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Research Article

Screening of bread wheat genotypes for identification of novel stripe rust resistance genes using molecular markers

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Abstract
Stripe Rust, caused by *Puccinia striiformis* Westend f. sp. *tritici* , is an important fungal disease of wheat worldwide. In this study, 41 wheat genotypes from various institutes across India were screened for adult plant resistance (APR) in field trials, and the presence of effective stripe rust resistance genes was evaluated in the laboratory using ten molecular markers.The bread wheat variety Agra local was used as a susceptible control. Molecular characterization studies revealed that the gene *Yr 2* was found in 29 entries, *Yr5* in 15 entries, *Yr9* in 7 entries, *Yr10* in 18 entries, *Yr15* in 18 entries, *Yr17* in 12 entries, *Yr18* in 29 entries, *Yr36* in 21 entries, *Yr40* in 31 entries while *Yr65* was absent in all the entries. The genotypes PBW780 and High Rainfall Wheat Screening Nursery (HRWSN) line 2105 had seven *Yr* genes conferring resistant phenotypes, suggesting that these were excellent sources of effectual stripe rust resistance. The coefficient of infection showed adequate genotypic variations for rust severity, 41 genotypes showed year-specific responses, suggesting strong G × E interactions.

Keywords: Bread wheat; Stripe rust; SSR; STS; APR.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is an allohexaploid (2n = 6x = 42, AABBDD) that evolved in South-Western Asia and spread globally for cultivation and consumption. Wheat is one of the main staple food of man and is grown in almost all the temperate and subtropical regions of the world (Rajesh *et al.,* 2013).To meet the growing demand for food, there is a need to improve wheat yields and enhance the genetic potential of this crop. (Sheera *et al.,* 2024). Its planting extends about 220 million hectares' worldover, making it a crop of immense importance (Morris *et al.,* 2015). In India, it is grown in an area of 31.76 million ha (ICAR-IIWBR 2021) with production of 314.51 million tons. Majority of areas under wheat cultivation encounter biotic and abiotic stresses, as well as soil nutrient deficiency, that results in low grain quality, ultimately reducing output (Grote *et al.,* 2021). Grain yield in wheat is a polygenic trait that is effected by different biotic and abiotic factors and can be refined by indirect selection based on yield attributes (Sandhu *et al.,* 2022). One of the various biotic factors that minimize productivity in wheat drastically is rusts (stripe, leaf and stem rust), (Beukert *et al.,* 2020). Among the three rust diseases *viz*, yellow, brown and black rust; yellow rust is a fierce disease of considerable significant in greatest proportion of the world where wheat is grown (Khan *et al.,* 2012; Singh *et al.,* 2014). The most successful strategy is breeding for resistance, because it doesn't upload enter value of farmers and is ecologically of low-risk (Yang and Liu, 2004). So far, about 83 stripe rust resistance genes have been reported with official and provisional designations in wheat (McIntosh 2022). The Northern Western Plains Zone of India which is considered the breadbasket of the country usually experiences a favourable climate for the genesis and spread of the wheat stripe rust

pathogen cycle and therefore poses extreme threat to the production goal of wheat. Genetic resistance is most effectual method for regulating the control of this disease. In order to produce resistant cultivars, resistance genes should be first identified in diverse germplasm and then transferred to suitable genetic background**.** This study is crucial for identifying yellow rust-resistant genes in wheat breeding lines and evaluating them in the field for adult plant resistance (APR).

MATERIALS AND METHODS

The present study was carried out in the research field of the Division of Plant Breeding and Genetics, Faculty of Agriculture, Sher-e-Kashmir University of Agriculture Sciences and Technology during the *Rabi* 2017- 2019 growing seasons. The experimental material comprised of 41 germplasm lines obtained from diverse eco-geographic regions available in the Division of Plant Breeding & Genetics, National Bureau of Plant Genetic Resources (NBPGR), International Maize and Wheat Improvement Center-International Bread Wheat Screening Nursery (CIMMYT- IBWSN), International Center for Agricultural Research in the Dry Areas (ICARDA- DBSYT) and germplasm repository from Indian Institute of Wheat and Barley Research (IIWBR), Karnal. A detailed listing of the genotypes and their corresponding serial numbers used in the gel plates is provided in **table 3**. Susceptible controls included Agra local, PBW 343, and Kharchia. The coordinates for the experimental site are 32°40N, 78°48E, and 336 meters above sea level.The field evaluation followed a randomized complete block design (RCBD) with recommended agronomic practices Artificial rust epidemics were induced through repeated spray inoculations using soap water solutions containing a mixture of races (46S119, 110S119 and 78S84).

Stripe rust severity on wheat plants was measured using the modified Cobb's scale (Peterson *et al.,* 1948) and categorized based on the field response scale (McIntosh and Arts, 1995) . To compute the coefficient of infection (CI), the severity of the disease and the host's reaction were combined. This involved multiplying severity values of 0.2, 0.4, 0.8, and 1.0 for host response ratings R, MR, MS, and S, respectively, as outlined by Pathan and Park (2006). Disease severity scoring data were gathered from five randomly chosen plants.The genotypes were screened for the presence of nine known *Yr* genes using SSR and STS markers. Agra local, susceptible to rust, served as the control.

Molecular Markers: The presence or absence of functional *Yr* genes in wheat lines was detected using closely linked (typically flanking) markers . This study used a total of 10 markers (7 SSR, 1 STS, 1 CAPS & 1 gene-based marker), as stated in **table 1**. Genomic DNA from the genotypes was extracted using 7-8 cm of young, actively growing fresh leaves, following the method proposed by Doyle, (1990). A gradient master cycler was used to carry out the PCR amplification reaction. The PCR cycle comprised of 5-minute denaturation stage, followed by a loop of 40 cycles of denaturation (at 94 ^{0}C for 1 minute), annealing (at 45-60 ^{0}C for 1 minute), & extension (at 72 ^{0}C for 2 minutes). The final extension lasted for 10 minutes at 72 ^{0}C. The PCR products were then kept at 40 ^{0}C

Table 1. List showing Gene, name of Marker, Marker type, sequence of Primer, Annealing (°C) and base pair size

Gene	Marker name	Marker type	Primer sequence	Annealing (℃)	+ (bp)\ -(bp)	References
Yr2	*Wmc364*	SSR	5'ATCACAATGCTGGCCCTAAAAC3′ 5′ CAGTGCCAAAATGTCGAAAGTC 3′	51	+ 207	(Feng *et al*., 2005)
Yr5	*STS7/8*	STS	5′GTACAATTCACCTAGAGT3′ 5′ GCAAGTTTTCTCCCTATT 3′	45	+308\-181	(Chen *et al.,* 2003
Yr 9	H20	SSR	5′ GTTGGAAGGGAGCTCGAGCTG 3′ 5′ GTTGGGCAGAAAGGTCGACATC 3′	56	+ 1598	(Liu *et al.,* 2008)
Yr10	*Xpsp3000*	SSR	5′GCAGACCTGTGTCATTGGTC3′ 5′ GATATAGTGGCAGCAGGATACG 3′	55	+260\-240	(Wang *et al.,* 2002)
Yr15	*Xbarc8*	SSR	5′GCGGGAATCATGCATAGGAAAACAGAA3′ 5′GCGGGGGCGAAACATACACATAAAAACA 3′	60	+250\-280	(Murphy *et al.,* 2009)
Yr17	*URIC/LN2*	CAPS	5′GGTCGCCCTGGCTTGCACCT3′ 5′ TGCAGCTACAGCAGTATGTACACA AAA 3′	64	+285\-275	(Jia *et al*., 2011)
Yr18	*Cssfr1*	Gene based	5′TTGATGAAACCAGTTTTTTTTCTA3′ 5′ GCCATTTAACATAATCATGATGGA 3′	58	+517	(Lagudah *et al.,* 2009)
Yr36	*Barc101*	SSR	5′GCTCCTCTCACGATCACGCAAAG3′ 5′GCGAGTCGATCACACTATGAGCCAATG 3′	52	+125	(Uuay *et al.,* 2005)
Yr 40	*Xgwm 382*	SSR	5′ GTCAGATAACGCCGTCCAAT 3′ 5′ CTACGTGCACCACCATTTTG 3′	60	+120	(Kuraparthy *et al.,* 2007)
Yr 65	Gwm18	SSR	5'TGGCGCCATGATTGCATTATCTTC3' 5'GGTTGCTGAAGAACCTTATTTAGG3'	53	+182/-188	(Cheng *et al.,* 2014)

Data Analysis: The location-specific and combined ANOVA for CI values of natural infection in wheat genotypes, along with the check variety (Agra Local), was performed using the PROC GLM procedure in the SAS (SAS Institute, 1988) statistical software package. The marker data was analysed using PAST 3 Software (Hammer, 2001). Molecular data thus obtained was used to identify wheat lines showing presence or absence of effective rust resistance genes.

RESULTS AND DISCUSSION

Genotypes with resistant phenotypes were identified using the two-year Average Coefficient of Infection, resulting in the selection of 15 promising genotypes for yellow rust resistance. The ANOVA (**Table 2**) revealed significant differences among genotypes across both years (2018 and 2019). The coefficient of infection demonstrated substantial genotypic variation in rust severity, and the 41 genotypes showed year-specific responses, indicating strong G × E interactions. This study identified genotypes with multiple Yr genes, which could serve as parent lines to enhance the genetic diversity of wheat breeding for resistance against *Puccinia striiformis* f. sp. tritici.

Molecular validation using WMC364 showed that 29 wheat lines carried the *Yr2* gene, while the remaining lines lacked this gene. Resistant lines displayed a 207 bp fragment, whereas susceptible lines showed a 201 bp fragment on a 3% agarose gel (Plate A). This finding aligns with the work of Feng *et al.* (2005), who reported the linkage of the WMC364 marker to *Yr2* at 5.6 cM, producing similar amplicon sizes. The STS7/8 primers amplified a 478 bp band in lines with *Yr5* and a 472 bp band in lines without *Yr5*. Digestion of STS7/8 amplification products with Dpn II produced distinct restriction fragments, visualized on 3% agarose gel (Plate B), revealing 15 wheat germplasm lines with the Yr5 gene. This gene shows high resistance levels to stripe rust in China (Chen, 2005) and Turkey (Zeybek and Yigit, 2004). Surveys in the Caucasian region, middle Asia (Ziyaev *et al.,* 2011), and Pakistan (Bux *et al.,* 2012) found Yr5 and Yr15 effective against all *Puccinia striiformis* f. sp. tritici races. In the present study the genotypes containing this gene showed resistance reaction.

Primer pair H20 F/R detected *Yr9* by amplifying a 1598 bp fragment (Plate C) in seven entries. Marker Xpsp3000, specific for *Yr10*, produced a 260 bp fragment in resistant lines and a 240 bp fragment in susceptible genotypes, including Agra local. Sixteen lines, including JAUW 649 and JAUW 667, had *Yr10* in heterozygous condition (Plate D). Validation of *Yr15* using SSR marker Xbarc8 showed a 250 bp fragment in 18 lines, with remaining lines lacking this gene (Plate E). A section of Triticum ventricosum chromosome 2NS, translocated to wheat chromosome 2A, contains rust resistance genes *Yr17*, *Lr37*, and Sr38 (Helguera *et al.,* 2003). Primers URIC and LN2 amplified 285 bp (N-allele) and 275 bp (A-allele) fragments. Restriction digestion with Dpn II separated these bands, with 285 bp undigested matching the N genome and 166 bp and 109 bp fragments matching the A genome. This primer validated *Yr17* in 12 wheat entries (Plate G). *Yr36*, co-segregating with markers Xucw 69 and Xbarc 101 on chromosome 6BS (Uauy *et al.,* 2005), was tracked using Xbarc101, producing a 125 bp fragment in 21 resistant genotypes (Plate H). *Yr40* on chromosome 5DS, derived from Ae. geniculata, was traced with Xgwm382, producing a 120 bp fragment in 31 resistant genotypes (Plate I).

The dendrogram based on Unweighted Paired Group Mathematical Average (UPGMA), revealed that the genotypes are clustered into two major groups based on the presence or absence of *Yr* genes. Cluster I contained 20 genotypes, further divided into sub-clusters, while the second main cluster had 21 genotypes, also divided into sub-clusters. Jaccard's similarity coefficients grouped

Table 2. Analysis of infection coefficient values for terminal rust severity over two consecutive years and pooled across different environments

Sources	d.f.	Mean Sum of Square	
		2017-18	2018-19
Replication	2	31.839**	8.503**
Genotypes	40	1098.939**	1495.663**
Error	80	2.621	0.453
Sources	d.f.		Mean Sum of Square
Replication	2		36.062**
Main effect:-			
Genotypes	40		2558.203**
Environment	1		1485.546**
G× E interactions	40		36.401**
Error	162		1.571

*,**significant at 5% and 1%level, respectively.

Table 3. Screening of germplasm for presence or absence of *Yr* genes considered in the study

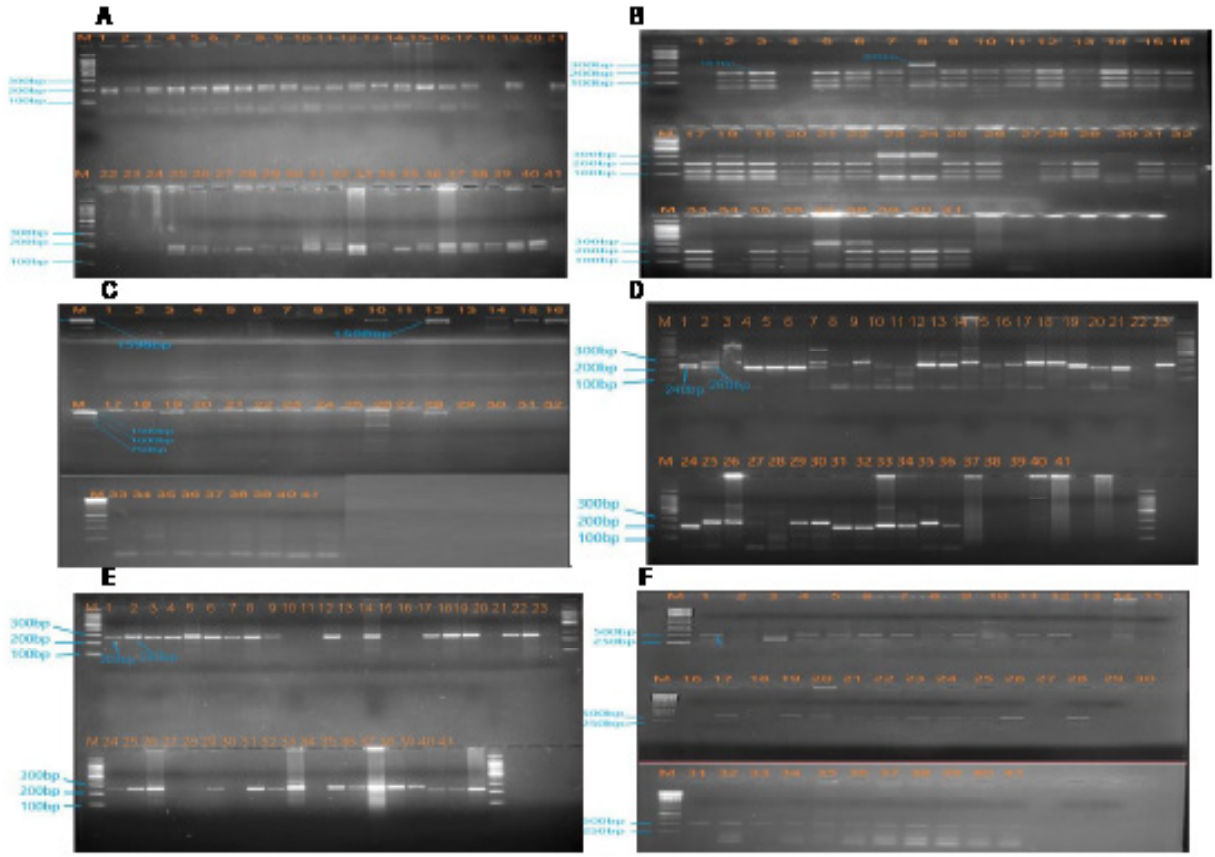
S.No	Genotypes	*Yr2*	*Yr5*	*Yr9*	*Yr10*	*Yr15*	*Yr17*	*Yr18*	*Yr36*	*Yr40*	Total	ACI over two successive years.
1	JAUW 584	0	0	0	0	1	1	1	0	0	3	8.46
2	JAUW 649	0	0	0	1	0	0	0	1	1	3	29.59
3	JAUW 650	1	0	0	1	1	1	0	1	1	6	4.76
4	JAUW 654	1	0	0	0	1	0	1	1	1	5	9.57
5	JAUW 665	1	0	0	0	0	0	1	1	1	4	29.58
6	JAUW 666	0	0	0	0	1	1	1	1	1	5	31.36
7	JAUW 667	1	0	0	1	1	0	1	0	1	5	31.15
8	JAUW 669	1	1	0	1	1	0	1	1	0	6	23.06
9	JAUW 672	1	0	0	1	0	0	1	1	1	6	2.21
10	RSP 561	1	0	1	0	0	0	1	0	0	3	22.21
11	WH 1080	1	0	1	0	0	0	1	1	0	4	2.09
12	PBW 779	1	0	1	0	1	0	1	1	1	6	2.65
13	PBW 750	1	0	0	0	0	1	1	0	1	4	2.70
14	PBW 780	1	0	0	1	1	1	1	1	1	7	1.55
15	PBW 723	1	0	1	0	0	1	0	0	1	4	11.99
16	HD 2967	1	0	0	0	0	0	0	0	0	1	50.00
17	WH 1184	1	1	0	1	0	0	0	0	1	4	2.48
18	PBW 752	0	1	0	1	0	1	0	0	1	4	0.45
19	WH 1142	0	0	1	0	0	1	1	1	0	4	8.30
20	IBWSN 1080	0	0	0	0	0	1	1	0	1	3	9.11
21	IBWSN 1057	1	1	0	0	1	0	1	0	1	5	29.16
22	HI 1619	0	0	0	0	0	0	1	1	1	3	2.09
23	RSP 81	0	1	0	0	0	0	1	0	1	3	30.07
24	13th HTWYT 719	0	1	0	0	1	0	1	1	0	4	0.61
25	35th ESWYT 113	1	1	0	1	1	0	1	0	1	6	10.29
26	25th HRWSN2105	1	1	1	1	1	0	1	0	1	7	0.63
27	47th IBWSN 1185	0	0	0	1	0	1	0	0	1	3	2.39
28	RAJ 3765	1	0	1	0	0	0	1	0	1	4	14.01
29	32nd SAWSN 3011	1	0	0	1	1	0	0	1	1	5	0.66
30	22nd SAWYT 323	1	1	0	1	0	0	0	1	1	5	4.88
31	JAUW 670	1	0	0	0	0	0	1	0	1	3	21.00
32	JAUW 671	1	0	0	0	1	0	1	0	1	4	28.26
33	JAUW 673	1	1	0	0	0	0	1	1	0	4	5.78
34	JAUW 674	1	0	0	0	0	1	0	0	0	2	35.28
35	MP 1318	1	1	0	1	0	0	1	1	1	6	4.76
36	HD 3086	1	0	0	0	1	0	1	1	1	5	2.77
37	22ndSAWYT 316	1	1	0	0	0	0	1	1	1	5	0.77
38	WH 1124	1	1	0	0	1	1	1	0	1	6	8.91
39	PBW 763	0	1	0	1	1	0	1	1	1	6	2.26
40	PBW 801	0	1	0	1	1	0	0	1	1	5	2.21
41	Agra local	1	0	0	0	0	0	0	0	0	1	85.36

genotypes, revealing similarities among certain genotypes such as JAUW 654 and HD 3086, and HD 2967 and Agra local. The marker data from nine genes separated all 41 lines into two distinct groups at the genomic level. A detailed illustartion of the results of molecular validation is presented in **Fig 1**.

This study identified genotypes with multiple *Yr* genes, serving as parent lines for broadening genetic diversity in wheat breeding for resistance against *Puccinia striiformis* f. sp. tritici. To enhance germplasm structure for crop development, more genotypes and markers are needed. The research aligns with Saini *et al.* (2002) and Zeng *et al.* (2014) in identifying yellow rust-resistant genes in wheat germplasm. Notably, *Yr5*, *Yr10*, and *Yr15* are potent, all-stage resistance genes. The study found Yr5 *effective* for North American and Iranian virulent races, and potent in the Caucasian, middle Asian, and Pakistani resistance gene surveys. *Yr10*, targeting all races in India, Pakistan, China, Iran, and the USA, was detected in 16 cultivars, aligning with Bariana *et al*. (2002).

To enhance germplasm structure for crop development, a broader range of genotypes and markers is necessary. Epidemiological studies showed that the *Yr5* gene was effective against North American and Iranian virulent races. In Caucasian, Middle Asian, and Pakistani resistance surveys, *Yr5* and *Yr15* were effectual against all *Puccinia striiformis* f. sp. tritici races. The efficacy of *Yr5* in Iran and its neighbouring regions makes it a valuable gene for wheat breeding initiatives. This study identified *Yr5* in 15 lines, confirming prior findings. The race-specific *Yr10* gene targets all races in India, Pakistan, China, Iran, and the USA. The present study detected *Yr10* in 16 cultivars, confirming the findings of Bariana *et al.* (2002), who reported that the gene *Yr10* amplifies a 258-260 bp segment, whereas lines without it amplify 240 bp.

Gene pyramiding, involving a combination of Yr genes, offers broad-spectrum, long-term protection against diseases and viruses (Fuchs, 2017). Additive and epistatic effects, prevalent in distinct genetic backgrounds, support the use of various Yr gene combinations in breeding programs (Ning, 2018). So, in any breeding program for disease resistance, using Yr genes like *Yr5, Yr10* and *Yr15* is important as they offer complete resistance to the new yellow rust strains. Other Yr genes should also be utilized, but they only provide partial resistance. The genotypes identified by this characterization research having several Yr genes may be valuable as parental lines for broadening *Puccinia striiformis* f. sp. tritici resistance sources in wheat breeding. However, more genotypes and markers are essential to give a clearer discernment of germplasm structure for crop development initiatives.



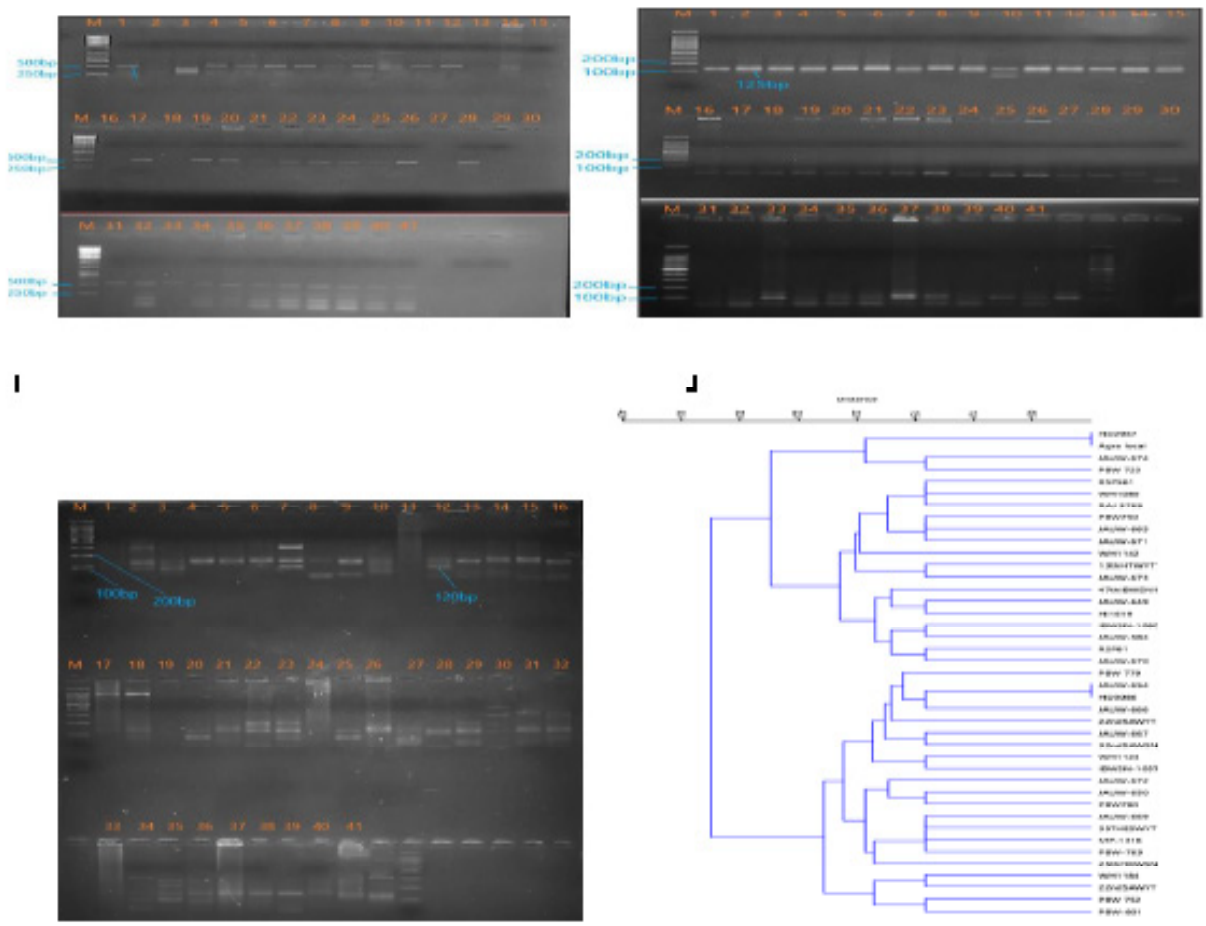


Fig 1. Electrophoretogram of the primers for different ***Yr*** resistance genes. Primers of ***Yr2, Yr5, Yr9, Yr10, Yr15, Yr17, Yr18, Yr36 and Yr40*** were used to screen the wheat genotypes (1–41). The postulated bands were easy to be discern according to previously reported base pairs of the PCR product. PCR amplification profile of these ***Yr*** genes are shown in separate plates (A to J). The presence or absence of gene is marked with an arrow. While fig J depicts dendogram showing clustering of 41 wheat lines based on Jaccard's similarity coefficient derived from the genotypic data of SSR and STS markers

Such evaluation data is significant and can be effectively used in developing future breeding techniques to produce long-lasting protection against the stripe rust fungus. The current study will aid future wheat improvement initiatives by facilitating gene pyramiding tactics against stripe rust. Future breeding programs may use these lines as donors to produce disease-resistant lines, which, after yield evaluation, can also be released as improved versions or new varieties.

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