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GENETIC VARIANCE, POPULATION STRUCTURE AND GENE FLOW AMONG *Triticum aestivum* FOR RUST RESISTANCE

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Bread wheat (*Triticum aestivum* L.) is among the top three cereal crops and major staple food as it accounts for 20% calories, consumed by humans in the world. The vulnerability of wheat to diseases is a risk to the global food security. Three types of rusts, caused by fungal strains, are the major yield limiting factors to reduce overall wheat production. In the present study, molecular markers were employed for genetic diversity analysis of wheat accessions regarding the presence of various rust resistance genes. Three hundred and sixty wheat accessions were screened against stem rust (Sr), leaf rust (Lr) and yellow rust (Yr) resistance genes. Eleven polymorphic SSRs detected 20 alleles, ranging from two to four alleles per locus. The allele frequency depicted that average number of alleles per locus was 2.55, 2.82, 2.82, 2.64 and 2.82 for population A, B, C, D and overall respectively. Gene diversity and heterozygosity were high for locus *Sr25* but its major allele frequency was low. The PIC value was high at loci *Lr34* and *Yr18*. Gene diversity, heterozygosity and PIC value were the lowest for locus *Sr39* but its major allele frequency was the highest. Gene diversity increased with the number of alleles, that had a low major allele frequency at a particular locus. The Polymorphic loci effectively discriminated 360 accessions into six major clusters via principal component and population structure analyses. Significant deviation of F_{ST} from zero in six suggested populations for seven loci indicated population differentiation and limited gene flow among them. A reduced median network was established, which suggested that taxon G54 (MaxiPak-65), G-42 (Chanab-70), G-66 (SA-42) and G-75 (Yecora-70) are the progenitors, which are sharing rust genes to Pakistani wheat. These SSRs appeared effective for estimating genetic diversity, population structure and gene flow for rust resistance and for marker assisted wheat breeding.
Keywords: Rust resistance, network analysis, principal component analysis, population structure, microsatellite marker

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the leading cereal, which is widely grown around the world to fulfill human food requirements (Sehgal *et al.,* 2012). Total world wheat production was 734.1 million tonnes in 2015 and it accounts about 20% of calories requirement of human beings (FAO, 2016). It is hexaploid with genome size of about 17.1 Gb (gigabase pairs), (Brenchley *et al.,* 2012). Wheat rusts, i.e., stem rust (Sr), stripe or yellow rust (Yr) and leaf rust (Lr) are most destructive diseases those seriously affect grain yield and quality throughout the world (Khanzada *et al.,* 2012; Pretorius *et al.,* 2011). The rusts can cause grain yield losses up to 100% (Kokhmetova *et al.,* 2011; Singh *et al.,* 2011a; Sharma *et al.,* 2013) and most commercial wheat varieties (80–90%) were found susceptible to stem rust race Ug99 (Jin and Singh, 2006; Jin *et al.,* 2007; Singh *et al.,* 2008). The incidence and severity of these diseases vary region to region throughout the world because the rust strains are area specific (Yu *et al.,* 2012). The rusts are becoming more dangerous and epidemic in Pakistan and worldwide (McIntosh, 1980; Singh *et al.,* 2004; Shah *et al.,* 2010).

The most effective, environment friendly and efficient method to prevent production losses caused by the rust pathogens is to develop resistant cultivars. Some problems are also associated with the phenotypic selection of rust such as inoculum availability, storage and multiplication facility, sophisticated quarantine chamber, skilled labor and etc. Therefore, the most accurate, efficient, cost effective and time saving method is molecular evaluation of wheat accessions for rust races (Kokhmetova *et al.,* 2011; Lopez-Vera *et al.,* 2014). Molecular characterization and genetic manipulation is the best way to screen wheat accessions against various genes conferring resistance to rust pathogens (Tsilo *et al.,* 2008; Hiebert *et al.,* 2010, 2011; Rouse *et al.,* 2012, Lopez-Vera *et al.,* 2014, Randhawa *et al.,* 2014). There is a need to evaluate wheat accessions against rust races to develop resistant varieties and prevent yield losses (Browning and Frey, 1981; Wolfe *et al.,* 1981, Qi *et al.,* 2007) and molecular markers are most effective tools for genetic studies of resistance (Bordes *et al.,* 2014). Microsatellites can predict

the genetic variability present in the crop accessions and can be used for direct selection in breeding programs. This study presents screening of wheat accessions with a set of microsatellite (SSR) markers to identify rust resistant genotypes to be utilized for wheat breeding program.

MATERIALS AND METHODS

***Plant material*:** A set of 360 wheat accessions (supplementary table S1) were collected from Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Three seeds of every collected accession were planted in trays, which were placed in the germinator. Temperature was maintained at 25±2°C and water was applied properly to ensure maximum germination of wheat seed. At 2 leaf stage, the plants were cut and washed with distilled water. Genomic DNA was extracted individually using CTAB method (Doyle and Doyle, 1987). The quality of extracted DNA samples was checked on 0.8% agarose gel and quantity was estimated by Image J software.

***PCR and electrophoresis*:** PCR was performed with a 25µL volume containing 16 µL of doubled distilled deionized water, 2.5 µL Taq buffer (10x), 2 µL of $MgCl_2$ (25 mM), 0.5 µL dNTPs (10 mM), 0.5 µL of forward and reverse primer (50 ng/µL), 0.5 µL *Taq* DNA polymerase (Fermentas) (2.5 U/µL) and 3 µL genomic DNA (15 ng/µL). Reported primers linked to different wheat rust resistant genes were synthesized (Table 1). Touchdown PCR was performed in thermal cycler (C-1000 thermal cycler, Bio Rad, USA) set with initial denaturation temperature of 94 °C for 10 min, first loop with 7 cycles of denaturation at 94 °C for 60 s, annealing at 62°C (-1 °C/cycle) for 60 s and extension at 72 °C for 60 s, and second loop of 34 cycles of denaturation at 94 °C for 60 s, annealing at 48-60 °C for 60 s and extension at 72 °C for 60 s followed by a final extension at 72 °C for 10 min. PCR products were analyzed on 2% agarose gel electrophoresis to confirm amplification and each band was considered as presence of single allele and each allele was scored as present (1) or absent (0) for each loci to develop binary data.

***Morphological performance under field conditions*:** Three hundred and sixty (360) accessions were arranged into four populations on the basis of their overall morphological performance for different traits like grain color, 1000 grain weight, plant height, days to heading, days to maturity, tillers per plant, response to leaf rust and yellow rust. Rust (Lr and Yr) response of different accessions was also scored and rated as resistant (R=4), moderately resistant (MR=3), moderately susceptible (MS=2) and susceptible (S=1) (Bariana *et al.*, 2009; McIntosh *et al.*, 1995). Scaling of accessions was done to construct populations. Then data was subjected for different analyses. Performance score of all accessions for these traits was averaged to scale the accessions in to four populations like the populations with average performance of 60-100%, 50-60%, 40-50% and 1-40% were grouped into four populations A, B, C and D respectively. Thus, of 360

Gene	Marker	Primer sequence (5'-----3')	Reference
Sr2	Barc133 SSR	F-AGCGCTCGAAAAGTCAG R-GGCAGGTCCAACTCCAG	Spielmayer *et al.* (2003)
Sr25	BF145935 EST	F-CTTCACCTCCAAGGAGTTCCAC R-GCGTACCTGATCACCACCTTGAAGG	Liu *et al.* (2010)
Sr26	BE518379 EST	F-AGCCGCGAAATCTACTTTGA R-TTAAACGGACAGAGCACACG	Mago *et al.* (2005)
Sr31	STS	F-CTCTGTGGATAGTTACTTGATCGA R-CCTAGAACATGCATGGCTGTTACA	Mago *et al.* (2002)
Sr36	Stm773 STS	F-ATGGTTTGTTGTGTTGTGTGTAGG R-AAACGCCCCAACCACCTCTCTC	Tsilo *et al.* (2008)
Sr39	*Sr39*#22r STS	F-AGAGAAGATAAGCAGTAAACATG R-TGCTGTCATGAGAGGAACTCTG	Gold *et al.* (1999)
Lr34	CsLV34 STS	F-GTTGGTTAAGACTGGTGATGG R-TGCTTGCTATTGCTGAATAGT	Kolmer *et al.* (2008)
Lr46	Xwmc44 SSR	F-GGTCTTCTGGGCTTTGATCCTG R-GTTGCTAGGGACCCGTAGTGG	Suenaga *et al.* (2003)
Yr10	Psp3000 SSR	F-GCAGACCTGTGTCATTGGTC R-GATATAGTGGCAGCAGGATAC	Temel *et al.* (2008)
Yr18	GWM295 SSR	F- GTGAAGCAGACCCACAACAC R- GACGGCTGCGACGTAGAG	Lagudah *et al.* (2006)
Yr29	Xgwm259/ WMS259 SSR	F- AGGGAAAAGACATCTTTTTTTTC R- CGACCGACTTCGGGTTC	Lagudah *et al.* (2006)

accessions, group A contained 95, group B 148, Group C 78 and group D comprised of 39 accessions.

***Statistical data analysis*:** Genetic divergence and cluster analysis was performed by using NTSYS-PC software (Exeter Software, NY, USA; Rohalf, 1998). Dendrogram was constructed by Unweighted Pair Group Method with Arithmatic Averages (UPGMA) method. Gene diversity (GD), heterozygosity (HET), polymorphism information content (PIC), and allele frequencies (AF) were calculated with PowerMarker v3.25 software following functions explained in Liu and Muse (2005) and manual of PowerMarker V3.0 (see URL; http://www.powermarker.net for manual and software). Effective loci discrimination was determined through principal component analysis (PCA) using PROC PRINCOMP in the SAS v 8 software. Inter-and intra-population structure depicting gene flow was constructed using the Structure V2.3.1 software (http://pritch.bsd.uchicago.edu/structure.html) according to Pritchard *et al.* (2000). Reduced median network was constructed between wheat accessions and loci using the software Network v 4.5.1.6 software (www.fuxus-engineering.com/network_terms.htm).

RESULTS AND DISCUSSION

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***Polymorphism, allele distribution and genetic diversity within and among populations*:** Molecular analysis of these accessions suggested six major populations and can also be subdivided into nine or more populations in the phylogenetic tree as studied comprehensively. Phylogenic tree showed the diverse pattern and similarity among the wheat accessions studied on the basis of presence and absence of rust genes (Fig. 1). Many accessions contained similar alleles for rust genes and have maximum similarity (G 17, G19 and G21) and some others were divergent (G24, G37 & G56 had 0% similarity) (data not shown).

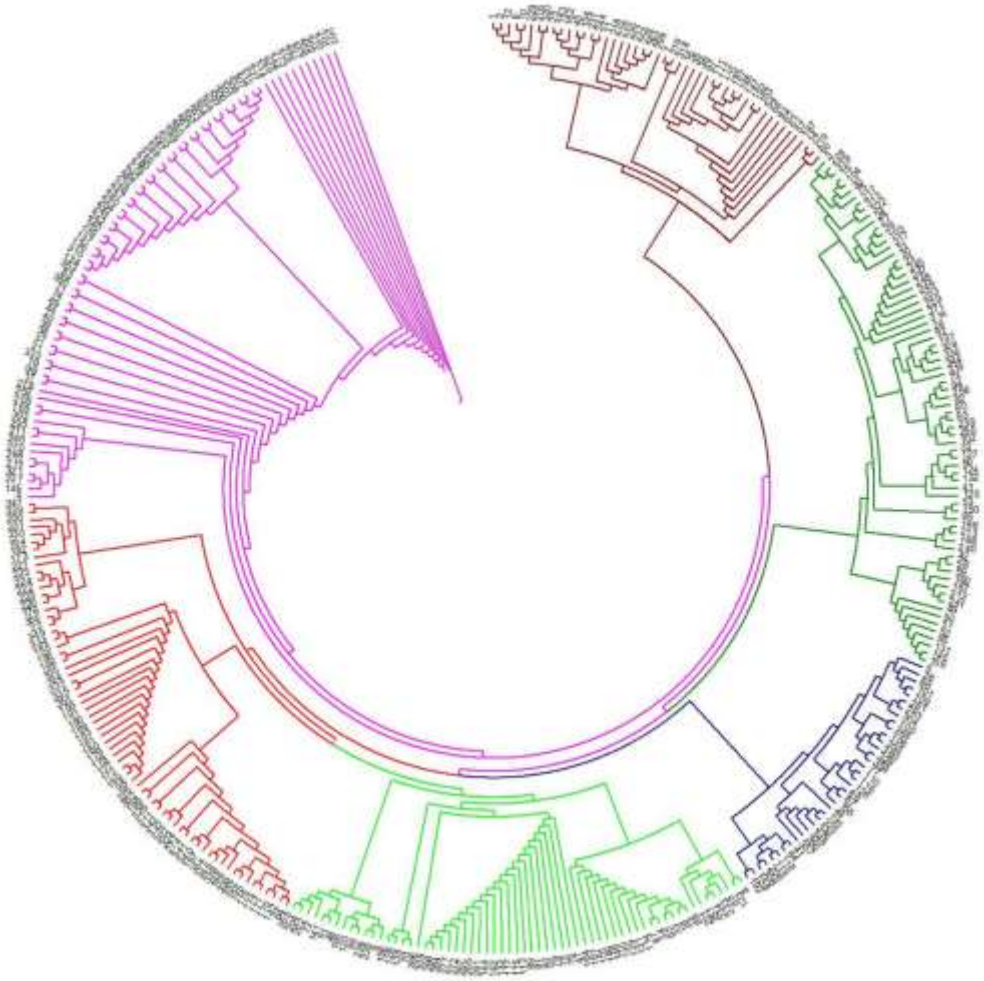

Figure 1. Phylogenetic tree of 360 wheat accessions on the basis of gene linked microsatellite markers. Accessions were distributed to six populations, indicated with different colors.

All polymorphic loci detected 20 alleles in total ranging from 2 (*Sr36* & *Sr39*) to 4 (*Lr46* & *Yr29*). The average allele numbers per locus within populations A,B,C,D and overall were 2.545, 2.818, 2.818, 2.636 and 2.818 respectively (Table 2). The allele D was only detected at respective loci of *Lr46* and *Yr29* in population A, B, C and D and overall in the populations. Frequency of major allele A (250 bp) at locus *Sr39*F was significantly ($P < 0.05$) higher than rest of the loci. Its frequency was1.000, 0.980, 0.987, 1.000 and 0.989 in population A, B, C, D and overall in the populations, respectively. Conversely, major allele A (114 bp) at locus *Sr2* in population A, B, C, D and overall in the populations, respectively were significantly less frequent among the all loci. Similarly, major allele B (250 bp) at loci *Lr46* and *Yr29* in population C was also significantly less frequent among all other loci. Null alleles were observed at locus *Sr2*, *Sr25*, *Sr26*, *Sr36*, *Lr34*, *Lr46*, *Yr18* and *Yr29* in 4 populations, at *Sr31* in three populations (B, C and D) and at *Sr39* and *Yr10* in two

Table 2. Allele frequency distribution within and between group A, B, C, D and overall in the populations.

Gene	Allele Name	Allele Size (bp)	Population A	Population B	Population C	Population D	Over all
Sr2	A	114	0.084	0.037	0.058	0.026	0.053
	B	120	0.737[a]	0.794[a]	0.801[a]	0.872[a]	0.789[a]
	C	Null	0.179	0.169	0.141	0.103	0.158
Sr25	A	180	0.500[a]	0.497[a]	0.487[a]	0.487[a]	0.494[a]
	B	198	0.489	0.476	0.487[a]	0.487[a]	0.483
	C	Null	0.011	0.027	0.026	0.026	0.022
Sr26	A	303	0.805[a]	0.787[a]	0.712[a]	0.872[a]	0.785[a]
	B	750	0.163	0.193	0.224	0.103	0.182
	C	Null	0.032	0.020	0.064	0.026	0.033
Sr31	A	250	1.000[a]	0.980[a]	0.987[a]	0.974[a]	0.986[a]
	B	Null	---	0.020	0.013	0.026	0.014
Sr36	A	155	0.979[a]	0.980[a]	0.949[a]	0.897[a]	0.964[a]
	B	Null	0.021	0.020	0.051	0.103	0.036
Sr39	A	210	1.000[a]	0.980[a]	0.987[a]	1.000[a]	0.989[a]
	B	Null	---	0.020	0.013	---	0.011
Lr34	A	150	0.295	0.277	0.256	0.385	0.289
	B	250	0.621[a]	0.669[a]	0.628[a]	0.590[a]	0.639[a]
	C	Null	0.084	0.054	0.115	0.026	0.072
Lr46	A	210	0.721[a]	0.767[a]	0.667[a]	0.654[a]	0.721[a]
	B	250	0.095	0.041	0.058	0.154	0.071
	C	290	0.153	0.145	0.212	0.115	0.158
	D	Null	0.032	0.047	0.064	0.077	0.050
Yr10	A	377	1.000[a]	0.973[a]	0.974[a]	1.000[a]	0.983[a]
	B	Null	---	0.027	0.026	---	0.017
Yr18	A	150	0.295	0.277	0.256	0.385	0.289
	B	250	0.621[a]	0.669[a]	0.628[a]	0.590[a]	0.639[a]
	C	Null	0.084	0.054	0.115	0.026	0.072
Yr29	A	210	0.721[a]	0.767[a]	0.667[a]	0.654[a]	0.721[a]
	B	250	0.095	0.041	0.058	0.154	0.071
	C	290	0.153	0.145	0.212	0.115	0.158
	D	Null	0.032	0.047	0.064	0.077	0.050
Average allele numbers per locus			2.545	2.818	2.818	2.636	2.818
Major allele's average frequency			0.791	0.806	0.772	0.781	0.792
SEM			0.358	0.365	0.344	0.356	0.357

[a]major allele = having highest frequency at each locus within population; SEM = standard error of the mean

Table 3. Gene diversity (GD), heterozygosity (HET) and polymorphism information content (PIC) of 11 markers in population A, B, C, D and Overall in whole germplasm/populations.

Gene	Population A			Population B			Population C			Population D			Overall		
	GD	HET	PIC	GD	HET	PIC	GD	HET	PIC	GD	HET	PIC	GD	HET	PIC
Sr2	0.418	0.021	0.375	0.340	0.020	0.302	0.335	0.013	0.305	0.229	0.051	0.212	0.350	0.022	0.315
Sr25	0.510	0.979	0.390	0.526	0.953	0.413	0.525	0.949	0.411	0.525	0.974	0.411	0.521	0.961	0.407
Sr26	0.324	0.326	0.288	0.343	0.358	0.296	0.439	0.372	0.384	0.229	0.205	0.212	0.350	0.336	0.308
Sr31	0.000	0.000	0.000	0.040	0.000	0.039	0.025	0.000	0.025	0.050	0.000	0.049	0.027	0.000	0.027
Sr36	0.041	0.000	0.040	0.040	0.000	0.039	0.097	0.000	0.093	0.184	0.000	0.167	0.070	0.000	0.067
Sr39	0.000	0.000	0.000	0.040	0.000	0.039	0.025	0.000	0.025	0.000	0.000	0.000	0.022	0.000	0.022
Lr34	0.520	0.147	0.447	0.473	0.176	0.401	0.526	0.179	0.462	0.504	0.667	0.400	0.503	0.222	0.430
Lr46	0.447	0.137	0.412	0.387	0.115	0.357	0.503	0.205	0.456	0.530	0.154	0.492	0.448	0.144	0.414
Yr10	0.000	0.000	0.000	0.053	0.000	0.051	0.050	0.000	0.049	0.000	0.000	0.000	0.033	0.000	0.032
Yr18	0.520	0.147	0.447	0.473	0.176	0.401	0.526	0.179	0.462	0.504	0.667	0.400	0.503	0.222	0.430
Yr29	0.447	0.137	0.412	0.387	0.115	0.357	0.503	0.205	0.456	0.530	0.154	0.492	0.448	0.144	0.414
Mean	0.293	0.172	0.256	0.282	0.174	0.245	0.323	0.191	0.284	0.298	0.261	0.258	0.298	0.187	0.260
SEM	0.231	0.286	0.199	0.197	0.281	0.165	0.225	0.280	0.194	0.225	0.344	0.190	0.214	0.282	0.182

populations (B and C) (Table 2). All populations A, B, C and D of the accessions deviated significantly from Hardy-Weinberg equilibrium (HWE) at all loci.

Mean major allele frequency (MAF) was 0.791, 0.806, 0.772, 0.781 and 0.792 for population A, B, C, B and over-all and highest MAF was indicated with a (Table 2). Gene diversity and heterozygosity remained highest at locus *Sr25* while PIC was highest at loci *Lr34* and *Yr18*. Ranges of respective gene diversity in Population A, B, C, D and overall in accessions were 0.000-0.520, 0.040-0.526, 0.025-0.526, 0.000-0.0530 and 0.022-0.521 respectively. Heterozygosity in populations A, B, C, D and overall ranged between 0.000-0.979, 0.000-0.953, 0.000-0.949, 0.000-0.974 and 0.000-0.961 respectively. While PIC in respective aforementioned populations ranged between 0.000-0.447, 0.039-0.413, 0.025-0.462, 0.000-0.492 and 0.022-0.430, respectively. Four loci *Sr2*, *Sr26*, *Lr46* and *Yr29* were statistically almost similar ($P < 0.05$) in their depiction of gene diversity and PIC (Table 3). The results of gene diversity, heterozygosity and PIC values revealed that there were almost dissimilar patterns of genetic response at all loci in each of populations A, B, C, D. Some markers were found to be linked to each other e.g. *Lr34* was linked to *Yr18* and *Lr46* with *Yr29* (Table 3). Highly significant ($P < 0.05$) positive association was observed between gene diversity and heterozygosity, gene diversity and polymorphism information content, heterozygosity and polymorphism information content and number of alleles and gene diversity while, major allele frequency showed strong negative association with genetic diversity, heterozygosity and polymorphism information content (Fig. 2).

***Gene flow between populations*:** All polymorphic loci effectively discriminated predefined four populations, based on molecular classification, into six clusters via PCA (Fig. 3). These clusters could be nine to be studied in more detail.

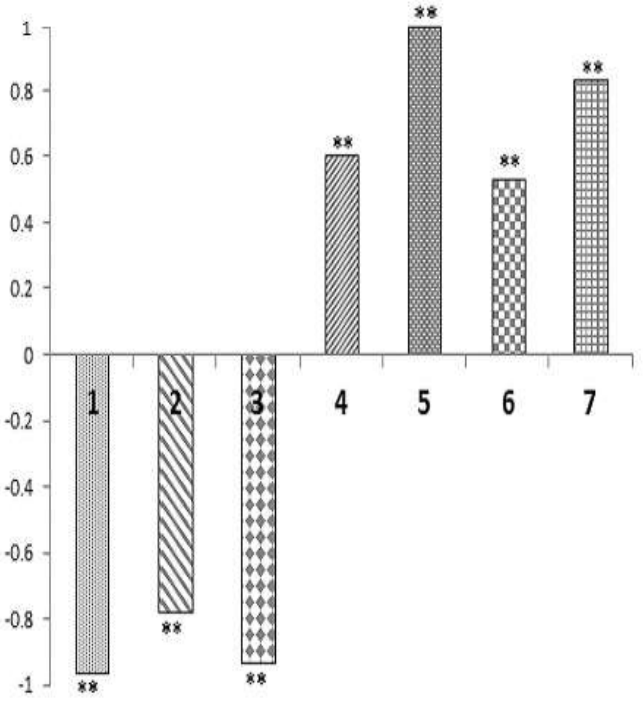

Figure 2. Correlation coefficients 'r' between selective parameters. 1 = Major allele frequency (MAF) vs genetic diversity (GD); 2 = MAF vs heterozygosity (HET); 3 = MAF vs polymorphism information content (PIC); 4 = GD vs HET; 5 = GD vs PIC; 6 = HET vs PIC; 7 = number of alleles vs GD. ** = highly significant

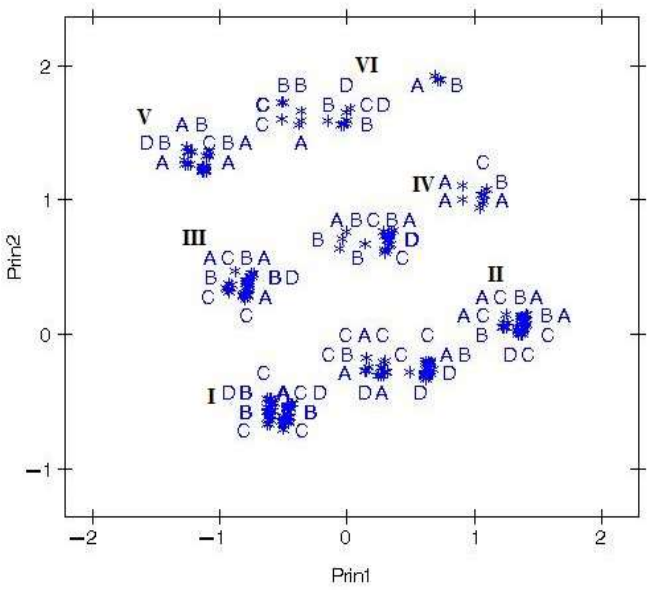

Figure 3. Effective loci discriminating predefined 360 accessions into six clusters revealed by principal component analysis.

The smaller clusters provided extensive information. On the other hand, more generalized classification provided only three populations/clusters which gave exclusive and incomplete information of each taxon. This was due to maximum overlapping of taxa in three clusters.

Six clusters were supported by the value of LnP(D) that was maximum (-1485.7) at K=6 (Fig 4 A). Considerable over-laps among A, B, C and D populations were evident. The majority of taxa from A, B and C grouped into six clusters I, II, III, IV, V and VI, while cluster III and IV contained only two taxa from D population. Effective loci discriminating predefined polymorphic markers into six clusters revealed by principal component analysis. Phylogenetic analysis and UPGMA tree view constructed via Ni's estimates also substantiated the results of PCA (Fig. 1). The pairwise global estimate of F_{ST} indicated significant differentiation between populations after correction for multiple comparisons, with F_{ST} values ranging from 0.0226 to 0.8845 (data not shown). The Bayesian clustering procedure detected the maximum likelihood for a model of six genetically distinct populations (K = 6, lnP(D) = -1485.7) via STRUCTURE 2.3 basic algorithm described by Pritchard *et al.,* (2000). For K >6, the clustering process failed to calculate a homogeneous posterior probability of the data between each iteration (Fig. 4B). The STRUCTURE algorithm showed that a partitioning of the genetic variation into six clusters was most probable when independent and correlated allele frequencies were applied (Fig 4B). A large proportion of the taxa have a genetic signature typical for one specific cluster. However, some taxa have a divided membership among two to six clusters.

***Detection of mutation in alleles and taxa phylogenies via network analysis*:** Reduced median (RM) network analysis of 360 accessions exhibited parallelism in 42 taxa only while 318 taxa were apparent in the network (Fig. 5A). Results revealed that G-54 (MaxiPak-65) is the reference accession and is most primitive. Thirteen of 20 alleles appeared in RM network analysis (Fig. 5B). *Yr10* appeared as consensus and/or reference allele, the rest of alleles showed deviations from the reference allele. *Sr25* and *Sr26* shared high similarity of nucleotide sequence and high similarity was shared between *Lr46*B and *Sr2*A as well.

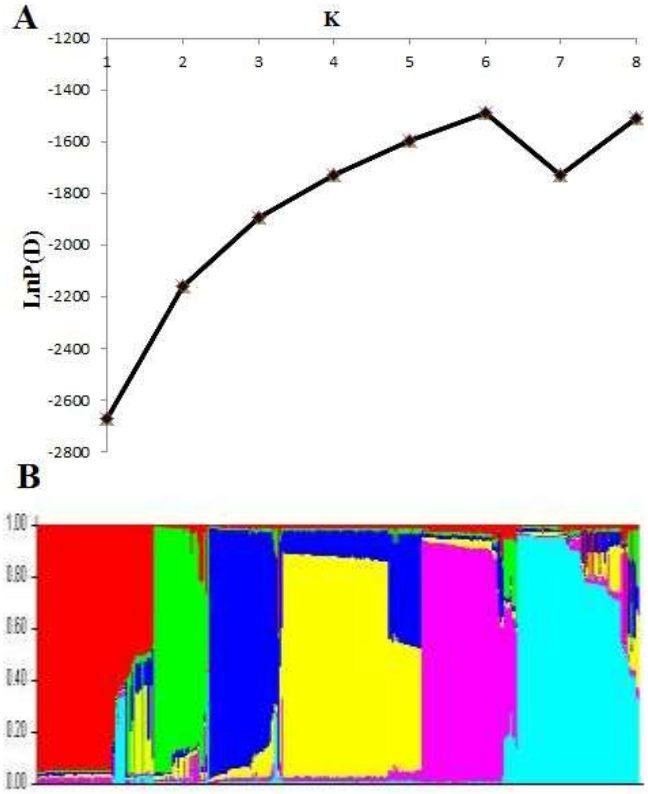

Figure 4. Structure of inter- and intra-populations. A. Estimated logarithms of data [Ln P(D)] against the number of populations tested (K). B. Estimated population structure inferred from the wheat germplasm for K = 6. Each taxon is represented by a thin vertical line, which is partitioned into K colored segments representing the taxon's estimated membership fraction in K clusters.

***Genetic diversity within and among populations*:** Gene diversity and heterozygosity are high at locus *Sr25* but its major allele frequency is less and PIC is high at loci *Lr34* & *Yr18*. Gene diversity, heterozygosity and PIC values are lowest at locus *Sr39* but its major allele frequency is highest (Table 3).

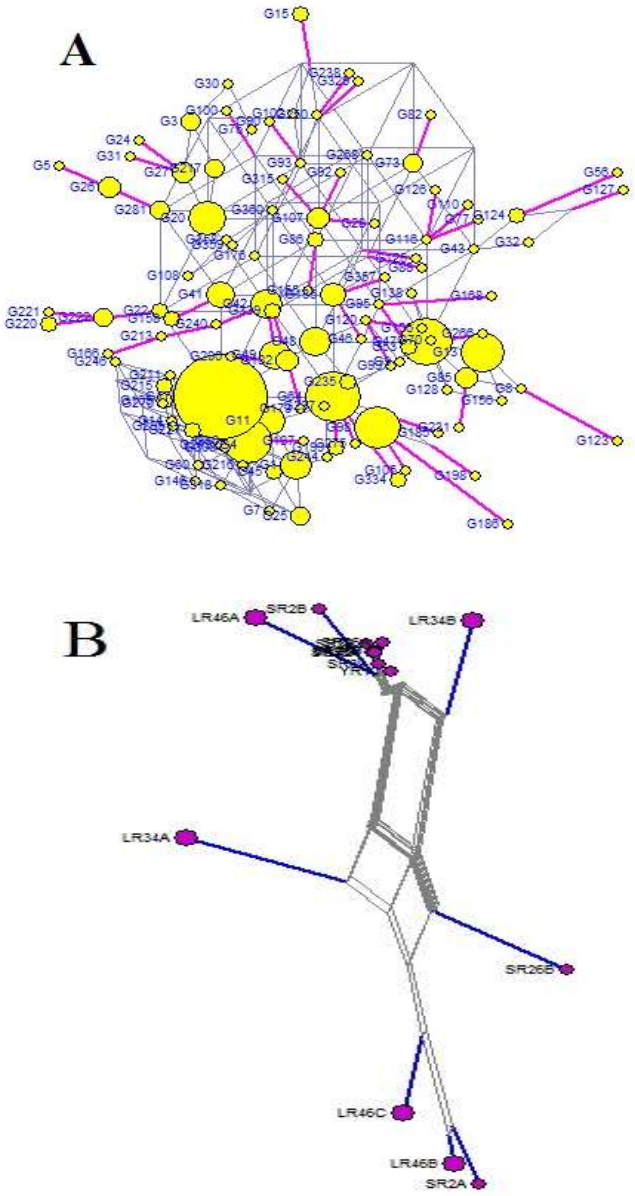

Figure 5. Reduced median network analysis. **A**. Networking among 360 wheat accessions, G stands for accession of wheat. **B**. Networking among 20 alleles; names contain the first two letter of rust type and the last 2 digits from the respective gene linked SSRs while the last letter of the name showed the respective allele number at the locus.

Former loci have more number of alleles (3) as compared to the latter ones (2 alleles). A positive significant association between number of alleles and gene diversity has been previously reported, and the use of number of alleles to evaluate the genetic diversity has been suggested (Salem *et al.,* 2010). The correlation between gene diversity and the number of alleles at all polymorphic loci is high (r= 0.835) and greater than r = 0.741 reported earlier in barley (Salem *et al.,* 2010), r = 0.533 in salicornia (Xu *et al.,* 2011). Results at two aforementioned loci agree with the suggestion of Salem *et al.* (2010) and Xu *et al.* (2011) but our cumulative data at all loci do not support these suggestions. However, large number of alleles with low major allele frequency at a locus produced high gene diversity and PIC values, and vice versa. Therefore, the use of the number of alleles together with their major allele frequency at a particular locus is suggested in order to evaluate the genetic diversity.

Average gene diversity, heterozygosity and PIC within each population were moderate and also remained comparable between four morphologically categorized populations (Table 3). Less number of alleles and moderate gene diversity/PIC confirm moderate or narrow genetic base of populations. This might be due to self-pollinating nature of the plants and equal distribution of rust resistance genes among the four populations. It is quite possible that these four populations have common ancestors and share many genes in common.

***Gene flowing between populations*:** Phenotypic variation in wheat plants might be caused by abiotic and biotic stresses like temperature, salinity, soil quality, plants density and diseases (Line, 2002; Brown and Howmoller, 2002; Chen, 2005). Morphological features were suggested to be obscure for classification of wheat plants. Molecular discrimination of four morphological distinct populations into six distinct clusters via PCA, phylogenetic analysis and UPGMA tree view, and considerable overlaps among A, B, C and D populations evidenced the involvement of common genes. Self-crossing nature of the plant, anthesis over a period of 115-130 days in most of accessions and co-existence of taxa within the same locality might have promoted the gene flow among and within the populations. There were several deviations within each population due to this gene flow. The gene flow also resulted in evolution of fifth and sixth molecular group whose members morphologically resemble the four populations. Some of the taxa appeared to be admixture of two to four populations. Previous studies reported deviation between molecular and morphological-based classification of salicornia plants, e.g. morphologically distinct two species of Salicornia in Japan consisted of five molecular populations (Sagane *et al.,* 2003).

Previously, genetic differentiation between the six homogeneous clusters of spotted gum species obtained from STRUCTURE mirrored geographically separated sampling localities (Ochieng *et al.,* 2010) and three in Salicornia (Xu *et al.,* 2011). In the present study, significant deviation of F_{ST} from zero over six populations and twenty loci indicated population differentiation and limited gene flow among the six suggested populations. Specific typical genetic signature of most of the members of each cluster supports division of 360 taxa into six distinct populations (Fig. 4B). However, divided membership of some taxa between four and/or six clusters substantiated the gene flow among the taxa and it

appeared to be more appropriate that these six clusters/populations are micro-species rather than distinct species.

RM network analysis showed six different clades/branches (Fig. 5A). These clades are highly overlapped. Major clade containing G-54 (MaxiPak-65) as reference taxon also contains G-75, which may be evolved from G-54. Other all clades appeared to be descendent from G-42, G-66 and G75. G-42 (Chanab-70), G-66 (SA-42) and G-75 (Yecora-70) were also found the progenitor of other clades. These were the post green revolution varieties. Chanab-70, SA-42 (Pakistani origin) and Yecora-70 (American origin) found sources of rust genes for wheat breeding in Pakistan. Most of the accessions tested in the present study contained blood of Maxi-Pak 65 (CIMMYT origin) while other contained Pakistani and American blood for rust resistance genes. G-17 (AARI 11- recently released variety) clade showed high gene diversity and PIC compared to the rest of clades and thus have broader genetic base. Distances of various taxa from the respective reference taxon showed the magnitude of mutations and deviations of respective taxon from the reference or consensus taxon (Bandelt *et al.*, 1995; Xu *et al.*, 2011). This analysis suggested the existence of six different clades or micro-species within wheat populations.

Wheat accessions (360), characterized in this study were the core breeding material for the development of rust resistant germplasm and varieties. The results of the study depicted that wheat accessions have variability and potential genes to confer resistance against rust strains. Information provided about the presence of rust genes in these accessions (Table S1) can be utilized for pyramiding of these genes in breeding programs. These results would be utilized and exploited for wheat breeding for rust resistance.

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