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DECIPHERING MOLECULAR DIVERSITY AND GENETIC RELATIONSHIPS IN BREAD WHEAT GERMPLASM USING SSR MARKERS

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ABSTRACT
Genetic diversity assessment is essential for the effective characterization and utilization of wheat germplasm in breeding programmes. The present study evaluated the molecular diversity and genetic relationships among 55 bread wheat (*Triticum aestivum* L.) genotypes using simple sequence repeat (SSR) markers. A total of 39 SSR markers were screened, of which 21 were polymorphic and used for molecular diversity analysis. The polymorphic markers produced 1–3 alleles per locus, with a mean of 1.62 alleles, while polymorphism information content (PIC) ranged from 0.106 to 0.847, with a mean of 0.462. Mean resolving power and marker index were 0.723 and 0.490, respectively. Jaccard's similarity coefficient ranged from 0.296 to 1.000, revealing substantial molecular variation among the genotypes. UPGMA analysis grouped the genotypes into six clusters, with three genotypes forming distinct singleton clusters. The observed molecular differentiation demonstrated the presence of genetically diverse material within the studied germplasm. The identified informative SSR markers and divergent genotypes may be useful for germplasm characterization and selection of diverse parents to broaden the genetic base of wheat breeding programmes.

KEYWORDS: Bread wheat, SSR markers, Molecular diversity, Jaccard similarity, UPGMA.

INTRODUCTION
Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and a major staple food crop. Worldwide, wheat is cultivated on over 220 million hectares and its production more than 798 million tonnes annually. India is the second-largest wheat producer after China, wheat cultivating on approximately 31.8 million hectares with an annual production exceeding 113.29 million tonnes with productivity of 35.6 quintal per hectare (FAO 2024). Bread wheat possesses one of the largest and most intricate genomes among cereal crops. It is an allohexaploid species with a chromosome number of 42 (2n = 6x = 42) (Bennett and Smith, 1976), comprising three genetically related genomes, designated as A, B, and D. The availability and effective utilization of genetic diversity are essential for broadening the genetic base of wheat and developing cultivars with improved adaptation and productivity. Characterization of germplasm at the molecular level provides valuable information on genetic relationships and facilitates the identification of diverse parental material for wheat improvement (Sharma *et al*., 2021; Türkoğlu *et al*., 2023). A wide range of molecular markers, including random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), and simple sequence repeats (SSR), are available for studying genetic diversity in crop plants. These markers provide useful information about genetic variation at the molecular level and are therefore widely used in crop genetic diversity and characterization studies (Palombi and Damiano, 2002; Tsonev *et al*., 2021). Among molecular markers, simple sequence repeats (SSRs) are valuable for genetic diversity analysis because of their reproducibility, codominant inheritance and ability to detect variation across the wheat genome. SSR markers have been successfully employed to characterize bread wheat germplasm, estimate genetic diversity and identify genetically distinct groups for breeding purposes (Devi *et al*., 2023; Türkoğlu *et al*., 2023). Recent studies have further demonstrated the effectiveness of SSR-based approaches for differentiating wheat genotypes and identifying informative markers for germplasm utilization (Nelwadker *et al*., 2025). Therefore, the present study was undertaken to assess the molecular diversity and genetic relationships among 55 bread wheat genotypes using 39 SSR markers, identify informative polymorphic markers, and determine the clustering pattern of the genotypes for their potential utilization in future wheat breeding programmes.

MATERIAL AND METHODS

Plant Material and DNA isolation

The Study comprised 55 bread wheat (*Triticum aestivum* L.) genotypes (Table- 1) collected from ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana and SVPUAT, Meerut U.P. Young, healthy leaf samples were collected from 25–30-day-old plants, transported to the laboratory in an insulated icebox and stored at −20°C until DNA isolation. Genomic DNA was extracted from approximately 300 mg leaf tissue using a modified CTAB method (Doyle and Doyle, 1987). The tissue was ground in liquid nitrogen and homogenized with pre-warmed CTAB extraction buffer, followed by incubation at 65°C for 1 h. DNA was purified using chloroform: isoamyl alcohol (24:1), precipitated with isopropanol, washed with 70% ethanol and dissolved in TE buffer. RNA was removed by RNase treatment. DNA concentration and purity were assessed spectrophotometrically at 260 and 280 nm, and DNA integrity was checked on 0.8% agarose gel.

Table 1. List of genotypes along with their pedigree/source used in the study

Sr. No.	Entry	Pedigree
1	NUR 55	21th HTDTSBWON
2	NUR 19	31th HRWSN
3	PYT4	HD 3086/DBW 86
4	Chiriya-1/UP-2382	Chirya-1/UP-2382
5	NUR-75	12th HPAN
6	BISA 3	GS/2021-22/2046
7	PYT 1	PBW 780/DBW-39
8	BISA 5	GS/2021-22/1028
9	PYT 3	LBP-2020-51
10	PYT 29	PBW 780/DBW-39
11	NUR 74	21th ESBWYT
12	ESWYT-2020-107	ESWYT-2020-107
13	BWL 6326/ QBP 1627	BWL 6326/ QBP 1627
14	PYT 14	30th ESWYT-144
15	NUR 93	21th ESBWYT
16	PYT 25	CHIRYA-1/ UP-2382
17	BISA 4	GS/2021-22/3042
18	QBP 1626/BWL 5190	QBP 1626/BWL 5190
19	PYT 8	BWL-6321/DBW 88
20	BISA 2	GS/2021-22/1001
21	NUR 16	21th ESBWYT
22	NUR 49	53th IBWSN
23	NUR 10	41th ESWYT
24	NUR 27	21th ESBWYT
25	NUR 38	53th IBWSN
26	NUR 71	38th IBWSN
27	PBW 765/RAJ 3765	PBW 765/RAJ 3765
28	PYT 28	HD 3086/DBW 86
29	NUR 56	21th ESBWYT
30	PYT 24	SAWSN-2019-3225
31	PYT 31	ESWYT-118
32	PYT 17	BWL-6320/QBP-1622
33	PYT 18	ESWYT-2020/107
34	PYT 6	HTWYT-2021/48
35	NUR 18	12th HPAN
36	BISA 8	GS/2021-22/6048
37	PYT 30	PBW 780/DBW-39
38	NUR 48	53th IBWSN

39	BISA 6	GS/2021-22/3048
40	BISA 1	GS/2021-22/3060
41	NUR 23	38th SAWSN
42	NUR 9	12th HPAN
43	PBW 780/RAJ 3765	PBW 780/RAJ 3765
44	PYT 20	SAWSN-2019-1135
45	PBW 765/DPW 621-50	PBW 765/DPW 621-50
46	BWL 6320/QBP 1622	BWL 6320/QBP 1622
47	NUR 29	38th IBWSN
48	NUR 39	8th WYCYT
49	NUR 86	38th IBWSN
50	PYT 7	GS-20-21-5028
51	K-1317 (C)	K-1317
52	DBW 222 (C)	DBW 222
53	DBW 303 (C)	DBW 303
54	DBW 327 (C)	DBW 327
55	DBW 187 (C)	DBW 187

SSR-PCR Amplification and Electrophoresis

A total of 39 SSR markers (Table- 2) were used for molecular characterization. PCR amplification was performed in a 15 µL reaction mixture containing 10× Taq buffer with $MgCl_2$, dNTPs, forward and reverse primers, Taq DNA polymerase, template DNA and Milli-Q water. Amplification consisted of initial denaturation at 94°C for 5 min, followed by 38 cycles of denaturation at 94°C for 1 min, annealing for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 10 min. The amplified products were separated on 2% agarose gel in 1x TAE buffer and visualized using a gel documentation system.

Table- 2. PCR Profile

Sr. No.	Marker	Forward Sequence (5'➔3')	Forward Sequence (5'➔3')	Tm (°C)
1	Xbarc352	CCCTTTCTCGCTCGCCTATCCC	CTGTTTGCCCAATCTCGGTGTG	58.8
2	Xwmc477	CGTCGAAAACCGTACACTCTCC	GCGAAACAGAATAGCCCTGATG	54.6
3	Xgwm319	GGTTGCTGTACAAGTGTTCACG	CGGGTGCTGTGTGTAATGAC	53.3
4	Xgdm87	AATAATGTGGCAGACAGTCTTGG	CCAAGCCCCAATCTCTCTCT	53.8
5	Xgwm37	ACTTCATTGTTGATCTTGCATG	CGACGAATTCCCAGCTAAAC	50.7
6	Xbarc8	GCGGGAATCATGCATAGGAAAACAGAA	GCGGGGGCGAAACATACACATAAAAACA	58.3
7	Xbarc71	GCGCTTGTTCCTCACCTGCTCATA	GCGTATATTCTCTCGTCTTCTTGTTGGT	58.3
8	Xwmc619	TTCCCTTTCCCCTCTTTCCG	TACAATCGCCACGAGCACCT	56.0
9	Xgwm124	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG	53.1
10	Xwmc432	ATGACACCAGATCTAGCAC	AATATTGGCATGATTACACA	46.2
11	Xgwm232	ATCTCAACGGCAAGCCG	CTGATGCAAGCAATCCACC	54.5
12	Xgwm312	ATCGCATGATGCACGTAGAG	ACATGCATGCCTACCTAATGG	52.3
13	Xgwm265	TGTTGCGGATGGTCACTATT	GAGTACACATTTGGCCTCTGC	51.1

14	Xgwm113	ATTCGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC	54.2
15	Xwmc617	CCACTAGGAAGAAGGGGAAACT	ATCTGGATTACTGGCCAACTGT	54.2
16	Xwmc74	AACGGCATTGAGCTCACCTTGG	TGCGTGAAGGCAGCTCAATCGG	57.2
17	Xwmc47	GAAACAGGGTTAACCATGCCAA	ATGGTGCTGCCAACAACATACA	54.7
18	Xwmc492	AGGATCAGAATAGTGCTACCC	ATCCCGTGATCAGAATAGTGT	53.9
19	Xwmc386	ATCACTGAAACGAAATGAGCGG	TGGTTGGCGGTTTTTCTCTACA	54.4
20	Xgwm190	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	54.2
21	Xwmc317	TGCTAGCAATGCTCCGGGTAAC	TCACGAAACCTTTTCCTCCTCC	55.2
22	Xcfd190	CAATCAGAAGCGCCATTGTT	CCCTGATGTTTTCTTTTTCTCC	50.9
23	Xwmc179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	55.4
24	Xwmc398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	50.5
25	Xgwm70	AGTGGCTGGGAGAGTGTCAT	GCCCATTACCGAGGACAC	54.9
26	Xgwm325	TTTCTTCTGTCGTTCTCTTCCC	TTTTTACGCGTCAACGACG	51.1
27	Xwmc469	AGGTGGCTGCCAACG	CAATTTTATCAGATGCCCGA	50.5
28	Xwmc307	GTTTGAAGACCAAGCTCCTCCT	ACCATAACCTCTCAAGAACCCA	52.1
29	Xwmc533	AATTGGATCGGCAGTTGGAG	AGCAAGCAGAGCATTGCGTT	54.9
30	Xwmc262	GCTTTAACAAAGATCCAAGTGG CAT	GTAAACATCCAAACAAAGTCGAA CG	53.7
31	Xgwm46	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA	53.0
32	Xwmc322	CGCCCCACTATGCTTTG	CGCAAACTTGTGTATCCTTATC	50.7
33	Xbarc340	GCAACCAAGGCAGCGTAAATG	GCGTGTAGCCGTCCATAAGCATC AT	57.6
34	Xwmc232	GAGATTTGTTCATTTCATCTTCG CA	TATATTAAAGGTTAGAGGTAGTC AG	48.8
35	Xuhw89	TCTCCAAGAGGGGAGAGACA	TTCCTCTACCCATGAATCTAGCA	53.6
36	Xwms30	ATCTTAGCATAGAAGGGAGTGG G	TTCTGCACCCTGGGTGAT	54.2
37	Xwms304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	51.4
38	Xwms169	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG	55.5
39	Xwmc153	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	57.3

Data Scoring and Molecular Diversity Analysis

Clear and reproducible SSR bands were scored manually, with the presence and absence of bands designated as 1 and 0, respectively, to generate a binary data matrix. Only unambiguous bands were considered for analysis. Genetic similarity among genotypes was estimated using Jaccard's similarity coefficient (Jaccard, 1908), and clustering was performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method in DARwin 6.0 and R. The Polymorphism Information Content (PIC) of each SSR marker was calculated according to Botstein *et al*. (1980) using allele frequencies. SSR-based molecular characterization and clustering have been widely applied for assessing genetic diversity and relationships among bread wheat genotypes (Devi *et al*., 2023; Nelwadker *et al*., 2025).

RESULTS AND DISCUSSION

Molecular diversity among 55 bread wheat (*Triticum aestivum L.*) genotypes was assessed using 39 SSR markers, of which 34 amplified successfully; 21 were polymorphic and 15 monomorphic. The polymorphic markers produced 1–3 alleles per locus, averaging 1.62 alleles. PIC ranged from 0.106 (Xuhw89) to 0.847 (Xgwm232), with a mean of 0.462; Xgwm232, Xwmc153 (0.753) and Xgwm124 (0.721) were the most informative markers. Resolving power ranged from 0.109–1.818 (mean 0.723), with Xwmc153 showing the highest value, while marker index ranged from 0.103–1.094 (mean 0.490), with Xwmc74 recording the maximum (Table 3). These results demonstrate the usefulness of SSR markers for discriminating wheat genotypes, consistent with recent SSR-based characterization of bread wheat germplasm (Ghazy *et al.,* 2025, Devi *et al*., 2023; Türkoğlu *et al*., 2023).

Table-3 Results of SSR markers across the fifty-five genotypes in Wheat

SR No.	**Primer**	**PIC**	**RP**	**MI**
1.	Xwmc469	0.640	0.800	0.480
2.	Xwmc477	0.238	0.255	0.222
3.	Xgwm325	0.501	1.345	0.967
4.	Xgwm190	0.389	0.436	0.341
5.	Xbarc71	0.597	0.109	0.103
6.	Xcfd190	0.206	0.402	0.309
7.	Xwmc153	0.753	1.818	1.066
8.	Xwmc74	0.565	1.636	1.094
9.	Xwmc617	0.206	0.218	0.194
10.	Xgwm124	0.721	0.236	0.137
11.	Xwmc322	0.537	1.673	1.074
12.	Xwmc432	0.206	0.453	0.316
13.	Xwmc386	0.174	0.182	0.165
14.	Xgwm37	0.140	0.145	0.135
15.	Xgwm232	0.847	1.382	0.987
16.	Xwmc398	0.492	0.109	0.103
17.	Xgdm87	0.597	1.782	1.086

18.	Xwmc179	0.635	0.293	0.276
19.	Xwms30	0.628	0.186	0.159
20.	Xuhw89	0.106	0.178	0.146
21	Xgwm46	0.529	1.564	0.949

Jaccard's similarity coefficient ranged from 0.296 to 1.000, with the highest similarity between BISA 8 and PBW 765/DPW 621-50, and the lowest between NUR-75 and PYT 28 and between PYT 30 and DBW 303. UPGMA analysis grouped the 55 genotypes into six clusters: Cluster I contained twenty nine genotypes, Cluster II twenty one, Cluster III two, and Clusters IV–VI one genotype each (Table- 4). The singleton clusters comprised NUR-75, BISA 5 and QBP 1626/BWL 5190, respectively. The wide similarity range and distinct clustering pattern indicate the presence of genetically differentiated material within the germplasm, as also reported in recent SSR-based wheat diversity studies (Devi *et al*., 2023; Nelwadker *et al*., 2025; Türkoğlu *et al*., 2023).

Table- 4 Clustering of 55 bread wheat genotypes based on SSR marker analysis

Cluster	**Number of Genotypes**	**Genotypes**
Cluster I	29	NUR 55, Chiriya-1/UP-2382, PYT 1, PYT 3, NUR 74, ESWYT-2020-107, BWL 6326/ QBP 1627, PYT 14, PYT 25, BISA 4, BISA 2, NUR 49, NUR 27, NUR 38, PYT 28, PYT 24, PYT31, PYT 17, PYT 18, NUR 18, PYT 30, BISA 6, NUR 9, PYT 20, BWL 6320/ QBP 1622, NUR 39, PYT 7, DBW 222, DBW 327
Cluster II	21	NUR 19, BISA 3, PYT 29, NUR 93, NUR 16, NUR 10, NUR 71, PBW 765/ RAJ 3765, NUR 56, PYT 6, BISA 8, NUR 48, BISA 1, NUR 23, PBW 780/ RAJ 3765, PBW 765/DPW 621-50, NUR29, NUR 86, K-1317, DBW 303, DBW187
Cluster III	2	PYT 4, PYT8
Cluster IV	1	NUR-75
Cluster V	1	BISA 5
Cluster VI	1	QBP 1626/BWL 5190

The molecular differentiation observed among the genotypes provides useful information for germplasm characterization and parental selection. Genotypes occupying different clusters, particularly those forming distinct clusters, may be useful for broadening the genetic base and developing diverse breeding populations, subject to confirmation of their agronomic performance. Recent wheat studies have similarly emphasized the value of molecular diversity analysis for identifying divergent parental material and improving the utilization of wheat germplasm (Alqurashi *et al*., 2026, Devi *et al*., 2023; Nelwadker *et al*., 2025).

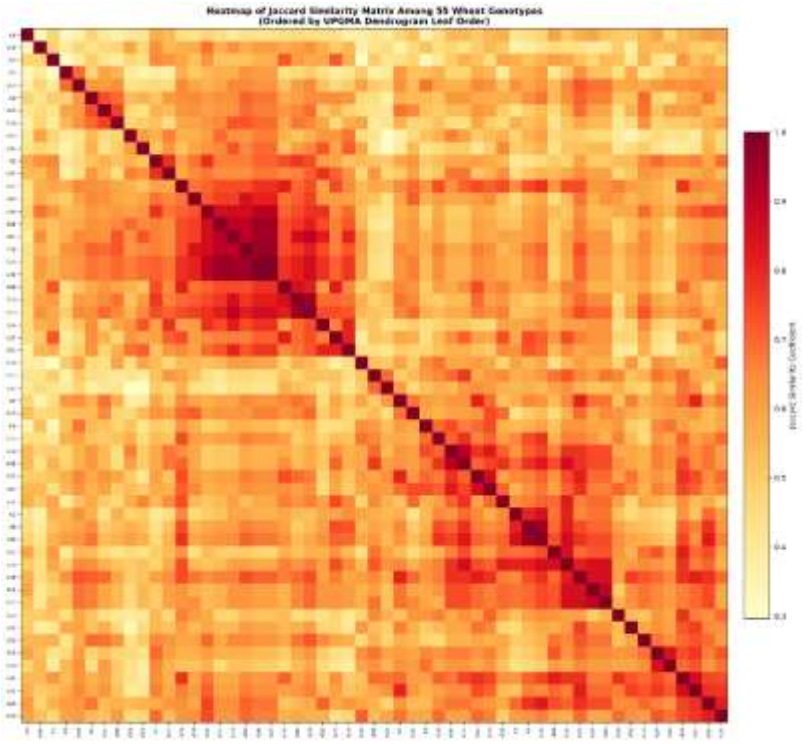

Fig.1 Heat-map of Jaccard similarity matrix among 55 wheat genotypes

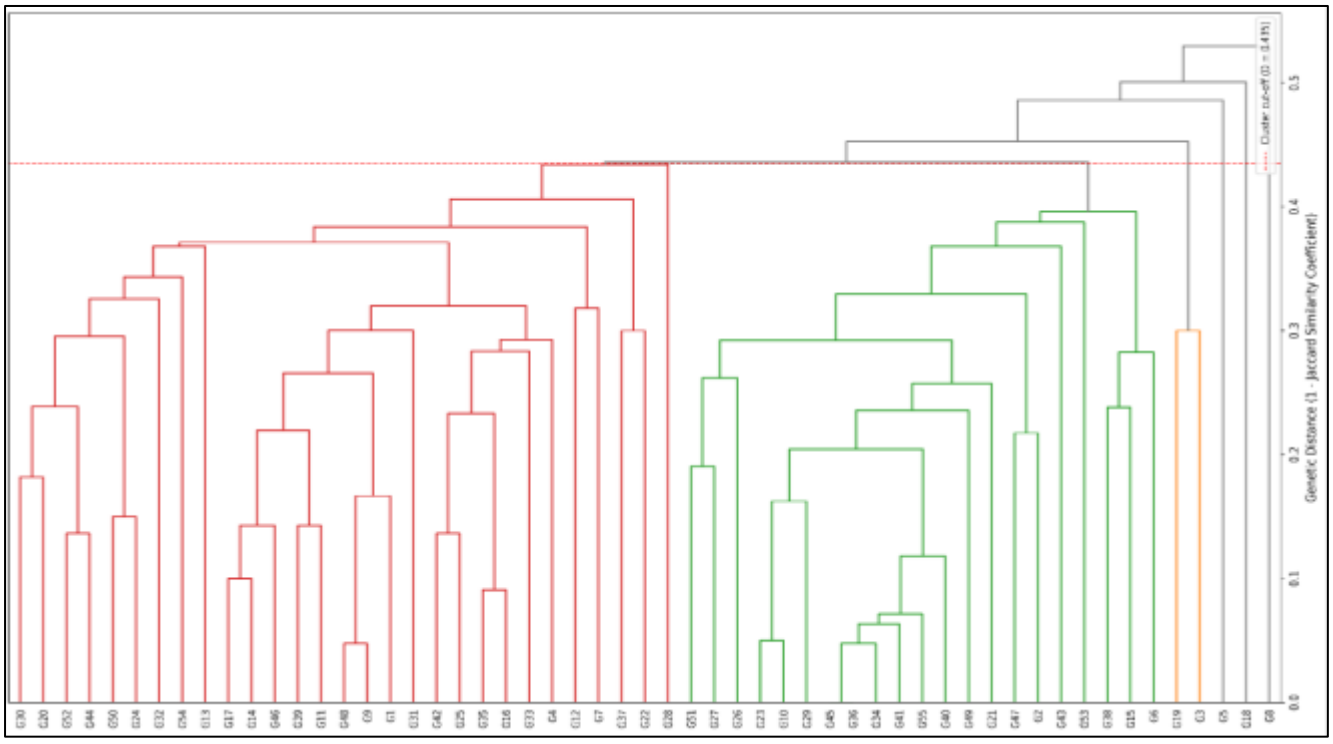

Fig. 2 UPGMA Dendrogram Based on Jaccard Similarity Coefficient for 55 Wheat Genotypes

CONCLUSION

The SSR-based analysis effectively revealed the molecular relationships and genetic structure of the studied bread wheat germplasm. The considerable variation in marker informativeness and the distinct clustering pattern indicated the presence of genetically differentiated material within the collection. The genetically distinct genotypes identified in the study, particularly those occupying separate clusters, represent valuable resources for broadening the genetic base of wheat. These materials can be considered in future hybridization programmes to generate diverse breeding populations and facilitate the development of improved wheat cultivars. Thus, SSR-based molecular characterization can complement conventional evaluation and provide a reliable basis for the conservation, characterization and strategic utilization of wheat genetic resources.

REFERENCES

1. Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32, 314–331.
2. Devi, S., Singh, V., Yashveer, S., Dalal, M. S., Paras, Chawla, R. K., Akbarzai, D. K., and Chaurasia, H. (2023). Molecular characterization of bread wheat (Triticum aestivum) genotypes using SSR markers. The Indian Journal of Agricultural Sciences, 93(9), 948–953.
3. Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11–15.
4. Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles, 44, 223–270.
5. Nelwadker, R. R., Rouf Mir, R., Kumar, S., and Khan, M. A. (2025). Genetic diversity analyses of bread wheat (Triticum aestivum L.) genotypes using microsatellite markers. Journal of Cereal Research, 17(3), 318–327. http://doi.org/10.25174/2582-2675/2025/169737.
6. Sharma, R., Kumar, S., Singh, S. K., Sharma, P., and Singh, G. P. (2021). Genetic diversity among bread wheat (Triticum aestivum L.) genotypes as assessed by SSRs. Journal of Cereal Research, 13(2).
7. Türkoğlu, A., Haliloğlu, K., Mohammadi, S. A., Öztürk, A., Bolouri, P., Özkan, G., Bocianowski, J., Pour-Booghdee, A., and Jamshidi, B. (2023). Genetic diversity and population structure in Türkiye bread wheat genotypes revealed by simple sequence repeats (SSR) markers. Genes, 14(6), 1182.
8. Faostat (2022). Food and Agriculture Organization of the United Nations.
9. Palombi, M.A. and Damiano, C. (2002). Comparison between RAPD and SSR molecular markers in detecting genetic variation in kiwi fruit (Actinidia deliciosa A. Chev). Plant Cell Reports, 20: 1061-1066.
10. Tsonev, S., Christov, N.K., Mihova, G., Dimitrova, A., and Todorovska, E.G. (2021). Genetic diversity and population structure of bread wheat varieties grown in bulgaria based on microsatellite and phenotypic analyses. Biotechnology and Biotechnological Equipment, 35(1), 1520–1533. DOI: 10.1080/13102818.2021.1996274.
11. Ghazy, A. I., Al Ateeq, T. K., Ibrahim, E. I., Abdel-Haleem, H., Attia, K. A., Azab, O., & Al-Doss, A. A. (2025). Selection of heat stress tolerant wheat genotypes for desert environments. Scientific Reports, 15(1), 36672.
12. Alqurashi, M., Aloufi, S., Albalawi, D. A., Al-Otaibi, A. S., Alharbi, B. M., Alzuaibr, F. M., & Abd El-Hakim, A. F. (2026). Molecular marker based assessment of wheat biodiversity for conservation and sustainable breeding. Biodiversity and Conservation, 35(3), 97.
13. Bennett, M. D., & Smith, J. B. (1976). Nuclear DNA amounts in angiosperms. Philosophical Transactions of the Royal Society, London, 274(933), 227-274.