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EVALUATION OF MAJOR AND ENVIRONMENTALLY DRIVEN GENES FOR RESISTANCE IN PAKISTANI WHEAT LANDRACES AND THEIR PROSPECTED POTENTIAL AGAINST YELLOW RUST

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ABSTRACT

Yellow rust is caused by *Pst* (*Puccinia striiformis tritici*), the most devastating wheat disease with continuous challenge of emerging virulences breaking vertical resistance worldwide resulting in epidemics. Vertical resistance genes incorporation is a sustainable, economical and environment-friendly approach to control rust diseases. Wheat landraces (WLR) acquired vertical resistance through long time exposure of host-pathogen survival competition in a specific area having unique agronomic traits, genetic base and resistance against biotic and abiotic agents can be an exploitable commodity for future food production. Fifty Pakistani WLRs already with known vertical resistance were screened against 7 potential Pakistani *Pst* races at seedling stage under glasshouse conditions to postulate resistance genes. Resistance magnitude was compared among the landraces. Six genes *Yr1, Yr8, Yr9, Yr43, Yr44,* and *YrTr1* were successfully postulated either singly or in combination along with unidentified genes in 45 landraces. Pakistani *Pst* races are avirulent to *Yr5, Yr10*, *Yr15*, *Yr24*, *Yr32, YrSp* and *YrTye*. Most frequently postulated genes are *Yr44* found in 22 genotypes, *YrTr1* in 21, *Yr9* in 19, *Yr43* in 18, *Yr8* and *Yr1* in 14 wheat landraces. Multiple *Yr* gene pyramiding was observed in (B-74, B-281, B-530) with the presence of *Yr8, Yr9, Yr43, Yr44,* and *YrTr1* and single gene were postulated from 12 genotypes. WLRs (B-03, B-158, B-160, B-171) reaction was immune showing presence of novel *Yr* genes. The study provides information regarding yellow rust resistance genes originated independently against localized *Pst* races with desirable agronomic traits since long and can be an option for food security in changing environmental challenges.

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INTRODUCTION

Yellow rust is caused by Pst (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) incurs heavy yield losses in the wheat worldwide including Pakistan. Although the disease was considered as targeting the wheat crop is cooler areas, however; during crop seasons, 2018-19 and 2019-20 yellow rust epidemic hit even tropical areas of Pakistan targeting the majority of the cultivars remaining a few. Epidemic resulted due to low prevailing temperature throughout the spring season

with frequent rains and favoured by the narrow gene pool especially in case of high yield wheat varieties (Ali *et al*., 1992). Moreover, the pathogen has adopted in warmer climate consequently area under stripe rust has expanded considerably. The epidemiology of stripe rust is influenced not only by the deployment of resistance genes in wheat varieties but also by climatic conditions affecting *Pst* infection and growth and by wind movements across the areas where wheat is grown (Wamalwa *et al.*, 2019; Wellings, 2011). Hence, regular virulence analysis is vital for disease management and the incorporation of resistant genes (Wan *et al*., 2016). Due to its obligate nature, the *Pst* race-specific resistance is described by Flor (1971) and provides the identification base of host-pathogen interaction. New virulent races of the yellow rust are continuously emerging worldwide, and in Pakistan, several races are characterized for their pathogenicity and virulences.

Various attempts had been made in search of resistance in commercial bread wheat cultivars and among synthetic hexaploid wheat as well to improve the genetic base of resistance against yellow rust disease (Rizwan *et al*., 2007; Tariq-Khan and Ul-Haque, 2011; Tariq-Khan *et al*., 2012). Attempts are not durable due to narrow genetic base of bread wheat cultivars and the presence of necrotic genes in synthetic hexaploid wheat making the desired traits transfer difficult. Wheat landraces (WLRs) are farmer's varieties or folk cultivars developed over thousands of years, human selection based on adaptation to a specific environment, taste, bread making quality and agronomic traits (Belay *et al*., 1995; Zeven, 1999). The morphological and genetic structure of WLRs are predominantly retained through selection, isolation, lack of migration, and restrictions on outcrossing and genetic recombination, developed multilocus structures, genetic drift or fragmentation (Brown, 2000) made them valuable sources for genetic base among cultivated wheat (Ehdaie and Waines, 1989; Jaradat, 2013; Masood *et al.*, 2005; Zencirci and Karagoz, 2005).

Gene postulation is a successful conventional technique to find desirable genes for resistance among diverse genotypes. An array of rust races with known specific virulence are subjected to determine the most probable genes for resistance present in host lines (Feng *et al.*, 2009; Ochoa *et al.*, 2006; Pathan and Park, 2006; Singh *et al.*, 2001). The postulation of 27 Australian spring wheat revealed the presence of *Yr7* and *Yr17* (Qamar *et al*., 2008), while *Yr1, Yr2, Yr2+YrHVII,* Yr3+unknown, *Yr3+Yr4, YrAlba, Yr5, Yr8, Yr19, Yr27*, and *Yr24* in 20 wheat cultivars. Ten probable genes for resistance in 13 WLRs with the common indication of *Yr9 (*Feng *et al*., 2009*)* and 22 Yr genes from 44 wheat cultivars were postulated in china (Xiaodan *et al.*, 2011). Dawit *et al*. (2012) and his colleagues postulated *Yr2, Yr3a, Yr4a, Yr6, Yr7, Yr8, Yr9, Yr27, Yr32*, and *YrSU* genes from 22 Ethiopian bread wheat cultivars; however, *Yr2, Yr6, Yr7, Yr8, Yr9, Yr27*, and *Yr32* were found ineffective against present African *Pst* races explaining the sufficient potential of the WLRs as a source of novel resistance genes.

During the cropping season, 2018-2019 yield losses due to yellow rust are estimated 13% and the most recent crop of 2019-2020 the disease is responsible for 15% yield reduction in Pakistan and most of the recently released varieties with resistance are attacked by the yellow rust (Personal communication with Agricultural authorities). Due to the serious nature of breakdown of resistance in wheat varieties which are originated from CIMMYT bloodlines; it was found imperative to go for landraces as conventional sources of resistance. WLRs are promising sources of resistance against diseases and genetic diversity due to their diverse origin and exploitable for new unidentified genes to improve bread wheat. New windows to acquire the genetic diversity and resistance against rusts are imperative in the current agricultural wheat production system by knowing above mentioned potential. Very little has been done to understand the genetic structure of WLRs and available inter-specific diversity existing in agro-ecosystems and is still dominating in parts of the Old World (Altieri and Merrick, 1987). Pakistani wheat landraces in special are very little explored for the presence of vertical resistance genes against abiotic and biotic stresses including yellow rust. This study aimed to answer the question that is their *Yr* gene present in WLRs durable and exploitable against yellow rust virulences to facilitate future wheat breeding.

MATERIALS AND METHODS

Wheat landraces (WLRs) seeds were collected from different wheat-growing areas of Pakistan and maintained at Crop Disease Research Institute (CDRI) Murree germplasm collection. The seedlings were grown under greenhouse conditions at CDRI, Murree-Pakistan. For the resistance genes, postulation 50

WLRs with known major gene resistance were used to screen against *Pst* races of Pakistani origin.

Pathogen culture

Seven Pakistani *Pst* physiological races, 434233, 474232, 534212, 414202, 434232, 574232 and 574212 were acquired from Yellow Rust Culture Collection, CDRI Murree (stored at -80 ^{O}C). The isolates were obtained from the samples collected during 05 growing seasons i.e., from 2012-2016 (Table 1). Their pathotypes were confirmed before use for screening on a set of yellow rust differential consisting of European, World, and Avocet near-isogenic lines (NILs). The seeds of the differential sets and NILs were obtained from the International Wheat and Maize Improvement Center (CIMMYT), Mexico.

Table 1. Yellow rust races collection from Pakistan and their avirulence and virulence of differential wheat hosts.

Sr. No.	Race	Avirulence	Virulence
1	434233	*Yr1, Yr5, Yr9, Yr10, Yr15, Yr24, Yr32, YrSp, YrTye*	*Yr6, Yr7, Yr8, Yr17, Yr27, Yr43, Yr44, YrTr1, YrExp2*
2	474232	*Yr1, Yr5, Yr10, Yr15, Yr24, Yr32, YrSp, YrTr1, YrTye*	*Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrExp2*
3	534212	*Yr5, Yr9, Yr10, Yr15, Yr24, Yr32, Yr44, YrSp, YrTr1, YrTye*	*Yr1, Yr6, Yr7, Yr8, Yr17, Yr27, Yr43, YrExp2*
4	414202	*Yr1, Yr5, Yr8, Yr9, Yr10, Yr15, Yr24, Yr32, Yr43, Yr44 YrSp, YrTr1, YrTye*	*Yr6, Yr7, Yr17, Yr27, YrExp2*
5	434232	*Yr1, Yr5, Yr9, Yr10, Yr15, Yr24, Yr32, YrSp, YrTr1, YrTye*	*Yr6, Yr7, Yr8, Yr17, Yr27, Yr43, Yr44, YrExp2*
6	574232	*Yr5, Yr10, Yr15, Yr24, Yr32, YrSp, YrTr1, YrTye*	*Yr1, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrExp2*
7	574212	*Yr5, Yr10, Yr15, Yr24, Yr32, Yr44, YrSp, YrTr1, YrTye*	*Yr1, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, YrExp2*

***Pst* races and cultures**

Immediately after the removal from -80 ^{O}C, the cultures of *Pst* races were exposed to heat shock for 15 minutes by dipping in hot water (45 ^{O}C). The urediniospores were then mixed with Mineral oil and Petroleum Ether (20:80 V/V) and sprayed on 10 days old seedlings of susceptible wheat cultivar Morocco. Inoculated plants were placed in the open air for 1 hour to evaporate the mixture of petroleum ether and mineral oil. Finally, the plants were transferred to a dew chamber maintained at 10 ^{O}C and 99% relative humidity for 24 hours. The next day the plants were transferred to a clean chamber of glasshouse set at 18 ^{O}C temperature to facilitate infections. After 12-15 days when the first pustule appeared on Morocco, the plants were transferred to the yellow rust infection development chamber set at 18 ^{O}C temperature. Upon sporulation, urediniospores were collected on the 20th day of inoculation on a piece of sterile butter paper by tapping the leaves of infected plants.

Evaluation of seedling resistance in Pakistani wheat landraces WLRs

The landraces along with yellow rust isogenic lines were screened under controlled glasshouse conditions. The landraces and isogenic lines were planted in 4″x4″ X 4″x4″ plastic pots filled with commercial peat moss (SAB Services, Germany). Susceptible cultivars Morocco and Avocet-S were included as checks. Five landraces were sown per pot with approximately 3-5 seeds per entry and the pots were watered from underneath and kept in clean glass house chamber till inoculation. Germinated seedlings were kept in a rust-free clean glasshouse chamber set at 30 ^{O}C for 10 days. The plants were fertilized after every 7 days, by irrigating them with a solution of NPK 1.6g per tray. After ten days when seedlings were at two-leaf stage (Feekes stage 1), were inoculated with yellow rust spores suspension using the aforementioned method and maintained in spore-proof glasshouse chamber with 10-12^{O}C temperature for the establishment of yellow rust infections. After 12-15 days when the first pustule appears on susceptible cultivars, the plants were transferred to the yellow rust infection development chamber maintained at 10-15 ^{O}C temperature.

Data recording

The resistance spectrum was based on a gene-for-gene hypothesis where the infection type (IT) produced by the *Pst* races on the wheat landraces was compared with

the ITs produced by the same races on the tester isogenic lines with known genes (differential lines) (Dubin, 1989; Hovmøller, 2007; Wamalwa *et al*., 2019). ITs were recorded three weeks after inoculation when the susceptible checks showed maximum infections (Line and Qayoum, 1992) according to 0-9 scale where IT 0 was regarded as immune while 9 was considered as highly susceptible (McNeal *et al*., 1971).

RESULTS

Fifty WLRs (Wheat landraces) of Pakistani origin having major genes for resistance were tested to all identified predominant *Puccinia striiformis* f. sp. *tritici* (*Pst*) races collected all over from Pakistan. Details of virulences and avirulences against yellow rust genes based on differential reaction infection types (IT) are summarized in Table (1) after 4 times continuous screening and infection type (IT) displayed by each WLRs to *Pst* race is given in Table (2). The IT produced by *Pst* on differential lines revealed that most of the races were virulent to genes *Yr1, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrTr1, YrExp2* while avirulent to *Yr5, Yr10, Yr15, Yr24, Yr32, YrSp,* and *YrTye* (Table 2). *Pst* race (434233) found most frequently virulent to 33 out of 50 screened WLRs*. Pst* race 574212 was second most aggressive which is virulent to 18 genotypes while the race 574232 was found mild with virulence to 10 landraces (Table 3). The screening results favour the presence of all type of single, double and double complementary genes in virulences existing in Pakistan. *Yr5* and *Yr15* are resistant against an extensive range of *Pst* isolates originated from different geographical regions (Dubin, 1989; Wamalwa *et al.*, 2019; Zhou *et al.*, 2014).

Table 2. Seedling stage infection types (IT) displayed by isogenic differential lines to 7 different pathotypes of *P. striiformis tritici.*

Landrace	Yr genes	Infection types produced by the *Pst* races						
		434233	474232	534212	414202	434232	574232	574212
AcSYr1NIL	*Yr1*	2	0	7	1	0	8	8
AcSYr5NIL	*Yr5*	2	0	2	2	0	0	1
AcSYr6NIL	*Yr6*	8	9	9	7	9	8	9
AcSYr7NIL	*Yr7*	8	9	9	8	9	9	8
AcSYr8NIL	*Yr8*	8	9	9	3	9	9	8
AcSYr9NIL	*Yr9*	4	9	6	3	2	9	8
AcSYr10NIL	*Yr10*	2	0	1	2	0	2	0
AcSYr15NIL	*Yr15*	0	0	0	0	0	0	0
AcSYr17NIL	*Yr17*	8	9	8	7	9	9	8
AcSYr24NIL	*Yr24*	3	0	3	3	3	2	3
AcSYr7NIL	*Yr27*	8	9	8	7	8	9	8
AcSYr32NIL	*Yr32*	3	0	3	3	2	0	3
AvSIDO377s(F3-41-1)	*Yr43*	7	9	7	6	9	9	7
AvSZak(1-1-35-91)	*Yr44*	8	9	4	2	9	8	4
AvSYrSPNIL	*YrSp*	0	0	0	1	2	0	0
AvSYrTres1NIL	*YrTr1*	8	0	6	3	5	1	5
AvSExp1/1-1974	*YrExp2*	8	9	9	7	9	9	8
Tyee	*YrTye*	2	0	3	4	0	4	1
Morocco	Moroc	9	9	9	9	9	9	9

Table 3. Seedling infection types displayed by Pakistani wheat landraces when tested with 7 pathotypes of *P. striformis tritici* and postulated genes.

Sr. No	Landraces	434233	474232	534212	414202	434232	574232	574212	Postulated genes
1	B-03	4	0	2	2	1	1	1	Resistant to all races
2	B-20	6	5	4	4	4	2	7	43, 44
3	B-22	9	2	4	5	5	7	5	44,Tr1
4	B-39	9	4	3	2	3	7	4	44
5	B-41	9	5	3	5	3	4	6	-
6	B-43	9	4	3	3	3	6	7	8, 9, 43, Tr1
7	B-46	8	4	5	4	4	3	7	8, 43, 44, Tr1
8	B-57	9	4	5	6	3	4	7	43
9	B-61	2	3	-	4	4	2	5	1, Tr1
10	B-64	2	5	6	4	4	6	7	1, 9, 43, Tr1
11	B-65	9	6	5	4	4	5	7	43
12	B-66	1	0	0	2	7	1	1	1, 44, Tr1
13	B-67	7	2	3	2	2	1	4	9, 43, 44, Tr1
14	B-74	9	4	4	3	3	3	7	8, 9, 43, 44, Tr1
15	B-79	4	6	2	2	4	7	2	9, 44
16	B-83	3	7	0	1	6	7	2	1
17	B-86	3	7	2	1	7	8	4	1, 44
18	B-87	4	6	1	1	7	7	1	9
19	B-124	9	5	4	1	3	3	5	1, 44, Tr1
20	B-142	8	4	4	3	3	4	6	8, 9, 44, Tr1
21	B-143	8	4	4	3	3	4	5	8, 9, 44, Tr1
22	B-148	9	4	3	1	4	3	4	1, 44
23	B-158	5	2	0	2	2	3	4	Resistant to all races
24	B-160	4	4	6	4	3	3	5	Resistant to all races
25	B-164	9	5	5	4	3	3	7	43
26	B-165	7	3	3	4	3	3	6	43
27	B-166	9	6	6	5	6	3	5	9, Tr1
28	B-171	5	3	4	3	3	3	6	Resistant to all races
29	B-179	9	5	4	5	3	4	6	44
30	B-199	9	5	5	4	4	4		-
31	B-201	9	5	6	4	5	6	5	Tr1
32	B-205	7	0	6	4	4	3	6	1, 43, Tr1
33	B-250	8	4	4	4	4	7	7	8, 43, 44, Tr1
34	B-263	9	6	5	5	3	-	6	-
35	B-276	9	4	4	4	3	4	8	1, 8, 9, 44
36	B-277	9	6	5	4	3	4	8	1, 8, 9
37	B-281	8	4	5	3	4	5	7	8, 9, 43, 44, Tr1
38	B-290	5	6	6	4	4	5	9	9, Tr1
39	B-308	9	0	3	1	5	6	5	1, Tr1
40	B-317	3	2	2	2	3	3	9	44
41	B-369	4	4	2	6	7	9	1	8, 9, 43
42	B-501	9	4	2	1	5	6	2	1, Tr1
43	B-507	9	5	1	1	4	-	8	1, 8, 9
44	B-527	9	5	1	1	6	9	4	1, 8, 9, 43, 44
45	B-530	9	4	4	3	2	5	7	8, 9, 43, 44, Tr1
46	B-532	9	4	5	3	3	4	7	8, 9, 43, Tr1
47	B-535	9	4	1	-	3	7	0	-
48	B-587	9	4	4	-	3	4	6	44
49	B-609	7	5	4	5	4	3	6	43, 44
50	B-673	4	-	5	7	3	5	9	9

Postulated Resistance genes during this study are *Yr1*, *Yr8*, *Yr9*, *Yr43*, *Yr44* and *YrTr1* in all tested WLRs at the seedling stage. Four WLRs (B-03, B-158, B-160, and B-171) were resistant to all the *Pst* isolates. Ten WLRs were resistant to the majority of the tested *Pst* races (Table 3). Each WLRs has been developed through a long-time evolution process in a specific environment, so these lines have developed resistance over the hundreds of years against the continuous disease attack. The *Yr* genes present in some WLRs like B-41, B-199, B-263, and B-535 could not be postulated because of non-matching virulence combinations with any of known genes for proper inferences. Six genes *Yr1, Yr8, Yr9, Yr43, Yr44*, and *YrTr1* were successfully postulated in Pakistani wheat genotypes among them *Yr44* and *YrTr1* found in 22 and 21 WLRs as most frequent while *Yr1* and *Yr8* in 14 each as least frequent. *Yr5, Yr10, Yr15, Yr24, Yr32, YrSp*, and *YrTye* could not postulate due to their low IT to *Pst* races; while *Yr6, Yr7, Yr17, Yr27*, and *YrExp2* were unable to found in any WLRs due to higher IT. WLRs B-41, B-199, B-263 and B-535 showed mixed IT (Table 3) so concluded with unidentified genes for resistance due to their extreme reactions. *Yr1* is postulated in 14 WLRs based on the multi-pathotype tests, 13 out of 14 landraces were postulated to have *Yr1* in combination with other genes. It was postulated in combination with *YrTr1* in 7 genotypes and with *Yr44* in 6 landraces. WLRs having *Yr1* in combination were found resistance to all virulences of Pakistani *Pst* that is even unique and provides an opportunity to the scientist for novel resistance (Table 3).

Yr8 was reported by Riley *et al*. (1968) which was originated from (*Aegilops speltoides*) was postulated in various combination with several resistance genes in 14 WLRs (28%) as multiple genes. This study confirms the presence of *Yr8* in multiple gene combinations with *Lr26*, *Yr9*, *Yr43*, *Yr44* and *YrTr1*. *Yr9* was found in 19 WLRs which is 38% of the total used for gene postulation (Table 3). The *Yr43* originated from IDO337s is a North American cultivar (Cheng and Chen 2010) was postulated in 18 WLRs (36%). It was found alone in WLRs B-57, B-164 and B-165 while in rest it was found in combination with *Yr1, Yr8, Yr9* & *YrTr1* (Table 3). The *Yr44,* originated from the US spring wheat cultivar Zak (Sui *et al.* 2009) was postulated in (42%) 21 WLRs (Table 3). It was found alone in genotypes B-39, B-179, B-317, and B-587 while among the rest of the landraces it was found in combination with other genes. *YrTr1* was first reported in winter club wheat variety Tres (Chen, 1992) and located on chromosome 6D (Chen, 1995b) postulated in 20 tested (42%) WLRs. It was found solitary in landrace B-201 while among the rest of the landraces it was found in combination with other postulated genes (Table 3).

Field screening of landraces AREA under Disease Progressive Curve (AUDPC) analysis against yellow rust

Field reaction of 36 wheat landraces (72% landraces with major genes for resistance) was resistant (R), 6 (12%) moderately resistant (MR), 8 (16%) moderately susceptible (MS) and 1 (2%) was found susceptible (S). The values of relative AUDPC ranged from 0-100% when universal susceptible check morocco shows 100% relative AUDPC. Resistant landraces (36) were found with relative AUDPC 0-10% indicating functional APR genes presence, seven landraces 14% displayed an intermediate type of resistance showed relative AUDPC 11-30%. Six landraces 12% were found susceptible showing relative AUDPC above 50% (Table 4).

Comparison between seedling and adult plant stage

Study comparison shows fifty wheat landraces gave variable results in glasshouse and field conditions. The susceptible reaction by B-290 and B-317 indicates the probability of resistant gene suppressor that can be explored to save our wheat crop from yellow rust losses. Moderately susceptible fifteen (15) landraces at the seedling stage were shown resistance at the adult plant stage, showing the presence of slow rusting minor genes for resistance or temperature-sensitive resistance against *Pst*. So, the study is giving an opportunity and windows for slow rusting genes and their incorporation to the elite wheat cultivars through direct breeding. The nature of APR needs to look into and needed to be analyzed further for the presence of minor genes if any in these and if they have minor genes in them they may serve as a good source of genes. Landrace B-535 shown immune at seedling stage and moderately susceptible at an adult stage which shown this landrace has only major genes for resistance. Landrace B-290 showed a moderately resistant reaction to highly aggressive *Pst* races and susceptible to second aggressive *Pst* at the seedling stage but shown resistance at the adult stage. The expression of resistance at seedling stage is MR to most of the *Pst* shows presence of many slow rusting genes which can express their presence at adult plant stage revealing an opportunity to the pathologist to go for new candidate minor genes through this landrace.

Table 4. Field reaction of wheat landraces with major genes for resistance against yellow rust and Disease progress translated as yellow rust coverage as Area Under Disease Progress Curve (%).

Sr. No.	Landrace	Seedling Reaction	Field Reaction	AUDPC	% AUDPC
1	B-03	1	30R	82.5	4.89
2	B-20	7	30R	87	5.16
3	B-22	5	20R	62	3.67
4	B-39	4	20R	80.5	4.77
5	B-41	6	30MS	348	20.65
6	B-43	7	20R	80.5	4.77
7	B-46	7	30R	75	4.45
8	B-57	7	20R	64	3.79
9	B-61	5	20R	57.5	3.41
10	B-64	7	30R	87	5.16
11	B-65	7	30R	93.5	5.54
12	B-66	1	30R	78	4.62
13	B-67	4	40R	115	6.82
14	B-74	7	30R	94.5	5.60
15	B-79	2	30MR	174	10.32
16	B-83	2	50MS	572	33.94
17	B-86	4	50MS	512	30.38
18	B-87	1	60MS	660	39.16
19	B-124	5	50R	152	9.02
20	B-142	6	40R	115	6.82
21	B-143	5	30MR	165	9.79
22	B-148	4	40R	115	6.82
23	B-158	4	30R	87	5.16
24	B-160	5	40R	115	6.82
25	B-164	7	30R	87	5.16
26	B-165	6	50R	152	9.02
27	B-166	5	40R	145.5	8.63
28	B-171	6	40R	115	6.82
29	B-179	6	30R	87	5.16
30	B-199	-	40R	115	6.82
31	B-201	5	30R	97.5	5.78
32	B-205	6	30R	93.5	5.54
33	B-250	7	40R	115	6.82
34	B-263	6	40R	124	7.35
35	B-276	8	40R	122.5	7.27
36	B-277	8	40R	115	6.82
37	B-281	7	30R	102	6.05
38	B-290	9	30R	87	5.16
39	B-308	5	40MR	233	13.82
40	B-317	9	40MS	460	27.29
41	B-369	1	40MR	230	13.64
42	B-501	2	60MR	378	22.43
43	B-507	8	70MR	452	26.82
44	B-527	4	60MS	756	44.86
45	B-530	7	50R	152	9.02
46	B-532	7	40R	115	6.82
47	B-535	0	60MS	756	44.86
48	B-587	6	70MS	808	47.95
49	B-609	6	50R	152	9.02
50	B-673	9	70S	1130	67.06
51	Morocco	9	100S	1685	100

R= Resistant, MR= Moderately Resistant, MS= Moderately Resistant and S= Susceptible

DISCUSSION

Yellow rust is an important wheat disease with challenging impact on national food security and agricultural economy worldwide in general and particularly in Pakistan. New yellow rust virulences resulted in outbreaks devastated the wheat crop by the breakdown of resistance in commercial bread wheat cultivars. The process is governed by fitness survival mechanism occurring in nature, and yellow rust pathogen *Puccinia striiformis* f. sp. *tritici* developed variety of physiological races around the world. Recent yellow rust outbreaks resulted in huge losses in Pakistan, it was estimated 13% for the 2018-19 crop and approximate expected estimates are even high for the crop year 2019-20. We tried to find the answer that if we have such genes in Pakistani WLRs having the capacity to save our food production. Six yellow rust *Pst* races with virulences (Table 1) were used to find the desired genes through Infection type virulence pattern (Table 2). Virulences were not effective to *Yr3, Yr5, Yr10, Yr15, Yr26, YrSp, YrCV* and moderately effective on *Yr18* (Awan *et al.*, 2017; Feng *et al.*, 2009; Rizwan *et al.*, 2010). Six genes for resistance Yr*1, Yr8, Yr9, Yr43, Yr44*, and *YrTr1* were postulated successfully in Pakistani WLRs. *Yr44* and *YrTr1* found in 22 and 21 WLRs respectively as most frequent while *Yr1* and *Yr8* in 14 each as least frequent.

Yr1 was originated from Chinese 166 and extensively used as a source of resistance in commercial bread wheat varieties (Stubbs, 1985) and virulences exist in most of the geographical areas around the world especially East Asian region and are no more effective source of resistance worldwide (Anmin *et al*., 2004). Gene was reported from Pakistani varieties released after 1969 up to 1995 (Rizwan *et al*., 2010). The presence of this *Yr8* in Pakistani landraces reveals that it developed after a long process and with diverse genes for resistance*.* It was found in combinations with other yellow rust postulated genes providing an opportunity to assess their role in combinations. Expressions of *Yr8* resistance in combination with other genes for resistance is highly variable, the presence of suspected minor genes for resistance and gene suppressors in WLRs which is needed to be explored (Table 3). *Yr8* was not commercially utilized in most of the breeding programs but Kirmani *et al.* (1984) reported the presence of this gene in Pakistani gene pool which was not accepted by the scientists (McIntosh *et al*., 1995). This study supports the finding of Kirmani regarding the presence of *Yr8* by the successful postulation of *Yr8* from Pakistani wheat landraces. However; the mechanism of expression of resistance of this gene is highly unpredictable. Cytological studies show that *Yr8* gene is located on 2D/2M chromosome and having fellow gene present on 5B chromosome (Chen, 1995a). It needs great insight into these landraces for not only yellow rust but also for other agronomic traits and other diseases and stresses. It is found with *YrTr1* in 14 WLRs and *Yr9, Yr43* and *Yr44* with varying combination and varying level of resistance. Our results are supported by the finding of other scientists working on gene postulation and found *YrYl* as associated gene in Chinese wheat landraces (Wu *et al*., 2016). *Yr9* was found in 19 WLRs which is 38% of the total used for gene postulation (Table 3) which is present on 1B/IR chromosome a homologous gene of *Yrlem* (Chen, 1995b). The gene has a lot of associated fellow postulated genes in Pakistani WLRs as an independent gene. Association makes the gene durable against yellow rust and even leaf rust due to *Lr28* gene association. The pleiotropic nature of *Yr9* got a great attraction in the past and many varieties were developed in different countries of the world carrying this gene complex. It was extensively incorporated by breeding programs exploiting yield advantage associated with 1B-1R translocation and its linkage with *Sr31* and *Lr26*. The leading variety of Pakistan Pak-81 lost its resistance due to the evolution of virulence to *Yr9*. It led to serious stripe rust epidemics during the 80s and 90s in many countries. In Pakistan, virulence against *Yr9* was observed in 1994 when the gene became ineffective when leading cultivars having this gene became susceptible (Chen *et al.*, 2014; Saari, 1995). Wheat varieties developed from CIMMYT blood containing *Yr9* were overcome by virulences resulting in resistance breakdown (Qamar *et al.*, 2012; Rizwan *et al.*, 2010).

Yr43 originated from IDO337s is a North American cultivar (Cheng and Chen, 2010) was postulated in 18 (36%). It was found alone in 3 WLRs and in rest, it was found in combinations with *Yr1, Yr8, Yr9* & *YrTr1*. Further analysis using linked molecular markers is needed to confirm the presence of this gene in these landraces. The gene was differentiated from all previously identified genes found on chromosome 2BL, based on the chromosomal location, reactions to the various races of the pathogen and tests of allelism

(Cheng and Chen, 2010; Sui *et al.*, 2009). Virulences to this gene were rare before the year 2000, but became common since 2000 in the US and many other countries including China, Ecuador, Ethiopia, Italy, Mexico, Nepal, Pakistan and Turkey (Wan *et al*., 2016). The *Yr44,* originated from the US spring wheat cultivar Zak (Sui *et al.*, 2009) was postulated (42%) in 21 WLRs (Table 3). It was found alone in 4 WLRs and in rest of genotypes combinations with other genes. The gene was effective against most of the races found prior to the year 2000 in the US Pacific Northwest but became ineffective against a majority of races since 2000 (Wan *et al*., 2016). Like *Yr43* virulence to *Yr44* has been detected in many other countries including Pakistan. In China, high virulence frequencies (>80%) were detected for *Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr44*, and *YrExp2*; moderate frequencies (20 to 80%) for virulences to *Yr1, Yr43, YrTr1*, and *YrTye* (Wan *et al*., 2016) and virulences and resistance in genotype with these genes are still performing against the existing *Pst* races of Pakistan. *Pst v*irulences to *Yr6*, *Yr43*, and *Yr44* each is controlled by a single dominant gene; those to *Yr7*, *Yr9*, *Yr17*, *Yr27* and *YrExp2* each was controlled by two dominant genes; and the virulence phenotype to *Yr8* was controlled by two complementary dominant genes (Siyoum *et al*., 2019). Virulence trend was found the same as that of previously reported from Pakistan. As some rust genes remain durable even upto more than half a century like *Lr34* against all emerged virulences (Krattinger *et al*., 2009). So these genes are worthwhile to be incorporated with other non-race specific genes in wheat breeding programs provided they are not associated with detrimental linkage drag (Gebreslasie *et al*., 2020). Tested 3 WLRs B-74, B-281 and B-530 (6%) are having genes *Yr8*, *Yr9*, *Yr43*, *Yr44*, *YrTr1* with a good level of resistance to yellow rust. Tested landraces against Pakistani yellow rust virulences show the opportunity for durable resistance. As these lines do not share ancestors to these lines where these genes were first reported and incorporated to the bread wheat. These wheat landraces can be used as a source of resistance at seedling and adult plant stage and easily exploited with their fixed properties for high-quality food economical crop production with resistance.

In this study, 4 phenotypically resistant wheat landraces were found having absolute resistance can have novel genes for resistance, 11 WLRs with single *Yr* gene, 15 WLRs with 2 and 3 *Yr* gene combination, 13 lines with more than 3 *Yr* genes against yellow rust were identified that endure over several decades to hundreds of years and generations. This richness of landraces as a source of resistance can be considered as an opportunity for enhancing the potential of wheat for *Pst* resistance in future improvement programs. Due to the seriousness of the disease spread in Pakistan where yellow rust is targeting leading wheat cultivar and breakdown of the resistance, these lines provide a potential option for breeding against yellow rust. Integration of adult plant resistance which is mostly due to minor genes at the adult plant stage and environmentally triggered genes and major genes can be a promising feature for future wheat breeding. Additionally, these landraces can be good starting material towards the identification of the gene through mapping, cloning, and functional characterization because of their nature. Moreover, landraces that contain a combination of *Yr* genes are potential targets for starting *Yr* gene pyramiding for quick deployment as naturally fixed traits in Pakistani wheat cultivars which will contribute to controlling large-scale epidemics of yellow rust**.** Besides, knowledge of the number and identity of the *Yr* rust resistance genes in these landraces will be useful in understanding their field reaction to changing *Pst* populations and it can be used as parents for improving future wheat cultivars.

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ETHICAL APPROVAL

This article is original and not published elsewhere. The authors discussed the results, read and approved the final manuscript. The authors confirm that there are no ethical issues in the publication of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.