



RESEARCH ARTICLE

Assessment and genetic characterisation of stripe rust resistance at seedling and adult plant stages in bread wheat germplasm

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Abstract

Stripe rust or yellow rust caused by *Puccinia striiformis* f. sp. *tritici* of wheat is a major biotic stress with rapid epidemics. Host-plant resistance is one of major breeding strategies in development of resistant cultivars, wheat germplasm showing great genetic diversity for resistance was screened for identifying potential resources for major and minor resistance genes. Forty-seven germplasms of advanced breeding materials were selected and seedling reaction test was conducted on germplasm to know potential race specific resistance against *Pst* stripe rust races. Relative susceptibility index assessed based on seedling infection type and their numerical response values. Adult plant screening under natural epiphytotic conditions recorded different quantitative disease parameter values viz., area under the disease progress curve (AUDPC), average coefficient of infection (ACI) and relative resistance index (RRI) which helps in categorising germplasm into durable non-specific ones. The phenotypic assessment of germplasm which was categorised as resistant germplasm, was validated through simple sequence repeat (SSR) markers for *Yr* race specific genes such as *Yr5*, *Yr10* and *Yr15* using different SSR markers viz., Xgmw501, Brac349, Xpsp300, Xgmw273 and Gms413. The gene *Yr5* was postulated in 14 germplasm lines, *Yr15* in 14 germplasm lines and *Yr10* in 11 germplasm lines. Four germplasm postulated with *Yr5+Yr15*, six germplasm postulated with *Yr15+Yr10* and *Yr5+Yr10* postulated in three germplasm and two germplasm showed immune response with other race specific resistance.

Keywords: phenotypic assessment; resistance; stripe rust; seedling; wheat

Introduction

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is a major biotic stress in wheat-growing regions worldwide and can cause up to 70 % yield loss (1). Broad adaptability of newly evolving virulent *Pst* races under shifting environmental conditions are considered as a vital factor of concern for stripe rust in wheat (2). The pathogen affects photosynthesis at any leaf stage until maturity; the infection continues on healthy plant leaf tissues under favourable conditions (3). Genetic diversity in wheat germplasm has been successfully exploited for management of stripe rust (4). Host plant resistance is being encouraged as most widely acceptable method in management of wheat rust due to emergence of new pathotypes poses a serious threat of stripe rust. Different stripe rust genes have been catalogued and temporarily designated as *Yr* genes under different quantitative trait loci (QTLs) (5). Raising threat of new pathotypes challenging the resistance in cultivars due to rapid susceptibility and slows down breeding process through conventional techniques (6).

Host-plant resistance is broadly categorised by two ways: one is major seedling resistance or race specific resistance and another is minor durable resistance or non-specific resistance; the former is broken down easily and responsible for the severe epidemics (7). Rust breeding programmes were initiated globally under the umbrella team of the Borlaug Global rust initiative identified various race specific resistance genes under different possible combinations for effective management of rust disease (8). *R*-genes encodes cytoplasmic proteins that recognise the pathogenic effector proteins (9), these proteins trigger immune response against pathogen leads to effector triggered immunity (ETI) associated with hypersensitive response leads to disruption of cellular components with subcellular changes (10). Race specific resistance exploited in breeding programmes of wheat and best explained with the deployment of *Yr2*, *Yr9* and *Yr27* (11–13). The susceptibility of these race specific genes were documented in varieties PBW343 and HD2967 (14). Many cultivars under cultivation are susceptible to prevalent pathotypes in present day situations, as cultivars shares common ancestral progeny genes which are

responsible for serious outbreaks of many rust epidemics in India (15). A new source of genetic resources was identified for exploitation where most of *Pst* races observed avirulent against *Yr5*, *Yr10* and *Yr15*. These are successfully deployed for the development of many resistant cultivars (16). Till date 86 (*Yr1*–*Yr86*) *Yr* stripe rust resistance genes have been designated, nearly 363 QTLs reported so far in wheat against stripe rust (17–19).

Different *R*-genes responsible for three different rusts 19 genes viz., *Sr60*, *Yr15/YrG303/YrH52*, *Lr1*, *Lr10*, *Lr21*, *Lr22a*, *Sr13*, *Sr21*, *Sr22*, *Sr33*, *Sr35*, *Sr45*, *Sr46*, *SrTA1662*, *Sr50*, *Yr5/YrSP*, *Yr7*, *Yr10* and *YrAS2388R*, are race specific genes, out of these 4 stripe rust resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr17* very effective and shows highly resistant to all prevalent stripe rust pathotypes in India (20). Additionally, the tagged markers for these genes are available and can be used to identify the presence or absence of these genes in the germplasm (<http://wheat.pw.usda.gov/>, accessed on 29 July 2023). *Yr5* a race specific gene for stripe rust was first identified in hexaploid wheat *Triticum aestivum* ssp. *spelta* var *album* (TSA), which is located on the 2 longarm chromosome which is 21 cM from the centromere (21). Wheat PI 178383 and Moro germplasm shows characteristic brown glume colour due to presence of *Yr10* on 1BS and 2 cM away from the *Rg1* locus, were very effective against many stripe rust pathotypes in countries like China, India, Pakistan, Iran, United States and Kazakhstan (22, 23). *Triticum dicoccoides* accession G-25 harboured the race specific resistance genes *Yr15*, at chromosome 1BS with wide-ranging resistance against 3000 genetically distinct *Pst* isolates globally (24). The present research investigation was conducted for screening of elite germplasm of wheat and validated through SSR markers for *Yr5*, *Yr10* and *Yr15* which are avirulent to *Pst* races showing good field response.

Materials and Methods

Planting materials

Fifty germplasm of bread wheat advance breeding material was obtained from the All India Coordinated Research Project (AICRP) - wheat and barley through the Division of Plant Breeding and Genetics, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST-Jammu) for seedling and field evaluation.

Pathotypes

Predominant pathotypes of the stripe rust had been procured from, Regional Research Station Flower Dale, Shimla, for testing the germplasm by inoculating under greenhouse conditions and open field evaluation, pathotypes of stripe rust races namely 46S119, 110S119, 238S119, 110S84 and 78S84 respectively, through the Division of Plant Pathology, Faculty of Agriculture, SKUAST-Jammu. Up to 2011, a total of 28 pathotypes had been identified in India (25). Besides these, five new pathotypes of stripe rust had been identified and confirmed in 2014. These have been designated as 46S117, 110S119, 238S119, 110S247 and 110S84. These pathotypes were more virulent than pre-existing pathotypes and appeared to have

mutation in existing pathotypes on Suwon x Omar and Riebesel 47/51. Virulence pattern of Indian stripe rust pathotypes used in the study are presented in Table 1 (26).

Seedling resistance

Four to five seeds of each genotype were sown separately for each pathotype of stripe rust. The seedlings were inoculated with individual *Pst* races using the standard inoculation procedure described in (27). Seedling infection was recorded 14–16 days post-inoculation on the first and second leaves independently for each pathotype. The qualitative infection type (IT) scores were then converted into a numerical scale for subsequent statistical and quantitative analysis. The seedling infection type of reaction was converted to continuous numerical data to calculate the susceptibility index to 0–9 scale in which 0-immune and 9-highly susceptible in reaction, the IT converted as follows 0, ;n, ;, 1-, 1, 1+, 2-, 2, 2+, 2++, 3-, 3 3+, 3++ and 4 were coded as 0, 0.5, 1, 2.5, 3, 3.5, 4, 5, 6, 6.5, 7, 8, 8.5, 9 (28). The susceptibility index was calculated from the converted data and further analysed statistically and the graphical representation are illustrated in Fig. 1 and 2 (29).

Field evaluation

Adult plant screening of germplasm conducted of the plant pathology under randomised complete block design (RCBD). Susceptible checks (Agra local, PBW343 and HD2967) were sown around the tested germplasm for even spread of the disease. The artificial epiphytotic were created by inoculating the spore mixture of *Pst* (46S119, 110S119, 238S119, 110S84 and 78S84) pathotypes on the susceptible germplasm. Quantitative disease parameters of the stripe rust such as AUDPC and coefficient of infection (CI) were calculated (12). The statistical analysis was performed for all the quantitative disease parameters; analysis of variance (ANOVA) was carried out to know the performance of different screened germplasm statistically significant over susceptible checks using *R*-statistical packages.

Molecular validation of resistance germplasm

DNA extraction

Screened germplasm, which showed the resistance germplasm was selected for the DNA extraction and the seeds of the selected germplasm were sown in growth chambers at 3–4 seeds per genotype and 100 mg of the leaf samples were at two leaf stages and crushed along with liquid nitrogen (N) and the DNA extraction protocol was followed using modified cetyltrimethylammonium bromide (CTAB) buffer method.

Polymerase chain reaction and electrophoresis

Thermal amplification of DNA was amplified in thermocycle master gradient polymerase chain reaction (PCR) system (Eppendorf, Germany). The PCR mixture of 100 ng of DNA template with reaction mixture volume contains 2 µL DNA sample, 6.25 µL of promega templates, 0.75 µL of selected primers and 10.75 µL of nuclease free water. Primer markers of each *Yr* gene linked SSR marker were synthesised by IDT Technologies Pvt Ltd. The PCR conditions initial denaturation step at 94 °C for 4 min, followed by

Table 1. Virulence pattern of Indian *Pst* pathotypes used in the study

S.No.	Pathotypes	European equivalent	Virulence spectrum
1	46S119	46E159	2, 3, 4, 6, 7, 8, 9, 11, 17, 18, 19, 21, 22, 23, 25, A, Avs, SD.
2	110S119	110E159	2, 3, 4, 6, 7, 8, 9, 11, 14, 17, 18, 19, 21, 22, 23, 25, 27, A, Avs, SD, SU.
3	238S119	238E159	2, 3, 4, 6, 7, 8, 9, 11, 14, 17, 18, 19, 21, 22, 23, 25, 27, A, Avs, SD, SU, Riebesel 47/51, HS507
4	110S84	110E22	2, 3, 6, 7, 8, 9, 17, 18, 19, 21, 22, 23, 25, 27, SD, SU
5	78S84	78E16	2, 3, 6, 7, 8, 9, 19, 21, 22, 23, 25, 27, SU
6	47S103	47E148	1, 2, 3, 4, 6, 7, 8, 11, 17, 18, 19, 22, 23, 25, A, Avs, SD

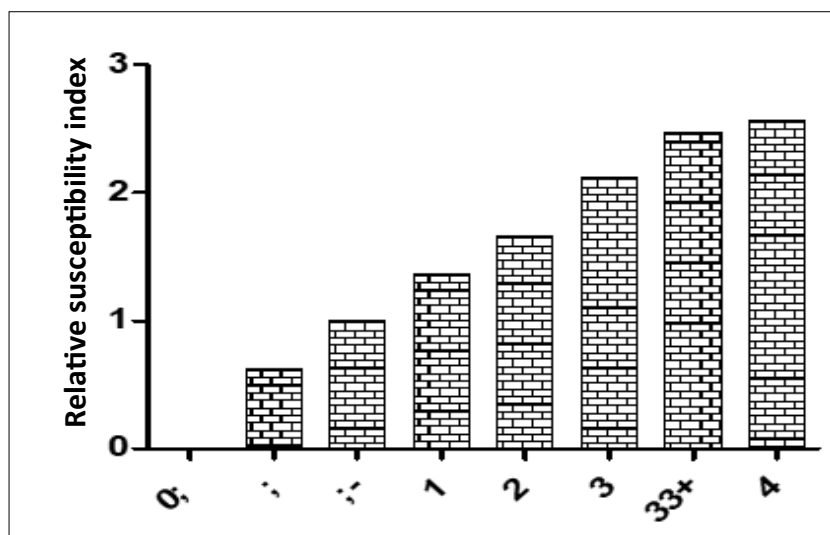


Fig. 1. Relative susceptible index.

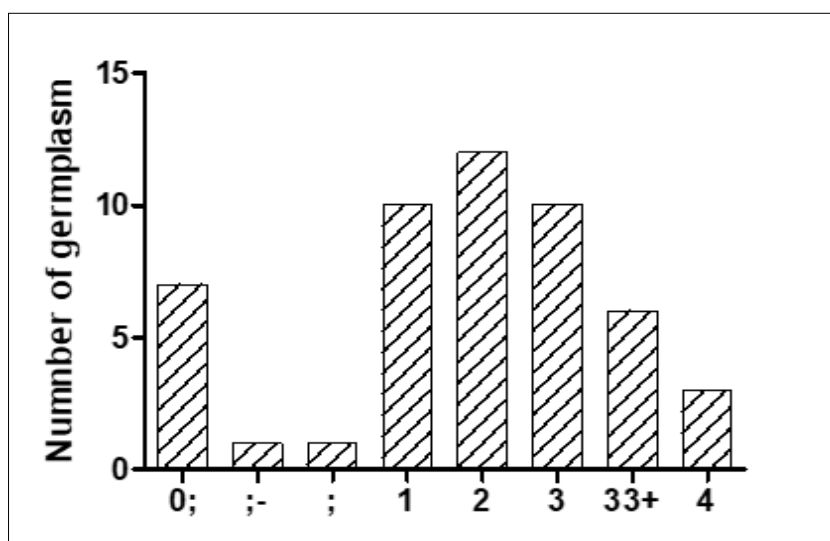


Fig. 2. Seedling response of wheat germplasm.

30–35 cycles (1 min 94 °C, 30 sec for 50–60 °C (based on primer annealing temperature), 1 min at 72 °C and final extension step for 10 min at 72 °C at 4 °C holding step for 24 hr. Master mix (PCR master mix is a ready-to-use 2× solution containing Taq DNA polymerase, Deoxynucleoside triphosphates (dNTPs), magnesium chloride (MgCl₂) and reaction buffers at optimal concentrations. The SSR marker employed in PCR of *Yr* gene specific SSR markers is given in Table 2.

Quantitative disease scoring

Quantitative disease scoring of the stripe rust parameters were calculated for characterisation of resistance patterns in wheat germplasm. Among the different disease scoring parameters, the modified Cobb's scale was used. Adult plant field observations were recorded as per modified Cobb's scale (32). Disease scores and their corresponding infection types at adult plants stage were followed based on disease scorings (33).

Relative resistance index

Germplasm adult plant disease scores were converted to relative resistance index (RRI) values between 0–9 scale, in which 0 represents susceptible and 9 represents highly resistant for stripe rust. Relative resistance index values 6 or 7 are considered as the acceptable levels and the values 6–7 are considered as the slow rusting lines and the RRI values were accepted as per previous studies (34).

$$RRI = \frac{100 - CARPA}{100} \times 9$$

Area under disease progress curve

Area under disease progress curve (AUDPC) is considered as an important parameter in the categorisation of the germplasm based on the AUDPC values. The values indicate that within the range of 0 as immune and 1–100, 100–200 and 200–400 are good sources of high, moderate and low levels of the slow rusting components (35).

$$A - \text{values} = \sum_{i=1}^k 0.5 \times (X_i + X_{i-1})D$$

where, X_i = the severity of rust at the end of time i , X_{i-1} = the severity of rust time $i-1$, k = number of rust evaluations, D = days of intervals between two observations.

Statistical analysis

The data of screened germplasm were subjected to statistical analysis through SPSS software to test the significance among the quantitative epidemiology of the screened germplasm. Field layout was followed by RCBD compared ANOVA values at standard statistical $p < 0.05$ for significant.

Table 2. SSR markers employed for amplification of different race specific linked genes

S. No	Yr gene	SSR marker	Forward primer	Reverse primer	Annealing temperature	Reference
1.	Yr5	Xgwm501	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTTCATCAGTCA	55	30
		Barc349	CGAATAGCCGCTGCACAAG	TATGCATGCCTTTCTTTACAAT	59	
2.	Yr10	Xpsp3000	GCAGACCTGTGTCATTGGTC	GATATAGTGGCAGCAGGATACG	60	5, 31
3.	Yr15	Xgwm273	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGAT C	60	30
		WMS413	TGCTTGCTCTAGATTGCTTGGG	GATCGTCTCGTCTTGGCA	57	30

Results

Seedling reaction test

Virulence and complexity of the *Pst* races to a particular gene are important and the seedling reaction at the seedling stage was considered as important to the effectiveness of the wheat cultivars for *Pst* races, the present research investigation was carried out with predominant pathotypes in Northwest plain zones (46S119, 110S119, 238S119, 110S84 and 78S84). The relative susceptibility index of tested germplasm and the range of virulence patterns were helpful in seedling resistance as a response to a combination of isolates was further inferred based on infection type scores (Table 3). This might be of great interest to breeders, as it maximises the number of virulences which a cultivar might face under natural conditions. Based on relative susceptibility (RS) values, 16 out of 50 germplasm lines exhibited resistance responses with RS index values based on IT scores from 0–1.5.

Adult plant screening

The ANOVA suggested a significant impact of block and treatments (years) on the study at p -value < 0.001 (Tables 4 and 5). Response to stripe rust infection substantially varied across the genotypes due to the significant effect of genetic factors. The tested genotypes were also found to differ significantly from the tested check lines.

Table 3. Categorisation of germplasm based on average coefficient of infection

Category	ACI
0	15, 39
1–10	1, 3, 5, 8, 9, 10, 11, 16, 17, 18, 19, 25, 26, 27, 30, 31, 32, 34, 35, 36, 37, 38, 41, 45
10–20	2, 12, 13, 21, 22, 28, 44, 46
20–40	4, 6, 7, 14, 23, 29, 40, 42, 43
> 40	20, 24, 33, 47, 48, 49, 50

Average coefficient of infection

Among 50 germplasm screened based on average coefficient of infection (ACI) values, there are 26 germplasm lines which showed the ACI values in between 0–10. Germplasm exhibited reactions ranging from 0 (immune) to MR-MS at the adult plant stage. Average coefficient of infection values 10–40 showed in 16 germplasm showed MR-MS to MS, 8 germplasm showed ACI values greater than 40 showed MS-S and highest ACI values was observed in one germplasm in Agra local and PBW 343 with susceptible (S) reaction (Fig. 3, Tables 6 and 7). Tested germplasms were compared as per critical difference at 5 % between two test treatments and control treatments (Table 8).

Area under disease progress curve

Area under disease progress curve values were calculated based on the disease severity values for two successive cropping seasons 2022–23 and 2023–24. Thirteen germplasm were recorded with AUDPC values 1–100, nine germplasm recorded 100–200, eight germplasm with an A-values 200–400, A-values 400–800 recorded in eleven germplasm and A-values greater than 800 recorded in 11

germplasm respectively. A-values up to 100 are considered the most important source of the major gene resistance and this germplasm also recorded the coefficient of infection value 0–10 represented in Table 7.

Table 4. Seedling response of the screened germplasm at seedling stage of bread wheat

S. No	Germplasm	Seedling Reaction	RS
1.	WH1	1	1.6
2.	WH2	33+	2.4
3.	WH3	3	2.0
4.	WH4	2	1.9
5.	WH5	0;	0.0
6.	WH6	2	1.9
7.	WH7	3	2.1
8.	WH8	1	1.4
9.	WH9	;	0.9
10.	WH10	0;	0.0
11.	WH11	1	1.4
12.	WH12	3	2.1
13.	WH13	2	1.7
14.	WH14	33+	2.4
15.	WH15	1	1.4
16.	WH16	0;	0.0
17.	WH17	2	1.9
18.	WH18	;-	1.0
19.	WH19	1	0.9
20.	WH20	3	2.2
21.	WH21	2	1.9
22.	WH22	2	1.9
23.	WH23	3	2.1
24.	WH24	3	2.1
25.	WH25	1	1.9
26.	WH26	0;	0.0
27.	WH27	0;	0.0
28.	WH28	33+	2.4
29.	WH29	3	2.3
30.	WH30	1	1.3
31.	WH31	2	1.8
32.	WH32	1	1.6
33.	WH33	4	2.4
34.	WH34	1	1.7
35.	WH35	;-	0.0
36.	WH36	2	1.9
37.	WH37	3	2.3
38.	WH38	2	2.0
39.	WH39	0;	0.0
40.	WH40	4	2.7
41.	WH41	2	2.3
42.	WH42	33+	2.4
43.	WH43	2	1.6
44.	WH44	2	1.4
45.	WH45	1	1.9
46.	WH46	3	2.3
47.	WH47	3	2.2
48.	AL	33+	2.4
49.	PBW343	4	2.6
50.	HD2967	33+	2.8

0;: immune (naught fleck), nearly immune (fleck minus), highly resistant (fleck); 1 (one): highly resistant; 2 (two): moderately resistant; 3 (three): moderately susceptible; 33+ (three three plus), 3+/4 (three plus/ four): highly susceptible.

Table 5. Adult plant disease scoring parameters of stripe rust during 2022–24 rabi season

S. No	Germplasm	Adult Plant screening response		FRS		ACI	RRI	AUDPC		Mean
		2022–23	2023–24	2022–23	2023–24			2021–22	2023–24	
1.	WH1	RMR	MRMS	3	9	6	8.3	54	202.5	128.25
2.	WH2	MRMS	MRMS	18	18	18	7.0	427.5	450	438.75
3.	WH3	RMR	MR	6	8	7	8.2	166.5	180	173.25
4.	WH4	MS	S	32	30	31	5.5	690	780	735
5.	WH5	TR	RMR	0	3	1.5	8.8	0	45	22.5
6.	WH6	MRMS	MS	18	24	21	6.6	390	506.25	448.12
7.	WH7	MS	S	16	30	23	6.4	630	675	652.5
8.	WH8	RMR	MR	3	8	5.5	8.4	99	142.5	120.75
9.	WH9	RMR	R	6	2	4	8.6	172.5	30	101.25
10.	WH10	R	RMR	2	3	2.5	8.7	45	112.5	78.75
11.	WH11	R	RMR	1	3	2	8.8	7.5	45	26.25
12.	WH12	MR	MS	8	24	16	7.2	240	667.5	453.75
13.	WH13	MSS	S	18	20	19	6.9	615	562.5	588.75
14.	WH14	S	MRMS	40	18	29	5.7	1200	540	870
15.	WH15	TR	0	0	0	0	0	0	0	0
16.	WH16	RMR	MRMS	6	12	9	8.0	202.5	420	311.25
17.	WH17	MRMS	MRMS	12	6	9	8.0	270	150	210
18.	WH18	R	TR	2	0	1	8.9	39	0	19.5
19.	WH19	RMR	5R	3	1	2	8.8	90	7.5	48.75
20.	WH20	MSS	S	54	30	42	4.3	1635	765	1200
21.	WH21	MRMS	MS	18	16	17	7.1	300	457.5	378.75
22.	WH22	RMR	S	3	20	11.5	7.7	60	412.5	236.25
23.	WH23	MSS	S	18	40	29	5.7	525	1237.5	881.25
24.	WH24	MS	S	80	70	75	0.6	990	1420.5	1205.2
25.	WH25	RMR	RMR	1.5	3	2.25	8.7	36.8	57	46.9
26.	WH26	RMR	RMR	6	6	6	8.3	180	195	187.5
27.	WH27	RMR	MR	6	4	5	8.4	180	82.5	131.25
28.	WH28	MR	MSS	12	18	15	7.3	285	540	412.5
29.	WH29	MS	S	24	40	32	5.4	750	1215	982.5
30.	WH30	RMR	MS	6	12	9	8.0	202.5	450	326.25
31.	WH31	RMR	MRMS	6	12	9	8.0	195	255	225
32.	WH32	RMR	RMR	9	6	7.5	8.2	189	166.5	177.75
33.	WH33	60S	30S	60	30	45	3.9	1837.5	750	1293.7
34.	WH34	0	RMR	0	3	1.5	8.8	0	60	30
35.	WH35	R	R	2	1	1.5	8.8	30	7.5	18.75
36.	WH36	RMR	RMR	3	1.5	2.25	8.7	67.5	11.25	39.375
37.	WH37	MR	RMR	12	3	7.5	8.2	300	45	172.5
38.	WH38	R	R	0.6	2	1.3	8.9	6	30	18.00
39.	WH39	0	0	0	0	0	9.0	0	0	0
40.	WH40	S	S	40	60	50	3.4	1312.5	1537.5	1425
41.	WH41	R	RMR	2	6	4	8.6	39	112.5	75.75
42.	WH42	MS	MRMS	32	12	22	6.5	990	337.5	663.75
43.	WH43	MS	S	16	40	28	5.9	480	727.5	603.75
44.	WH44	MR	MRMS	8	18	13	7.5	229.5	420	324.75
45.	WH45	MR	RMR	8	3	5.5	8.4	259.5	90	174.75
46.	WH46	MRMS	MRMS	18	12	15	7.3	382.5	270	326.25
47.	WH47	S	S	60	30	45	3.9	2175	750	1462.5
48.	AL	S	S	60	80	70	2.3	1987.5	2550	2268.75
49.	PBW343	S	S	80	70	75	0.0	2580	2400	2490.5
50.	HD2967	S	S	70	70	70	1.1	1987.5	1912.5	1950

MR: moderately resistant; MRMS: moderately resistant to moderately susceptible; MS: moderately susceptible; MSS: moderately susceptible to susceptible; R: resistant; RMR: resistant to moderately resistant; S: susceptible.

Table 6. Adult plant resistance response of screened germplasm and postulated -race specific gene through SSR markers

S. No	Germplasm	ACI	Yr5	Yr10	Yr15	Postulated genes
1	WH1	6	+	-	+	Yr5+Yr15+
2	WH2	18	-	-	-	-
3	WH3	7			+	Yr15+
4	WH4	31	-	-	-	-
5	WH5	1.5			+	Yr15+
6	WH6	21	-	-	-	-
7	WH7	23	-	-	-	-
8	WH8	5.5	+	+	-	Yr5+Yr10+
9	WH9	4	+	-	+	Yr5+Yr15+
10	WH10	2.5	-	+	+	Yr10+Yr15+
11	WH11	2	+	+	-	Yr5+Yr10+
12	WH12	16	-	-	-	-
13	WH13	19	-	-	-	-
14	WH14	29	-	-	-	-
15	WH15	0	+	+	-	Yr5+Yr10+

16	WH16	9	-	-	-	-
17	WH17	9	-	-	-	-
18	WH18	1	+	+	+	Yr5+Yr10+Yr15+
19	WH19	2	+	+	-	Yr5+Yr10+
20	WH20	42	-	-	-	-
21	WH21	17	-	-	-	-
22	WH22	11.5	-	-	-	-
23	WH23	29	-	-	-	-
24	WH24	75	-	-	-	-
25	WH25	2.25	+	+	+	Yr5+Yr10+Yr15+
26	WH26	6	-	+	+	Yr10+Yr15+
27	WH27	5	-	-	-	-
28	WH28	15	-	-	-	-
29	WH29	32	-	-	-	-
30	WH30	9	-	-	-	-
31	WH31	9	-	-	+	Yr15+
32	WH32	7.5	-	-	+	Yr15+
33	WH33	45	-	-	-	-
34	WH34	1.5	+	-	-	Yr5+
35	WH35	1.5	-	-	+	Yr15+
36	WH36	2.25	-	+	+	Yr10+Yr15+
37	WH37	7.5	+	+	-	Yr5+Yr10+
38	WH38	1.3	-	+	+	Yr10+Yr15+
39	WH39	0	-	-	-	-
40	WH40	50	-	-	-	-
41	WH41	4	+	-	-	Yr5+
42	WH42	22	-	-	-	-
43	WH43	28	-	-	-	-
44	WH44	13	-	-	-	-
45	WH45	5.5	+	-	-	Yr5+
46	WH46	15	-	-	-	-
47	WH47	45	-	-	-	-
48	AL	70	-	-	-	-
49	PBW343	75	-	-	-	-
50	HD2967	70	-	-	-	-

Table 7. Mean sum of squares of disease intensity and area under the disease progress curve for the growing seasons 2021–22 and 2023–24

Source	D. f.	Mean. Sq.			
		ACI		AUDPC	
Block (ignoring treatments)	3	2130.60	**	2547445.84	**
Treatment (eliminating blocks)	49	734.32	*	437100.77	ns
Treatment: Check	2	108.33	ns	59855.25	ns
Treatment: Test and test vs. check	47	760.96	*	453153.77	ns
Residuals	6	119.44		192711.92	

ns $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$.

Table 8. Critical difference at 5 % level of significance comparison type

Comparison	ACI
A test treatment and a control treatment	34.52
Control treatment means	18.91
Two test treatments (Different blocks)	43.67
Two test treatments (Same block)	37.82

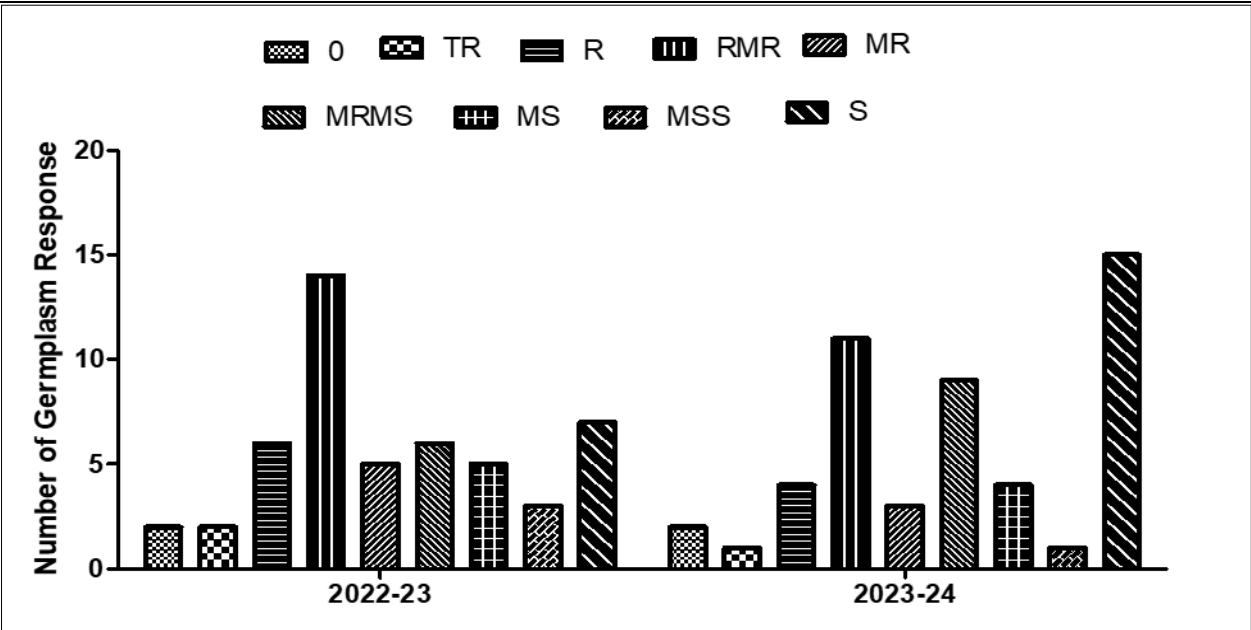


Fig. 3. Adult plant disease response of screened germplasm from 2022–24.

Relative resistance index

Relative resistance index of germplasm was determined to know the resistance pattern at adult plant screening. Relative resistance index values 0 represents the most susceptible one and RRI values for stripe rust greater than 6 or 7 are desirable for stripe rust resistance (Table 1). The RRI value 9 recorded in only one germplasm respectively. Eleven germplasm recorded RRI values 6–8 and 25 germplasm, recorded; RRI value more than 8–9 and finally 13 germplasm recorded the RRI values less than 6 indicate that these are not potential sources of the resistance. The germplasm RRI values are based on final disease severity of germplasm, based on the AUDPC, CI and RRI molecular validation of resistant germplasm with SSR markers was carried out to unlock the potential source of the genetic background with known *Yr* gene linked SSR markers adult plant response of different quantitative parameters was detailed in the Table 7.

Molecular validation of *Yr* resistance genes

Three *Yr* resistance genes linked SSR markers represented in Table 1 are deployed among the resistant germplasm to know their presence or absence, which are presently under the avirulence nature for the predominate *Pst* pathotypes (46S119, 110S119, 238S119, 110S84 and 78S84), while the avirulence and virulence pattern are given in Table 2.

Marker based analysis

In the current study 50 germplasm lines were screened for two successive cropping seasons, the final rust severity (FRS) values based on two cropping seasons categorised and selected for molecular validation with help of SSR markers to know the presence or absence of resistant gene combinations in single or multiple represented in Table 8 and graphical representation of gene combinations in Fig. 4.

Molecular validation of *Yr5*

Molecular validation of *Yr5*, a gene specific SSR marker Xgmw501 amplified a positive band of 175 bp (Fig. 5). Among the screened germplasm, the germplasm such as WH11, WH15, WH18, WH31, WH34 and WH35 germplasm were amplified at 175 bp among 25 resistant germplasm with frequency of 24 %. Another marker Barc349 amplified in WH1, WH8, WH 9, WH18, WH19, WH25, WH34, WH37, WH41 and WH45, with frequency of 40 among resistant germplasm at 105 bp are represented in Fig. 6.

Molecular validation of *Yr10*

Simple sequence repeats marker Xpsp3000 marker which was closely linked to *Yr10* amplified at 260 bp as 220 bp, 260 bp, 270 bp, 280 bp. Resistance germplasm like WH8, WH10, WH11, WH15, WH18, WH19, WH25, WH26, WH36, WH 37 and WH38 of wheat are amplified at 260 bp with a frequency of 43.47 % represented in Fig. 7.

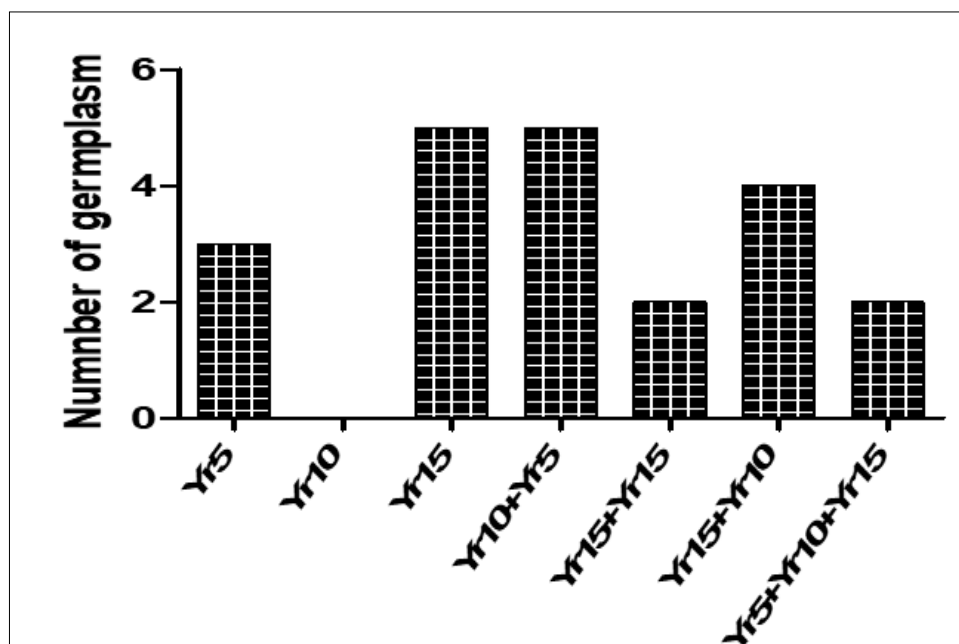


Fig. 4. Postulated gene combinations.

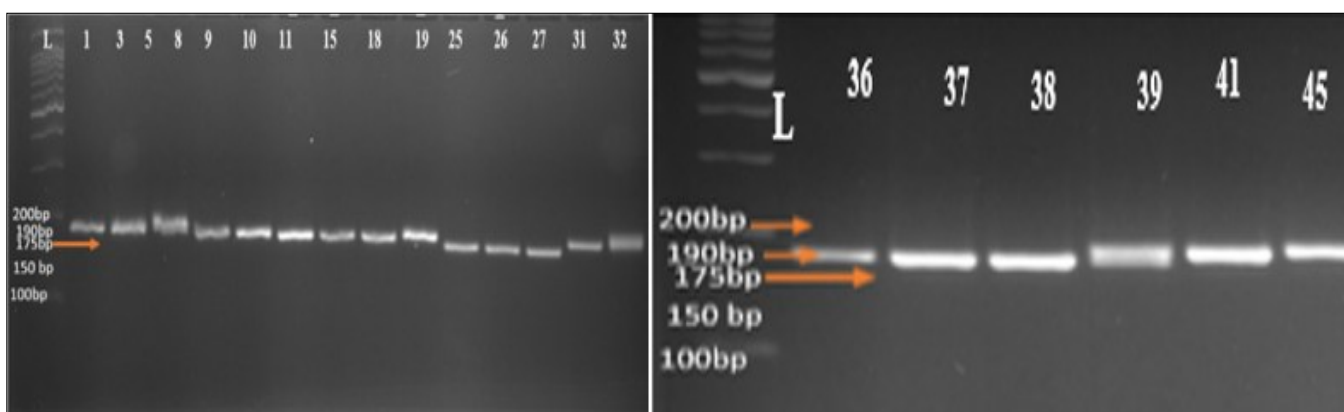


Fig. 5. Depicting presence of *Yr5*-Xgmw501 SSR marker amplified at 175 bp in the screened germplasm.

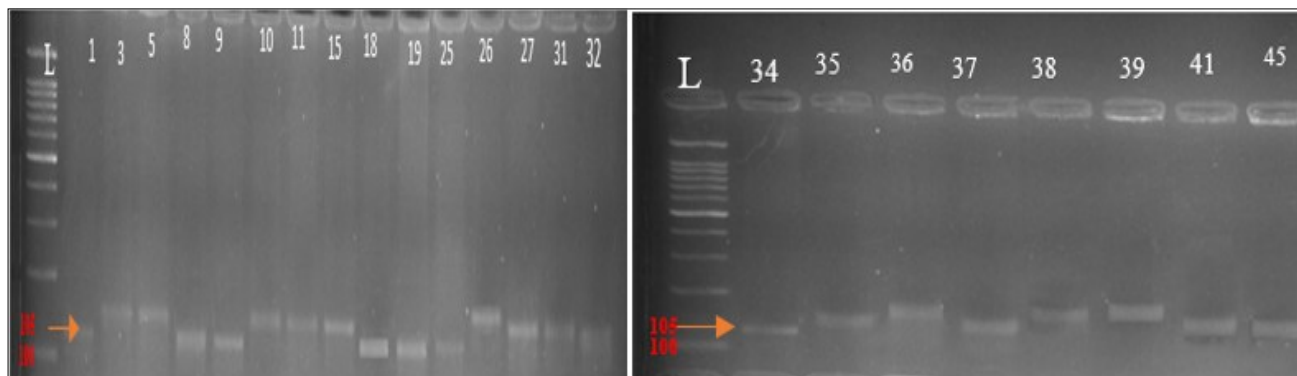


Fig. 6. Depicting presence of Yr5- Xbrac 349 SSR marker amplified at 105 bp in the screened germplasm.

Molecular validation of Yr15

Molecular validation of *Yr15* with the help of two SSR markers Xgwm273 and Xgwm273 was amplified at 150 bp, 170 bp and 200 bp (Fig. 7). The lines which are amplified at 150 bp are closely linked to *Yr15* gene, amplified among the following germplasm viz., WH1, WH5, WH9, WH10, WH18, WH26, WH31, WH35 and WH37 with a frequency of 39.13 and GMS413 from grain genes as represented in Fig. 8. GMS413 SSR marker amplified at 96 bp and 100 bp SSR marker *GMS413* showed the presence of *Yr15* at 96 bp in WH1, WH3, WH5, WH9, WH10, WH25, WH26, WH31, WH32, WH36 and WH38 with frequency of 47.82 % represented in Fig. 9.

Discussion

Stripe rust is a major biotic stress responsible for significant yield losses due to its aggressive and widespread nature. In India the menace of stripe rust is a serious threat particularly in North India where it covers an area of more than 10 million hectares (31). Extensive use of breeding techniques and field experiment under natural and field epiphytotic conditions identify potential sources of resistance in wheat (30). Race specific resistance or major resistance

deployed in the development of narrow genetic base cultivars confer resistance to few or particular races of the pathogens. Because of narrow genetic base, the host-pathogen recognition is based on pathogen derived molecule patterns and which is overcome by the new evolving races (36). Quantitative resistance of stripe rust based on numerical disease scoring rates of screened germplasm helpful in identifying of promising source of genetic material. To ascertain resistance, the germplasm was categorised based on the AUDPC, RRI and FRS values at adult plant stage (37).

Relative susceptibility index of the screened germplasm at the seedling stage reveals the rate susceptibility of germplasm to mixture of pathotypes. Forty-seven germplasm along with three susceptible checks Agra local, PBW343 were accessed for comparing of resistance patterns in test lines under natural epiphytotic conditions. Twenty-three germplasm are resistant among 47 screened germplasm. Based on IT, resistance diversity was observed among screened germplasm, FRS values from 0–80 for different ITs. Twenty-three germplasm are resistant with FRS 0–10 with AUDPC values from 19–174.5, relative resistance index values were observed above 8.5–9 as major race specific, RRI values above 6–7 are good potential source for adult plant resistance or

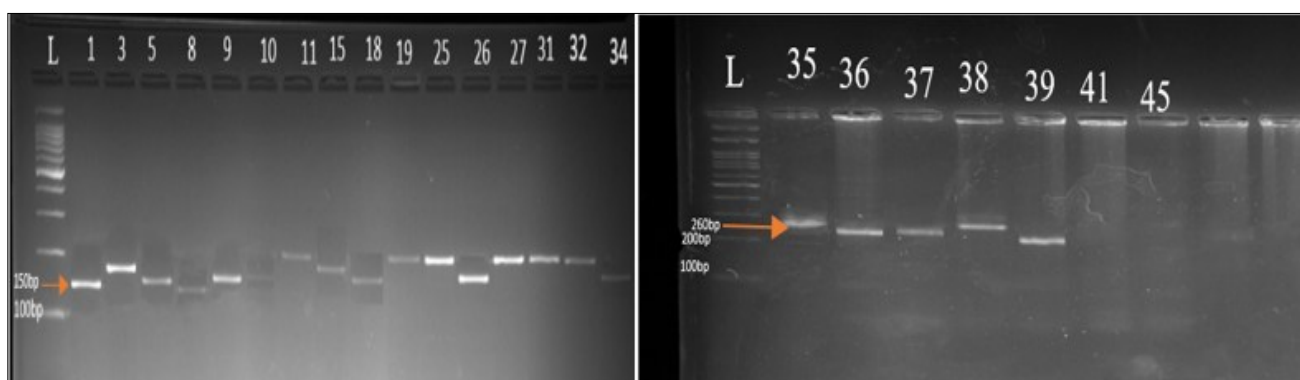


Fig. 7. Depicting presence of Yr10- Xpsp3000 SSR marker amplified at 260 bp in the screened germplasm.

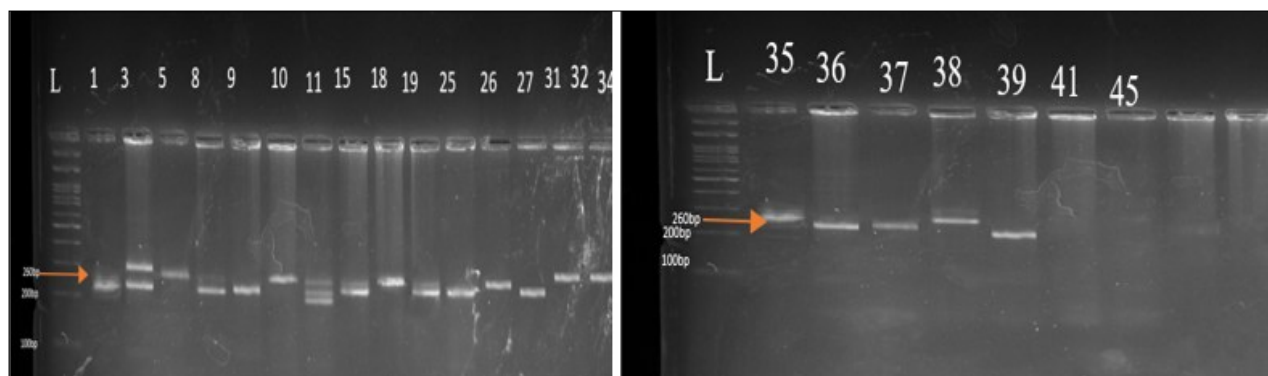


Fig. 8. Depicting presence of Yr15- Xgwm501 SSR marker amplified at 150 bp in the screened germplasm.

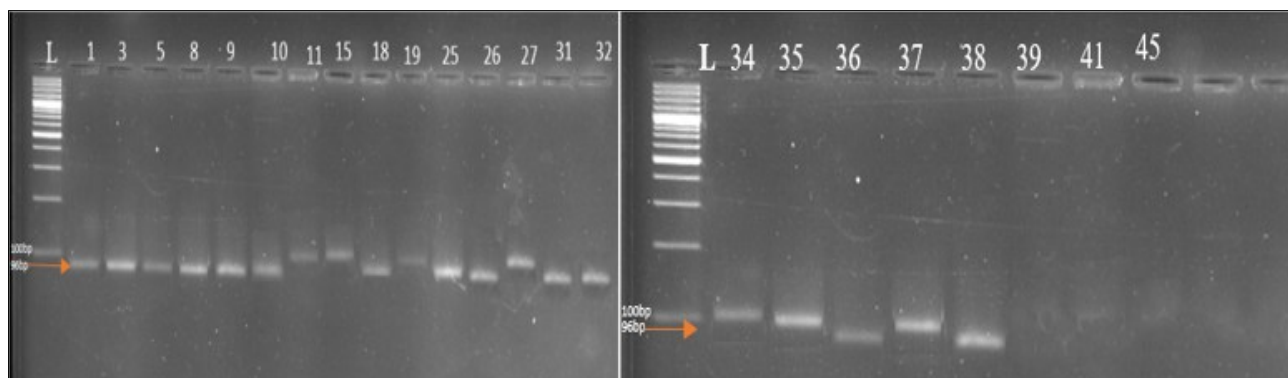


Fig. 9. Depicting presence of *Yr15*-GMS413 SSR marker amplified at 105 bp in the screened germplasm.

horizontal resistance and germplasm which shows an RRI value of 0 have highest susceptibility. Final rust severity values at adult plant stage categorised the germplasm with 0 infection as immune with strong race specific, FRS values 1–20 % contain both race specific and adult plant resistance, values within the range of 20–40 % moderate levels of slow rusting genes and values with 40–60 % low levels of slow rusting genes and > 60 % refers high susceptible (38). Thirteen germplasm were recorded AUDPC values 1–100, nine germplasm recorded 100–200, eight germplasm with A-values 200–400, A-values 400–800 recorded in eleven germplasm and A-values greater than 800 recorded in 11 germplasm respectively. Area under disease progress curve is considered an important parameter in the categorisation of the germplasm based on the AUDPC values. The values indicate that within the range of 0 as immune and 1–100, 100–200 and 200–400 are good source of high, moderate and low levels of the slow rusting components (30). Relative resistance index values of the different germplasm based on 0–9 where 0 values recorded as the highly susceptible recorded in Agra local and RRI in other susceptible checks such as PBW343 recorded RRI values as 1–2 represents as susceptible ones. Nine represents the more resistant and lines in the range of 8.5–9 are more resistant and RRI values in between 6 and 8 are slow rusting. The RRI values above 6–7 and 7–8 which indicates strong source of slow rust with Adult plant resistance genes (APR) genes (39).

Characterisation of germplasm resistance in response to different rust pathotypes determined the level of host susceptibility and resistance pattern for germplasm. Seedling reaction revealed that out of 47 germplasm, 23 tested germplasm lines of susceptibility index 0–2 with IT of 0-RMR, which means they have highly resistant and high levels of vertical resistance and tested lines shows the relative susceptibility index from 2.1–2.8 with MR-S. The susceptibility index of the tested germplasm helps in revealing the nature of resistance pattern at seedling stage and help in the development of the resistance varieties during the breeding programmes.

Field-based screening and molecular validation of resistant germplasm with SSR markers are helpful in confirming the race specific resistance based on the quantitative disease parameters, *Yr5* with SSR linked markers Xgwm501 amplified a positive band of 175 bp, Xbrac349 another SSR linked marker amplified at 105 bp (40). The lines with *Yr5* observed FRS with ACI values during 2022–23, A-values observed under different postulated genes of wheat resistant germplasm with ACI values 1–10, the AUDPC values in germplasm postulated with *Yr5* from 26.5–174.5. Gene *Yr15* was validated with two SSR markers such as GMS413 and Xgwm 273 amplified at 96 bp and 15 bp, the resistant germplasm lines with

AUDPC values from 45–180. Xpsp3000 linked SSR marker amplified at 260 bp in resistant germplasm with 0-RMR IT (41, 42). The combination of the *Yr5*, *Yr15* and *Yr10* under different combinations but two germplasm where the three postulated genes were showed ACI and AUDPC values 0. The postulated gene combinations reveals that the field population of *Pst* pathotypes also developed resistance races against *Yr5*, *Yr10* and *Yr15* except in two germplasm where resistance was governed by other genes than *Yr5*, *Yr10* and *Yr15*, these types of quantitative disease parameters based molecular validation was similar to the previous results of bread wheat molecular characterisation (43).

Conclusion

Durability is an important breeding strategy for effectiveness of rust resistance in bread wheat against stripe rust deployed through plant breeding principles. Diversity characterisation of wheat germplasm for stripe rust is typically more important in breeding methods through rapid seeding and field-based assessment. In this investigation, wheat lines conferring a high level of race specific resistance, partial and adult plant resistance were characterised and described their genetic ability for disease progress. Molecular validation of resistance lines was validated through gene linked SSR markers through for *Yr5*, *Yr10* and *Yr15* for identification of narrow genetic base, identification of adult plant resistance germplasm further needs to be deployed for development of major race specific and durable resistance. Application marker assisted breeding which helps in minimising the duration of breeding period and also with complete documentation of developing cultivars.

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Authors' contributions

LVR and BR performed the experimental work. AKM and VJ wrote the manuscript. MS conducted the statistical analysis. LVR and HP reviewed and revised the manuscript draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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