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Full Length Article

Assessment of Bread Wheat Genotypes for Resistance to *Puccinia striiformis* f. sp. *tritici*

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Abstract

Stripe rust, caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the major diseases that threaten wheat production worldwide. This study aimed to assess the resistance of 100 bread wheat genotypes to stripe rust by multiple evaluations *via* field testing, seedling stage testing and molecular marker analysis. A greenhouse seedling evaluation, in which virulent isolates of *Pst* were used for artificial inoculation, revealed that Parwaz, Parula 73, Tatara and GA 2002 were resistant wheat varieties. The field experiments were conducted in a randomized complete block design and the disease severity data were obtained *via* the modified Cobb scale. The coefficient of infection revealed significant variations in resistance among the varieties, with low resistance values in Dirk, Blue Silver and Marvi-2000. Molecular marker analysis *via* Kompetitive Allele Specific PCR (KASP) genotyping revealed the resistance genes *Yr17* and *Yr46* in C-228, C-217, C-250, C-271, C-273, Chenab-70, Blue Silver, Tatara and Khatakwal. Genotyping data are suggestive of a high variability towards resistance, complemented with molecular markers, when the genetics of resistance are analyzed. The findings demonstrate the significance of integrating field evaluations, seedling testing and molecular markers to identify resistant wheat varieties, ensuring the more efficient selection of resistant varieties for maximum wheat yield.

Keywords: Molecular markers; *Puccinia striiformis* f. sp. *tritici*; Stripe rust; Wheat

Introduction

Wheat stripe rust is caused by the destructive fungus *Puccinia striiformis* f.sp. *tritici* (Jamil *et al*. 2020). Its attack increases production costs and significantly lower grain quality and output, causing billions of dollars in annual economic losses worldwide (Afzal *et al*. 2024). In susceptible cultivars, it can reduce yield by 10–70%, with potential losses of 100%, especially during periods of severe epidemics (Kokhmetova *et al.* 2021). Various experts have reported various *Pst* pathotypes in Pakistan. During 1978, 1997-98 and 2005, there were four significant stripe rust outbreaks that cost the Pakistani economy $244, $33 and $100 million, respectively (Bahri *et al*. 2011). The most cost-effective and environmentally friendly way to combat the continuous and rapid spread of stripe rust, particularly in regions such as China and Pakistan, where outbreaks are frequent, is to develop resistant cultivars (Abbas *et al*. 2024).

Field evaluation of wheat genotypes for resistance to stripe rust is critical for identifying cultivars resistant to stripe rust that can sustain disease outbreaks under natural circumstances. Such evaluations are usually performed by planting a number of different wheat genotypes in areas prone to disease and recording the presence and intensity of stripe rust over several growing seasons (Lan *et al*. 2010). Field experiments should be conducted in diverse geographic areas to better understand genotype–environment interactions. In other words, it is vital to perform field assessments when creating and identifying stripe rust-resistant cultivars for wheat varieties suitable for particular agro-ecological zones (Ali *et al*. 2017). Seedling stage evaluation enables how resistant wheat genotypes are against stripe rust by observing the development of the disease under controlled conditions, thereby reducing environmental variations that might impact the results obtained from field trials. Recent advancements in greenhouse screening methods have improved the specificity and reliability of resistance assessments *via* techniques such as the use of molecular markers and precision phenotyping (Milus and Seyran 2004).

Kompetitive allele-specific PCR (KASP) is a new competitive allele-specific PCR for single nucleotides

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polymorphism genotyping assays in which allele-specific primers are used to amplify the sample DNA *via* a heat cycler. The 5′ end of each allele-specific primer was coupled to the fluorometric dyes HEX and FAM (Zhao *et al*. 2017). KASP can achieve high-precision allele genotyping of SNPs for a limited number of target markers in large-scale segregating or natural populations. It also offers great flexibility in terms of the number of SNP and samples used for determination (Chen *et al.* 2024). Approximately 83 *Yr* resistance genes dispersed across 21 chromosomes have been identified to genetically control stripe rust (Mcintosh *et al*. 1995; Maccaferri *et al*. 2015). Only eight *Yr* genes, *Yr36* (Fu *et al*. 2009), *Yr18* (*Lr34*) (Lagudah *et al*. 2009), *Yr46* (*Lr67*) (Hiebert *et al*. 2015), *Yr15* (Klymiuk *et al*. 2018), *Yr5/YrSp* (Marchal *et al*. 2018), *Yr79*, *YrAS2388* and *YrU111*, have been functionally validated (Abbas *et al*. 2024). The *Yr17* gene, which confers resistance to stripe rust in wheat, has been used by breeders worldwide (Helguera *et al*. 2003). The goals of this study were to identify resistance genes using associated molecular markers and to identify resistant cultivars through field and greenhouse evaluations.

Materials and Methods

Experimental material

One hundred wheat varieties were evaluated for the identification of varieties resistant to stripe rust. These varieties include historically significant cultivars that present diverse genetic diversities (Table 1). Field evaluations were conducted at the Crop Disease Research Institute of the National Agriculture Research Institute, Islamabad, Pakistan. The inoculum was prepared and applied to all the wheat varieties. The modified Cobbs scale was used to record disease severity. Additionally, resistance at the seedling stage was assessed in a greenhouse. Molecular markers were used to identify specific disease-resistance genes, providing a comprehensive evaluation of the resistance profiles of several wheat varieties.

Field evaluation

Field evaluation was carried out in the field area of the Crop Disease Research Institute at the National Agricultural Research Center, Islamabad. The experimental design was a randomized complete block design. Each wheat variety was sown at a row-to-row distance of 30 cm with three replications, which is an ideal condition for optimum growth, as well as sufficient space for the disease to spread uniformly in the field. The weather at the time of sowing was favorable for the natural growth of stripe rust. A universally sensitive wheat variety, Morocco, was planted as a control at the border.

One hundred historical wheat varieties were inoculated with *Pst*. For uniformity of infection, an inoculum was prepared by mixing paraffin oil and petroleum spirit at a ratio of 30:70. To aid in the dissemination of spores, striped rust spores from the gelatin capsule were added to the mixture. Pathogen inoculum was sprayed onto the field using a sprayer. Morocco-a universally susceptible variety was planted at the borders as a check. Symptoms of stripe rust have been consistently observed in the field.

Every two weeks, data were collected from various wheat varieties to assess the resistance levels by recording the severity and prevalence of *Pst*. Disease severity was assessed *via* the modified Cobb scale (Peterson *et al.* 1948). The coefficient of infection was calculated by multiplying the response value by the constant value of the infection type, as shown in Table 2.

To determine whether the average infection index of resistant versus nonresistant cultivars differed significantly, an ANOVA was performed. By comparing the means of the CI for the two groups, ANOVA was used to assess the variability between groups and within groups. The results can be used to understand the effectiveness of resistance in different lines.

Seedling stage testing

Following field evaluation, seedling stage screening further clarified the resistance profiles of the wheat varieties. Five seeds of each variety, with three replications, were sown in plastic pots with dimensions of $7 \times 7 \times 7$ cm^3. For optimal growing conditions and appropriate drainage, a 2:1:1 (v/v/v) mixture of soil, sand and compost was added to each pot (Rizwan *et al*. 2007).

An extremely virulent *Pst* isolate *Yr 210* was used to create the urediospore suspension. For urediospore suspension, a 70:30 petroleum ether to mineral oil ratio was used. The suspension was thoroughly shaken to ensure uniform spore dispersal. One week after planting, the first fully grown leaves were inoculated. An atomizer was used to spray the seedlings with urediospore solution for uniform coverage. The inoculated plants were left to dry for five minutes before being lightly misted with water to promote spore germination. The inoculated seedlings were placed in a wet cage with a small water supply at the bottom to generate an extremely humid environment. The cages were later replaced with a dew chamber maintained at 10°C. Therefore, initiating an infection process was important.

Following the dew-chamber incubation, the seedlings were placed in a greenhouse at 18°C. Adequate lighting was provided in the greenhouse while ensuring that proper ventilation was maintained to allow cooling. Three weeks after inoculation, infection symptoms were recorded on plants on a 9 scale as shown in Table 2 (McNeal *et al.* 1971).

Evaluation through molecular markers

Genomic DNA was extracted from young leaf tissues of 100 wheat varieties *via* a CTAB-based extraction method,

Table 1: A list of wheat cultivars used in this study with parentage

Varieties	Year of Release	Pedigree/Parentage
T-9	1918	Selection from landrace
T-11	?	-
Dirk	?	FORD/DONDEE (I)
C-518	1933	T9 × 8A
C-591	1934	T9 × 8A
C-228	1941	HARD FEDERATION X 9D
C-217	1944	C 516 X C 591
C-250	1944	Hard Federation x 9D
C-271	1957	C 230 × IP 165
C-273	1957	C 209 × C 591
Mexipak 65	1965	PJ62/GB55 II8156-0PAK
Khushal-69	1969	Y50/4/C271PK937-17F-3K-2F; 21931-CHAPINGO53; ANDES-SIB/3;
Chenab-70	1970	C271/WI(E)//SON64 PK 146-12A-4A-0A
Barani-70	1970	PIT/GB//C271 PK50-18A-4A-13A-0A
Blue silver	1971	1154388/NA/3/ YT54/N10B/ LR64 II 18427-0PAK
SA-42	1971	NAI60/CB151//S949/ 3/MEXIPAK
Lylpur-73	1973	BB/NOR67 II27100-307M-100A-0A-OPAK
Pari-73	1973	CNO67//SN64/ KLRE/3/ 8156 II23584-303M-0Y-11A-1A-1436-OPAK
Parula-73	1973	-
Yacora	1975	CNO67//SN64/KLRE/3/8156 II23584-303M-0Y-11A-1A-1436-OPAK
LU-26	1976	BLS/KHUSHAL
Punjab-76	1976	NAI60/CB151//S949/3/MEXIPAK PK6841-2A-2A-1A-0A
WL-711	1978	S308/CHRIS//KAL
Jauhar-78	1978	Nayab* (Fast neutran 600rads (Mutagensis)
Zarghoon	1979	CC-Inia/Tobari-C.fon/BB CM8237-G-1M-3Y-2M -4Y-0M
Pak-81	1981	KVZ//BUHO//KAL/BB CM33027-F-15M-500Y-0M-76B-OY-OPAK
Punjab-81	1981	KVZ//BUHO//KAL/BB CM33027-F-15M-500Y-0M-76B-OY-OPAK
Sindh-81	1983	Norento x Mexipak
Faisalabad-83	1983	FURY/KAL/BB CM37138-48Y-1M-5Y-1M -4V-5Y-0A-0PAK
Kohinoor-83	1983	OREF1158/FDL/MFN/ 2*TIBA63/3/ COC CM37987-I-1Y-5M-OY-0PAK
Barani-83	1983	BB/GLL/ 3/ GTO/7C//BB/CN0 CM32347-3M -1Y-1M-1Y-1K-0A-0PAK
Faisalabad-85	1985	MAYA/MON//KVZ/TRM CM44083-N-3Y-1M -1Y-1M-1Y-OB
Sarsabz	1985	PI/FRND//MXP/3/PI/M20/ 70
Tandojam-83	1985	BLUEJAYHS CM-5287-J-1J-2M-2Y-3M-0Y
Pirsbak-85	1985	KVZ/BUHO/ BB CM33027F-1SM-4Y-4M-2Y-1M -1Y-0M
Punjab-85	1985	KVZ/TRM//PTM/ANA CM43903-H-4Y-1M-1Y-3M-3Y-OB-0PAK
Wadank-85	1985	-
Chakwal-86	1986	Fln/ACS//ANA SWM4578-56M-3Y-3M-0Y-PAK
Rawal-87	1987	MAYA/MON//KVZ/TRM CM 44083-N-2Y-2M -1Y-1M-1Y-2M-0Y
Khyber-87	1987	KVZ/TRM//PTM/ANA-CM 43930 CM 43903-H-4Y-1M -1Y-3M-2Y-0B
Sutluj-86	1987	-
Shalimar-88	1988	-
Zardana	1989	Ciano"S" 67-8156 x Tobari-66-CNO 67/NOV-66/ 11-12300/ LR6408156-PAN-76 CM40577-6M -2Y-4M-4M-1Y-8M-1Y-0Y
Mehran-89	1989	VEERY 'S' CM38027-F-15M-500Y-0M-87B-0Y
Rohtas-90	1990	INIA F 66/ A.DISTCHUM//INIA66/ 3/ GEN W.8461-R-0PAK(PAK)
Inqilab-91	1991	WL-711/CROW"S"
Pasban-90	1991	INIA66/A. DISTT//INIA66/3/GEN
Soghat-90	1991	Pavon Mutant-3
Bakhtawar-92	1992	JUP/BJYG//URES CM 67458-4Y-1M -3Y-1M-5Y-B
Kaghan-93	1993	TTR/JUN CM59123-3M -1Y-2M-1Y-2M-2Y-0M -0PAK
Anmol-91	1993	LIRA 'S' CM-43903-H-4Y-1M -1Y-3M -3Y-0B
Shaheen-94	1994	MLT"S"
Parwaz-94	1995	V5648/PRL"S" = V-87189
Shahkar-95	1995	WL 711//F3.71/TRM Pb 20371-20A-4A-0A-0K-0A
Kohsar-95	1995	PSN/BOW CM69560-1M -1Y-1M-2Y-0M-0PAK(PAK)
Kirin-95	1996	WL-711/CROW"S"
Nowshera-96	1996	BUCK/FLK//MYNA/VUL CM91575-34Y-0M-0Y-1M -0Y
Punjab-96	1996	SA 42 *2/3/CC/INIA4// BB/4/INIA/HD832 Pb1352-B-4K-36A-0A
Suleman-96	1996	F-6.74/BUN//SIS/3/ VEE #7 (F6) CM86141-62M-0Y-0M-4Y-0M
Abadgar-93	1996	YAK54/NORIN10/BREVOT/SON64LIRA"S"
Soorab-96 (B)	1996	-
Tatara	1996	JUP/ALO"S"//KLT"S"/3/VEE"S" M79510-024Y-2M-05Y-01M -1Y-0B
ARZI-96	1996	MEN" SIB/MY48//4/14/3/ YAYLA 305
Punjab-96	1996	SA 42 *2/4CC/INIA//BB/3/ INIA/HD832 Pb1352-B-4K-36A-0A
Bahawalpur-97	1997	MTI „S" CM47634-1-2M -3Y-1M-2Y-1Y-1M-0Y
MH-97	1997	ATTILA = ND/VG9144/ KAL/ BB/3/ YACO/4/ VEE#5
Kohistan-97	1997	V-1562//CHRC`S'/HORK/ 3/KUFRA-I/ 4/CARP`S'/BJY`S' Pb. 24883-B-1A.OA.
Fakhar-e-Sarhad-97	1997	PFAU"S"/SERI/BOW "S" CM85295-010-TOPY-2M-0Y-0M-3Y-0M
Chakwal-97	1997	BUC'S' / FCT'S' CM84663-7M -0Y-0M-7Y-0M
Durum-97	1997	-
Darawar-97	1997	SASONO KOMUGI/NORIN// BOB"S" Pb18551-2B-1B-1B-2B-1B-0B
Daman-98	1998	BOW"S"/3/CAR853/ COC// VEE" S", CP02274-4C-0C-0Y-5M-ORES
Dera-98	1998	F12-71/COC//CNO79 CM76688-9Y-03M-02Y-2B-0Y
Ghaznavi	1998	-
Zarlashata	1999	Ures"s" / Bow CM78108-1M-02Y-02M-22Y-3B-0Y
Magalla-99	1999	OPATA/BOW'S' CM 83398-2M-0Y-0M -5Y-0M
Bahalwalpur-2000	2000	AU/UP301//GLL/ Sx/ 3/PEW „S"/4/MAI „S"//MAY A „S"//PEW"S" CM.67245-C-2M-OY
Marvi-2000	2000	CMH-77 A917/KPV-1600//RL-6010/ 6*SKA
Takbeer	2000	-
Iqbal-2000	2000	BURGUS/SORT 12-13//KAL/BB/3/PAK 81 PB 21912-11A-0A-0A-59A-0A -0A
Auqab-2000	2000	CROW'S'/NAC//BOW'S' PB 22138-3A-0A-0A-231A-0A
Amin-2000	2000	PASTOR/OPATA CM 110624-7M -020Y-010M-010SY-010M-0M -0Y
Saleem-2000	2000	CHAM-6//KITE/PGO ICW 93-0032-7F-0K-0F
Punjnad-1	2000	Pb85/NKT BR2194-12B-OB
Khatakwal	2000	-
Raj	2000	KAUZ* 2/TRAP//KAUZ CRG 742-6Y-010M-0Y
Chenab-2000	2000	CHUM18/BAU CM91045
Waqaf-01	2001	OPATA/RAYON//KAUZ CMBW 90Y3180-0TOPM-3Y-010M-010M-010Y-1M-015Y-0Y
SH-2002	2002	INQ91/FINK"S"
Bahkhar-2002	2002	P20102//PIMA/SKA/3/TTR 'S' /BOW PB-23826-D-1A-1A-1T-1T-0T
AS-2002	2002	KHP/D31708// CM74A370/3/ CIAN079/ 4/RL6043/*4NAC PBD 795-23A-1A-0A.
GA-2002	2002	DWL5023/SNB//SNB CM84986-H-1M -3M -2B-0M
Ufaq	2002	V.84133/ V83150
Manthar-2003	2003	KAUZ//ALTAR84/AOS CM 111633-6M -20Y-10M -10M-2Y-0M-0B.
KT-2000	2003	GEN#WHETON SWMI11508-LAP-1AP-1AP-4AP-1AP-5AP-0AP
Pirsabak-2004	2004	KAUZ/STAR
Bhittai	2004	VEE/TRAP#1//SOGHAT-90
Zam-04	2004	KAUZ* 2/OPATA//KAUZ CRG 732-11Y-010M-0Y
TD-1	2004	H-68XMAISXNORTENO
Pirsabak-2005	2005	MUNIA/SHTO//AMSEL

Table 2: Coefficient of infection scale for stripe rust severity assessment

Reaction	Observation	Response value
No Disease	O	0
Resistant	R	0.2
Resistant- Moderately Resistant	R-MR	0.3*
Moderately Resistant	MR	0.4
Moderately Resistant - Moderately Susceptible	MR-MS	0.6
Moderately Susceptible	MS	0.8
Moderately Susceptible - Susceptible	MS-S	0.9*
Susceptible	S	1

Table 3: Sequence of primers used in this study

Gene	Forward Primer 1 (Allele G)	Forward Primer 2 (Allele T)	Common Reverse Primer	Reference
Yr 46	5'-[FAM]-AGGTTCTGGCGATCGTGT-3'	5'-[HEX]-AGGTTCTGGCGATCGTGA-3'	5'-CGCAGCATCACGATCTGAC-3'	(Foessel *et al*. 2011)
Yr 17	5'-[FAM]-AGGTTCTGGCGATCGTGT-3'	5'-[HEX]-AGGTTCTGGCGATCGTGA-3'	5'-CGCAGCATCACGATCTGAC-3'	(Helguera *et al*. 2003)

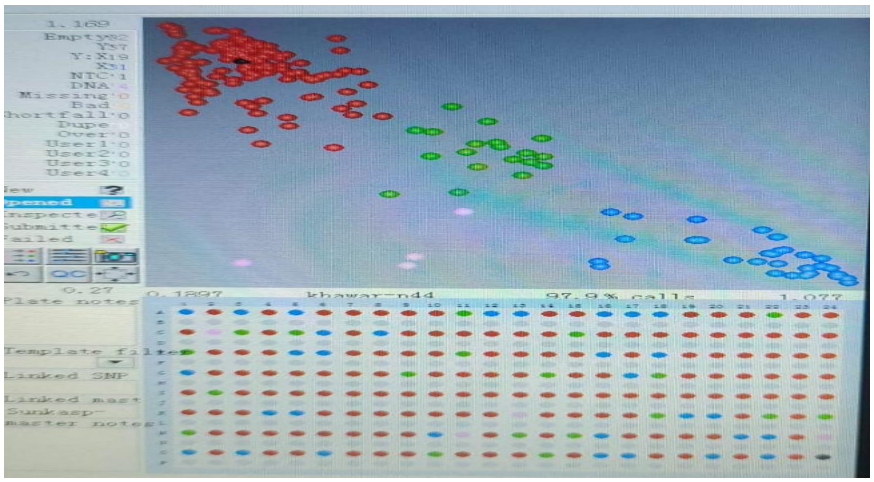

Fig. 1: KASP marker amplification at PCR cycle 49, illustrating genotype clustering

as described by (Ain *et al.* 2015). Briefly, leaf tissues were isolated, quickly frozen in liquid nitrogen and crushed into fine powder using a mortar and pestle. CTAB extraction was then performed on the powdered tissue, after which it was purified with chloroform alcohol (24:1). DNA was precipitated using isopropanol, washed with 70% ethanol and resuspended in TE buffer.

Kompetitive allele-specific PCR (KASP) genotyping of all the wheat varieties, specifically genes *Yr17* and *Yr46,* was performed to identify stripe rust resistance. For this purpose, the specific SNP markers *Yr17* and *Yr46* were used (Herrera-Foessel *et al*. 2011). To prepare the master mixture for each KASP reaction, the subsequent components were combined; 2.5 μL of 2X KASP master mixture (LGC Genomics, UK), 0.07 μL of KASP assay mixture (including SNP-specific primers), 2.5 μL of template DNA (50–100 ng μL^{-1}) and 0.07 μL of nuclease-free water. The total amount of the reaction mixture was 5.07 μL.

PCR amplification was performed *via* an Eppendorf Master Cycler pro 384 (Eppendorf, Germany) with the following thermocycling conditions: initial denaturation at 95°C for 15 min; touchdown cycles; for 20 sec, 10 cycles at 95°C were carried out and then annealing began at 65°C and was reduced by 1°C for 25 sec period. The amplification cycle was as follows: 30 cycles of 95°C for 10 sec and 57°C for 60 sec. The amplification was followed by reading the plates *via* the PHERASTAR plate reader to capture fluorescence signals, which are indicative of the presence of SNP markers. The primers used were allele-specific and were intended for the detection of SNPs related to resistance genes (Table 3). The PCR cycle 49 is an example of a selected marker. The top portion shows clustering and the bottom portion shows the sample type. The group of known resistant individuals in the testing panel separated well from the heterozygous and known susceptible individuals (Fig. 1).

Results

Field evaluation

A low coefficient of infection value of 9 in the wheat variety Dirk, 2 for blue silver and 9 for Marvi-2000 against stripe rust was indicative of strong resistance potential.

Table 4: Combined phenotypic and molecular marker data of the wheat varieties used in this study

Cultivars	Disease Response	CI	*Yr210*	*Yr17*	*Yr46*
T-9	0	0	6	-	+
T-11	80 - Susceptible	80	6	-	-
DIRK	30 - Resistant to moderately resistant	9	5	-	-
C-518	80 - Susceptible	80	6	-	-
C-591	80 - Susceptible	80	5	-	-
C-228	0	0	5	-	+
C-217	0	0	6	-	+
C-250	0	0	6	-	+
C-271	80 - Susceptible	80	5	-	+
C-273	0	0	5	-	+
MEXIPAK 65	80 - Susceptible	80	7	-	-
KHUSHAL-69	80 - Susceptible	80	6	-	-
CHENAB-70	80 - Susceptible	80	7	-	+
BARANI-70	80 - Moderately susceptible to susceptible	72	5	-	-
BLUE SILVER	10 - Resistant	2	6	-	+
SA-42	70 - Moderately Resistant to Moderately Susceptible	42	6	-	-
LYLPUR-73	80 - Moderately susceptible to susceptible	72	6	-	-
PARI-73	80 - Moderately susceptible to susceptible	72	4	-	-
PARULA-73	30 - Moderately susceptible to susceptible	27	2	-	-
YACORA	90 - Susceptible	90	4	-	-
LU-26	80 - Susceptible	80	5	-	-
PUNJAB-76	70 - Susceptible	70	4	-	-
WL-711	80 - Susceptible	80	6	-	-
JAUHAR-78	70 - Susceptible	70	3	-	-
ZARGHOON	90 - Susceptible	90	6	-	-
PAK 81	40 - Moderately susceptible to susceptible	36	7	-	-
PUNJAB-81	80 - Susceptible	80	7	-	-
SIND-81	90 - Susceptible	90	6	-	-
FAISALABAD-83	80 - Susceptible	80	7	-	-
KOHINOOR-83	80 - Susceptible	80	6	-	-
BARANI-83	70 - Moderately susceptible to susceptible	63	6	-	-
FAISALABAD-85	80 - Moderately susceptible to susceptible	72	5	-	-
SARSABZ	90 - Susceptible	90	6	-	-
TANDOJAM-83	90 - Susceptible	90	7	-	-
PIRSBAK-85	90 - Susceptible	90	5	-	-
PUNJAB-85	20 - Moderately susceptible to susceptible	18	5	-	-
WDANK-85	50 - Susceptible	50	5	-	-
CHAKWAL-86	20 - Moderately resistant to moderately susceptible	12	5	-	-
RAWAL-87	70 - Moderately susceptible to susceptible	63	6	-	-
KHYBER-87	80 - Susceptible	80	7	-	-
LOCAL WHITE	90 - Susceptible	90	6	-	-
SHALIMAR-88	80 - Moderately susceptible to susceptible	72	5	-	-
ZARDANA	90 - Susceptible	90	6	-	-
MEHRAN-89	80 - Moderately susceptible to susceptible	72	6	-	-
ROHTAS-90	40 - Moderately susceptible to susceptible	36	5	-	-
INQILAB-91	80 - Moderately susceptible to susceptible	72	5	-	-
PASBAN-90	80 - Susceptible	80	6	-	-
SOGHAT-90	80 - Susceptible	80	5	-	-
BAKHTAWAR-92	80 - Susceptible	80	6	-	-
KAGHAN-93	40 - Moderately resistant to moderately susceptible	24	5	-	-
ANMOL-91	70 - Susceptible	70	6	-	-
SHAHEEN-94	80 - Susceptible	70	6	-	-
PARWAZ-94	20 - Moderately susceptible to susceptible	18	1	-	-
SHAHKAR-95	70 - Moderately susceptible to susceptible	63	5	-	-
KOHSAR-95	80 - Susceptible	80	5	-	-
KIRIN-95	90 - Susceptible	90	7	-	-
NOWSHERA-96	90 - Susceptible	90	6	-	-
PUNJAB-96	70 - Moderately susceptible to susceptible	63	6	-	-
SULEMAN-96	60 - Moderately susceptible to susceptible	54	4	-	-
ABADGAR-93	90 - Moderately susceptible	72	6	+	-
SOORAB-96 (BARLEY)	0	0	1	+	-
TATARA	60 - Moderately susceptible to susceptible	54	2	+	+
AZRI-96	70 - Moderately susceptible to susceptible	63	5	-	-
PUNJAB-96	60 - Moderately susceptible to susceptible	54	4	-	-
BAHAWALPUR-97	20 - Moderately resistant to moderately susceptible	12	5	-	-
MH-97	90 - Susceptible	90	6	-	-

Table 4: Continued

Table 4: Continued

KOHISTAN-97	90 - Susceptible	90	6	-	-
FAKHR-E-SARHAD	40 - Moderately susceptible to susceptible	36	6	-	-
CHAKWAL-97	40 - Susceptible	40	6	-	-
DURUM-97	0	0	7	-	-
DARAWAR-97	60 - Moderately susceptible to susceptible	54	6	+	-
DAMAN-98	70 - Susceptible	70	5	-	-
DERA-98	70 - Moderately susceptible to susceptible	63	6	-	-
GHAZNAVI	30 - Moderately susceptible to susceptible	27	7	-	-
ZARLASHATA	80 - Moderately susceptible to susceptible	72	5	-	-
MAGALLA-99	0	0	6	-	-
BAHAWALPUR-2000	70 - Susceptible	70	6	-	-
MARVI-2000	10 - Moderately susceptible to susceptible	9	6	-	-
TAKBEER	20 - Moderately resistant to moderately susceptible	12	5	-	-
IQBAL-2000	70 - Susceptible	70	NA	+	-
AUQAB-2000	70 - Susceptible	70	6	-	-
AMIN-2000	80 - Susceptible	80	6	-	-
SALEEM-2000	70 - Susceptible	70	6	-	-
PUNJNAD-1	30 - Susceptible	30	7	-	-
KHATAKWAL	0	0	6	-	+
RAJ	60 - Moderately susceptible to susceptible	54	6	-	-
CHENAB-2000	70 - Susceptible	70	5	-	-
WAFAQ-01	60 - Susceptible	60	6	-	-
SH-2002	70 - Susceptible	70	6	-	-
BAHKHAR-2002	80 - Susceptible	80	6	-	-
AS-2002	70 - Susceptible	70	6	-	-
GA-2002	80 - Susceptible	80	2	-	-
UFAQ-2002	90 - Susceptible	90	5	-	-
MANTHAR-2003	60 - Moderately susceptible to susceptible	54	6	-	-
KT-2000	50 - Moderately susceptible to susceptible	45	5	-	-
PIRSBAK-2004	60 - Susceptible	60	6	-	-
BHITTAI	40 - Moderately resistant to moderately susceptible	24	6	-	-
ZAM-04	80 - Susceptible	80	5	-	-
TD-1	90 - Susceptible	90	6	-	-
PIRSABAK-2005	0	0	4	-	-

Table 5: Statistical analysis of field experiment data

ANOVA: Single Factor						
SUMMAY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Resistant	3	20	6.6666667	16.333333		
Nonresistant	87	5739	65.965517	459.96391		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10197.426	1	10197.426	22.66692	7.519E-06	3.949321
Within Groups	39589.563	88	449.8814			
Total	49786.989	89				

A wide range of CI values from 1–9 indicates that wheat varieties are resistant to stripe rust. These resistant lines included Dirk, Blue silver and Marvi-2000. These varieties presented minimum disease response, indicating that they were most resistant to stripe rust in wheat (Table 4).

There is strong evidence from the ANOVA results, which indicate that the resistance levels are differ between susceptible and resistant varieties. If an F-value exceeds the critical value of 3.95, there is more variance in the mean group scores than in the individual differences (Table 5). This implies that the dissimilarities observed in the infection index cannot be attributed solely to chance. A low P-value indicates that the chances of attaining such far-fetched outcomes are small; hence, the null hypothesis is rejected. Therefore, it can comfortably be argued that there was a meaningful difference in the average infection index between the resistant and nonresistant lines (Table 5).

Greenhouse evaluation

According to the seedling stage data, Parwaz-94, Parula-73, Tatara, GA-2002, Jauhar-78, Pari-73, Yacora, Punjab-76, Punjab-96, Suleman-96 and Pirsabak-2005 were the varieties most resistant to stripe rust according to the seedling stage data. Other analyzed varieties are given in (Table 6). The greenhouse screening at the seedling stage revealed notable differences in susceptibility to stripe rust among various bread wheat cultivars. The most resistant variety was Parwaz-94 without any signs of infection - this resistance level was termed ‘very resistant’.

Table 6: Patterns of host response variation among selected wheat genotypes to *Puccinia striiformis* f. sp. *tritici* at the seedling stage

Scale	Host response	Number of Cultivars	Cultivars
1	Very resistant	1	Parwaz-94
2	Resistant	3	Parula-73, Tatara, GA-2002
3	Resistant to moderately resistant	1	Jauhar-78
4	Moderately resistant	6	Pari-73, Yacora, Punjab-76, Punjab-96, Suleman-96, Pirsabak-2005
5	Moderately resistant to moderately susceptible	26	Dirk, C-591, C-228, C-271, C-273, Barani-70, Faisalabad-85, Pirsbak-85, Punjab-85, Wdank-85, Chakwal-86, Shalimar-88, Rohtas-90, Inqilab-91, Soghat-90, Kaghan-93, Shahkar-95, Kohsar-95, Azri-96, Punjab-96, Bahawalpur-97, Daman-98, Zarlashata, Chenab-2000, Ufaq-2002, KT-2000, Zam-04
6	Moderately susceptible	47	T-9, T-11, C-518, C-217, C-250, Khushal-69, Blue silver, SA-42, Lylpur-73, WL-711, Zarghoon, Sind-81, Kohinoor-83, Barani-83, Sarsabz, Rawal-87, Local white, Zardana, Mehran-89, Pasban-90, Bakhtawar-92, Anmol-91, Shaheen-94, Nowshera-96, Abadgar-93, MH-97, Kohistan-97, Fakhr-e-Sarhad, Chakwal-97, Darawar-97, Dera-98, Magalla-99, Bahawalpur-2000, Marvi-2000, Auqab-2000, Amin-2000, Saleem-2000, Khatakwal, Raj, Wafaq-01, SH-2002, Bahkhar-2002, AS-2002, Manthar-2003, Pirsbak-2004, Bhittai, TD-1
7	Moderately suceptible	10	Mexipak 65, Chenab-70, Pak 81, Punjab-81, Faisalabad-83, Tandojam-83, Khyber-87, Durum-97, Ghaznavi, Punjnad-1
8	Susceptible		
9	Very Susceptible		
NA		1	Iqbal-2000

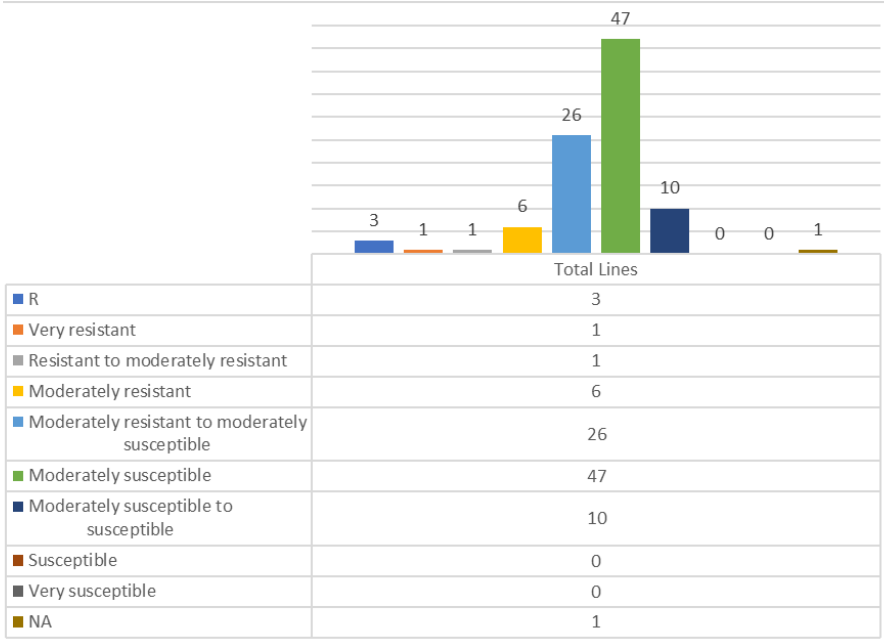

Fig. 2: Distribution of wheat varieties on the basis of symptom expression at the seedling stage

Others that fall under this category include Parula-73, which has yellow lesions all over its leaves, Tatara, which has necrotic spots; and while GA-2002, which has small necrotic flecks on some parts of the blade. Several lines such as Pari-73, Yacora, Punjab-76, Punjab-96, Suleman-96 and Pirsabak-2005, exhibited less sporulation, which makes them moderately resistant. Fig. 2 illustrates the total number of wheat varieties by their symptom expression at the seedling stage.

Molecular marker screening

Kompetitive allele-specific PCR (KASP) markers revealed distinct patterns of *Yr17* and *Yr46* gene presence among the wheat genotypes tested. A subset of 9 varieties- C-228, C-217, C-250, C-271, C-273, Chenab-70, Blue Silver, Tatara and Khatakwal, were found to contain both *Yr17* and *Yr46* markers. This indicates the potential for dual resistance (Table 7). These Abadgar-93, Soorab-96, Darawar-9 and Iqbal-2000 varieties contained the *Yr17* marker but lacked the *Yr46* marker, indicating that resistance associated with *Yr17* only. The T-9 variety presented a *Yr46* marker but lacked a of *Yr17* marker, indicating that resistance was associated with *Yr46* only.

Discussion

We identified many susceptible wheat genotypes, thereby emphasizing the urgent need for resistant varieties

Table 7: Molecular marker-assisted wheat varieties carrying yr17 and yr46 to stripe rust

Markers	Number of cultivars	Names of cultivars
Yr 17+ and *Yr 46*+	9	C-228, C-217, C-250, C-271, C-273, Chenab-70, Blue Silver, Tatara and Khatakwal.
Yr17+ and *Yr46*-	4	Abadgar-93, Soorab-96, Darawar-9 and Iqbal-2000
Yr17- and *Yr46*+	1	T-9
Yr17- and *Yr46*-	84	T-11, Dirk, C-518, C-591, Mexipak 65, Khushal-69, SA-42, Lylpur-73, Pari-73, Parula-73, Yacora, LU-26, Punjab-76, WL-711, Jauhar-78, Zarghoon, Pak-81, Punjab-81, Sindh-81, Faisalabad-83, Kohinoor-83, Barani-83, Faisalabad-85, Sarsabz, Tandojam-83, Pirsbak-85, Punjab-85, Wadank-85, Chakwal-86, Rawal-87, Khyber-87, Sutluj-86,Shalimar-88, Zardana, Mehran-89, Rohtas-90, Inqilab-91, Pasban-90, Soghat-90, Bakhtawar-92, Kaghan-93, Anmol-91, Shaheen-94, Parwaz-94, Shahkar-95, Kohsar-95, Kirin-95, Nowshera-96, Punjab-96, Suleman-96, ARZI-96, Bahawalpur-97, MH-97, Kohistan-97, Fakhar-e-Sarhad-97, Chakwal-97, Durum-97, Daman-98, Dera-98, Ghaznavi, Zarlashata, Magalla-99, Bahalwalpur-2000, Marvi-2000, Takbeer, Auqab-2000, Amin-2000, Saleem-2000, Punjnad-1, Raj, Chenab-2000, Waqaf-01, SH-2002, Bahkhar-2002, AS-2002, GA-2002, Ufaq, Manthar-2003, KT-2000, Pirsabak-2004, Bhittai, Zam-04 TD-1 and Pirsabak-2005

against the newly evolving pathotypes of *Puccinia striiformis* f. sp. *tritici*. Many wheat varieties had high CI values, suggesting a greater risk of susceptibility. The CI values for Mexipak 65 and Yacora were 80 and 90, respectively, clearly indicating that these strains were highly susceptible and exhibited limited resistance. This information highlights the resistance of wheat varieties to stripe rust and highlights the need to choose and cultivate resistant varieties as a tactic to reduce the consequences of the disease. According to recent studies, genotypes lack effective resistance genes against current and future *Pst* races. Furthermore, the high CI values obtained could be a sign of favorable environmental factors for disease expression throughout the experiment. The degree of stripe rust infection may be significantly affected by changes in the temperature, humidity and other environmental factors. According to the latest research, the high CI values for these controls serve as crucial benchmark, validating the experimental setup and enabling a reliable comparison of the resistance levels of the examined wheat varieties (Afzal *et al*. 2022).

According to the ANOVA results, the P-value was less than the significance threshold of 0.05 and the F-value was greater than the critical value (3.95). These results indicate a significant difference between the average infection indices of both the resistant and non-resistant varieties. This shows that- the change in the CI is likely due to the difference in the varietal types and not to random choice. Therefore, resistance appeared in different varieties. This finding also emphasized that the variation in CI recorded was mainly due to genetic divergence among wheat cultivars with some random experimental error. This confirms that genetic factors chiefly cause varying levels of resistance to stripe rust. The identification of significant differences between resistant and susceptible varieties provides a solid basis for marker-assisted selection (MAS) in breeding programs. By identifying and utilizing molecular markers with resistance genes, breeders can select resistant progenies in an easier and faster manner, expediting the development of improved cultivars (Soriano and Royo 2015). Statistical validation of resistance differences will also allow more precise discrimination of the tested wheat lines so that recommendations can be made for their deployment across various agricultural areas according to their resistance profiles.

Some varieties, such as Dirk and C-591, have moderate sporulation and are thus considered moderately resistant to moderately susceptible. However, there was a large set of varieties that displayed moderate sporulation, including T-9 and T-11 and hence fell into the category of moderately susceptible varieties. Varieties such as Mexipak 65, Chenab-70 and others were observed to have relatively high levels of sporulation and were classified as moderately susceptible to susceptibility. Because some varieties are relatively prone to yield losses, their increased susceptibility requires prompt care. A high sporulation rate suggests either insufficiently efficient resistance genes or genes that are rendered ineffective owing to the emergence of novel *Pst* pathotypes. These cultivars are particularly vulnerable to stripe rust outbreaks, which can significantly impact both yield and quality (Chen 2005). Identifying such varieties highlights the critical importance of cultivar selection for reducing disease incidence and maintaining yield stability. The genetic basis of the differential susceptibility observed in this study requires further investigation, especially *via* genome-wide association studies (GWASs) and analyses of gene expression, to discover resistance mechanisms and to inform future breeding strategies.

The most concerning category was extreme sporulation, indicating a high to very high susceptibility to infection. At the seedling growth level, the rating indicates the importance of choosing resistant genotypes to enhance crop health and guarantee yield consistency. Some strains exhibit excessive sporulation, which suggests that they lack effective resistance genes, making them highly vulnerable to stripe rust infection. This observation highlights the lack of effective resistance gene expression in these varieties, which explains their extreme vulnerability to infection, probably even at the seedling stage. This extreme susceptibility, in turn, poses a substantial threat to wheat production, increasing yield and economic losses for farmers. Owing to the rapid evolution of *Pst* into new and more virulent races, the pressure on resistance breeding is increasing (Johnson *et al*. 2023). To improve crop health, stabilize productivity and safeguard food security in areas with stripe rust, it is crucial to breed resistant cultivars. Molecular screening revealed variations in the presence of *Yr17* and *Yr46* resistance genes

among the tested wheat genotypes, with a significant proportion devoid of these key markers. For example, C-228, C-217, C-250 and Blue silver were genotyped with *Yr17* and *Yr46* markers thought to confer perhaps a high level of resistance because they work together. Abadgar-93 and Soorab-96 contained a single *Yr17* marker, suggesting that the resistance control gene was located mainly at this locus, whereas T-9 only contained *Yr46,* indicating another genetic pool of resistance. There were 9 varieties with both markers, 4 with *Yr 17* and without *Yr 46* and 84 without both markers. In particular, *Yr17* has been widely employed, although the emergence of virulent pathotypes has reduced its efficacy (Ali *et al*. 2020). In contrast, *Yr46* is a slow-term resistance gene that provides partial resistance and when combined with *Yr17*, may increase resistance levels (Suenaga *et al*. 2003). The presence of both genes in these genotypes suggests a potentially effective resistance mechanism for breeding programs aimed at producing cultivars with long-lasting resistance to stripe rust. The high number of genotypes with no *Yr17* or *Yr46* highlights the susceptibility of many of the germplasms to stripe rust. This discovery highlights the need to include diverse sources of resistance in breeding to expand the genetic basis of resistance. The lack of known resistance genes calls for the search for new resistance mechanisms, including quantitative trait loci (QTLs) and emerging resistance genes (Singh *et al*. 2016). Furthermore, genomic selection techniques are being rapidly used to improve the breeding of stripe rust-resistant wheat varieties by focusing on multiple resistance loci (Klymiuk *et al*. 2019). The integration of molecular-assisted breeding methods will be key to the development high-yielding wheat cultivars that are resistant to various diseases, including emerging stripe rust. In addition, the roles of gene stacking and complementary resistance mechanisms should form a part of breeding programs for long-lasting resistance. Studies have indicated that such durability and broad-spectrum resistance against stripe rust results from the integration of slow-term genes with major resistance genes (Khan *et al*. 2022). The effectiveness of *Yr17* and *Yr46* in different genetic backgrounds and varying agro-climatic conditions should be evaluated in future studies to determine their stability and adaptability. Sustainable management of stripe rust could be gained from broader resistance gene diversity achieved through wild wheat relatives and novel resistance locus combinations.

Conclusion

This research provides a thorough assessment of the resistance of wheat genotypes to stripe rust *via* field tests, seedling tests and molecular marker technology. Integration of these approaches resulted in detection of highly resistant varieties such as Dirk, Blue Silver and Marvi-2000, while molecular screening validated probable dual resistance in some genotypes. These findings are essential for breeding programs to develop resistant cultivars to guarantee stable wheat production and food security in stripe rust-susceptible areas. The integration of traditional and molecular approaches provides a promising pathway for improving wheat resilience and adaptability to changing environmental challenges.

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Author Contributions

AH carried out research, including planning and wrote the manuscript. SM provided guidance as supervisor and also helped in formatting of manuscript. MF facilitated the research work and provided valuable expertise. MJ helped in carrying out the field experiments.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

The data presented in this study will be available upon a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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