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Genetic identification of yellow rust disease resistance in soft wheat (Triticum Aestivum l.) Samples using DNA markers

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Abstract: This study assessed the genetic polymorphism of wheat samples in relation to yellow rust disease resistance through DNA markers genetically linked to this trait. According to the analysis, the markers Xgwm140 (PIC = 0.72) and Xgwm340 (PIC = 0.53) exhibited the highest levels of polymorphism, playing a significant role in the identification of yellow rust-resistant alleles, with 305 and 220 base pairs, respectively. Phylogenetic analysis revealed genetic diversity among the genotypes and indicated that resistant genotypes tended to cluster into distinct groups. The findings of this study provide a reliable tool for identifying resistant genotypes, which can be effectively utilized in the selection process during wheat breeding programs aimed at enhancing resistance to yellow rust disease. This version reflects a more detailed and formal scientific tone, maintaining the essence of your original text while providing further clarity on the methods and outcomes. Let me know if you need any further adjustments. In 2024 field trials, wheat varieties were tested for yellow rust resistance using molecular markers for Yr genes. Varieties with Yr5 and Yr15 showed full resistance, while those with Yr6, Yr9, Yr7, and Yr27 were susceptible. Yr62 alone was weak but enhanced resistance when combined with other genes. Yr5 and Yr15 were identified as the most effective for

breeding resistant varieties.

Keywords: Wheat (Triticum aestivum L.), DNA markers, PCR, yellow rust, genetic polymorphism, resistance alleles.

Introduction: Wheat is a major staple crop, covering more than 219 million hectares worldwide, with annual production exceeding 760 million tons. Wheat provides approximately 20% of the daily caloric needs of the global population. Today, due to the negative consequences of climate change in agriculture, several issues related to both biotic and abiotic factors have emerged. Factors such as high temperatures, drought, and rust diseases are creating significant challenges in wheat production. Biotrophic pathogens, particularly fungi, are the primary cause of rust diseases, which result in substantial economic damage to wheat cultivation. Each year, fungi and insects contribute to a 21.5% reduction in wheat yield worldwide. Yellow rust infection can develop at any stage of the plant's life cycle, from the seedling stage to maturity, meaning it can progress throughout the entire vegetative period of the plant. Cases of yellow rust are frequently observed in over 60 countries, and the disease is found on every continent except Antarctica [1].

Wheat stripe rust, caused by Puccinia striiformis f. sp. tritici, is recognized as one of the most significant biotic stresses impacting wheat (Triticum aestivum L.) on a global scale [2]. The disease typically induces damage to wheat crops within the range of 0.1% to 5.0%, with potential yield losses extending from 5% to 25% [3]. Under unfavorable environmental conditions or during severe outbreaks, the extent of yield loss can reach up to 100%. Genotypes possessing a limited number of resistance genes, such as Yr5 and Yr15, have been identified as highly effective against Pst (Puccinia striiformis) and are widely adopted worldwide as part of integrated disease management strategies [4]. The genetic variability of the Yr gene family plays a pivotal role in controlling the dynamics of yellow rust epidemics. Moreover, non-race-specific resistance, exemplified by genes such as Yr18, which confer resistance in older plant stages, contributes significantly to the overall durability and effectiveness of wheat resistance. These resistance mechanisms have been extensively employed in wheat breeding programs for several decades [5,6].

Wheat (Triticum aestivum L.) is one of the most important cereal crops globally and holds particular agricultural significance in Uzbekistan. Improving wheat productivity and quality, particularly by developing and introducing cultivars resistant to major diseases such as yellow rust, is of paramount scientific and practical importance [7]. The yellow rust pathogen represents a critical constraint to wheat production, severely affecting plant development and reducing yields. Puccinia striiformis f. sp. tritici is considered the principal biotic stress factor in wheat cultivation across Central Asia, where fungicide applications are the primary control method, particularly in winter wheat fields [8]. This widespread use of fungicides in response to the pathogen underscores the ongoing challenge of managing this disease across the region, as indicated by various studies and field observations [9]. To assess the impact of yellow rust (Puccinia striiformis f. sp. tritici) on wheat yield, a field experiment was conducted with three treatments: an untreated control, a bio-treatment background, and a fungicide-treated background. Urediniospores of the pathogen were inoculated to ensure consistent disease pressure. Results showed that the untreated control experienced the highest disease severity, while both bio-treatment and fungicide applications significantly reduced infection rates and improved plant health. Yield analysis confirmed that both treatments resulted in higher yields compared to the control, demonstrating the effectiveness of bio-based and chemical treatments in managing yellow rust and enhancing crop productivity. [10].

In recent years, the emergence of yellow rust in major wheat-producing countries has led to significant crop losses. Recent advancements in molecular marker technologies have created effective tools to address such complex problems. For example, the use of DNA molecular markers based on polymerase chain reaction (PCR) offers several advantages over traditional phenotypic trait selection [11]. Marker-assisted selection (MAS) has been widely applied to target rust resistance genes in various generations. These methods can improve selection efficiency in plant breeding, particularly when applied to overcome some of the challenges associated with classical phenotypic screening approaches. When MAS is used in the early hybrid generations of plants, multiple DNA markers are employed simultaneously to check several genes at once.

METHODS

Molecular research was conducted at the Molecular and Biochemical Genetics Laboratory of the Institute of Genetics and Experimental Plant Biology, Academy of Sciences of Uzbekistan. The list of samples taken from this collection is presented in Table 1.

Plant Materials. Within the scope of this study, the soft wheat (Triticum aestivum L.) samples listed in the table below were used as plant materials for analysis.

Table 1

List of soft wheat (*Triticum aestivum* L.) varieties and samples used in the study

№	Research samples	№	Research samples	№	Research samples	№	Research samples	№	Research samples
1	Ezoz ,.	15	Heine's Kolben (S;Yr6+1)	29	Yr10/6 Avoset S	43	Yelanchik	57	Avocet-YRA 3/3/ ALTAR84/ AESQ//APATA
2	Pervitsa	16	Heine's Peko (S;Yr6+?)	30	Bobur	44	Yr18/3 Avoset S	58	Krasnadar
3	Yr 1/6 avocet S	17	Fielder	31	Moro (W;Yr10)	45	Zamin 1	59	Lal Bahadur/Pavon 1BL
4	Yr 1/6 avS	18	Yr7/6 Avoset S	32	Yr15/6 Avoset S	46	Hamkor	60	AVOCET YRA*3/PASTOR
5	Yr 15	19	Tanya	33	Yr17/6 Avoset S	47	Vexa	61	PASTOR
6	216	20	Morocco	34	Do'stlik	48	Evelena	62	DAVR
7	Kalyansoma (S)	21	Reichersberg 42 (W;Yr7+?)	35	Yuka	49	Bezostiya	63	TEMIRYAZOVKA 150
8	Grom	22	Thatcher	36	Yr32/6 Avoset S	50	Lemhi	64	ANTANINA
9	Xisorach	23	Yr8/6 Avoset S	37	Carstens(W;Yr32)	51	TP 981	65	SABRBOSH
10	Vassa	24	Compair(S;Yr8)	38	Yr SP/6 Avoset S	52	TP 1295	66	Yr10
11	Hybrid 46 (W;Yr4)	25	Yr9/6 Avoset S	39	Spaldings prolific W;Yr SP	53	Yr27/6 Avoset S	67	Andijon 2
12	Yr 5/6 Avocet S	26	Fed4/Kavkaz (Yr9)	40	Asr	54	Ciano 79	68	G'ozg'on
13	TRITICUM spelta (Inter Yr 5)	27	Clement(W;Yr9+ Yr2+?)	41	Yaksart	55	ATTILA CM 85836-50Y	69	Andijon 4
14	Yr 6/6 Avocet S	28	Grut	42	Starshina	56	OPATA 85	70	Alekseyevich

Genomic DNA Extraction. Genomic DNA was extracted from young leaf tissues of wheat plants using a slightly modified version of the CTAB method developed by Paterson et al. (1993) [12].

Polymerase Chain Reaction (PCR) Analysis. For molecular analysis, a total of 11 highly reliable microsatellite (SSR) markers associated with yellow rust resistance were selected based on peer-reviewed publications in international journals. PCR reactions were carried out using the T100 Thermal Cycler (BIO-RAD, USA), employing the "Hot Start" protocol to enhance specificity and efficiency.

Gel Electrophoresis and Genotyping. Genotyping of the samples was performed based on the molecular weight of PCR products using gel electrophoresis. The electrophoresis was conducted on 2.5–3.0% agarose gels (CondaLab, Spain). Results were documented using the GelDoc Go Gel Imaging System (BIO-RAD, USA).

Characterization of Polymorphic Markers. For each polymorphic marker, the Polymorphism Information Content (PIC) and heterozygosity (He) values were calculated using the iMEC web-based software. PIC values indicate the discriminatory power of a marker, while He reflects the degree of genetic variation within the population.

RESULTS

To assess the genetic polymorphism of the wheat varieties using the panel of DNA markers, genomic DNA was extracted using the CTAB method. The quality and quantity of DNA samples were evaluated using 0.9% agarose gel electrophoresis and spectrophotometric analysis, after which PCR amplification was performed.

Genotyping and PCR Screening. PCR screening was performed on the extracted DNA samples using primers genetically linked to yellow rust resistance. Genotyping was conducted with the help of the GelAnalyzer software. According to the analysis results, among the 11 polymorphic DNA markers associated with yellow rust resistance, two markers exhibited a Polymorphism Information Content (PIC) value higher than 0.5. The primer pairs Xgwm340 (PIC = 0.53) and Xgwm140 (PIC = 0.72) demonstrated the highest levels of polymorphism, indicating their strong discriminatory power (Table 2).

Table 2

Panel of DNA markers genetically associated with yellow rust resistance in soft wheat samples and their characteristics

№	Marker	Primer sequences 5'-3'	PIC	He
1	Xgwm140	AAGGCAAAGGCAAAGTGG	0.72	0.73
		TGATCTTTACCAAGCATTCG		
2	Xgwm501	AAGAATACTTTAATGAA	0.44	0.46
		CAAACTTATCAGGATTAC		
3	Xgwm340	TAATTGGGACCGAGAGACG	0.53	0.55
		TTCTTGCAGCTCCAAAACCT		
4	barc0187	CGAATAGCCGCTGCACAAG	0.41	0.42
		TATGCATGCCTTTCTTTACAAT		
5	gwm413	GGTCGCCCTGGCTTGCACCT	0.36	0.34
		TGCTTGTCTAGATTGCTTGGG		
6	XPSP3000	GATCGTCTCGTCCTTGGCA	0.29	0.31
		GATATAGTGGCAGCAGGATA		
7	XGWM493-3BS	TACAATTCACCTAGAGT	0.39	0.37
		GCAAGTTTTCTCCCTATT		
8	xgwm268	CAAACTTATCAGGATTAC	0.35	0.39
		GGTCGCCCTGGCTTGCACCT		
9	barc008	CAGACAAACAGAGTACGGGC	0.47	0.46
		GGTGCAATTTGAGTTTGGAGT		
10	S23M41	TCAACGGAACCTCCAATTTC	0.28	0.30
		AGGTAGGTGTTCCAGCTTGC		

11	Barc349	CGAATAGCCGCTGCACAAG		
		TATGCATGCCTTTCTTTACAAT	0.33	0.34

According to the results, the Gwm340 marker was genetically associated with yellow rust resistance through the presence of a 220 bp allele, while the Gwm140 marker was associated with yellow rust resistance through the 305 bp allele (Figure 1).

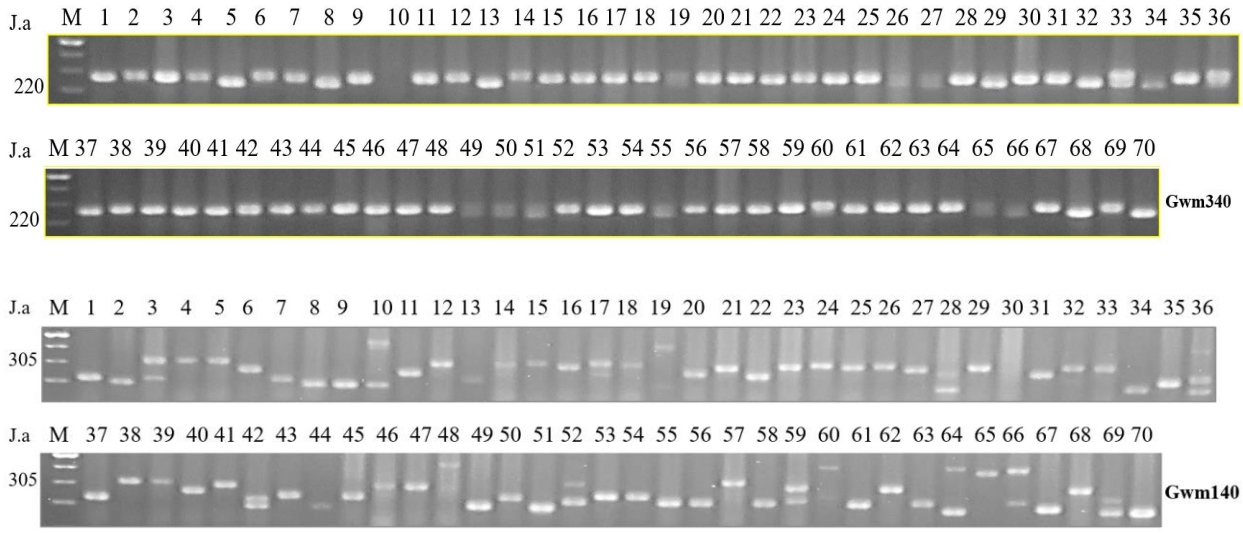

Figure 1.

Electropherogram of PCR amplicons using polymorphic DNA markers genetically associated with yellow rust resistance in soft wheat samples. M – molecular weight marker; bp – base pairs. Sample order (1–70) corresponds to Table 1.

As indicated by the results of the study, analysis based on the Gwm340 DNA marker revealed the presence of two distinct alleles (260 bp and 220 bp) associated with yellow rust resistance in wheat populations. These alleles primarily reflect genetic variations within the wheat genome that confer resistance to yellow rust.

During the study, out of 70 wheat samples, the resistance allele (260 bp) was found in only 10 samples, representing a relatively small proportion of individuals with yellow rust resistance traits. This suggests that resistance alleles are present in low frequencies within the overall wheat population, underscoring the need for focused attention on selection processes to increase the prevalence of this trait in breeding programs. Furthermore, the Gwm140 DNA marker was used to study yellow rust resistance. Based on the results of the PCR analyses, the yellow rust resistance-specific 305 bp allele was found in the genomes of 16 wheat samples. This suggests the presence of genetic markers associated with yellow rust resistance within the wheat genome. Consequently, it highlights the existence of potential genetic resources for promoting and selecting this allele to further enhance yellow rust resistance in wheat. Such studies are essential for developing more effective strategies to combat yellow rust and play a crucial role in improving wheat resistance.

A phylogenetic analysis of the yellow rust resistance alleles was conducted using 11 DNA markers obtained in the study. According to the results of the phylogenetic analysis, divergence and differences were observed among these markers, reflecting the genetic diversity of wheat and the distribution of yellow rust resistance alleles across various populations and genetic groups. Phylogenetic divergence, in turn, illustrates the varying degrees of genetic variation in the genetic resources conferring resistance to yellow rust and their genetic relationships. Studying this analysis will help identify which genetic variants and alleles need to be incorporated into breeding programs to ensure resistance (Figure 2).

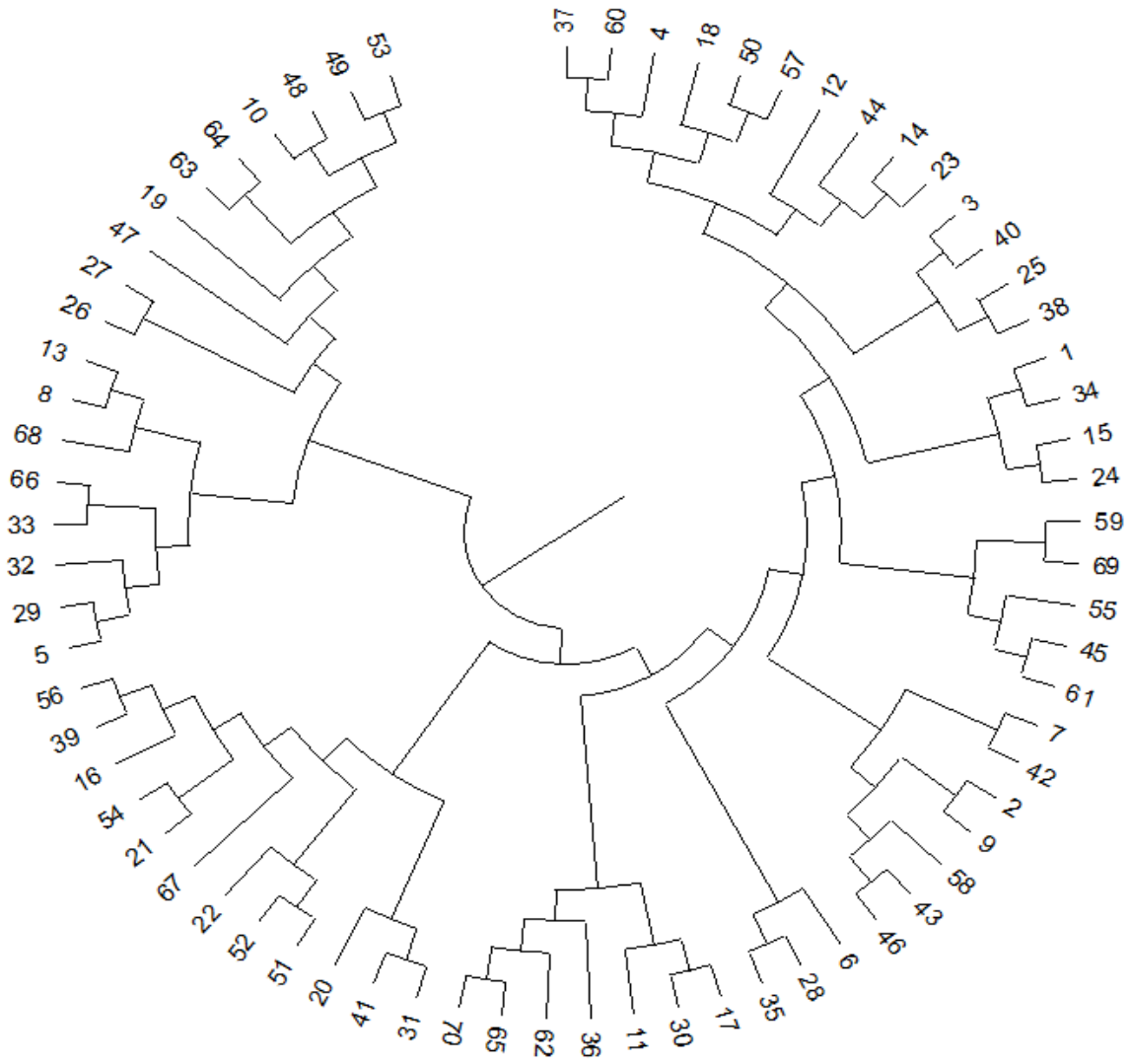

Figure 2

Phylogenetic tree based on the polymorphism of DNA markers genetically associated with yellow rust resistance alleles in soft wheat samples.

In this study, a phylogenetic analysis was conducted to determine the level of yellow rust (Puccinia striiformis f. sp. tritici) resistance in wheat genotypes using molecular markers. As a result of this analysis, the genotypes were grouped according to their genetic similarity, and the results were presented in the form of a radial phylogenetic tree (dendrogram).

The overall structure of the dendrogram clearly indicates the genetic diversity among the genotypes. Several major clusters are distinguished in the tree, reflecting a high level of genetic similarity between certain genotypes. Notably, some genotypes are closely grouped in small branches, suggesting their allele composition is similar. Other genotypes, however, are placed in independent branches, indicating they are genetically distinct from the rest.

The clustering observed in the phylogenetic tree confirms the presence of varying levels of resistance or susceptibility to yellow rust disease. Specifically, certain clusters contain genotypes that are closely grouped, with resistant samples potentially dominating these groups. This provides an opportunity to identify potential resistant genotypes based on molecular analyses and use them in breeding programs.

Additionally, the presence of genetically distinct genotypes in the tree highlights their significance as unique genetic resources. These samples are considered promising for expanding genetic diversity in new hybridization programs.

This phylogenetic analysis served to identify genetic differences among wheat genotypes based on yellow rust resistance alleles. The results have significant implications for selecting high-resistance genotypes in breeding programs, grouping genetic resources, and organizing breeding efforts effectively. Notably, working with genetic clusters identified through this analysis will facilitate more targeted and purposeful breeding.

Table 3

Disease severity to stripe rust and presence of *Yr* genes in wheat genotypes from Uzbekistan

№	Research samples	Yr-gen	Yellow Rust Severity %, RT [b]	S23M41	gwm413	P6M12	P6M12	xgwm526	gwm192
			2024 year	Yr5	Yr15	Yr9	Yr9	Yr7	yr62
1	Ezoz		5MR	0	1	0	0	0	1
2	Pervitsa		80MS	0	0	1	1	1	1
3	Yr 1/6 avocet S	Yr1	80MS	0	0	0	0	1	0
4	Yr 1/6 avS	NIL 1	90MS	0	0	0	0	1	0
5	Yr 15		0	0	1	0	0	1	0
6	216		50S	0	0	0	0	1	1
7	Kalyansoma (S)	Yr 2	90S	0	0	0	0	1	1
8	Grom		50MS	0	0	0	0	1	1
9	Xisorach		R	1	1	0	0	1	1
10	Vassa		80S	0	0	1	1	1	1
11	Hybrid 46 (W;Yr4)	(W;Yr4)	10MR	1	1	0	0	1	1
12	Yr 5/6 Avocet S	Yr 5	0	1	1	0	0	1	1
13	TRITICUM spelta (Inter Yr 5)	Yr 5	0	1	1	0	0	1	1
14	Yr 6/6 Avocet S	Yr 6	100S	0	0	0	0	1	0
15	Heine's Kolben (S;Yr6+1)	(S;Yr6+1)	70MS	0	0	0	0	1	0
16	Heine's Peko (S;Yr6+?)	(S;Yr6+1)	10MR	1	0	0	0	1	1
17	Fielder	Yr6,Yr20	100MS	0	0	0	0	1	0
18	Yr7/6 Avoset S	Yr7	90MS	0	0	0	0	1	0
19	Tanya		50MS	0	0	1	1	0	1
20	Morocco			1	0	0	0	0	1
21	Reichersberg 42 (W;Yr7+?)	(W;Yr7+?)	40MS-MR	0	0	0	0	1	1
22	Thatcher	Yr7	60MS-MR	0	0	0	0	1	1
23	Yr8/6 Avoset S	Yr8	50MS-S	0	0	0	0	1	0
24	Compair(S;Yr8)	(S;Yr8)	70MS	0	0	0	0	1	1
25	Yr9/6 Avoset S	Yr9		0	1	0	0	1	1
26	Fed4/Kavkaz (Yr9)	Yr9	80S	0	1	1	1	1	0

27	Clement(W;Yr9+ Yr2+?)	(W;Yr9+ Yr2+?)	70MS	0	0	1	1	1	1
28	Grut		70MS-MR	0	0	1	1	1	0
29	Yr10/6 Avoset S	Yr10	0	0	1	0	0	1	0
30	Bobur		70MS-MR	1	1	0	0	0	1
31	Moro (W;Yr10)	(W;Yr10)	0	0	1	0	0	1	1
32	Yr15/6 Avoset S	Yr15	0	0	1	0	0	1	0
33	Yr17/6 Avoset S	Yr17	70MS-S	0	0	0	0	1	0
34	Do'stlik		50MS-MR	0	0	0	0	1	1
35	Yuka		40MS	1	1	1	1	0	1
36	Yr32/6 Avoset S	Yr32	50MS	1	0	1	1	1	0
37	Carstens(W;Yr32)	(W;Yr32)	90S	0	0	0	0	1	1
38	Yr SP/6 Avoset S	Yr SP	0	0	1	0	0	1	1
39	Spaldings prolific W;Yr SP	W;Yr SP	20MS	1	0	0	0	1	0
40	Asr		50MS	0	1	0	0	1	0
41	Yaksart		60S	0	1	0	0	1	1
42	Starshina		60MS	0	0	0	0	1	0
43	Yelanchik		60MR	1	0	0	0	0	1
44	Yr18/3 Avoset S	Yr18	90S	0	1	0	0	1	0
45	Zamin 1		90MS	0	0	0	0	0	1
46	Hamkor		30MS	1	0	0	0	0	1
47	Vexa		80MS	0	1	1	1	0	0
48	Evelena		70MS	0	1	1	1	0	1
49	Bezostiya		70MS	0	0	1	1	0	1
50	Lemhi	yr21	100S	0	1	0	0	1	1
51	TP 981	-	60MS	0	1	0	0	1	0
52	TP 1295		50MS	0	1	0	0	1	0
53	Yr27/6 Avoset S	Yr27	50MS	1	0	1	1	1	0
54	Ciano 79	Yr27	100S	0	0	0	0	1	1
55	ATTILA CM 85836-50Y	Yr27+?	80MS	0	0	0	0	1	0
56	OPATA 85	Yr27+ Yr18	100S	0	0	0	0	1	0
57	Avocet-YRA 3/3/ ALTAR84/ AESQ//APATA	Yr 28	90S	0	1	0	0	0	0
58	Krasnadar		40MS	0	0	0	0	0	0

59	Lal Bahadur/Pavon 1BL	Yr29	50S	0	0	0	0	0	1
60	AVOCET YRA*3/PASTOR	Yr31	100S	0	1	0	0	1	0
61	PASTOR	Yr31+APR	80MS	1	0	0	0	0	1
62	DAVR		70S	0	0	0	0	0	0
63	TEMIRYAZOVKA 150		20MS	1	0	1	1	1	1
64	ANTANINA		80MS	0	0	1	1	1	0
65	SABRBOSH		40MS	1	0	0	0	0	1
66	Yr10		0	1	0	0	0	0	1
67	Andijon 2		0	1	0	0	0	1	1
68	G'ozg'on		70S	0	0	0	0	1	1
69	Andijon 4		70MS	1	0	0	0	0	1
70	Alekseyevich		30MS	1	0	0	0	0	1

b—Values indicate severity, RT—reaction type. "1", "0" indicate the presence, absence of corresponding gene, respectively.

Identification of Genes Based on Molecular Markers

Molecular markers are regarded as highly efficient tools for detecting and combining multiple resistance genes, as accomplishing this process based solely on phenotypic data is often complex and, at times, unfeasible [13]. Several scientific studies have successfully identified the sources of key Yr resistance genes (Yr9, Yr5, and Yr15) within winter wheat breeding materials, underscoring their importance in developing disease-resistant cultivars [14,15,16].

Molecular markers linked to the Yr15 gene were first identified by Sun et al. (1997), Peng et al. (2000), and Murphy et al. (2009) [17–19]. This gene was mapped to a genetic interval of 6.4 centimorgans (cM), flanked by the markers Xgwm413. The Xgwm413 marker is positioned 2.5 cM on the proximal (closer to the center) side. Research by Murphy et al. (2009) Xgwm413 are highly reliable diagnostic tools for identifying the Yr15 gene across nearly all genetic backgrounds tested [19].

In the field trials of 2024, wheat varieties were tested for resistance to yellow rust (Puccinia striiformis f. sp. tritici). Additionally, molecular markers were used to identify the presence of Yr genes (e.g., Yr5, Yr15, Yr9, Yr7, Yr62) conferring resistance to yellow rust in these varieties. Varieties such as Yr5/6 Avocet S, Yr15/6 Avocet S, Yr10/6 Avocet S, and Yr SP/6 Avocet S showed no signs of the disease, and they are considered fully resistant. These varieties possess Yr5, Yr15, or Yr10 genes, which are highly effective in suppressing the disease either completely or very strongly. In contrast, varieties like Fielder, Avocet-YRA/PASTOR, Ciano 79, and OPATA 85 were 100% susceptible (completely affected by the disease). The Yr6, Yr31, Yr27 genes present in these varieties did not provide adequate protection, casting doubt on their potential for disease resistance. The results indicated that Yr5 and Yr15 genes are the most effective in providing protection against yellow rust. Varieties possessing these genes showed almost no signs of the disease. On the other hand, varieties carrying Yr9, Yr7, and Yr27 genes showed intermediate (MS) or full (S) susceptibility. These genes alone are not sufficiently protective and, therefore, are recommended for use in combination with other more effective genes. The Yr62 gene, although found in many varieties, was not highly effective when used alone. However, when combined with other Yr genes, it can provide strong resistance.

CONCLUSION

The results of the study demonstrate the effectiveness of using DNA markers to evaluate the yellow rust resistance levels in wheat samples. The markers Xgwm140 and Xgwm340, which exhibited the highest polymorphism, were genetically associated with resistance alleles, making them valuable tools for marker-assisted selection (MAS) in breeding programs. The relatively low frequency of resistant alleles underscores their importance as genetic resources and

emphasizes the need to strengthen selection efforts. Phylogenetic analyses identified the genetic diversity within the population, enabling effective genotype grouping and laying the foundation for future selection strategies. These approaches provide a scientific basis for developing wheat varieties with enhanced resistance to yellow rust.

Recommended Genes for Breeding: Yr5, Yr15, Yr10 – these genes have shown consistent and stable resistance in both field and marker-based results. Marker-Assisted Selection (MAS): Through the use of these markers, it is possible to accelerate the breeding process by identifying the genes at an early stage (during the seedling stage). This allows for faster and more efficient selection of resistant varieties.

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