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MOLECULAR MARKER-ASSISTED SELECTION OF YELLOW RUST–RESISTANT, HIGH-QUALITY WHEAT GENOTYPES.

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Abstract. *Yellow rust (Puccinia striiformis f. sp. tritici) is one of the most destructive diseases affecting wheat (Triticum aestivum L.) worldwide, causing significant yield and quality losses. This study aimed to identify wheat genotypes resistant to yellow rust and possessing superior grain quality using molecular marker-assisted selection (MAS). A total of 48 wheat genotypes, including international and local varieties, were evaluated using 17 SSR markers linked to yellow rust resistance and additional DNA markers associated with grain quality traits. Genomic DNA was extracted using a modified CTAB method, and PCR amplification was performed. Gel-electrophoresis and GelAnalyzer software were used to assess genotypic polymorphism. The Wmc177 (150 bp) and Xgwm120 (156 bp) markers were identified as highly reliable for yellow rust resistance. Furthermore, UMN19 (367 bp) and UMN25 (299 bp) markers were linked to superior grain and flour quality. Cluster analysis based on resistant alleles revealed groups of genotypes with high genetic similarity, highlighting promising candidates for future breeding programs. The study demonstrates the effectiveness of MAS in selecting wheat genotypes with combined disease resistance and grain quality traits, providing valuable genetic resources for breeding strategies.*

Keywords: *Triticum aestivum L., yellow rust, molecular markers, SSR, grain quality, MAS, genetic polymorphism*

Introduction

Wheat (*Triticum aestivum* L.) is one of the world’s most important staple crops, and its yield and grain quality are crucial for global food security. Among the major constraints affecting wheat production, yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is particularly destructive, leading to substantial yield losses and deterioration of grain and flour quality. Developing and selecting cultivars resistant to yellow rust is therefore a central objective in wheat breeding programs. Traditional breeding methods rely on phenotypic selection, which is time-consuming, labor-intensive, and heavily influenced by environmental conditions. In contrast, molecular marker-assisted selection (MAS) offers a precise and efficient approach, allowing early detection of target alleles associated with disease resistance and quality traits. SSR and other DNA markers have been widely applied to detect genetic polymorphisms linked to yellow rust resistance and grain/flour quality, enabling the identification of resistant alleles and promising genotypes for breeding. This study aims to identify wheat genotypes carrying alleles associated with yellow rust resistance and superior quality traits using molecular markers. Specifically, Wmc177 and Xgwm120 were employed for yellow rust resistance, while UMN19 and UMN25 were used to assess grain and flour quality. This integrated molecular approach provides a reliable framework for developing high-yielding, disease-resistant, and high-quality wheat cultivars.

Materials and Methods.

The study was conducted on various cultivars of bread wheat (Triticum aestivum L.).

Plant material was obtained from the collection of the Laboratory of Cereal and Legume Crop Selection, Institute of Genetics and Experimental Biology of Plants, Academy of Sciences of the Republic of Uzbekistan. Genomic DNA extraction was performed using a modified CTAB method (Paterson et al., 1993) to obtain high-quality DNA from young leaf tissues. The quality and concentration of DNA samples were assessed using a Drawell K5600 micro-spectrophotometer. Gel electrophoresis was employed for genotyping PCR products using 1.5–2% agarose gels (CondaLab, Spain), and results were documented with the GelDoc Go Gel Imaging System (BIO-RAD, USA). Polymorphism analysis was carried out using SSR markers.

The polymorphism information content (PIC) and expected heterozygosity (He) of each marker were calculated using the iMAC web tool, according to formulas described by Botstein et al. (1980) and Nei (1973). A set of 17 SSR markers associated with yellow rust resistance and grain quality traits was used in the study to assess genetic polymorphism.

Statistical analysis was performed using one-way ANOVA to determine significant differences among cultivars, and phylogenetic relationships were visualized using MEGA 11 software. This integrated molecular approach provides an effective and reliable framework for genotyping and assessing genetic polymorphism in wheat.

The samples used in this study were of the common wheat species Triticum aestivum.L., whose names are listed in the table below.

No.	Genotype	No.	Genotype
1	25th IWWYT-IRR № 9806	25	25th IWWYT-IRR № 9830
2	25th IWWYT-IRR № 9807	26	Saberbash
3	25th IWWYT-IRR № 9808	27	Temiryazovka-150
4	25th IWWYT-IRR № 9809	28	Alekseevich
5	25th IWWYT-IRR № 9810	29	225
6	25th IWWYT-IRR № 9811	30	232
7	25th IWWYT-IRR № 9812	31	238
8	25th IWWYT-IRR № 9813	32	243
9	25th IWWYT-IRR № 9814	33	256
10	25th IWWYT-IRR № 9815	34	273
11	25th IWWYT-IRR № 9816	35	274
12	25th IWWYT-IRR № 9817	36	281
13	25th IWWYT-IRR № 9818	37	286
14	25th IWWYT-IRR № 9819	38	292
15	25th IWWYT-IRR № 9820	39	Yonbosh
16	25th IWWYT-IRR № 9821	40	Xisorak
17	25th IWWYT-IRR № 9822	41	Yuka
18	25th IWWYT-IRR № 9823	42	Gurt
19	25th IWWYT-IRR № 9824	43	Xamkor
20	25th IWWYT-IRR № 9825	44	Velena
21	25th IWWYT-IRR № 9826	45	Pervitsa
22	25th IWWYT-IRR № 9827	46	Bobur
23	25th IWWYT-IRR № 9828	47	Yelanchik
24	25th IWWYT-IRR № 9829	48	Andijon-2

Table 1. List of varieties and specimens of Triticum aestivum L.

A number of scientific publications worldwide have reported on the investigation of wheat’s resistance to yellow rust using molecular-genetic approaches.

Based on articles published in Scopus and Web of Science, as well as utilizing the GrainGenes database, a panel of DNA markers was developed to assess genetic polymorphism in the studied samples. This panel included 17 SSR markers previously associated with yellow rust resistance, along with DNA markers linked to grain quality traits (Table 2).

No.	**Marker name**	**Primer sequence 5'-3'**	Allele Range (bp)	**Number of alleles**	**Reference**
1	Xb arc8	gcgggaatcatgcataggaaaacagaa gcggggcgaaacatacacataaaaaaa	260, 280	2	Rosewarne et al . , (2006)
2	Xgwm95	gatcaaacacacaccccctcc aatgcaaagtgaaaaacccg	160, 250	2	Rosewarne et al . , (2006)
3	Xg wm533	g aaggcgaatcaaacggaata gttgctttaggggaaaagcc	110, 130	2	Rosewarne et al . , (2006)
4	Xgwm582	aaccacaaaaagatccaacgacac cathatagaaggattttgagag	90, 100	2	Rosewarne et al . , (2006)
5	Xwmc 177	g agggctctctttaattcttgct ggtctatcgtaatccacctgta	175, 185, 190	3	Rosewarne et al . , (2006)
6	X barc 181	cgctggagggggtaagtcatcac cgcaaatcaagaacacgg gagaaagaa	165, 180	2	Khlestkina et al., 2007
7	X gwm 120	gatccaccttcctctctctc gattatactggtgccgaaac	110, 135, 145	3	Khlestkina et al., 2007
8	X gwm 374	atagtgtgttgcatgctgtgtg tctaattagcgttggctgcc	220, 290	2	Khlestkina et al., 2007
9	X gwm 437	gatcaagactttgtatctctc gatgtccaacagttagctta	80, 90, 100	3	Khlestkina et al., 2007
10	X wmc 198	cacgctgccatcacttttac ttgaagtggtcattgttgct	240, 250	2	Rosewarne et al . , (2006)
11	X wmc 44	ggtcttctgggctttgatcctg tgttgctagggacccgtagtgg	200, 260, 270	3	Rosewarne et al . , (2006)
12	Xgwm374	atagtgtgtttgcatgctgtgtg tctaattagcgttggctgcc	180,195	2	Khlestkina et al., 2007
13	Xgwm190	gtgcttgctgagctatgagtc gtgccacgtggtacctttg	120,145	2	Khlestkina et al., 2007
14	Xgwm249	caaatggatcgagaaaggga ctgccatttttctggatctacc	150,170,200	3	Khlestkina et al., 2007
15	Xgwm268	aggggatatgttgtcactcca ttatgtgattgcgtacgtaccc	220,245, 260	3	Khlestkina et al., 2007
16	Xgwm297	gcgtaggagagatgccccaaaggtt gcgtgcggactcgtgaatcattac	175, 205	2	Khlestkina et al., 2007
17	Xgwm413	tgcttgtctagattgcttggg gatcgtctcgtccttggca	160, 185, 210	3	Khlestkina et al., 2007
	High molecular weight glutenin subunits				
2	UMN 19	cgagacaatatgagcagcaag ctgccatggagaagttgga	367, 385	2	Lei et al., 2006
4	UMN 25	gggacaatacgagcagcaaa cttgttccggttgttgcca	300, 315	2	Lei et al., 2008

Table 2. Characterization of DNA markers genetically linked to wheat yellow rust resistance

Results

Genomic DNA was extracted from the experimental samples using a modified CTAB method to evaluate the genetic polymorphism of the cultivars through a panel of DNA markers.

The quality and quantity of the extracted DNA samples were assessed by gel electrophoresis on 0.9% agarose gel and using a spectrophotometer. Following this procedure, PCR amplification was performed (Figure 1).

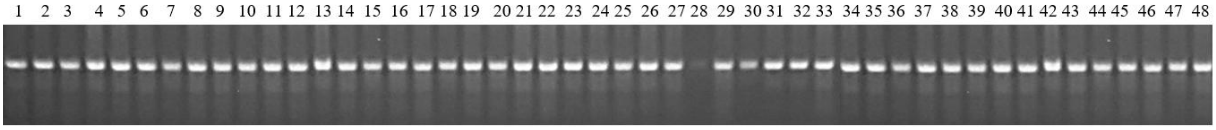

Figure 1. Electropherogram of genomic DNA from the experimental samples. The sample IDs (1–48) correspond to those listed in Table 1.

PCR screening of the isolated DNA samples was performed using primers linked to yellow rust resistance, and genotyping was conducted with the GelAnalyzer software. Out of the 34 primer pairs used to assess genetic polymorphism, 11 pairs revealed genetic differences among the experimental samples. According to Botstein et al. (1980), markers with a PIC value greater than 0.5 are considered highly polymorphic and reliable for molecular-genetic studies. In the present study, two of the polymorphic markers exhibited PIC values above 0.5, with Xgwm120 (0.84) and Wmc177 (0.62) showing the highest levels of polymorphism (Table 3.3).

№	Marker Name	Allele Range (bp)	Resistance Allele (bp)	PIC	He	Reference
1	Xgwm120	110, 156	156	0,84	0,87	Sun, Q., et al., 2002
2	Barc8	260, 280	280	0,42	0,43	Kumari et al., 2019
3	csLV34	160, 250	160	0,40	0,41	Helguera M., et al., 2003
4	Gwm533	110, 120	110	0,32	0,32	Spielmeyer, W., 2003
5	IAG95-STS	90, 100	90	0,21	0,22	Kumari et al., 2019
6	Wmc177	105, 125, 150	150	0,62	0,67	Spielmeyer, W.,2003
7	Xbarc181	165, 180	180	0,28	0,29	Wang et al., 2008
8	Xgwm374	220, 230, 250, 260, 280, 290	260	0,23	0,24	Gupta et al., 2020
9	Xgwm437	80, 90, 100	90	0,42	0,49	Gupta et al., 2020
10	Xwmc198	240, 250	250	0,45	0,47	Kumari et al., 2019
11	Xwmc44	200, 260, 270	270	0,14	0,16	Kumari et al., 2019

Table 3. Description and analysis of DNA markers

During the study, the electrophoregrams of the most reliable DNA markers, Wmc177 and Xgwm120, were analyzed. According to the results, the Wmc177 marker revealed a total of three alleles — 105 bp, 125 bp, and 150 bp, among which the 150 bp allele was clearly associated with resistance to yellow rust (Puccinia striiformis). Genotypes carrying the Wmc177_150 bp allele exhibited a high level of genetic resistance to the disease. This resistance-associated allele was detected in 25 wheat samples, which are as follows: 1, 2, 3, 6, 8, 9, 11, 12, 13, 15, 16, 18, 19, 20, 22, 25, 28, 31, 32, 34, 35, 43, 45, 46, and 48.

These samples possess the resistant allele of the Wmc177 marker, indicating their genetic potential for resistance to yellow rust. Moreover, in samples 10 and 47, in addition to the Wmc177_150 bp allele, the 105 bp allele was also detected, indicating that these genotypes are heterozygous. This may reflect an intermediate level of resistance to the disease or possible interactions between the alleles. In contrast, samples 5, 14, and 42 carried only the 105 bp allele, which is not associated with resistance to yellow rust. These samples may be classified as susceptible genotypes. The remaining 18 wheat samples exhibited the 125 bp allele. The association of this allele with resistance to yellow rust is currently unknown, and further molecular and agrobiological studies are required to clarify its role (Figure 2).

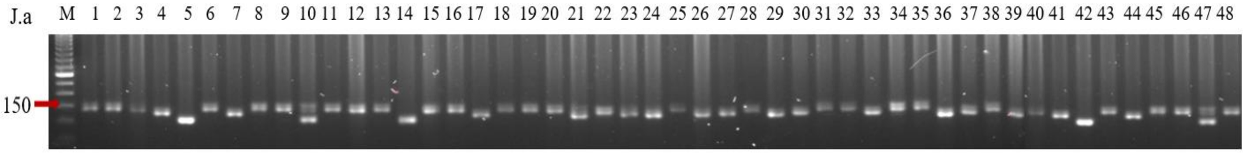

Figure 2. Gel electropherogram of the Wmc177 DNA marker genetically associated with yellow rust resistance. See Table 1 for the list of research samples (1–48).

In our study, the Xgwm120 DNA marker, which was found to be reliable for detecting genetic resistance to yellow rust (Puccinia striiformis), revealed two alleles — 110 bp and 156 bp based on PCR (polymerase chain reaction) results. Analysis indicated that the 156 bp allele is genetically associated with resistance to yellow rust, and genotypes carrying this allele exhibited stable resistance to the disease. Specifically, the Xgwm120_156 bp allele was detected in 22 wheat samples: 1, 2, 6, 7, 8, 9, 14, 16, 18, 20, 23, 24, 25, 27, 29, 30, 31, 32, 33, 43, and 48.

Among these, 12 samples were homozygous (carrying two copies of the resistant allele), while the remaining 10 samples were heterozygous (carrying one resistant and one susceptible allele). These results indicate that these genotypes possess high to moderate genetic potential for resistance to yellow rust.

In the remaining 26 samples, only the 110 bp allele was detected. Since this allele is not associated with yellow rust resistance, these samples are considered genetically susceptible (Figure 3).

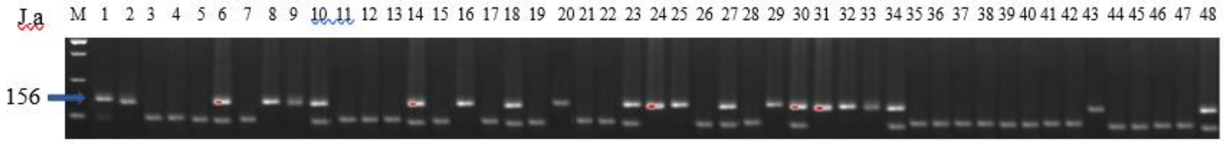

Figure 3. Gel electropherogram of the Xgwm120 DNA marker genetically associated with yellow rust resistance. See Table 1 for the list of research samples (1–48).

Based on the conducted study, PCR analyses were performed using 11 DNA markers to identify genetic resistance to yellow rust (Puccinia striiformis). These markers were previously described in the scientific literature as being associated with resistance to this disease, and they were used to evaluate the potential for marker-assisted selection (MAS). Analysis showed that the 150 bp allele of the Wmc177 marker and the 156 bp allele of the Xgwm120 marker are reliably and genetically associated with resistance to yellow rust. Based on the presence of these resistance alleles, 13 genotypes were identified as resistant. Furthermore, phenotypic evaluations conducted under both laboratory and field conditions were taken into account.

Comparison of molecular (genotypic) and phenotypic data indicated that samples 1, 2, 8, 9, 16, 20, 29, 31, and 32 were consistently resistant to yellow rust, as confirmed by both marker analysis and phenotypic expression. Based on these results, these genotypes can be recommended as genetic sources of yellow rust resistance in future breeding programs. In particular, genotypes confirmed by molecular markers as resistant can serve as key donors for developing durable resistant wheat varieties. The main objective of this study was to identify genetic factors influencing grain and flour quality in wheat (Triticum aestivum L.) and to select promising genotypes suitable for use in breeding programs. For this purpose, PCR analyses using five different primers were conducted on the selected 48 wheat samples. Specifically, PCR analysis using the UMN19 DNA marker is shown in the electropherogram in Figure 4. A molecular weight marker (M) was used, and all 48 samples were tested. Results indicated that seven samples (7, 9, 14, 18, 23, 24, and 25) carried the 367 bp specific UMN19 allele, which reflects the presence of alleles associated with grain and flour quality in these genotypes. These results not only highlight the distribution of the UMN19 marker among genotypes but also serve as a reliable molecular indicator for detecting genetic variation related to grain and flour quality.

In future breeding programs, genotypes carrying this marker may serve as an important genetic resource for developing high-quality wheat varieties.

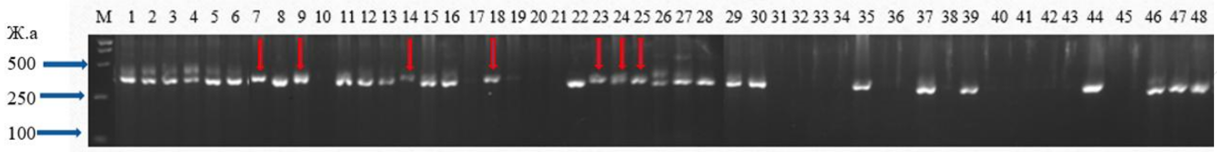

Figure 4. PCR results using the UMN19 DNA marker. M – molecular weight marker; (bp 367), samples 1–48.

During this study, special attention was given to identifying the key molecular-genetic factors influencing wheat grain quality and derived products. For this purpose, PCR analyses using the UMN25 DNA marker were performed on the selected 48 wheat samples to detect genetic traits associated with flour and grain quality. The results of the analysis are presented in the electropherogram shown in Figure 5. PCR products were evaluated relative to a molecular weight marker, and alleles in each sample were identified. The results revealed that in nine samples (1, 10, 15, 20, 25, 30, 33, 35, and 47), the UMN25 marker displayed a specific 299 bp allele, which is presumed to be associated with key genetic determinants of flour quality and grain composition.

The presence of the UMN25 allele in these samples indicates that these genotypes carry alleles influencing flour and grain quality. This makes these genotypes valuable as genetic donors in breeding programs aimed at developing wheat varieties with superior quality traits.

The use of marker-assisted selection (MAS) enables breeders to identify such genotypes efficiently, providing a genetically informed and reliable selection strategy.

Therefore, genotypes carrying the UMN25 DNA marker represent important genetic resources that may positively affect flour quality, technological properties of the grain, and overall nutritional value in future breeding programs. Identification of molecular markers associated with these genotypes significantly enhances the efficiency and precision of selection efforts.

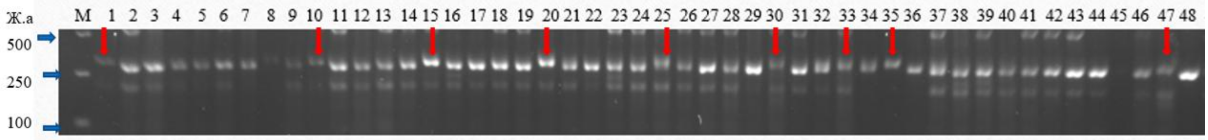

Figure 5. PCR results using the UMN25 DNA marker. M – molecular weight marker; (bp 299). Samples 1–48.

Phylogenetic tree analysis based on DNA markers proved to be a crucial tool for assessing the genetic diversity among the wheat samples. This analysis revealed the genetic relationships among 47 wheat genotypes, which were grouped according to their genetic distances. The network structure observed in the tree reflects the presence of varying levels of genetic divergence among the samples.

During the analysis, it was observed that the wheat genotypes 1, 16, 20, 30, 31, 32, and 33, identified as resistant to yellow rust (Puccinia striiformis f. sp. tritici), clustered closely together. This indicates a high degree of genetic similarity among these genotypes, confirming that resistance traits can be reliably detected through DNA markers. Notably, the very low phylogenetic distance values among these genotypes (ranging from 0.000 to 0.142) further substantiate their close genetic relatedness. This observation demonstrates a clear correlation between molecular marker-based analysis and phenotypic resistance to yellow rust. The overall structure of the phylogenetic tree confirms high genetic diversity across the wheat samples. Some genotypes exhibited relatively large genetic distances, indicating significant genotypic differences. This highlights the richness of genetic resources within the studied population, offering substantial opportunities for future wheat breeding programs. It is particularly noteworthy that the yellow rust-resistant genotypes clustered together, confirming that the observed resistance traits have a genetic basis. These findings are especially valuable for marker-assisted selection (MAS), enabling the early identification and selection of resistant genotypes.

Overall, the phylogenetic analysis based on DNA markers provided a robust methodological foundation for evaluating genetic diversity, identifying disease-resistant genotypes, and selecting promising candidates for wheat breeding programs (Figure 6).

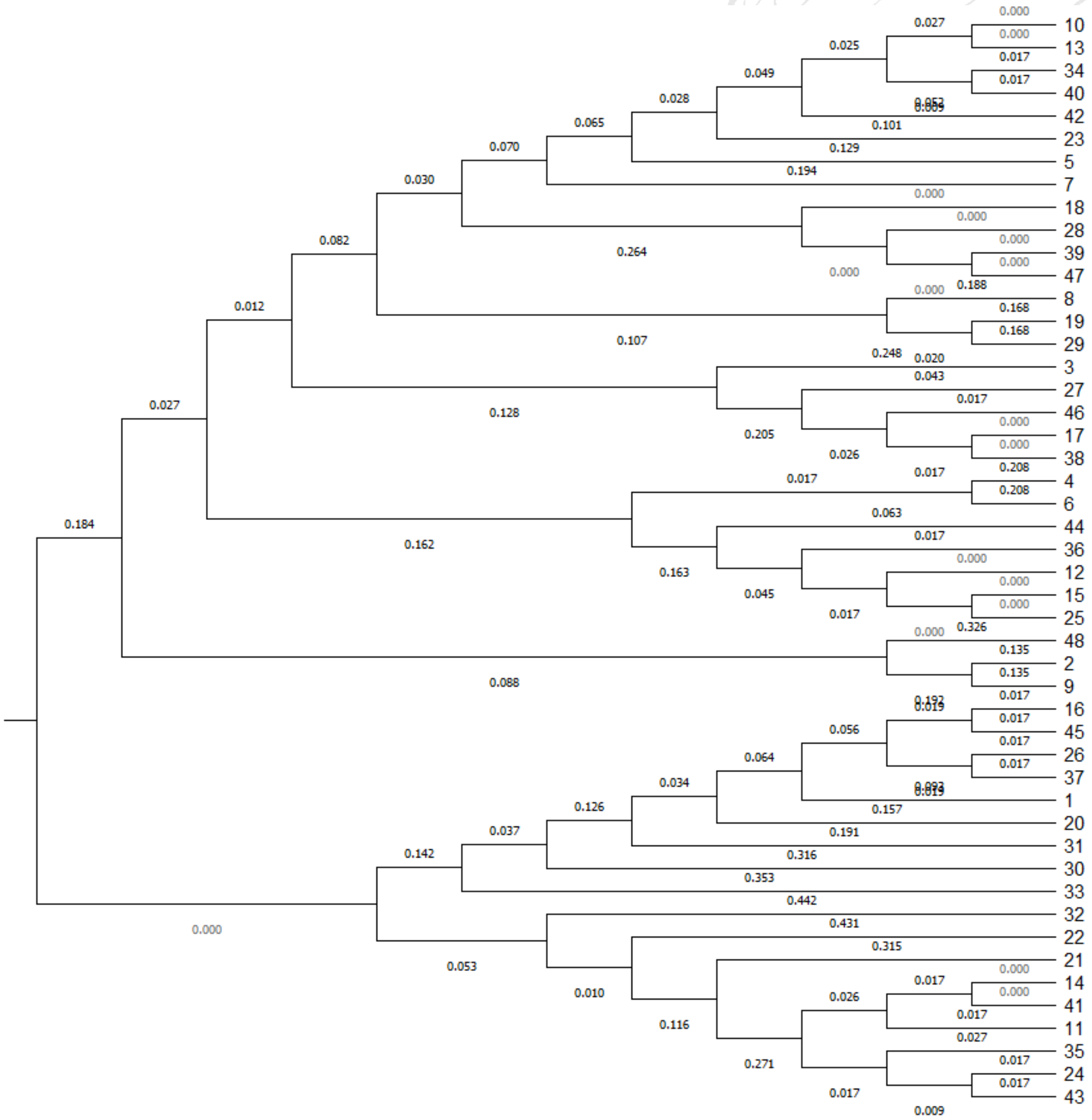

Figure 6. Cluster analysis of wheat samples based on yellow rust resistance alleles.

In this study, bread wheat (Triticum aestivum L.) genotypes were evaluated at the molecular-genetic level to determine their resistance to yellow rust (Puccinia striiformis), as well as their genetic traits associated with grain and flour quality, in order to identify promising genotypes for use in breeding programs. For this purpose, 48 wheat samples were selected, and PCR (polymerase chain reaction) analyses were conducted using several reliable DNA markers.

For assessing resistance to yellow rust, the Wmc177 and Xgwm120 markers were recognized as reliable molecular indicators. The analysis revealed that the 150 bp allele of Wmc177 and the 156 bp allele of Xgwm120 are genetically associated with resistance. Genotypic evaluation based on these two markers confirmed that samples 1, 2, 8, 9, 16, 20, 29, 31, and 32 exhibited both genotypic and phenotypic resistance to yellow rust. These genotypes are therefore highly valuable as donor lines for developing rust-resistant wheat varieties.

Furthermore, genetic traits influencing the technological and nutritional properties of grain and flour were assessed using the UMN19 and UMN25 DNA markers. The 367 bp allele of UMN19 was detected in samples 7, 9, 14, 18, 23, 24, and 25, indicating its association with high flour quality. Similarly, the 299 bp allele of UMN25 was observed in samples 1, 10, 15, 20, 25, 30, 33, 35, and 47, suggesting its relevance as a molecular indicator of grain quality.

Based on the overall evaluation, samples 1, 2, 8, 9, 16, 20, 25, 29, 31, and 32 were identified as promising genotypes, combining both yellow rust resistance and genetic advantages for flour and grain quality. These genotypes can serve as key genetic resources for developing new, high-quality, disease-resistant wheat varieties through marker-assisted selection (MAS).

In summary, the conducted molecular-genetic analyses provide a solid scientific basis for accelerating selection processes, enhancing breeding efficiency, and guiding the development of superior wheat varieties. Based on these findings, the Wmc177 and Xgwm120 markers are recommended as reliable indicators of yellow rust resistance, while UMN19 and UMN25 markers are validated as effective markers for genetic traits associated with flour and grain quality.

Conclusion

The present study comprehensively evaluated 48 bread wheat (Triticum aestivum L.) genotypes at the molecular-genetic level to elucidate their resistance to yellow rust (Puccinia striiformis) and allelic variation associated with grain and flour quality traits. Using a panel of reliable DNA markers, including Wmc177, Xgwm120, UMN19, and UMN25, PCR-based genotyping revealed distinct allelic polymorphisms that correlate with phenotypic performance and agronomically relevant traits. The 150 bp allele of Wmc177 and the 156 bp allele of Xgwm120 were unequivocally associated with genetic resistance to yellow rust. Genotypes 1, 2, 8, 9, 16, 20, 29, 31, and 32 carrying these alleles exhibited both homozygous and heterozygous configurations, confirming their stable resistance and demonstrating their potential as primary donor lines in marker-assisted selection (MAS) programs for rust-resistant cultivars. Allelic distribution analyses further indicated that heterozygous genotypes may confer intermediate resistance, while genotypes lacking these alleles are genetically susceptible, highlighting the practical utility of these markers for early-stage screening in breeding pipelines.

Concurrently, the UMN19 (367 bp) and UMN25 (299 bp) alleles were identified as robust molecular indicators of superior flour and grain quality traits. Selected genotypes harboring these alleles displayed favorable genetic predispositions for high-quality end-use products, establishing these markers as valuable tools for quality-oriented breeding.

Phylogenetic and cluster analyses based on combined marker data delineated the genetic structure and diversity of the evaluated germplasm. Yellow rust-resistant genotypes clustered together, reflecting their genetic coherence, while the overall population retained considerable genetic variability, ensuring ample scope for future breeding interventions targeting both disease resistance and grain quality improvement. In summary, this integrative molecular-genetic characterization enables the precise identification of wheat genotypes combining rust resistance with superior flour and grain quality, providing a scientifically rigorous foundation for MAS-driven breeding strategies. The study demonstrates that Wmc177 and Xgwm120 are reliable markers for yellow rust resistance, whereas UMN19 and UMN25 effectively capture allelic variation underlying grain and flour quality, thereby facilitating the development of high-yielding, disease-resistant, and quality-enhanced wheat cultivars.

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