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DIAGNOSIS OF YELLOW RUST DISEASE RESISTANCE IN BREAD WHEAT (*TRITICUM AESTIVUM* L.) USING SSR MARKERS

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SUMMARY

Yellow rust (*Puccinia striiformis* f. sp. *tritici*) is the primary disease affecting wheat (*Triticum aestivum* L.) crop productivity worldwide. The latest study aimed to evaluate the 68 wheat cultivars and advanced lines for resistance to yellow rust under field conditions using both morphological assessment and SSR molecular markers. The results revealed eight wheat genotypes (*Yr15/6 Avocet S, Xisorak, Yr5/6* Avocet S, *Triticum spelta*, Yr10/6* Avocet S, Yr SP/6* Avocet S, Spaldings Prolific, and Andijon-2**) were distinctly highly resistant. The two SSR markers Barc008 and Gwm140 proved to be the most reliable for detecting resistance, whereas four other markers (Gwm340, Gwm111, Xgwm131, and Gwm251) showed variable results across the genotypes. The integration of morphological and molecular data highlighted the genetic diversity in yellow rust resistance and demonstrated their potential to efficiently screen the wheat germplasm. The results provide a valuable base for selection of resistant parental lines and employment of marker-assisted selection (MAS) in wheat breeding programs aimed at improving the resistance to yellow rust disease.

Keywords: Wheat (*T. aestivum* L.), yellow rust, SSR markers, *Yr* genes, resistance, marker-assisted selection (MAS), genotypes evaluation

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Key findings: Eight promising wheat (*T. aestivum* L.) genotypes (*Yr15/6 Avocet S, Xisorak, Yr5/6* Avocet S, *Triticum spelta*, Yr10/6* Avocet S, Yr SP/6* Avocet S, Spaldings Prolific, and Andijon-2) showed complete resistance to yellow rust under field conditions. The SSR markers Barc008 and Gwm140 were successful as highly reliable for detecting this resistance, demonstrating robust concordance with morphological evaluation.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the backbone of global food security and one of the most strategically important cereal crops in the international market due to its high economic value and consumption (Curtis *et al*., 2002). In the present era, fungal diseases affecting wheat production, particularly the rust diseases that caused substantial reduction in global yield, further widen the gap between wheat grain supply and demand. Among these diseases, stripe, or yellow rust (*Puccinia striiformis* f. sp. *tritici*, or Pst), has become the most devastating, accounting for the loss of millions of tons of wheat grains annually and frequently triggering regional epidemics that threaten wheat production worldwide (Omara *et al*., 2022). Although the first report of the said rust disease was in Lower Egypt, it has emerged as a major hindrance in wheat-growing regions across the globe.

The most economical, environmentally safe, and sustainable method of controlling stripe rust is the development of genetically resistant wheat cultivars through different breeding programs. However, for breeding highly effective and durable resistant cultivars, comprehensive knowledge about the existing pathogen races, genetic structure of pathogen populations, their virulence spectra, and the dynamics of virulence shifts across the different agroecological zones is crucial (Shahin *et al*., 2020a). Moreover, the knowledge and ability to predict the emergence of new virulent races—capable of overcoming existing resistance genes in wheat genotypes—remains a viable component of effective disease management strategies (Shahin, 2020b).

Over the past decades, immense research has laid the foundation for identifying major stripe rust resistance *Yr* genes, i.e., *Yr5*, *Yr15*, *Yr37*, *Yr51*, and *Yr57*. These showcased a great distribution across the wheat genome and conferred varying degrees of resistance (Shahin and Ragab, 2015). In addition, numerous quantitative trait loci (QTL) and temporarily designated resistance genes have been notable, which contribute to a broad-spectrum and durable resistance against diverse pathogen races. However, on the other side, the maximum evolutionary potential of *P. striiformis* included frequent mutation, genetic recombination, and rapid shifts in virulence patterns. Consequently, the yellow rust resistance based solely on single major genes often breaks down within a short period after deployment (Chen, 2005; Jin *et al*., 2010). Therefore, pyramiding multiple durable resistance genes into elite cultivars is considerably one of the most efficient approaches to achieving long-term and stable protection against stripe rust (Peterson *et al*., 1948).

In the last decade, significant advancements have resulted in the development of reliable molecular markers for detecting stripe rust resistance genes. Among these molecular markers, simple sequence repeat (SSR) markers are immensely applicable due to their high polymorphism, reproducibility, and co-dominant inheritance (Qulmamatova *et al*., 2022). The SSR markers have proven to be highly effective for mapping genetic distances, discriminating the wheat genotypes, and performing precise screening of *Yr* genes without requiring labor-intensive pathogenicity assays (Turakulov *et al*., 2024). Therefore, marker-assisted selection (MAS) has become an integral component of modern wheat breeding programs and attained authentication for its precision, efficiency, and capacity to accelerate the development of stripe rust-resistant wheat cultivars.

From this perspective, the primary objective of the presented study was to assess the stripe rust resistance in soft wheat genotypes through molecular analysis using

SSR markers and compare the resistance levels of 68 wheat genotypes examined under field conditions. In this study, the identified *Yr* genes and the genetic profiles generated through SSR marker analysis provide valuable insights for breeding programs aiming to develop wheat cultivars with stripe rust resistance. Furthermore, these results can be effectively beneficial in marker-assisted backcrossing strategies to introgress resistant alleles into susceptible genotypes, thereby strengthening the genetic base for stripe rust resistance in wheat crops.

MATERIALS AND METHODS

Plant materials

In this study, the experimental material comprised 68 bread wheat (*T. aestivum* L.) cultivars and advanced lines (Table 1). These wheat genotypes came from two main sources: a subset from the Institute of Genetics and Experimental Plant Biology, Academy of Sciences, Uzbekistan, while other genotypes, including advanced lines carrying well-characterized stripe rust (*Yr*) resistance genes, came from the International Maize and Wheat Improvement Center (CIMMYT), Mexico. The management of plant material was in accordance with international guidelines as well as intellectual property standards. All wheat genotypes evaluated transpired under field conditions at the adult plant stage for resistance to stripe rust using artificial inoculation, allowing for precise assessment of various resistance levels. The combined set of wheat genotypes enabled a thorough phenotypic and molecular evaluation of stripe rust resistance, providing crucial data for selecting resistant lines and supporting marker-assisted breeding programs.

Table 1. Bread wheat (*Triticum aestivum* L.) cultivars used in the study.

No.	Research samples	No.	Research samples	No.	Research samples	No.	Research samples	No.	Research samples
1	Ezoz	15	Heine's Kolben (S;Yr6+1)	29	Yr10/6 Avoset S	43	Yr18/3 Avoset S	57	Krasnadar
2	Pervitsa	16	Heine's Peko (S;Yr6	30	Bobur	44	Zamin 1	58	Lal Bahadur/Pavon 1BL
3	Yr 1/6 avocet S	17	Fielder	31	Moro (W;Yr10)	45	Hamkor	59	AVOCET YRA*3/PASTOR
4	Yr 1/6 avS	18	Yr7/6 Avoset S	32	Yr17/6 Avoset S	46	Vexa	60	Pastor
5	Yr 15	19	Tanya	33	Do'stlik	47	Evelena	61	Davr
6	216	20	Morocco	34	Yuka	48	Bezostoya	62	Temiryazovka 150
7	Kalyansoma (S)	21	Reichersberg 42 (W;Yr7+?)	35	Yr32/6 Avoset S	49	Lemhi	63	Antanina
8	Grom	22	Thatcher	36	Carstens (W;Yr32)	50	TP 981	64	Sabrbosh
9	Xisorach	23	Yr8/6 Avoset S	37	Yr SP/6 Avoset S	51	TP 1295	65	Andijon 2
10	Vassa	24	Compair (S;Yr8)	38	Spaldings prolific W;Yr SP	52	Yr27/6 Avoset S	66	G'ozg'on
11	Hybrid 46 (W;Yr4)	25	Yr9/6 Avoset S	39	Asr	53	Ciano 79	67	Andijon 4
12	Yr 5/6 Avocet S	26	Fed4/Kavkaz (Yr9)	40	Yaksart	54	ATTILA CM 85836-50Y	68	Alekseyevich
13	TRITICUM spelta (Inter Yr 5)	27	Clement (W;Yr9+ Yr2+?)	41	Starshina	55	OPATA 85		
14	Yr 6/6 Avocet S	28	Grut	42	Yelanchik	56	Avocet-YRA 3/3/ ALTAR84/ AESQ//APATA		

Field experiments

The conduct of field experiments was under open field conditions with optimal irrigation using 68 bread wheat genotypes, with each genotype sown in a 2 m² subplot to evaluate resistance to yellow rust. The experimental layout followed a randomized complete block design with three replications, ensuring considerable statistical analysis of the disease responses (Kurbanbaev *et al*., 2024; Erjigitov *et al*., 2025).

The wheat plots underwent artificial inoculation with urediniospores of *Puccinia striiformis* f. sp. *tritici* (Pst) to simulate natural disease conditions. The preparation of inoculum continued by mixing a predetermined amount of urediniospores with water at a standardized concentration and uniformly spraying across the plants in all the subplots at the tillering stage, ensuring even coverage of the entire plots. This methodology allowed for consistent infection pressure across all wheat genotypes while maintaining reproducibility of the disease assessment.

Disease assessment

In wheat genotypes, the stripe rust infection level evaluation used a modified version of the Cobb scale. Infection types' classification was as follows: 0 for no visible symptoms, R (resistant) for plants exhibiting necrotic lesions with or without small pustules, and MR (moderately resistant) for plants showing small pustules surrounded by necrotic tissues. Meanwhile, MS (moderately susceptible) describes genotypes with medium-sized pustules, minimal necrosis, and occasional chlorosis, and S (susceptible) indicates plants with large pustules, lacking necrosis or chlorotic reactions (McIntosh *et al.,* 2013).

Disease intensity entailed quantification by estimating the percentage of leaf area covered by pustules, with values ranging from 0% (no infection) to 100% (complete coverage of the leaf). This standard approach allowed for a standardized assessment of the disease severity across all tested genotypes. The said technique facilitated the classification of wheat cultivars and advanced lines into resistant or susceptible categories, enabling subsequent analysis and comparison of the genotype responses under field conditions.

Genomic DNA isolation

Genomic DNA extraction proceeded from fresh wheat leaves using a modified CTAB (cetyltrimethylammonium bromide) protocol with slight adjustments, including tissue disruption under liquid nitrogen to improve cell lysis (Tolibova *et al*., 2025). The quality and concentration of the isolated DNA received assessment using a NanoDrop Eight spectrophotometer (Thermo Fisher Scientific, USA). The studied wheat samples with sufficient DNA yield underwent subsequent standardization to a working concentration of 50 ng/µL for downstream molecular analysis.

SSR markers analysis and gel electrophoresis

The SSR-based wheat genotyping continued by following the modified protocol, as described with adaptation for resistance to stripe rust. A total of six SSR markers linked to known Yr-resistant genes succeeded in their selection from publicly available databases (Table 2).

The PCR reactions took place in a T100 Thermal Cycler (Bio-Rad, USA) with a total reaction volume of 10 µL, comprising 2.0 µL of 5x Screen Mix (Evrogen, Russia), 1.0 µL of genomic DNA (50 ng/µL), 1.0 µL of primer pair mix, and 6.0 µL of DNase-free distilled water. The PCR program comprised an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 20 s (denaturation), 55 °C–60 °C for 30 s (annealing), and 72 °C for 50 s (extension), with a final extension at 72 °C for 5 min.

The PCR products incurred separation by 2.5% agarose gel electrophoresis and gained visualization after staining with ethidium bromide. The capture of gel images used the GelDoc Go Gel Imaging System (Bio-Rad, USA), with fragment sizes estimated utilizing the AmpliSize Molecular Ruler (Bio-Rad, USA). The resulting SSR profiles served to construct a phylogenetic tree, achieving

Table 2. Panel of SSR markers associated with yellow rust–resistant *Yr* genes.

DNA markers	Primer Forward/Reverse (5'->3')	Associated gene	Associated allele (Reference)
Barc008	GCGGGAATCATGCATAGGAAAACAGAA GCGGGGCGAAACATACACATAAAAACA	Yr15	220 bp (Eriksen *et al*., 2004)
Gwm340	GCAATCTTTTTTCTGACCACG ACGAGGCAAGAACACACATG	Yr18	250 bp (Sun *et al*., 2002)
Gwm140	ATGGAGATATTTGGCCTACAAC CTTGACTTCAAGGCGTGACA	Yr29	152 bp (Sun *et al*., 2002)
Gwm111	TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCACTCG	Yr33	210 bp (Sun *et al*., 2002)
Xgwm131	AATCCCCACCGATTCTTCTC AGTTCGTGGGTCTCTGATGG	Yr39	160 bp (Sun *et al*., 2002)
Gwm251	CAACTGGTTG CTACACAAGCA GGGATGTCTGTTCCATCTTAG	Yr62	300 bp (Sun *et al*., 2002)

visualization and analysis with NCSS 12 software, allowing classification of wheat genotypes based on their genetic similarity and presence of stripe rust resistance alleles.

Data analysis

Genetic diversity assessment among wheat genotypes used SSR markers based on the polymorphism information content (PIC) and expected heterozygosity (He) indices. For each SSR marker, PIC and He values sustained calculations based on allele frequencies. The PIC, which reflects the level of marker polymorphism, entailed calculations using the following formula:

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Where pip_ipi is the frequency of the iii-th allele in the population, and nnn is the number of alleles. According to Botstein *et al*. (1980), markers with PIC values above 0.5 tend to be highly polymorphic, markers in the range of 0.25–0.5 are moderately polymorphic, and markers below 0.25 are low polymorphic.

The expected heterozygosity (He), upon calculation, used the same formula:

$$He = 1 - \sum_{i=1}^{n} p_i^2$$

He reflects the probability that two randomly selected alleles at a given locus are different from each other and was used to evaluate the level of genetic diversity in the wheat population (Nei and Roychoudhury, 1974). Higher He values indicate a higher level of genetic diversity in the population.

Statistical analysis

In this study, the performed statistical analysis assessed the genetic diversity and relationship among the examined wheat genotypes based on molecular marker data and morphological traits. A randomized complete block design with three replications was the layout employed in field experiments. The analysis of variance (ANOVA) sought to determine the significant differences among the wheat cultivars and advanced lines (Qulmamatova *et al*., 2022).

Cluster analysis used the UPGMA algorithm (unweighted pair group method with arithmetic mean), with a dendrogram illustrating the phylogenetic relationship among the wheat genotypes generated (Kurbanbaev *et al*., 2024). All the analyses related to phylogeny and marker informativeness proceeded using NSCC (NTSYS) software packages.

RESULTS AND DISCUSSION

Wheat genotypes comprising cultivars and advanced lines received evaluation for yellow rust (*Puccinia striiformis* f. sp. *tritici*) resistance under field infection conditions. Disease

severity assessment was per international standards (R, MR, MS, and S). The results revealed that, out of 68 wheat genotypes, eight genotypes (Yr15/6* Avocet S, Xisorak, Yr5/6* Avocet S, Triticum spelta, Yr10/6* Avocet S, Yr SP/6* Avocet S, Spaldings Prolific, and Andijon-2) were remarkably and completely stripe rust disease-free. These findings indicate the presence of major resistance genes, such as *Yr5*, *Yr10*, and *Yr15*, remains highly effective under natural epidemic pressure, which is consistent with previous studies reporting their stable performance across environments (Turakulov *et al*., 2024). Additionally, 15 genotypes enunciated high levels of resistance to yellow rust, showing some infection while maintaining overall resistance. Such partial but durable resistance suggests the possible contribution of adult-plant resistance (APR) genes, which are common to enhance long-term protection against stripe rust in breeding lines. In contrast, six wheat genotypes (Morocco, Yr9/6* Avocet S, Lemhi, Ciano-79, AVOCET-YRA3/PASTOR, and Avocet-YRA3) appeared as highly susceptible to stripe rust disease (Table 3). The susceptibility of the wheat cultivar Morocco has also been confirmed in studies conducted by Ochilov *et al*. (2025) and other researchers.

The PCR analysis of 68 wheat genotypes using six SSR markers associated with *Yr* genes revealed a total of 20 alleles. The average number of alleles per locus was 3.3, ranging from two alleles observed at the locus Xgwm131 to five alleles detected at the locus gwm340 (Table 4). The polymorphism information content (PIC) varied between 0.495 and 0.745, with an average of 0.610, revealing the highest level of informativeness of the selected markers. These PIC values indicate the SSR markers used in this study possess strong discriminatory power, which aligns with previous findings where SSR markers targeting rust-resistance loci showed high polymorphism and effectiveness in differentiating wheat germplasm (Shahin *et al*., 2020). Similarly, expected heterozygosity (He) ranged from 0.529 to 0.783, with an average of 0.658, suggesting substantial genetic variability among the studied wheat genotypes. This considerable heterozygosity further reflects the diverse genetic background of the evaluated materials, consistent with reports demonstrating that SSR markers linked to *Yr* genes efficiently capture allelic diversity in wheat breeding populations (Shewabez *et al*., 2022).

Comparative analysis with previous studies exhibited consistency in marker informativeness. Sun *et al*. (2002) reported that each marker, gwm251 and gwm340, amplified four alleles, with PIC values of 0.5812 and 0.6833, respectively, reflecting their largest discriminatory power. In contrast, Bakhadirov *et al*. (2024) observed five alleles amplified by the marker Xgwm111, highlighting some variation in allele numbers across different wheat populations. The results collectively demonstrated the selected SSR markers proved to be effective tools for evaluating the genetic diversity and recognizing resistance alleles associated with *Yr* genes in wheat genotypes.

The marker Barc008 displayed an association with the *Yr15* gene, having been reported to confer resistance to yellow rust when the resistant allele corresponds to a fragment length of 220 bp (Eriksen *et al*., 2004). The PCR analysis of the 68 wheat genotypes revealed all morphologically resistant genotypes owned a 220 bp resistant allele, while this allele was completely absent in susceptible wheat genotypes. This robust concordance confirmed that Barc008 was distinctly the highly reliable marker for gene Yr15-mediated resistance in the evaluated wheat material, supporting its use in marker-assisted selection (MAS) for identifying resistant wheat genotypes without relying solely on field evaluation.

Past studies revealed the *Yr15* gene had initial detection in wild wheat (*Triticum dicoccoides*) genotypes, with the said gene closely associated with the markers Xgwm413, Barc008, and Xgwm273 (Murphy *et al*., 2009). The results indicated these markers provide the highest accuracy for the detection of the *Yr15* gene and can be effective for selection programs across different wheat cultivars. Similarly, Kokhmetova *et al*. (2021) successfully utilized these markers to identify

Table 3. Morphological and molecular analysis of yellow rust resistance in wheat samples.

No.	Wheat genotypes	Phenotypic analysis of yellow rust resistance	Yr15	Yr18	Yr29	Yr33	Yr39	Yr62
			Barc008	Gwm340	Gwm140	Gwm111	Xgwm131	Gwm251
		2024-2025	220 bp.	250 bp.	152 bp.	210 bp.	160 bp.	300 bp.
1	E'zoz	5MR	0	0	1	0	0	1
2	Pervitsa	70MS	0	0	0	1	1	1
3	Yr 1/6* Avocet S	50MR	0	0	1	0	1	1
4	Yr 1/6* AvS	50MS-S	0	0	0	0	0	0
5	Yr15/6* Avocet S	0	1	1	1	0	0	0
6	216	20MS	0	0	0	0	0	0
7	Kalyansona (S)	50MS-S	0	0	0	0	0	1
8	Grom	40MS	0	1	0	0	0	0
9	Xisorak	0	0	0	1	0	0	0
10	Vassa	60MS	0	0	0	0	1	1
11	Hybrid 46 (W;Yr4)	R	1	0	1	0	0	0
12	Yr 5/6* Avocet S	0	1	0	1	0	1	0
13	Triticum spelta (Inter, Yr 5)	0	0	1	1	1	1	0
14	Yr 6/6* Avocet S	70MS	0	0	0	1	1	0
15	Heine's Kolben (S;Yr6+1)	40MS-MR	0	0	0	0	1	0
16	Heine's Peko (S;Yr6+?)	5MR	0	0	1	0	1	0
17	Fielder	60MS-S	0	0	0	0	0	0
18	Yr7/6* Avocet S	50MS	0	0	0	1	1	0
19	Tanya	50MS-MR	0	0	0	0	1	0
20	Morocco	100S	0	0	0	0	0	0
21	Reichersberg 42 (W;Yr7+?)	5MR	0	0	1	0	1	0
22	Thatcher	30MS-MR	0	0	0	0	0	0
23	Yr8/6* Avocet S	30MS	0	0	0	0	1	0
24	Compare (S;Yr8)	40MS	0	0	0	1	0	0
25	Yr9/6* Avocet S	S	0	0	0	0	1	0
26	Fed.4/Kavkaz (Yr9)	40MS	0	0	0	0	1	0
27	Clement (W;Yr9+ Yr2+?)	60MS	0	0	0	0	0	0
28	Grut	30MS	0	0	0	0	0	0
29	Yr10/6* Avocet S	0	1	1	1	0	0	0
30	Bobur	30MS	0	0	0	0	1	0
31	Moro (W;Yr10)	5MR	0	0	0	1	0	0
32	Yr17/6* Avocet S	R	0	1	1	0	1	0
33	Do'stlik	30MR	0	0	0	0	0	0
34	Yuka	10MS	0	0	0	0	1	0
35	Yr32/6* Avocet S	60MS	0	1	0	0	1	0
36	Carstens V (W;Yr32)	30MS	0	1	0	0	1	0
37	Yr SP/6* Avocet S	0	1	1	1	0	1	0
38	Spaldings Prolific (W;YrSP)	0	0	1	1	0	1	0
39	Asr	30MS	0	1	0	0	1	1
40	Yaksart	30MS	0	1	0	0	1	0
41	Starshina	30MR	0	1	0	0	1	0
42	Yelanchik	20MR	0	1	0	0	1	0
43	Yr18/3* Avocet S	60MS-S	0	1	0	0	1	0
44	Zamin 1	50MS	0	1	0	0	0	0
45	Hamkor	5MR	0	1	1	0	0	0

Note* 1—Amplification for molecular markers; 0—no amplification. Percentage of rust severity assessment used a modified Cobb scale (Peterson *et al*., 1948) 0 – no visible disease symptoms (immune response); R (Resistant) – small hypersensitive necrotic flecks with no or very limited sporulation; MR (Moderately resistant) – small to medium uredinia with limited sporulation, often associated with chlorosis or necrosis; and S (Susceptible**)** – large uredinia with abundant sporulation and no necrosis or chlorosis.

Table 3. (cont'd.)

No.	Wheat genotypes	Phenotypic analysis of yellow rust resistance	Yr15	Yr18	Yr29	Yr33	Yr39	Yr62
			Barc008	Gwm340	Gwm140	Gwm111	Xgwm131	Gwm251
		2024-2025	220 bp.	250 bp.	152 bp.	210 bp.	160 bp.	300 bp.
46	Vexa	50MS	0	0	0	0	0	0
47	Evelena	50MR	0	1	0	0	1	0
48	Bezostoya	50MS-MR	0	0	0	0	1	0
49	Lemhi	80S	0	1	0	0	1	0
50	TP 981	40MR	0	1	0	0	0	0
51	TP 1295	20MR	0	1	0	0	1	0
52	Yr27/6* Avocet S	20MS-MR	0	0	0	0	1	0
53	Ciano 79	90S	0	1	0	0	0	0
54	ATTILA CM 85836-50Y	60MS-S	0	1	0	0	0	0
55	OPATA 85	80MS	0	1	0	0	1	0
56	Avocet-YRA *3/3.	70S	0	1	0	1	0	0
57	Krasnodarskaya-99	30MS	0	1	0	0	1	0
58	Lal Bahadur/Pavon 1BL	30S	0	1	0	0	1	0
59	AVOCET -YRA*3/PASTOR	80S	0	1	0	0	1	0
60	PASTOR	60MS-S	0	1	0	0	0	0
61	DAVR	50MS	0	1	0	0	1	0
62	TEMIRYAZOVKA 150	20MS	0	0	0	0	0	0
63	ANTANINA	50MS-MR	0	0	0	0	1	0
64	SABRBOSH	R	0	1	1	0	0	0
65	Andijon 2	0	1	1	1	0	0	0
66	G'ozg'on	20MR	0	0	0	0	0	0
67	Andijon 4	50MS	0	1	0	0	0	0
68	Alekseyevich	10MR	0	1	1	0	0	0

Note* 1—Amplification for molecular markers; 0—no amplification. Percentage of rust severity assessment used a modified Cobb scale (Peterson *et al*., 1948) 0 – no visible disease symptoms (immune response); R (Resistant) – small hypersensitive necrotic flecks with no or very limited sporulation; MR (Moderately resistant) – small to medium uredinia with limited sporulation, often associated with chlorosis or necrosis; and S (Susceptible**)** – large uredinia with abundant sporulation and no necrosis or chlorosis.

Table 4. SSR markers and their polymorphism information content (PIC) and heterozygosity (He) values.

DNA markers	Molecular weight (pair bases, pb)	Number of alleles	PIC	HE	*P*-Value
Barc008	220-310	3	0.678	0.731	0.0043
Gwm340	180-260	5	0.543	0.641	0.0174
Gwm140	130-255	4	0.745	0.783	0.0147
Gwm111	170-210	3	0.578	0.611	0.0328
Xgwm131	160-180	2	0.495	0.529	0.0132
Gwm251	220-300	3	0.622	0.656	0.0129

the resistant wheat genotypes, confirming their practical application in breeding for yellow rust resistance.

In contrast, the SSR markers Gwm340, Gwm111, Xgwm131, and Gwm251, appearing to be associated with *Yr18*, *Yr33*, *Yr39*, and *Yr62* genes, revealed that resistance alleles were evident in both resistant and susceptible wheat genotypes. Notably, the cultivar Morocco emerged as highly susceptible to yellow rust as per morphological evaluation; however, it also possessed these resistance alleles. This inconsistency suggested that these markers may detect non-functional alleles and also incur effects from recombination events that decouple the marker from the actual resistance

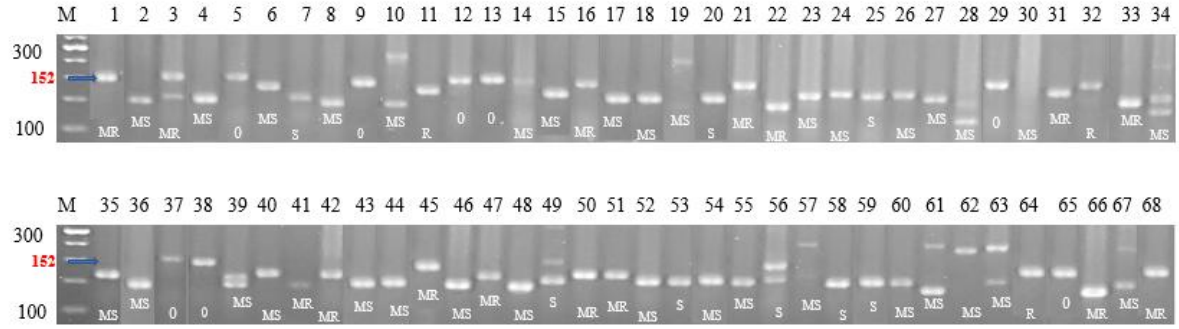

Figure 1. Electropherogram of Gwm140 primers associated with Yr18 genes. M – Molecular weight size marker (base pairs, 50 bp). See Table 1 for the identification of the 68 wheat genotypes (1–68) used in this study. 0 – no visible disease symptoms (immune response); R (Resistant) – small hypersensitive necrotic flecks with no or very limited sporulation; MR (Moderately resistant) – small to medium uredinia with limited sporulation, often associated with chlorosis or necrosis; and S (Susceptible) – large uredinia with abundant sporulation and no necrosis or chlorosis.

phenotype. Reports of similar limitations have resulted in previous studies, indicating the predictive value of these markers can vary depending upon the genetic background of the wheat germplasm (Bakhadirov *et al*., 2024). Therefore, these markers alone were insufficient for accurate selection, and their interpretation should be complementary with morphological assessment in a broader sense.

On the other hand, the marker Gwm140, which occurred to be associated with Yr18, exhibited the highest level of reliability. The said resistant allele (152 bp) succeeded in its detection in 16 wheat genotypes (1, 3, 5, 9, 11, 12, 13, 16, 21, 29, 37, 38, 45, 64, 65, and 68), with all these genotypes also morphologically confirmed as resistant to yellow rust disease (Figure 1). This perfect agreement between molecular and phenotypic data underscores the robustness of the marker Gwm140 for Yr18-mediated resistance and demonstrates its effectiveness for selecting resistant wheat genotypes in breeding programs.

Overall, in this study, the integration of molecular marker analysis with morphological evaluation allowed the confirmation of truly resistant genotypes and the identification of markers found to be less reliable. Markers like Barc008 and Gwm140 provide robust predictive power, while the other four markers, such as Gwm340, Gwm111, Xgwm131, and Gwm251, require cautious interpretation. These results highlighted the importance of validating SSR markers across diverse wheat germplasm and demonstrated that combining molecular and phenotypic approaches offers a reliable strategy for improving yellow rust resistance in wheat through different breeding programs. In recent studies, Guo *et al*. (2025) expressed that the SSR marker Barc008 is a reliable molecular tool for evaluating resistance to yellow rust (*Puccinia striiformis* f. sp. *tritici*) in bread wheat. Their results confirmed a strong association between the presence of the Barc008 allele and phenotypic resistance to yellow rust under both controlled and field conditions. This finding supports the use of Barc008 in marker-assisted selection programs aiming to develop wheat varieties with enhanced resistance to stripe rust.

CONCLUSIONS

Eight wheat genotypes exhibited complete resistance to yellow rust disease under field conditions. The SSR analysis revealed the markers Barc008 and Gwm140 proved to be highly reliable, which considerably aligned with observations based on morphological evaluation for stripe rust resistance. Four

markers, such as Gwm340, Gwm111, Xgwm131, and Gwm251, showed inconsistent results and emerged as less effective in wheat screening. The validated markers Barc008 and Gwm140 can be effectively favorable in marker-assisted selection to improve wheat resistance. The recognized resistant wheat genotypes can serve as valuable parental lines in future breeding programs.

REFERENCES

Bakhadirov USh, Turaev OS, Erjigitov DSh, Dolimov AA, Tursunmurodova BT, Fayzullaev AZ, Matkarimov FI, Qulmamatova DE, Baboev SK, Ziyaev ZM, Kushanov FN (2024). Determining aphid resistance genes in bread wheat (*Triticum aestivum* L.) cultivars using DNA markers. *SABRAO J. Breed. Genet*. 56(2): 582–590. http://doi.org/10.54910/ sabrao2024.56.2.11.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American J. Genetics*, 32(3), 314.

Chen XM (2005). Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. tritici] on wheat. *Can. J. Plant Pathol.* 27(3): 314–337. https://doi.org/10.1080/ 07060660509507230.

Curtis BC, Rajaram S, Macpherson HG (2002). Bread wheat improvement and production. *Rome*: 30: 1–18.

Eriksen L, Afshari F, Christiansen MJ, McIntosh AA, Jahoor A, Wellings CR (2004). Yr32 for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. *Theor. Appl. Genet.* 108: 567–575. https://doi.org/10.1007/s00122-003-1456-0.

Erjigitov DSh, Turaev OS, Dolimov AA, Mamatkulova GF, Mukhammadiev OA, Tursunmurodova BT, Kushanov FN (2025). Molecular characterization of heat tolerance in bread wheat (*Triticum aestivum* L.) cultivars/lines using genomic SSR markers. *Plant Interactions*. 20(1). https://doi.org/10.1080/17429145.2025.25 46997.

Guo H, Wang L, Bai X, Wu L, Zhang X, Zhang S, Yang Z, Yang E, Chang Z, Li X, Qiao L (2025). Mapping of a major locus for resistance to yellow rust in wheat. *Agronomy 15*(11): 2511. https://doi.org/10.3390/agronomy15112511.

Jin Y, Szabo LJ, Carson M (2010). Century-old mystery of *Puccinia striiformis* life history solved with the identification of Berberis as an alternate host. *Phytopathology* 43: 2–5. https://doi.org/10.1094/PHYTO-100-5-0432

Kokhmetova A, Rsaliyev A, Malysheva A, Atishova M, Kumarbayeva M, Keishilov Z (2021) Identification of stripe rust resistance genes in common wheat cultivars and breeding lines from Kazakhstan. *Plants* 10: 2303. https://doi.org/10.3390/ plants10112303.

Kurbanbaev I, Abdushukirova S, Fayzullaev A, Ziyadullaev Z, Sanaev N, Kulmamatova D (2024). Morpho-yield contributing traits and correlation of soybean parental and F1 hybrids*. J. Wildlife Biodivers.* 4: 129–137. https://doi.org/10.5281/zenodo.13823818

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Somers DJ, Appels R, Devos KM (2013). Catalogue of gene symbols for wheat. *Annual Wheat Newsletter*, 59: 1–98.

Murphy LR, Santra D, Kidwell K, Yan GP, Chen XM, Campbell KG (2009). Linkage maps of wheat stripe rust resistance genes Yr5 and Yr15 for use in marker-assisted selection. *Crop Sci*. 49(5): 1786–1790. doi: 10.2135/cropsci2008.10.0621.

Nei M, Roychoudhury AK (1974). Sampling variances of heterozygosity and genetic distance. *Genetics*, 76(2), 379–390. https://doi.org/10.1093/genetics/76.2.379.

Ochilov BO, Turakulov KS, Meliev SK, Melikuziev FA, Aytenov IS, Murodova SM, Khalillaeva GO, Chinikulov BK, Azimova LA, Urinov AM, Turaev OS, Kushanov FN, Salakhutdinov IB, Ma J, Awais M, Bozorov TA (2025). Development of yellow rust-resistant and high-yielding bread wheat (*Triticum aestivum* L.) lines using marker-assisted backcrossing strategies. *Int. J. Mol. Sci.* 26(15): 7603. https://doi.org/10.3390/ijms26157603.

Omara RI, Mazrou YSA, Elsayed A, Moawad N, Nehela Y, Shahin AA (2022). Mentoring interactive system for stripe rust. *Agronomy* 12(10): 2416. https://doi.org/10.3390/agronomy12102416.

Peterson RF, Campbell AB, Hannah AE (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereal. *Can. J. Res*. 60: 496–500. https://doi.org/10.1139/cjr48c-033.

Qulmamatova DE, Baboev SK, Buronov AK (2022). Genetic variability and inheritance pattern of yield components through diallel analysis in spring wheat. *SABRAO J. Breed. Genet*. 54(1): 21–29. http://doi.org/10.54910/sabrao2022.54.1.3.

Shahin A, Ashmawy M, El-Orabey W, Esmail S (2020a). Yield losses in wheat caused by stripe rust (*Puccinia striiformis*) in Egypt. *Am. J. Life Sci.* 8(5): 127–134. https://doi.org/10.11648/j.ajls.20200805.17

Shahin AA (2020b). Occurrence of new races and virulence changes of the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. tritici) in Egypt. *Arch. Phytopathol. Plant Prot*. 53(11–12): 552–569. https://doi.org/10.1080/03235408.2020.1767330

Shahin AA, Ragab KE (2015). Inheritance of adult plant stripe rust resistance in wheat cultivars Giza160 and Giza168. *J. Plant Prot. Path Mansoura Univ*. 6(4): 587–596. https://doi.org/10.21608/jppp.2015.53659

Shewabez E, Bekele E, Alemu A (2022). Genetic characterization and genome-wide association mapping for stem rust resistance in spring bread wheat. *BMC Genom* Data 23, 11. https://doi.org/10.1186/s12863-022-01030-4

Sun Q, Wei Y, Ni Z, Xie C, Yang T (2002). Microsatellite marker for yellow rust resistance gene Yr5 in wheat introgressed from spelt wheat. *Plant Breed.* 121: 539–541.

Tolibova Z, Adilova S, Matkarimov F, Kholliyev O, Ikromova U, Sobirov F, Baboev S, Alloberganova Z, Qulmamatova D (2025). Evaluation of salt tolerance in local varieties and foreign collection samples of chickpea (*Cicer arietinum* L.). *J. Wildl. Biodivers.* 9(2): 380–387.

Turakulov K, Bozorov T, Meliev S, Chinniqulov B, Shokirova D (2024). Genes conferring resistance to yellow rust in local bread wheat varieties. *Acad. Res. Edu. Sci*. 5(5): 411–417.