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Research article

Characterization of some local and commercial bread wheat (*Triticum aestivum* L.) genotypes with allele-specific DNA markers

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Abstract

Landraces play a significant role as genetic reservoirs in wheat breeding studies. Advances in functional marker technology have facilitated early and more precise selection processes. This study involved the characterization of a total of 96 bread wheat genotypes, comprising 76 landraces and 20 registered cultivars, utilizing allele-specific DNA markers targeting various genes including those for gluten strength, yellow rust resistance, stem rust resistance, dwarfness, rye translocation, hardiness. Molecular analysis revealed the presence of 148 alleles, with an average of 21.14 alleles per marker, and an average polymorphic information content (PIC) value of 0.5625. Specific genes such as the rye translocation gene were identified in genotypes 161 and 884, while the grain hardiness gene was found in genotypes 672, 3088, 3384, 3414, and 3541. The stem rust resistance gene was detected in the cultivar Adana-99, the yellow rust resistance gene in genotypes 1635 and 2115, and the grain hardiness gene in 31 genotypes including the cultivar Masaccio. Based on the dendrogram analysis, genotype 3652 exhibited around 93% genetic similarity with the cultivar Masaccio, while genotypes 2190, 2715, and 2897 showed similarity to genotype 2946. Genotypes 2959 and 2960 and genotypes 3334 and 3359 shared approximately 91% genetic similarity.

Keywords: *Allele specific marker; bread wheat; genetic diversity; landraces*

1. Introduction

Grains constitute one of the primary sources of protein and calories globally, serving as crucial food staples. Among grain products, wheat stands out as a fundamental food source for many countries worldwide due to its ease of cultivation, transportation, nutritional value, storage convenience, and broad adaptability (Rao and Poonia, 2023; Sertse et al., 2023).

Wheat is categorized into three groups based on chromosome numbers: diploid (2n=2x=14), tetraploid (2n=4x=28), and hexaploid (2n=6x=42). Diploid wheat contains only one A, B, or D genome. *Triticum durum* (durum wheat), classified as tetraploid, possesses both A and B genomes. *Triticum aestivum* (bread wheat), a hexaploid species, harbors the A, B, and D genomes together (Kaya, 2018; Afshari-Behbahanizadeh et al., 2024).

As time progresses, our country's wheat cultivation areas have expanded and reached their maximum capacity. With no further room for expansion, the focus has shifted towards enhancing yield per unit area. However, a mere yield increase is perceived as inadequate under current wheat consumption conditions. In addition to high productivity per unit area, there is a growing demand for crops demonstrating resilience against biotic and abiotic stress factors, along with superior quality characteristics. Given our country's diverse soil and climatic conditions, developing varieties capable of adapting to various climate conditions is crucial while maintaining high-quality standards. This is because wheat production is significantly influenced by factors such as variety, agricultural practices, and environmental conditions (Aktas et al., 2017; Gungor and

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Dumlupinar, 2019; Filip et al., 2023).

Breeding programs are labor-intensive, costly, and time-consuming studies. The fundamental principles of breeding emphasize the importance of clear objectives, meticulous observation, suitable locations, and appropriate parent selection to develop superior varieties. To succeed in breeding programs, the presence of potential sources of variation and their effective utilization are essential. Over the past quarter-century, the advent of DNA technologies and molecular markers in breeding programs has led to the rapid advancement of marker-assisted selection (MAS). Particularly in cereal crops, studies on quality, yield, and resistance to pests and diseases have increased. These efforts have yielded successful results. Marker-assisted selection (MAS) has enabled breeding programs to be completed with less labor and shorter timeframes. Additionally, MAS offers the advantage of requiring smaller population sizes than traditional breeding methods (Gupta and Varshney, 2000; Babu et al., 2004; Song et al., 2023; Habib et al., 2024).

With the adoption of molecular marker technologies, breeding durations have been reduced in recent years. One commonly used marker technology is Simple Sequence Repeats (SSR) (Dede, 2007; Turkoglu et al., 2023). Short Tandem Repeats (STRs) refer to the consecutive repeat sequences randomly occurring in the genetic material of a locus. Markers consisting of 1-6 base pair repeats are also known as SSRs or microsatellites (Ozsensoy and Kurar, 2012). Allele sizes in microsatellite markers can be determined through electrophoresis. Microsatellites are co-dominant, meaning they can distinguish heterozygous characters from homozygous characters. Consequently, SSR markers are easily standardized, exhibit high polymorphism rates and repeatability, and are co-dominant. Due to these superior characteristics, microsatellites are frequently preferred in characterization studies (Gebologlu and Furan, 2017; Merga and Getu, 2023; Undal and Ahir, 2023).

This study characterized 96 bread wheat genotypes (76 local genotypes and 20 standard varieties) using seven allele-specific DNA markers.

2. Materials and methods

The research used 76 landrace bread wheat genotypes from different provinces of Turkey and ten winter and ten summer registered bread wheat cultivars from the National Seed Gene Bank within the Plant Genetic Resources of the Aegean Agricultural Research Institute. Information about genotypes is presented in Ocaktan (2021).

2.1. Leaf sampling procedure

The wheat varieties planted in pots were sampled during the two-leaf stage, and leaf samples were collected. These samples were then placed into 2 ml Eppendorf tubes and stored at -80°C until DNA isolation. DNA isolation was carried out using the cetyl trimethyl ammonium bromide (CTAB) method described by Oliver et al. (2010).

2.2. DNA isolation

The leaf samples from bread wheat varieties were stored at -80°C and then crushed in 2 ml Eppendorf tubes using a sterile rod immersed in liquid nitrogen. Following this, 1 ml of isolation solution consisting of 1 M Tris-HCl (pH 8.0), 0.5 M EDTA (pH 8.0), 5 M NaCl, 2% w/v cetyltrimethylammonium bromide (CTAB), 2% Polyvinylpyrrolidone 40, and 5% sarcosine was added to the ground leaf samples in the Eppendorf tubes. The tubes were then incubated in a water bath at 65°C for one hour, and were homogenized by inversion for every 20 minutes. After incubation, a mixture of 1 ml chloroform and isoamyl alcohol (24:1) was added to the samples and mixed by inversion in the dark for 30 minutes. Subsequently, the samples were centrifuged at 10,000 rpm for 20 minutes. The clear liquid supernatant was carefully pipetted into empty sterile tubes, and then 1 ml of isopropanol, pre-chilled at -20°C, was slowly added and mixed gently by inversion. After centrifugation at 10,000 rpm for 30 minutes, the supernatant was removed without disturbing the pellet. The remaining pellets in the tubes were washed by adding 2 ml of 70% ethanol and centrifuged at 13,000 rpm for 2 minutes. The ethanol was then carefully removed without disturbing the pellet. The dried pellets were dissolved by adding ten mM Tris-HCl (pH 8.0) and RNAse to eliminate RNA. Finally, the quantity and quality of the extracted DNA were measured using a Nanodrop device.

2.3. DNA primers

The study utilized DNA markers, including Bx7OE (Gluten Strength gene), Sun104 (Yellow Rust Yr51 gene), Sun209 (Stem Rust Sr49 gene), DF-MR2 (Dwarfness Rht-D1 gene), RYE-NOR (Rye Translocations gene), Pina-D1 (Grain hardiness Pina gene), and Sun1 (Waxy Wx-A1 gene), to determine quality-related traits, certain diseases, and degrees of relatedness in 96 bread wheat varieties. The DNA markers used in the study are provided in Table 1 below.

Table 1
DNA primers used in molecular characterization.

No	Primer Name	Primer Sequence (5′-3′)	Gene Region	Reference	Expected Band Length (bp)	Marker Type
1	Bx7OE_F Bx7OE_R	CCTCAGCATGCAAACATGCAGC CTGAAACCTTTGGCCAGTCATGTC	Gluten Strength	Butow et al., 2003	563	Co-dominant
2	Sun104_F Sun104_R	TGCTATGTGCGTGATGATGA TTACATGCTCCAGCGACTTG	Stripe Rust *Yr51*	Randhawa et al., 2014	225	Dominant
3	Sun209_F Sun209_R	AG CTATGAGCTTCGCTATTG GTGATTGGTTCGGATTACTTA	Stem Rust *Sr49*	Bansal et al., 2015	148	Co-dominant
4	DF-MR2_F DF-MR2_R	CGCGCAATTATTGGCCAGAGATAG CCCCATGGCCATCTCGAGCTGCTA	Dwarfness *Rht-D1*	Ellis et al., 2002	254	Dominant
5	RYE-NOR_F RYE-NOR_R	GCATGTAGCGACTAACTCATC CCCAGTTTTCCATGTCGC	Rye Translocation	Koebner, 1995	400, 600, 700, 800	Dominant
6	Pina-D1_F Pina-D1_R	CCCTGTAGAGACAAAGCTAA TCACCAGTAATAGCCAATAGT	Grain Hardiness Pina	Gautier et al., 1994	330	Dominant
7	Sun1_F Sun1_R	CGCTCCCTGAAGAGAGAAAGAA ATAGGCACAACCCCTAAC	Waxy *Wx-A1*	Shariflou and Sharp, 1999	Xsun-7A, 219, 233, 260, 271, 275, 285 and 289	Co-dominant

2.4. Polymerase chain reactions (PCR) and fragment analysis

The polymerase chain reaction (PCR) procedure involved preparing a 20 µl solution with the following components added to 96-well PCR plates: 1 µl of dNTP mixture (2.5 mM mixture of A+T+G+C), 2 µl of 10x buffer, 0.1 µl of $MgCl_2$, DNA primer pair (5 µl each of forward and reverse primers), 5 µl of genomic DNA (60 ng), 1.8 µl of ddH_2O, and 0.1 µl of DNA polymerase (5U/µl, Fermentas). PCR reactions were performed using an ABI-brand PCR machine. The reactions commenced with an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes. Finally, the reactions were completed with a final extension step at 72°C for 10 minutes. The finished PCR products were stored at -20°C until further use. Following the PCR process, the obtained products were utilized for fragment analysis. The analysis was conducted using the QIAxcel Advanced System, a fragment analysis device from Qiagen, to obtain DNA bands corresponding to the varieties.

2.5. Evaluation of data

According to the fragment analysis results, scoring precision was accepted as ± 4 base pairs, and alleles were evaluated accordingly. The similarities of these varieties were calculated using the Dice index (Dice, 1945) in the NTSYSpc 2.21q (Rohlf, 2005) program. DNA bands of the obtained varieties were coded as "0" or "1" and a binary data matrix was created. With the help of the created matrix, a dendrogram showing the similarities of the varieties was obtained using UPGMA (Unweighted Pair Group Method Arithmetic Average).

2.6. Calculation of polymorphism information contents

Polymorphism information contents for each DNA marker to be used in molecular analyses were calculated with the formula given below according to Weir (1996);

$$PIC=1-\sum Pi^2$$

Pi; is the frequency of the i^{th} allele in 96 bread wheat genotypes studied in the research.

3. Results and discussion

3.1. Polymorphism information content (PIC) and allele numbers of the markers used

This study used seven functional DNA markers to screen 76 landraces and 20 standard bread wheat cultivars for polymorphism. As a result of the screenings, a total of 148 alleles were detected, all of which exhibited polymorphic characteristics. The average number of alleles was determined to be 21.14. The primer "RYE-NOR" produced the highest number of alleles, generating 43 polymorphic alleles, while the primers DF-MR2 and SUN104 produced the lowest number of alleles, generating six polymorphic alleles each.

After the study, the average Polymorphic Information Content (PIC) value was determined to be 0.5625. The highest PIC value calculated was 0.9651, while the lowest PIC value was found to be 0.1172. Table 2 presents the calculation of PIC values for 96 bread wheat genotypes, the information content of DNA primers used in DNA screening, and the number of alleles.

Table 2
DNA primers, allele numbers and polymorphism information content (PIC) values used in screening genotypes.

No	Primer Name	Allele Numbers	Expected Band Length (bp)	PIC Value
1	$Bx7^{OE}$	35*	563	0.9651
2	Sun104	6	225	0.1172
3	Sun209	16	148	0.9206
4	DF-MR2	6*	254	0.1172
5	RYE-NOR	43	400, 600, 700, 800	0.4946
6	Pina-D1	31	330	0.4373
7	Sun1	11	Xsun-7A, 219, 233, 260, 271, 275, 285 and 289	0.8855
8	**Mean**	**21.14**		**0.5625**

***** PIC value has been calculated using non-specific alleles.

Bx7OE primer is a marker used to detect genes related to gluten strength. Butow et al. (2003) stated that the Bx7OE marker corresponds to a co-dominant marker for the encoded gene region of 750 bp, with alleles of 563 bp obtained for lines lacking the Glu-B1al (520 bp) gene, with a difference of 43 bases. In the study, scanning 96 bread wheat genotypes (76 local and 20 standard) using the Bx7OE primer did not yield the desired gene region. Previous studies by Kocyigit et al. (2021) and Uysal and Dumlupinar (2022) also failed to obtain the desired gene region using the Bx7OE primer. The Sun104 primer is a marker to detect genes resistant to yellow rust (Yr51) disease.

Randhawa et al. (2014) utilized the Sun104 primer for detecting yellow rust (Yr51) disease, obtaining an allele of 225 bp and determining that this band length was associated with the gene for resistance to yellow rust disease. Our study detected the yellow rust disease resistance gene in the varieties 1635 and 2115 (Fig.1; Table 3). In previous studies regarding the Sun104 primer, Kocyigit et al. (2021) reported obtaining the desired gene region in two hybrid combinations. Our study appears to be consistent with the findings of the previous research.

The Sun209 primer is a marker used to detect the black rust trait. Bansal et al. (2015) utilized the Sun209 marker to detect the Sr49 gene associated with black rust, determining the desired band length to be 148 bp. In our study, the presence of the gene for resistance to black rust disease was detected in the genotype Adana-99 (Table 3). In studies on the Sun209 primer, Uysal and Dumlupinar (2022) reported obtaining the desired gene region in 33 genotypes, while Kocyigit et al. (2021) reported success in two genotypes.

The DF-MR2 primer is a marker used to detect dwarfness (short) traits. Ellis et al. (2002) reported an allele of 254 bp length in the gene region encoded by the DF-MR2 marker and noted its association with dwarfness (Short). However, the desired gene region was not obtained in our study when scanning 96 wheat genotypes (76 local and 20 standard cultivars) using the DF-MR2 primer. In studies related to the DF-MR2 primer. Kocyigit et al. (2021) also reported not obtaining the desired gene region, indicating consistency with our findings. The RYE-NOR primer is a marker used to detect rye translocation traits. Koebner (1995) identified alleles of 400, 600, 700, and 800 bp length using the RYE-NOR marker. Our study detected the rye translocation gene in varieties 161 and 884 (Table 3). Kocyigit et al. (2021) reported obtaining the desired gene region in 3 hybrid combinations, consistent with our findings in studies

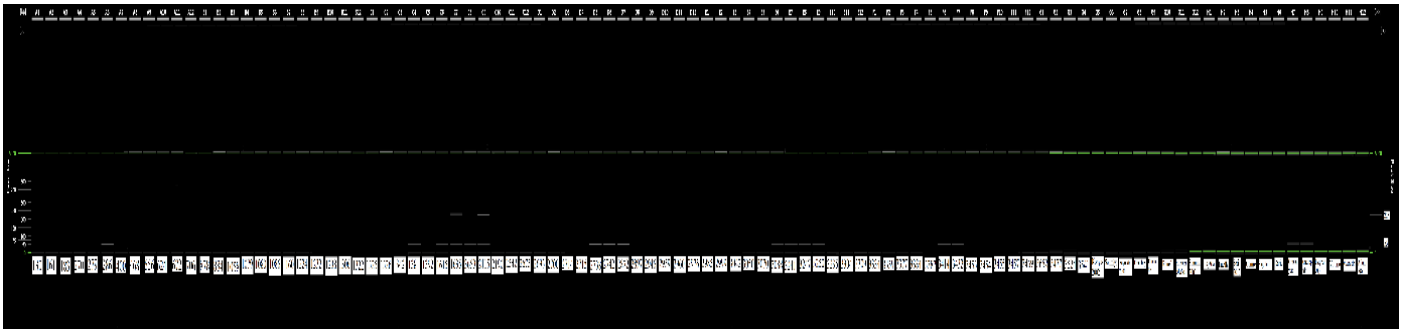

Fig. 1. Gel image of Sun 104 primer.

Table 3
Distribution of bands giving specific allele genes as a result of DNA marker screening of 96 bread wheat genotypes.

No	Genotypes	Sun1 219 bp Waxy Wx-A1	Sun104 Yr51, 225 bp Stripe Rust	Sun209 Sr49, 148 bp Stem Rust	Pina-D1 330 pb Grain Hardiness	Bx7OE 563 bp Gluten Strength	DF-MR2 254 bp Dwarfness (Short)	RYE-NOR 400, 600,700, 800 bp Rye Translocations
1	130							
2	161							+
3	183							
4	270							
5	279							
6	286							
7	400							
8	468							
9	556							
10	624							
11	672				+			
12	706							
13	878							
14	884							+
15	1058							
16	1079							
17	1080							
18	1086							
19	1160	+						
20	1224							
21	1232	+						
22	1238							
23	1306							
24	1322							
25	1325							
26	1326							
27	1342							
28	1381							
29	1392							
30	1615	+						
31	1635	+	+					
32	3659	+						
33	2115		+					
34	2190	+						
35	2542							
36	2675	+						
37	2693	+						
38	2700	+						
39	2714							
40	2715	+						
41	2738							
42	2742							
43	2872	+						
44	2897	+						
45	2946	+						
46	2959	+						
47	2960	+						
48	2976							

49	2985						
50	2999	+					
51	3042						
52	3050	+					
53	3070	+					
54	3088	+				+	
55	3241	+					
56	3247	+					
57	3250						
58	3295	+					
59	3304	+					
60	3309	+					
61	3334	+					
62	3359	+					
63	3377						
64	3384	+				+	
65	3387						
66	3414					+	
67	3432						
68	3453	+					
69	3454						
70	3455	+					
71	3457						
72	3469						
73	3652	+					
74	3477						
75	3524						
76	3541					+	
77	Konya-2002						
78	Selimiye						
79	Bayraktar - 2000						
80	Tosunbey						
81	Flamura-85						
82	Rumeli						
83	Krasunia odes'ka						
84	Sönmez- 2001						
85	Esperia						
86	Lucilla						
87	Seri-2013						
88	Cemre						
89	Sagittario						
90	Dariel						
91	Osmaniyem						
92	Karatopak						
93	Ceyhan-99						
94	Vittorio						
95	Masaccio	+					
96	Adana-99			+			

related to the RYE-NOR primer. The Pina-D1 primer is a marker used to detect grain hardiness traits. Gautier et al. (1994), Tranquilli et al. (1999), and Teniente Pérez et al. (2017) reported alleles of 330 bp, 331 bp, and 260 bp, respectively, associated with grain hardiness. Our study detected the grain hardiness gene in varieties 672, 3088, 3384, 3414, and 3541 (Table 3). However, Kocyigit et al. (2021) reported not obtaining the desired gene region in studies on the Pina-D1 primer, suggesting potential differences in genetic properties among the studied varieties. The Sun1 primer is a marker to detect the Waxy (Wx-A1) trait. Maryami et al. (2014) reported alleles of 230 bp and 265 bp, while Shariflou and Sharp (1999) reported alleles of various lengths (219 bp, 233 bp, 260 bp, 271 bp, 275 bp, 285 bp, and 289 bp) associated with the Waxy gene. Our study detected the Waxy gene in genotypes 1160, 1232, 1615, 1635, 3659, and others (Table 3). Like other markers, Kocyigit et al. (2021) reported not obtaining the desired gene region with the Sun1 primer, suggesting potential differences in genetic properties among the studied genotypes.

3.2. Dendrogram created based on obtained data

After screening 96 bread wheat genotypes using seven different DNA markers, the collected data were utilized to construct a phylogenetic tree employing the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method based on Weir's (1996) genetic similarity matrix (Fig.2). According to the resulting dendrogram, genotype 3652 shares approximately 93% genetic similarity with the genotype Masaccio. Genotypes 2190, 2715, and 2897 demonstrate about 91% similarity with genotype 2946. Similarly, genotypes 2959 and 2960, as well as genotypes 3334 and 3359, exhibit approxi-

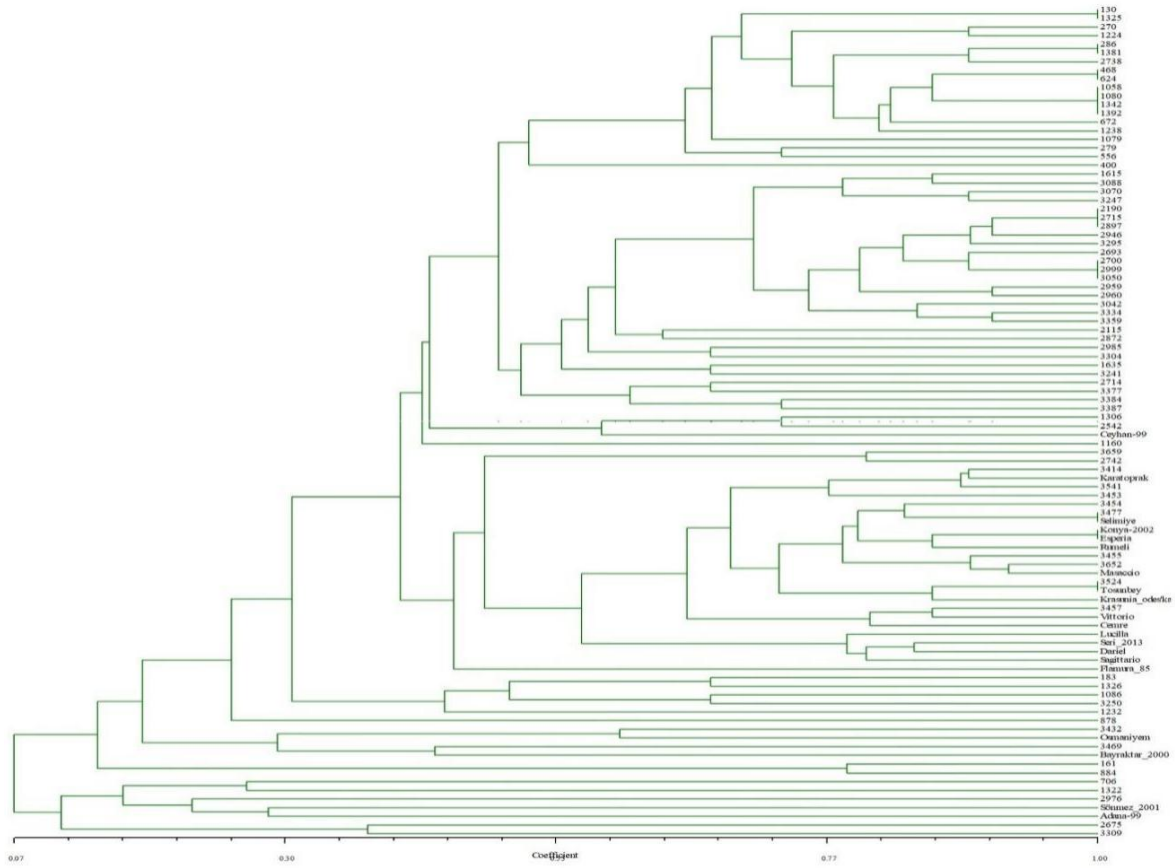

Fig. 2. Dendrogram constructed with 148 alleles obtained from 7 DNA markers of bread wheat genotypes.

mately 91% similarity. Moreover, genotypes 270 and 1224 and 286 and 1381 are similar to genotype 2738. Genotypes3070 and 3247, 2700, 2999, and 3050, as well as genotype 3414, also share approximately 89% similarity with genotype 3652 and the genotype Masaccio. Furthermore, genotypes Konya-2002 and Esperia are similar to the genotype Rumeli, while genotypes 3524 and Tosunbey are similar to the genotype Krasunia odes'ka. Similarly, genotypes 3457 and Vittorio, as well as genotypes 3042, 3334, and 3359, are similar, along with genotypes 1058, 1080, 1342, and 1392, to genotypes 468 and 624, and genotypes 1615 and 3088 share about 84% similarity. Besides, genotypes Seri-2013 and Dariel demonstrate approximately 83% similarity, while genotypes 1238 and 672, as well as genotypes 1058, 1080, 1342, and 1392, show about 80% similarity to genotypes 3659 and 2742, as well as genotype 3457 and Vittorio, and genotypes Seri-2013 and Dariel with Sagittario, respectively.

4. Conclusion and recommendations

In this study, local and commercial bread wheat genotypes were characterized using functional DNA markers, resulting in the identification of 148 alleles and the determination of polymorphism information content. The dendrogram created allowed the determination of the relatedness among genotypes and the identification of genotypes carrying genes for rye translocation, Pina-D1 grain hardiness, black rust Sr49, yellow rust Yr51, and Wx-A1 waxy traits. The results obtained in this study are likely effectively utilized in breeding programs.

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Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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