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MOLECULAR MARKER-BASED EVALUATION OF MICRO YIELD TRIAL FOR LEAF RUST AND YELLOW RUST

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Abstract

Like in many other wheat-producing regions of the world, Pakistan's wheat crops are susceptible to two common fungal diseases: leaf (Lr) and yellow rust (Yr). The present study was conducted to screen rust resistance potential of 50 advance lines of spring wheat. In this work, DNA markers associated with various genes that provide resistance to leaf rusts and yellow rust were used. A total of 10 genes, consisting of seven Lr (Lr10, Lr19, Lr28, Lr29, Lr34, Lr46, Lr67) and three Yr (Yr5, Yr10, Yr15) were studied through linked DNA markers. Marker Lr10 and SCS265 identified the presence of the Lr10 and Lr 19 gene in 30 and one advance lines out of the 50 advances lines correspondingly, indicating their resistance to leaf rust. Marker SCS421 for Lr 28 and Lr 29 for Lr 29 amplified in 34 and 50 wheat advance lines. Marker csLV34 and XMC44 detected the presence of the Lr34/YR18/Pm38 and Lr46/YR29/Pm39 gene in 5 and 13 lines respectively. The Yr-5/Yr43 and Yr 10 genes were detected in 9 and 31 wheat lines by Wms501 and Xpsp3000, suggesting that these lines may be resistant to rust. There was no amplification of markers CFD 23 for Lr67/Yr46/Sr55/ and Xgwm413 for Yr 15 in any wheat line. This particular subset of wheat lines, including AKHBAR-19, 10141, V-20330, PGMB-20-48, V-19080, INDUS-21, NR-564, and WVH-1214, attributed to selective breeding practices or spontaneous allelic accumulation.

Keywords: Leaf or brown rust, Rust percentage**,** SSR markers, Strip or yellow rust, Wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important staple food in over 40 countries that constitute about 27% of the world's population (Sharma *et al*., 2019). It is known as king of cereal due to the high economic value (Sunil kumar *et al*., 2022). Wheat was grown on 9.6 million hectares (mha) in 2023-24, a 6.6% increase from the previous year's area of 9.0 mha. Wheat adds 2.2% of the GDP and 9% of the total value added in agriculture. Wheat yield production increased by 11.6% to 31.4 million tonnes, from 28.2 million tonnes in 2023-24 (Economic Survey of Pakistan, 2023-24).

Different types of pathogens are the sources of commercially significant diseases in wheat. There are three types of rusts: wheat stem rust, also referred to as black rust; wheat leaf rust, often referred to as brown rust; and wheat stripe rust, frequently referred to as yellow rust. The causing agents behind these diseases are *Puccinia graminis*, *P. triticina*, and *P. striiformis*, respectively. Wheat leaf rust is comparatively more widely distributed than stem rust and yellow rust (Khan *et al*., 2021; Ismail *et al*., 2021). In the event of a severe outbreak, major wheat-producing countries in Asia, such as Pakistan, could experience production losses of up to 70%. Even a yield reduction of only 10% is estimated to result in economic losses exceeding 80 million USD. In regions like Central and Northern Punjab, where generally warm weather creates ideal conditions for the disease's spread, the disease continues to pose a serious threat to Pakistan's wheat crop. Effective disease control

could ensure minimal losses in the coming years with the widespread use of resistant cultivars (Ismail *et al*., 2021).

Rust disease is easily controllable for a period by using resistant varieties. However, due to the constant changes in the rust population, this is a never-ending battle. According to previous reports, Pakistan, India, France, and Mexico have been facing variations in pathogenicity and evolving pathogens that can infect previously known resistant varieties. Breeding for long-term resistance is challenging due to continuous variations in pathogens, as rust infections can produce distinct races with related virulence. As a result, the effectiveness of varieties based on widely used resistance genes may last only a few years until the disease acquires equivalent pathogenicity (Ali *et al*., 2017; Khan *et al*., 2021).

Because marker-assisted selection (MAS) improves the parent plant genome and enables the precise transfer of genes of interest, researchers are closely monitoring this technique (Parveen *et al*., 2014). Besides resistance genes that work at the seedling stage, some genes provide resistance at the adult plant stage, such as Lr34, Lr46, and Lr67. Other well-known leaf rust resistance genes that function in adult plants include Lr68, Lr74, Lr75, Lr77, and Lr78. In certain situations, these genes collaborate with seedling resistance genes such as Lr10, Lr13, Lr16, and Lr18, which give resistance when combined with other genes, notably Lr34 (Bokore *et al*., 2020). DNA SSR markers for several yellow rust resistance genes have been reported. These include Yr5, Yr9, Yr15, Yr17, Yr26, Yr33, and YrH52. These markers are available for marker-assisted selection (MAS) in wheat. They help in identifying and breeding rust-resistant varieties. SSR markers are frequently used for yellow rust resistance gene pyramid because of their highly polymorphic and co-dominant nature. A co-dominant marker allows the detection of both alleles in a genotype. It helps to distinguish homozygous and heterozygous individuals in a population. (Jamil *et al*., 2020). The present study was conducted to explore the potential of 50 advanced lines of spring wheat.

MATERIALS AND METHODS

Wheat leaves of 50 genotypes were collected from the field of Agricultural Biotechnology Research Institute, Faisalabad (Table 1). For DNA isolation from leaves, CTAB method with ISO-17025 protocol-based modifications was used. DNA quantified with the Nanodrop 2000 Spectro photo meter. A total of 10 genes, consisting of seven Lr (Lr10, Lr19, Lr28, Lr29, Lr34, Lr46, Lr67) and three Yr (Yr5, Yr10, Yr15) were studied through linked DNA markers as shown in table 2 (MASWheat, 2021).

PCR Reaction and gel electrophoresis

2.0 µL of 10X PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 1.5 µL of 2.0 mM dNTPs, 1.0 µL of each of the forward and reverse primers at 1.0 µM, 0.2 µL of 5 U/µL Taq DNA polymerase, 2.0 µL of 30 ng/µL of isolated DNA, and 10.8 µL of ddH_2O were the components of the master mix for the PCR analysis with the final volume of 20 µL. The thermal cycler PCR profile was as follows as shown in table 1. 2% agarose gel was used to separate the PCR products. A 50 bps DNA ladder was loaded to estimate the fragment size. The gel was visualized using an Ultra Nyc Photonyx gel documentation system.

Table 1: Advance lines used for screening against rust

Sr. No.	Entries	Sr. No.	Entries	Sr. No.	Entries	Sr. No.	Entries	Sr. No.	Entries
1	V-20337	11	V-19590	21	V-20352	31	EV-19103	41	NR-564
2	V-19532	12	20C207	22	10HP-428	32	19BT022	42	WV-1196
3	AKBAR-19	13	PGMB-20-43	23	V-20330	33	BF-7799	43	NR-560
4	V-19559	14	TWS-1902	24	PGMB20-48	34	INDUS-21	44	19C160
5	V-20395	15	IS-18565	25	RUSTAM-21	35	PAKISTAN-13	45	WVH-1214
6	V-20355	16	10141	26	NW-74	36	IS-18363	46	191297
7	HYT-10-95	17	180007	27	V-19080	37	V-20241	47	JM-1683
8	V-20418	18	NR-559	28	AZP-21	38	AZRI-8	48	JM-1215
9	BF-20105	19	BF-7792	29	19C166	39	B-19261	49	18FJ-01
10	TWS-1907	20	TWS-1926	30	NW-103	40	NR-561	50	GOLDEN-100

Table 2: Primers sequences of 10 linked SSR markers to screen against rust disease

Markers	Gene	Forward (F:) and Reverse (R:) SSR Primer	T_n(ºC)
Lrh10	Lr10	F: GAAGCCCTTCGTCTCATCTG R: TTGATTCATTGCAGATGAGATCACG	56
SCS265	Lr19	F: GGC GGA TAA GCA GAG CAG AG R: GGC GGA TAA GTG GGT TAT GG	56
SCS421	Lr28	F: ACA AGG TAA GTC TCC AAC CA R: AGT CGA CCG AGA TTT TAA CC	56
Lr29	Lr29	F24: GTG ACC TCA GGC AAT GCA CAC AGT R24: GTG ACC TCA GAA CCG ATG TCC ATC	60
csLV34	Lr34/YR18/ Pm38	F: GTT GGT TAA GAC TGG TGA TGG R: TGC TTG CTA TTG CTG AAT AGT	56
XMC44	Lr46/YR29/ Pm39	F: GGT CTT CTG GGC TTT GAT CCT G R: GTT GCT AGG GAC CCG TAG TGG	56
CFD23	Lr67/Yr46/Sr55/ Pm46/Ltn3	F: CAA TAA GTA GGC CGG GAC AA R: TGT GCC AGT TGA GTT TGC TC	60
Wms501	Yr5/Yr43	F: CTC ACG CAT TTG ACC ATA TAC AAC T R: TAT TGC ATA ACA TGG CCT CCA GT	60
Xpsp3000	Yr10	F: TCAAAGACATCAAGAGCCGC R: TGGCCTACATGAACTCTGGAT	45
Xgwm413	Yr15	F: TGCTTGTCTAGATTGCTTGGG R: GATCGTCTCGTCCTTGGCA	56

Table 3: PCR thermal cycle profile

Steps	Temperature	Time	Cycles
Initial Denaturation	94°C	5 minutes,	1
Final Denaturation	94°C	1 minute	35
Annealing	according to the primer as specified in table 1.1	1 minute	
Initial Extension	72°C	1 minute	
Initial Extension	72°C	7 minute	1

RESULTS

Among the markers used, Lrh10 revealed the presence of Lr10 gene in 30 out of the 50 wheat lines as shown in table 1.3 and figure 1. This indicated that these specific lines possess resistance against leaf rust, a valuable trait in wheat breeding programs. Similarly, marker SCS265 confirmed the presence of the Lr19 gene, responsible for leaf rust resistance, in only one of the 50 tested lines, showing an amplified fragment of 512 bps. The Lr28 gene was found in 34 of the wheat lines under consideration using marker SCS421. (Table 1.4 and figure 1). The presence of this gene suggested resistance to leaf rust in these lines, with an amplified fragment of the expected 570 bps length. Additionally, marker Lr29 indicated that all 50 lines possess the Lr29 gene, signifying resistance against leaf rust, as demonstrated by the presence of a 150 bps amplified fragment.

Five out of fifty wheat lines had the Lr34/Yr18/Pm38 gene, according to marker csLV34 (Figure 2). Because the marker is co-dominant, gel electrophoresis revealed two bands, 150 bps and 229 bps. Some lines were still susceptible in the field because the 150 bps band denotes resistance and the 229 bps band indicates susceptibility.

The Lr46/Yr29/Pm39 gene was found in 13 wheat lines using marker XMC44. Due to the co-dominant nature of the marker, the gel also displayed two fragments, 242 bps (resistant) and 240 bps (susceptible). Since the marker CFD23 did not result in any amplification in the advanced lines, the gene Lr67/Yr46/Sr55/Pm46/Ltn3 was not found in any wheat line (Table 4).

Marker Wms501 provided crucial insights into the genetic composition of the 50 advanced wheat lines, confirming the presence of genes associated with rust resistance. Specifically, it identified the presence of Yr-5/Yr43 gene in nine of these lines as shown in table 1.5 and figure 1. This information highlights the potential rust resistance of these lines, which is a valuable trait in wheat breeding programs. Another marker, Xpsp3000, yielded insightful results by amplifying in 31 of the wheat lines, confirming the presence of the Yr10 gene as shown in figure 3. This gene is associated with rust resistance and significant interest to wheat breeders. Agarose gel electrophoresis of Xpsp3000 revealed four fragments, underscoring the co-dominant nature of the Yr-10 gene.

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Table 4: Presences of Lr gene in the advance lines

Markers	**Gene**	**Advance lines**	**Lr gene**	**Fragment size (bps)**
Lrh10	Lr10	V-20337, V-19532, V-19559, V-20395, V-20355, HYT-10-95, V-20418, BF-20105, TWS-1907, V-19590, 20C207, 180007, NR-559, 10HP-428, V-20330, PGMB20-48, RUSTAM-21, V-19080, 19C166, BF-7799, INDUS-21, PAKISTAN-13, B-19261, NR-561, WV-1196, NR-560, 19C160, WVH-1214, JM-1683, JM-1215	+	310
SCS265	Lr19	TWS-1902	+	512
SCS421	Lr28	V-20337, V-19532, AKBAR-19, V-19559HYT-10-95, V-20418, BF-20105, TWS-1907, V-19590, 20C207, TWS-1902, 10141, 180007, NR-559, TWS-1926, V-20352, V-20330, PGMB20-48, AZP-21, NW-103, EV-19103, 19BT022, BF-7799, INDUS-21, PAKISTAN-13, IS-18363, AZRI-8, B-19261, NR-561, NR-564, WV-1196, NR-560, 19C160, WVH-1214	+	570
Lr29	Lr-29	Present in all genotypes	+	150
csLV34	Lr34/YR18/ Pm38 (codominant)	V-20355, TWS-1907, TWS-1902, 10141, WV-1196	+ (150bps) - (229bps)	150
XMC44	Lr46/YR29/ Pm39 (codominant)	V-19532, AKBAR-19, TWS-1926, V-20352, V-20330, PGMB20-48, V-19080, 19C166, NW-103, V-20241, NR-564, JM-1683, 18FJ-01	+ (242) -(240)	242
CFD23	Lr67/Yr46/Sr55/ Pm46/Ltn3	Absent in all genotypes	-	211

Table 5: Presences of Yr gene in the advance lines

Markers	Gene	Advance lines	Lr gene	Desire fragment size (bps)
Wms501	Yr5/Yr43	AKBAR-19, V-20418, 10141, BF-7792, RUSTAM-21, NW-74, V-19080, 19BT022, INDUS-21	+	176
Xpsp3000	Yr10 (codominant)	V-20337, AKBAR-19, V-19559, V-20355, HYT-10-95, TWS-1907, 20C207, PGMB-20-43, IS, 18565, 10141, 180007, NR-559, BF-7792, TWS-1926, V-20352, 10HP-428, V-20330, PGMB20-48, RUSTAM-21, V-19080, AZP-21, 19C166, NW-103, EV-19103, INDUS-21, IS-18363, B-19261, NR-561, NR-564, WV-1196, NR-560	+(220bps) -(240bps) +(260bps) +(286bps)	260
Xgwm413	Yr-15 (codominant)	Absences in all advance lines	+(90,95bps) -(100bps)	90, 95

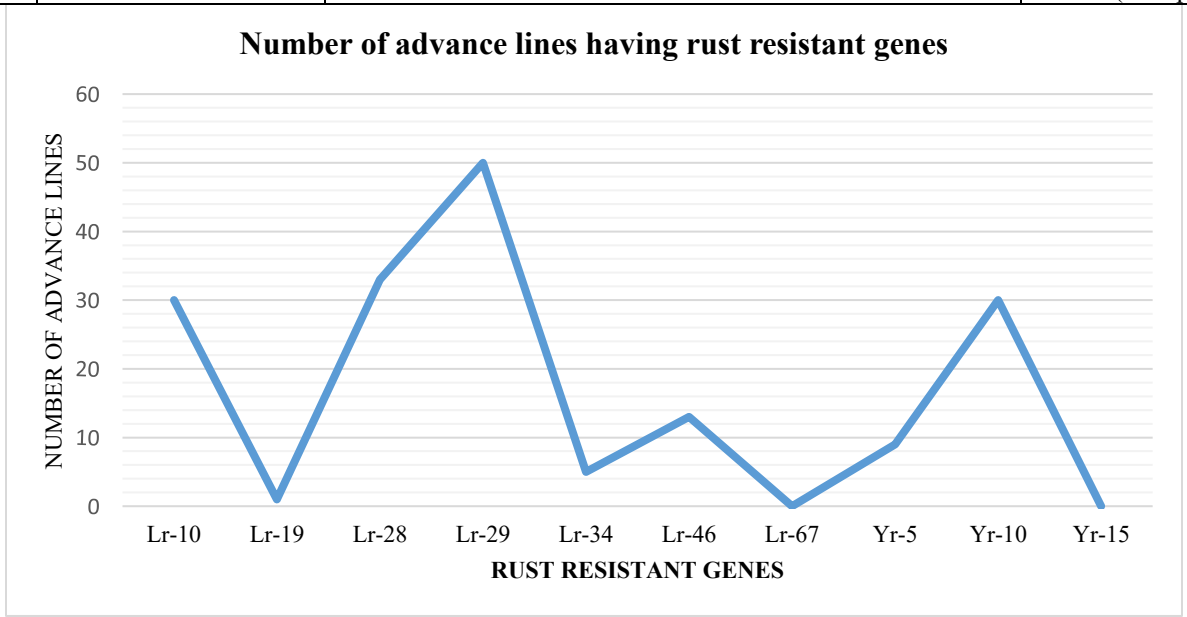

Figure 1: Number of advance lines having rust resistant genes

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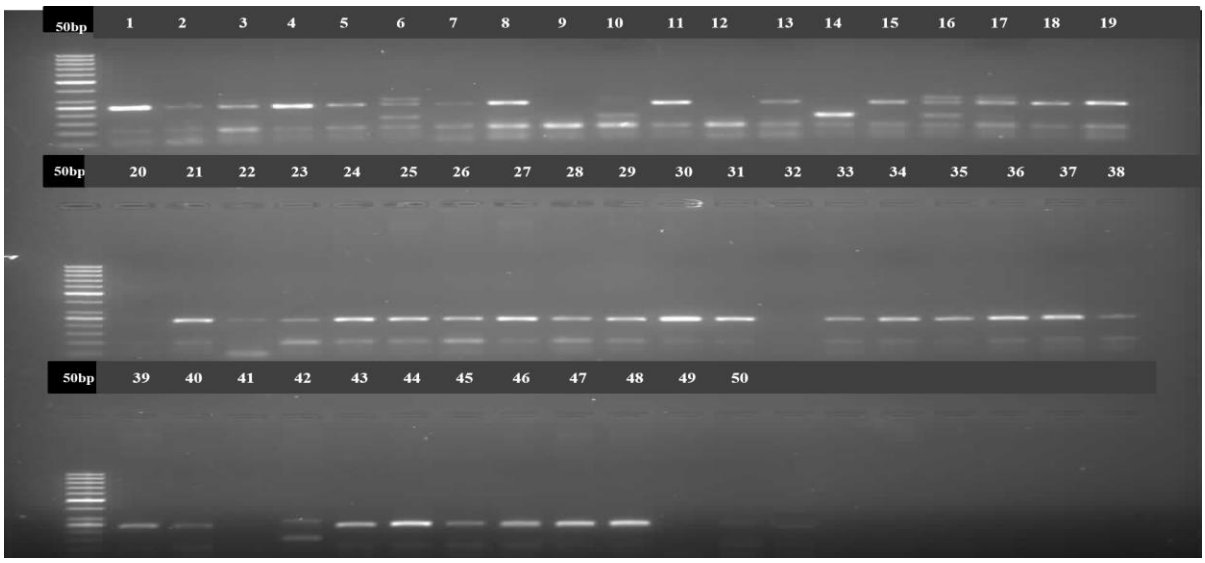

Figure 2: Amplification of Lr34 in 50 advance lines

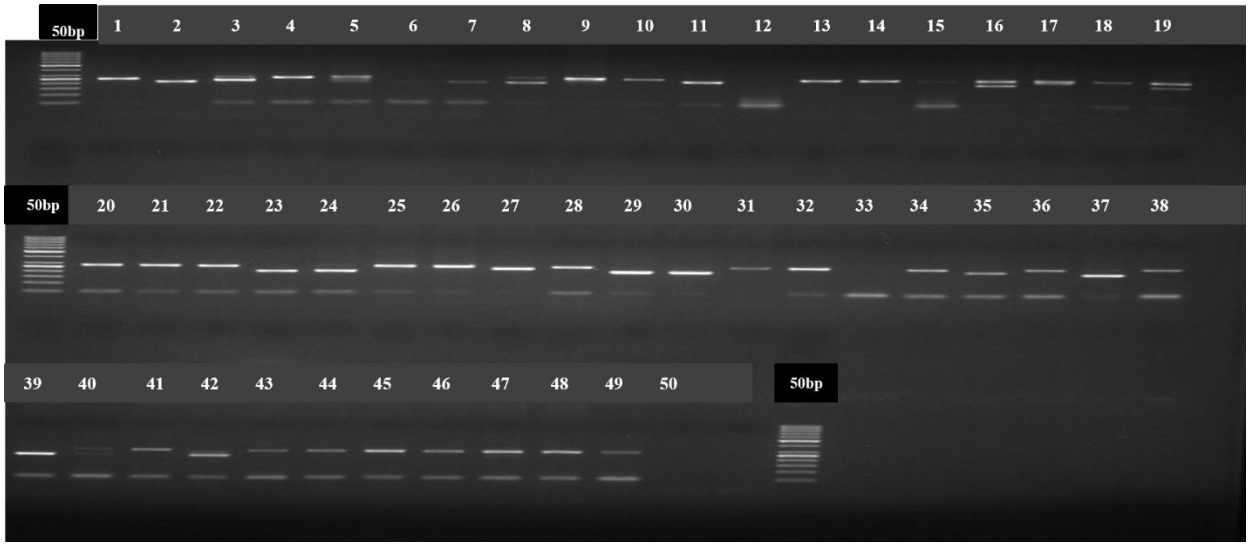

Figure 3: Amplification of Yr-10 in 50 advance lines

Three of these fragments measured 220 bps, 260 bps, and 286 bps, which are indicative of resistance, while one fragment was 240 bps, signifying susceptibility to rust. Conversely, marker Xgwm413 did not amplify in any of the tested lines, providing evidence for the absence of the related Yr15 gene in these advanced wheat lines (Table 5). This absence of the gene underscores the importance of genetic diversity and the potential need for further breeding efforts to introduce rust resistance into these lines. These findings provided critical information about the rust resistance profiles of the advanced wheat lines, offering valuable insights into future breeding efforts aimed at developing rust-resistant wheat varieties.

In a comprehensive assessment of 50 advanced wheat lines, a noteworthy observation emerged as only 8 of these lines displayed a markedly elevated allelic frequency of Lr (leaf rust) and yr

(yellow rust) resistance genes. This particular subset of wheat lines, including AKHBAR-19, 10141, V-20330, PGMB-20-48, V-19080, INDUS-21, NR-564, and WVH-1214, exhibited a distinctive genetic profile in terms of rust resistance, as visually depicted in Figure 4. This observation carries significant scientific implications, reflecting the complex genetic landscape of wheat. It is suggestive of a genetic enrichment that may be attributed to selective breeding practices or spontaneous allelic accumulation. Furthermore, these results underscore the intricate multigenic nature of rust resistance in wheat, where combinations of resistance alleles in these lines may confer robust immunity to both leaf rust and yellow rust.

DISCUSSION

In this study, a comprehensive screening of 50 advanced wheat lines was conducted for evaluating the presence of genes associated with resistance against leaf rust and yellow rust by utilizing 10 diseases linked SSR markers. Our results shed light on the genetic composition of these wheat lines and their potential for rust resistance, which is of great significance in wheat breeding programs.

Among the SSR markers used, Lrh-10 was instrumental in identifying the presence of the Lr-10 gene in 30 out of the 50 wheat lines. The presence of Lr-10 in these specific lines is a promising indication of their resistance against leaf rust, a highly desirable trait in wheat breeding according to the Parveen *et al*., 2014. Similarly, screening with marker SCS265 confirmed the presence of the Lr19 gene, known for its role in conferring leaf rust resistance. The observed amplified fragment of 512 bps in only one line is consistent with the expected length reported in the literature, provides further validation of this finding (Gupta *et al*., 2006). According to the Parveen *et al*., 2014, marker SCS265 with gene Lr19 amplified 130 bps fragment which is contrary to the finding of this study.

Marker SCS421 was employed to identify the Lr28 gene in 34 out of the 50 wheat lines which is a substantial number. The presence of Lr28 in these lines strongly suggests resistance to leaf rust, supported by the detection of an amplified fragment of the anticipated 570 bps length in agarose gel electrophoresis analysis (Revathi *et al*, 2020). Additionally, screening using marker Lr29 revealed that all 50 lines having the Lr29 gene with 150bps fragment size, indicative of their resistance against leaf rust. But it was differently demonstrated by the presence of a 900 bps amplified fragment for Lr29 (Błaszczyk *et al*., 2008).

The results obtained from marker csLV34 were intriguing, as they unveiled the presence of the Lr34/YR18/Pm38 gene in five of the 50 lines. However, it is noteworthy that these lines remain susceptible to rust, as agarose gel electrophoresis analysis revealed the co-dominant nature of the Lr34/YR18/Pm38 gene, resulting in two distinct bands one at the desired 150 bps length and the other at 229 bps, which is associated with susceptibility (Tomkowiak *et al*., 2021).

Marker XMC44 played a pivotal role in the identification of the Lr46/YR29/Pm39 gene in 13 lines. Similar to csLV34, our gel electrophoresis analysis displayed two fragments, one at the expected 242 bps length, indicating resistance, and the other at 240 bps, signifying susceptibility due to the co-dominant nature of Lr46/YR29/Pm39 (Chemayek *et al*., 2020).

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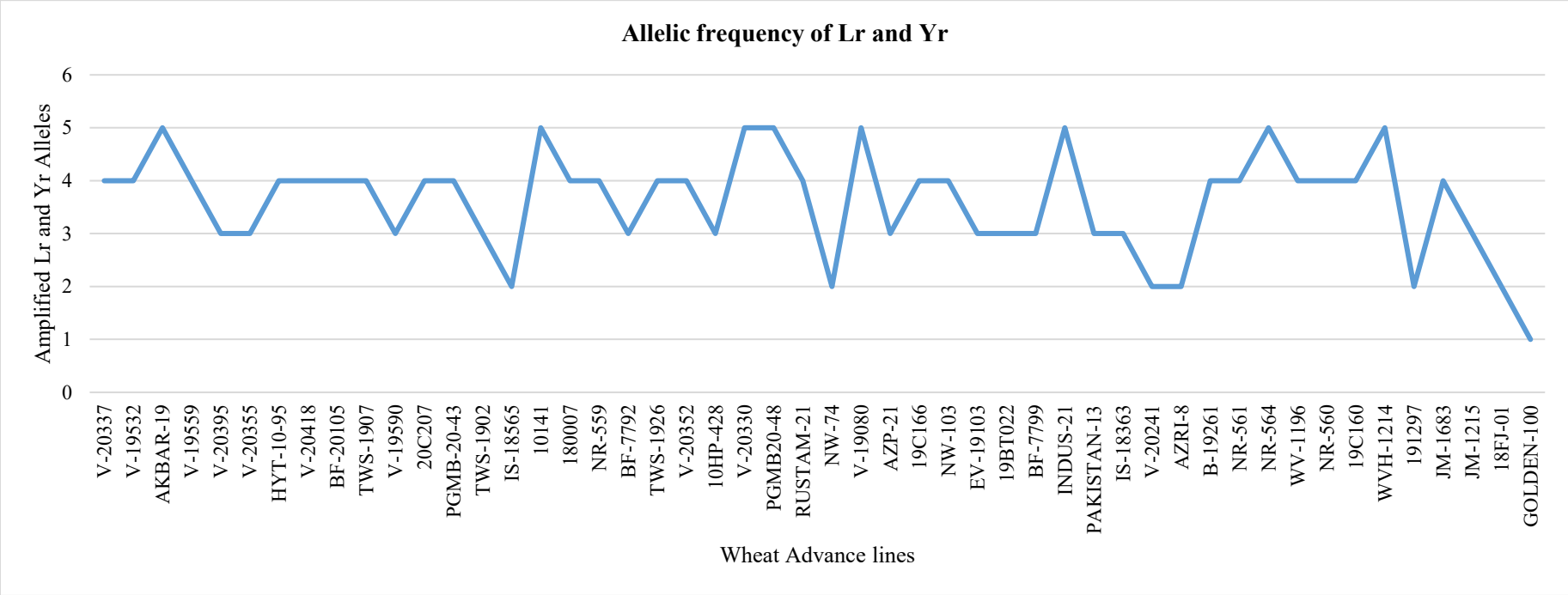

Figure 4: Allelic frequency of Lr and Yr per advance line

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Notably, genes such as Lr67/Yr46/Sr55/Pm46/Ltn3 were conspicuously absent in all lines, as indicated by the failure of the related marker CFD23 to amplify in any of the advanced lines. In our investigation, the utilization of Marker Wms501 proved to be invaluable in unraveling the genetic underpinnings of rust resistance in the 50 advanced wheat lines. This marker confirmed the presence of the Yr-5/Yr43 gene in nine of these lines. This finding serves as a significant indicator of the potential rust resistance harbored by these lines, which holds great promise for their incorporation into wheat breeding programs (Skowrońska *et al*., 2020).

Equally compelling were the results obtained through Marker Xpsp3000, which was successfully amplified in 31 of the wheat lines, demonstrating to the presence of the Yr10 gene. The Yr10 gene is a well-known player in rust resistance and is of significant interest to wheat breeders (Kokhmetova *et al*., 2021). Notably, the agarose gel electrophoresis analysis of Xpsp3000 yielded four distinct fragments, a clear reflection of the co-dominant nature of the Yr-15 gene. Three of these fragments, measuring 220 bps, 260 bps, and 286 bps, stand as strong indicators of resistance, while the presence of one fragment at 240 bps suggests susceptibility to rust. This intriguing result highlights the complexity of rust resistance mechanisms within these lines (Kokhmetova *et al*., 2021).

Conversely, the outcomes of Marker Xgwm413 were distinctive, as it failed to amplify in any of the tested lines. This lack of amplification provides compelling evidence for the absence of the related Yr-15 gene in these advanced wheat lines. The absence of this gene underscores the importance of genetic diversity and signals a potential avenue for further breeding efforts to introduce rust resistance attribute into these lines (Kokhmetova *et al*., 2021).

The subset of wheat lines, which includes AKHBAR-19, 10141, V-20330, PGMB-20-48, V-19080, INDUS-21, NR-564, and WVH-1214, stands out with a unique genetic profile regarding rust resistance as similar discussed by Akhtar *et al*., 2021. This finding holds profound scientific significance, shedding light on the intricate genetic intricacies within the realm of wheat. It implies the existence of a genetic enrichment phenomenon that could be ascribed to either deliberate selective breeding efforts or spontaneous accumulation of allelic variants.

CONCLUSION

This subset of wheat lines, including AKHBAR-19, 10141, V-20330, PGMB-20-48, V-19080, INDUS-21, NR-564, and WVH-1214, exhibited a distinctive genetic profile in terms of rust resistance. This observation carries significant scientific implications, reflecting the complex genetic landscape of wheat. It is suggestive of a genetic enrichment that may be attributed to selective breeding practices or spontaneous allelic accumulation.

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CONFLICT OF INTEREST:

No potential conflict of interest was reported by the author(s).

AUTHOR'S CONTRIBUTION

All authors equally contributed to the design and execution of the experiment, data collection, analysis, and preparation of the manuscript. All authors read and approved the final version of the manuscript.

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