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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF WHEAT (*Triticum aestivum* L.) GENOTYPES USING RAPD MARKERS

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ABSTRACT

In this study, Random Amplified Polymorphic DNA (RAPD) analysis was employed to assess the genetic variability among sixteen wheat genotypes using 28 RAPD primers. A total of 212 bands were amplified, of which 109 were polymorphic, resulting in an average polymorphism of 55.62%. The number of bands per primer ranged from 4 to 14, with Polymorphism Information Content (PIC) values between 0.731 and 0.911 (average 0.820) and RAPD Primer Index (RPI) values ranging from 2.99 to 12.75 (average 6.288), indicating high primer informativeness. Several primers, including OPA-18, OPE-09, and OPZ-15, were particularly effective in discriminating among genotypes. Sixteen genotype-specific markers were identified, with GW-366 exhibiting the highest number of unique bands. Jaccard's similarity coefficients ranged from 0.633 to 0.881, indicating moderate to high genetic relatedness, with GW-11 and GW-173 being the most similar, and HD-3086 and GW-366 the most divergent. Cluster analysis grouped the genotypes into two major clusters, with GW genotypes clustering closely together and HD-3086 and AG-30-1 showing greater divergence. Scatter plot analysis further confirmed genetic variability among genotypes. The results demonstrate substantial genetic diversity among the studied wheat genotypes and highlight the importance of RAPD markers for germplasm diversity, selection of diverse parents, and marker-assisted breeding programs. Thus, understanding genetic diversity among wheat genotypes is crucial for effective breeding and germplasm conservation.
***Keywords*:** Wheat (*Triticum aestivum*), Genetic diversity, RAPD markers, Polymorphism, Genotype-specific markers, Jaccard similarity, Cluster analysis.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops in the world and plays a central role in global food security. As a staple food for billions of people, wheat provides essential nutrients such as carbohydrates, proteins, vitamins, and minerals, making it a vital component of human diets (Niaz *et al.*, 2025). The global demand for wheat continues to rise due to population growth, urbanization, and changing dietary preferences, particularly in developing countries. Consequently, enhancing wheat production and quality has become a major goal for plant breeders, agronomists, and researchers worldwide.

Genetic diversity is the foundation of plant breeding and crop improvement (Gajera *et al.*, 2024; Hirpara *et al.*, 2017a). It provides the raw material for selection and allows breeders to develop new varieties that are more productive, resilient, and adaptable to diverse environmental conditions. Diverse germplasm ensures that crops can withstand biotic stresses, such as pests and diseases, as well as abiotic stresses, including drought, salinity, and temperature extremes (Xu *et al.*, 2023). In wheat, genetic variation manifests in traits such as grain yield, quality, disease resistance, and tolerance to environmental stresses. Therefore, understanding the extent of genetic variability among wheat genotypes is critical for identifying superior parents for breeding programs, conserving valuable germplasm, and maintaining sustainable crop production (Verma *et al.*, 2024).

Traditional approaches to evaluating genetic diversity in wheat have relied primarily on morphological and agronomic traits, such as plant height, spike length, grain size, and yield components. While these traits are informative, they are often influenced by environmental factors (Khan *et al.*, 2026; Savani *et al.*, 2023), making it difficult to accurately assess genetic differences. Morphological evaluation also requires extensive field trials over multiple locations and seasons, which can be time-consuming and resource-intensive (Purohit *et al.*, 2022; Hirapara *et al.*, 2022). As a result, there has been increasing interest in molecular markers as tools to complement traditional breeding approaches and provide a more precise understanding of genetic relationships.

Molecular markers are DNA-based tools that detect polymorphisms at the genetic level (Bhadani *et al.*, 2018; Hirpara *et al.*, 2017b). Unlike morphological traits, molecular markers are stable across environments and provide direct insight into the genetic makeup of plants. They allow researchers to distinguish between closely related genotypes, identify unique alleles, and track the inheritance of desirable traits. Several types of molecular markers are available, including Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) (Hirpara and Gajera, 2018), Single Nucleotide Polymorphisms (SNPs), Sequence Characterized Amplified Region (SCAR) (Gajera *et al.*, 2023), Sequence-Related Amplified Polymorphism (SRAP) (Hirpara *et al.*, 2016), Start Codon Targeted (SCoT) marker (Gajera *et al.*, 2016), Inter-Simple Sequence Repeat (ISSR) (Patel *et al.*, 2023) and Random Amplified Polymorphic DNA (RAPD) markers (Bhadani *et al.*, 2018).

Among these, RAPD markers are particularly advantageous due to their simplicity, cost-effectiveness, and ability to generate multiple polymorphic loci without prior knowledge of the genome sequence. RAPD involves the amplification of random DNA fragments using short, arbitrary primers in a polymerase chain reaction (PCR). The presence or absence of amplified fragments reflects genetic differences among individuals. RAPD markers have been widely applied in wheat and other crops to evaluate genetic diversity, identify genotype-specific markers, detect polymorphism, and assist in marker-assisted selection (Nazarzadeh *et al.*, 2020).

Despite the availability of diverse wheat germplasm, limited information is available regarding the genetic relationships among several commonly grown genotypes in certain regions. Therefore, the present study aimed to evaluate the genetic diversity and phylogentic relationship among sixteen wheat genotypes using RAPD markers, identify informative primers and genotype-specific markers, and generate insights for future wheat breeding and germplasm management programs.

Materials and Methods

Plant materials and collections

Sixteen wheat (*Triticum aestivum* L.) genotypes, namely GW-11, GW-173, GW-190, GW-273, GW-322, GW-366, GW-496, HD-2932, HD-3086, J-24, KRL-210, Lok-1, AG-30-1, WH-1154, WH-1156, and HS-590, were procured from Wheat Research Station, Junagadh Agricultural University, Junagadh. Young, healthy leaf tissues were collected for DNA extraction.

DNA Extraction

Genomic DNA was extracted from young, healthy leaves of sixteen wheat (*Triticum aestivum* L.) genotypes using the CTAB method using method described by Stein *et al.* (2001) with minor modifications. The integrity of the isolated genomic DNA was verified using 0.8 % agarose gel electrophoresis. Quantification of DNA was performed using spectrophotometry (NanoDrop, Qiagen 200463), measuring absorbance at 260 and 280 nm (Hirpara *et al.*, 2017c). The ratio of A260/A280 was used to assess purity, with values between 1.8 and 2.0 considered optimal for PCR-based applications. DNA samples were diluted to a working concentration of 20 ng/µL for PCR-RAPD amplification (Maeruf et al., 2023)

RAPD Analysis

A total of 28 oligonucleotide RAPD primers (Merck bioscience, Bangalore) were used for PCR amplification. Polymerase chain reactions (PCRs) were performed using method developed by Das *et al.* (2002) in the Veriti 96 well Thermal Cycler for amplification. The reaction mixture (15 µl) contained 1.5 µl of 10X TaqPCR buffer,3unit/µl of Taq DNA polymerase (0.15 µl), 10mM/µl of each dNTP mix (1.2 µl), 25 pmoles.μl^{-1} Primer (1.2 µl). The reaction profile was preceded by an initial denaturing step of 94^{o}C for 4 min., than subjected to 38 cycles of a 1 min at 94^{o}C;1 min at 36^{o}C and 1.5 min at 72^{o}C and final extension step for 7 min at 72^{o}C was given for polishing the ends of PCR products (Nazarzadeh *et al.*, 2020).

Gel Electrophoresis and Scoring

Amplified PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized under UV light. The amplified fragments were scored as `1' for the presence and `0' for the absence of a band from higher to lower molecular

weight products in binary matrix and utilized for polymorphic pattern and phylogenetic relationship (Hirpara *et al.*, 2017c).

Data Analysis

Polymorphism Information Content (PIC) and RAPD Primer Index (RPI) were calculated for each primer. Genotype-specific markers were identified by detecting unique bands in individual genotypes. The molecular size of each fragment was estimated using 100 bp DNA ladder plus as standard. Genetic similarity among genotypes was estimated using Jaccard's similarity coefficients, and a dendrogram was constructed using the unweighted pair-group method with arithmetic average (UPGMA) (Rohlf, 1998). This similarity matrix was analyzed using numerical taxonomy and multivariate analysis system (NTSYS-pc 2.2) and clustered with UPGMA algorithm to determine the genetic relationships among the 16 wheat genotypes. A scatter plot of pairwise similarity values was also generated to visualize genetic relationships among genotypes (Hesam *et al.*, 2026).

Interpretation

The genetic diversity among wheat genotypes was assessed based on the number of polymorphic bands, genotype-specific markers, PIC and RPI values, similarity coefficients, cluster patterns, and scatter plot distribution, providing insights for germplasm characterization, selection of genetically diverse parents, and marker-assisted breeding.

Results and Discussion

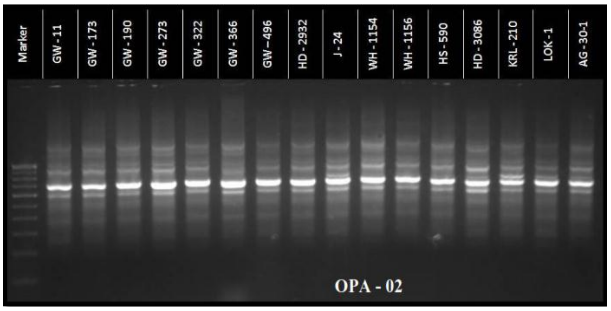
The RAPD analysis of sixteen wheat genotypes using 28 primers revealed substantial genetic variation. A total of 212 bands were amplified, ranging from 113 to 2845 bp, with the number of bands per primer varying from 4 to 14 (Table 1). Among these, 109 bands were polymorphic and 88 were monomorphic, resulting in an average polymorphism of 55.62%. Several primers, including OPA-18, OPE-09, and OPO-06, exhibited 100% polymorphism, indicating their strong discriminatory power, while others such as OPD-03 and OPA-07 produced no polymorphic bands. The Polymorphism Information Content (PIC) values ranged from 0.731 to 0.911, averaging 0.820, highlighting the high informativeness of the primers. Similarly, the RAPD Primer Index (RPI) varied from 2.99 to 12.75, with an average of 6.288, demonstrating the primers' differential capacity to distinguish among genotypes. Overall, primers such as OPZ-15, OPA-18, and OPE-09 were particularly effective in detecting genetic diversity, making them valuable tools for wheat germplasm characterization and marker-assisted selection (Table 1 and Fig. 1).

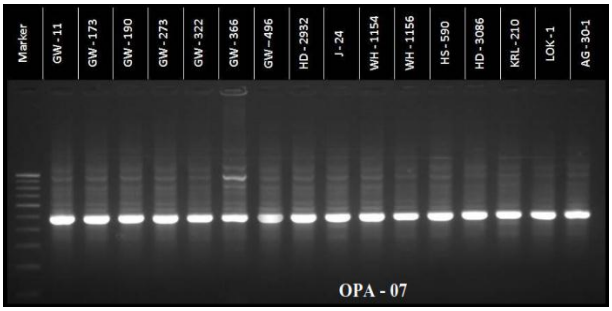
Table 1: Size, number of amplified bands, percent polymorphism and PIC obtained by RAPD primers.

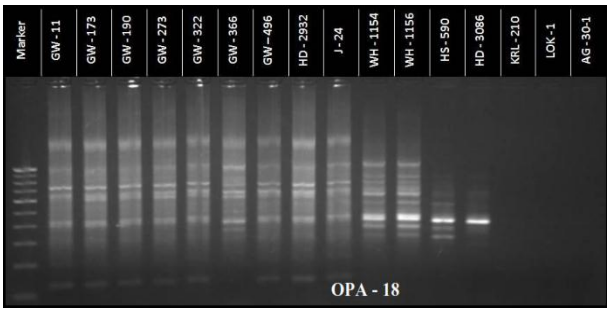
Sr. No.	RAPD primers	Allele/ Band Size (bp)	Total no. of Allele/ Bands	Poly-morphic Bands			Mono-mophic Bands	% Poly-morphism	PIC	RPI
				S	U	Total Bands				
1	OPA-02	452-1600	6	1	0	1	5	16.66	0.809	4.85
2	OPA–07	436-1107	4	0	0	0	4	0.00	0.750	3.00
3	OPA–16	145-1498	7	2	2	4	3	57.14	0.806	5.64
4	OPA–18	296-1111	9	9	0	9	0	100	0.866	7.79
5	OPB–07	233-920	7	6	0	6	1	85.71	0.826	5.78
6	OPC–05	271-1167	6	3	1	4	2	66.66	0.746	4.47
7	OPD–02	241-1772	6	1	0	1	5	16.66	0.828	4.96
8	OPD–03	209-1473	7	0	0	0	7	0.00	0.857	5.99
9	OPD–05	316-1980	6	5	0	5	1	83.33	0.763	4.57
10	OPD–07	275-1920	8	0	0	0	8	0.00	0.875	7.00
11	OPE–09	127-2845	11	11	0	11	0	100	0.850	9.35
12	OPF– 06	113-2161	10	5	2	7	3	70.00	0.843	8.43
13	OPF– 15	190-1613	9	6	0	6	3	66.66	0.861	7.74
14	OPG–12	182-2162	7	4	0	4	3	57.14	0.802	5.61
15	OPH–15	270-857	5	4	0	4	1	80.00	0.736	3.68
16	OPM-05	258-1628	8	2	0	2	6	25.00	0.870	6.96
17	OPM-14	370-2209	7	1	1	2	5	28.57	0.813	5.69
18	OPN–04	402-1641	4	2	0	2	2	50.00	0.748	2.99
19	OPN–10	250-2495	7	6	0	6	1	85.71	0.787	5.50
20	OPN–13	197-1072	7	6	0	6	1	85.71	0.830	5.81
21	OPO–06	176-1403	9	8	1	9	0	100	0.854	7.68
22	OPO–13	177-1213	7	1	0	1	6	14.28	0.856	5.99
23	OPO–15	181-1014	9	5	1	6	3	66.66	0.821	7.38
24	OPQ–12	395-1702	6	2	0	2	4	33.33	0.811	4.86

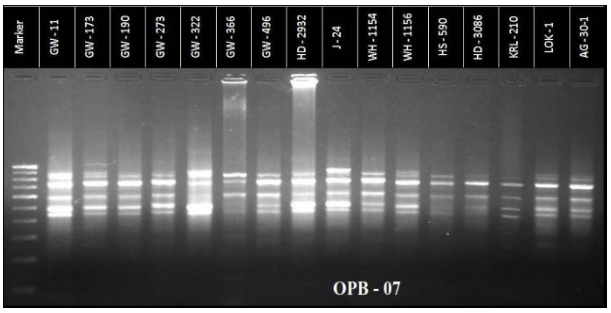
25	OPQ–14	247-2493	10	8	0	8	2	80.00	0.884	8.84
26	OPR–14	264-2066	9	4	2	6	3	66.66	0.838	7.54
27	OPZ–10	208-2307	7	2	3	5	2	71.42	0.731	5.11
28	OPZ-15	150-1386	14	5	2	7	7	50.00	0.911	12.75
Total			212	109	15	124	88	-	-	-
Average			-	-	-	4.42	-	55.62	0.820	6.288

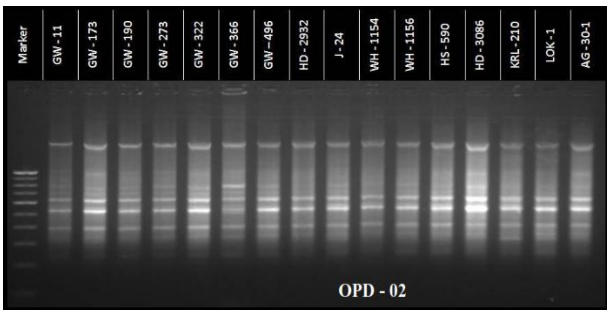
S=Shared**; U**=Unique**; T**=Total Polymorphic Bands; **PIC**=Polymorphism information content; **RPI** = (RAPD Primer Index) = Total number of Bands X PIC

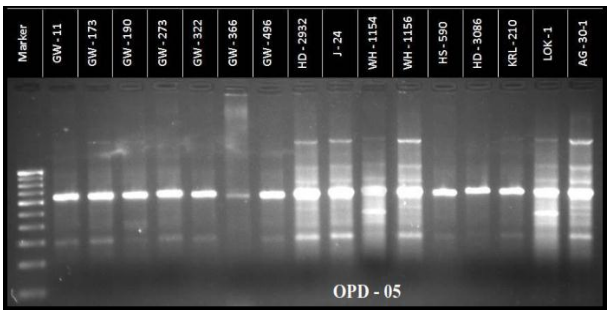


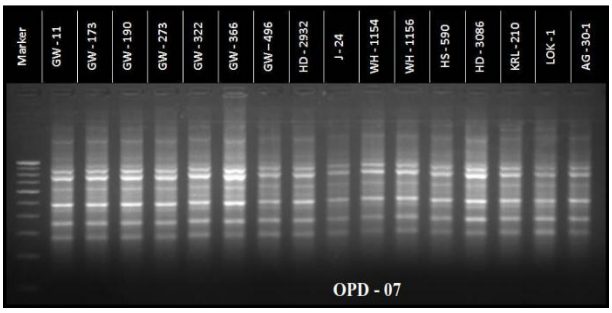


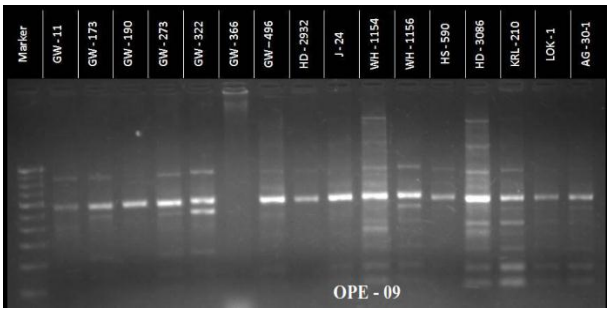


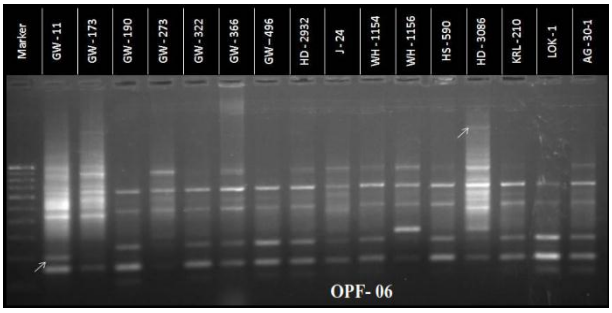


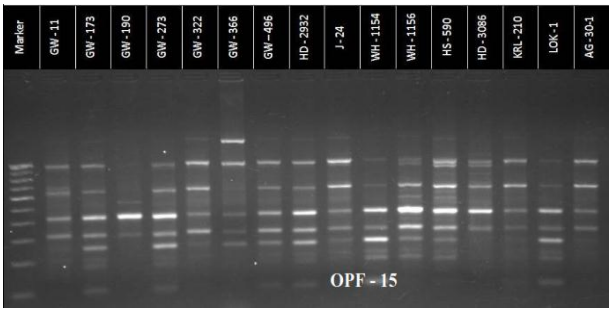












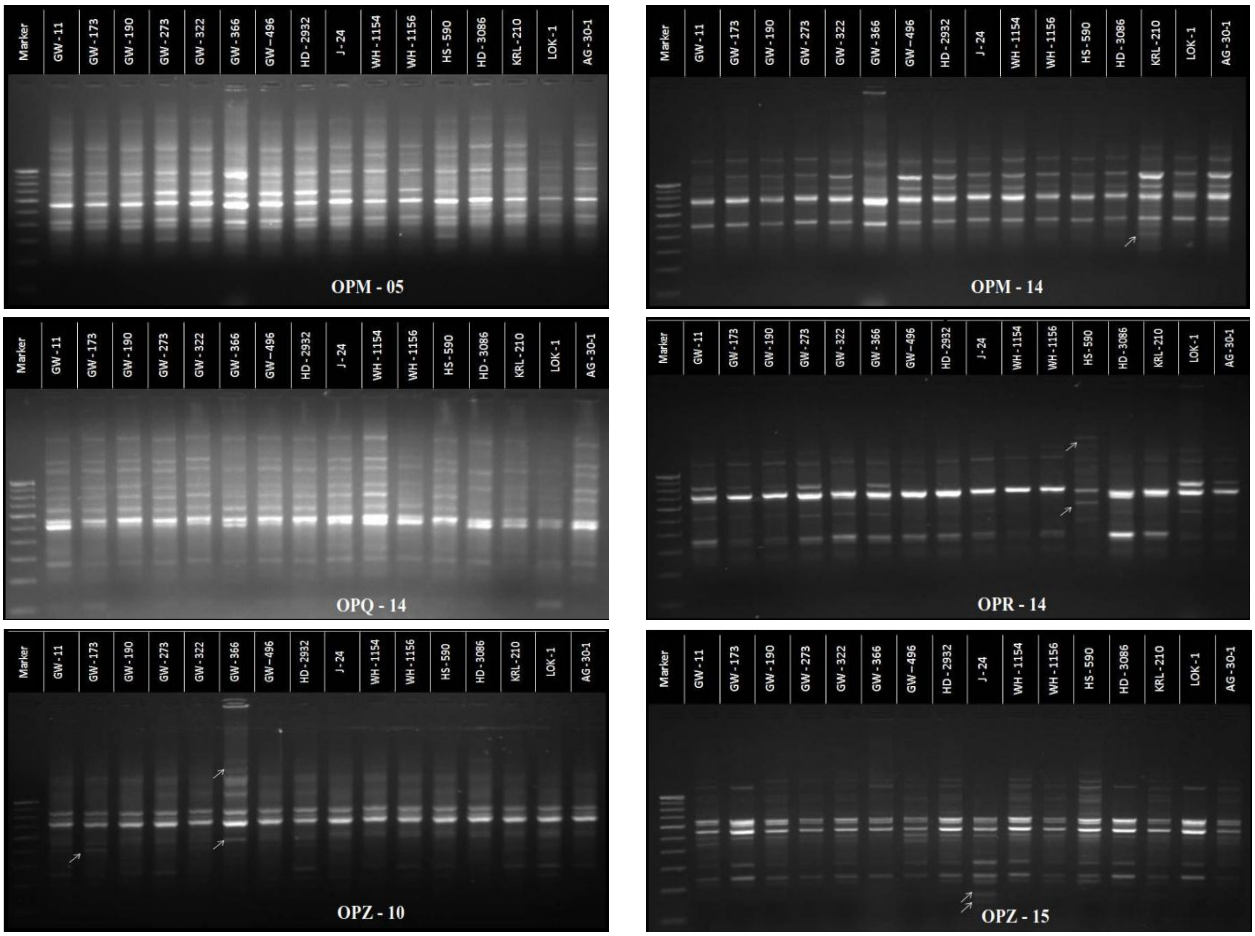


Fig. 1 : RAPD primers amplified across sixteen wheat genotypes

Analysis of genotype-specific RAPD markers revealed that certain primers produced bands unique to individual genotypes, underscoring their potential for genotype identification. For instance, OPA-16 amplified two unique bands in Lok-1 (220 and 358 bp), OPC-05 produced a 510 bp fragment in GW-366, and OPF-06 generated 178 bp and 2161 bp bands in GW-11 and HD-3086, respectively. Other primers such as OPM-14, OPO-06, OPO-15, OPR-14, and OPZ-15 produced unique bands in KRL-210, GW-366, J-24, HS-590, and HD-2932. In total, sixteen genotype-specific bands were identified, with GW-366 exhibiting the highest number (4 bands), followed by GW-173, J-24, HS-590, HD-3086, Lok-1, and HD-2932, each with 1–2 unique markers (Table 2). These findings demonstrate the utility of RAPD primers in distinguishing individual wheat genotypes for germplasm characterization and breeding programs.

Table 2: Molecular weight(bp) of genotypes specific markers generated by RAPD primers.

Primer	Genotypes															
RAPD	GW-11	GW-173	GW-190	GW-273	GW-322	GW-366	GW-496	HD-2932	J-24	WH-1154	WH-1156	HS-590	HD-3086	KRL-210	Lok-1	AG-30-1
OPA-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	358	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	220	-
OPC-05	-	-	-	-	-	510	-	-	-	-	-	-	-	-	-	-
OPF-06	178	-	-	-	-	-	-	-	-	-	-	-	2161	-	-	-
OPM-14	-	-	-	-	-	-	-	-	-	-	-	-	-	370	-	-
OPO-06	-	-	-	-	-	1272	-	-	-	-	-	-	-	-	-	-
OPO-15	-	214	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPR-14	-	-	-	-	-	-	-	-	-	-	-	2066	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	651	-	-	-	-

OPZ-10	-	391	-	-	-	2307	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	499	-	-	-	-	-	-	-	-	-	-
OPZ-15	-	-	-	-	-	-	-	-	169	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	150	-	-	-	-	-	-	-
Total	**1**	**2**	**-**	**-**	**-**	**4**	**-**	**-**	**2**	**-**	**-**	**2**	**1**	**1**	**2**	**-**

Jaccard's similarity coefficients calculated from RAPD data ranged from 0.633 to 0.881, indicating moderate to high genetic similarity among the genotypes. The highest similarity was observed between GW-11 and GW-173 (0.880), while the lowest was between HD-3086 and GW-366 (0.633), highlighting the most genetically divergent pair. Genotypes such as GW-273, GW-496, and HD-2932 consistently showed high similarity (0.8–0.86) with other genotypes, whereas HS-590 and HD-3086 generally exhibited lower similarity, reflecting greater genetic distinctness. These results confirm substantial genetic variability among the wheat genotypes, providing valuable information for selecting diverse parents in breeding programs and for germplasm conservation (Table 3).

The dendrogram constructed from RAPD data using Jaccard's similarity coefficients grouped the sixteen genotypes into two major clusters. Group I was further divided into subgroups A1 and A2. Subgroup A1(a) included GW-11, GW-173, GW-190, GW-273, GW-322, and GW-496, showing high genetic similarity, while A1(b) comprised HD-2932 and J-24, moderately related to A1(a). Subgroup A2 contained GW-366, WH-1154, WH-1156, and HS-590, displaying moderate similarity and some distinctness from A1. Group II included KRL-210, Lok-1, AG-30-1, and HD-3086, representing the most genetically divergent genotypes. Overall, the dendrogram indicates that GW genotypes cluster closely together, whereas HD-3086 and AG-30-1 are the most distinct, corroborating the patterns observed in the similarity coefficients (Fig. 2).

Table 3: Jaccard's similarity coefficient between the wheat genotypes based on the RAPD data.

	GW-11	**GW-173**	**GW-190**	**GW-273**	**GW-322**	**GW-366**	**GW-496**	**HD-2932**	**J-24**	**WH-1154**	**WH-1156**	**HS-590**	**HD-3086**	**KRL-210**	**Lok-1**	**AG-30-1**
GW-11	1.000															
GW-173	0.880	1.000														
GW-190	0.828	0.867	1.000													
GW-273	0.860	0.876	0.859	1.000												
GW-322	0.812	0.807	0.837	0.869	1.000											
GW-366	0.796	0.746	0.776	0.805	0.772	1.000										
GW-496	0.831	0.801	0.820	0.852	0.828	0.824	1.000									
HD-2932	0.797	0.826	0.822	0.852	0.841	0.757	0.860	1.000								
J-24	0.753	0.781	0.777	0.783	0.806	0.726	0.811	0.881	1.000							
WH-1154	0.737	0.766	0.763	0.790	0.748	0.721	0.784	0.820	0.755	1.000						
WH-1156	0.722	0.751	0.738	0.785	0.754	0.696	0.768	0.815	0.761	0.833	1.000					
HS-590	0.688	0.717	0.745	0.750	0.740	0.692	0.743	0.738	0.689	0.775	0.792	1.000				
HD-3086	0.678	0.727	0.705	0.678	0.670	0.633	0.660	0.659	0.679	0.713	0.689	0.705	1.000			
KRL-210	0.748	0.714	0.763	0.725	0.737	0.668	0.729	0.714	0.715	0.720	0.705	0.733	0.743	1.000		
Lok-1	0.672	0.693	0.701	0.704	0.664	0.677	0.696	0.746	0.704	0.709	0.705	0.680	0.660	0.741	1.000	
AG-30-1	0.704	0.692	0.721	0.757	0.716	0.687	0.740	0.756	0.714	0.730	0.725	0.732	0.690	0.785	0.789	1.000

The scatter plot of pairwise genetic similarity further illustrates the genetic relationships among the genotypes. Most points cluster between 0.70 and 0.83, indicating moderate to high similarity, while a few reach approximately 0.88, representing highly similar pairs. Points between 0.63 and 0.70 reflect genetically divergent pairs. The plot visually confirms the variation in genetic relatedness, supporting the results from both Jaccard's similarity coefficients and the dendrogram, and highlighting the presence of both closely related and genetically distinct genotypes within the study set (Fig. 3).

To assess the goodness of fit of the RAPD data clustering, a matrix of cophenetic values was computed using the program COPH (Fig. 3). The cophenetic matrices were then compared with the original similarity matrices generated by SIMQUAL. Plots of one matrix

against the other were constructed, and the association statistics were calculated using MAXCOMP. In the present study, the Mantel test statistic Z was normalized, and the degree of goodness of fit for the cluster analysis (matrix correlation r=0.87) was classified as a "good fit," according to the criteria of Rohlf (1998).

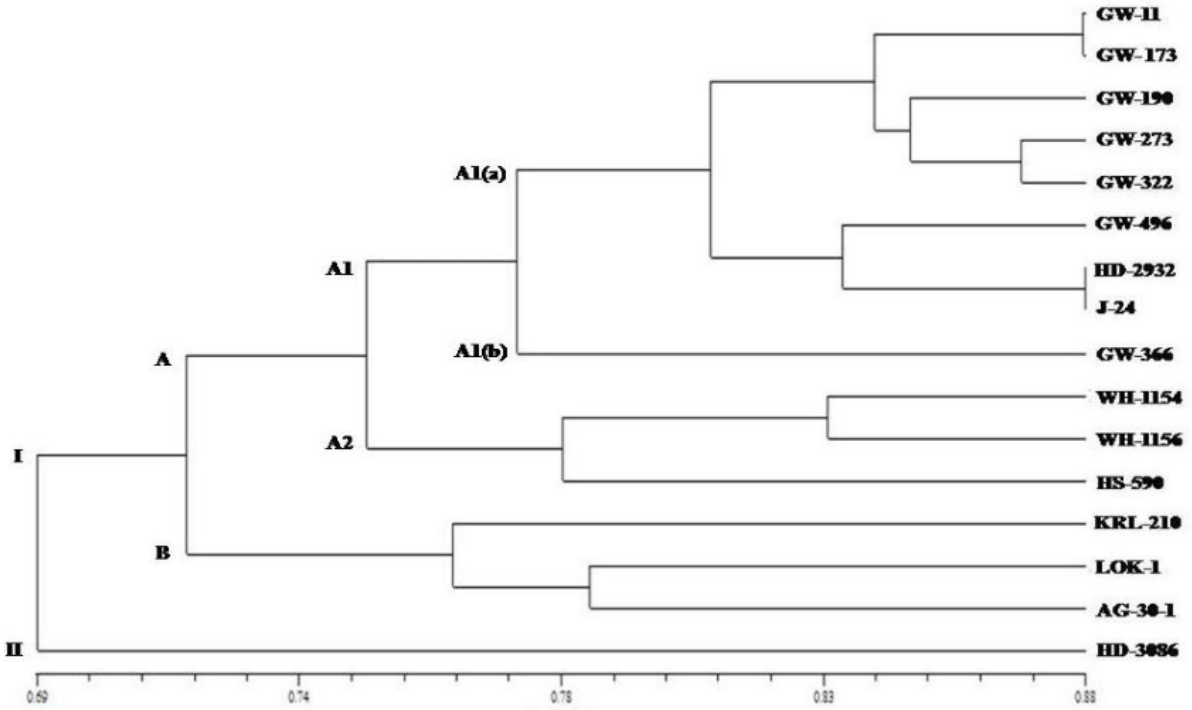
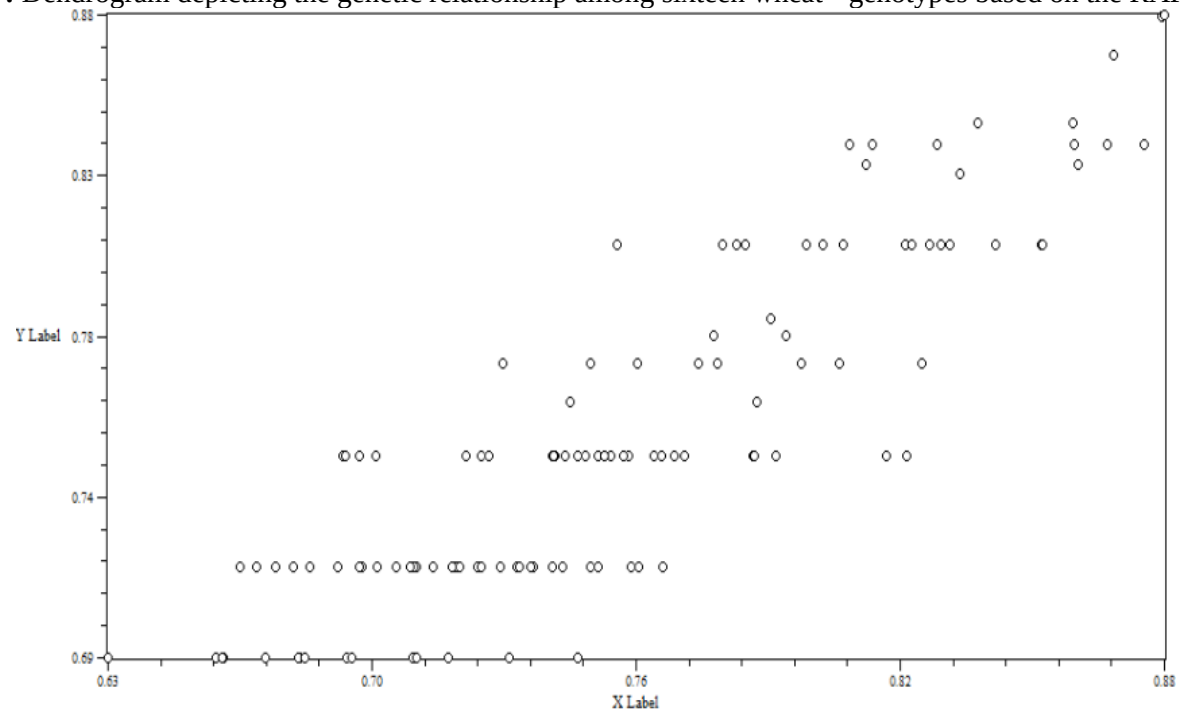


Fig. 2 : Dendrogram depicting the genetic relationship among sixteen wheat genotypes based on the RAPD data.



Matrix correlation: r=0.87

Fig. 3 : Cophenetic values against Jaccard's similarity coefficient from RAPD data of sixteen wheat genotypes.

Moghaieb *et al.* (2009) demonstrated that genetic makeup significantly influences rust tolerance in wheat. By analyzing seedlings of seven *Triticum aestivum* and two *T. durum* cultivars after fungal infection for two weeks, they identified 82 polymorphic RAPD markers out of 118 tested (69.5%), highlighting their utility in

distinguishing genotypes. This finding aligns with prior studies that have reported the effectiveness of RAPD markers in differentiating resistant and susceptible genotypes across various crops. For example, Sandhu *et al*. (2003) successfully tagged rust resistance genes in Brazilian wheat cultivars, while Boora (2003) identified multiple RAPD markers associated with resistance to anthracnose, leaf blight, and oral leaf spot in sorghum. Similarly, Gogoi *et al.* (2005) used RAPD to distinguish between Karnal bunt-resistant and susceptible wheat cultivars.

These studies collectively underscore the versatility of RAPD markers in evaluating genetic variation within germplasm and identifying disease-resistant genotypes. RAPD markers, due to their simplicity, rapidity, and cost-effectiveness, remain a valuable tool for early-stage screening in breeding programs. Despite the shift toward high-throughput markers, RAPD remains relevant for preliminary screening of germplasm, especially in resource-limited settings. Mumtaz *et al*. (2009) further expanded this approach, using 150 RAPD primers to amplify products ranging from 250 bp to 1000 bp, demonstrating the marker's capacity to reveal extensive molecular diversity in wheat.

Sharma *et al.* (2021) evaluated a set of bread wheat genotypes and reported a high degree of polymorphism across the SSR loci examined. The study detected multiple alleles per locus, with polymorphic information content (PIC) values ranging from moderate to high, indicating that SSR markers are reliable tools for distinguishing closely related genotypes. Cluster analysis grouped the genotypes into distinct clusters that reflected their genetic relationships, suggesting the potential of SSR markers for parental selection in breeding programs.

Similarly, Tahir *et al.* (2022) combined agronomic performance assessment with SSR-based molecular analysis in selected wheat lines. The results showed significant genetic variability among the lines, with SSR markers detecting polymorphic loci that correlated with key agronomic traits such as plant height, grain yield, and stress tolerance. This study emphasized the complementary role of molecular markers alongside phenotypic evaluation, reinforcing that integrating SSR data with field performance can accelerate the identification of superior genotypes for breeding programs.

Türkoğlu *et al.* (2023) analyzed bread wheat genotypes from Türkiye using SSR markers to evaluate both genetic diversity and population structure. Their results revealed clear genetic differentiation among the genotypes, as confirmed by cluster analysis and principal coordinate analysis (PCoA). The study also identified subpopulations corresponding to geographic origin, highlighting the impact of regional adaptation on genetic structure. Such findings underscore the utility of SSR markers in germplasm characterization, conservation, and selection of diverse parents to broaden the genetic base in breeding programs.

In conclusion, RAPD markers laid the groundwork for understanding molecular variation in wheat and identifying rust-resistant genotypes. The integration of advanced molecular markers and genome-wide tools now complements RAPD, allowing breeders to accelerate marker-assisted selection and develop wheat cultivars with durable resistance to rust diseases.

Conclusion

The present study demonstrates that RAPD analysis of sixteen wheat genotypes using 28 primers revealed substantial genetic variability, highlighting the effectiveness of RAPD markers for germplasm characterization and breeding. A total of 212 bands were amplified, ranging from 113 to 2845 bp, of which 109 were polymorphic, resulting in an average polymorphism of 55.62%. The number of bands per primer varied from 4 to 14, with PIC values ranging from 0.731 to 0.911 (average 0.820) and RPI values from 2.99 to 12.75 (average 6.288). Primers such as OPA-18, OPE-09, OPZ-15, and OPO-06 exhibited 100% polymorphism, reflecting their high discriminatory power and effectiveness in assessing genetic diversity. Genotype-specific markers were detected across multiple primers, with GW-366 showing the highest number of unique bands, while other genotypes exhibited 1–2 unique fragments. These markers are valuable for varietal identification, marker-assisted selection, and breeding programs targeting desirable traits. Jaccard's similarity coefficients ranged from 0.633 to 0.881, with GW-11 and GW-173 being the most similar, and HD-3086 and GW-366 the most divergent. Dendrogram and scatter plot analyses confirmed these genetic relationships, grouping closely related genotypes together and distinguishing genetically distinct ones. Overall, the results demonstrate significant genetic diversity among wheat genotypes, providing a reliable basis for germplasm evaluation, selection of diverse parental lines, and development of high-yielding, disease-resistant, and adaptable wheat cultivars.

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