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FIELD ASSESSMENT AND MOLECULAR MARKERS-BASED CHARACTERIZATION OF YELLOW RUST RESISTANCE IN WHEAT HYBRID PROGENIES

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ABSTRACT

Yellow rust is one of the major production constraints of wheat in Pakistan. To accelerate development of rust resistant cultivars, field testing may be accompanied with molecular genotyping. In the present studies, 56 F_1 wheat hybrids were developed through 8×8 full diallel crosses during 2014-15. All the 56 F_1 wheat hybrids along with parental genotypes were evaluated during 2015-16 under rainfed and irrigated conditions to identify yellow rust resistant genotypes. Pooled analysis of variance revealed highly significant ($P < 0.01$) differences among the genotypes for final rust severity, relative area under disease progress curve and average co-efficient of infection. Under both environments, 41 genotypes showed high, 7 moderate while remaining genotypes showed low level of partial resistance. Under rainfed condition, genotypes PS-05×AH, PS-05 and PS-13×PS-05 while under irrigated condition, Lal-13×JB, PS-05×Lal-13, PS-05×Tat-96 and JB×PS-05 were partially resistant in the field. The presence of yellow rust resistance genes, *Yr5*, *Yr17* and *Yr18*, were confirmed in 76%, 78% and 37% genotypes respectively, using molecular markers, which were present either individually or in combinations of two or three genes. Among the parents, PS-13, JB and PS-05 performed well under both conditions. Under irrigated condition, cross combinations, Lal-13×JB, KW×PS-05 and JB×AH while under rainfed condition, genotypes PS-05×PJ-11, Tat-96×AH and JB×AH, showed best performance in terms of yield and rust resistance. Cluster analysis grouped majority of partial resistance genotypes into sub cluster G1. Field testing and molecular markers analysis, revealed the presence of variability in resistance among the studied genotypes.

Keywords: Stripe rust, Wheat Hybrids, Genetic improvement, Peshawar

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INTRODUCTION

Wheat is the most important cereal crop of the world. Yield stability of wheat is continuously challenged by several biotic and abiotic stresses. Among the biotic stresses, fungal diseases are the major wheat production constrains in majority of wheat-cultivated areas of the world (Ali *et al*., 2014a; Mansfield *et al*., 2012). In fungal diseases, three rusts viz. leaf, stem and yellow rusts caused by *Puccinia* spp. are more devastating (Hovmøller *et al*., 2010). Stripe/yellow rust is a biotrophic airborne pathogen (Ali *et al*., 2014a) and can disperse over hundreds of kilometers (Vergara-Diaz *et al*., 2015). Rust causes significant yield losses in wheat globally (Lin and Chen, 2007), while its magnitude depends on crop developmental stage, initial infection and relative resistance/susceptibility of the host cultivar (Kolmer *et al*., 2007; Wellings, 2011). The pathogen is extremely diverse and recombinant in the Himalayan region of Pakistan (Ali *et al*., 2014b; Khan *et al*., 2019) with even higher risk of losses and rapid acquisition of virulence against the resistant cultivars (Ali *et al*., 2017). Managing diseases below a certain level to achieve high yield is possible only through resistant genes or chemical controls but chemicals are expensive and unsafe to human health and environment (Asad *et al*., 2012). Exploitation of genetic resistance is the most efficient, cost effective and environment friendly approach to control yellow rust (Ali *et al*., 2014b; Oliver, 2014).

Several resistance genes have been identified and mapped (Yuan *et al*., 2012) but only few of them exhibit adult plant resistance (APR) which often provide durable resistance to rust disease (Uauy *et al*., 2005). Varieties carrying effective rust resistance genes can be used to transfer these genes into other susceptible varieties (Ma and Singh, 1996). Several wheat rust resistance genes have been introgressed through interspecific crosses but a large number are not yet in commercial varieties due to linkage with unknown chromosome segments which carry negative characters linked to resistant gene (Ellis *et al*., 2014). Additionally, the limited number of genes deployed in a large number

of wheat varieties result in loss of their effectiveness on their exploitation under the field conditions over a long period of time (Brar and Kutcher, 2016). Loss of effectiveness of resistance genes is due to aggressiveness of the pathogen (Agenbag *et al*., 2012) and acquisition of virulence to resistance (Ali *et al*., 2009a). Genetic improvement of existing varieties is thus indispensable, particularly with an aim to look for partial resistance (Ali *et al*., 2009b). Partial resistance is generally expressed at adult plant stage, and could be potentially associated with high temperature (Chen, 2013). Breeders must have to discover, characterize and incorporate new sources of adult plant resistance based on minor genes to protect wheat crop from disease (Ma and Singh, 1996). This will necessitate field screening using partial resistance parameters and investigating progression of host disease response over time (Ali *et al*., 2009c), along with the exploitation of molecular markers (Bai *et al*., 2010). Molecular markers have made it convenient to screen for resistance genes and transfer them into target breeding material (Suenaga *et al*., 2003).

In addition to biotic stresses, abiotic stress such as drought is also one of the most significant plant production limiting factor in wheat (Kilic and Yagbasanlar, 2010). Wheat is grown on different climatic conditions such as arid and semi-arid areas, but its yield is severely limited by water-deficit stress (Alderfasi and Nielsen, 2001). Climatic changes and global warming have imparted an increased water scarcity due to uncertain rainfalls and plant breeders have the challenge to develop new varieties and hybrids for changing climate (Ullah *et al*., 2013). The ability of a variety to produce high and satisfactory grain yield over a wide area with stress and non-stress conditions is very important (Ahmad *et al*., 2003). Additionally, the highly irrigated plots vs. drought faced crops could show variable level of disease incidence and thus should be considered while evaluating the disease resistance in wheat germplasm. High humidity encourages the infestation and multiplication of what rusts (Ali *et al*., 2009a), while high temperature impacts the exhibition of field resistance (Chen, 2013).

This study was thus designed to assess the yellow rust resistant in wheat germplasm developed through crossing of elite varieties based on field testing, under irrigated and rainfed conditions, and molecular characterization.

MATERIALS AND METHODS

Selection of parents and generation of crosses: To study yellow rust resistance in wheat, a set of eight promising varieties i.e., Atta Habib (AH), Lalma-13 (Lal-13), Tatara-96 (Tat-96), Punjab-11 (PJ-11), Pirsabak-2005 (PS-05), Pirsabak-2013 (PS-13), Janbaz (JB) and land race Khatakwal (KW) were crossed in 8×8 full diallel technique during 2014-15. In the succeeding wheat crop growing season (2015-16), parents and their F_1 hybrids were evaluated for yield and rust resistance under rainfed and irrigated conditions (with four irrigations, as per routine practice) at Shirin Khan Research Farms (latitude 34° 1' N, longitude 71° 28' E), The University of Agriculture, Peshawar.

Field testing and disease scoring: The experiment was conducted in randomized complete block design (RCBD) under irrigated and rainfed conditions. All the 64 genotypes were grown in three replications under both environments. Each entry consisted, 2 rows of 2 meters length. Inter-row and inter-plant space as maintained, 30 and 15 cm, respectively. Standard cultural practices and recommended inputs were applied. Precipitation data during the crop growth season were obtained from Meteorology department, regional office Peshawar. All 64 genotypes including 8 parents and 56 F_1 wheat hybrids were evaluated in the field and molecularly characterized for yellow rust resistance. The tested location is a hotspot for yellow rust disease and the spreader line "Morocco" was severely infected and therefore considered as susceptible check. Natural infection under field condition was relied because the experimental site is a hot spot for yellow rust (Ali *et al*., 2009c; Ali *et al*., 2014b). Disease scores were made through assessment of disease severity and host reaction, along with estimation of co-efficient of infection (Ali and Hodson, 2017). Yellow rust host reaction and disease severity data were further utilized to compute final rust severity (FRS), relative area under disease progress curve (rAUDPC) and average co-efficient of infection (ACI) as explained by Ali *et al* (2009a), Pathan and Park (2006) and Safavi and Afshari (2012). Cluster analysis was carried out according to Ward (1963) to identify overall grouping of genotypes and summarize their partial resistance as described earlier by Ali *et al*. (2009a).

Molecular screening for rust resistance genes: All 64 wheat genotypes were screened for the presence and absence of yellow rust resistance genes through molecular markers at The University of Sydney Australia after extracting DNA and preliminary tests at IBGE Peshawar. DNA was extracted following the protocol as described by Ali *et al*. (2017). The extracted DNA was quantified with Thermo Scientific Nanodrop-2000c, and Polymerase chain reaction (PCR) was performed for three primers linked with yellow rust resistance genes. Primer sequence from 5′→3′ of STS-7 was GTACAATTCACCTAGAGT; GCAAGTTTTCT CCCTATT linked with *Yr*5 gene, for Sc-Y15 AGGGGCTACTGACCAAGGCT; GCAGCTAC AGCAGTATGTACACAAAA linked with *Yr17* gene and for csLV34 GTTGGTTAAGACTG GTGATGG; TGCTTGCTATTGCTGAATAGT linked with *Yr18* gene. For optimization of annealing temperatures and

running the PCR, Thermo Scientific PCR kit was used. PCR amplification was done by incubating the DNA samples for 3 minutes at 95° C for initial denaturation followed by 35 cycles comprising denaturation at 95° C for 60 seconds; annealing temperature of primer STS-7, Sc-Y15 and csLV34 at 90 sec were 52.7° C, 53.7° C and 60° C, respectively; and extension at 72° C for 30s. The final extension step was carried out at 60° C for 30 minutes. The PCR amplification was carried out using a Biorad thermo cycler. After PCR amplification the products were run on 2% agarose gel at 110 V electrophoresis for 90 minutes. For staining 2 µl of GelRed was used for 100 ml of gel solution. Fragments were visualized under UV light unit fitted with a GelDoc-IT UVP camera. The presence (+) and absence (−) of the expected bands was noted to infer the presence or absence of the resistance gene and for subsequent genetic study each band was considered as a single locus.

Data analyses: The collected data were subjected to analysis of variance (ANOVA) for the studied traits according to Steel *et al*. (1997). The data was compiled in MS Excel for further analyses and interpretation. Both ANOVA and cluster analyses were done in R-software using R studio version 3.2.2.

RESULTS

Pooled statistical analysis of the data indicated significant ($p<0.01$) differences among the genotypes across the environments. Genotype by environment (G×E) interactions was also significant for ACI and grain yield plant^{-1} while non-significant for FRS and rAUDPC (Table 1A). Significant ($p<0.01$) variations were observed among the genotypes for all the studied traits under both conditions (Table 1B).

Table 1A. Mean squares of pooled analysis of variance for various traits under rainfed and irrigated conditions.

Character	Environment (E)	Reps (Env)	Genotypes (G)	G×E	Pooled error
	(d.f.=1)	(d.f.= 4)	(d.f.=63)	(d.f.= 63)	(d.f.= 52)
FRS	2395.0	765.4	541.4**	67.39^{ns}	55.06
ACI	620.8	229.6	217.8**	27.04**	21.67
rAUDPC	888.9	2254.1	1141.8**	192.9^{ns}	197.26

$p < 0.05$ = * $p < 0.01$ = ** ns = non-significant
FRS = Final rust severity, ACI = Average coefficient of infection, rAUDPC = relative area under disease progress curve

Table 1B. Analysis of variance (ANOVA) for various parameters under rainfed and irrigated conditions.

	Irrigated				Rainfed			
Character	Reps (d.f.= 2)	Genotypes (d.f.= 63)	Error (d.f.=126)	CV%	Reps (d.f.= 2)	Genotypes (d.f.= 63)	Error (d.f.=126)	CV%
FRS	1280	356.91**	56.15	56	250.75	251.8**	53.9	88
rAUDPC	1239	734**	196	142	3268.21	662.0**	206.0	110
ACI	401	135.26**	18.83	60	58.42	109.54**	24.50	105

$p < 0.05$ = * $p < 0.01$ = ** ns = non-significant
FRS = Final rust severity, ACI = Average coefficient of infection, rAUDPC = relative area under disease progress curve.

Disease pressure and its progress over time: The disease outbreak was started in the second week of March (average temperature 19°C) till 1st week of April (average temperature 23.9°C) due to favorable temperature and comparatively maximum precipitation during March 2016 (Fig. 1). The disease severity increased over time and reached to maximum at the 3rd scoring done 135 days after sowing (Fig. 1B). In April 2016 after 3rd scoring, a decreasing trend in rust severity was observed at high temperature (30.6°C) and comparatively low precipitation which is relatively unfavorable for spread of the stripe rust pathogen. While considering the irrigated vs. rainfed conditions, there was limited differences in yellow rust severity under the two conditions till the 2nd scoring done 125 days after sowing, though the differences were more evident at the 3rd scoring date. The overall disease was higher in the irrigated trial than the rainfed conditions (Fig. 1B).

Variability for yellow rust severity: Relative AUDPC estimate for the parental lines revealed high yellow rust severity on Punjab-11 under both rainfed and irrigated conditions (Fig. 2), followed by Tatara-96 under rainfed conditions, and Khatakwal under irrigated conditions. The rest of the parental lines had low rAUDPC values, with least value for Janbaz under both rainfed and irrigated conditions during crop growing season 2016 (Fig. 2). This was further reflected by the severity of various parental combinations, where genotypes having Punjab-11 as maternal or paternal parent, showed comparatively maximum value of susceptibility, while

Janbaz showed better performance as paternal parent and PS-13 as maternal parent (Fig. 3).

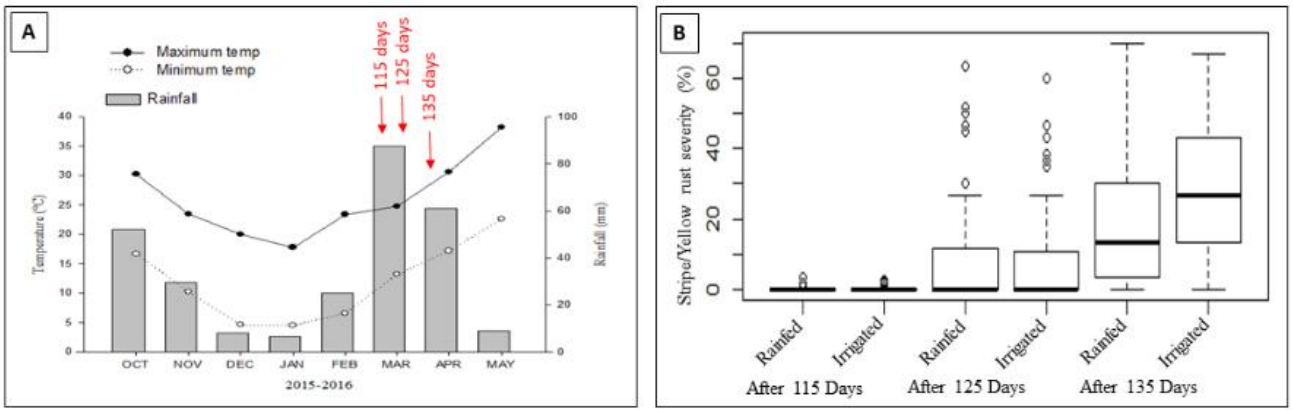

Fig. 1. Temporal variability in weather conditions (A) and yellow rust prevalence as observed for 64 wheat genotypes (B) during wheat growing season 2015-16.

Average yellow rust data showed an increasing trend after 115 days of sowing when yellow rust appeared 3% on Khatakwal and 1% on Punjab-11 while during second scoring after 125 days its severity was increased to a maximum of 61% for cross PJ-11×KW (Fig. 3). At the third scoring, maximum rust severity of 67% was observed for PJ-11×KW. In third scoring increasing trend of disease severity was observed on majority of the genotypes except JB×AH. These results clearly indicated increasing disease trend overtime which reached up to 67% at the last scoring as shown in the box plot (Fig. 1B). The slow yellow rusting performance of the tested genotypes were scored for final rust severity (FRS), Average coefficient of infection (ACI) and relative area under disease progress curve (rAUDPC) in both conditions. Crosses combination PS-05×Lal-13 and JB×PS-05 showed immune response under both conditions. Several genotypes like Lal-13×Tat-96, Tat-96×PS-13, PS-05×PS-13 and KW×PS-05 were identified with high level of partial resistance under both environments (Table 2). Maximum (67%) FRS was recorded for susceptible line PJ-11×KW under both rainfed and irrigated conditions (Table 2). Under rainfed condition based on FRS values, 11 genotypes were grouped as immune having 0 value, 41 in high, 7 in moderate while remaining five genotypes were grouped as low level of partial resistance genotypes. Similarly, under irrigated condition, 4 genotypes were grouped as immune, 33 in high, 17 in moderate, while remaining 10 lines were grouped as low level of partial resistance genotypes (Table 2).

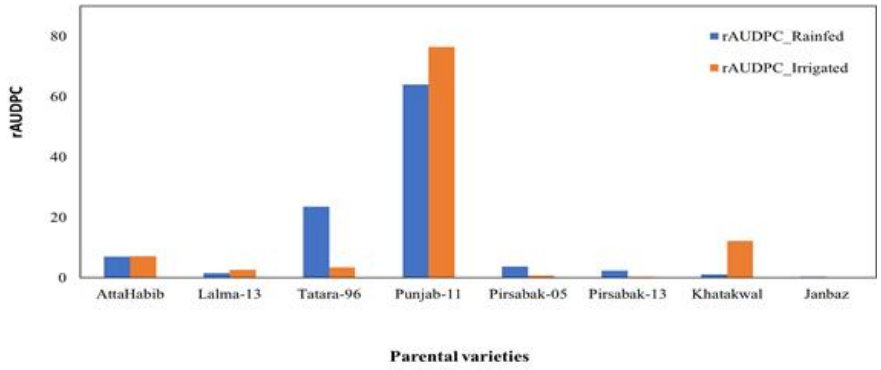

Fig. 2. Relative area under disease progress curve (rAUDPC) recorded for eight parental wheat (*Triticum aestivum* L.) genotypes tested during wheat growing season 2015-16 at Peshawar.

Genotypes having ACI value 0-20, 21–40 and 41–60 were clustered into high, moderate and low levels of adult plant resistance (APR), respectively. Under both conditions, ACI values less than 20 was recorded for 61 genotypes and considered as high APR genotypes while moderate for remaining three genotypes (Table 2). Minimum ACI of zero was observed for genotypes JB×PS-05, PS-05×Lal-13 and JB under both conditions (Table 2). Based on rAUDPC majority of the genotypes showed high to moderate level of yellow rust resistance

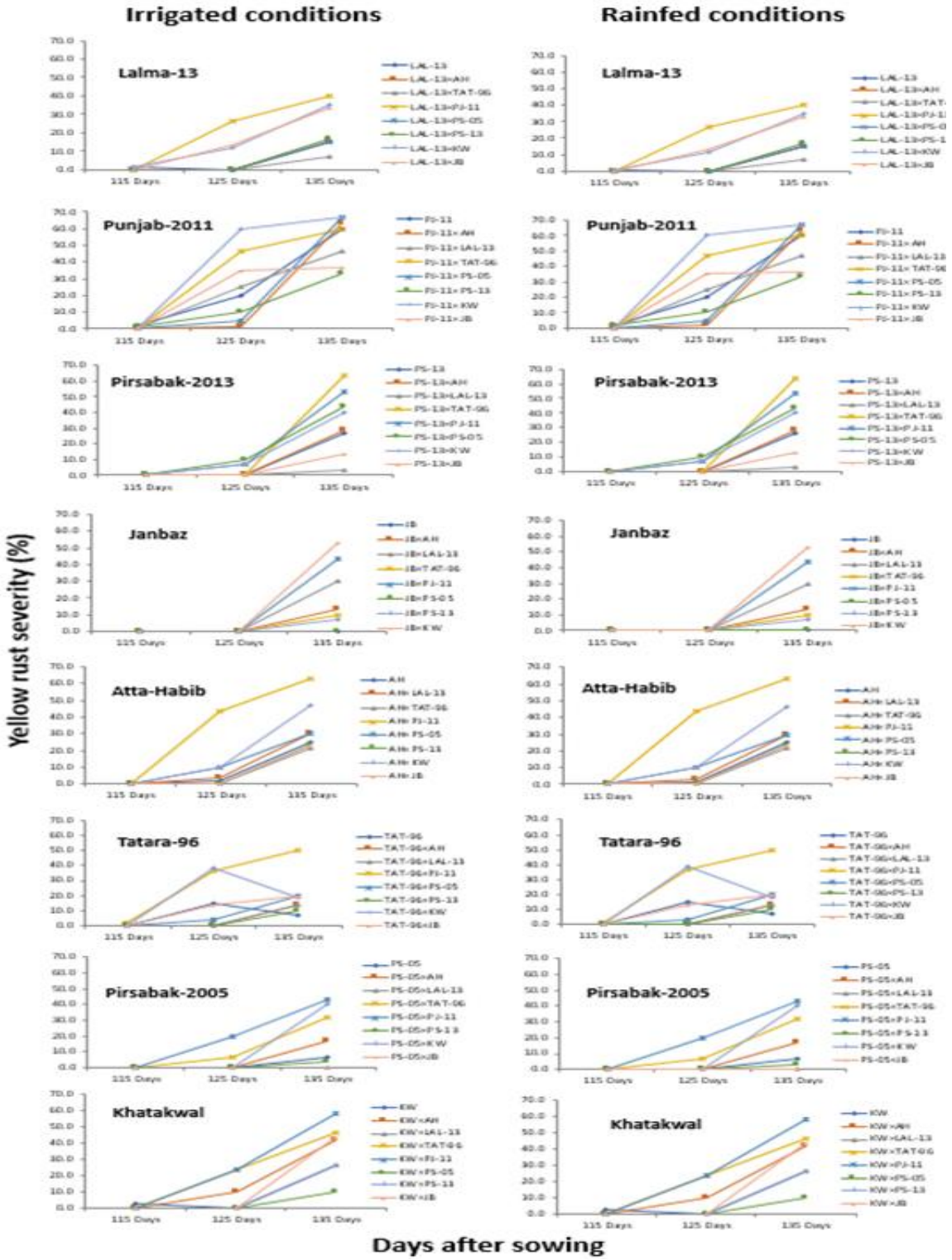

Fig. 3. Yellow rust progression curve for cross combination of eight parental lines of wheat (*Triticum aestivum* L.) genotypes during wheat growing season 2015-16.

under both environments (Table 2). Under both conditions, rAUDPC and maximum susceptibility (100%) were observed for cross PJ-11×KW (Table 2). Under rainfed condition 30 genotypes while under

irrigated condition 12 genotypes exhibited rAUDPC values up to 2 and considered as high level of partial yellow rust resistance genotypes. Similarly, 14 genotypes under rainfed and 21 genotypes under irrigated conditions were moderately resistant with rAUDPC values up to 10. The remaining genotypes were grouped into low level of partial resistance with rAUDPC value up to 100% (Table 2). Based on partial resistance parameters, genotypes such as JB×PS-13, JB×PS-05, PS-13×JB, KW×PS-05, KW×PS-13, PS-05×AH, AH×Tat-96, PS-05×JB, Lal-13×Tat-96, Tat-96×AH, and PS-13×Lal-13 showed partial resistance behavior under both rainfed and irrigated environments (Table 2). Molecular genotyping confirmed the presence of all three resistance genes in several genotypes such as AH×Tat-96, PS-05×JB, Tat-96×AH, JB×Tat-96 and JB×PS-05 (Table 2).

Clustering based on pooled data of partial resistance parameters grouped all genotypes into 5 sub clusters (Fig. 4A). Cluster 1 consisted of 39 genotypes with immune or high partial yellow rust resistance response and majority of these genotypes had ACI values less than 20. Second cluster consisted of 5 genotypes, i.e., AH×KW, PS-13×Tat-96, PJ-11×AH, PJ-11×Lal-13 and PS-13×KW having moderate to low level of partial resistance. Third cluster consisted of 13 genotypes and majority of these genotypes had moderate partial resistance response. Fourth cluster consisted of most susceptible cross, PJ-11×KW while, 5th cluster had 6 genotypes having low level of partial resistance (Fig. 4 A).

Molecular genotyping for yellow rust resistance genes: Molecular markers i.e. STS-7, ScY15 and CsLV34 linked with YR resistance genes (*Yr5, Yr17* and *Yr18*) were variably present among these genotypes (Table 3). Molecular marker STS-7 linked with *Yr5* amplified specific bands of 500 bp in 49 out of 64 genotypes and confirmed the presence of *Yr5* genes in 76% of the tested material. Similarly, marker ScY15 amplified bands of 290 bp in 50 out of 64 genotypes, which indicated the presence of resistant gene *Yr17* in 78% of the studied material. Marker csLV34 amplified allele of 150 bp and 230 bp in several genotypes which indicate the presence of resistance gene *Yr18*. Band of 150 bp was amplified in 24 genotypes which is 37% of the studied genotypes. Cluster analysis on molecular markers-based loci of 56 F_1 progeny genotypes along with parental genotypes resulted in detection of four groups (Fig. 4B). The G1 group consisted of 19 genotypes along with parent Atta Habib which consist resistant genes *Yr*17 and *Yr*18. Most of the parents were grouped in G2 and was largest group consisting of 31 genotypes carrying resistant genes *Yr5* and *Yr17*. G3 consisted of 10 genotypes having resistant genes *Yr*5 and *Yr*18. G4 was smallest group composed of only four genotypes consisting one resistant gene *Yr*5. This study confirmed the presence of all three resistant genes in several lines such as Lal-13×JB and JB×PS-05 and found partially resistant in the field which was the primary objective of this investigation.

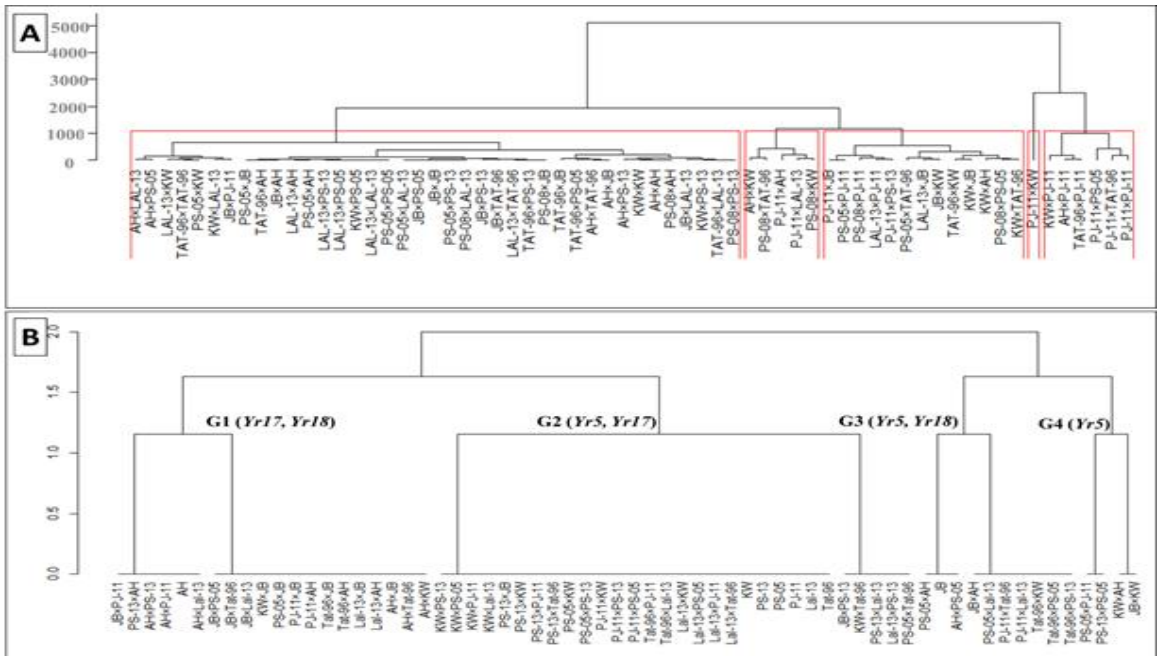

Fig. 4. Cluster analyses of 64 genotypes based on disease response parameters (A) and molecular marker genotyping (B), tested during wheat growing season 2015-16.

Table 2. Means of final rust severity (FRS %), average co-efficient of infection (ACI %) relative area under disease progress curve (rAUDPC) and Yellow rust resistant genes in 64 wheat genotypes during wheat growing season 2015-16.

Genotypes	FRS		ACI		rAUDPC		Rust resistant genes		
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	*Yr18*	*Yr17*	*Yr5*
AH	13	25	5	4.6	7	7	+	+	-
Lal-13	7	15	1	2.3	2	3	-	+	+
Tat-96	27	7	4	3.1	24	3	-	+	+
PJ-11	63	60	21	18.9	64	77	-	+	+
PS-05	13	7	2	0.8	4	1	-	+	+
PS-13	13	3	3	0.3	2	0	-	+	+
KW	3	27	2	5.5	1	12	-	+	+
JB	3	0	0	0	0	0	+	-	+
AH×Lal-13	7	30	2	3.9	2	18	+	+	-
AH×Tat-96	7	22	1	1.8	2	7	+	+	+
AH×PJ-11	47	63	11	25	39	68	+	+	-
AH×PS-05	0	30	0	5.1	0	20	+	-	+
AH×PS-13	13	23	2	4.8	6	9	+	+	-
AH×KW	40	47	10	10.3	30	33	+	+	+
AH×JB	3	23	0	3.9	0	9	+	+	+
Lal-13×AH	0	15	0	2.5	0	4	+	+	+
Lal-13×Tat-96	3	7	0	0.6	0	1	-	+	+
Lal-13×PJ-11	37	40	9	12.8	20	26	-	+	+
Lal-13×PS-05	3	15	0	2.1	0	4	-	+	+
Lal-13×PS-13	7	17	1	2.2	2	3	-	+	-
Lal-13×KW	20	35	3	8.8	5	20	-	+	+
Lal-13×JB	27	33	4	8.9	9	24	+	+	+
Tat-96×AH	0	13	0	2.2	0	3	+	+	+
Tat-96×Lal-13	13	13	2	1.1	6	5	-	+	+
Tat-96×PJ-11	57	50	18	16.8	57	52	-	+	+
Tat-96×PS-05	17	20	4	4.2	5	5	-	-	+
Tat-96×PS-13	3	10	0	1.1	0	2	-	-	+
Tat-96×KW	30	18	5	5.1	24	14	-	-	+
Tat-96×JB	0	20	1	4.4	0	7	+	+	+
PJ-11×AH	30	63	8	17	15	59	+	+	+
PJ-11×Lal-13	47	47	15	11.4	43	29	-	-	+
PJ-11×Tat-96	57	60	23	20.3	66	69	-	-	+
PJ-11×PS-05	60	67	12	20	44	79	-	+	+
PJ-11×PS-13	30	33	8	10	22	26	-	+	+
PJ-11×KW	67	67	29	28	100	100	-	+	+
PJ-11×JB	37	37	12	6	34	18	+	+	+
PS-05×AH	7	17	2	4	1	3	+	-	+
PS-05×Lal-13	0	0	0	0	0	0	-	-	+
PS-05×Tat-96	37	32	8	9	18	12	-	+	-
PS-05×PJ-11	30	43	9	13	17	33	-	-	-
PS-05×PS-13	0	3	0	0	0	0	-	+	+
PS-05×KW	7	40	1	6	1	24	-	+	+
PS-05×JB	17	0	1	0	3	0	+	+	+
PS-13×AH	15	28	2	4	5	9	+	+	-
PS-13×Lal-13	0	63	0	15	0	44	-	+	-
PS-13×Tat-96	33	53	6	13	14	34	-	+	+
PS-13×PJ-11	23	43	7	12	10	32	-	+	+
PS-13×PS-05	27	27	4	4	8	9	-	-	-
PS-13×KW	30	40	11	11	39	30	-	+	+
PS-13×JB	0	13	0	3	0	2	-	+	+

KW×AH	12	42	2	12	2	37	+	-	-
KW×Lal-13	20	27	3	5	13	10	-	+	+
KW×Tat-96	13	47	2	12	3	38	-	+	-
KW×PJ-11	13	58	3	24	5	94	-	+	+
KW×PS-05	3	10	1	2	1	4	-	+	+
KW×PS-13	3	27	1	5	0	10	-	+	+
KW×JB	30	43	4	7	12	23	+	+	+
JB×AH	0	13	5	3	0	3	-	-	+
JB×Lal-13	10	30	1	5	2	10	+	+	+
JB×Tat-96	7	10	1	2	1	2	+	+	+
JB×PJ-11	13	43	4	7	3	20	+	+	-
JB×PS-05	0	0	0	0	0	0	+	+	+
JB×PS-13	0	7	0	1	0	1	-	+	-
JB×KW	3	53	4	9	0	31	+	-	-

(+) and (–) Sign shows presence and absence, respectively
AH= Atta Habib, Lal-13 = Lalma-13, Tat-96 =Tatara-96, PJ-11= Punjab-11,
PS-05=Pirsabak-2005, PS-13 = Pirsabak-2013, JB = Janbaz and KW = Khatakwal

DISCUSSION

Our results revealed the status of rust resistance in hybrid progenies of major wheat varieties across two environments (rainfed vs. irrigated conditions) and for confirmation, molecular markers were used. The study aimed to decipher the disease resistance in hybrid progenies using both field testing and molecular markers for the parents and their hybrid progenies, which must be helpful for yellow rust disease management, which is a significant threat to wheat production (Ali and Hodson, 2017).

The disease outbreak started in the second week of March till first week of April due to favorable condition for yellow rust disease, the pathogen being favored by the low temperatures and thus, infected wheat crop relatively in early growth stage (Vergara-Diaz *et al*. 2015). Average yellow rust data showed an increasing trend after 115 days of sowing while during second scoring after 125 days its severity increased up to maximum level, due to favorable environment for disease as previously suggested by Ali *et al*. (2014a). In April 2016 decreasing trend in rust severity was observed due to unfavorable environmental condition for yellow rust. High rainfall and low average temperature in the growing season contribute significantly to the establishment and spreading of stripe rust in wheat crop (Agenbag *et al*. 2012; Chen 2013).

Considering the impact of micro-environment particularly in terms of humidity and temperature (Wan and Chen 2012), screening of the hybrid progenies was done at variable environments i.e., irrigated and rainfed conditions. The resistance response varied across the two tested environments, as revealed significant genotype-by-environment (G×E) interaction for ACI, which reflects on the role of micro environment in terms of humidity and temperature for onset of the rust diseases (Ali *et al*., 2009a). Indeed evaluation of wheat germplasm across different environments must be done for assessment of adult plant resistance (Ma and Singh, 1996), which is considered more durable (Ali *et al*., 2014b, Shah *et al*., 2010).

The hybrid progenies revealed significant variability for yellow rust resistance, which could be attributed to the variability at genetic level as influenced by the environment (Ahmad *et al*.2016; Farshadfar and Amiri 2015). Several genotypes showed moderate (M) to moderately susceptible (MS) reaction which indicated lack of high level of resistance among the tested hybrid progenies, which has been common in Pakistani wheat germplasm (Afzal *et al*. 2008; Ali *et al*., 2009a; Lillemo *et al.,* 2008).

Slow rusting has been studied based on FRS, ACI and rAUDPC which categorize the lines into four groups of partial yellow rust resistance i.e., immune, high, moderate and low levels of partial yellow rust resistance as categorized in previous work (Ali *et al*., 2009a; Pathan and Park, 2006). In our study, majority of the studied hybrid progenies exhibited high level of partial resistance having FRS value up to 30. Field based variability in these parameters reflect on the level of partial resistance to yellow rust in different wheat genotypes (Taye *et al*., 2014; Pathan and Park, 2006; Ali *et al*., 2009c; Safavi and Afshari, 2012). Partial resistance is due to several minor genes which prevents development of newly virulent race of the pathogen because multiple point mutations are very rare in nature (Ali *et al*., 2009a; Pathan and Park, 2006).

Under both irrigated and rainfed conditions, genotypes carrying partial rust resistance genes, comparatively performed well. Genotypes having similar partial resistance, were further grouped using cluster analyses (Ward 1963), based on FRS, ACI and rAUDPC. Lines having partial resistance phenotype possessed minor resistance genes which could be accumulated and utilized in a breeding program for durable rust resistance

(Ali *et al*. 2009b; Pathan and Park 2006; Brar *et al*., 2018).

Significant variability was observed through molecular genotyping for yellow rust resistance genes. Yellow rust resistance gene *Yr5* specific marker amplified in 49 out of 64 genotypes which is 76% of the studied material. Similarly, *Yr17* specific marker was amplified in 50 out of 64 genotypes, which indicate the frequent presence (78%) of resistant gene *Yr17* in the studied material. Marker csLV34 amplified allele of 150 bp and 230 bp in several genotypes which indicate the presence of resistance gene *Yr18*. Band of 150 bp was amplified in 24 genotypes which is 37% of all the studied genotypes. Shah *et al*. (2010) also reported amplification of two marker alleles for csLv34, in which 150bp was closely linked with marker resistance gene *Yr18* and 230bp was not associated with resistance. Cluster analyses grouped related genotypes in to four clusters based on presence of resistance genes. The first cluster G1 consisted of those lines which had either all the three or two genes, while G2 consisted those lines which have two genes and was the largest group among all clusters. Similarly, G3 consisted of genotypes which has only one resistance gene. Group 4 genotypes were comprised of only one or none of the studied gene.

Many rust resistance genes *Yr5, Yr7, Yr8, Yr9, Yr10, Yr17* and *Yr18* have been mapped in several wheat breeding program, to combine it with other genes of all-stage rust resistance (Chen, 2013 and Mallard *et al*., 2005). These results would be helpful in transferring *Yr5* genes in commercial cultivars and combination with other *Yr* resistance genes. Similarly, *Yr17* gene provides yellow rust resistance both at seedling and adult plant stages and is present in many European wheat lines (Ali *et al*. (2014a) which is introgressed into wheat from *Aegilops ventricosa* along with linked genes *Lr37* and *Sr38* (Boyd, 2005) which could be incorporated into deficient varieties. Genes *Yr36* and *Yr18* provide non race-specific durable resistance to wheat yellow rust (Yuan *et al*. 2012). *Yr18* gene is linked with powdery mildew (*Pm38*), stem rust resistance (*Sr57*), leaf rust resistance (*Lr34*) and leaf tip necrosis phenotype (Ellis *et al.,* 2014). This study confirmed the presence of all three resistant genes in several lines and found partially resistant in the field which was the primary objective of this investigation. Thus, combination of field testing along with molecular study is an important strategy to identify genes conferring partial resistance. Both field testing and molecular markers results showed variation in resistance and the identified genotypes could be utilized in a wheat breeding program to develop yellow rust resistant varieties and to reduce wheat yield losses due to yellow rust.

Conclusions: Considerable variability among the F_1 population, for yellow rust resistance and yield potential, was observed. Based on partial resistance parameters, genotypes JB×PS-13, JB×PS-05, PS-13×JB KW×PS-05, KW×PS-13, PS-05×AH, AH×Tat-96, PS-05×JB, Lal-13×Tat-96, Tat-96×AH, and PS-13×Lal-13 showed partial yellow rust resistance having chlorotic and necrotic response. Under rainfed condition, genotypes PS-05×AH, PS-05 and PS-13×PS-05 while under irrigated condition, Lal-13×JB, PS-05×Lal-13, PS-05×Tat-96 and JB×PS-05 showed partial resistance response in field. Molecular genotyping confirmed the presence of all three resistance genes in genotypes AH×Tat-96, PS-05×JB, Tat-96×AH, JB×Tat-96 and JB×PS-05. The yellow rust resistance gene *Yr5* specific marker was amplified in 76%, *Yr17* in 78% and *Yr18* in 37% of the studied wheat genotypes. These genotypes may be used in a breeding program and could be further evaluated for the development of disease resistant and high yielding varieties.

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REFERNCES

Afzal, S.N., M.I. Haque, M.S. Ahmedani, A. Rauf, M. Munir, S.S. Firdous, A. Rattu, and I. Ahmad(2008). Impact of stripe rust on kernel weight of wheat varieties sown in rainfed areas of Pakistan. Pakistan J. Bot. 40(2): 923-929.

Agenbag, G.M., Z.A. Pretorius, L.A. Boyd, C.M. Bender and R. Prins (2012). Identification of adult plant resistance to stripe rust in the wheat cultivar Cappelle-Desprez. Theor. Appl. Gen. 125: 109-120.

Ahmad, I., N. Mahmood, I. Khaliq and N. Khan (2016). Genetic analysis for five important morphological attributes in wheat (*Triticum aestivum* L.). J. Ani. Plant Sci. 26(3): 725-730.

Ahmad, R., S. Qadir, N. Ahmad and K.H. Shah (2003). Yield potential and stability of nine wheat varieties under water stress conditions. Int. J. Agric. Biol. 5(1): 7-9.

Alderfasi, A.A and D.C. Nielsen (2001). Use of crop water stress index for monitoring water status and scheduling irrigation in wheat. Agric. Water Manag. 47: 69-75.

Ali, S., P. Gladieux, M. Leconte, A. Gautier, A.F. Justesen, M.S. Hovmøller, J. Enjalbert and C. De-Vallavieille-Pope (2014a). Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis f. sp. tritici*. PLoS Pathog. 10(1): 1-12.

Ali, S., M. Leconte, H. Rahman, M.S. Saqib, P. Gladieux,J. Enjalbert and C. De Vallavieille-Pope (2014b). A high virulence and pathotype diversity of *Puccinia striiformis f. sp. tritici* at its centre of diversity, the Himalayan region of Pakistan. Eur. J. Plant Pathol. 140: 275-290.

Ali, S., H. Rahman, S.J.A. Shahand F. Ullah (2009a). Assessment of field resistance using host-pathogen interaction phenotype for wheat yellow rust. Afric. Crop Sci. J. 17(4): 213-221.

Ali. S., S.J.A. Shah, H. Rahman. M.S. Saqib, M. Ibrahim and M. Sajjad (2009b). Variability in wheat yield under yellow rust pressure in Pakistan. Turk. J. Agric. Fores. 33: 537-546.

Ali, S., S.J.A. Shah, I.H. Khalil, H. Raman, K. Maqbool and W. Ullah (2009c). Partial resistance to yellow rust in introduced winter wheat germplasm at the north of Pakistan. Aus. J. Crop Sci. 3: 37-43.

Ali, S., and D. Hodson (2017). Wheat rust surveillance: Field disease scoring and sample collection for phenotyping and molecular genotyping. *In* "Wheat Rust Diseases", pp. 3-11. Springer.

Asad, M.A., X. Xia, C. Wang and Z. He (2012). Molecular mapping of stripe rust resistance gene *YrSN104* in Chinese wheat line Shaannong 104. Hereditas.149: 146-152.

Bai, Y.-L., C.-Y. Zhang, S. Quan, L. Feng, C. Na, S.-C. Xu, G. Yang, and X.-D. Xu (2010). Molecular screening and resistance evaluation of American wheat cultivars to Chinese stripe rust races. Agric.Sci. China **9**, 1455-1461.

Boyd, L. 2005. Can Robigus defeat an old enemy?– Yellow rust of wheat. J. Agric. Sci. 143: 233-243.

Brar, G., and H. Kutcher (2016). Race characterization of *Puccinia striiformis f. sp. tritici*, the cause of wheat stripe rust, in Saskatchewan and southern Alberta, Canada and virulence comparison with races from the United States. J. Plant Disease. 100. 1744-1753.

Brar, G.S., S. Ali D. Qutob S. Ambrose K. Lou R. Maclachlan, C.J. Pozniak, Y-B. Fu, A.G. Sharpe and H.R. Kutcher (2018). Genome re-sequencing and simple sequence repeat markers reveal the existence of divergent lineages in the Canadian Puccinia striiformis f. sp. tritici population with extensive DNA methylation. Envi. Microbiol.. 20, 1498-1515.

Chen, X. (2013). High-temperature adult-plant resistance, key for sustainable control of stripe rust. Americ. J. Plant Sci. 4: 608-627.

Ellis, J.G, E.S. Lagudah, W. Spielmeyer and P.N. Dodds (2014). The past, present and future of breeding rust resistant wheat. Front. Plant Sci. 5: 1-13.

Farshadfar, E and R. Amiri (2015). Genetic analysis of physiological indicators of drought tolerance in bread wheat using diallel technique. Genetika. 47(1): 107-118.

Hovmøller, M.S., S. Walter and A.F. Justesen (2010). Escalating threat of wheat rusts. Science 329: 369-369.

Kilic, H. and T. Yagbasanlar (2010). The effect of drought stress on grain yield, yield components and some quality traits of durum wheat (*Triticum turgidum ssp. durum*) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 38(1): 164-170.

Kolmer, J.A., Y. Jin and D.L. Long (2007). Wheat leaf and stem rust in the United States. Aus. J. Agric. Res. 58: 631-638.

Khan M.R., Z.U. Rahman, S.N. Nazir, S. Tshewang, S. Baidya, D. Hodson, M. Imtiaz and S. Ali (2019). Genetic divergence and diversity in Himalayan Puccinia striiformis populations from Bhutan, Nepal and Pakistan. Phytopathol. 109:1793-1800.

Lillemo, M., B. Asalf, R.P. Singh, J. Huerta-Espino, X.M. Chen, Z.H. He and Å. Bjørnstad (2008). The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor. Appl. Genet. 116: 1155-1166.

Lin, F. and X.M. Chen (2007). Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor. Appl. Genet. 114: 1277-1287.

Ma, H., and R.P. Singh (1996). Contribution of adult plant resistance gene Yr18 in protecting wheat from yellow rust. Plant Disease. 80(1): 66-69.

Mallard, S., D. Gaudet, A. Aldeia, C. Abelard, A.L. Besnard,P. Sourdille and F. Dedryver. 2005. Genetic analysis of durable resistance to yellow rust in bread wheat. Theor. Appl. Genet. 110: 1401-1409.

Mansfield, J. S. Genin, S. Magori, V. Citovsky, M. Sriariyanum, P.Ponald, M. Dow, V. Verdier, S.V. Beer, M.A. Machodo, A. Toth, G. Salmand

and G.D. Foster (2012). The Top 10 fungal pathogens in molecular plant pathology. Mole. Plant Patho. 13:414-430.

Oliver, R.P. (2014). A reassessment of the risk of rust fungi developing resistance to fungicides. Pest Manag. Sci. 70: 1641-1645.

Pathan, A.K. and R.F. Park (2006). Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica. 149: 327-342.

Safavi, S.A. and F. Afshari (2012). Identification of resistance to *Puccinia striiformis* f. sp. *tritici in* some elite wheat lines. J. Crop Protec. 1(4): 293-302.

Shah, S. J. A., M. Imtiaz and S. Hussain (2010). Phenotypic and molecular characterization of wheat for slow rusting resistance against *Puccinia striiformis Westend. f. sp. tritici*. J. Phytopathol. 158: 1-10.

Steel, R. G. D., J. H. Torrie and D. A Dickey. (1997). Principle and procedures of statistic: A biometrical approach. New York: McGraw-Hill.

Suenaga, K., R.P. Singh, J. Huerta-Espino and H. William (2003). Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathol. 93(7): 881-890.

Taye, T., C. Fininsa and G. Woldeab (2014). Evaluation of wheat cultivars for slow rusting resistance in Guji zone, Southern Oromia. Afric. J. Agric. Res. 9(46): 3388-3392.

Uauy, C., J.C. Brevis, X. Chen, I. Khan, L. Jackson, O. Chicaiza, A.Distelfeld, T. Fahima and J. Dubcovsky (2005). High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from *Triticum turgidum* ssp. dicoccoides is closely linked to the grain protein content locus Gpc-B1. Theor. Appl. Genet. 112: 97-105.

Vergara-Diaz, O., S.C. Kefauver, A. Elazab, M.T. Nieto-Taladriz and J.L. Araus (2015). Grain yield losses in yellow-rusted durum wheat estimated using digital and conventional parameters under field conditions. Crop J. 3: 200-210.

Wan, A and X. Chen (2012). Virulence, frequency, and distribution of races of Puccinia striiformis f. sp. tritici and P. striiformis f. sp. hordei identified in the united states in 2008 and 2009. Plant Disease. 96: 67-74.

Ward, J.H. (1963). Hierarchical grouping to optimize an objective function. J. Americ. Statis. Associa. 58(301): 236-244.

Wellings, C.R. (2011). Global status of stripe rust: a review of historical and current threats. Euphytica. 179: 129-141.

Yuan, C., H. Jiang, H. Wang, K. Li, H. Tang, X. Li and D. Fu. (2012). Distribution, frequency and variation of stripe rust resistance loci *Yr10, Lr34/Yr18* and *Yr36* in Chinese wheat cultivars. J. Genet. Geno. 39: 587-592.