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Original article

Genetic Characterization and Brown Rust Resistance Screening of Gamma Irradiated Bread Wheat M4 Populations by DNA Markers

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Abstract

The rapid increase in the world population has increased the demand for wheat, and breeding studies that enable the development of high-yield varieties resistant to various stress conditions have accelerated to meet this demand. Brown rust disease poses a serious threat to sustainable wheat cultivation. Classical breeding studies are effectively used in variety development in important agricultural plant species, supported by mutation and molecular breeding studies. In recent years, retrotransposon-based molecular markers have been widely used to better understand the variation in genotypes obtained in breeding processes. In this study, 15 retrotransposon based molecular markers, iPBS primers, were used for molecular characterization in M4 bread wheat populations exposed to different doses of gamma radiation. In addition, the presence of 8 Lr genes was examined in order to determine resistance to brown rust disease, which is one of the important causes of yield loss in various wheat varieties. A total of 102 bands belonging to iPBS primers were observed in 35 genotypes belonging to the seven bread wheat varieties analyzed. The average polymorphism rate of the bread wheat populations used in the study was calculated as relatively low (5.88%). It was observed that the studied wheat varieties had six Lr gene regions (Lr13, Lr19, Lr22a, Lr24, Lr37, and Lr47) except for the Lr14a and Lr34 genes from brown rust resistance genes. The molecular data obtained from the study will provide significant information for using mutation breeding and molecular breeding in classical breeding studies in bread wheat.

Keywords: iPBS, Lr Genes, Molecular Markers, Retrotransposons, *Triticum aestivum*.

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INTRODUCTION

Wheat has a complex history of dispersal, adaptation, and selection since its introduction into the Fertile Crescent approximately 8,000 to 10,000 years ago. Triticum aestivum L. germplasm developed along ancient human migration routes and was spread by early farmers into both Western Europe and Eastern Asia. Following this dispersal, cultivated wheat populations adapted to local environments and became known as "landraces." Since the transition from a hunter-gatherer lifestyle to an agricultural one, wheat has played an indispensable role in the rapid development of civilizations. It has also been repeatedly shaped through selection to meet human nutritional needs and adapt to diverse environments (Balfourier et al., 2019).

Due to the rapid growth of the world's population, it is estimated that the population will reach approximately 10 billion by 2050. Therefore, a significant increase in wheat yield is needed to meet future global food needs (Li et al., 2020). Wheat's rapid growth, broad adaptability, productivity, and ability to meet the nutritional, trade, and socioeconomic needs of many countries allow for a global production of approximately 770 million tons (Kiraz et al., 2019). According to 2022 data from the Food and Agriculture Organization of the United Nations (FAO), the world's wheat production area is 219,153,830 hectares, and wheat production is 808,441,568.18 tons. Looking at the world's largest wheat producing countries based on average production from 1994 to 2022, the top 10 are China, India, the United States, Russia, France, Canada, Germany, Pakistan, Australia, and Ukraine. In terms of average wheat production between 1994 and 2022, wheat ranks fourth globally after sugar beet, corn, and rice, while it ranks first in Turkiye. Türkiye's share of wheat production area is 3%, and its share in total production is around 2% on average. According to 2022 data, 19,750,000 tons of wheat were produced in Turkey on 6.60 million hectares of land (FAO, 2023). This production amount has fallen below the level required to meet the rapidly growing population's seed and food needs. Due to the gradual decrease in agricultural land, wheat breeding primarily aims to increase yield per unit area (Sağır and Kara, 2021). In the 2021/22 production season, Türkiye's self-sufficiency rate in wheat production was 87.26%. However, wheat production areas have been shrinking over the years (Çelik and Sarıoğlu, 2023).

Breeding programs exist to improve wheat quality. The need for higher yields with fewer inputs, the emergence of new pests and diseases, changes in environmental conditions, and market demand are driving breeders to develop varieties that are disease-resistant, high-yielding, and resistant to biotic and abiotic stresses (Guzman and Ibba, 2023). To ensure global food security, the genetic improvement of crops is crucial, as it facilitates the development of new genotypes that can tolerate both biotic and abiotic stresses. However, conventional genetic improvement requires more time than other strategies (such as mutation breeding) due to the higher number of crop cycles required to achieve homozygosity in conventional breeding and reproductive barriers that limit the transfer of favorable alleles across

species. On the other hand, mutation breeding is an effective tool for developing new crop varieties with desired traits, such as heat stress-tolerant crops that adapt to high temperatures to maintain crop yields. Mutation is widely used in crop improvement as a source of phenotypic and genotypic diversity and also as a driving force for evolutionary diversity (Ahumada-Flores et al., 2021).

Molecular markers are used to increase biodiversity and create variation, which are among the most important factors in breeding studies. They are used in the creation of linkage maps and the tracking of individuals or lines carrying specific genes. DNA molecular markers essentially detect nucleotide sequence variation at a specific locus in the genome (Kalendar et al., 2011). Molecular markers are traits that enable the acquisition of genetic information about an organism. Markers help directly identify genetic variation of species independent of many diverse factors (i.e. tissue and organ type, developmental stage, and habitat conditions) (Shi et al., 2023). Molecular markers are indispensable tools in agricultural genetics because they create the DNA fingerprint of varieties and identify differences, as well as determining genus and species phylogeny, selecting lines harboring genes with desired traits for breeding studies, and creating linkage maps (Sipahi & Yumurtacı, 2020; Mirzaei, 2021).

There are some studies on mutation breeding in wheat and the studies are based on generally morphological characterization (Ahumada-Flores et al., 2021; Kiani et al., 2022; Bakar, 2023). It has been observed that molecular characterization-based studies on genotypes obtained through mutation breeding are limited (Aly et al., 2019). Furthermore, there are molecular studies using retrotransposon markers on various wheat genotypes or varieties (Tagimanova et al., 2014; Alkan et al., 2019; Arystanbekkyzy et al., 2019; Marzang et al., 2020; Demirel, 2020; Nadeem, 2021; Safiyar et al., 2022; Kızılgeci et al., 2022; Haliloğlu et al., 2023).

Brown rust disease causes yield losses in wheat, so chemicals are used to prevent and treat the disease. The chemical structure of synthetic fungicides has adverse effects on the health and metabolic life of humans and animals. Therefore, the use of disease-resistant wheat varieties is becoming more widespread (Afzal et al., 2023). More than 80 leaf rust (Lr) genes have been identified spread across all 21 chromosomes of wheat. While breeding studies are carried out in wheat, determining resistance to brown rust diseases with the help of molecular markers makes great contributions to the acceleration and effectiveness of the breeding process (Bilgen et al., 2023).

This study aimed to genetically characterize bread wheat (Triticum aestivum) mutant lines (M4 population) treated with gamma irradiation and developed through mutation breeding using 15 iPBS primers. Furthermore, the brown rust resistance genes Lr13, Lr14a, Lr19, Lr22a, Lr24, Lr34, Lr37, and Lr47 were analyzed using molecular markers to identify potential differences in the presence of resistance genes in wheat lines exposed to gamma irradiation.

MATERIALS and METHODS

Plant materials and DNA extraction

In this study, leaf samples of bread wheat M4 genotypes generated by gamma irradiation of seven different wheat varieties were obtained from Tekirdağ Namık Kemal University, Faculty of Agriculture, and Department of Field Crops (Table 1). Leaf tissue samples belonging to each genotype were stored at -20ºC till DNA extraction. DNA extraction protocols of Dellaporta et al., (1983) and, Doyle and Doyle (1990) were performed. The Nanodrop® LITE spectrophotometer was used to quantify and qualify the extracted DNA samples. 10 ng/µL diluted DNA samples were preserved at +4°C for downstream polymerase chain reaction (PCR) analysis.

Table 1. Wheat genotypes and gamma doses used in the study

Genotype number	Variety name	Gamma Dose (Gy)	Genotype number	Variety name	Gamma Dose (Gy)
1	Tekirdağ	0 (Control)	19	Sagittario	300
2	Tekirdağ	100	20	Sagittario	400
3	Tekirdağ	200	21	Ergene	0 (Control)
4	Tekirdağ	300	22	Ergene	100
5	Tekirdağ	400	23	Ergene	200
6	Sana	0 (Control)	24	Ergene	300
7	Sana	100	25	Ergene	400
8	Sana	200	26	Flamura 85	0 (Control)
9	Sana	300	27	Flamura 85	100
10	Sana	400	28	Flamura 85	200
11	Lider	0 (Control)	29	Flamura 85	300
12	Lider	100	30	Flamura 85	400
13	Lider	200	31	Flamura 80	0 (Control)
14	Lider	300	32	Flamura 80	100
15	Lider	400	33	Flamura 80	200
16	Sagittario	0 (Control)	34	Flamura 80	300
17	Sagittario	100	35	Flamura 80	400
18	Sagittario	200			

Molecular characterization studies with iPBS primers

In this study, primers of 15 retrotransposon-based iPBS markers selected from the study of Kalendar et al. (2011) were used in the genetic characterization of wheat genotypes (Table 2). For the

iPBS primers used in genetic characterization in the study, the PCR conditions and cycles determined in the study of Kalendar et al. (2011) were tried and the necessary optimizations were made and used. After the PCR process, electrophoresis was performed to verify whether the desired products were obtained. A 1.5% agarose gel containing RedSafe Nucleic Acid Staining Solution (2.5 µl/100 ml) was prepared for electrophoresis. The PCR products were run in 1X TBE (Tris-Borate-EDTA, pH=8.2) buffer for approximately 100 minutes at 100 volts.

Table 2. iPBS primer information used in genetic characterization of bread wheat genotypes

No	Primer Name	Primer Sequence (5' → 3')	Annealing Temperature (°C)	Observed band number	Observed band range (bp)
1	iPBS2237	CCCCTACCTGGCGTGCCA	54	4	400-1310
2	iPBS2249	AACCGACCTCTGATACCA	51	10	570-1555
3	iPBS2373	GAACTTGCTCCGATGCCA	52	6	480-1440
4	iPBS2400	CCCCTCCTTCTAGCGCCA	51	8	295-1360
5	iPBS2077	CTCACGATGCCA	50	7	220-985
6	iPBS2278	GCTCATGATACCA	48	8	325-1210
7	iPBS2375	TCGCATCAACCA	52	6	250-800
8	iPBS2377	ACGAAGGGACCA	50	8	310-1400
9	iPBS2378	GGTCCTCATCCA	50	6	150-910
10	iPBS2387	GCGCAATACCCA	50	8	195-1150
11	iPBS2390	GCAACAACCCCA	54	6	150-1070
12	iPBS2392	TAGATGGTGCCA	50	4	360-1065
13	iPBS2393	TACGGTACGCCA	50	4	375-1200
14	iPBS2095	GCTCGGATACCA	48	10	340-2135
15	iPBS2388	TTGGAAGACCCA	48	7	250-2180

Molecular marker analysis for brown rust resistance

A literature study was conducted for markers that could be used in the selection of Lr genes that can determine resistance to brown rust in wheat (Vida et al., 2010; Liu et al., 2021). Detailed information on the Lr gene regions in wheat identified as a result of the literature study and the primers used to identify these regions are given in Bilgen et al., (2023). PCR conditions and PCR cycles related to Lr gene identification were performed as described by Bilgen et al., (2023).

Data analysis

For each sample in the study, statistical analyses were performed by scoring bands identified as a result of iPBS marker analyses as present (1) if they were observed, or as absent (0) if they were not. To determine the diversity of wheat M4 populations, percentages of polymorphic loci , the number of bands observed at polymorphic loci (Na), the number of effective bands (Ne), Nei's (1987) genetic diversity

value (uh), Shannon's constant (I), and standard errors were calculated. The data obtained from the statistical analyses were evaluated using the statistical package program GenAlEx Version 6.3 (Peakall and Smouse, 2006).

In determining resistance to brown rust; the observed allele sizes for each Lr gene were compared in base pairs (bp) with both the control and the sizes of Lr gene-specific amplification alleles given in the literature to determine whether there was resistance to the Lr gene in mutant lines of each wheat variety (Röder et al., 1998; Helguera et al., 2000, 2003; Prins et al., 2001; Mustafa et al., 2013; El-Orabey et al., 2019; Bilgen et al., 2023).

RESULTS and DISCUSSION

The 15 iPBS primers generated 102 bands in 35 samples of seven bread wheat varieties analyzed. The number of bands varied from 4 to 10 bands per iPBS primer (Table 2). The lowest number of band was observed at the iPBS2237, iPBS2392, and iPBS2393 whereas the highest number of band was observed at the iPBS2249 and iPBS2095. The average polymorphism rate of the bread wheat genotypes used in the study was calculated as relatively low (5.88%). Genetic diversity parameters for the seven wheat cultivars used in this study and the percentage of polymorphic loci are given in Table 3.

The means of Nei's (1987) unbiased genetic diversity value (uh) and Shannon's constant (I) were calculated as 0.026 and 0.032, respectively. When the bands observed in all iPBS loci were evaluated, the highest polymorphism rate was observed in the Flamura80 cultivar with 13.73%. The mean observed number of bands per locus in each population (Na) was highest in the Flamura80 population (1.029) and lowest in the Sana population (0.853). The mean effective alleles per locus in each population (Ne) was also highest in the Flamura80 population (1.078) and lower in the other populations. The Shannon constant (I) was calculated with the highest value in the Flamura80 population (0.074). The highest genetic diversity values of Nei (uh) were also observed in the Flamura80 population (0.061). According to the obtained data, Flamura80 was a population richer in genetic diversity compared to the other populations, while the Sana and Tekirdağ populations were determined to be more limited in terms of genetic diversity (Table 3).

In a study conducted by Baran (2024), genetic diversity and population structure were analyzed using 12 iPBS primers (selected from 30 iPBS primers) showing high polymorphism in 70 bread wheat genotypes. A total of 170 bands were reported for the 12 iPBS primers in the study (an average of 14.17 bands per primer). Of the primers used in the study, iPBS2375 was also used in our study. The mean effective number of bands per locus was calculated as 1.37, the Shannon constant as 0.23, and the Nei's value for genetic diversity as 0.35. The results of the genetic diversity parameters obtained in the study were observed to be similar to those in our study. The study emphasized that the iPBS marker system is one of the ideal marker systems for the evaluation of genetic diversity in wheat.

Table 3. Genetic diversity parameters of the studied bread wheat varieties

Cultivar name	Na*	Ne*	I*	uh*	%P
Tekirdağ	0.863	1.014	0.012	0.010	%1.96
Sana	0.853	1.014	0.012	0.010	%1.96
Lider	0.892	1.023	0.025	0.020	%4.90
Sagittario	0.902	1.018	0.020	0.016	%3.92
Ergene	0.931	1.037	0.033	0.027	%5.88
Flamura85	0.971	1.055	0.049	0.041	%8.82
Flamura80	1.029	1.078	0.074	0.061	%13.73
Mean	0.920	1.034	0.032	0.026	%5.88
SE*	0.016	0.005	0.005	0.004	%1.59

*Na=Mean number of bands per locus, Ne=Effective number of bands, I=Shannon's information index, uh=Nei's (1987) unbiased genetic diversity value, %P=Percentage of polymorphism, SE=Standard error

In a study conducted by Haliloğlu et al. (2023), the diversity of 63 Turkish bread wheat genotypes was analyzed using iPBS-retrotransposon primers. A total of 1231 polymorphic bands were obtained from the 34 primers used. The number of bands per primer varied between 8 (iPBS2375) and 60 (iPBS2381), with an average of 36 bands. Some of the iPBS primers used in the study (iPBS2077, iPBS2278, iPBS2375, iPBS2377, iPBS2387, iPBS2390, iPBS2392, and iPBS2388) overlapped with the primers used in our study. The band numbers found in this study differ from those in our study due to the different genotypes used. The results of the studies highlight that the iPBS system is a powerful tool for the identification and classification of genetic resources.

In a study conducted by Nadeem (2021), 13 iPBS primers were used for the molecular characterization of 74 Turkish bread wheat varieties. A total of 152 bands were reported in the 13 polymorphic primers used for molecular characterization. The two primers used in this study were identical to the primers used in our study. In this study, 13 bands were observed with the iPBS2095 primer, while 10 bands were observed in our study. Similarly, 15 bands were found in the iPBS2237 primer used in the study, but only four bands were found in our study, and this primer was determined to be monomorphic in the samples used. This primer was evaluated as monomorphic because the same banding structure was observed at all loci of this primer in all studied samples regardless of variety and gamma radiation dose.

In the study of Pour et al. (2019), 15 iPBS primers were used to determine DNA methylation and genomic instability in wheat genotypes exposed to aluminum stress. In the control Haymana, Kılçıksız and Bezostaja 1 genotypes that were not exposed to the stress factor, a total of 206, 195 and 180 bands were observed for the 15 iPBS primers, respectively. The average number of bands per primer was calculated as 6.80. After different doses of aluminum application, a total of 109, 108 and 102 bands were observed to disappear in the 15 iPBS primers for Haymana, Kılçıksız and Bezostaja 1 varieties,

respectively. It was also reported that the number of polymorphic bands observed differed among aluminum application doses. According to the study conducted by Pour et al. (2019), it can be concluded that the iPBS marker system is a suitable marker for determining genetic diversity in genotypes exposed to stress factors.

In a 2022 study, 54 barley cultivars from Turkey and Bulgaria were examined for genetic diversity and population structure using 18 iPBS and 4 SCoT markers. A total of 560 bands were identified, of which 530 were reported to be polymorphic (438 iPBS and 92 SCoT). The study demonstrates that the genetic diversity of barley cultivars from Turkey and Bulgaria is high and that iPBS and SCoT markers are powerful tools in genetic diversity analyses (Güngör et al., 2022). The iPBS2400 primer used in the study reported 39 bands, but only 8 were found in our study.

According to heatmap analysis, the presence of genetic bands is expressed in red and their absence in blue tones, and the dendrogram structure reveals that the genotypes are gathered under two main genetic groups (Figure 1). Furthermore, genotypes outside the clusters that exhibited different patterns were considered genetically divergent individuals, which can be interpreted as indicators of possible mutational effects or genetic admixture. Overall, the heatmap provided a detailed observation of the genetic structure across both genotypes and bands, confirming that iPBS markers successfully demonstrated genetic structure across varieties. Furthermore, the analysis indicated that different gamma-ray doses caused variation at the genomic level. The clusters observed in the heatmap reveal difference in the genetic structure of individuals exposed to high doses, particularly 300 and 400 Gy. This suggests that radiation may affect retrotransposon activity, leading to heritable polymorphism. Furthermore, the different genetic responses of different cultivars to the same doses indicate that genetic background plays an important role in responses to radiation stress.

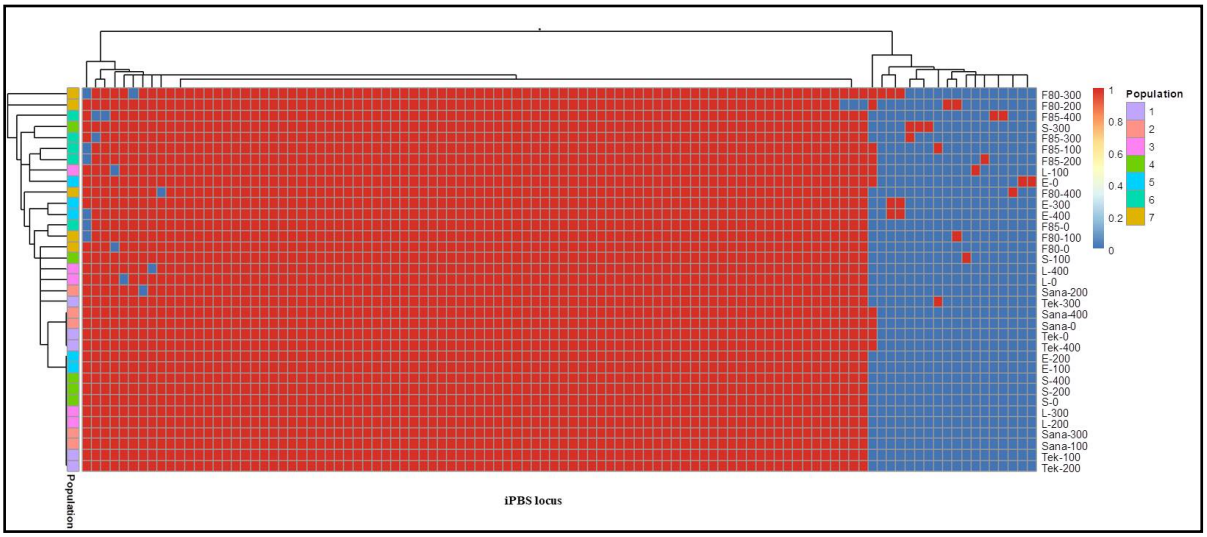

Figure 1. Heatmap showing the genotype-based distribution of genetic variation identified with iPBS data

The presence (+) and absence (-) of brown rust resistance genes in the genotypes of the studied wheat varieties to which different gamma doses were applied are presented in Table 4. As a result of the research, the band size associated with resistance in each Lr gene region is presented in Table 4. As a result of PCR amplification performed on 35 wheat varieties, bands belonging to the Lr13, Lr19, Lr22a, Lr24, Lr37 and Lr47 gene regions were observed in individuals of the control (0) and all gamma-irradiated varieties. For Lr14a gene region, a band was observed at 174 bp (Röder et al., 1998; Başer et al., 2020; Bilgen et al., 2023). While bands were observed in the Lr14a gene region in control (0) genotypes of seven variety and 26 gamma-irradiated samples, no bands were observed in the 300 Gy and 400 Gy gamma-irradiated samples of the Lider variety. Also in the study, a 150 bp band associated with Lr34 was observed in 23 of 35 samples. No bands belonging to the Lr34 gene region were observed in the control and gamma-irradiated Sana and Sagittario varieties, or in the Flamura80 samples treated with 200 Gy and 400 Gy gamma irradiation. It can be concluded that the applied gamma radiation dose can convert brown rust resistant genotypes into non-resistant genotypes in some cultivars. However, since there is no correlation between gamma ray dose and the presence/absence of brown rust resistance genes in all studied samples, the study needs to be investigated in detail with more samples and different molecular marker systems to reach a definitive conclusion.

In Mustafa et al., (2013), the genetic structure of important Lr genes (Lr10, Lr13, Lr21, Lr24, Lr26, Lr27, and Lr31) was evaluated using molecular markers in 38 commercial wheat cultivars. It was determined that Lr genes were mostly prevalent (e.g., Lr27 and Lr31), while Lr24 and Lr21 were absent. Analysis showed that the ABI 3730 capillary array method and STS (sequence tagged site) and SSR (simple sequence repeat) markers were effective in gene identification and validation. A band was visualized at 123 bp in the Lr13 gene region and at 310 bp in the Lr24 gene region. The results suggested that Lr genes can be used for the accumulation of resistance-enhancing genes through marker-assisted selection (MAS), and that the integration of these genes into future wheat cultivars is a critical step for achieving resistance to leaf rust. The allele sizes determined in the Lr13 and Lr24 gene regions in this study and the results of our study are the same in the Lr13 gene region, while the allele was observed at 310 bp in the Lr24 gene region, but in our study, the allele was observed at 300 bp. Differences in the electrophoresis technique and the programs used to determine the band sizes may have caused the band sizes to differ. Comparison with control samples confirmed that the bands reported in different studies were the same for the gene region.

As a result of the research, a PCR band at 123 bp was observed in lines containing the Lr13 gene region (Seyfarth et al., 2000; Mustafa et al., 2013; Bilgen et al., 2023). In our study, as a result of PCR amplification performed on 35 wheat varieties, the band belonging to the Lr13 gene region was observed in individuals of the control (0) and all gamma-irradiated varieties.

Table 4. Resistance of wheat genotypes to brown rust disease (Lr gene present +, Lr gene absent -)

Genotype Code	Genotype Name*	*Lr13*	*Lr14a*	*Lr19*	*Lr22a*	*Lr24*	*Lr34*	*Lr37*	*Lr47*
		123 bp	174 bp	130 bp	147 bp	300 bp	150 bp	285 bp	282 bp
1	Tek-0	+	+	+	+	+	+	+	+
2	Tek-100	+	+	+	+	+	+	+	+
3	Tek-200	+	+	+	+	+	+	+	+
4	Tek-300	+	+	+	+	+	+	+	+
5	Tek-400	+	+	+	+	+	+	+	+
6	Sana-0	+	+	+	+	+	-	+	+
7	Sana-100	+	+	+	+	+	-	+	+
8	Sana-200	+	+	+	+	+	-	+	+
9	Sana-300	+	+	+	+	+	-	+	+
10	Sana-400	+	+	+	+	+	-	+	+
11	L-0	+	+	+	+	+	+	+	+
12	L-100	+	+	+	+	+	+	+	+
13	L-200	+	+	+	+	+	+	+	+
14	L-300	+	-	+	+	+	+	+	+
15	L-400	+	-	+	+	+	+	+	+
16	S-0	+	+	+	+	+	-	+	+
17	S-100	+	+	+	+	+	-	+	+
18	S-200	+	+	+	+	+	-	+	+
19	S-300	+	+	+	+	+	-	+	+
20	S-400	+	+	+	+	+	-	+	+
21	E-0	+	+	+	+	+	+	+	+
22	E-100	+	+	+	+	+	+	+	+
23	E-200	+	+	+	+	+	+	+	+
24	E-300	+	+	+	+	+	+	+	+
25	E-400	+	+	+	+	+	+	+	+
26	F85-0	+	+	+	+	+	+	+	+
27	F85-100	+	+	+	+	+	+	+	+
28	F85-200	+	+	+	+	+	+	+	+
29	F85-300	+	+	+	+	+	+	+	+
30	F85-400	+	+	+	+	+	+	+	+
31	F80-0	+	+	+	+	+	+	+	+
32	F80-100	+	+	+	+	+	+	+	+
33	F80-200	+	+	+	+	+	-	+	+
34	F80-300	+	+	+	+	+	+	+	+
35	F80-400	+	+	+	+	+	-	+	+

*Tek: Tekirdağ; Sana: Sana; L: Lider; S: Sagittario; E: Ergene; F85: Flamura85; F80; Flamura80

In a study conducted for the Lr34 gene region, partial resistance levels of 39 wheat genotypes to brown rust were evaluated during three growing seasons (2016/2017, 2017/2018 and 2018/2019). Giza 171, Misr 3, and Sohag 5 showed full resistance, while 28 genotypes exhibited low or moderate resistance. 8 genotypes showed rapid rust. In addition, monogenic lines Lr34, Lr46, Lr67, and Lr68 were identified by molecular markers, and phenotypic results were confirmed by these analyses. A 150 bp band associated with resistance was identified in the Lr34 gene region (El-Orabey et al., 2019). In our study, a 150 bp band was observed in 23 of 35 control (0) and gamma-irradiated samples. The results in samples containing the Lr34 gene region are the same as in this study.

The allele size of the Lr37 leaf rust resistance gene was determined to be 285 bp using the URIC/LN2 primer (Helguera et al., 2003). The same result was observed in our study. Xu et al., (2017) also reported that a 285 bp allele was observed for leaf rust resistance in commercial wheat varieties using the same primers, while no PCR amplification band was observed in the negative control.

The Xabc465 RFLP locus was converted into a PCR-based marker to facilitate transfer of the Lr47 leaf rust resistance gene into *T. aestivum* in the Helguera et al., (2000). Additionally, a CAPS marker was created for Lr47, and a 282-bp band was observed in this marker after PCR amplification with the primer PS10LF/R (Helguera et al., 2000). In our study, a 282-bp band was also observed after PCR amplification with the same primer.

In this study, we have obtained significant information regarding both the genetic diversity and brown rust resistance of mutated bread wheat M4 populations. Based on the marker analysis results obtained in our study, wheat genotypes from the Flamura85 (all genotypes) and Flamura80 variety (especially F80-100 and F80-300), which has been identified as having relatively high genetic diversity and resistance to brown rust, can be selected and used as parents in the design of breeding hybridization programs.

CONCLUSION

The agricultural sector faces various challenges, including climate change, drought, pests, diseases, and diminishing agricultural land. Breeding is crucial to address these challenges and minimize yield losses. Marker-assisted selection (MAS), in particular, enables the effective use of genetic diversity in plant breeding and allows for the more rapid and precise development of varieties with desired traits. Molecular characterization techniques, on the other hand, provide rapid and accurate results in identifying disease resistant genotypes by analyzing genetic diversity. Therefore, incorporating molecular markers into breeding programs is critical for shortening breeding times and rapidly identifying suitable genotypes. Although there are numerous molecular studies on wheat in the literature, a recent increase in studies on the iPBS marker system has been observed.

The iPBS system in the characterization of lines developed through mutation breeding in the M4 population and to identify potential differences in the presence of the brown rust resistance gene in wheat lines exposed to gamma irradiation is significant. Genetic characterization in breeding programs, identifying plant species resistant to diseases and environmental stresses, is critical for sustainable agriculture and preserving biodiversity. Furthermore, identifying genetic variations that increase adaptability allows the development of species more resilient to environmental stresses such as climate change and diseases. Therefore, this study highlights the importance of using productive, resistant varieties in breeding programs, increasing genetic diversity, shortening the breeding process, and using molecular markers to obtain precise information about the genotype of the plant used.

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Additional Declaration

Author Contributions

In this study, Conceptualisation, DA and BBB; Laboratory Analysis and Data Collection, DA; Interpreted data; BBB, Writing original draft preparation, DA and BBB, Writing, review, and editing, DA and BBB. All authors have read and agreed to the published version of the manuscript.

Artificial Intelligence Statement

No artificial intelligence support was used in any part of this study.

Conflict Of Interest Statement

The authors declare that they have no conflict of interest regarding the publication of this study.

Ethical Statement

This study was conducted in accordance with the principles of Pen Academic Publishing Research Ethics Policy throughout all stages of the process.

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