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Research Article

ASSESSMENT OF GENETIC VARIABILITY FOR WHEAT YELLOW RUST RESISTANCE AND *PUCCINIA STRIIFORMIS* F.SP. *TRITICI* PATHOTYPES FROM PAKISTAN

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ABSTRACT

After their release, commercial wheat cultivars often experience a notable decline in effectiveness against yellow rust, highlighting the crucial importance of genetic resistance studies for developing durable solutions for sustainable wheat production. This study characterized 205 wheat genotypes for seedling and adult plant (*Yr18*) resistance genes, using 14 diverse races of *Puccinia striiformis* f.sp. *tritici* (*Pst*). Additionally, 75 *Pst* isolates sampled during 2008 were also analyzed. Seedling resistance in 16 genotypes remained undetermined. Twelve resistance patterns emerged: 82 genotypes showed susceptibility across all pathotypes, while 10 displayed consistent resistance. Fifty-seven genotypes were inferred to carry *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr9*, and an unknown gene. *Yr18* prevalence was observed in 29 cultivars but was absent in landraces. Eight resistance spectra were shared between landraces and cultivars, and four were cultivar-specific (*Yr6+Yr7*, *Yr9+Yr17*, and *Yr18*). Many landraces carried defeated resistance genes, as evident from prevalent pathotypes in the 2006 and 2008 surveys in Khyber Pakhtunkhwa, Pakistan. The low frequency of resistance genes in Pakistani cultivars underscores the need for increased use of undefeated seedling resistance genes and *Yr18*-associated cultivars for effective disease control.

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INTRODUCTION

Yellow rust, caused by *Puccinia striiformis* Westend. f.sp. *tritici* (*Pst*), is a serious constraint to global wheat production, including Pakistan, where wheat covers 9 million hectares with a production of 28 million tonnes (Anonymous, 2024a). The majority of cultivated land employs commercial cultivars released in the past, with traditional landraces cultivated on 0.42 million hectares. Approximately 70% of the wheat area is susceptible to yellow rust, leading to recurrent epidemics and substantial economic losses. In the 2018-2019 cropping season, yellow rust caused estimated yield losses of approximately 13%. In the subsequent 2019-2020 season, Pakistan experienced a further 15% reduction in yield due to the disease (Tariq-Khan et al., 2020). Despite national and provincial breeding efforts for disease-resistant germplasm, many old cultivars were abandoned and recently released were delisted due to yellow rust susceptibility (Anonymous, 2024b).

The periodic outbreak of yellow rust in Pakistan is attributed to insufficient knowledge about host-pathogen genetic variability and unreliable resistance sources. Breeding programs aim to provide cultivars with effective resistance gene combinations against prevalent pathogen populations. Information about resistance genes and virulence factors enhances germplasm diversification. Analyzing resistance genes in breeding programs facilitates the development and deployment of resistant cultivars. Over 80 yellow rust resistance genes have been reported globally (Anonymous, 2023), with *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr27,* and *YrSu* dominating in 40 bread wheat cultivars from Pakistan, which included 19 cultivars released during the period 1991-2006, whilst the remaining 21 were candidate varieties (Bahri et al., 2011). However, the resistance profile of germplasm released after this period and traditional landraces remains unexplored. *Yr18*, a non-hypersensitive resistance gene, was detected in 12 out of 59 tested Pakistani wheat genotypes. Alone, *Yr18* lacks sufficient resistance but, when combined with 3-4 minor genes, it proves effective across diverse environments. The csLV34 marker, located near the *Lr34/Yr18* gene complex, was used to screen 61 genotypes (including 27 CIMMYT lines, 13 released varieties, 17 NARC lines and 4 checks) in Pakistan, revealing its presence in only 12 genotypes (Qamar et al., 2012).

This study characterized and compared 205 bread wheat cultivars and landraces to enhance knowledge of resistance genes in Pakistan. Seedling-stage assessments and molecular screening for the *Yr18* gene were conducted. Surveying prevalent pathotypes in the Peshawar, Nowshera, and Mingora regions using 75 isolates aimed to guide breeding programs for more effective resistance in the country.

MATERIALS AND METHODS

Wheat germplasm and pathotypes

Seedling resistance genes were identified in 205 wheat genotypes sourced from various regions and elevations in Pakistan, as outlined in Tables 1a, 1b and 1c. This dataset comprised 118 landraces meticulously collected by the Plant Genetic Resource Institute (PGRI) across diverse geographical areas in Pakistan. Additionally, the genotypes encompassed a mix of old and recent cultivars, with candidate cultivars derived from various breeding programs undergoing national multilocation testing over the past five years. Seeds for the current and future cultivars, excluding landraces, were sourced from the Plant Protection Division (PPD) of the Nuclear Institute for Food and Agriculture, Peshawar.

For the analysis, twelve French and two Pakistani *Pst* pathotypes, each purified through a single urediospore isolation process as described by de Vallavieille-Pope et al. (2012), were employed. These test pathotypes were crucial for postulating the resistance spectra of Pakistani bread wheat germplasm in this study. In Table 2, the *Pst* pathotypes are presented, along with their corresponding virulence and avirulence factors, confirmed on thirty-five differentials, including a set of genotypes reported by Johnson et al. (1972).

Seedling tests for resistance genes

Seedling resistance genes were determined using twelve French (i.e. 6E16, 6E16V27, 237E141V17, 169E136V17, 40E8, 43E138, 106E139, 109E141, 232E137, 233E169V17, 237E141, and 239E141V17) and two Pakistani pathotypes (i.e. P1 and P2) with complementary virulence patterns reported by Bahri et al. (2011) and de Vallavieille-Pope et al. (2012). Given that several of these test races demonstrate multiple avirulence factors, deciphering precise resistance gene combinations was not always straightforward. Fourteen sets, each comprising 205 wheat genotypes, were established for the seedling resistance genes assessment study. The experimental procedure involved planting 6-8 seeds/genotype/set in 7×7 cm plastic pots filled with standard glasshouse soil. Each experiment was replicated twice, with 5-7 plants per replicate.

After two weeks of sowing, all sets were systematically inoculated with 5 mg fresh spores of each yellow rust pathotype, suspended in 300 µl Soltrol 170 (mineral oil), at the emergence of the 2nd leaf. Each set of cultivar/landrace/pathotype combinations was then kept in a dew chamber at 8°C with 100% relative humidity for about 24 hours, facilitating fungus penetration as described by de Vallavieille-Pope et al. (1995). Subsequently, sets were shifted to a climatic chamber (day: 16 hours, 300 µmol quanta m-2, 17°C; night: 8 hours, 14°C). Before inoculation, a robust light treatment of up to 8 hours was applied to enhance successful infection as reported by de Vallavieille-Pope et al. (2002).

Individual plant assessments were conducted after 15-17 days of inoculation using a 0-9 rating scale of McNeal et al. (1971), considering factors such as chlorosis/necrosis and the severity of sporulation. Host-pathogen interaction phenotypes/infection types (ITs) were categorized as Resistant (IT = 0-3), Intermediate (IT = 4-6), and Susceptible (IT = 7-9).

Table 1a. The code[a] for resistance spectrum and postulated resistance (PR) genes in Pakistani wheat (Released cultivars) after testing with fourteen Pst races and a molecular test.

Genotypes	Spectrum code	PR genes	Genotypes	Spectrum code	PR genes	Genotypes	Spectrum code	PR genes
Parwaz-94	0	Ni	Faisalabad-85*	4	*Yr7+, Yr18*	Zargoon	6	*Yr27*
Tandojam-83	1	*Yr2+*	Auqab-2000	4	*Yr7+*	Pasban	8	*Yr6+Yr9*
Maxi-Pak	1	*Yr2+*	Faisalabad-83*	4	*Yr7+, Yr18*	Bahawalpur-00*	10	*Yr6+Yr7+Yr9, Yr18*
WL-711	1	*Yr2+*	Sariab-92	4	*Yr7+*	Pirsabak 2005	10	*Yr6+Yr7+Yr9*
Sind-81*	1	*Yr2+ Yr18*	Kohistan-97	4	*Yr7+*	Bahawalpur-95*	10	*Yr6+Yr7+Yr9, Yr18*
Pak-81	1	*Yr2+*	GA-2002*	5	*Yr9+*, *Yr18*	Rohtas-90	10	*Yr6+Yr7+Yr9*
KT-2003	1	*Yr2+*	Kohinoor-83[8]	5	*Yr9+*, *Yr18*	Iqbal 2000	11	resistant
Sehar 2006	2	*Yr3* or *Yr25*	SH-2002	6	*Yr27*	Manthar-3	11	resistant
Chakwal-86	2	*Yr3* or *Yr25*	Marvi-2000	6	*Yr27*	Pirsabak 2004*	11	resistant, *Yr18*
Zardana	3	*Yr6+*	Shafaq 2006*	6	*Yr27, Yr18*	Gaznavi-98*	11	resistant, *Yr18*
Kaghan-93	3	*Yr6+*	Punjab-96*	6	*Yr27, Yr18*	AS-2002*	12	susceptible, *Yr18*

***=** indicates the presence of *Yr18* linked allele of 150bp

[a] Code for the resistance spectrum is mentioned in Table 2

NI: Non-identified; the resistance spectra found did not facilitate to postulation of any single or combination of resistance genes;

Spectrum code 11: Found resistant to all tested races; it corresponds to either a novel resistance gene or a combination of genes that could be present but not detectable with the current set of used races.

Spectrum code 12 (Susceptible): susceptible to all tested races; it corresponds to either no resistance gene or a combination of genes overcome by the current set of used races.

Molecular tests for *Yr18*

Seeds of 205 wheat genotypes were grown separately as mentioned above and from one-week-old leaves, DNA was extracted using the CTAB protocol (Imtiaz et al., 2008). PCR amplification using csLV34F + R primers, diagnostic marker for *Yr18* was performed in 10-µl reaction. The reaction mixture composition was 1.0-ml (10 picomol) each of R and F primers, 1.0-ml (2 mM) dNTPs, 1.0-ml 10X PCR buffer, 0.1-ml (5 unit/ml) Tag-polymerase, 2.0-ml DNA (60-70ng DNA template) and 4.9ml dH20. The PCR was done in an Eppendorf Gradient Thermal Cycler, at 94^0C for 3 minutes, followed by 45 cycles of 15 seconds at 94°C, 15 seconds at 58°C, 15 seconds at 72°C, and a final extension step of 10 minutes at 72°C. The PCR products were separated via electrophoresis in a 1.2% agarose gel and subsequently visualized under UV light after staining with ethidium bromide (500 µl/l).

Pathotype analysis

Seventy five *Pst* isolates were sampled from the Peshawar (east and west) area in 2008, Nowshara and Mingora. Spore revival and production were performed within a tightly controlled spore-proof climatic room, ensuring strict containment to prevent any unintended dispersion of foreign spores into the environment. Rusted leaves were used for inoculation which was carefully performed by gently rubbing them onto the first leaf of 7-day-old seedlings of the susceptible cultivar Michigan Amber. Post-inoculation, seedlings were incubated as mentioned above. One week after inoculation, each pot was enclosed in a cellophane bag to prevent any potential cross-contamination. After 18 days post-inoculation, spores were harvested, subjected to drying in a desiccator at 4°C for 3 days, and subsequently stored in microtubes at -80°C. The virulence combinations of the isolates were characterized

using a panel of 15 differential varieties (Johnson et al., 1972), supplemented with 18 additional wheat lines: Kalyansona (*Yr2*), Federation × 4 Kavkaz (*Yr9*), Clement (*Yr9+*), VPM1 (*Yr17+*), TP981 (*Yr25*), Anza (*YrA*), Early Premium (*YrEp*), Jubilejina 2 (*YrJu*), Victo (*YrVic*), and Australian Avocet isogenic lines (*Yr1*, *5*, *6*, *7*, *8*, *15*, *24*, *26*, and *27*) (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Resistancegenes/Yrgene.xls). This differential set effectively discerns between 24 distinct virulence factors. Each isolate was inoculated by spraying 5 mg of uredospores, suspended in 300 µl of mineral oil (Soltrol), onto five seedlings at the two-leaf stage of each cultivar, following the aforementioned incubation conditions. Individual plant assessments were conducted 15-17 days post-inoculation, as outlined previously. Virulence against a specific YrX gene was inferred when any of the cultivars carrying that particular gene displayed a susceptible reaction. Throughout the text, virulence against the YrX resistance gene is denoted as vX (AvX for avirulence).

Table 1b. The code[a] for resistance spectrum and postulated resistance (PR) genes in Pakistani wheat (Future cultivars) after testing with fourteen *Pst* races and a molecular test.

Genotypes	Spectrum code	PR genes	Genotypes	Spectrum code	PR genes	Genotypes	Spectrum code	PR genes
Maria	0	NI	MPT-7	4	*Yr7+*	PR-89*	9	*Yr6+Yr1,, 7, Yr18*
93T347*	0	NI, *Yr18*	CT-03457	4	*Yr7+*	NR-285*	9	*Yr6+Yr17, Yr18*
V-9316*	0	NI, *Yr18*	V-00BT034	4	*Yr7+*	9247	9	*Yr6+Yr17*
V-02192	0	NI	Q.S-1	4	*Yr7+*	CT-99022*	10	*Yr6+Yr7+Yr9, Yr18*
V-032862*	0	NI, *Yr18*	V-03138	4	*Yr7+*	V-04189	10	*Yr6+Yr7+Yr9*
PR-94	0	NI	V-04022	4	*Yr7+*	SN-122	10	*Yr6+Yr7+Yr9*
NIA-8/7	0	NI	SM-07018*	5	*Yr9+*, *Yr18*	DN-38	11	resistant, *Yr18*
93T347	0	NI	V-002495-A	5	*Yr9+*	B-07	11	resistant
V-15-10	1	*Yr2+*	V-022668	6	*Yr27*	V-04112*,	11	resistant, *Yr18*
NR-270	1	*Yr2+*	MALIR	6	*Yr27*	NRDW-1	11	resistant
V-01078	1	*Yr2+*	V-9244	6	*Yr27*	V-03158	12	susceptible
V-03079	1	*Yr2+*	MSH-14*	6	*Yr27, Yr18*	AUP-4606	12	susceptible
V-04188	2	*Yr3* or *Yr25*	45006	6	*Yr27*	TW0107	12	susceptible
PR-90	2	*Yr3* or *Yr25*	V-00183*	6	*Yr27, Yr18*	LSRR2006*	12	susceptible, *Yr18*
Ras I	3	*Yr6+*	V-99022	6	*Yr27*	99B2237	12	susceptible
L 886	3	*Yr6+*	L 1076	7	*Yr6+Yr7*	Ras II*	12	susceptible, *Yr18*
L 41	3	*Yr6+*	V-04178*	8	*Yr6+Yr9, Yr18*	NRL 0320*	12	susceptible, *Yr18*
2KC050	4	*Yr7+*	PR-88*	9	*Yr6+Yr17, Yr18*	V-033010*	12	susceptible, *Yr18*

***=** indicates the presence of *Yr18* linked allele of 150bp; [a] Code for the resistance spectrum is mentioned in Table 2.
NI: Non-identified; the resistance spectra found did not facilitate to postulation of any single or combination of resistance genes;
Spectrum code 11: Found resistant to all tested races; it corresponds to either a novel resistance gene or a combination of genes that could be present but not detectable with the current set of used races.
Spectrum code 12 (Susceptible): susceptible to all tested races; it corresponds to either no resistance gene or a combination of genes overcome by the current set of used races.

Table 1c. The code[a] for resistance spectrum and postulated resistance (PR) genes in Pakistani wheat (Land races) after testing with fourteen *Pst* races and a molecular test.

Genotypes	Spectrum code	PR genes	Genotypes	Spectrum code	PR genes	Genotypes	Spectrum code	PR genes
12231	0	NI	11928	10	*Yr6+Yr7+Yr9*	11793	12	-do-
11586	0	NI	12206	10	*Yr6+Yr7+Yr9*	11795	12	-do-
11616	0	NI	12207	10	*Yr6+Yr7+Yr9*	11247	12	-do-
11618	0	Ni	12212	10	*Yr6+Yr7+Yr9*	12248	12	-do-
11393	0	Ni	12254	11	resistant	12253	12	-do-
11791	0	Ni	12213	11	resistant	11484	12	-do-
11792	0	Ni	11345	12	susceptible	11566	12	-do-
11390	0	Ni	11480	12	susceptible	11567	12	-do-
11489	1	*Yr2+*	11505	12	susceptible	11810	12	-do-
12234	1	*Yr2+*	11572	12	susceptible	11811	12	-do-
12284	1	*Yr2+*	11574	12	susceptible	11764	12	-do-
11583	1	*Yr2+*	11581	12	susceptible	12276	12	-do-
11588	1	*Yr2+*	11746	12	susceptible	12296	12	susceptible
12268	1	*Yr2+*	11747	12	susceptible	12297	12	-do-
12271	1	*Yr2+*	11763	12	susceptible	12298	12	-do-
11565	1	*Yr2+*	11765	12	susceptible	12299	12	-do-
12292	1	*Yr2+*	11788	12	susceptible	11486	12	-do-
12320	1	*Yr2+*	11807	12	susceptible	11579	12	-do
11561	1	*Yr2+*	11808	12	susceptible	11580	12	-do-
11562	1	*Yr2+*	11812	12	susceptible	11766	12	-do-
12203	2	*Yr3* or *Yr25*	12223	12	susceptible	12278	12	-do-
11893	2	*Yr3* or *Yr25*	12246	12	susceptible	12301	12	-do-
11894	3	*Yr6+*	12285	12	susceptible	12302	12	-do-
11582	4	*Yr7+*	12287	12	susceptible	12303	12	-do-
12054	4	*Yr7+*	12289	12	susceptible	12305	12	-do-
12193	4	*Yr7+*	12293	12	-do-	12306	12	-do-
11394	5	*Yr9+*	12295	12	-do-	12307	12	-do-
11794	5	*Yr9+*	12311	12	-do-	12308	12	-do-
12269	5	*Yr9+*	12313	12	-do-	13178	12	-do-
11391	6	*Yr27*	12316	12	-do-	11929	12	-do-
12270	6	*Yr27*	12961	12	-do-	12290	12	-do-
12208	6	*Yr27*	11481	12	-do-	12291	12	-do-
12209	6	*Yr27*	11584	12	-do-	12294	12	-do-
11614	10	*Yr6+Yr7+Yr9*	11585	12	-do-	12310	12	-do-

11787	10	*Yr6+Yr7+Yr9*	11617	12	-do-	12312	12	-do-
12009	10	*Yr6+Yr7+Yr9*	11619	12	-do-	12314	12	-do-
12194	10	*Yr6+Yr7+Yr9*	11620	12	-do-	12315	12	-do-
11612	10	*Yr6+Yr7+Yr9*	11621	12	-do-	12317	12	-do-
12252	10	*Yr6+Yr7+Yr9*	11614	12	-do-	12319	12	-do-
12257	10	*Yr6+Yr7+Yr9*						

***=** indicates the presence of *Yr18* linked allele of 150bp ; [a] Code for the resistance spectrum is mentioned in Table 2

NI: Non-identified; the resistance spectra found did not facilitate to postulation of any single or combination of resistance genes; Spectrum code 11: Found resistant to all tested races; it corresponds to either a novel resistance gene or a combination of genes that could be present but not detectable with the current set of used races ; Spectrum code 12 (Susceptible): susceptible to all tested races; it corresponds to either no resistance gene or a combination of genes overcome by the current set of used races.

RESULTS

Yellow rust resistance genes were postulated in 205 wheat genotypes which included 118 landraces, 54 future, and 33 released cultivars. ITs of *Pst* pathotypes produced on these genotypes were compared to ITs produced on standard yellow rust differentials, each containing a specific yellow rust resistance gene. Resistance and susceptibility patterns of 205 genotypes, following inoculation with 14 *Pst* races, yielded twelve resistance spectra (Table 3) possessing single, double, or multiple genes. Out of 12 resistance spectra, 8 were common in both landraces and cultivars while the remaining 4 were cultivars specific (Figure 1). Adult plant resistance gene *Yr18* was not found in landraces while it was prevalent in a total of 29 modern and few old cultivars (Figure 2).

Unidentified resistance

No specific resistance gene related to any particular race was identified among the 14 pathotypes tested across sixteen different genotypes. These genotypes comprised a released cultivar (Parwaz-94), seven potential future cultivars (Maria, V-9316, V-02192, V-032862, PR-94, NIA-8/7, and 93T347), and eight accessions of landraces (12231, 11586, 11616, 11618, 11393, 11791, 11792, and 11390), as listed in Table 3.

Single gene spectra

Seventy-three genotypes exhibited six monogenic resistance spectra, labeled Spectrum 1 to 6, with frequencies ranging from 3% to 11% (Table 3). Among these, *Yr2* was identified in five released cultivars (Tandojam-83, Maxi-Pak, WL-711, Sind-81, and Pak-81), three potential future cultivars (V-15-10, NR-270, and V-03079), and twelve landrace accessions (11489, 12234, 12284, 11583, 11588, 12268, 12271, 11565, 12292, 12320, 11561, and 11562), as indicated in Table 3, due to their matching resistance spectrum with the differential cultivar 'Kalyansona'. Likewise, *Yr3* or *Yr25* was hypothesized to be present in two released cultivars (Sehar 2006 and Chakwal-86), two potential future cultivars (V-04188 and PR-90), and two landrace accessions (12203 and 11893), as their resistance spectra matched those of Vilmorin 23, Nord Desprez, and TP 981 (except 6E16). Moreover, two released cultivars (Zardana-89 and Kaghan-93), three potential future cultivars (Ras I, L 886, and L 41), along with one landrace accession (11894), were suggested to carry *Yr6*+ (Table 3), showing an identical resistance spectrum to the differential cultivar 'Heines Kolben'. Yr7, possibly with an additional unknown gene, was inferred in fifteen genotypes, with resistance spectra closest to the differential variety 'Reichersberg 42'. These genotypes include Faisalabad-83, Faisalabad-85, Sariab-92, Kohistan-97, Auqab-2000, 2KC050, MPT-7, CT-03457, V-00BT034, Q.S-1, V-03138, V-04022, 11582, 12054, and 12193 (Table 1a, 1b and 1c). *Yr9* was postulated for seven genotypes (Table 3), with resistance spectra akin to that of the differential variety 'Federation X 4 Kavkaz'. Among these genotypes, two each were released cultivars (Kohinoor-83 and GA-2002) and potential future cultivars (V-002495-A and SM-07018), while three were landraces (11394, 11794, and 12269), as listed in Table 1a, 1b and 1c. Interestingly, the resistance spectrum 6, displayed by sixteen genotypes (Table 3), did not perfectly align with any specific seedling resistance gene in the differential cultivar/line. Finally, only race 70E16-V27 and 70E0-V27 showed virulence on a subset of cultivars and lines, including SH-2002, Marvi-2000, Shafaq 2006, Punjab-96, Zargoon-79, V-022668, MALIR, V-9244, MSH-14, 45006, V-00183, V-99022, 11391, 12270, 12208, and 12209 (Table 1a, 1b and 1c), suggesting a possible presence of *Yr27* in these varieties.

Table 2. Races of *Pst* along with their virulence factors utilized to postulate the resistance spectra of Pakistani wheat.

Differential cultivars	*Yr* genes	Yellow rust races/pathotypes													
		6 E 0	6 E 16	40 E 8	43 E 138	45 E 140	106 E 139	169 E 136V17	232 E137	233 E 169 V17	237 E 141	237 E 141 V17	239 E 143V17	70E16- V27 (P1)	70E0- V27 (P2)
Clement	*Yr9+Yr2+*	-	-	-	-	-	-	V	V	V	V	V	V	-	-
Suwon 92 x Omar	*YrSu*	-	-	-	-	-	V	-	V	V	V	V	V	V	V
Strubes Dickkopf	*Yr25+YrSd*	-	-	V	V	V	V	V	V	V	V	V	V	-	-
Moro	*Yr10+*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vilmorin 23	*Yr3+*	-	-	V	V	V	V	V	V	V	V	V	V	-	-
Heines Kolben	*Yr6+Yr2*	V	V	-	-	V	-	-	-	-	V	V	V	V	V
Lee	*Yr7+*	V	V	-	V	-	V	-	-	-	-	-	V	V	V
Chinese 166	*Yr1*	-	-	-	V	V	-	V	-	V	V	V	V	-	-
Heines VII	*Yr2+*	-	-	-	V	V	V	V	V	V	V	V	V	-	-
Spaldings Prolific	*YrSp*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carstens V	*Yr32*	-	-	-	-	-	-	-	-	V	-	-	-	-	-
Compair	*Yr8+*	-	V	-	-	-	-	-	-	-	-	-	-	V	-
Nord Desprez	*Yr3+*	-	-	V	V	V	V	V	V	V	V	V	V	-	-
Heines Peko	*Yr6+Yr2*	-	-	-	-	V	-	-	-	-	V	V	V	-	-
Reichersberg 42	*Yr7+*	-	I	-	V	-	V	-	-	-	-	-	V	-	-
Hybrid 46	*Yr4+*	-	-	-	-	-	V	-	V	V	V	V	V	-	-
Federation X4 Kavkaz	*Yr9*	V	-	-	-	-	-	V	V	V	V	V	V	-	-
Kalyansona	*Yr2*	V	V	-	V	V	V	V	V	V	V	V	V	V	V
Anza	*YrA*	-	-	-	-	V	-	V	-	-	-	-	-	V	V
VPM1	*Yr17* +	-	-	-	-	-	-	V	-	V	-	V	V	-	-
TP 981	*Yr25*	-	V	V	V	V	V	V	V	V	V	V	V	-	-
Funo	*YrA*	-	-	-	-	V	-	V	-	-	-	-	-	V	V
Jubilejina 2	U	V	-	U	V	V	V	V	V	V	V	V	V	-	-
Victo	*YrVic*	V	V	V	V	V	V	V	V	V	V	V	V	-	-
Early Premium	*YrEp*	U	-	U	V	V	V	V	V	V	V	V	V	-	U
Michigan	Susceptible	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Yr1/6*Avo.S	*Yr1*	U	-	U	V	V	V	V	-	V	V	V	V	-	-
Yr5/6*Avo.S	*Yr5*	U	-	U	-	-	-	-	-	-	-	-	-	-	U
Yr6/6*Avo.S	*Yr6*	U	V	U	-	V	-	-	-	-	V	V	V	V	V
Yr7/6*Avo.S	*Yr7*	U	V	U	V	-	V	-	-	-	-	-	V	V	V
Yr8/6*Avo.S	*Yr8*	U	V	U	-	-	-	-	-	-	-	-	-	V	V
Yr15/6*Avo.S	*Yr15*	U	-	U	-	-	-	-	-	-	-	-	-	-	-
Yr24/6*Avo.S	*Yr24*	U	-	U	-	-	-	-	-	-	-	-	-	-	-
Yr26/6*Avo.S	*Yr26*	U	-	U	-	-	-	-	-	-	-	-	-	-	-
Yr27/6*Avo.S	*Yr27*	-	-	U	V	U	V	V	V	V	V	V	U	V	V

V: virulence (Infection types 7-9); -: Avirulence (Infection types 0-4); I: Intermediate reactions (Infection types 5-6) ; U: unknown.

Multiple resistance gene spectra

Five pathotypes exhibited a shared virulence for Heines Kolben (*Yr6*) and Lee (*Yr7*), suggesting the potential presence of both *Yr7* and *Yr6* in the future candidate cultivar 'L-1076' (Table 1b). In another scenario, two genotypes (Pasban-90 and V-04178) displayed susceptibility to four pathotypes that shared virulence for Heines Kolben (*Yr6*) and Federation X 4 Kavkaz (*Yr9*) (Table 2), indicating a combination of *Yr6* and *Yr9*.

Table 3. Resistance spectra of Pakistani wheat genotypes to 12 French and two Pakistani (P1 and P2) *Pst* races.

Resistance spectrum code	Resistance genes	No of genotypes	% of genotypes	*Pst* races/pathotypes													
				6 E 0	6 E 16	40 E 8	43 E 138	45 E 140	106 E 139	169 E 136V17	232 E137	233 E 169V17	237 E 141	237 E 141 V17	239 E 143V17	70E16V27(P1)	70E0V27(P2)
0	NI	16	7.61	R	R	R	R	R	R	R	S	S	R	S	S	R	R
1	*Yr2+*	23	10.95	S	S	R	S	S	S	S	S	S	S	S	S	S	S
2	*Yr3* or *Y25*	6	2.85	R	R	S	S	S	S	S	S	S	S	S	S	R	R
3	*Yr6+*	6	2.85	S	S	R	R	S	R	R	R	R	S	S	S	S	S
4	*Yr7+*	15	7.14	R	R	R	R	R	R	R	R	R	R	R	S	R	R
5	*Yr9+*	7	3.33	S	R	R	R	R	R	S	S	S	S	S	S	R	R
6	*Yr27*	16	7.61	R	R	R	R	R	R	R	R	R	R	R	R	S	S
7	*Yr6+Yr7*	1	0.47	S	S	R	R	R	R	R	R	R	R	R	S	S	S
8	*Yr6+Yr9*	2	0.95	S	R	R	R	R	R	R	I	R	S	S	S	R	R
9	*Yr6+Yr17*	4	1.90	R	R	R	R	R	R	R	R	R	R	S	S	R	R
10	*Yr6+Yr7+Yr9*	18	8.57	S	R	R	R	R	R	R	R	R	R	R	S	R	R
11	Resistant to all races	10	4.76	R	R	R	R	R	R	R	R	R	R	R	R	R	R
12	Susceptible to all races	82	39	S	S	S	S	S	S	S	S	S	S	S	S	S	S

R: Resistance (infection types 0-4); I: Intermediate reactions (infection types 5-6); S: susceptibility (Infection types 7-9)
NI: Non-identified; the resistance spectra found did not facilitate to postulation of any single or combination of resistance genes for code 0 is an example of non-identified resistance genes, there were other spectra found not given here.

Similarly, four future candidate cultivars, namely 'PR-88', 'PR-89', 'NR-285', and '9247' (Table 1b), showed susceptibility to 237E141V17 and 239E143V17, which shared virulence for Heines Kolben (*Yr6*) and VPM1 (*Yr17*). Hence, it was suggested that these cultivars might carry a combination of *Yr6* and *Yr17*. Furthermore, eighteen genotypes were susceptible to 6E0 and 239E143V17 but resistant to the remaining 12 pathotypes (Table 3). These two pathotypes shared virulence for Heines Kolben (*Yr6*), Lee (*Yr7*), and Federation X 4Kavkaz (*Yr9*), indicating a potential combination of *Yr6, Yr7*, and *Yr9*. Consequently, it was postulated that four released cultivars (Pirsabak 2005, Bahawalpur-2000, Rohtas-90, and Bahawalpur-95) and three future candidate cultivars (CT-99022, SN-122, and V-04189), along with eleven landraces (11614, 11787, 12009, 12194, 11612, 12252, 12257, 11928, 12206, 12207, and 12212) (Table 1a, 1b and 1c), might carry a combination of *Yr6*, *Yr7*, and *Yr9*.

Non-conclusive pattern

Among 92 genotypes tested, 10 demonstrated resistance while 82 showed susceptibility to all 14 yellow rust test pathotypes (Table 3). The resistant group comprised 'Gaznavi-98', 'Pirsabak 2004', 'Iqbal 2000', 'Manthar-3', 'DN-38', 'B-07', 'V-04112', 'NRDW-1', '12254', and '12213' (Table 1a, 1b and 1c). Conversely, the susceptible category included 72 landraces, one released cultivar, and eight potential future cultivars (Table 1a, 1b and 1c). No specific gene could be postulated in either the resistant or susceptible genotypes. However, it is conceivable that the resistant genotypes might carry either novel yellow rust resistance genes or a combination thereof, which could not be identified with the current set of pathotypes. Similarly, it is plausible that susceptible genotypes either lack yellow rust resistance genes entirely, possess a combination of such genes, or have succumbed to the virulence of the present pathotypes.

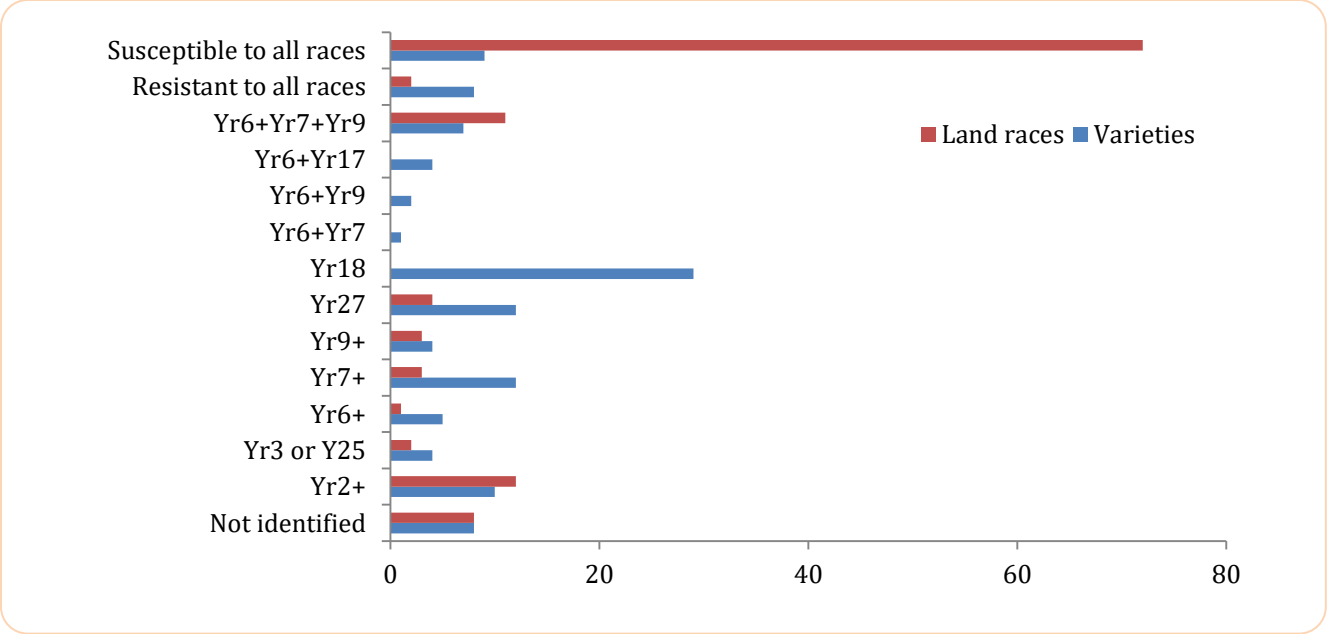

Figure 1. Comparison of yellow rust resistance gene frequencies of wheat cultivars and landraces of Pakistan.

Molecular analyses for *Yr18*

Among the 205 genotypes examined, 12 released cultivars (including Pirsabak 2004, Gaznavi-98, Bahawalpur-2000, AS-2002, GA-2002, Shafaq 2006, Faisalabad-83, Faisalabad-85, Bahawalpur-95, Punjab-96, Kohinoor-83, and Sind-81) and 17 potential future cultivars (such as DN-38, CT-99022, PR-88, PR-89, LSRR2006, SM-07018, Ras II, NRL 0320, 93T347, V-9316, V-033010, V-032862, V-04178, V-04112, V-00183, MSH-14, and NR-285) were found to carry a marker allele of 150 bp, indicating the presence of *Yr18*. These genotypes were spread across all spectra except 2 and 3. In the remaining genotypes, a non-*Yr18* allele of 230 bp was detected (Figure 3 and Table 1a, 1b and 1c).

Pathotype analysis

Out of 75 isolates, virulence spectra of 55 were defined which were grouped into four pathotypes i.e. P1, P27, P28, and P29 (Table 4). The virulences *Vr1*, *2, 6, 7, 9, 27* were frequent and the corresponding genes *Yr1*, *2, 6, 7, 9, 27* were considered defeated (Table 4). The *Vr17* is not detected, the cultivar carrying *Yr17* should be resistant. Virulences *Vr5* and *Vr10* were not associated with the pathotypes identified while P27 to P29 also lacked virulences *Vr26*, *Vr32,* and *VrSp*. Two major pathotypes (P1 and P27), represented 96% of the population and shared virulences *Vr1*, *2*, *6*, *7*, *9*, *27*, *A* (except P1) and *VrSu* (Table 4). Both P1 and P27 displayed distinct virulence patterns compared to the other pathotypes: all were avirulent on *Yr25*, *YrEp*, and an exceptionally susceptible cultivar, Victo (*YrVic*), for which no known resistance gene exists against North European, Mediterranean, and Chinese isolates. Both P28 and P29 had common virulences *Vr2*, *6*, *7*, *27,* and *Su* but differed for single virulences *Vr9* and *VrA*. The virulence frequency of *Pst* pathotypes 2008 for resistance genes *Yr1*, *2, 6, 7, 9*, *27* and *Su* was around 100% (Table 5).

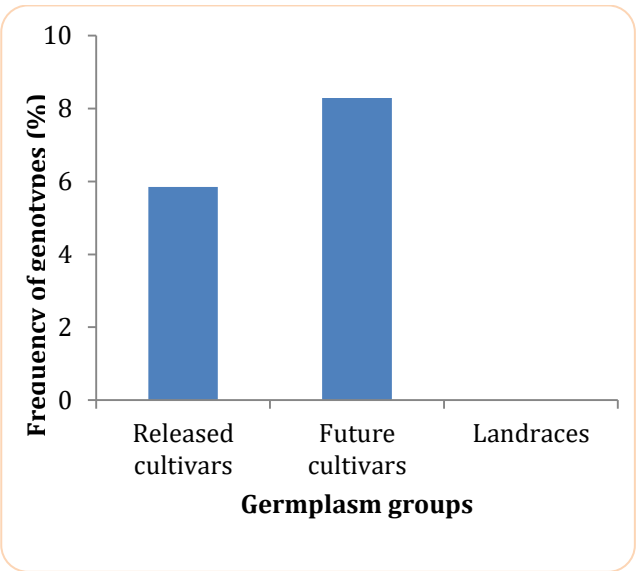

Figure 2. Frequency of *Yr18* gene in 205 Pakistani wheat genotypes identified with molecular marker csLV34.

Table 4: Number of *Pst* pathotypes and their virulence factors sampled from different geographical areas of Pakistan during 2006-08.

Pathotypes	No of isolates	Virulence Factors																												Abbotabad			East of Peshawar			West of Peshawar						Mingora			Nowshara		
		1	2	3	4	5	6	7	8	9	10	15	17	21	24	25	26	27	32	A	Sp	Su	Sd	Ep	Ju	Vic	Pr	Tr	Exp	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
P1	51	1	2	-	-	-	6	7	-	9	-	-	-		-	-	-	27	-	-	-	SU	-	-	-	-				10	-	-	3	-	4	5	-	12	-	-	-	2	-	15
P2	14	-	2	-	-	-	6	7	8	-	-	-	-		-	-	-	27	-	A	-	SU	-	-	-	-				4	-	-	-	-	-	2	-	-	-	-	-	8	-	-
P3	2	-	2	-	-	-	6	7	8	-	-	-	-		-	-	-	27	-	-	-	SU	-	-	-	-				-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
P4	1	-	2	-	-	-	6	7	8	9	-	-	-		-	25	-	27	-	A	-	-	-	EP	-	V				1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P5	2	-	2	-	-	-	6	7	-	9	-	-	-		-	25	-	-	-	A	-	-	-	EP	-	V				-	-	-	-	-	-	1	-	-	-	-	-	1	-	-
P6	4	-	2	-	-	-	6	7	8	9	-	-	-		-	25	-	-	-	A	-	-	-	EP	-	V				2	-	-	-	-	-	2	-	-	-	-	-	-	-	
P7	1	-	2	-	-	-	6	7	-	9	-	-	-		-	25	-	27	-	A	-	-	-	EP	-	V				-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
P8	1	-	2	-	-	-	6	7	8	-	-	-	-		-	25	-	27	-	A	-	-	-	EP	-	V				-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
P9	1	1	2	-	-	-	6	7	-	9	-	-	-		-	25	-	27	-	A	-	SU	-	EP	-	V				-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
P10	1	1	2	3	-	-	6	7	8	9	-	-	-		-	25	-	27	-	A	-	SU	-	EP	JU	V				-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
P11	1	1	2	-	-	-	-	7	-	9	-	-	-		-	25	-	27	-	A	-	SU	-	EP	JU	V				-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
P12	1	1	2	3	4	-	6	-	-	9	-	-	17		-	25	-	27	-	-	-	SU	SD	EP	JU	V				-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
P13	2	1	-	3		-	6	7	-	9	-		-	21								SU					Pr	-	Exp	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P14	2	1	-	3		-	6	7	8	9	-		-	21								SU					Pr	-	Exp	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P15	1	1	2	3		-	6	7	8	9	-		-	21								SU					Pr	-	Exp	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P16	1	1	2	3		-	6	7	8	9	-		-	21								-					Pr	Tr	Exp	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P17	1	1	2	3		-	6	7	8	9	-		-	21								SU					Pr	Tr	Exp	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
P18	2	-	2	3		-	6	7	-	-	-		-	21								-					Pr	-	Exp	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-
P19	1	-	2	3		-	-	-	-	-	10		-	21								SU					-	Tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
P20	1	-	2	3		-	6	7	-	-	-		-	21								SU					-	-	Exp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P21	1	1	-	3		-	6	7	8	9	-		-	-								SU					Pr	-	Exp	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P22	1	1	-	3		-	-	7	8	-	-		-	21								SU					Pr	-	Exp	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
P23	1	1	-	3		-	-	-	-	-	-		-	21								SU					-	Tr	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
P24	2	-	-	3		-	6	7	8	9	-		-	21								SU					Pr	-	Exp	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P25	1	-	-	3		-	6	7	8	-	-		-	21								SU					Pr	Tr	Exp	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P26	1	-	-	3		-	6	7	-	-	-		-	21								SU					Pr	-	Exp	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P27	22	1	2	-	-	-	6	7	-	9	-	-	-	U	-	-	-	27	-	A	-	SU	-	-	-	-				-	-	-	-	-	-	-	-	13	-	-	-	-	-	9
P28	1	-	2	-	-	-	6	7	-	-	-	-	-	U	-	-	-	27	-	A	-	SU	-	-	-	-				-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
P29	1	-	2	-	-	-	6	7	-	9	-	-	-	U	-	-	-	27	-	-	-	SU	-	-	-	-				-	-	-	-	-	1	-	-	-	-	-	-	-	-	-

Results of P1 to P12 belonging to 2006 were reported by Bochra et al., 2011 while P13 to P26 belonging to 2007 were analyzed by Dr. Xianming Chen, USDA, USA- Unpublished data (empty cells)

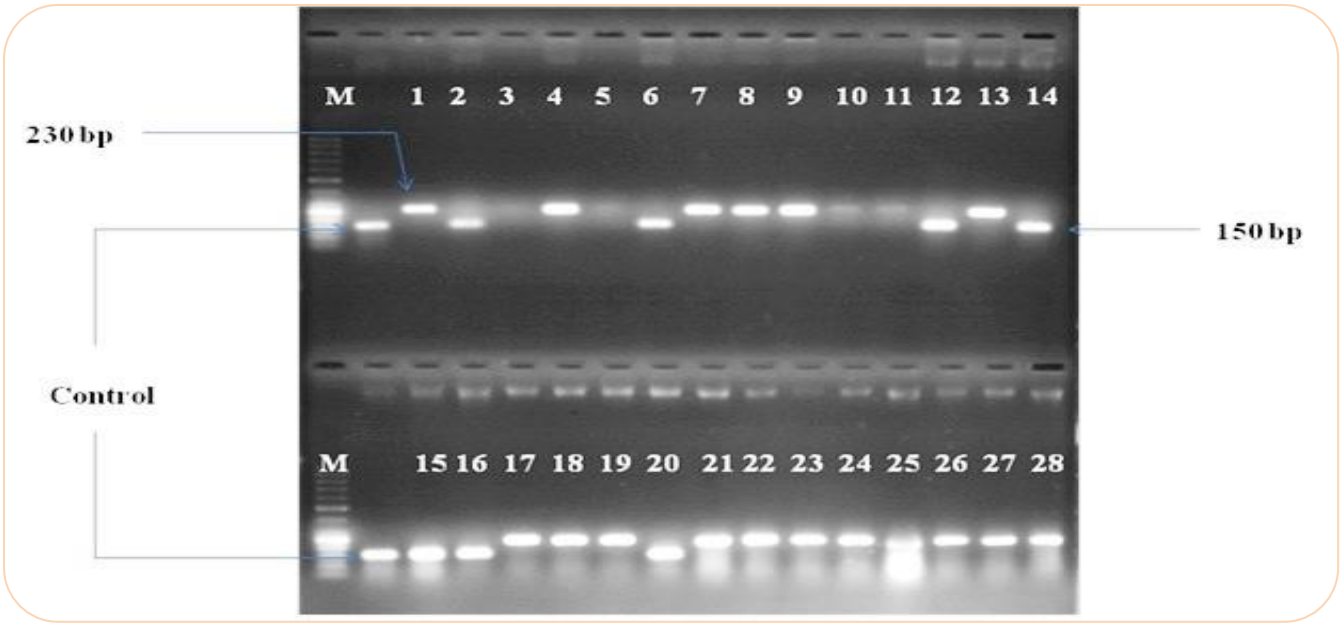

Figure 3. csLV34 PCR amplification products of 28 varieties along with positive control; resistant varieties 2. Bahwalpur-95; 6. Bakhtawar-93; 12. Sind-81; 14. CT-00231; 15. V-01180; 16. MSH-14; 20. Ras-II, susceptible varieties 4. Inqilab-91; 7. Kiran-95; 8. KT-2000; 9. Pirsabak-2005; 10. Tatara; 11. Zargoon-79; 13. AUP-4606; 17. CT-99022; 18. 99B4012; 19. MALIR; 21. 11345; 22. 11389; 23. 11480; 24. 11489; 25. 11505; 26. 11572; 27. 11574; 28. 11581, Missing samples 3. Bhakhar-2002 and 5. Iqbal-2000. M stands for size standar.

Table 5: Percentage of Pst isolates with virulence for individual resistance genes over three years in northwest Pakistan.

Yellow rust	*Pst* isolates virulence frequency (%)		
resistance genes	2005-06	2006-07	2007-08
Yr1	49	56	96
Yr2	100	39	100
Yr3	4	100	0
Yr4	2	0	0
Yr6	98	83	100
Yr7	98	89	100
Yr8	47	67	0
Yr9	65	56	98
Yr10	0	6	0
Yr17	2	0	0
Yr21(Lemhi)	0	94	0
Yr25	26.5	Not Tested	0
Yr27	87.8	-do-	100
YrVic	26.5	-do-	0
YrA	53.1	-do-	41.8
YrSu	82	83	100
YrSd	2	0	0
YrEp	27	Not tested	0
YrJu	6	-do-	0
YrPr	Not Tested	83.3	Not Tested
YrTr	-do-	27.8	-do-
YrExp	-do-	88.9	-do-
Total Isolates	49	18	55

[1] Virulence for the pathotypes *A*, *Su*, *Sd*, *Vic*, *Pr*, *Tr,* and *Exp* indicate resistance genes carried by the cultivars Anza, Suwon92 x Omar, Strubes Dickkopf, Victo, Produra, Tres, and Express.
Chinese varieties (i.e. Early Premium (Ep) and Jubiliena (Ju)), carrying unidentified *Yr* resistance genes were included.

DISCUSSION

In the past twenty years, the rise of new races of *Pst* has presented a significant challenge to global wheat production, as wheat yellow rust becomes increasingly menacing. To combat this threat, extensive international endeavors are in progress to meticulously track the development and spread of *Pst* races, identify the pathotypes of isolates, assess disease-resistant genetic resources, and develop wheat cultivars endowed with enduring resistance to the disease (Perronne et al., 2021). This study efficiently discerned the genetic basis of rust resistance across a spectrum of landraces, upcoming varieties, and already released cultivars using 14 *Pst* pathotypes set along with a bi-allelic STS marker. Traceable pedigrees of the wheat genotypes were also examined to corroborate postulated genes along with pathotyping of the prevalent yellow rust population.

The resistance spectrum of 16 genotypes did not align with the known genes targeted by the *Pst* pathotypes utilized in this study. Further exploration of these genotypes through inoculation with *Pst* pathotypes harboring different virulence/avirulence factors could aid in identifying genes that were not postulated in the current investigation. Twenty-two genotypes were suggested to carry *Yr2*+, although the pedigree information supporting the presence of these genes was unavailable for 12 landraces and one upcoming cultivar. Among the remaining nine, the *Yr2* donor in various pedigrees was traced back to 'Kalyansona', 'WL-711', and 'Lermo-Rajo-64', all known to possess *Yr2* according to previous studies (Nagarajan et al., 1987; Singh and Johnson, 1988; Badebo et al., 1990; McIntosh et al., 1992). Similarly, *Yr3* or *Yr25* was inferred in six genotypes, of which the pedigree information for two future cultivars and one released cultivar is documented. *Yr3* or *Yr25* was identified in only one line, 'V-04188', as a parent, according to available sources (Line, 1990; McIntosh et al., 1997). Likewise, *Yr6*+ was proposed in six genotypes, with pedigree information available for two released cultivars (Zardana-89 and Kaghan-93), which supported the postulation of *Yr6* in both cultivars (www.cdl.umn.edu/database/wheatgene.html).

Yr7+ presence was detected in 15 genotypes. Among these, one future cultivar and five released cultivars had 'CIANO-67' in their pedigrees, suggesting it might be the donor of *Yr7* in these six genotypes (www.cdl.umn.edu/database/wheatgene.html). Similarly, two other future cultivars, '2KC050' and 'CT-03457', with 'Veery-5' in their pedigrees, carried *Yr7* (McIntosh et al., 1990). However, the pedigrees of three future cultivars (Q.S-1, V-03138, and V-04022) did not align with the presence of *Yr7* as inferred in the present study. Meanwhile, MPT-7, with cv. Pavon-76 in its pedigree, was confirmed to carry *Yr7* (Badebo et al., 1990). Presence of *Yr9*+ was hypothesized in seven genotypes. While the pedigree of one cultivar (GA-2002) did not support the postulation results, *Yr9* was confirmed in another cultivar (Kohinoor-83), as previously reported (www.cdl.umn.edu/database/wheatgene.html). Future cultivars 'SM-07018' and 'V-002495-A' had two genotypes, 'Veery-5' and 'Sutlej-86', in their pedigrees, which may be the sources of the *Yr9* gene (Hussain et al., 1988; McIntosh et al., 1990) in these cultivars. Sixteen genotypes were inferred to carry *Yr27*, with seven lacking pedigree information. In another five (Punjab-96, Zargoon-79, V-9244, 45006, and MSH-14), the gene was not traceable in their pedigrees. Inqilab-91 was identified as the source of *Yr27* (McIntosh et al., 2005) in the parentage of two future and one released cultivar (V-00183, V-99022, and SH-2003) inferred to carry *Yr27*. Additionally, both 'MALIR' and 'V-022668' had 'CIANO-67' and 'Kauz', respectively, as one of the parents and both carried *Yr27* (McIntosh et al., 1998, 2005).

A combination of *Yr6* and *Yr7* was postulated in only one future cultivar (L1076) lacking pedigree information. One of the released (Pasban-90) and future cultivar (V-04178) were inferred to carry *Yr6* and *Yr9* combination, however, only *Yr9* presence was supported by each pedigree (www.cdl.umn.edu/database/wheatgene.html). Four future cultivars were inferred to carry a combination of *Yr6* and *Yr17*; however, this gene pair cannot be traced in the NR-285 background. In the other three future cultivars (i.e. 88, PR-89, and 9247) pedigree in case of each was supportive of only *Yr6* (www.cdl.umn.edu/database/wheatgene.html). A combination of *Yr6*, *Yr7* and *Yr9* was inferred in 18 genotypes, of which, eleven landraces and one cultivar lack parental information. Two future cultivars, 'CT-99022' and 'SN-122' have 'Kauz' as a common parent in each which might be a donor of *Yr6*, *Yr7* and *Yr9* (www.cdl.umn.edu/database/wheatgene.html). Parentage of Bahawalpur-95 only supported *Yr9* presence which is known to be present in one of its parents 'Avroa' (Borojevic and Dencic, 1988). Similarly, 'CIANO-67' is a common ancestor in each of Rohtas-90 and V-04189 which may be the donor of *Yr6* and *Yr7*

(www.cdl.umn.edu/database/wheatgene.html) but evidence for *Yr 9* could not be established. 'Amsel' stands as one of the parental contributors, while 'CIANO-67' adds to the genetic background of Pirsabak-2005 (Badebo et al., 1990, www.cdl.umn.edu/database/Wheatgene.html), the pedigree details were, therefore, supportive to inferre *Yr6*, *Yr7* and *Yr9* combination in this cultivar.

A combination of *Yr6* and *Yr7* was postulated in only one future cultivar (L1076), which lacked pedigree information. Similarly, one released cultivar (Pasban-90) and one future cultivar (V-04178) were inferred to carry a combination of *Yr6* and *Yr9*. However, only the presence of *Yr9* was supported by their pedigrees (www.cdl.umn.edu/database/wheatgene.html). Four future cultivars were inferred to carry a combination of *Yr6* and *Yr17*; however, this gene pair could not be traced in the background of NR-285. In the case of the other three future cultivars (PR-88, PR-89, and 9247), the pedigree information was supportive of only *Yr6* (www.cdl.umn.edu/database/wheatgene.html). A combination of *Yr6*, *Yr7*, and *Yr9* was inferred in 18 genotypes, with eleven landraces and one cultivar lacking parental information. Two future cultivars, 'CT-99022' and 'SN-122', share 'Kauz' as a common parent, which might be a donor of *Yr6*, *Yr7*, and *Yr9* (www.cdl.umn.edu/database/wheatgene.html). The parentage of Bahawalpur-95 only supported the presence of *Yr9*, known to be present in one of its parents, 'Avroa' (Borojevic and Dencic, 1988). Similarly, 'CIANO-67' is a common ancestor in the pedigrees of both Rohtas-90 and V-04189, which may be the donor of *Yr6* and *Yr7* (www.cdl.umn.edu/database/wheatgene.html), although evidence for *Yr9* could not be established. 'Amsel' is listed as one of the parental contributors, while 'CIANO-67' adds to the genetic background of Pirsabak-2005 (Badebo et al., 1990, www.cdl.umn.edu/database/Wheatgene.html), making the pedigree details supportive of inferring a combination of *Yr6*, *Yr7*, and *Yr9* in this cultivar.

Since 10 genotypes exhibited resistance against all 14 yellow rust pathotypes, it suggests the possibility of novel *Yr* resistance genes, or combinations thereof, within these genotypes. These genes might have escaped detection with the *Pst* pathotypes used in the current study, underscoring the need for further investigation. Conversely, 81 genotypes displayed susceptibility to all test pathotypes, indicating either a lack of *Yr* resistance genes or the presence of a combination of such genes. Alternatively, these genotypes may be susceptible to the current set of pathotypes.

The *Yr18* marker allele of 150 bp was amplified in 29 released and future cultivars, all of which were either direct selections from CIMMYT or derived from CIMMYT germplasm. The Brazilian cultivar Frontana is a well-known source of the gene pair *Yr18/Lr34* in many CIMMYT-originated cultivars (Kolmer et al., 2008). Developed in South America, Frontana was created by crossing Mentana, an Italian introduction, with Frontiera. Mentana carried the *Yr18/Lr34*-associated allele of 150 bp, while Frontiera had a non *Yr18/Lr34* associated allele of 230 bp (Kolmer et al., 2008). Mentana, serving as the donor of the *Yr18/Lr34* associated allele identified in Frontana, played a crucial role in this genetic lineage. This allele was propagated through subsequent crosses with Frontana within the CIMMYT program, leading to the development of numerous cultivars such as Penjamo 62, Lerma Rojo, and Nainari 60. These cultivars, in turn, became foundational components of CIMMYT germplasm, including Kauz, Yaco, Fret 2, Norteno, Nocozari, Yaquai-50, Lerma Rajo-64/Tezanos Pintos Precoz, Nadadores 63, Ciano 67, Jupateco 73, and Torim 73. The presence of *Yr18/Lr34* in these foundational cultivars likely contributed to its presence in the pedigrees of the 29 Pakistani cultivars mentioned (McIntosh et al., 2005; Kolmer et al., 2008; Shah et al., 2010; www.cdl.umn.edu/database/wheatgene.html).

The virulences *Vr1*, *Vr2*, *Vr6*, *Vr7*, *Vr9*, and *Vr27* were frequently associated with the four pathotypes P1, P27, P28, and P29 detected in the current study, indicating that the corresponding host resistance genes *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr9*, and *Yr27* have been defeated. These pathotypes do not match the virulence pattern of the widely dispersed pathotype *PstS2*, known for its high-temperature aggressiveness and its erosion of the *Yr9* gene in the Middle East and South Asia (Singh et al., 2004; Hovmøller et al., 2008; Ali et al., 2017). The extensive use of the *Yr2*, *Yr6*, and *Yr7* genes regionally led to increased genetic vulnerability, causing the erosion of these resistance genes in Africa, the Middle East (Bamdadian, 1984; El-Daoudi et al., 1996; Hakim et al., 2002; Yahyaoui et al., 2002), and Pakistan. In Pakistan, this vulnerability resulted in a major epidemic on wheat cultivars Mexipak, Chenab 70, Barani 70, Khushal, and Mangla in 1973 (Ahmad, 2002; Hussain et al., 2004). The popularity of cultivars with the 1B/1R translocation carrying the *Yr9* gene in various Asian

countries led to a scenario of monoculture, which rendered *Yr9* resistance ineffective. This resistance breakdown was first documented in Ethiopia in 1986 (Badebo et al., 1990) and later in the Middle East (Afshari, 2008; Bahri et al., 2009). In Pakistan, the *Yr9*-based resistance of two widely grown cultivars, Pirsabak-85 and Pak-81, broke during the 1994-95 growing season in Khyber Pakhtunkhwa and Punjab, leading to an epidemic caused by race 134E150 (Ahmad, 2002; Hussain et al., 2004). Following these epidemics, many resistant cultivars protected by the *Yr27* gene were released. Two major cultivars, Inqilab-91 (*Yr27*) and PBW343 (*Yr9* and *Yr27*), covered over 11 million hectares in yellow rust-prone areas of Pakistan and India, and were also grown under different names in other countries in the region (Singh et al., 2004). Evidence of *Yr27* virulence began to appear in Pakistan in 2003 and was confirmed in 2004. This virulence was also documented in Afghanistan, Iran, Kyrgyzstan, Tajikistan, and India (Duveiller et al., 2007). The cultivation of a limited number of cultivars with race-specific resistance genes increased genetic uniformity, resulting in greater vulnerability, which likely led to the erosion of Inqilab-91's resistance in Pakistan during 2005 (Duveiller et al., 2007).

Virulence and SSR diversity of *Pst* population sampled in Pakistan during 2005-2006 and the ongoing selective pressures in major wheat cultivars were reported by Bahri et al (2011). Analyses of 49 isolates from the Northwest Frontier Province (now Khyber Pakhtunkhwa) of Pakistan were characterized into 12 distinct pathotypes. Frequencies of virulence against *Yr6, 7*, *8*, *9*, *SU,* and *EP* ranged between 62 and 97%, while virulence against *Yr2* was consistently present across all pathotypes. No virulences for *Yr5*, *10*, *15*, *24*, *32,* and *Sp* were detected while virulences against *Yr3*, *4*, *17*, and *SD* were rare, whereas they are common in North West Europe. The described pathotypes are defined into 27 distinct genotypes. The genotypes were classified into five groups, with one hybrid isolate, delineating three distinct lineages based on the SSR-based phylogenetic tree. The majority of the studied isolates (80%), characterized by three predominant pathotypes (P1-P3), belong to a single lineage, while the remaining isolates exhibit proximity to either the Mediterranean or Northern European lineages. Genetic recombination was hypothesized among P2 isolates. Yellow rust resistance genes inferred in 41 Pakistani genotypes showed the frequent prevalence of *Yr2*, *6*, *7*, *8*, *9*, *27,* and *Su*. Only 11 genotypes displayed resistance to P1-P3. This study revealed high diversity at the genetic level in the Pakistani *Pst* population and suggested genetic exchanges with Western Mediterranean countries. Factors including pathotypes migration and varietal diversity might have contributed to maintaining the current high genetic diversity in Pakistani *Pst*. The situation in Pakistan has not changed concerning the presence of virulences for *Yr2, 6*, *7*, *9,* and *27* as it was reported recently from Pakistan (Bahri et al, 2011; Dr. Xianming unpublished data, Ali et al., 2014; Sufiyan et al., 2021). All the isolates lack the virulence on Victo, which is characteristic of Pakistani populations and not mentioned elsewhere.

The study's findings underscore a critical need to reassess wheat cultivation practices in Pakistan. Relying on cultivars and landraces with compromised seedling resistance genes, coupled with the limited protection offered by the adult plant resistance gene *Yr18*, presents significant challenges against yellow rust. The prevalence of virulent *Pst* pathotypes targeting inferred resistance genes (Bahri et al., 2011) further highlights the potential shortcomings of solely relying on *Yr18*-containing cultivars under field conditions. A decisive shift toward a more resilient strategy is imperative. Prioritizing the incorporation of additional resistance genes lacking corresponding pathotypes will bolster the resilience of studied genotypes and others under cultivation. Diversifying the genetic defenses against yellow rust will equip cultivars with a broader array of unmatched genes, heightening the pathogen's genetic adaptation hurdles for successful infection. This multifaceted resistance approach, endorsed in previous studies, shows promise for enhancing durability and sustainability in wheat production amidst the evolving threat of yellow rust in Pakistan.

Continuously broadening the gene pool for yellow rust resistance through global wheat breeding programs is paramount. Additionally, achieving resistance durability through gene pyramiding is urgent to keep pace with the relentless evolution of the pathogen. Anticipating climate change and fungal evolution's potential to spawn new pathotypes or races underscores the need to streamline and genetically characterize novel genes for swift mobilization when necessary. Moreover, this study plays a pivotal role in informing cultivar development and deployment

strategies for rust management. By identifying current cultivar limitations and emphasizing the necessity for additional resistance genes, the study guides breeders toward creating more resilient wheat varieties. The insights into the genetic basis of resistance to yellow rust stress the importance of diversifying the genetic arsenal against this pathogen. As breeders strive to develop and release new cultivars, prioritizing the incorporation of a broader range of resistance genes will enhance durability and sustainability in wheat production. Additionally, strategically deploying these newly developed cultivars will maximize their effectiveness in combating rust diseases. By integrating the findings of this study into cultivar development and deployment practices, stakeholders can better manage rust diseases and mitigate their impact on wheat yields and global food security.

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AUTHORS' CONTRIBUTIONS

SH coordinated material arrangements in Pakistan and developed initial draft manuscript; SJAS planned and conducted field surveys for rust sampling and wheat germplasm from Pakistan and managed its transportation to France and conducted yellow rust pathotyping and gene postulation experiments in France and contributed to the manuscript's improvement; ML carried out pathotyping and gene postulation experimental arrangements, spore increase, and data interpretation in France; CP provided resources for the study in France, designed and supervised the whole study and improved and finalized the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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