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MOLECULAR AND FIELD-BASED CHARACTERIZATION OF YELLOW RUST RESISTANCE IN EXOTIC WHEAT GERMPLASM

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Field based assessment of wheat rust resistance was conducted for 29 advanced CIMMYT wheat lines and local checks across three locations of Pakistan vz. Peshawar, Mansehra and Bannu, complemented with molecular marker-based screening. A high disease pressure was prevalent across all locations associated with the favorable cold and wet climatic conditions prevalent during the year (2016). The maximum severity was recorded at Mansehra (up to 90%) followed by Peshawar (up to 50%) and Bannu (up to 45%). Significant variability existed amongst the tested wheat lines for yellow rust severity (ranging from 0% to 90%) and yield potential (ranging from 263 g to 757 g per 4.5 m^2 plot). Cluster analysis grouped 29 lines and three checks into four clusters. A better grain yield among the advanced lines was recorded for W-SA-104 (565 g per 4.5 m^2 plot), W-SA-115 (452 g) and W-SA-118 (447 g). None of the lines was resistance at every location and thus none had average coefficient of infection "ACI" = 0, though 26 lines were identified to possess partial resistance to yellow rust (with ACI < 20). Genotyping for the presence of resistance genes with molecular markers STS-7 (linked with *Yr5*), SC-Y15 (linked with *Yr17*) and Xwmc-44 (linked with *Yr29*) revealed the highest frequency of *Yr17* (90.60%) detected in 29 wheat lines, followed by *Yr29* (87.5%) detected in 28 wheat lines and then by *Yr5* (50%) detected in 16 lines. Among 15 wheat lines (46.87%), three resistant genes were detected together. Variability detected in resistance response based on both field testing and molecular markers could potentially be exploited in wheat breeding to develop better resistance varieties for deployment at field level.
Keywords: Molecular markers, field testing, *Puccinia striiformis*, partial resistance.

INTRODUCTION

Wheat production is threatened by various diseases, particularly wheat rusts, which may reduce production by 10-50%, depending on crop stage at which the disease occurs and the level of host resistance (Beddow *et al.,* 2015). Among various wheat diseases, the wheat yellow rust caused by *Puccinia striiformis* is an important wheat disease throughout the wheat growing regions of the globe (Ali *et al.,* 2017). It can cause poor seedling germination, shortened height, slow growth, foliar injury, reduced floweret set, low forage quality and wrinkle grains, ultimately leading to reduced grain yield (Singh *et al.,* 2004). The disease is prevalent in major wheat growing areas, when the favorable conditions prevail. In Pakistan yellow rust is mostly found in 70% of the total area where wheat is grown (Singh *et al.,* 2004). Several widespread outbreaks of yellow rust have been reported in various areas of Baluchistan, Khyber Pakhtunkhwa and Punjab. The drivers for these outbreaks were the prevalent climatic conditions favoring the disease and the acquisition of virulence by the pathogen to the resistance in cultivated varieties (Ali *et al.,*2014; Bahri *et al.,*2011). Thus, strategy based on regular efforts to develop resistant cultivars is the only important economic and environmentally friendly measure.

Several yellow rust resistance genes are known in wheat crop where the number of genes with official and provisional designations now exceeds 70, however, release of many wheat verities in Pakistan is based on only few race-specific vertical resistance genes (Ali *et al.,*2014; Bahri *et al.,* 2011; McIntosh *et al.,* 2010). Any wheat breeding programme considers incorporation of resistance genes into improved genetic background as an important objective. Several wheat varieties that show resistance to stripe rust have been developed in Pakistan and worldwide (Bahri *et al.,* 2011; de Vallavieille-Pope *et al.,*2012; Hovmøller, 2007; Singh *et al.,*2016). The approach in the past was to create resistance using race specific major genes. Moreover, a large portion of the released varieties depended on few major genes which results in a type of mono-culturing with respect to resistance genes (Ali *et al.,* 2017; Perronne *et al.,* 2017). For example, Inqilab released in Pakistan and PBW343 in India were both based on Yr27 genes (Singh *et al.,* 2004) which has been broken down bringing about severe losses (Kisana *et al.,* 2003). The pathogen becomes virulent to these genes after some years and thus the majority of race specific resistance is

lost rapidly (de Vallavieille-Pope *et al.,* 2012; Hovmøller, 2001). Thus, a continuous effort is required to use diverse sources of germplasm to reduce the pace of this virulence acquisition by the pathogen.

On a global scale, various resistance genes can give effective resistance if they are used in certain combinations in individual cultivars and deployed in regions with consideration of pathogen virulence's (Ali *et al.,* 2017; Perronne *et al.,* 2017). Cultivars mixture having different resistance genes has also increased the life of the resistance genes effectiveness (Perronne *et al.,* 2017). However, the phenotype-based identification of multiple resistance genes could be troublesome, while the molecular markers could be an option in such cases. Thus, along with molecular characterization, field-based testing must be carried out across multiple locations to confirm the utility of resistance under field conditions (Ali *et al.,* 2007; Ali *et al.,* 2009). Development of new varieties with novel sources of resistance, particularly based on race non-specific resistance requires both field-based and molecular characterization of wheat germplasm for yellow rust resistance. In case of field testing, multilocation assessment of resistance status is essential and important for breeders during the development of wheat varieties. Different parameters are used for assessing field resistance of genetic resources. These include co-efficient of infection (CI), infection rate (IR), area under rust progress curve (AURPC) and final rust severity (FRS), which enables assessment of slow rusting form of field resistance (Ali *et al.,* 2009). Using the co-efficient of infection (CI) and average co-efficient of infection (ACI) may be used to identify field resistance on the basis of single scoring.

As the field resistance is influenced by climatic conditions and the prevalent pathogen populations, it must be complemented with molecular characterization for resistance genes (Hammer *et al.,* 2000). Various types of molecular markers are available for molecular characterization of wheat germplasm, including markers linked with resistance genes. The molecular markers includes markers linked with resistance genes like Yr5 (Yan *et al.,* 2003), Yr15 (Peng *et al.,* 2000), Yr17 (Seah *et al.,* 2001) and Yr29 (Rosewarne *et al.,* 2006). These resistance genes are not yet fully overcome in Pakistan (Ali *et al.,* 2014; Ali *et al.,* 2017; Bahri *et al.,* 2011), and could be potential sources for resistance breeding in Pakistan.

Little efforts have been made in Pakistan to exploit diversity in resistance genes for further breeding and deployment of resistance genes based on multi-location trials complemented with molecular markers characterization. The present study was thus designed to characterize resistance in introduced wheat germplasm across locations as well as using molecular markers. The main objectives were: i). to assess the yellow rust status across major wheat growing regions of Khyber Pakhtunkhwa (Pakistan) during yellow rust epidemics season 2016, ii). to assess the field resistance response of 29 introduced wheat lines along with three check varieties across major wheat growing regions, iii). to molecularly characterize yellow rust resistance genes in these wheat lines and correlate their presence with field resistance.

MATERIALS AND METHODS

To characterize yellow rust resistance in introduced wheat lines, the present study was designed to conduct multi-location trial and molecular genotyping, under the regular wheat breeding trails of Institute of Biotechnology & Genetic Engineering (IBGE), the University of Agriculture, Peshawar, Pakistan. A total of 29 advanced CIMMYT wheat lines along

Table 1. The set of 29 exotic wheat lines along with three check varieties selected for testing their yellow rust resistance through multi-location testing and molecular markers.

Sr.	Genotype	Detail	Sr.	Genotype	Detail
1	W-SA-91	Introduced line	17	W-SA-107	Introduced line
2	W-SA-92	Introduced line	18	W-SA-108	Introduced line
3	W-SA-93	Introduced line	19	W-SA-109	Introduced line
4	W-SA-94	Introduced line	20	W-SA-110	Introduced line
5	W-SA-95	Introduced line	21	W-SA-111	Introduced line
6	W-SA-96	Introduced line	22	W-SA-112	Introduced line
7	W-SA-97	Introduced line	23	W-SA-113	Introduced line
8	W-SA-98	Introduced line	24	W-SA-115	Introduced line
9	W-SA-99	Introduced line	25	W-SA-116	Introduced line
10	W-SA-100	Introduced line	26	W-SA-117	Introduced line
11	W-SA-101	Introduced line	27	W-SA-118	Introduced line
12	W-SA-102	Introduced line	28	W-SA-119	Introduced line
13	W-SA-103	Introduced line	29	W-SA-120	Introduced line
14	W-SA-104	Introduced line	30	Atta-Habib	Check variety
15	W-SA-105	Introduced line	31	Ghanimat-e-IBGE	Check variety
16	W-SA-106	Introduced line	32	Siran	Check variety

Table 2. Sowing, yellow rust scoring and harvesting dates for testing of wheat lines trail during crop season 2015-16.

Location	Institute/Farmer field	GPS	Sowing date	Yellow rust scoring date	Harvesting date
Peshawar	IBGE farm, UAP	34° 1'N, 71°28'E	November 24, 2015	April 14, 25, 2016	May 17, 2016
Lakki Marwat	ARS Sarainaurang	32° 36' N, 70°54' E	November 30, 2015	March 24, 2016	April 29, 2016
Mansehra	Farmer field, Labbarkot	34.33° N, 73.2° E	December 10, 2015	May 05, 2016	May 21, 2016

with three check varieties (Atta-Habib, Ghanimat-e-IBGE and Siran) were selected to be tested across three contrasting wheat growing regions: Peshawar, Mansehra and Bannu during wheat rust season 2016 (Table 1).

***Field testing and rust scoring*:** The selected wheat lines were tested at the selected locations using a randomized complete block design (RCBD) with three replications. Each plot within each replication consisted of three rows of 1.5 m length with row-to-row distance of 0.3m. The block to block distance was kept one meter. Crop production strategy at each location was followed according to crop recommendations for respective locations (Table 2).

All the 29 wheat lines along with three check varieties (Atta-Habib, Ghanimat-e-IBGE and Siran) in three replications were harvested separately and data was collected on biological yield, grain yield and harvest index according to the standard descriptors (Ali *et al.,* 2009).

Disease severity and host reaction was considered to characterize host resistance in wheat lines under field conditions (Ali and Hodson, 2017). Numerical values for host reaction were obtained by assigning values to each host reaction category which were multiplied with yellow rust severity to estimate the co-efficient of yellow rust infection (CI). Across all three locations, average CI value for each wheat line was calculated to get ACI value, which was used to categorize the field-based partial resistance levels (Ali *et al.,* 2009).

***Molecular genotyping for Yr resistance genes*:** DNA was extracted from leaves samples (1-2 g) using a modified CTAB (Cetyltrimethyl ammonium bromide) method (Ali *et al.,* 2017). The subsequent isolated DNA was diluted in 70 µL 1x TBE (Tris/Borate/EDTA) buffer, quantified with nanodrop technique to check the concentration and quality of extracted DNA and then stored at -20 °C until the samples were used to amplify the molecular markers.

The polymerase chain reaction (PCR) was performed for primers linked with yellow rust resistance genes. Thermo Scientific PCR kit was used for performing PCR reactions. Reaction volume for each PCR reaction was 10 µL containing, 5 µL master mix, 0.3 µL Taq polymerase, 0.5 µL of each forward and reverse primers (SSR markers), 1 µL of template DNA and at last ddH2O was added to make final volume of 10 µL. PCR conditions were optimized for each marker. The PCR amplification of DNA was done by incubating the DNA samples for 3 minutes at 95°C for initial denaturation followed by 34 cycles comprising denaturation at 95°C for 1 minute; annealing of primer STS-7, SC-Y15 and Xwmc-44 for 90 sec. at 52.7°C, 53.7°C and 59°C, respectively; and extension at 72°C for 30 sec. A final extension step was carried out at 60°C for 30 minutes. PCR amplifications were done using Biorad thermocycler. The amplified products after successful completion of PCR reactions were checked on 1.5% Agarose gel and the band size of different isolates was compared with the described sizes in original papers reporting development of the markers.

***Analyses of field and molecular genotyping data*:** Both molecular and phenotypic data was compiled in MS Excel and analyses were made using R-statistical environment. Field data was analyzed using analysis of variance technique appropriate for RCBD and correlation was estimated between yield and disease parameters (Ali *et al.,* 2009) in R-statistical environment. Box plots and cluster analysis based on field and molecular genotyping data using R-statistical environment.

RESULTS

Significant variability was observed across location, among genotypes and its interaction as assessed for the 29 exotic wheat lines and three local checks when assessed at three contrasting wheat growing regions; Peshawar, Mansehra and Bannu. Molecular characterization with Yr-linked markers i.e., STS-7, SC-Y15 and Xwmc-44 revealed variable presence of these resistance genes in the tested molecular markers.

Table 3. Mean square values and their significance based on combined ANOVA for yellow 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	11052.5**	8669.8**	0.80582**	2.02522**	743.62**
Replication within location	6	578.1	542.6	0.10188**	0.46526**	99.27
Genotypes	31	959.2**	631.5**	0.05031**	0.25012**	77.56**
G x L	62	444.0**	262.5**	0.02407**	0.18586**	90.59**
Error	186	429.2	307.1	0.01215	0.04355	29.21

** refers to highly significant differences ($p<0.001$)

***Disease severity & coefficient of infection (CI) across locations*:** Data on disease severity of 29 exotic wheat varieties along with three check varieties (Atta-Habib, Ghanimat-e-IBGE and Siran) tested across three locations of Khyber Pakhtunkhwa, are shown in Figure 1. Across locations, significant differences were detected among wheat lines (Table 3). A high disease pressure was evident at all three locations. The mean maximum disease severity was recorded at Mansehra (90%), followed by Peshawar (50%) and Bannu (45%). At Mansehra the maximum value of disease was 90% whereas the minimum disease severity was 2%. Majority of the wheat lines at Mansehra showed 20-50% disease severity values. At Peshawar the maximum disease value recorded was 50% whereas the minimum was 0%. The disease severity of majority of the wheat lines was in the range of 5-25%. At Bannu the maximum disease value was 45% and the minimum disease severity value was 0%. The disease severity for majority of the wheat lines at Bannu was in the range of 0-20%.

Considering, the genotype-location interaction, the maximum Yr-co-efficient of infection (55) was recorded at Mansehra, followed by Peshawar and Bannu; whereas, minimum CI value was recorded (0) at Peshawar and Bannu. The maximum CI value (55) at Mansehra was recorded for wheat lines W-SA-102, W-SA-106, W-SA-109 and W-SA-97. None of these wheat lines showed resistance at every location (i.e., ACI=0). Therefore, levels of partial resistance could be measured for all of these. The maximum ACI (42.8) was calculated for W-SA-109 whereas the minimum ACI (0.9) was calculated for W-SA-105. Twenty-six of these wheat lines with their ACI values below 20, were identified as being useful in having high partial resistance to yellow rust. Seven wheat lines had ACI values between 0-5 and were considered to have major gene based resistance rather than partial resistance. Four of the wheat lines had moderate levels of partial resistance (ACI = 21–40), and these could be considered desirable lines, if their performance is superior in other respects. W-SA-109 with ACI (42.8) was considered to have a low level of partial resistance.

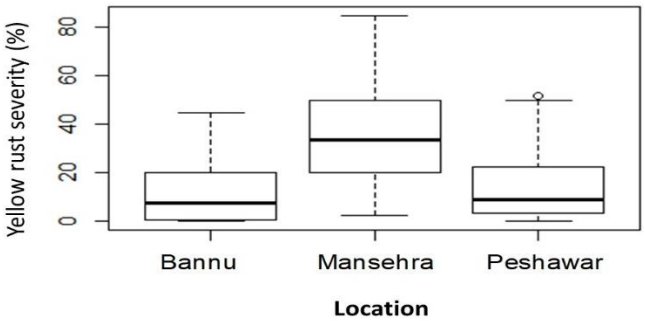

Figure 1. Yellow rust severity (%) across three locations of Khyber Pakhtunkhwa with contrasting climatic conditions, as revealed on 32 wheat lines during wheat crop season 2015-16.

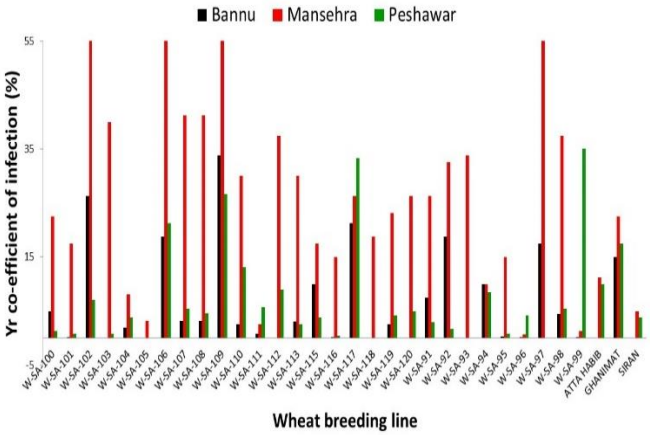

Figure 2. Co-efficient of yellow rust infection of 29 exotic wheat lines along with three local check varieties at three contrasting locations of Khyber Pakhtunkhwa during 2015-16.

***Grain and biological yield (g per 4.5 m^2 plot) and harvest index (%)*:** Highly significant differences were observed across locations for grain yield, biological yield and harvest index (Table 3 & 4). The mean grain yield among the wheat lines ranged from 263 g per plot to 757 g per plot. The maximum mean grain yield was produced by Atta-Habib (757 g), followed by Ghanimat-e-IBGE (625 g) and Siran (596 g), along with W-SA-104 (565 g), W-SA-115 (452 g) and W-SA-118 (447 g), which had comparatively better grain yield.

Mean maximum biological yield was produced at Mansehra (1401 g), followed by Peshawar (1171 g) and Bannu (978 g), respectively (Table 4). The maximum mean biological yield was 1684 g, which was recorded for Atta-Habib, followed by W-SA-104 (1551 g), Ghanimat-e-IBGE (1482 g), W-SA-111 (1441 g per plot) and Siran (1438 g), respectively. At Bannu, the maximum biological yield 2500 (g) was observed for W-SA-104; while at Mansehra, the maximum biological yield (2501 g) was of Atta-Habib; while at Peshawar, the maximum biological yield of 1550 g was recorded for check variety Atta-Habib.

Among the tested locations, the maximum mean harvest index was observed at Mansehra (36%); followed by Peshawar (32%), while the minimum was observed for Bannu (30%; Table 4). Data on mean harvest index of the tested wheat lines ranged from 26% to 50%. The maximum mean harvest index across locations was calculated 50% for Atta-Habib, while the minimum mean harvest index was calculated 26% (for W-SA-107). Harvest index of tested wheat genotypes varied significantly across locations with significant location x genotype interaction (Table 3).

***Association and clustering based on field parameters*:** Association of yellow rust severity was non-significant with grain yield, biological yield and harvest index (Fig. 3). Among the yield parameters, there was a strong correlation between grain yield, biological yield and harvest index.

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

Full Code	Grain Yield (g) per plot*				Biological Yield (g) per plot*				Harvest index (%)			
	Lakki Marwat	Mansehra	Peshawar	Mean	Lakki Marwat	Mansehra	Peshawar	Mean	Lakki Marwat	Mansehra	Peshawar	Mean
W-SA-100	234	366	451	373	500	1279	1500	1212	47	28	30	33
W-SA-101	204	336	373	333	800	994	1067	998	26	33	35	33
W-SA-102	148	436	370	355	800	1243	1200	1148	19	35	30	30
W-SA-103	230	453	410	394	600	1014	1300	1088	38	44	31	37
W-SA-104	725	605	433	565	2500	1504	1267	1551	40	41	34	37
W-SA-105	100	782	416	430	600	1973	1300	1408	18	40	32	32
W-SA-106	148	412	303	314	1100	1189	1067	1113	13	34	28	28
W-SA-107	163	465	334	322	1000	1633	1200	1311	16	29	28	26
W-SA-108	401	492	315	390	500	1293	1100	1064	46	38	27	34
W-SA-109	297	395	418	377	1700	1308	1367	1403	17	30	31	28
W-SA-110	403	609	322	427	1500	1690	1033	1330	21	36	30	31
W-SA-111	424	522	364	426	1600	1923	1067	1441	29	27	34	31
W-SA-112	220	407	384	343	500	1500	1133	1150	21	26	34	29
W-SA-113	378	457	335	383	2000	1366	1067	1322	19	33	31	30
W-SA-115	182	653	407	452	1300	1393	1133	1248	14	47	36	36
W-SA-116	138	722	374	406	500	1487	1033	1096	27	49	37	40
W-SA-117	241	557	452	422	800	1209	1400	1236	42	46	32	38
W-SA-118	342	597	417	447	1400	1300	1233	1283	36	46	34	38
W-SA-119	274	457	497	422	800	1284	1367	1245	44	36	36	37
W-SA-120	148	310	364	287	400	967	1033	906	31	31	35	33
W-SA-91	112	557	325	367	600	1432	1033	1094	19	39	31	32
W-SA-92	270	461	406	383	2000	1376	1167	1375	19	34	35	32
W-SA-93	84	308	398	316	600	843	1167	964	14	37	34	32
W-SA-94	146	311	310	263	500	1124	933	925	20	28	33	29
W-SA-95	132	306	399	296	700	1145	1067	1032	15	26	38	30
W-SA-96	200	416	317	312	500	841	1133	930	20	49	28	34
W-SA-97	226	407	330	338	1500	1469	933	1206	15	29	35	30
W-SA-98	271	407	331	335	500	1225	1133	1058	32	33	30	31
W-SA-99	154	404	325	323	500	1370	1067	1073	31	29	31	30
ATTA-HABIB	756	1052	463	757	1000	2501	1550	1684	76	43	30	50
GHANIMAT-e-IBGE	626	787	462	625	1000	2047	1400	1482	61	39	33	44
SIRAN	597	757	434	596	1000	1915	1400	1438	60	39	31	43
Mean	**253**	**506**	**379**	**390**	**978**	**1401**	**1171**	**1213**	**30**	**36**	**32**	**34**

Cluster analysis based on field disease parameters and grain yield, for 29 wheat lines along with three checks resulted in the formation of four clusters (Fig. 4). The first cluster comprised of sixteen lines and was further divided into five sub-clusters; in which the first sub-cluster consisted of four lines; the second of three wheat lines; third of four lines; fourth of three lines; the fifth of two lines. The second cluster contained five wheat lines and was further divided into two sub-clusters; the first sub-cluster consisted of two wheat lines whereas the second consisted of three lines. The third cluster consisted of two wheat lines. The fourth cluster consisted of nine wheat lines and was further divided into four sub-clusters. All the wheat lines in the first cluster were identified to possess high partial resistance to yellow rust based on their ACI values which were below 20. The wheat lines in the second cluster also showed ACI value less than 20 and were identified to possess high partial resistance to yellow rust except one wheat line W-SA-117 having ACI value greater than 20 had moderate level of partial resistance. The third cluster consisted of only two wheat lines in which one line showed moderate levels of partial resistance with ACI value less than 40, while the second line in this group showed low level of partial resistance with ACI value 42.8 but both having less variation in grain yield. All of the wheat lines in the fourth cluster showed high level of partial resistance to yellow rust except one wheat line W-SA-97 which showed moderate level of partial resistance with ACI value greater than 20.

***Molecular genotyping to screen for resistance genes*:** The tested 29 lines and three local checks were analyzed for the presence and absence of yellow rust resistance genes by using gene specific molecular markers viz. STS-7 linked with Yr5 gene; SC-Y15 linked with Yr17 gene; Xwmc-44 linked with Yr29 gene (Table 5). In case of yellow rust resistance gene Yr5 SSR marker produced specific band of 500 bp in 15 lines i.e., 46.87% out of 32 wheat lines. The PCR amplification band of 290 bp was obtained for Yr17 marker in 29 lines viz. 90.6% out of 32 wheat lines. The band of 210 bp was amplified for Yr29 in 24 wheat lines viz. 75% out of 32 wheat lines.

Cluster analysis on molecular markers-based loci of 29 exotic wheat lines along with three checks i.e., Atta-Habib, Ghanimat-e-IBGE and Siran resulted in the formation of four

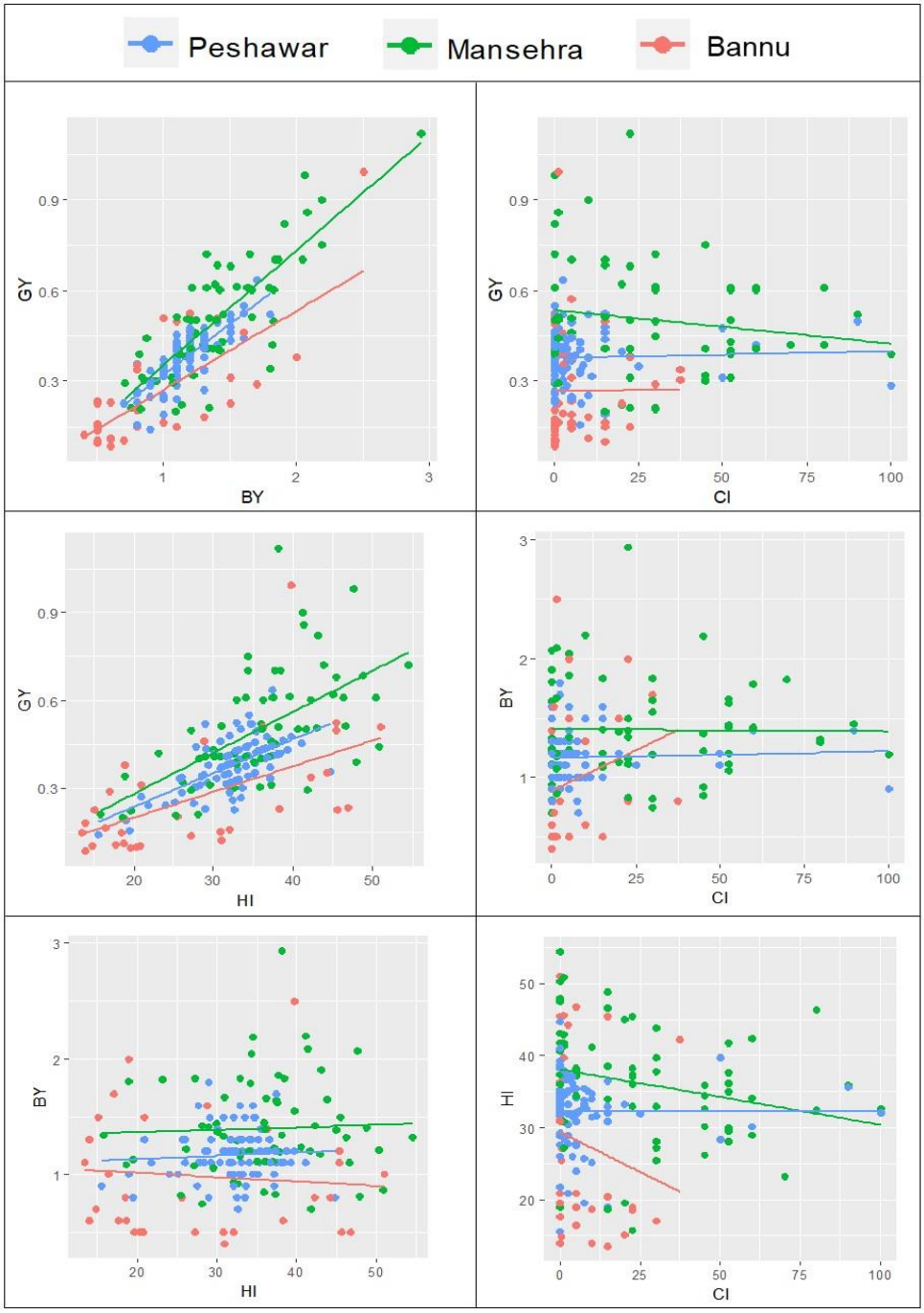

Figure 3. Association of yellow rust severity with yield parameters i.e., grain yield per plot (g), biological yield per plot (g) and harvest index (%).

groups (Fig. 5). The first group contained two wheat lines W-SA-93 and W-SA-110. These two wheat lines contain Yr5 resistance gene. The second group contained 11 wheat lines, which carried three yellow rust resistance genes i.e., Yr5, Yr17 and Yr29 except for W-SA-109, W-SA-107, W-SA-106 and W-SA-96. The third group contained six wheat lines and Yr29 gene was present in these lines. In the fourth group 13 wheat lines were present and these lines contained Yr17 yellow rust resistance gene, except for W-SA-98 and W-SA-101 that showed presence of bands for Yr29 gene.

Table 5. Presence and absence of yellow rust resistance genes in exotic wheat lines tested to characterize their resistance at molecular markers level.

Line	SC-Y15 (*Yr17*)*	STS-7 (*Yr5*)*	Xwmc-44 (*Yr29*)*
W-SA-91	-	-	+
W-SA-92	+	+	+
W-SA-93	+	+	-
W-SA-94	+	+	+
W-SA-95	+	+	+
W-SA-96	+	+	+
W-SA-97	+	-	+
W-SA-98	-	-	-
W-SA-99	+	-	-
W-SA-100	+	-	+
W-SA-101	-	-	+
W-SA-102	+	-	+
W-SA-103	+	-	+
W-SA-104	+	-	+
W-SA-105	+	+	+
W-SA-106	+	+	-
W-SA-107	+	+	-
W-SA-108	+	-	-
W-SA-109	+	+	+
W-SA-110	+	+	+
W-SA-111	+	+	+
W-SA-112	+	+	+
W-SA-113	+	-	+
W-SA-115	+	-	-
W-SA-116	+	-	+
W-SA-117	+	-	+
W-SA-118	+	-	-
W-SA-119	+	-	+
W-SA-120	+	-	+
ATTA-HABIB	+	+	+
GHANIMAT-e-IBGE	+	+	+
SIRAN	+	+	+

* The sign + and – shows presence and absence of genes, respectively.

Figure 4. Cluster analysis of exotic wheat lines based on yellow rust and yield parameters tested across three locations of Khyber Pakhtunkhwa during 2015-16.

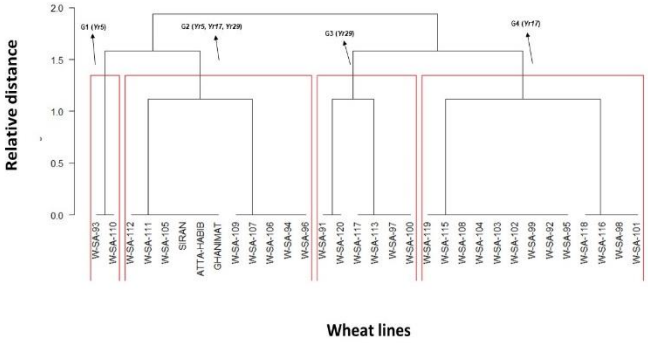

Figure 5. Dendrogram for exotic wheat lines made through cluster analyses on molecular markers-based resistance loci.

DISCUSSION

The present study characterized introduced wheat lines for yellow rust resistance and yield potential through the use of multilocation trials and molecular genotyping at Peshawar, Mansehra and Bannu. Our results revealed a highly significant variability among the tested varieties for yellow rust resistance, which we discuss in the context of their potential for further breeding and wheat improvement for this geographical region with high diversity and recombinant pathogen structure (Ali *et al.*, 2014).

***Yellow rust severity across locations*:** An overall high yellow rust pressure was observed with the maximum up to 90% severity on some lines, though this was non-uniform across locations as observed on various wheat lines with significant locations effect. Yellow rust severity could vary across locations, due to variability in pathogen population and climatic conditions (Vallavieille-Pope *et al.*, 1995). In this study, the maximum disease severity was recorded at Mansehra, followed by Peshawar and Bannu. The occurrence of yellow rust at Mansehra was relatively high due to the cold climate of Mansehra (Ali *et al.*, 2009). The climatic conditions of Mansehra are favorable for yellow rust disease as compared to Peshawar and Bannu, as this pathogen requires a cold and humid climate for its development (Ali and Hodson, 2017; de Vallavieille-Pope *et al.*, 1995). At Bannu relatively low level of yellow rust severity was due to the warm climatic condition which is considered to be unfavorable for yellow rust development (de Vallavieille-Pope *et al.*, 1995). Due to the moderate temperature of Peshawar, yellow rust severity was present in moderate severity at this location. Climatic conditions and surrounding environment has a great influence on the growth and spread of various diseases, including wheat rusts. Cold climatic conditions are favorable for yellow rust disease and the disease epidemics are greatly influenced by climatic factors (Bahri, 2008). Climatic conditions could also favor selection of certain strains of yellow rust, as revealed in the case of invasive strains which could infect wheat under relatively

warm climatic conditions (Hovmøller *et al.,* 2008; Markell and Milus, 2008). These factors together result in variable disease pressure on the same host genotypes across various locations.

***Variability in wheat germplasm for rust resistance*:** We also observed significant variability in field resistance status of tested wheat lines with significant genotype x location interaction. The interaction of several factors, such as prevalent climate, variable host genetic background and pathogen virulence profile could be responsible for variability in severity and infection efficiency of various host genotypes, both within and across locations (de Vallavieille-Pope *et al.,* 2012). The interaction between climate, host and pathogen is responsible for disease onset, as per disease triangle (Agrios, 2004). Variability in the prevalent races at different locations is shown to influence various resistance genes at different locations (Perronne *et al.,* 2017). Similarly, the expression of resistance itself could directly be influenced by prevailing climates. This is even more important in the case of field level partial resistance, where temperature has been shown to influence adult plant resistance expression (Chen, 2005; Sun *et al.,* 2002). Thus, variability in pathogen populations and climatic conditions could explain the variability in disease pressure and host resistance expression at the field level.

Significant variability was observed for host resistance in the tested wheat lines as inferred from their severity, host reaction and coefficient of infection (Pathan and Park, 2007). A calculation of coefficient of infection (CI) can be achieved by combining disease severity and host reaction data. These could describe the partial/adult plant resistance (APR), when consider the average CI across different sites (Ali *et al.,* 2009). The use of field based partial resistance testing is essential for a durable resistance based yellow rust control (Singh *et al.,* 2004). However, the field level partial resistance needs to be cross checked with greenhouse studies, and molecular marker-based data. Combination of both major genes based and partial resistance in this manner is necessary prior to large scale agricultural deployment (Brown, 2015).

***Variability in yield potential in association with rust severity*:** The higher grain and biological yield and harvest index at Mansehra could be attributed to longer crop duration and more rainfall during wheat season compared to Peshawar and Bannu (Khalil and Jan, 2002). At Mansehra the crop is sown almost the same time as at Peshawar and Bannu, however, it is harvested at least one and half month later than Bannu. Thus, the crop has more growing degree days as well as more grain filling duration, which would have enabled a better accumulation of photosynthates in the leaf and a better assortment of resources from leaf and stem into grains, resulting in high grain yield and grain weight.

Considering the impact of disease on yield potential, yellow rust severity showed negative association with grain yield and harvest index whereas a weak positive association was observed between yellow rust severity and biological yield. A strong negative relationship between disease severity with yield and yield components had been described by multiple researchers when conducting controlled condition experiments (Allan *et al.,*1963; Sunderman and Wise, 1964). However, under field conditions and with diverse germplasm, a weaker negative relationship was observed in breeding lines with partial resistance in a study from Pakistan (Ali *et al.,* 2007). A negative linear relationship was estimated between yellow rust severity and yield potential traits, for just one location, CCRI, Nowshera where yellow rust severity was the maximum (Ali *et al.,* 2009). Due to lower yellow rust severity the varieties showed relatively higher 1000-grain yield and grain weight and vice versa. In the case of more diversity in germplasm, however, some of the lines even with low rust severity, may produce low grain yield, and vice versa, mainly due to their genetic yield potential, rather than the effect of rust disease.

A similar pattern of association of rust severity with biological yield and harvest index was observed, the former too being strongly correlated with grain yield. Biological yield is remarkable not only to reflect on overall capacity of any variety to accumulate photosynthates, but also in some traditional agro-systems, where biomass of wheat is used as a commercial product and provides suitable income to farmers (Arif *et al.,* 2006; Naveed *et al.,* 2015). Between biomass and grain yield a balance should be sought, because if biomass is improved then it could generally make the plant more susceptible to the pathogen attack. In our results, cluster analysis of 29 wheat lines along with three checks i.e. Atta-Habib, Ghanimat-e-IBGE and Siran resulted in four clusters, based on partial resistance parameters and grain yield. Crossing among distant individuals of any crop would generate further variation which could be exploited in subsequent breeding programmes (Singh *et al.,* 2004; Tabassum *et al.,* 2010). Thus, this information of clustering based on phenotypic profile could be useful for intercrossing of various individuals, considering other agronomic traits. Similarly, cluster analysis has previously been used to group lines based on morphological data, particularly on disease data to identify group of lines with various level of field resistance (Ali *et al.,* 2009; Ali *et al.,* 2009).

***Molecular markers-based screening*:** Our results revealed significant variability among the tested lines as revealed through molecular genotyping for yellow rust resistance genes. The yellow rust resistance gene Yr5 specific marker was amplified in 15 lines; the Yr17 marker was amplified in 29 lines; and the Yr29 was amplified in 24 wheat lines. Cluster analysis on the results of these molecular markers-based loci identified four groups. The first group was consisted of two wheat lines, which contained Yr5 resistance gene. The second group was consisted of 11 wheat lines and contained three yellow rust resistance genes i.e. Yr5, Yr17 and Yr29 except for four lines (W-SA-109, W-SA-107, W-SA-106 and W-SA-96). Whereas the third group contained

six wheat lines and Yr29 gene was present in these lines that showed resistance to yellow rust. In the fourth group 13 wheat lines were present and these lines contained Yr17 yellow rust resistance gene, except for W-SA-98 and W-SA-101 that showed additional presence of bands for Yr29 gene. A clear description is attained through the use of inoculation procedures in the identification of Lr, Yr and Sr resistance genes; this however is associated with certain limitations (McIntosh *et al.,* 2010). Thus, identification of molecular markers linked with resistance genes and their exploitation in molecular breeding must be useful to develop resistant varieties (Singh *et al.*, 2004).

Among the tested genes, the Yr5 is one of the most effective at worldwide scale (Ali *et al.*, 2014; Ali *et al.,* 2017; de Vallavieille-Pope *et al.,* 2012; Hovmøller *et al.,* 2016). Yr5 resistance genes has been mapped on chromosome 2B, where many other resistance genes i.e., Yr7, Yr27, Yr31, YrV23, YrSp, YrQz, YrTp1, YrCN19 have also been mapped (Yin *et al.,*2006). Utility of some of these genes has been reported in China and worldwide (Ali *et al.,* 2017; Sun *et al.,* 2002). Three resistance gene analog polymorphism (RGAP) markers, co-segregated with the Yr5 locus, and four markers tightly linked to the locus have been developed (Sun *et al.,* 2002). The focus of which was to combine Yr5 with other seedling resistance genes, and also with high temperature adult plant (HTAP) resistance, with the intention of providing durable and superior resistance (Yan *et al.,* 2003). Other valuable markers have also been developed, such as sequence tagged sites (STS) and cleaved amplified polymorphic sequence (CAPS) markers (Chen *et al.,* 2003). The results of these studies would be influential in the move to transfer Yr5 into commercial cultivars, and the combination of it with other Yr genes through marker assisted selection. In North America (Bux *et al.,* 2012; Chen *et al.,* 2003) and Iran (Afshari, 2010), epidemiological research shows that Yr5 is effective against all virulent rust strains. In China (Chen, 2005) and Turkey (Zeybek and Yigit, 2004), this gene has been shown to be highly resistant to stripe rust. In the Caucasian region, middle Asia (Ziyaev *et al.,* 2011) and Pakistan (Bux *et al.,* 2012) Yr5 and Yr15 were shown to be resistant against all *P. striiformis* races they tested. However, the virulence has been observed in low frequency in natural yellow rust populations of the Himalayan region (Ali *et al.,* 2014).

Similarly, the Yr17 gene provides yellow rust resistance at both the seedling and adult plant stages and is found in many cultivars of European wheat (Vallavieille-Pope *et al.,* 2012). The gene was introduced from *Aegilopsventricosa* into chromosome 2A, and was earlier found to be closely linked (0.5 cM) to leaf and stem rust resistance genes Lr37 and Sr38, correspondingly (Robert *et al.,*1999). The identification of molecular markers linked to the Yr17 gene in these introduced lines was the purpose of this study. The gene Yr17 is not widely reported in South Asia (Ali *et al.,* 2014), though it is already overcome in Europe (de Vallavieille-Pope*et al.,* 2012) and recently emerged important in East Africa and Central Asia (Ali *et al.,* 2017).

As far as Yr29 considered, using the monosomic series of Lal Bahadur, slow rusting and tightly linked genes Lr46 and Yr29 were identified in the cultivar Pavon and located on chromosome 1B (Singh *et al.,*1998). In the investigation of such race non-specific genes effective at the adult plant stage (William *et al.,* 2003), the SSR marker Xwmc-44 located on 1BL chromosome was found to be 3.6 cM to Yr29 (Rosewarne *et al.,* 2006). The gene is not widely deployed and thus its virulence is not reported over large area till now. Being an adult plant stage resistance gene, it has a good potential for further deployment in resistance breeding.

Thus, field-based testing to identify partial resistance and molecular markers to confirm the presence of resistance genes would enable to identify lines with improved resistance, which could be further used in breeding programmes or for development of varieties. A combined approach is likely to provide durable assistance i.e., combining race specific seedling resistance genes to be used alongside other effective genes and/or with race non-specific adult-plant resistance genes (Yan *et al.,* 2003). Our results thus revealed the presence of variation in resistance response based on both field testing and molecular markers which could be utilized in wheat breeding to develop better resistance varieties to be exploited at field level, thus reducing wheat yield losses due to wheat yellow rust.

***Conclusion*:** The present work revealed the presence of substantial variability among the tested lines for yellow rust resistance and yield potential, as assessed through field testing and molecular markers screening. The yellow rust resistance gene Yr5 specific marker was amplified in 15 lines; the Yr17 marker was amplified in 29 lines; and the Yr29 was amplified in 24 wheat lines. These lines represented a potential resource for further breeding and wheat improvement for sustainable disease control through genetic resistance.

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