0.46	19.54	4.42	13.56	20.52	33.1	24.94	*19.27*	6.70
4-leaf length	20.5 ± 3.89	19.36 ± 2.61	0.7	0.79	16.4	7.54	11.7	15.32	28.1	22.86	*18.96*	13.47
Biomass (g)	18.63 ± 6.91	19.96 ± 5.40	1.24	1.63	28.84	17.12	9.2	14.40	38.04	31.52	*37.08***	27.07
100-grain weight	4.64 ± 0.35	5.10 ± 0.43	0.06	0.13	1.57	1.50	3.79	4.19	5.36	5.69	7.53	8.49
Grain yield	5.42 ± 1.9	6.33 ± 1.01	0.34	0.30	7.79	3.50	2.6	4.77	10.39	8.27	*35.09***	15.90
Harvest index	30.29 ± 8.04	32.79 ± 5.89	1.44	1.78	41.2	18.53	18.75	25.06	59.96	43.60	*26.53***	17.97

Standard error-St-EE; Standard deviation-St-dev; CV %- Coefficient of variance in percent ** Genotypes represent significant variation with respect to cultivars; highlighted box showed high level of %CV in comparison to checks/cultivars/improved lines

negatively correlated with 100-grain weight and harvest index. Biomass had significant correlation with grain yield, while it was negatively correlated with 100-grain weight and harvest index. The 100-grain weight had negative correlation with grain yield, whereas grain yield had highly significant correlation with harvest index (Table 3).

Disease response

The genotypes collected from Mirali (ECDOCK) NWA, Mirali North Waziristan Agency-1, FR-Bannu-1, Mirali North Waziristan Agency-2, Bannu Proper1 and FR-Bannu Domel-1, were resistance to rust caused by *Puccinia triticina*, while the remaining were affected at the range of 9.2% and considered as escaping lines. All the check varieties (from serial # 31-44) were highly susceptible to rust disease and they were averagely affected at 78.5% (Table 4). After artificial inoculation, the lines showed immunity to pathogen was genetically resistant and hence genotypes (Mirali (ECDOCK) North Waziristan Agency, Mirali North Waziristan Agency-1, FR-Bannu-1, Mirali North Waziristan Agency-2, Bannu Proper-1 and FR Bannu Domel -1) were selected as immune lines.

Molecular characterization

The SDS-PAGE was carried out on 15% polyacrylamide gel concentration. To check reproducibility of the polypeptide bands, the experiment was repeated twice and only consistent markers were reported. The binary data was analyzed for similarity indices to investigate proteomic homology using Jacquard's similarity index. The genotypes at 83% coefficient of similarity were divided into two main lineages, while the genotypes at 90% coefficient of similarity were divided into five clusters. In cluster analysis, cluster 1 and 2 explained 89%, cluster 1 with 2 and 3 indicated 84%, and cluster 3 and 4 resolved 86%; whereas cluster 4 with 3 and 5 explained 86% genetic similarity in the glutenin protein profile (Figure 1). Based on HMW glutenin, Jaccard's similarity coefficient was calculated, which showed that the genotypes of Bannu Proper-1, FR Bannu Marghali Pirbakhel and Mirali North Waziristan Agency-4 were similar to Raj. While the genotypes of Mirali (ECDOCK) North Waziristan Agency, Mirali North Waziristan Agency-1, Bannu Plain Areas-2, FR Bannu Domel-1, FR Bannu Domel-3, Miranshah North Waziristan Agency-1, Miranshah North Waziristan Agency-2, Bannu Proper-2 Waziristan Agency-2 and Waziristan North Waziristan Agency were less (80%) similar to Raj. Among the germplasm, the genotype of Bannu Proper-1, Mirali (ECDOCK) North Waziristan Agency, Mirali North Waziristan Agency-1, and FR Bannu Domel-1

Table 3. Correlation coefficient of 44 wheat genotypes/cultivars among quantitative traits cultivated during 2008 to 2009.

S/No.	Correlation	Plant height	Spike length	Awn length	1 leaf length	2 leaf length	3 leaf length	4 leaf length	Biomas/5	100-grain wt.	Grain yield/5
1	Spike length	0.25*									
2	Awn length	0.32*	0.28*								
3	1 leaf length	0.64**	0.23*	0.31*							
4	2 leaf length	0.58**	0.22*	0.15*	0.76**						
5	3 leaf length	0.61**	0.13*	0.09*	0.66**	0.94**					
6	4 leaf length	0.31*	0.13*	0.05	0.32*	0.65**	0.77**				
7	Biomas/5	0.71**	-0.01	0.15*	0.70**	0.79**	0.77**	0.54**			
8	100-grain wt.	-0.32*	-0.23*	0.08	-0.35*	-0.59**	-0.56**	-0.36*	-0.29*		
9	Grain yield/5	0.79**	0.07	0.14	0.58**	0.61*	0.64**	0.53**	0.83**	-0.19	
10	Harvest index	-0.02	0.08	-0.06*	-0.31*	-0.35*	-0.30*	-0.11*	-0.40**	0.1	0.13

*significant at the 0.05 probability level; ** significant at the 0.01 probability level.

were resistant to disease. Therefore, attention is needed to recommend these varieties for cultivation. The genotypes ofFR Bannu-1, FR Bannu-2, FR Bannu (Dry Area), FR Bannu Domel-2, North Waziristan Agency-1, Mirali North Waziristan Agency-2, FR Bannu Marghali Pirbakhel (Inqilab), Bannu Plain Areas-1, FR Bannu-3, Mirali North Waziristan Agency-3, North Waziristan Agency-3, Mirali North Waziristan Agency-5, Bannu-1 and Bannu-2 were 60% similar with Raj. Among these germplasm, the genotypes of Mirali North Waziristan Agency-2 and FR Bannu-1 were resistant to disease. Therefore, genes should be transferred from these resistance varieties to high yielding susceptible varieties through conventional breeding (Table 5).

The Jaccard's similarity coefficient for Bakhtawar-92 was also calculated for proteomic homology. It was calculated that the genotypes of FR Bannu (Dry Area), FR Bannu Marghali Pirbakhel, FR Bannu Domel-1, FR Bannu Domel-2, Mirali North Waziristan Agency-3, North Waziristan Agency-3, Bannu-1 and Bannu-2 displayed 100% similarity with Bakhtawar-92. Similarly, the genotypes of FR Bannu Domel-3, Bannu Proper-2, North Waziristan Agency-2 and Waziristan North Waziristan Agency showed 80% homology. Among them, the genotypes of Mirali North Waziristan Agency-2 and FR Bannu Domel-1 were resistant. Genotypes of the North Waziristan Agency-1, Mirali North Waziristan Agency-2, Bannu Proper-1, Bannu Plains Areas-1, FR Bannu-3, FR Bannu Marghali Pirbakhel (Inqilab), FR Bannu Marghali Pirbakhel, Mirali North Waziristan Agency-4, Mirali North Waziristan Agency-5 , FR Bannu-1 and FR Bannu-2, showed 60% and that of Mirali (ECDOCK) North Waziristan Agency-1, Mirali North Waziristan Agency-1 and Bannu Plain Areas-2 showed 40% proteomic homology with Bakhtwar-92. Among these varieties Mirali (ECDOCK) North Waziristan Agency-1, Mirali North Waziristan Agency-1, Mirali North Waziristan Agency-2, FR Bannu-1 and Bannu Proper-1 were resistant (Table 5).

DISCUSSION

A low level of allelic variation was observed in qualitative traits, while the quantitative traits showed higher magnitude of variation among landraces as compared to improved lines. Green plant colour existed predominantly in landraces, while the improved lines were comparatively dark green. The qualitative traits are frequently used for varietals identification and germplasm cataloging. Due to plant vigour, all the improved lines were vigorous and similarly, five genotypes were identified. Wheat plants with better vigour have been reported as promising and high yielding. Sixty eight percent of the total population had a plant height ranging from 42 to 60 cm, while 23% were 61.1 to 68.9 cm height. Plant height exhibited a positive correlation with leaf length, biomass and grain yield. Thirty percent of the total population had 8.06 to 8.6cm spike length and 50% ranged from 6.1 to 7.94 cm. Spike length showed a positive correlation with awn length (Spagnoletti and Qualset, 1987).

Table 4. Immune response against rust disease reported in Pakistani wheat germplasm collected from North Waziristan Agency.

Row No.	DS after 87 days	DS after 120 days	Plants resistance /row	Susceptible plants/row	% Susceptibility during harvesting
1	Absent	Absent	34	0	0
2	Absent	Absent	23	0	0
3	Absent	Present	36	2	5.26
4	Present	Present	34	1	2.85
5	Absent	Absent	16	0	0
6	Absent	Absent	37	0	0
7	Absent	Present	32	2	5.88
8	Absent	Absent	21	0	0
9	Absent	Present	28	2	6.66
10	Present	Present	42	5	10.6
11	Absent	Present	36	10	21.7
12	Absent	Present	42	3	6.66
13	Absent	Absent	38	0	0
14	Absent	Present	56	10	15.15
15	Absent	Present	44	4	10
16	Absent	Present	38	8	17.39
17	Absent	Present	40	3	6.97
18	Absent	Present	42	7	14.28
19	Absent	Present	23	18	43.9
20	Absent	Present	42	1	2.32
21	Absent	Present	27	11	28.94
22	Present	Present	31	2	6.06
23	Absent	Present	33	7	17.5
24	Absent	Present	23	9	28.12
25	Present	Present	19	8	29.62
26	Present	Present	13	9	34.61
27	Present	Present	19	8	29.62
28	Present	Present	13	12	48
29	Present	Present	NA	25	53.19
30	Present	Present	14	17	54.83
31	Present	Present	6	14	70
32	Present	Present	11	16	59.25
33	Present	Present	NA	22	61.11
34	Present	Present	13	21	61.76
35	Present	Present	2	20	90.9
36	Present	Present	2	21	91.3
37	Present	Present	0	17	100
38	Present	Present	NA	25	100
39	Present	Present	0	23	100
40	Present	Present	0	24	100

DS-disease status; absent - rust disease absent; present - rust present after specific days.

The leaf length showed positive correlation with biomass and grain yield that indicated influence of photosynthesis and thus providing the greatest proportion of photosynthates to the developing grain that had also been reported by Dammania et al. (1996). High level of genetic diversity was observed in leaf length, 100 grain mass and harvest index that indicated the scope of improvement for these traits and the selected genotypes from this germplasm could be used for wheat improvement program. Due to positive linear relationship of biomass with grain yield, five percent germplasm ranging from 35.42 to 38.04 gm for biomass is suggested to exploit

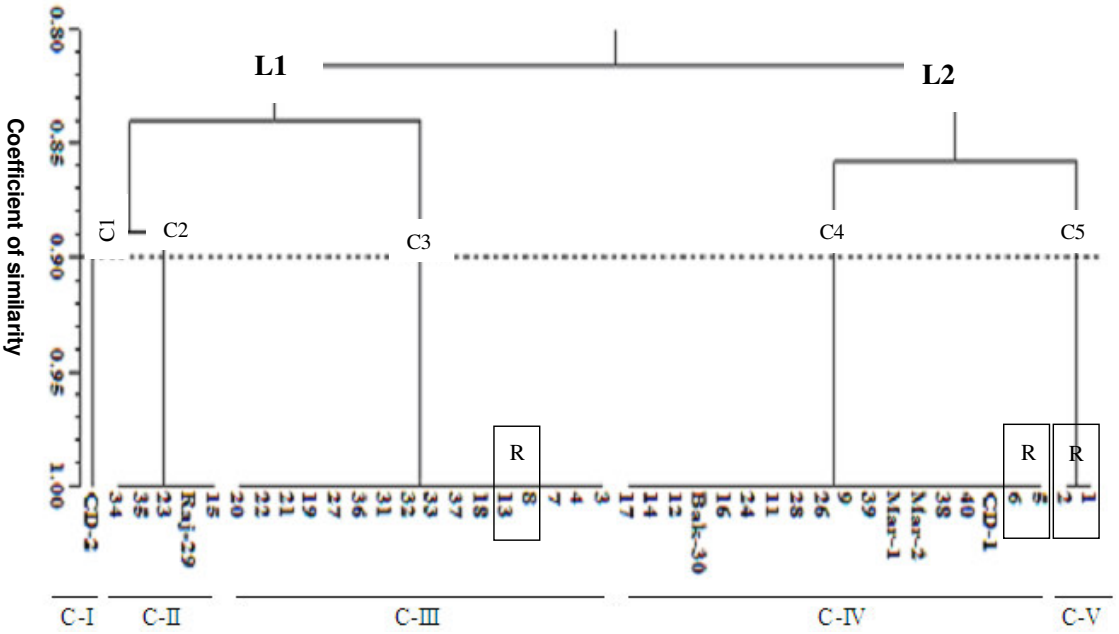

Figure 1. Cluster analysis of 43 lines collected from different parts of Pakistan based on high molecular weight HMW glutenin protein.

Table 5. Jaccard's similarity coefficient (genetic similarity-GS) between genotypes Raj and Bakhtawar-92 on the basis of HMW glutenin protein.

S/No.	Genotypes	GS-Raj (%)	GS- Bakh-92 (%)
1	Mirali (ECDOCK) North Waziristan Agency	80	40
2	Mirali North Waziristan Agency-1	80	40
3	North Waziristan Agency-1	60	60
4	FR Bannu Marghali Pirbakhel (Inqilab)	60	60
5	FR Bannu-1	60	60
6	Mirali North Waziristan Agency-2	60	60
7	FR Bannu-2	60	60
8	Bannu Proper-1	100	60
9	Bannu plain areas-1	60	60
10	Bannu plain areas-2	80	40
11	FR Bannu-3	60	60
12	FR Bannu (dry area)	60	100
13	FR Bannu Domel -1	60	10
14	FR Bannu Domel -2	60	10
15	FR Bannu Marghali Pirbakhel	100	60
16	Mirali North Waziristan Agency-3	60	100
17	FR Bannu-4	60	100
18	FR Bannu Domel -3	80	80
19	Miranshah North Waziristan Agency-1	80	80
20	Miranshah NW North Waziristan Agency-2	80	80
21	Bannu Proper-2	80	80
22	North Waziristan Agency-2	80	80
23	Mirali North Waziristan Agency-4	100	60
24	North Waziristan Agency-3	60	100
25	Mirali North Waziristan Agency-5	60	60

Table 5. Continued.

26	Bannu-1	60	100
27	Waziristan North Waziristan Agency	80	80
28	Bannu-2	60	100
31	Check 31 (Bakkar)	80	80
32	Check 32 (Watan)	80	80
33	Check 33 (Saleem-2000)	80	80
34	Check 34 (Yecura)	100	60
35	Check 35	100	60
36	Check 36	80	80
37	Check 37	80	80
38	Check 38	60	100
39	Check 39	60	100
40	Check 40	60	100
41	Mardan-1 (cultivars)	60	100
42	Mardan-2 (cultivars)	60	100
43	Chakdara-1 (cultivars)	60	100
44	Chakdara-2 (cultivars)	80	80

for breeding wheat cultivars with higher biomass that could effectively partition in to economic yield. In the present study, new source of rust resistance genotypes namely *Mirali* (ECDOCK) North Waziristan Agency, Mirali North Waziristan Agency-1, *FR Bannu-1*, *Mirali North Waziristan Agency-2*, *Bannu Proper-1and FR Bannu Domel-1*, were identified. These lines are suggested to investigate against various races of rust for their wider utilization that will ultimately help to combat serious threats due to rust for wheat production. It was also suggested that molecular characterization of this germplasm may be accorded to select new markers against rust disease.

The analysis of HMW glutenin subunits in wheat is useful tool not only for diversity studies but also to optimize the variation in germplasm collections and, to breed cultivars with improved bread-making quality (Sultana et al., 2007). Our results revealed a preliminary investigation on HMW glutenin genes in the landraces collected from a remote area and several novel alleles detected at all the *Glu-1* loci could help in improving wheat quality. Generally, the “null” allele at the *Glu-A1* locus is predominantly observed in hexaploid wheat cultivars and the germplasm accessions (Cross and Guo, 1993). We also observed a similar pattern in wheat landraces possessing “null” allele followed by the allele 2* and 1. This contrasting pattern of *Glu-A1* allele distribution between wheat cultivars and the landraces is an example of genetic erosion caused by man (Martin et al., 2008; Caballero et al., 2009). Detection of novel *Glu-1* alleles in the germplasm in both the hexaploid and tetraploid wheat had been reported by Lagudah et al. (1987) and Ciaffi et al. (1993)

Another interesting allelic variant encoded at the *Glu-D1* locus was the subunit pair 2**+12’ with slow mobility as compared to the standard subunits (2+12) at this locus and, was found frequently distributed in the accessions collected from the Baluchistan province of Pakistan (Tahir et al., 1996). The *Glu-D1xy* (D1x+ D1y) variants with slower mobility as compared to 2+12 were also detected in some hexaploid landraces from Afghanistan and this protein was quite expected in the present landraces. The allelic variants (*Glu-D1xy*) detected in the germplasm from Afghanistan seem similar to the subunits 2**+12’ detected in our study on the basis of description in the Cross and Gou (1993). The material being cultivated in Afghanistan might share common parentage/landraces.

In conclusion, wheat germplasm reported in this manuscript was collected from a very remote area of Pakistan that has never been explored. These genotypes showed high range of genetic divergence and significant response to rust diseases. New wheat genotypes, resistant to rust disease, were identified, which posses’ new allele for rust response and further investigation of the sources through molecular markers is needed. Polymorphism for HMW glutenin loci was observed that could be associated with collection site of landraces. Since the HMW glutenin subunits indicated higher bread-making quality, hence the variation at these loci is essential for the plant breeders to develop cultivars with improved bread-making quality. The preliminary information was quite encouraging and genetic markers must employ to broaden the horizon for germplasm utilization.

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