International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 847-851
www.biochemjournal.com
Received: 30-11-2025
Accepted: 28-12-2025

P Saisupriya
Department of Vegetable Science, (COH). (SKLTSHU), Rajendranagar, Hyderabad, Telangana, India

P Saidaiah
Department of Genetics and Plant Breeding, (COH), (SKLTSHU), Mojerla, Telangana, India

SR Pandravada
Department of Economic Botany, NBPGR Regional Station, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
P Saisupriya
Department of Vegetable Science, (COH). (SKLTSHU), Rajendranagar, Hyderabad, Telangana, India

Molecular divergence analysis of chilli (*Capsicum annuum* L.) germplasm using SSR markers

P Saisupriya, P Saidaiah and SR Pandravada

DOI: https://www.doi.org/10.33545/26174693.2026.v10.i1Sk.7090

Abstract
An investigation was carried out to study the molecular characterization of chilli genotypes through SSR markers. PCR analysis of the thirty five genotypes using 40 SSRs ensured polymorphism with 39 markers and monomorphic with 1 marker. A total of 203 alleles were observed and the number of alleles varied from 1 (monomorphic) to 11 with an average of 5.07. Highest number of alleles were observed with HpmsE 074 marker. Genetic diversity among the genotypes was assessed on the basis of Polymorphic information content (PIC) value. PIC values of the markers varied from 0.09 to 0.89 with average value of 0.53. Based on cluster analysis done using 40 SSRs two diverse genotypes (IC-570408 and IC-561648) and two highly similar genotypes (IC-215012 and IC-572498) were identified which can be deployed in chilli breeding and crop improvement programmes.

Keywords: Chilli, genotypes, SSR markers, polymorphism, alleles, genetic diversity

Introduction
Among the 25 species of the genus capsicum of chilli crop only five species are being grown worldwide (Bosland and Botava, 2000; Costa *et al*., 2009) [2, 4]. It is cultivated for its fruits which are utilized in various forms such as vegetable, condiment, spice, and in processed forms like powders, sauce, paste, pickle, flakes, etc. (Timmarao *et al*., 2025) [19]. It is mainly valued for its pungency, which is due to crystalline acrid volatile alkaloid capsaicin, present in the placenta of fruits. Due to the numerous benefits offered by capsaicin, it is used widely in various pharmaceutical products. It is used for industrial purpose by extraction of oleoresin. In food processing factories, oleoresin extracted from chilli is used for better colour distribution in food. From global perspective, chilli crop is an major spice cum vegetable crop (Saisupriya *et al*., 2024) [17]. The diverse utilization of chilli from culinary, to health and industrial uses has driven its cultivation worldwide (Lozada *et al*., 2022) [12]. For optimal growth, chilli plants thrive in daytime temperatures between 20 °C and 30 °C and prefer well-drained loamy soils with a pH between 5.5-6.8 (Khan *et al*. 2025) [10].
Genetic diversity is the total variety of genes present in a single population and it estimates the inherent genetic potential of a cross in subsequent advanced generations. It can be evaluated at various levels such as genotypic, molecular and phenotypic level. Such studies facilitate understanding of genetic relationships between accessions, identification of germplasm excesses and admixtures, and identification of genitor pairs with sufficient genetic distance (Delfini *et al*., 2021) [6]. DNA markers are prominent tools for identification of genotypes and diverse parents for hybridization. Given that these molecular markers are not influenced by environmental variability, they are crucial tools for genetic mapping and assessing the diversity.
The markers play a very important role in determining the position of genes and that gene can be used for targeted traits and novel varieties can be developed by using this genetic makeup (Bashir *et al*., 2022) [1]. With the development of molecular biology technique, many DNA markers have been emerged that are useful in identification of polymorphism at genetic level. Various DNA markers advented for chilli include random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs). SSR have numerous benefits among genetic markers such as high reproducibility and useful in analysis of genetic diversity in various spp. (Jamir *et al*., 2022) [9].

In cultivated species with low levels of variation, simple sequence repeats are the most widely used for analysis of molecular diversity (Geleta *et al*., 2004) [7]. They are highly polymorphic in nature and used to predict heterosis. They are powerful tools for estimating genetic similarities and diversity (Sharmin *et al*., 2018) [18]. Thus, SSR markers preferred to perform the molecular characterization research due to its adaptation to automation and in particular, a quite reliable system, as the results cannot change in the repetition of the reactions (Polat *et al*., 2025) [13]. They are used for gene tagging, marker-assisted selection and gene or population mapping (Hasan *et al*., 2021) [8]. Profiling DNA sequences using molecular markers can reveal genetic differences among accessions. Successful characterization supports the development of improved cultivars and sustains crop diversity, particularly under diverse environmental conditions (Khan *et al.*, 2025) [10]. Hence the present experiment was conducted with an aim to capture the potential genetic diversity of different chilli genotypes at molecular level by using SSR markers.

Materials and Methods

The present experiment was carried out with 35 chilli accessions as treatments at College of Horticulture, SKLTSHU, Rajendranagar, Hyderabad. Forty (40) SSRs primers were selected from the map of chilli genome for the amplification of genomic DNA. From each genotype, genomic DNA was isolated was performed by cetyl trimethyl ammonium bromide (CTAB) method, (Clarke 2009) [3] with specific changes. DNA was quantified by using agarose gel electrophoresis (Dean *et al*., 1999) [5]. DNA was amplified in 0.2ml PCR tubes holding reaction mixture of 25 µl. PCR products were separated on 3% agarose gel electrophoresis.

Scoring of Bands

Ultra violet transilluminator was used for studying the SSR-PCR bands. They were later photographed in gel documentation unit. Scoring of SSR bands was done by with presence scored as 1 and absence as 0. Standard marker of 100bp is used for assessment of sizes of the bands. This data is used to generate Bi-nomial data for all the genotypes using excel sheet. Against each primer the banding patterns of all genotypes were differentiated. Bands were considered variable if they are present in one genotype and absent in another. Number of alleles and polymorphic information content were calculated to check the informativeness level and discriminatory efficiency of SSR primers used in this study. PIC value was estimated based on the method proposed by Roldan-Ruiz *et al.* (2000) [15].

Diversity Analysis

Similarity matrix and cluster analysis of 35 genotypes was done using the collected data. The cluster analysis and a dendrogram were constructed with the help of NTSYS software based on unweighted paired group of arithmetic mean average (UPGMA). Linkage distance was calculated among these 35 genotypes and they were divided into various clusters, sub-cluster and sub-sub clusters.

Results and Discussion

Molecular Diversity of Chilli Accessions

Genetic diversity analysis of 35 chill genotypes was carried out using 40 SSR primers. PCR analysis of the genotypes using 40 SSRs ensured polymorphism with 39 markers and monomorphic with 1 marker. Using the allelic data of 39 polymorphic markers, binary matrix data was prepared. A total of 203 alleles were observed and the number of alleles varied from 1 (monomorphic) to 11 with an average of 5.07. Highest number of alleles were observed with HpmsE 074 and the same marker was used by Nanda *et al.*, (2016) for tagging genomic regions controlling anthracnose resistance and reported polymorphic to F_2 mapping population parents (PBC 80 and SB 1) in *Capsicum baccatum*.

Polymorphism Information Content (PIC)

Genetic diversity among the genotypes was assessed on the basis of Polymorphic information content (PIC) value. PIC values among SSRs varied widely from 0.09 to 0.89 with an average of 0.53 (Table 1). The observation of the present study suggests the polymorphism in the genotypes which is reflected by the PIC values. Highest PIC value of 0.89 was observed with HpmsE140, HpmsE066 followed by HpmsE071 (0.85), Epms331 (0.80) implying their further use in genetic variability studies of chilli germplasm. Whereas lowest PIC value of 0.09 was observed with Gpms 178 marker. The observed average PIC value is similar to the PIC value outlined by Yong sham *et al*., 2005 [20] (0.53).

Table 1: List of polymorphic primers with sequence, annealing temperature and PIC

S.no	Primer	Sequence	Annealing temperature (ºC)	PIC value
1.	HpmsE143	F- CCATTCAGCTAGGGTTCAGTCCA R- CGACCAAATCGAATCTTCGTGA	53	0.26
2.	HpmsE065	F-TGAAATAGGCCAATCCCTTTGC R- ATTCCCTGGGATTCCTGCATTA	53	0.62
3.	HpmsE013	F-GCGCCAAGTGAGTTGAATTGAT R- CACCAATCCGCTTGCTGTTGTA	52	0.69
4.	HpmsE096	F- CGGGTCAAACAAAAACCGAAGT R- GCTTGTGGTTGAGCTCGCTCTT	50	0.61
5.	HpmsE012	F- AAACGCTGAAAAAGGCGTTGAC R- TGCACCAACTTCTTCCATGCAC	50	0.61
6.	HpmsE046	F- TCCTCAAGATTGTTGTCATCATCA R- AAAGGCATTTTCATTTTCGACTTT	50	0.55
7.	HpmsE080	F- TGAGGGTGAGAACAAGTGTGGA R- GTTATGGTGGACGGGGTACGAA	55	0
8.	Epms-331	F- AACCCAATCCCCTTATCCAC R- GCATTAGCAGAAGCCATTTG	52	0.80
9.	HpmsE024	F- CGAGCCTAACCACCCAAATCAG R- AAGGGAACGGAGGGACGACTAC	55	0.48

10.	HpmsE054	F- GCCACCCCTCACCTCTCTCTCT R- GTTGTTCGCTGGGCTCTTTCTC	58	0.79
11.	HpmsE064	F- CCCTCCTTTTACCTCGTCAAAAA R- ATGCCAAGGAGCAATGAGAACC	52	0.58
12.	HpmsE074	F- CGCCAGTTAACTTCCGACCTGT R- GGAGCGGGTTGAGAGAGTTGAG	53	0.65
13.	Gpms-101	F- CCTATCACCCTCTTTGAGCC R-TAAAGACCAGCCCTGGATGA	53	0.31
14.	HpmsE075	F- GCGGCTCAGCAGAAAGAGAGAG R- TGCCACAGCTGGAGAACGTAAA	52	0.37
15.	Gpms178	F- GATTTTTGACATGTCACATTCATG R- AACGTTGAAAAATAAAGTAAGCAAG	50	0.09
16.	HpmsE021	F- CACACTAAGCATTCTGCTTTCACA R- GGAGGGAATAGTAGCGGTTTGGA	53	0.52
17.	HpmsE022	F- GCACCAGCATCAACATCAGCAT R- CAGCAGGTGAAGGACTTGCAGA	56	0.78
18.	HpmsE026	F- CCAAAGTCCATCGACGTCTCAA R- ATCAAATGGCAAACCAGGAGGA	52	0.49
19.	HpmsE033	F- TGGATCCTCCTTTCTACTTCAACA R- AAGGGTGGTGAAAAGGGGATTT	53	0.42
20.	HpmsE104	F- TCCAATCATCGCTCCTTTACGG R- CGAGCAACATGAGACGGTGAAT	52	0.65
21.	HpmsE010	F- GCTATTTTCCGGCGTGTGAGAG F- CCAAGTTCAGGCCCAGGAGTAA	52	0.45
22.	HpmsE119	F- TGTTCTGGACTGCTGCTCTTCG R- TGACCAACAACCTCATCATCCAA	52	0.41
23.	HpmsE081	F- TTGGCTATTGCCTCTCCAGACC R- CATGTTGGGGAGAATGTGAACG	52	0.45
24.	HpmsE111	F- CCATCATTTCTCCCCAATTCCA R- GAGAGCAGAAGAAGGGGTGGTG	52	0.16
25.	HpmsE140	F- GGCTCTGCCTCTCGTCTCCTC R- AGGATCAGAAGCAGCGCATTTC	58	0.89
26.	HpmsE066	F- AAGCGCGGTCACTGGAACTAAC R- CGCCGGTTTTTCATCAATTACA	50	0.89
27.	HpmsE071	F-CCCCTTCTCCTCCCTCATAAGC R-TTCCATGATGTTACCGGAGCAA	56.5	0.85
28.	HpmsE120	F- GGGGGAGGAAGAGAAGAAGTCG R- CCGGACTTTACGAGCACAACCT	50	0.54
29.	Hpms1-5	F- CCAAACGAACCGATGAACACTC R- GACAATGTTGAAAAAGGTGGAAGAC	52	0.42
30.	HpmsE068	F- TGTTCCTTTTGTTGTTACCTTTTG R- CGTCTAGGAATGGAAGAAGAGC	50	0.59
31.	HpmsE103	F- ATTGTGACCCGACTCCTCCAT R- TGCTAATGGTGCTAATGCGGTA	52	0.53
32.	HpmsE114	F- GGTGAGGGAGGTGTGAGCAAA R- GATCCACATACGCCATCACTGC	52	0.38
33.	Gpms-161	F- CGAAATCCAATAAACGAGTGAAG R- CCTGTGTGAACAAGTTTTCAGG	50	0.66
34.	Epms342	F- CTGGTAGTTGCAAGAGTAGATCG R- ATGATCTTTGACGACGAGGG	53	0.64
35.	HpmsE025	F- TGAGCATCCCGTTATCTCAAATCA R- CCCAATTCTTCAGGCAATCTCC	52	0.29
36.	HpmsE084	F- GCCAGAAGATCCATACTCTCATCA R- GGAATGAGCAAAAACAAGAGTCC	52	0.68
37.	HpmsE150	F- CCCTCTTCCCCGACTCTCTCTT R- AAGCCAATGACTGCATGACCAC	55	0.46
38.	HpmsE149	F- CGGAAACTAAACACACTTTCTCTC R- GACTGGACGCCAGTTTGATT	50	0.60
39.	Epms-386	F- ACGCCAAGAAAATCATCTCC R- CCATTGCTGAAGAAAATGGG	50	0.41
40.	HpmsE102	F- AAGGTGGTGGTATGGACTGCGTA R- TTTAAGCGTTCAGTTGGCGAAA	50	0.73

Cluster Analysis

The binary matrix data derived from the 40 SSR markers analysis was used for diversity assessment. Jaccard's similarity coefficient values were calculated for 35 chilli accessions using NTSYS-pc version 2.02e (Rohlf 1998) [14] and the dendrogram was constructed by UPGMA (Unweighted pair group method with arithmetic mean) method. Thirty five chilli genotypes were grouped into 9 clusters in the dendrogram based on similarity coefficients which are in the range of 0.48 to 0.865. Clustering of the genotypes is depicted in Figure 1. Cluster I was comprised of 5 genotypes *viz.*, IC-528433, IC-526448, IC-561655, EC-

399567 and EC-402113. Cluster II was with 4 genotypes namely, LCA 625 IC-334383, IC-526737 and Pusa jwala. Cluster III has 4 genotypes viz., IC-410423, IC-214965, IC-570408 and IC-572459. Cluster IV was with three genotypes IC-447018, IC-505237 and IC-347044. Cluster V with 2 genotypes *viz.*, IC-561648 and EC-390030. Cluster VI with 2 genotypes IC-561622 and IC-561676, cluster VII with 2 genotypes EC-378688 and EC-399535. Cluster VIII was also with 2 genotypes IC-572498 and IC-215012. Cluster IX with one genotype Sindhur, cluster X with EC-399581, cluster XI with IC-394819, cluster XII with IC-319335, cluster XIII with 214966, cluster XIV with EC-378632, cluster XV with IC-528442, cluster XVI with IC-610383, cluster XVII with IC-610381, cluster XVIII with IC-363993 and cluster XIX with IC-363918.

Highest similarity (86.5%) was observed between IC-215012 and IC-572498 which were placed in cluster VIII. The genotypes IC-214965 and IC-410423 were also found to be very closely related (85%), which belong to cluster III. Out of 35 genotypes, two genotypes IC-570408 and IC-561648 were found to be the most diverse, grouping separately with least similarity. The diversity of these two genotypes was also seen in phenotypical cluster analysis by Tocher's method. Similarly other 2 genotypes IC-610383 and IC-399581 were also most diverse with less similarity. Thus, from the present study two diverse genotypes (IC-570408 and IC-561648) and two highly similar genotypes (IC-215012 and IC-572498) were identified which can be deployed in chilli breeding and crop improvement programmes. The results confirmed that the rich diversity existing in chilli genotypes could be used as a genetic resource in designing breeding programs.

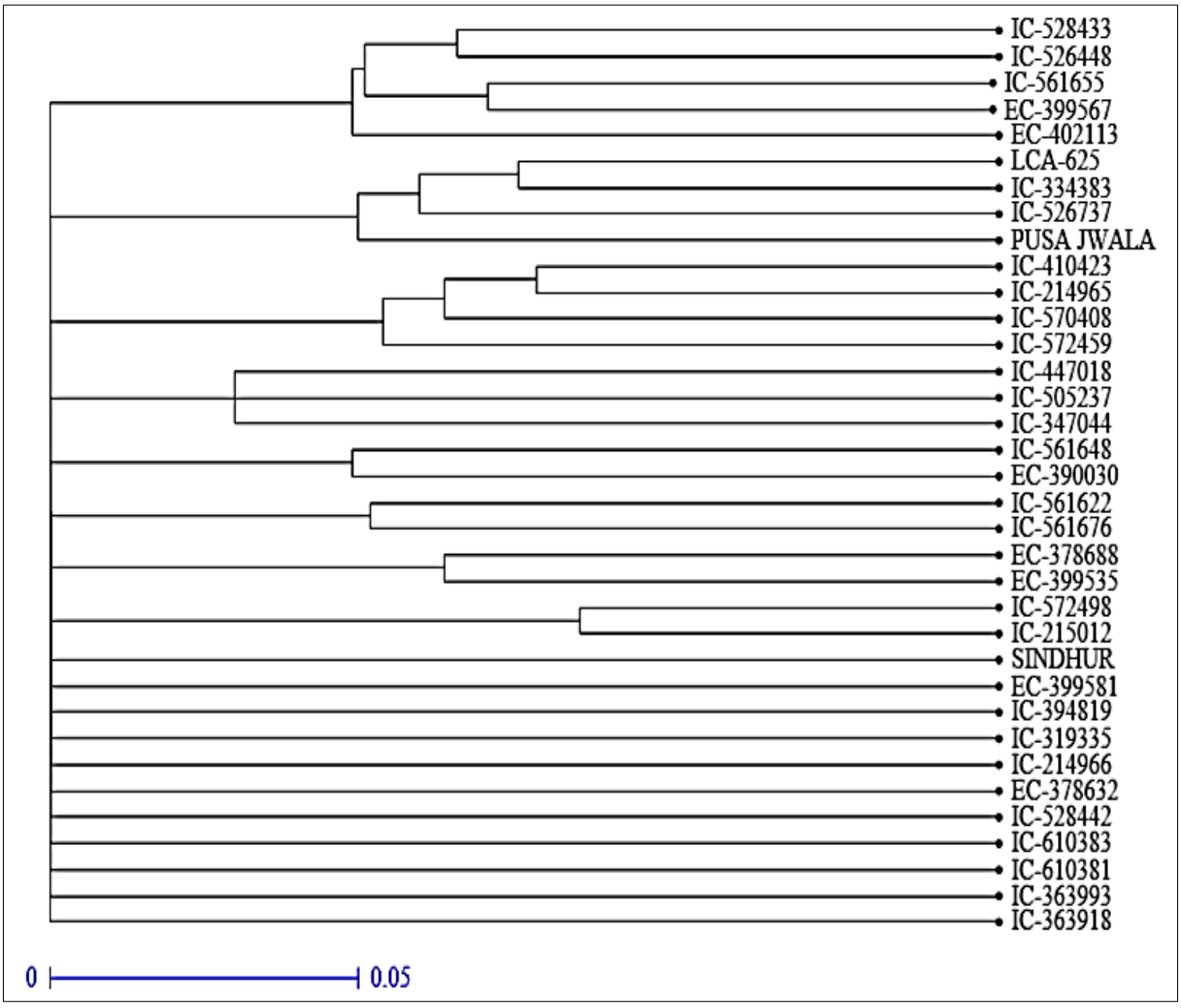

Fig 1: Cluster analysis of 35 genotypes of chilli based on UPGMA based on 40 SSR primers

Conclusion

Detection of genetic diversity provides valuable information in selecting superior genotypes for plant breeding. Among the 35 chilli genotypes, SSR markers showed genetic variability. These markers are important tools for calculating the genetic similarities and diversity. The genetic relationships presented among the studied genotypes are useful for selection of parents for future breeding programs. Based on cluster analysis done using 40 SSRs diverse genotypes have been identified for developing mapping populations, crop improvement and heterosis breeding.

The present study revealed that SSR analysis is quick and reliable procedure for genetic diversity analysis as they show sufficient polymorphism within the experimental population. The polymorphic markers with more PIC values are highly informative SSR markers for genetic studies. Markers with high PIC value identified in the present investigation can be used for varietal identification, germplasm conservation, DUS testing, DNA fingerprinting and analysis of genetic purity. Therefore, the information yielded from this study will be useful for further chilli breeding studies.

Declaration

There is no competing of interest amongst the authors.

Acknowledgment

Authors acknowledge SKLTGHU for the invaluable support and provision of resources during the research.

Reference

1. Bashir MH, Afzal M, Nawaz MS, Khan AZ, Aziz S, Ullah F, *et al*. Characterization and advancement of microsatellite (SSR) markers for various stresses in wheat. International Journal of Agriculture and Biosciences. 2022;11(2):131-138. doi:10.47278/journal.ijab/2022.018
2. Bosland PW, Botava EJ. Peppers: vegetable and spice capsicums. Wallingford (UK): CABI Publishing; 2000. p. 204.
3. Clarke J. Cetyltrimethyl ammonium bromide (CTAB) DNA miniprep for plant DNA isolation. Cold Spring Harbor Protocols. 2009;10:1101.
4. Costa LV, Lopes R, Lopes MTG, Figueiredo AF, Barros WS, Alves SRM. Cross compatibility of domesticated hot pepper and cultivated sweet pepper. Crop Breeding and Applied Biotechnology. 2009;9:37-44.
5. Dean RE, Dahlberg JA, Hopkins MS, Mitchell SE, Kresovich S. Genetic redundancy and diversity among 'Orange' accessions in the U.S. national sorghum collection as assessed with simple sequence repeat (SSR) markers. Crop Science. 1999;39:1215-1221.
6. Delfini J, Moda-Cirino V, Santos Neto J, Ruas PM, Sant'Ana GC, Gepts P, *et al*. Population structure, genetic diversity and genomic selection signatures among a Brazilian common bean germplasm. Scientific Reports. 2021;11:1-12.
7. Geleta LF, Labuschagne MT, Viljoen CD. Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. Plant Breeding. 2004;123:467-473.
8. Hasan N, Choudhary S, Naaz N, Sharma N, Laskar RA. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. Journal of Genetic Engineering and Biotechnology. 2021;19(1):1-26. doi:10.1186/S43141-021-00231-1
9. Jamir M, Pankaj KS, Daiho L. Genetic diversity analysis using SSR marker in Naga King chilli (*Capsicum chinense* Jacq.) genotypes. Annals of Plant and Soil Research. 2022;24(4):618-629. doi:10.47815/apsr.2022.10218
10. Khan N, Khaqan K, Ahmed MJ. Genetic diversity exploration in *Capsicum annuum* L. sweet pepper germplasm through chemical and morphological characterization. Pure and Applied Biology. 2025;12:692-709.
11. Khan N, Khaqan K, Ahmed MJ, Awan SI, Begum S, Khattak SH. Microsatellite (SSR) markers application in the characterization of genetic diversity in *Capsicum annuum* germplasm. International Journal of Agriculture and Biology. 2025;34:340513. doi:10.17957/IJAB/15.2398
12. Lozada DN, Bosland PW, Barchenger DW, Haghshenas-Jaryani M, Sanogo S, Walker S. Chile pepper (*Capsicum* spp.) breeding and improvement in the multi-omics era. Frontiers in Plant Science. 2022;13:879182. doi:10.3389/fpls.2022.879182
13. Polat Y, Karc H, Çelik F, Salih K, Nesibe EK. SSR marker-based molecular characterization and genetic diversity in pomegranate (*Punica granatum* L.) genotypes. Genetic Resources and Crop Evolution. 2025. doi:10.1007/s10722-025-02336-9
14. Rohlf FJ. NTSYS-pc: numerical taxonomy and multivariate analysis system. New York: Applied Biostatistics; 1998.
15. Roldan-Ruiz I, Dendauw J, Van Bockstaele E, Depicker A, De Loose M. AFLP markers reveal high polymorphic rates in rye grasses (*Lolium* spp.). Molecular Breeding. 2000;6:125-134.
16. Nanda C, Mohan Rao A, Ramesh S, Hittalmani S, Prathibha VH. Tagging SSR markers associated with genomic regions controlling anthracnose resistance in chilli (*Capsicum baccatum* L.). Vegetos. 2016;29(3):1-5.
17. Saisupriya P, Saidaiah P, Harikishan S, Pandravada SR. Studies on aflatoxin resistance in chilli (*Capsicum annuum* L.) germplasm. Annals of Phytomedicine. 2024;13(1):1-7. doi:10.54085/ap.2024.13.1.66
18. Sharmin A, Ekramul H, Masudul H, Fahima K. Molecular diversity analysis of some chilli (*Capsicum* spp.) genotypes using SSR markers. American Journal of Plant Sciences. 2018;9(3). doi:10.4236/ajps.2018.93029
19. Timmarao KS, Ponnam N, Lakshmanareddy DC, Reddy MK, Venkataravanappa V, Roshini P, *et al*. Molecular mapping and development of SSR markers associated with chilli leaf curl virus resistance in chilli (*Capsicum annuum* L.). Genomics. 2025;117:111015. doi:10.1016/j.ygeno.2025.111015
20. Yong-Sham K, Lee JM, Yi GB, Yi SI, Kim KM, Soh EH, *et al*. Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum* L.) varieties. Molecules and Cells. 2005;19(3):428-435.