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MOLECULAR MARKERS AND FIELD-BASED SCREENING OF WHEAT GERMPLASM FOR LEAF RUST RESISTANCE

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Abstract

Leaf rust disease in wheat (caused by *Puccinia triticina*), is the best controlled through sustainable deployment of genetic resistance, which requires rigorous testing through field testing and marker assisted selection. A set of 28 exotic lines and three local checks were screened for leaf rust resistance using three *Lr* genes linked molecular markers and field testing at three locations (Lakki-Marwat, Peshawar and Mansehra). Overall leaf rust pressure was low during the wheat season of 2015-16, with maximum at Lakki-Marwat (up to 70%), followed by Peshawar (up to 50%) and minimum at Mansehra (up to 30%). The tested germplasm had variable resistance level as revealed through ACI (average co-efficient of infection); where 16 out of 28 genotypes were completely resistant, while few genotypes showed partial resistance. The maximum CI value was recorded for wheat line W-SA-87, which was 55 at Lakki Marwat, 33 at Peshawar and 15 at Mansehra, while several (18) lines had CI value of zero across the three locations. Variability existed in yield parameters with W-SA-84 (466 g per 4.5 m^2 plot), W-SA-78 (443 g) and W-SA-79 (431 g) producing the better grain yield among the advance lines. Molecular genotyping revealed that STS-7 (linked with *LrPr*) was the most frequent (83.8%), present in 26 lines; followed by SC-Y15 (linked with *Lr37*) present in 24 lines (77.4%), while csLV34 (linked with *Lr34*) was present in 16 lines (71.1%). Interestingly, in 45% of the studied germplasm all three of the resistant genes were identified. Cluster analysis resulted in four clusters, grouping different wheat lines on the basis of both phenotypic (disease severity and yield parameters) and molecular genotypic data. These results would be useful for crossing and selection of resistance lines to reduce the leaf rust disease and ensure higher wheat yield.

Key words: Field resistance; Marker assisted selection; *Puccinia triticina*.

Introduction

Different pathogens cause economically important diseases on wheat, among those the three rust diseases are the most threatening worldwide (Beddow *et al*., 2015). These three rusts are wheat stem rust also called black rust; wheat leaf rust also known as brown rust and wheat stripe rust or yellow rust, which are caused by *Puccinia graminis*, *P. triticina* and *P. striiformis*, respectively. Unlike stem rust and yellow rust, distribution of wheat leaf rust is relatively widespread (Gupta *et al.,* 2006; Khan *et al.,* 2021). The leaf rust disease strongly reduce grain yield by reducing grains per spike and grain weight (Reynolds *et al*., 2004). Lower yield per unit area results in low wheat production and thus threaten food security in many parts of the world (Huerta-Espino *et al*., 2011). Asian Countries including Pakistan, which produce most of the wheat of the world could face up to 70% yield losses in case of severe epidemics (Singh *et al.*, 2004), where a loss of up to 10% in yield have been projected of the worth more than 80 million dollars (Hussain *et al*., 1980). In Pakistan, the disease remains a serious threat to wheat production in Central and Northern Punjab, where the prevailing warm climate makes the conditions favorable for this disease. Efficient control of disease could ensure limited losses during years with extensive use of resistant varieties (Hussain *et al*., 1980).

Breeding resistant varieties is a solution to overcome the leaf rust disease, while the ever changing rust population has made this resistance breeding a continuous struggle (Ali *et al*., 2017; Khan *et al.,* 2021). Variation in virulence of the pathogen population may cause disease on previously known resistant varieties as observed in India (Bhardwaj *et al*., 2005), France (Goyeau *et al*., 2006) and Mexico (Singh *et al*., 2004). Pathogen variability makes breeding for long-term resistance difficult because of the capability of rust pathogens to generate diverse races with corresponding virulences (Pathan & Park, 2006). Consequently, the effectiveness of varieties based on extensively used resistance genes could last only few years, after which the corresponding virulence is acquired by the pathogen. This makes the varieties with resistance genes increasingly susceptible to rust and the farmers avoid to cultivate such varieties (Park & Felsenstein, 1998). Alternative measures would thus be required for a more sustainable deployment of resistance genes in different wheat lines and their appropriate cultivation at the field, locations and/or regional level (Ali *et al*., 2017; Vallavieille-Pope *et al*., 2012).

Numerous resistance genes effective against the pathogen of leaf rust have been discovered and selected so far (McIntosh *et al*., 2005), a large number of these resistence genes have their origin in wild relatives of wheat and rye. Exploitation of these resistance genes for genetic improvement of wheat against leaf rust pathogen with specific emphasis on race-non-specific, partial and quantitative resistance components should be encouraged to ensure higher wheat yields and thus food security. Previous studies have suggested the long lasting effects of certain resistance genes like *Lr34* (Singh *et al*., 2000),

which stayed effective for several years of deployment under field conditions (Kolmer & Oelke, 2006). Thus resistance status of both indigenous and exotic germplasm must be exploited for genetic improvement of wheat to attain a durable genetic resistance of wheat against rusts pathogens (Singh *et al*., 2000). Genetic characterization should not only be based on field testing but also accompanied by molecular markers-based screening. Molecular markers of different types have been developed to assess the diversity. For resistance breeding in wheat, screening of germplasm is achieved through molecular markers. Several molecular markers were made available to carry out screening of breeding material for resistance against leaf rust pathogen. Inclusion of these molecular markers during germplasm screening must thus enable us to breed genetic resistance in wheat with more precision and efficiency, if applied along with field testing at diverse climatic conditions.

The resistance level of cultivars is generally assessed at field level through their infection response/reaction, a phenotype coming from wheat-leaf rust pathogen interaction. This interaction is, however, influenced by both surrounding climatic conditions and the variability in pathogen population, which possibly will give a variable response of host for the same wheat lines across locations (Ali *et al*., 2010). Even expression of genes giving partial resistance remains variably influenced by the temperature (Agarwal *et al*., 2003). With the presence of various pathotypes the expression of resistance might be more complex under realistic field weather conditions, which will thus require screening with molecular markers for selection (Iqbal *et al.,* 2020).

This study was thus designed with an aim to screen exotic wheat lines against leaf rust resistance through multi-location testing across different areas of Pakistan along with the use of leaf rust resistance linked markers alongwith the following objectives: i). to assess the leaf rust situation across tested locations of Khyber Pakhtunkhwa and assess the tested cultivars for leaf rust resistance and yield potential, ii). to characterize the germplasm with molecular markers and correlate it with the field disease response and iii). to study the association of yield related traits with disease parameters and check the overall diversity based on these parameters.

Material and Methods

Field experimentation: Assessment was made for 28 wheat lines selected from advanced CIMMYT germplasm along with three local check varieties. These check varieties were Siran, Atta-Habib and Ghanimat-e-IBGE, the three varieties developed at IBGE, University of Peshawar (Table 1). For multilocation testing, three locations viz. Peshawar, Mansehra and Lakki-Marwat were selected which represented diverse wheat growing regions of Khyber Pakhtunkhwa (Fig. 1). The experiment was carried out in randomized complete block (RCB) design composed of three replications, containing individual plots with three rows of 1.5 m length and 0.3 m row space. Block to block distance was kept at 1 m.

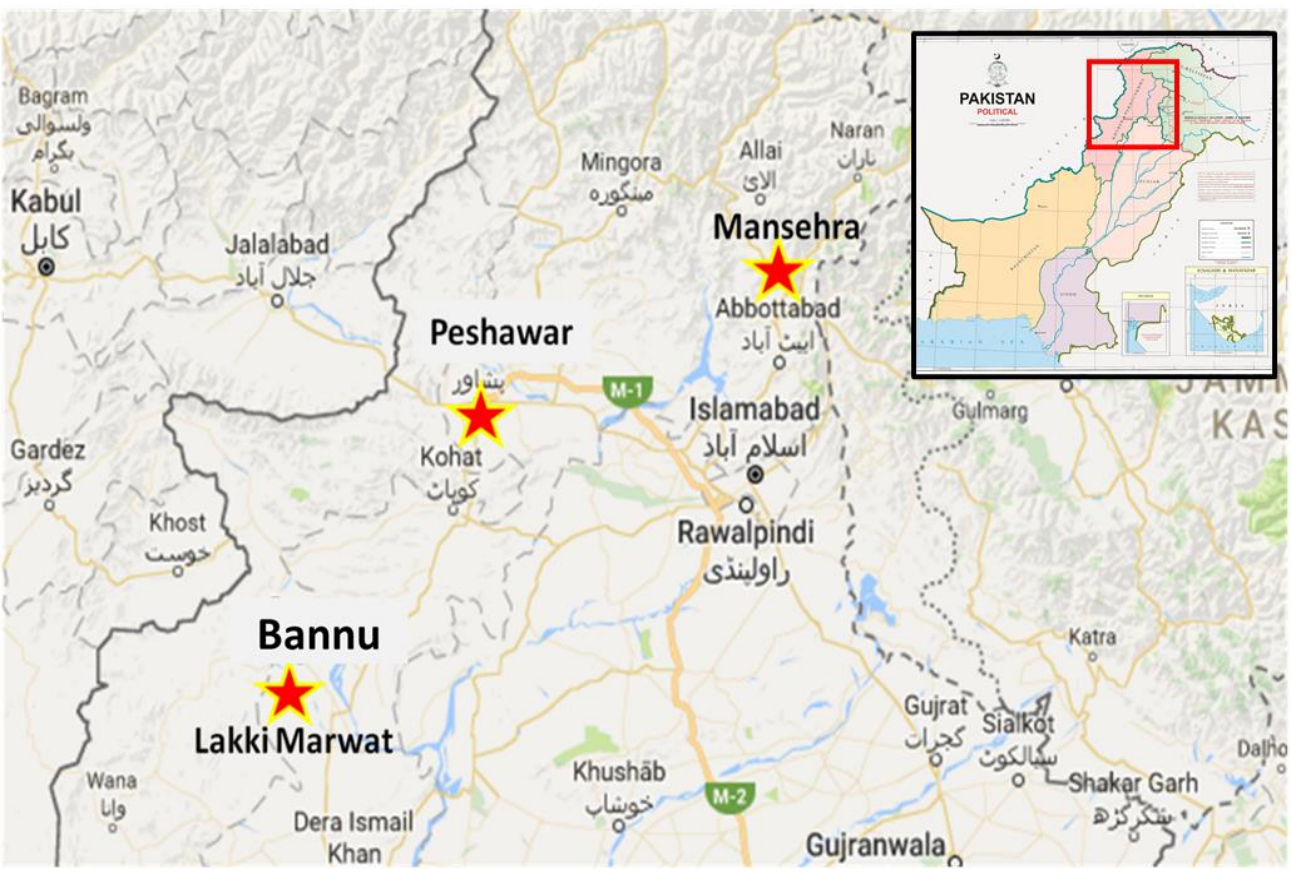

Fig. 1. Three contrasting climatic locations selected for testing multilocation leaf rust resistance status of exotic wheat lines during crop season 2015-16.

Table 1. A set of 28 exotic wheat lines along with three local check varieties selected for testing their leaf rust resistance through multilocation testing and molecular markers.

Breeding line	Origin	Breeding line	Origin
W-SA-61	Exotic line	W-SA-79	Exotic line
W-SA-63	Exotic line	W-SA-80	Exotic line
W-SA-64	Exotic line	W-SA-81	Exotic line
W-SA-65	Exotic line	W-SA-82	Exotic line
W-SA-66	Exotic line	W-SA-83	Exotic line
W-SA-67	Exotic line	W-SA-84	Exotic line
W-SA-68	Exotic line	W-SA-85	Exotic line
W-SA-69	Exotic line	W-SA-86	Exotic line
W-SA-70	Exotic line	W-SA-87	Exotic line
W-SA-72	Exotic line	W-SA-88	Exotic line
W-SA-73	Exotic line	W-SA-89	Exotic line
W-SA-74	Exotic line	W-SA-90	Exotic line
W-SA-75	Exotic line	ATTA-HABIB	Local check
W-SA-76	Exotic line	GHANIMAT-e- IBGE	Local check
W-SA-77	Exotic line	SIRAN	Local check
W-SA-78	Exotic line		

Field data collection – disease scoring and yield parameters: The host resistance is assessed in terms of host reaction and disease severity (Ali *et al*., 2017). The host reaction represents the response of host-pathogen interaction in terms of its susceptibility or resistance, while the severity represents the degree of susceptibility in terms of leaf area covered by the rust spores. The severity was based on percent of leaf area covered while considering the overall plot and the host reaction was assessed as host response categories (Ali *et al*., 2017). The host reaction was converted into a numerical value to estimate co-efficient of infection (CI) and for cluster analyses purposes i.e., Immune (I) = 0, Resistant (R) = 0.10, Moderately Resistant (MR) = 0.25, Moderate (M) = 0.50, Moderately Susceptible (MS) = 0.75 and Susceptible (S) = 1.00. CI was calculated through multiplication of severity by the corresponding numerical value of host reaction observed, while the average co-efficient of infection was calculated as average over the three locations (Ali *et al*., 2017).

For yield parameters, data was taken on grain yield, biological yield and harvest index to assess the yield potential of these lines in consideration of their leaf rust resistance status. Data on these parameters were recorded across all the three locations after harvest for all these parameters.

Molecular markers-based screening: Molecular genotyping was done with three resistance genes linked molecular markers i.e., STS-7 (linked with partial resistance to leaf rust, here designated as "*LrPr*"), SC-Y15 (linked with *Lr37*) and csLV34 (linked with *Lr34*). Liquid nitrogen was used to crush fresh leaves samples (1-2 g) of all genotypes and modified CTAB method of DNA extraction was used to extract DNA. TBE buffer (1x) was used to dilute the extracted DNA and stored at -20°C for further use in PCR reactions. The PCR was performed for leaf rust resistance gene markers using Thermo Scientific PCR kit. The PCR conditions were calibrated with various annealing temperatures, no. of cycles and DNA and primer concentrations to attain suitable amplification for further separation and scoring on gel electrophoresis (Table 2). After achieving the desired calibration of PCR conditions, PCR products were checked on 1.5% agarose gel. Scoring was made following the original publication of respective marker.

Data analyses: Data on both morphological parameters and molecular markers were compiled in MS Excel and analyzed using appropriate statistical analyses procedure. Yield and morphological parameters were analyzed with ANOVA technique appropriate for RCBD design in the statistical software "R" in the R-studio environment. Similarly, R – software was also used for cluster analysis (Ali *et al*., 2009a).

Results

Our results revealed a highly significant variability among wheat lines for all parameters, while location effect and the genotype/line x location interaction were significant only for grain yield, biological yield, and harvest index (Table 3), along with substantial variability in yellow rust resistance as assessed through field testing and molecular markers.

Prevalence of wheat leaf rust and status of resistance in wheat: Wheat leaf rust severity varied across three locations of Khyber Pakhtunkhwa, as assessed for the 28 wheat lines and three local check varieties (Fig. 2). An overall low leaf rust pressure was observed across all the three locations during the season, compared to some high intensity years previously reported. The box plot showed that the average value was close to the minimum disease severity i.e., 0% at all the three locations, with some lines showing high severity. Among the studied locations, relatively high leaf rust severity (up to 70% for some lines) was observed at Lakki-Marwat, with lower leaf rust severity observed at Peshawar (Fig. 2). In contrast, the leaf rust incidence at Mansehra was the least, with 0% leaf rust severity on most of the tested lines. Majority of the genotypes had low severity at all locations.

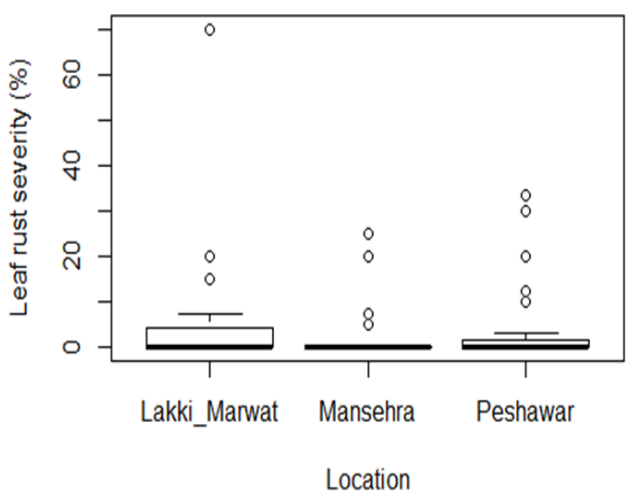

Fig. 2. Leaf rust severity (%) across three locations of Khyber Pakhtunkhwa with contrasting climatic conditions, as revealed on 32 wheat lines tested during leaf rust epidemics season 2016.

Table 2. Details on PCR primers sequences and their optimized PCR thermal profiles, used for molecular markers-based screening of leaf rust resistance in wheat germplasm.

Primer name	STS-7/STS-8 (*LrPr)*	SCY-15 (*Lr37)*	CsLV34 (*Lr34)*
Sequence	R 5`GCAAGTTTTCTCCCTATT3` F GTACAATTCACCTAGAGT	5`TGCAGCTACAGCAGTATGTACACAAAA3` R F AGGGGCTACTGACCAAGGCT	5`TGCTTGCTATTGCTGAATAGT3 R F GTTGGTTAAGACTGGTGATGG`
Initial denaturation	95°C for 15min	95°C for 15min	95°C for 15min
Denaturation	94°C for 15sec	94°C for 15sec	94°C for 15 sec
Annealing	32°C for 45sec	32°C for 45sec	32°C for 45sec
Extension	72°C for 30 sec	72°C for 30sec	72°C for 30sec
PCR Cycles	34	34	34
Final Extension	72°C for 7 min	72°C for 7 min	72°C for 7min

Table 3. Mean square values and their significance based on combined ANOVA for leaf rust and yield parameters of exotic wheat lines evaluated across three locations of Khyber Pakhtunkhwa, during 2015-16.

Source of variance	Df	Severity	CI	Grain yield	Biological yield	Harvest index
Location	2	41.000^{ns}	51.250^{ns}	0.828**	1.940**	757.800**
Replication within location	6	31.117^{ns}	32.850^{ns}	0.014^{ns}	0.039^{ns}	63.550^{ns}
Genotypes	30	383.337**	247.120**	0.050**	0.174**	108.603**
G x L	60	45.357^{ns}	30.537^{ns}	0.023**	0.155**	116.192**
Error	180	35.144	27.978	0.005	0.024	19.718

ns = Non-significant; ** = Significant at <0.01

The resistance level as inferred from coefficient of infection (CI) values for the 28 wheat lines along with three checks revealed significantly among lines (Fig. 3), though the location effect and the genotype x location interaction were non-significant (Table 3). CI value of W-SA-87 was comparatively high across locations compared to other wheat lines. CI value of W-SA-87 was 55 at Lakki Marwat, 33 at Peshawar and 15 at Mansehra. W-SA-64 had the second largest CI value across all three locations. At Peshawar CI value of W-SA-64 was 30, at Lakki Marwat the CI value was 10, while at Mansehra this value for W-SA-64 was 7. The minimum CI value was recorded for W-SA-68 and W-SA-77 which were 2 and 1 at Lakki Marwat and Peshawar respectively, and at Mansehra CI value for these two wheat lines were zero. CI value for W-SA-84 at Peshawar was zero, while at Lakki Marwat at Mansehra this value was one. At Peshawar CI value for W-SA-61 was 1, while at Mansehra and at Lakki Marwat the CI value for this genotype was zero. Among all the wheat lines, 18 lines (including Atta-Habib and Ghanimat-e-IBGE) had a co-efficient of infection (CI) value of zero for all the three locations. High ACI values were recorded in case of following genotypes, W-SA-87 (30.35), W-SA-64 (18.92). Local check Siran had ACI value of 0.5.

Yield potential of the tested lines: Statistical analysis of the data on yield parameters revealed significant differences among the tested locations, among genotypes and their interaction (Table 4). Mean biological yield produced at Lakki-Marwat was 1041 g, while it was 1401 g per 4.5 m^2 plot at Mansehra and 1186 g at Peshawar. The maximum mean biological yields were recorded for the three checks i.e. Atta-Habib (1684 g), Ghanimat (1482 g) and Siran (1438 g; Table 4). Besides these check varieties the highest mean biological yield was recorded for W-SA-86 (1536 g), followed by W-SA-88 (1400) and W-SA-85 (1379 g). Similarly, W-SA-81, W-SA-72 and W-SA-61, also had high mean biological yield. The minimum mean biological yield was produced by W-SA-66 and W-SA-63 which was 975 g and 992 g, respectively.

The highest grain yield per plot was produced at Mansehra (526 g per 4.5 m^2 plot) followed by Peshawar (384 g). Lakki-Marwat had the minimum average grain yield (315 g). Grain yield per plot among the tested lines ranged from 276 g to 606 g. The highest grain yield was obtained by W-SA-73 (606 g) followed by W-SA-86 (510 g) and W-SA-85 (477 g). However, W-SA-84 (466 g), W-SA-78 (443 g), W-SA-79 (431 g) and W-SA-73 (427 g) also had relatively better grain yield. Performance of the tested wheat lines in terms of grain yield varied significantly across the three locations. The maximum grain yield was recorded for W-SA-86 (760 g) followed by W-SA-81 (657 g), W-SA-85 (597g) and W-SA-80 (547 g) at Mansehra. Grain yield at Peshawar ranged from 249 g (for W-SA-73) to 550g (for W-SA-84). Highest grain yield at Mansehra was 760 g (for W-SA-86), while the minimum was 357 g (for W-SA-77), still higher than Peshawar and Lakki-Marwat. Grain yield of these genotypes at Lakki-Marwat, on the other hand, ranged from 78 g for (W-SA-87) to 506 g (for W-SA-78).

Harvest index significantly varied across the locations and among wheat lines with considerable genotypes/line x location interaction. Among the tested locations, the maximum mean of harvest index was observed at Mansehra 38%, followed by Peshawar 33%, while the minimum was observed at Lakki-Marwat 28% (Table 4). The mean harvest index of the tested wheat lines ranged from 25% to 40% (Table 4). The maximum harvest index across locations was calculated for W-SA-84 and W-SA-85 (40%), followed by W-SA-79, W-SA-82 (39%) and W-SA-80 (37%). The minimum mean harvest index was calculated for W-SA-69 (25%), followed by W-SA-64 (26%) and W-SA-88 with a harvest index value of 28%. Harvest index of the tested wheat lines varied significantly across the locations, which ranged from 11% to 56%.

Table 4. Grain yield, biological yield and harvest index of exotic wheat lines across three locations of Khyber Pakhtunkhwa during wheat season of 2015-16.

Full code	Biological yield per plot (g)				Yield data per plot (g)				Harvest index			
	Lakki Marwat	Mansehra	Peshawar	Mean	Lakki Marwat	Mansehra	Peshawar	Mean	Lakki Marwat	Mansehra	Peshawar	Mean
W-SA-61	1200	1575	1167	1308	396	532	370	424	40	34	32	34
W-SA-63	950	1122	933	992	188	407	287	293	20	36	30	29
W-SA-64	600	1171	1067	1024	100	367	273	276	17	31	26	26
W-SA-65	1000	1227	1067	1109	241	472	326	343	23	38	30	32
W-SA-66	700	1074	1000	975	187	332	351	298	43	32	35	35
W-SA-67	400	1261	1133	1054	110	407	379	310	19	32	34	31
W-SA-68	700	1270	1333	1207	204	511	371	363	23	40	28	31
W-SA-69	1100	1247	1167	1182	172	290	337	277	18	24	29	25
W-SA-70	600	1419	1200	1173	276	514	362	381	43	36	30	35
W-SA-72	900	1184	1500	1295	173	537	458	399	13	46	31	33
W-SA-73	2300	1522	867	1563	875	693	249	606	38	46	30	38
W-SA-74	1000	1376	1267	1259	371	562	373	426	36	41	29	34
W-SA-75	600	1145	1067	1015	132	442	349	313	19	38	33	32
W-SA-76	1000	1410	1133	1203	239	540	331	365	14	39	29	30
W-SA-77	2000	909	1067	1170	481	357	373	399	27	38	35	35
W-SA-78	1500	1463	1233	1354	506	426	413	443	30	29	33	31
W-SA-79	1100	1305	1133	1185	437	512	373	431	53	39	33	39
W-SA-80	500	1504	1067	1118	131	547	435	380	27	37	41	37
W-SA-81	1500	1656	1033	1319	150	657	326	370	11	40	32	31
W-SA-82	900	1064	1100	1046	377	411	381	390	47	38	35	39
W-SA-83	800	1649	1200	1283	344	572	383	426	20	35	32	31
W-SA-84	400	1101	1433	1150	134	507	550	466	34	46	39	40
W-SA-85	1600	1187	1433	1379	346	597	485	477	24	56	35	40
W-SA-86	1500	1891	1200	1536	322	760	448	510	26	41	37	36
W-SA-87	400	1145	1233	1065	78	562	378	389	20	49	30	35
W-SA-88	2000	1251	1250	1400	412	362	380	385	21	29	31	28
W-SA-89	1000	1722	1000	1241	186	413	401	369	19	24	40	31
W-SA-90	1000	1123	1133	1108	223	429	416	364	17	40	37	35
ATTA-HABIB	1000	2501	1550	1684	756	1052	463	757	53	43	30	42
GHANIMAT-e-IBGE	1000	2047	1400	1482	626	787	462	625	50	39	33	41
SIRAN	1000	1915	1400	1438	597	757	434	596	51	39	31	40
Location mean	1041	1401	1186	1236	315	526	384	415	28	38	33	34

* Plot area was 4.5 m^2

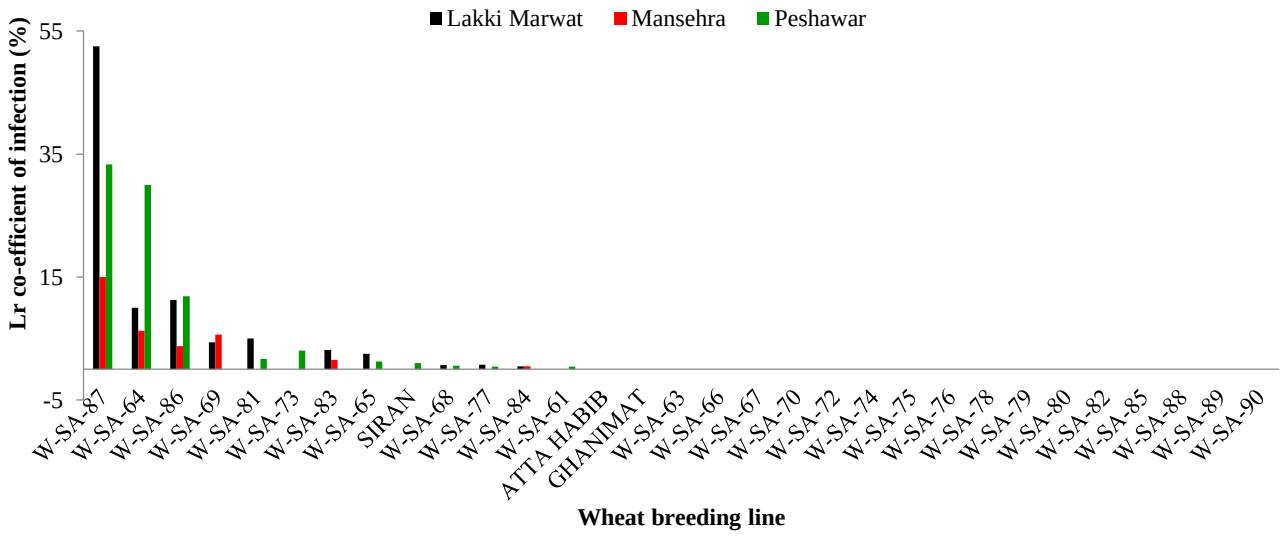

Fig. 3. Co-efficient of leaf rust infection (%) of exotic wheat breeding lines along with three local check varieties at three contrasting locations of Khyber Pakhtunkhwa during 2015-16.

Association of leaf rust severity with yield related traits: The strength of relationship of leaf rust with yield related traits was negative (Fig. 4), however, it remained non-significant with very low R^2 values i.e., 0.061 (grain yield), 0.050 (biological yield) and 0.045 (harvest index). This non-significance could be the result of diversity in the tested germplasm and an overall low disease pressure. Wheat lines with low leaf rust severity showed relatively higher values for yield related traits. Similarly, some other lines with relatively higher disease severity had lower yield parameters. When the association was assessed for each location, the strength of this association was the maximum at Lakki Marwat, a location with more disease incidence (Fig. 4). An overall low disease pressure at most of the locations with many disease values of 0% could also result in this lack of clear association. Further studies would be required to elucidate this relation in more controlled condition experiments.

Phenotypic parameters-based diversity in the tested lines: Cluster analysis of 28 wheat lines along with three local checks resulted in four clusters, based on yield related and partial resistance linked traits (Fig. 5). The first cluster comprised of one variety i.e., W-SA-87. The 2nd cluster was also consisted of one variety i.e. W-SA-64. The third cluster was consisted of 26 varities and this cluster was sub-divided into 6 clusters; in which the first sub-cluster contained two varities (W-SA-69 and W-SA-83); the second sub-cluster contained five varities (W-SA-84, W-SA-85, W-SA-80, W-SA-79 and W-SA-82); the third sub-cluster had three varities (W-SA65, W-SA81, W-SA68 and W-SA77); the fourth sub-cluster had seven varities (W-SA-61, Atta-Habib, Ghanimat, W-SA-66, W-SA-74, W-SA-70 and W-SA-90); the fifth sub-cluster had three varities (W-SA-88, W-SA-63 and W-SA-76); and the last sub-cluster had 5 varities (W-SA-72, W-SA-75, W-SA-67, W-SA-78 and W-SA-89). The fourth and last cluster consisted of three varities i.e. W-SA-86, SIRAN and W-SA-73.

Molecular markers-based screening for resistance genes: The tested 28 exotic wheat lines along with three checks were screened with molecular markers linked with resistance genes *Lr34*, *Lr37* and one other *Lr* genes conferring partial resistant (here designated as *LrPr*). All the four markers produced distinct, reproducible bands (Table 5). The leaf rust resistance genes *Lr34* associated marker csLV34 band (380bp) was amplified in 23 lines (74.1%). The PCR amplification of *Lr37* associated marker (290bp) was obtained in 24 lines (77.4%). The *LrPr* associated marker (of 500bp) associated with partial leaf rust resistance was amplified in 26 lines (83.8%). Comparison of the data on the presence of these markers with the average co-efficient of infection (ACI) reflected that most of the lines had low leaf rust infestation. Among the two lines having more than 10 ACI, W-SA-87 carried only LrPr, while W-SA-64 carried LrPr and Lr34. All the lines carrying all the three tested leaf rust resistance genes had a very low ACI value (less than 1). These results must however, be considered in the context of low disease pressure (Fig. 6).

Cluster analyses were based on the resistance genes presence to assess the grouping based on these resistance genes, resulting in at least four clusters. All the 28 exotic wheat lines along with three checks were grouped into four clusters on the basis of similarity for the presence of these genes. Some of the wheat lines had all resistance genes; some had one or two resistance genes while few had no resistance genes. The first cluster (G1) consisted of all those lines which had all the three genes (i.e., *Lr37*, *Lr34*, and partial resistance leaf rust genes; *LrPr*). G1 was the largest group among all clusters, consisting of 14 lines. G2 consisted of seven lines and had only one resistance genes "*Lr34*". G3 contained seven lines, possessing two resistance genes, while G4 consisted of three lines having no resistance gene among the tested markers.

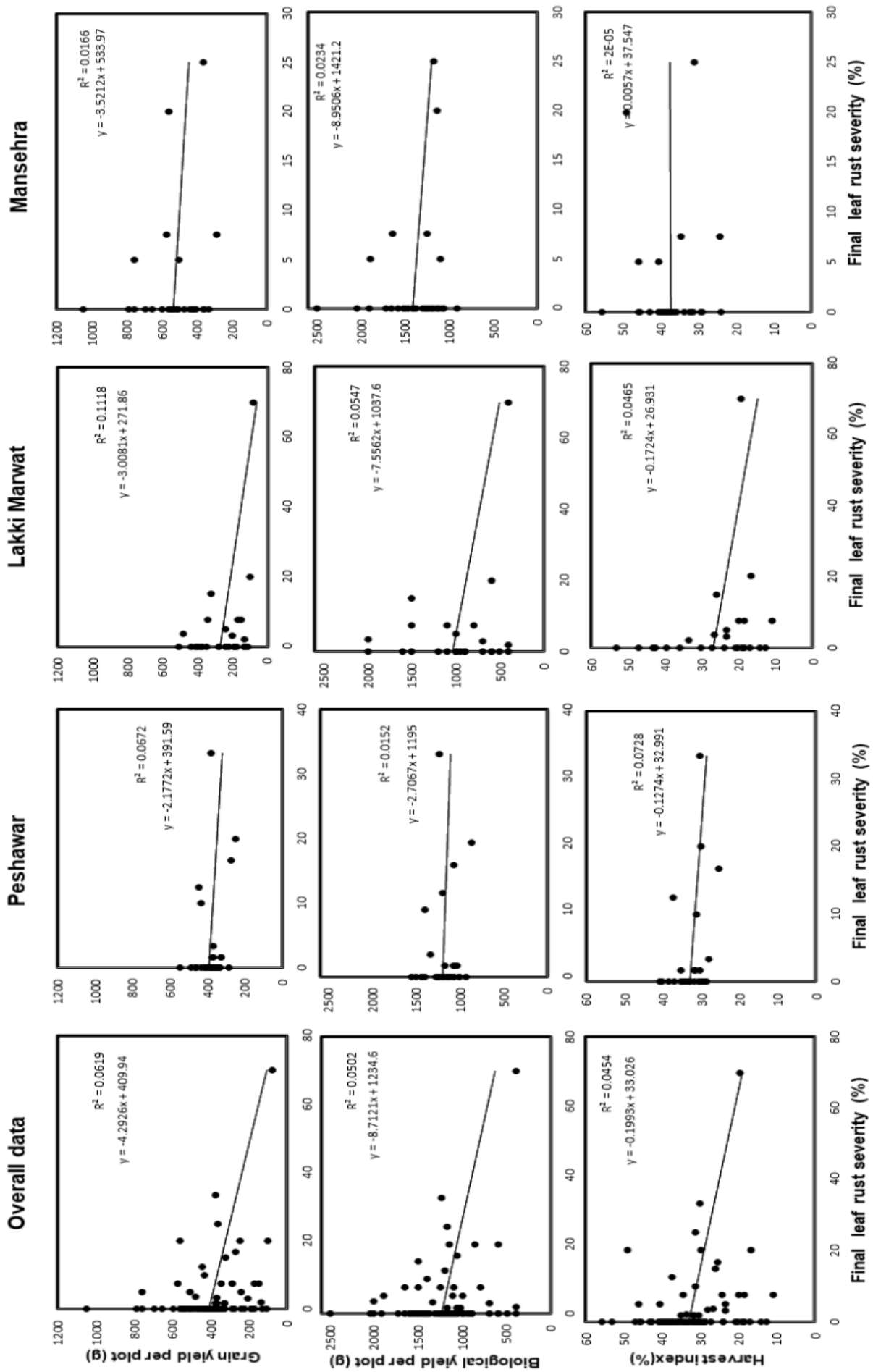

Fig. 4. Association of leaf rust severity with yield parameters, yield per plot (g), biological yield per plot (g) and harvest index (%), as assessed based on overall data and across each location.

Table 5. Presence and absence of leaf rust resistance genes in exotic wheat lines and three local check varieties (+ and – sign shows presence and absence of resistance genes associated markers).

Line	SCY-15 (Lr37)	STS*7 (LrPr)	csLV34 (Lr34)	ACI
W-SA-61	+	+	+	0.18
W-SA-63	+	+	+	0.00
W-SA-64	-	+	+	18.75
W-SA-65	+	+	-	1.25
W-SA-66	+	+	+	0.00
W-SA-67	-	+	+	0.00
W-SA-68	+	+	+	0.44
W-SA-69	-	-	+	2.86
W-SA-70	-	-	+	0.00
W-SA-72	+	-	+	0.00
W-SA-73	-	+	+	1.80
W-SA-74	-	+	+	0.00
W-SA-75	-	+	+	0.00
W-SA-76	-	+	+	0.00
W-SA-77	-	+	+	0.39
W-SA-78	-	+	-	0.00
W-SA-79	+	+	+	0.00
W-SA-80	+	-	+	0.00
W-SA-81	+	-	+	2.14
W-SA-82	-	-	-	0.00
W-SA-83	+	-	+	1.32
W-SA-84	+	-	+	0.25
W-SA-85	+	+	-	0.00
W-SA-86	-	+	+	8.96
W-SA-87	-	+	-	30.42
W-SA-88	+	+	-	0.00
W-SA-89	+	+	+	0.00
W-SA-90	+	+	-	0.00
ATTA-HABIB	+	+	-	0.00
GHANIMAT-e-IBGE	+	+	-	0.00
SIRAN	+	+	-	0.50

Discussion

Our work identified relatively low disease pressure of wheat leaf rust across three different locations of Khyber Pakhtunkhwa i.e., Peshawar, Mansehra and Lakki-Marwat, during the leaf rust epidemics season 2016. The study also confirmed the presence of variability in response to leaf rust in field resistance of exotic wheat germplasm. The results identified a negative but relatively weak correlation between leaf rust severity and wheat yield parameters. Finally, the study also enabled us to identify resistance genes in the germplasm through molecular markers-based genotyping.

Leaf rust prevalence and resistance variability: Leaf rust prevalence was low and variable at all the tested locations as revealed by its severity on the tested wheat lines, although it was still high at Lakki Marwat. This could be explained by the climatic condition of Lakki-Marwat with warm temperature (Ali *et al*., 2009b), more favorable for leaf rust infection than that of Peshawar. Very low leaf rust occurrence and 0% severity was observed for many genotypes at Mansehra which could be mainly due to relatively very cold climate of Mansehra (Khalil & Jan, 2002), not favoring the development of leaf rust at this location along with the overall resistance in these genotypes. Indeed, warmer environment could lead to serious leaf rust epidemic if a susceptible wheat variety is deployed (Dubin & Torres, 1981). The wheat crop year 2015-16 was relatively cold with more rains, resulting in high yellow rust infestation than leaf rust (Ali *et al*., 2016; Khan *et al*., 2021). All the three rusts require different types of temperature for disease development; yellow rust develops in cool temperature environment, leaf rust requires moderate temperature for its development, while stem rust requires warmer environment (Line & Chen, 1995). The growth of leaf rust pathogen requires specific climatic conditions (Roelfs, 1992), and thus it infects wheat in growing areas worldwide with that temperature range. Although, yellow rust requires cooler environment (Eversmeyer & Kramer, 2000), leaf rust require comparatively warmer environment and stem rust develops in environment in which temperature is high (Khan *et al.,* 2021).

Across locations the tested genotypes had variable severity and CI values. The variability in infection efficiency and severity of various host genotypes across all locations could be attributed to the influence of both prevalent climatic condition and variation in host genetic background and pathogen virulence profile (Ali *et al*., 2009b). Leaf rust severity depends on prevailing environmental conditions and on geographic location (Kolmer, 2005). The disease onset is the result of host-pathogen interaction as influenced by the climate (Agrios, 2004). The observed variability in disease response for the selected lines could be the result of variability in pathogen population in terms of virulence factors, coupled with the differential climatic conditions prevalent across locations with the implication of crop microclimate and disease escape (Ali *et al*., 2009b). Indeed the expression of resistance itself could be influenced by the temperature conditions (Agarwal *et al*., 2003). Further research under the greenhouse conditions with variable races and climatic conditions would be helpful to further elucidate this variation.

In our results for most of the tested lines across three locations, the CI value was zero. The maximum CI value ‘55’ for W-SA-87 was recorded at Lakki-Marwat followed by Peshawar and Mansehra where the maximum CI value was 33 and 15, respectively. The ACI value reflected multi-location based resistance status as evidenced through severity of leaf rust and the response of host (Pathan & Park, 2006). In complement of major resistance genes with the partial resistance, like *Lr34* or *Lr13*, could increase the long-lasting of resistance genes under field conditions (Kolmer, 1992). However, before these partial resistance conclusions could be verified, such field-based inferences must, however, be complemented with greenhouse and molecular markers-based confirmation of complete and partial resistance before further deployment at large scale farmer field levels.

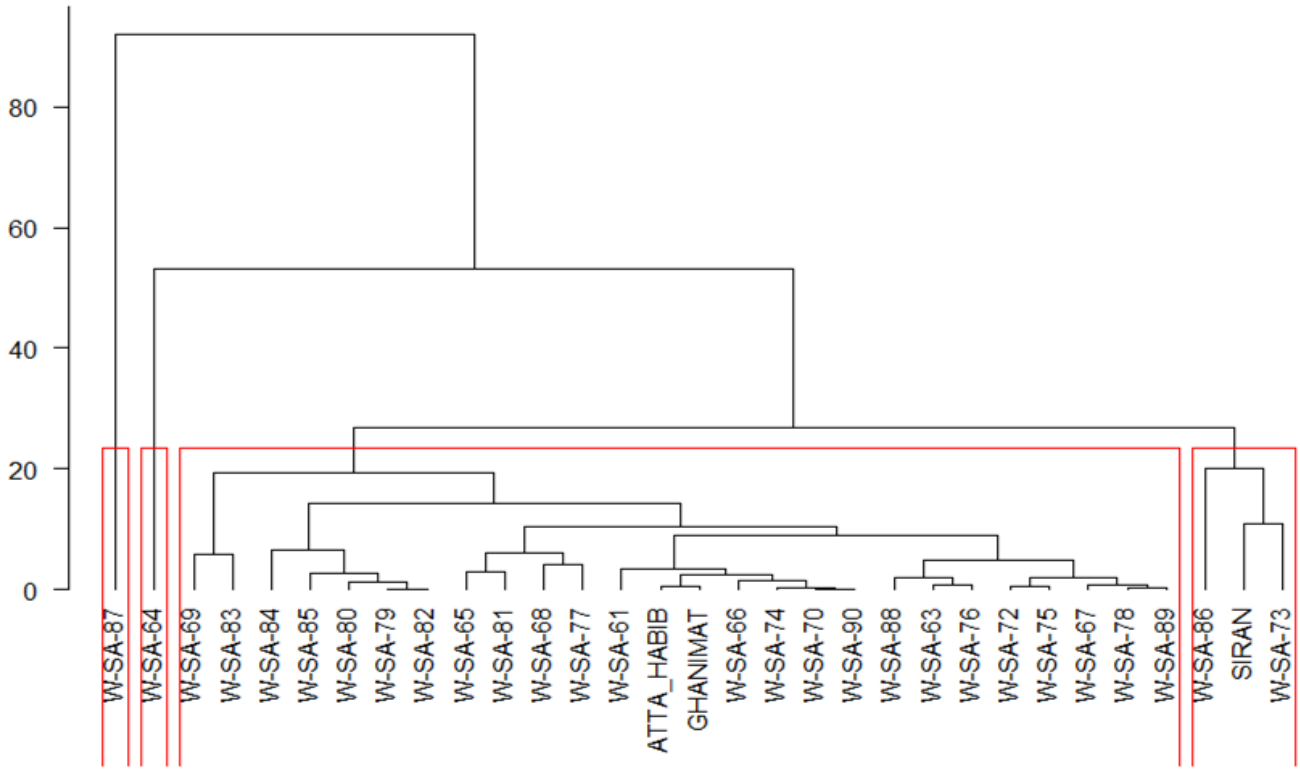

Fig. 5. Dendrogram for exotic wheat lines made through cluster analyses on multilocation based leaf rust and yield parameters during crop season 2015-16.

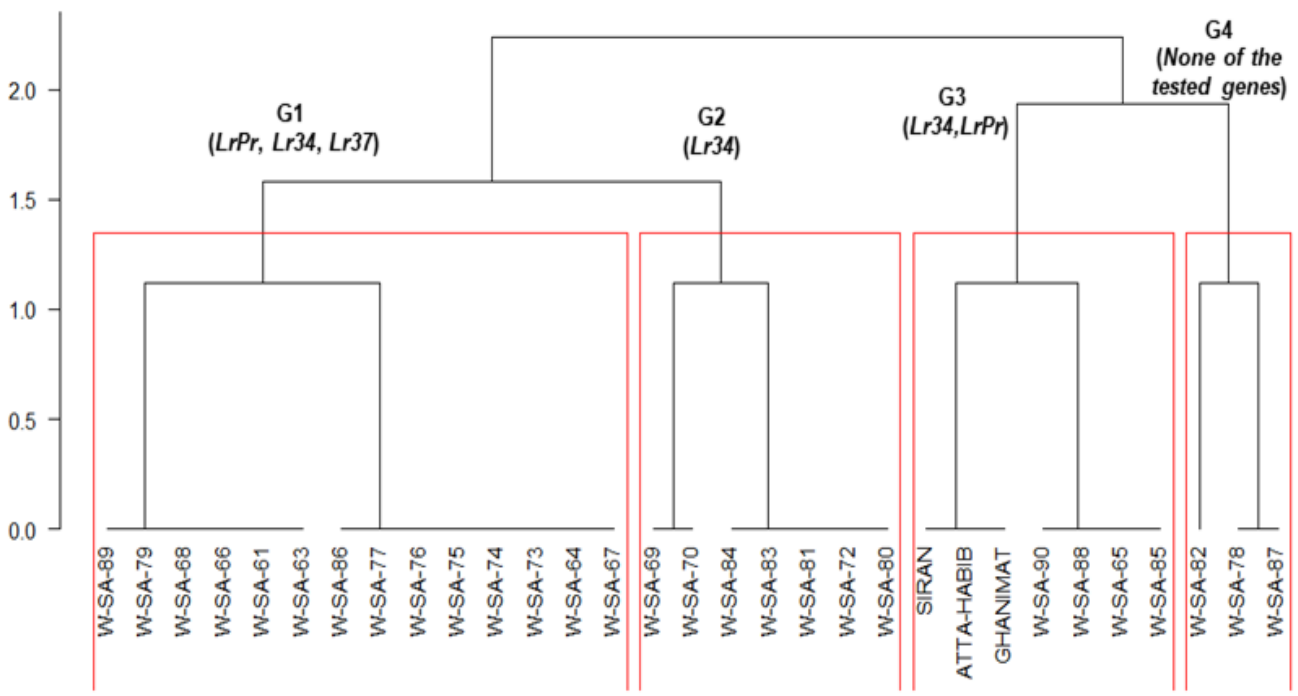

Fig. 6. Dendrogram for exotic wheat lines made through cluster analyses on molecular markers-based resistance loci.

Yield potential of introduced lines: In our study mean grain and biological yields and harvest index were maximum at Mansehra and the minimum at Lakki-Marwat. This high yield potential at Mansehra could be attributed to long crop duration at Mansehra, where the crop is sown in mid of November and harvested in late May, compared to Lakki Marwat where despite the same sowing date, the crop is usually harvested about a month earlier than Mansehra (Ali *et al*., 2009b). The low grain and biological yield at Lakki Marwat could be further attributed to low availability of water. Biological yield reflects on the overall biomass, which has commercial value in case of fodder and dual purpose wheat (Allan *et al*., 1963). Though, increased biomass could result in more favorable microclimate for crop pathogens.

A negative relationship was observed between leaf rust severity and yield related traits. Disease severity has been shown to be negatively correlated with yield related traits (Allan *et al*., 1963; Sunderman & Wise, 1964), with sometimes up to 50% losses in the final yield (Germán *et al*., 2007). A weaker negative relationship was reported in breeding lines with partial resistance (Ali *et al*., 2007). The lack of a clear relationship could be attributed to the diversity in tested genotypes.

Diversity revealed by cluster analysis of 28 wheat genotypes along with three local checks resulted in identification of four clusters, based on partial resistance and yield parameters. Among the four clusters, the fourth cluster had three wheat lines having greater value of grain yield amongst all lines. The third cluster consisted of many wheat lines and it was the second highest yielding cluster. In third cluster, W-SA-84 was the only line which showed a little bit susceptibility, while all other wheat lines were resistant and this could be an explanation to their high production. Cluster one and two were susceptible with low yields. Cluster analyses based on yield and rust parameters have been used previously in exotic and local wheat varieties (Ali *et al*., 2009b; Zaefyzadeh *et al*., 2009) which was mainly explained by their partial resistance behavior.

Molecular marker-based variability for resistance: Our work reflected on the utility of molecular markers in complementation with field testing, in line with previous work where screening was based on molecular markers in various genetic stocks for crop improvement (Gale *et al*., 1995). Some of these were specific to particular genes/loci (Blake *et al*., 1996), other were associated with quantitative trait loci (Weising *et al*., 1995). Molecular markers-based genotyping for the tested lines for resistance genes *Lr34*, *Lr37* and one other *Lr* genes conferring partial resistant (*LrPr*), revealed the amplification of *Lr34* in 23 lines (74.1%); *Lr37* in 24 wheat lines (77.4%); and *LrPr* in 26 lines (83.8%). Cluster analyses based on the resistance genes presence also resulted in four clusters. The first cluster G1 consisted of all those lines which had all the three genes, and was the largest group among all clusters, consisting of 14 lines. G2 consisted of seven lines which carried only one resistance genes *"Lr34"*. G3 contained seven lines, possessing two resistance genes, while G4 consisted of three lines having no resistance gene among the tested markers.

Among the tested markers, *Lr34* was present in 21 wheat lines out of 31 (74.1%), but *Lr34* reduced the level of infection to almost half and was not fully resistant (Singh & Rajaram, 1992). However, this was reported to be durable (Caldwell, 1968), and excellent resistance would be very effective when partial resistance gene (*Lr34*) was complemented with other resistance genes (Singh & Rajaram, 1992). Numerous leaf rust resistance analysis reported that *Lr34* showed complete and durable resistance if it was combined with adult plant resistance or seedling resistance genes (Schnurbusch *et al*., 2004).

The distribution of *Lr37* varied in various germplasm. For example, in Egyptian germplasm, it was suggested to be in-effective to reduce leaf rust (Imbaby *et al*., 2014). Another study found out that *Lr37* was present in 10 genotypes out of 37 (27.02%), higher than our studied germplasm (Stepień *et al*., 2002). It was also present at a high frequency in the UK cultivars (Park *et al*., 2001). At adult plant stage it was identified that *Lr37* was susceptible against particular pathotypes (Chicaiza *et al*., 2006), In another set of genotypes, *Lr37* was present in 2 genotypes out of 66 genotypes (Vanzetti *et al*., 2011).

In our results the *LrPr* marker (of 500bp) attributed as partial resistance was amplified in 26 lines with prevalence of 83.8%. Several studies have identified partial leaf rust resistance in wheat genetic stock (Ittu, 2000) and has been advocated for durable resistance (Stepień *et al*., 2002). In spring wheat populations, partial resistance has been reported to show additive genetic variability (Das *et al*., 1992). Molecular mapping and identification of partial rust resistance genes was conducted to identify locations of these partial resistance genes (Herrera-Foessel *et al*., 2012).

Conclusions

This work concluded on the prevalence of a low leaf rust pressure across locations during the year 2015-16. Among the tested locations, relatively high leaf rust pressure was observed in Lakki-Marwat, while its prevalence was low at Peshawar and Mansehra. It was noticed as previously reported that the pathogen *Puccinia triticina* is highly adaptable in warmer environment. The study also revealed that susceptible reaction were also present in case of some wheat lines, while others with resistant and even partial resistant reaction which could be recommended for further breeding. Among the tested germplasm, *Lr34* was present in 23 lines (74.1%); *Lr37* in 24 lines (77.4%); and *LrPr* in 26 lines (83.8%). Only 45% of the study wheat lines contained all three of the resistant genes identified. It is concluded that there was potential variability for resistance against leaf rust among the tested lines, which could be exploited in future after further testing. The available diversity of variation in the germplasm can be utilized for further breeding purpose.

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