RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (2) 635-642, 2026
http://doi.org/10.54910/sabrao2026.58.2.14
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

HIGH-RESOLUTION DNA FINGERPRINTING AND GENETIC DIVERSITY ASSESSMENT IN SORGHUM (*SORGHUM BICOLOR* L.) USING SSR MARKERS

S. KANWAL[1*], M. AKRAM[2], S. JAMIL[1], AREEBA[2], and Q. SHAKIL[1]

[1]Agricultural Biotechnology Research Institute, University of Agriculture, Faisalabad, Pakistan
[2]University of Agriculture, Faisalabad, Pakistan
[*]Corresponding author's email: Kanwalshamsa32@yahoo.com
Email addresses of co-authors: ranashahbaz436@gmail.com, Shakrajamil@yahoo.com, areebaqadir2@gmail.com, Shakil.qamar@yahoo.com

SUMMARY

Genetic diversity is a key driver of crop improvement and resilience against environmental stresses. This study aimed to evaluate genetic diversity and develop DNA fingerprints of 13 sorghum (*S. bicolor* L.) genotypes using highly polymorphic simple sequence repeat (SSR) markers. SSR markers used totaled 50 to amplify genomic regions, with genetic relationships analyzed using unweighted pair group method with arithmetic mean (UPGMA) clustering, principal coordinate analysis (PCoA), and heatmap visualization based on binary allele scoring. The markers exhibited high informativeness, with PIC (polymorphism information content) values ranging from 0.49 to 0.94, and classifying the majority as highly informative (PIC ≥ 0.80), effectively discriminating among the genotypes. Multivariate analysis consistently revealed three major genetic clusters, highlighting both closely related and highly divergent genotypes. These findings underscore the presence of genetically distinct lines, representing valuable resources for broadening the genetic base in sorghum breeding. Overall, the study demonstrates that SSR-based DNA fingerprinting is a reliable, cost-effective, and efficient tool for molecular characterization, diversity assessment, and strategic parent selection. Such approaches are essential for optimizing sorghum breeding programs and enhancing crop resilience under changing environmental conditions.

Keywords: Sorghum (*S. bicolor* L.), genetic diversity, DNA fingerprinting, SSR markers, UPGMA clustering, principal coordinate analysis, heatmap tree, genetic relationship

Key findings: Highly informative SSR markers, including XTXP-294, XTXP-210, and XTXP-218, effectively distinguished the sorghum (*S. bicolor* L.) genotypes, forming three major clusters that reveal patterns of genetic divergence. Several genotypes, AK-113, CMS-7B, and F-01-2017, were highly divergent, indicating their potential for introducing novel alleles.

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: September 24, 2025; Accepted: March 12, 2026.

Citation: Kanwal S, Akram M, Jamil S, Areeba, Shakil Q (2026). High-resolution DNA fingerprinting and genetic diversity assessment in sorghum (*Sorghum bicolor* L.) using SSR markers. *SABRAO J. Breed. Genet. 58 (2) 635-642. http://doi.org/10.54910/sabrao2026.58.2.14*.

INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench), a climate-resistant crop, is a lifeline for small farmers in Pakistan’s arid regions, where droughts and heatwaves reduce yields of staple crops like wheat and maize. Despite its natural drought tolerance, sorghum’s productivity remains constrained due to limited genetic diversity in commercial varieties. With its C4 photosynthetic pathway and drought tolerance, sorghum serves as a vital genetic resource for breeding climate-resilient cultivars to enhance food security in challenging environments. Exploiting the abundant genetic variation within sorghum germplasm collections and their wild relatives is crucial for developing improved cultivars that are adapted to both biotic and abiotic stresses (Hao *et al*., 2021).

In overcoming this gap, genetic diversity serves as the foundation for developing improved crop varieties, enabling breeders to combine desirable traits, uncover valuable alleles, and conserve key agronomic characteristics. Among molecular tools, SSR markers have become crucial for DNA fingerprinting due to their reliability, high polymorphism rates, and ability to differentiate even closely related genotypes (Kumar *et al*., 2011). Recent applications in germplasm characterization illustrate how SSR-based profiling accelerates the identification of genetically diverse parents, supports trait introgression, and enhances conservation efforts (Cuevas *et al*., 2018).

In sorghum, these markers have revealed broad variation among landraces, breeding lines, and wild relatives. This study addresses a critical limitation in Pakistani sorghum breeding by using SSR markers to assess genetic diversity. We provide genetic information for 13 genotypes, disclosing substantial divergence among them. Notably, some advanced lines exhibit exceptional genetic distance, highlighting their potential as diverse parental sources. Breeders can apply these results to protect traditional varieties using verifiable SSR profiles under national seed laws and exploit the identified genetic divergence for developing high-yielding, stress-resistant hybrids.

This study delivers the genetic characterization of Pakistan's traditional sorghum diversity through SSR-marker analysis, establishing essential foundational data for both conservation and breeding applications. We generate reproducible molecular fingerprints for 13 sorghum genotypes, enabling precise varietal identification and revealing valuable allelic variation. These findings provide breeders with genetically verified parental selection to develop climate-resilient hybrids suited to Pakistan’s arid regions, linking genetic resource conservation with modern crop improvement.

MATERIALS AND METHODS

Thirteen sorghum genotypes surveyed used SSR markers for genetic diversity assessment (Table 1). The cultivars’ growing transpired at the Agricultural Biotechnology Research Institute (ABRI), Faisalabad, Pakistan, under standard growth conditions.

DNA extraction

Genomic DNA extraction occurred from fresh young leaves using the CTAB method with minor modifications for cereals (Doyle and Doyle, 1990). Leaf tissues underwent grinding in liquid nitrogen (−196 °C) to obtain a fine powder for extraction. The DNA extraction buffer (CTAB buffer-2x) (for 1 l: Tris base 12.11 g, polyvinyl pyrrolidone [PVP] 20 g, sodium ethylenediamine tetraacetic acid [Na-EDTA] 7.44 g, sodium chloride [NaCl] 81.8 g, 2% β-mercaptoethanol, and cetyltrimethylammonium bromide, or CTAB, 30 g) served to homogenize and digest the sample. The mixture received incubation at 65 °C for 20 min with occasional swirling before emulsification with phenol:chloroform:isoamyl alcohol (25:24:1). DNA precipitation utilized absolute ethanol and sodium acetate, then proceeded to make pellets before washing with 70% ethanol and resuspension in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Finally, the DNA incurred treatment with RNase to remove RNA contaminants. The purity and concentration of DNA, when measured,

Table 1. List of sorghum genotypes used in the study.

No.	Genotypes	Species
1	YSH-134	*Sorghum bicolor* (L.) Moench
2	CMS-7B	*Sorghum bicolor* (L.) Moench
3	YSS-98	*Sorghum bicolor* (L.) Moench
4	CMS-7A	*Sorghum bicolor* (L.) Moench
5	YS-16	*Sorghum bicolor* (L.) Moench
6	Pak Sorghum	*Sorghum bicolor* (L.) Moench
7	Ausaf	*Sorghum bicolor* (L.) Moench
8	AK-113	*Sorghum bicolor* (L.) Moench
9	Fakhr-e-Punjab	*Sorghum bicolor* (L.) Moench
10	YS-17	*Sorghum bicolor* (L.) Moench
11	YS-42	*Sorghum bicolor* (L.) Moench
12	F-01-2017	*Sorghum bicolor* (L.) Moench
13	No. 1572	*Sorghum bicolor* (L.) Moench

employed a NanoDrop spectrophotometer (Thermo Scientific, U.S.A.).

PCR amplification

PCR reactions succeeded in a thermal cycler with a total reaction volume of 25 µL, including 20 ng/µL genomic DNA of each variety, 0.6 µM of each forward and reverse primer, and 13 µL of green master mix for different SSR markers. The following temperature conditions applied for amplification comprised initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at variable primer-specific temperatures for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. The amplified PCR products remained in storage at 4 °C (Rahman *et al*., 2022).

Gel electrophoresis

PCR products' separation on 6% polyacrylamide gels used the electrophoresis system model POWERPRO-3AMP (Cleaver Scientific Limited). Electrophoresis took place at 90 W for 1 h with the gels stained with silver nitrate (Jamil *et al*., 2021). Capturing images engaged a Syngene transilluminator, with the banding patterns recorded under UV transilluminator for further analysis. (Figure 1).

Statistical analysis

The use of UPGMA clustering served to construct a dendrogram using R software (R v4.3.1). Moreover, polymorphism information content (PIC) for each SSR marker, along with the number of alleles, also attained calculations in Microsoft Excel using the formula by Botstein *et al*. (1980). The study also employed the principal coordinate analysis (PCoA) and heatmap visualization using the R software (R v4.3.1) (Liu *et al*., 2003).

RESULTS AND DISCUSSION

Polymorphism information content (PIC) analysis

A considerable level of polymorphism resulted among the 13 sorghum genotypes using 50 SSR loci, confirming the effectiveness of these markers in revealing genetic diversity within the analyzed panel (Table 2). The PIC values ranged from 0.49 to 0.94, indicating substantial variability in marker informativeness across loci. Among these, 43 loci showed high PIC values (PIC ≥ 0.80), with XTXP-294 (0.94) and XTXP-210 and XTXP-218 (0.93) being the most polymorphic. Moderately informative markers included XTXP-183 (0.71), XTXP-358 (0.72), and XTXP-63 (0.77), while OL-4 (0.49) was the only low-informative locus.

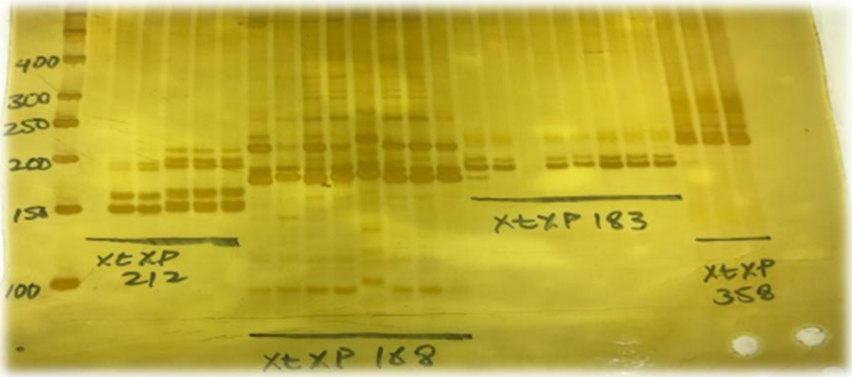

Figure 1. SSR banding pattern of sorghum genotypes on PAGE, used for allele scoring.

Table 2. Polymorphism information content (PIC) values and informativeness of 50 SSR markers used for genetic diversity analysis in sorghum genotypes.

No.	Primers	Number of alleles	PIC value	No.	Primers	Number of Alleles	PIC value
1	XTXP-65	13	0.88	26	XTXP-168	17	0.92
2	XTXP-218	25	0.93	27	XTXP-183	6	0.71
3	XTXP-50	12	0.87	28	XTXP-46	24	0.92
4	XTXP-18	7	0.84	29	XTXP-227	13	0.89
5	XTXP-63	7	0.77	30	XTXP-273	10	0.82
6	XTXP-61	10	0.86	31	XTXP-96	17	0.92
7	XTXP-47	16	0.91	32	OL-4	3	0.49
8	XTXP-343	17	0.92	33	XTXP-350	8	0.79
9	XTXP-36	12	0.87	34	XTXP-358	5	0.72
10	XTXP-159	13	0.89	35	XTXP-217	13	0.89
11	XTXP-215	19	0.92	36	XTXP-33	11	0.89
12	XTXP-302	10	0.86	37	XTXP-176	5	0.67
13	XTXP-92	17	0.92	38	XTXP-295	9	0.81
14	XTXP-10	9	0.83	39	XTXP-23	14	0.89
15	XTXP-312	14	0.87	40	XTXP-51	11	0.88
16	XTXP-278	14	0.86	41	XTXP-104	10	0.83
17	XTXP-13	8	0.82	42	XTXP-145	11	0.89
18	XGAP-42	13	0.89	43	XTXP-149	13	0.87
19	XTXP-210	22	0.93	44	XTXP-303	11	0.81
20	XTXP-114	9	0.87	45	XTXP-6	8	0.82
21	XTXP-294	23	0.94	46	XTXP-60	9	0.84
22	XTXP-225	11	0.87	47	XTXP-208	11	0.89
23	XGAP-34	9	0.81	48	XTXP-325	10	0.88
24	XTXP-69	21	0.92	49	XTXP-57	13	0.89
25	XTXP-212	8	0.81	50	XTXP-228	7	0.82

The predominance of highly informative loci indicates that the selected SSR panel is effective for differentiating the studied genotypes. The overall high PIC values reflect a strong genetic base among the selected genotypes and the suitability of SSRs for genetic diversity assessment (Cuevas *et al*., 2018). Markers, such as XTXP-294 and XTXP-210, exhibited superior allele detection and are particularly useful for varietal identification and marker-assisted selection**,** consistent with earlier findings (Santhiya *et al*., 2020). These results demonstrate the potential of SSR-based fingerprinting as a reliable and cost-effective tool for identifying diverse parental lines and guiding future sorghum breeding programs. Reports on similar findings have appeared in other cereals, where highly polymorphic SSRs effectively captured allelic variation across diverse germplasm (Hu *et al*., 2019).

Cluster analysis

Cluster analysis based on the unweighted pair group method with arithmetic mean (UPGMA) revealed clear genetic differentiation among the 13 sorghum genotypes, forming three distinct clusters at a genetic distance ranging from 1 to 4 (Figure 2). Cluster III, represented in red, comprised the most divergent genotypes, AK-113, Fakhr-e-Punjab, YS-17, YS-12, F-01-2017, and NO.1572, which were separated by longer branch lengths, indicating the highest degree of genetic variability. This broad genetic distance suggests these genotypes possess unique allelic combinations and can serve as valuable parents. Similar patterns have been noted by Melo *et al*. (2021) in genotypes from contrasting agroecological origins. Cluster II, represented in yellow, including CMS-7A, YS-16, Pak Sorghum, and Ausaf, showed moderate diversity. Comparable clusters have been notable for containing useful phenotypic and stress-tolerant variation in sorghum (Navyashree *et al*., 2024). Using these genotypes in crosses could contribute alleles not present in the most divergent or the most related clusters, helping to maximize heterosis while retaining agronomic adaptability.

Cluster I, represented in blue, comprising YSH-134, CMS-7B, and YSS-98, was the most closely related group, suggesting shared selection history or common parental lines. This pattern aligns with Borrell *et al*. (2014), who found that elite breeding lines with similar genetic backgrounds cluster tightly. Together, these clustering results reflect the underlying genetic constitution of the studied genotypes and affirm the effectiveness of SSR markers in detecting genetic differentiation. They also provide strong guidance for selecting parental genotypes from different clusters, particularly from Clusters III and II, for future breeding of stress-resilient, high-yielding sorghum varieties.

Principal coordinate analysis (PCoA)

Principal coordinate analysis (PCoA) helped visualize genetic relationships among the 12 sorghum genotypes based on SSR markers using two axes, representing the main proportion of genetic variation. The PCoA plot revealed three distinct clusters (Figure 3).

Cluster 1 included YSH-134, YSS-98, CMS-7A, CMS-7B, and YS-16, with YS-16 positioned slightly further from the other members, indicating relative intra-cluster divergence. Cluster 2, comprising Ausaf, Pak Sorghum, and AK-113, showed moderate separation from Cluster 1. These observations align with those of Nemera *et al*. (2022), who reported weak clustering in the Ethiopian sorghum population due to continuous gene flow and shared agroecological conditions. Cluster 3 comprised YS-42, YS-17, F-01-2017, and Fakhr-e-Punjab, with F-01-2017 positioned furthest from the remaining members, highlighting significant allelic differences. These results agree with Shahbazi *et al*. (2025), who performed SSR-based PCoA analysis, confirming its reliability for identifying diverse parental lines in crop improvement programs. The distinct positioning of YS-16 and F-01-2017 signifies these genotypes are relatively more genetically divergent within the studied panel, which may be of interest for further evaluation in breeding programs after phenotypic and agronomic validation (Morris *et al*., 2013).

Heatmap tree analysis

The heatmap (Figure 4) provided a visual representation of pairwise genetic similarity among the genotypes based on SSR-marker data. Heatmaps offer an intuitive and effective method for visually interpreting genetic similarity and supporting marker validation in molecular studies (Hu *et al*., 2019). This approach has wide recognition for its efficacy in revealing intricate genetic relationships and population substructures that might be missed in a simple dendrogram analysis.

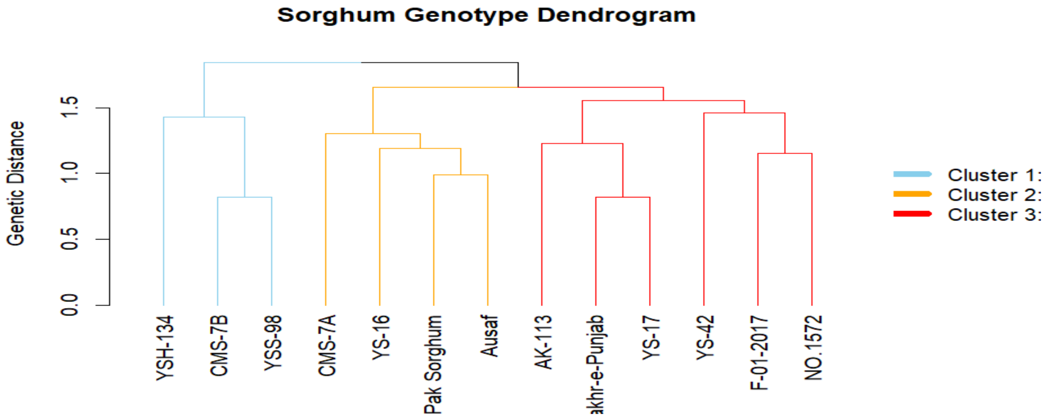

Figure 2. Dendrogram showing genetic clustering of 13 sorghum genotypes based on SSR data.

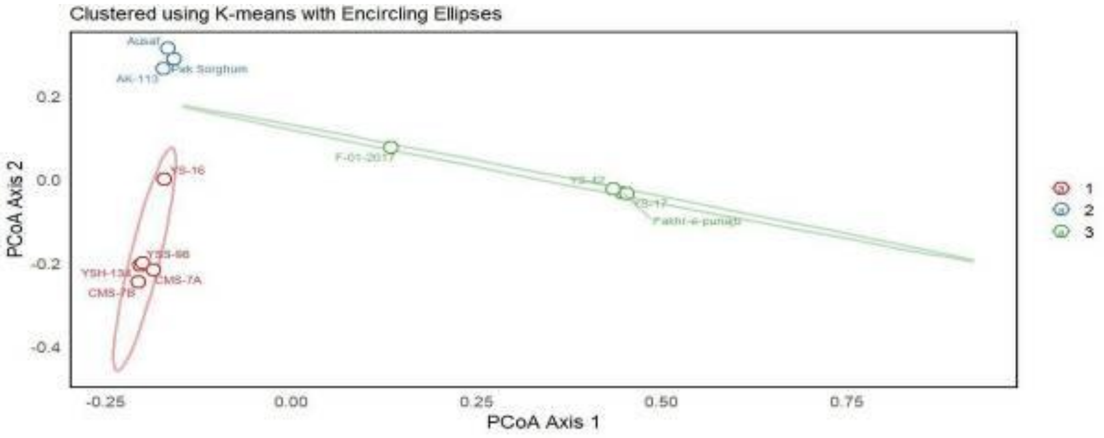

Figure 3. Principal coordinate analysis (PCoA) supporting dendrogram results showing three different clusters.

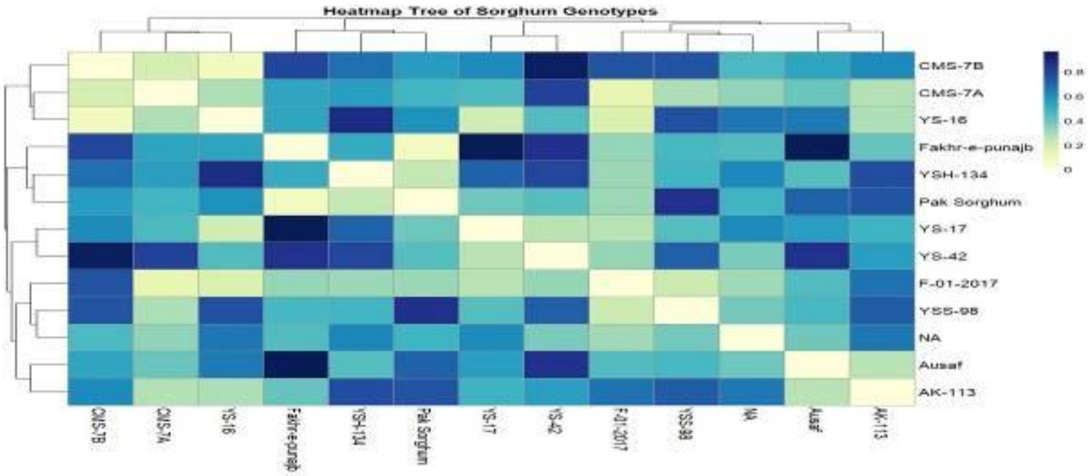

Figure 4. Heatmap tree representing genetic similarity among 13 sorghum genotypes based on SSR marker profiles.

Genotypes, such as CMS-7A and CMS-7B, as well as YS-16 and YS-17, appeared closely clustered, indicating high genetic similarity. In contrast, AK-113 and CMS-7B displayed lighter-colored intersections, reflecting lower similarity with other genotypes, while Fakhr-e-Punjab and YS-16 showed relatively darker intersections, suggesting higher similarity between them. Overall, the clustering pattern detailed at least three to four genetically distinct groups among the studied genotypes. The similarity patterns observed in the heatmap corresponded with the relationships identified through UPGMA clustering, supporting the consistency of SSR markers in resolving genetic variations among the analyzed genotypes. Comparable SSR-based analyses have likewise reported substantial variability within sorghum germplasm collections (Ahmed *et al*., 2025).

AK-113 and CMS-7B, carrying unique alleles, indicate the genetic divergence within the studied panel, which may be essential for sustaining yield stability under climate change. Such genetic diversity is important for enhancing traits like drought tolerance, disease resistance, and overall crop resilience (Lasky *et al*., 2015).

CONCLUSIONS

SSR markers proved effective for assessing genetic diversity and relationships among 13 sorghum genotypes. Multivariate analysis identified distinct lines that could be useful to broaden the genetic base. This study delivers a reliable fingerprinting platform that is immediately applicable for cataloging germplasm, selecting parental lines, and prioritizing genetic resources for conservation. The path forward lies in the seamless integration of this molecular dataset with deep phenotypic profiling. We envision that this will catalyze a new phase of precision breeding, where genetic markers directly inform the development of tailored sorghum varieties equipped to thrive in a changing climate. Future integration of phenotypic evaluation and complementary molecular markers will further enhance the precision of diversity studies and support the development of climate-resilient sorghum varieties.

REFERENCES

Ahmed A, Elsafy M, Zhourghane A, Abdalla AAA, Abreha KB, Geleta M, Rahmatov M, Abdelhalim TS (2025). Exploring the genetic variability of Sudanese wild sorghum (*Sorghum bicolor* [L.] Moench) germplasm for post-attachment *Striga hermonthica* resistance mechanisms using single sequence repeat (SSR) primers. *Genet. Resour. Crop Evol.* 72(4): 4583-4595. https://doi.org/10.1007/s10722-024-02230-w.

Borrell AK, Mullet JE, George-Jaeggli B, Van-Oosterom EJ, Hammer GL, Klein PE, Jordan DR (2014). Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *J. Exp. Bot.* 65(21): 6251-6263. https://doi.org/10.1093/jxb/eru232.

Cuevas HE, Prom LK, Cooper EA, Knoll JE, Ni X (2018). Genome-wide association mapping of anthracnose (*Colletotrichum sublineolum*) resistance in the U.S. sorghum association panel. *Plant Genome* 11(2): 170099. https://doi.org/10.3835/plantgenome2017.11.0099.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Hammer GL, Jordan DR, Chapman SC (2020). Crop adaptation to climate change: Opportunities through genomics and systems modelling. *Plant Cell Environ.* 43(6): 1630-1642. https://doi.org/10.1111/pce.13705.

Hao H, Li Z, Leng C, Lu C, Luo H, Liu Y, Hasenstein KH, Chen J (2021). Sorghum breeding in the genomic era: Opportunities and challenges. *Theor. Appl. Genet.* 134(7): 1899-1924. https://doi.org/10.1007/s00122-021-03789-z.

Hu Z, Olatoye MO, Marla S, Morris GP (2019). An integrated genotyping-by-sequencing polymorphism map for over 10,000 sorghum genotypes. *Plant Genome* 12(1): 180044. https://doi.org/10.3835/plantgenome2018.06.0044.

Jamil S, Shahzad R, Iqbal MA, Yasmeen E, Rahman SU (2021). DNA fingerprinting and genetic diversity assessment of GM cotton genotypes for protection of plant breeders' rights. *Int. J. Agric. Biol.* 25(4): 768-776. https://doi.org/10.17957/IJAB/15.1706.

Kanwal S, Jamil S, Shahzad R, Rahman SU, Iqbal MZ (2021). Standardization of different protocols for genomic DNA isolation from *Phoenix dactylifera*. *Pak. J. Bot.* 53(5): 1665-1668. https://doi.org/10.30848/PJB2021-5(30).

Kumar S, Choudhary AK, Solanki RK, Pratap A (2011). Towards marker-assisted selection in pulses: A review. *Plant Breed.* 130(3): 297-313. https://doi.org/10.1111/j.1439-0523.2011.01851.x.

Lasky JR, Upadhyaya HD, Ramu P, Deshpande S, Hash CT, Bonnette J, Juenger TE, Hyma K, Acharya C, Mitchell SE, Buckler ES, Brenton Z, Kresovich S, Morris GP (2015). Genome-environment associations in sorghum landraces predict adaptive traits. *Sci. Adv.* 1(6): e1400218. https://doi.org/10.1126/sciadv.1400218.

Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J (2003). Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. *Genetics* 165: 2117-2128. https://doi.org/10.1093/genetics/165.4.2117.

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Acharya CB, Brown PJ, Mitchell SE, Glaubitz JC, Buckler ES, Kresovich S (2013). Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proc. Natl. Acad. Sci. U.S.A.* 110(2): 453–458. https://doi.org/10.1073/pnas.1215985110.

Navyashree R, Mummigatti UV, Nethra P, Basavaraj B, Hanamaratti NG (2024). Evaluation of genetic diversity in sorghum genotypes via SSR markers. *Plant Cell Biotechnol. Mol. Biol.* 25(11-12): 1–9. https://doi.org/10.56557/pcbmb/2024/v25i11-128833.

Nemera B, Kebede M, Enyew M, Feyissa T (2022). Genetic diversity and population structure of sorghum (*Sorghum bicolor* [L.] Moench) in Ethiopia as revealed by microsatellite markers. *Acta Agric. Scand. Sect. B Soil Plant Sci.* 72(1): 873–884. https://doi.org/10.1080/09064710.2022.2117078.

Rahman SU, Jamil S, Shahzad R, Yasmeen E, Sattar S, Iqbal MZ (2022). Genetic diversity and DNA fingerprinting of potato varieties using simple sequence repeat (SSR) markers. *J. Anim. Plant Sci.* 32(3): 775–783. https://doi.org/10.36899/JAPS.2022.3.0467.

Santhiya S, Kowshiga K, Abisha D, Sarankumar C, Kavithamani D, Selvi B, Senthil N (2020). SSR marker-based DNA fingerprinting and morphological characterization for varietal identification in popular sorghum varieties of Tamil Nadu. *Electr. J. Plant Breed.* 11(2): 521–527. https://doi.org/10.37992/2020.1102.088.

Shahbazi S, Ghaffarian S, Razinataj M, Zangi MR, Hamid R, Panahi B (2025). SSR-based molecular characterization of *Verticillium* wilt resistance in Iranian cotton cultivars. *Biochem. Biophys. Rep.* 42: 102059. https://doi.org/10.1016/j.bbrep.2025.102059.

Silva KJD, Pastina MM, Guimarães CT, Magalhães JV, Pimentel LD, Schaffert RE, Menezes CBD (2021). Genetic diversity and heterotic grouping of sorghum lines using SNP markers. *Sci. Agric.* 78(6): e20200039. https://doi.org/10.1590/1678-992X-2020-0039.

Tao Y, Zhao X, Mace E, Henry R, Jordan D (2019). Exploring and exploiting pan-genomics for crop improvement. *Mol. Plant* 12(2): 156–169. https://doi.org/10.1016/j.molp.2018.12.016.

Xing X, Hu T, Wang Y, Li Y, Wang W, Hu H, Wei Q, Yan Y, Gan D, Bao C (2024). Construction of SNP fingerprints and genetic diversity analysis of radish (*Raphanus sativus* L.). *Front. Plant Sci.* 15: 1329890. https://doi.org/10.3389/fpls.2024.1329890.

Yin J, Zhao H, Wu X, Ma Y, Zhang J, Li Y, Shao G, Chen H, Han R, Xu Z (2023). SSR marker based analysis for identification and genetic diversity of non-heading Chinese cabbage varieties. *Front. Plant Sci.* 14: 1112748. https://doi.org/10.3389/fpls.2023.1112748.