

Molecular analysis of genetic variability and relationship among Pearl Millet (*Pennisetum glaucum*) genotypes by using SSR markers

ABSTRACT

Pearl millet ~~belongs to genus~~ ~~has a place with~~ ~~sort~~ *Pennisetum*, and family *Poaceae*. Pearl millet is ~~the most broadly~~ ~~widely~~ developed grain crop in Asia and Africa representing close to half of the worldwide millet creation. In India, pearl millet is the fourth most broadly developed food crop after rice, wheat, and maize. ~~We used~~ ~~m~~ ~~Molecular techniques~~ ~~were~~ ~~used~~ to investigate the genetic diversity and relatedness of six genotypes ~~of pearl millet~~ viz: ICMV155, Dhanshakti, PC-612, Sampada, HHB-67, and HHB-197. ~~The~~ ~~the~~ six genotypes ~~were~~ ~~as~~ collected from National ~~A~~griculture ~~R~~esearch ~~P~~roject Aurangabad. The present study was conducted at Department of Plant Biotechnology at K.K.Wagh College of Agricultural Biotechnology, Nashik.

DNA was isolated by fixing a sample in alcohol without using liquid nitrogen. ~~S~~, ~~six~~ genotypes were analyzed through SSR primers to determine the extent of molecular characterization. PCR amplification using 10 SSR primers generated a total ~~of~~ 111 ~~no.~~ bands ~~which~~ were scored corresponding to average of 11 bands per primer ~~s~~ with 77 bands showing polymorphism (67%) and 34 bands showing monomorphism (30%). The PIC value ranged from 0.35 to 0.68 with average of 0.4. Jaccard's coefficient based of SSR analysis 0.38 to 0.90. The dendrogram constructed using UPGMA method. It has main two clusters. Cluster-1 consisted of C1- HHB 67, V6-ICMV-155, C2- HHB 197 and C4- Sampada. Cluster-2 comprised C3-Dhanshakti and V5-PC 612 as an out group. C2- HHB 197 and C4- Sampada genotypes had ~~ve~~ highest similarity coefficient 0.76. Among all the genotypes, C1- HHB 67 and V5- PC 612 ~~were~~ ~~as~~ found most diverse as ~~they~~ separated from all other genotypes at a very low similarity coefficient of 0.6. The identified markers can prove useful for identification of diverse germplasm and future DNA fingerprinting studies.

Keywords: Pearl millet, DNA, RNase, Gel Electrophoresis, SSR Markers, DNA Profiling

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) 2n = 2x = 14] commonly referred to as Bajra is otherwise called bulrush millet, cattail, or spiked millet [12]. Pearl millet belongs to genus *Pennisetum*, and family *Poaceae*. ~~It~~ ~~Pearl millet~~ is the most broadly developed grain crop in Asia and Africa representing close to half of the worldwide millet creation. In India, pearl millet is the fourth most broadly developed food crop after rice, wheat, and maize [1]. ~~The~~ ~~Pearl millet~~ spikelets ~~of pearl millet~~ are uniquely protogynous. ~~the~~ ~~majority~~ of the styles ~~have begun~~ ~~begin~~ to evaporate before dust shed. ~~It is a profusely cross pollinated crop, so the yield is generally profoundly cross pollinated. It is a dual purpose crop being utilized as feed and grain for domesticated animals. It is a double reason crop as it very well may be utilized as both feed and grain for domesticated animals.~~ The family *Pennisetum* contains around 140 species. The term 'millet' incorporates numerous types of different genera, among which the more conspicuous are pearl millet (*Pennisetum glaucum* (L.) R. Br.), Finger millet (*Eleusine coracana* (L.) Gaertn), Foxtail millet (*Setaria italica* (L.) Beauv) and Proso millet (*Panicum miliaceum* L.). Pearl millet assumes a basic part in food security [15]. It has amazing properties like high photosynthetic proficiency, high dry matter creation limit, early development, dry season resilience, the necessity of insignificant buy inputs, and general liberation from biotic and abiotic stresses [1]. It can endure the most unfavorable agro-climatic circumstances where different harvests like sorghum and maize neglect to create monetary yields [1]. It can be designated as "Perfect resilient crop for the future" [11]. ~~In that capacity, it very well may be assigned as the "Ideal tough yield for what's to come" [11].~~ The dietary benefit of pearl millet is considerably more prevalent than some other harvests since, it is profoundly nutritious, non-corrosive framing, no glutinous food having a few nutraceutical and useful

well-being properties. Pearl millet is the great wellspring of energy, carb, rough filaments (impervious to starch, solvent, and insoluble dietary strands), dissolvable and insoluble fat, proteins (8-19%), debris, dietary filaments (1.2g/100g), cancer prevention agents, fat (3-8%) with better fat edibility, iron, and zinc. It is likewise a rich wellspring of nutrients like riboflavin, niacin, thiamine, and minerals (2.3mg/100g) like potassium, phosphorous, magnesium, iron, zinc, copper, and manganese [1]. It displays a superior fundamental amino ~~acid~~ profile of protein in contrast with maize and rice. It contains lesser crosslinked prolamins prompting higher absorbability of the millet proteins. It has 74% polyunsaturated ~~unsaturated~~ fat (PUFA) and ~~wealthy in healthfully significant and~~ crucial unsaturated fat like omega-3 unsaturated fats ~~that is~~ oleic ~~corrosive~~ (25%), linoleic ~~corrosive~~ (45%) and linolenic ~~corrosive~~ (4%). ~~These PUFA are viewed for good health. that are viewed as best for wellbeing.~~ It is a gluten-free grain holding soluble properties even after being cooked. ~~It is suitable for individuals having gluten energy. and is hence really great for individuals having gluten energy.~~ It contains a higher amount of gradually edible starch (SDS) and safe starch (RS) which represent a lower glycemic file (GI) ~~and is quite it is widely~~ pursued in the new seasons of changing weight control plans, food propensities, food industry, and diabetics. Pearl millet has a C: N proportion more noteworthy than 50. Pearl millet is tall growing crop and produces 3-5 tons/acre of dry matter biomass, like sorghum-Sudan grass. Rotting pearl millet ~~establishes~~ left on the dirt surface ~~holdsing~~ 60-80% of potassium in the straw ~~for the accompanying harvest.~~ (www.aicrp.icar.gov.in/pearl).

Pearl millet has higher fiber content. It goes about as probiotic nourishment for miniature vegetation present in our body and along these lines gets us far from blockage. It is additionally fit for bringing cholesterol down to cancer prevention agent movement with phytates, polyphenols, and so on (www.aicrp.icar.gov.in/pearl). These assume a critical part in counteraction of significant human illnesses like diabetes, malignant growth, and cardiovascular and neurodegenerative sicknesses [2]. Due to its various unique features, it is a very useful and important crop for food and nutritional security and is rightly termed as nutri-cereal. Because of its different extraordinary elements, it is an exceptionally helpful and significant harvest for food and nourishing security and is properly named nutri-oat [1]. In any case, yield is low and variable, seldom coming to 1000kg/ha. Besides, creation is compromised by fleece mold sickness, and striga parasitic weed predation by bugs [6]. India is the biggest maker of pearl millet on the planet with an area of 6.93 million ha., creation of 8.61 million tones, and an efficiency of 1243kg/ha (<http://www.agricoop.nic.in>). To bring millets into the standard for taking advantage of the nourishing rich properties and advancing their development, Govt. of India has declared year 2018 as the "Year of Millet" and the year 2023 as "International Year of Millets" by FAO committee on agriculture (COAG) forum. The significant pearl millet developing states are Rajasthan, Uttar Pradesh, Gujarat, Madhya Pradesh, Karnataka, Tamil Nadu, and Andhra Pradesh adding to 90% of complete creation in the country. The greater part of pearl millet in India is filled in the stormy season (Kharif) season (June/July-September/October). It is likewise developed during the summer season (February-May) in ~~some places of pieces of~~ Gujarat, Rajasthan, and Uttar Pradesh, and during the post-blustery (rabi) season (November-February) at a limited scale in Maharashtra and Gujarat.

In the early days, crop breeders used morphological markers for the assessment of genetic diversity and choosing parents for developing new cultivars. Morphological characterizations were mainly used to select superior genotypes, but different studies revealed ~~that~~ that morphological markers are not appropriate for trait exhibiting lower heritability. Morphological markers were greatly influenced by the interaction of the genotype with the environment in which it is expressed. Commonly known genotype environment interaction (GXE) occur when two or more genotypes perform differently in different environments, and are thus described as differential genotypic sensitivities to environments [6]. Moreover, due to the high out-crossing breeding nature and structure of genetic diversity in pearl millet species, the morphological data/markers are inadequate in providing reliable ~~information.~~ ~~information~~ [2]. Such constraints gave rise to the evolution of molecular markers which cannot only be used to distinguish various germplasm accessions, but can also characterize and estimate genetic distances among different groups of genotypes ultimately enhancing the power of conventional plant breeding and genetic methods [2]. Thus, molecular markers put forward significant advantages over morphological markers for assessment of genetic diversity [2].

DNA based molecular markers have been successfully employed in quantification of genetic diversity, genotype identification, delineation and Marker assisted selection. These have been effectively utilized

for the assessment of genetic diversity as compared to the morphological or biochemical due to their abundance, high level of polymorphism, reproducibility and being independent of the environment. Pearl millet exhibits tremendous amount of diversity at both phenotypic and genotypic level [14]. Molecular markers namely RFLP, RAPD, ISSR and SSRs are mostly used, among these microsatellite or Simple sequence repeats (SSRs) have been found as more reliable because they are highly polymorphic, informative, and co-dominant markers which are often broadly applicable since loci are frequently conserved between related species. ~~and sometimes.~~ Sometimes the integration of genomic technologies in pearl millet breeding will improve the efficiency of breeding programs in the development of better cultivars and reduce the time required for cultivar development. Thus, in the present investigation, SSR markers were used to characterize the pattern of diversity among Pearl millet genotypes.

2. MATERIAL AND METHODS

2.1 PLANT MATERIAL

Seeds of six genotypes (ICMV155, Dhanshakti, PC-612, Sampada, HHB-67, and HHB-197) of Pearl millet were procured from National Agriculture Research Project, Aurangabad.

2.2 DNA Isolation and Purification

Total genomic DNA was isolated from fresh leaf tissues by the method of Doyle and Doyle [5] with minor modifications as explained below.

The samples were prepared from by putting approximately 200 mg of fresh leaf tissue ground to a fine powder in a mortar and pestle. The tissues were ground into fine powder. To this fine ground powder, 600 µl of preheated plant extraction buffer was added and the extractant was taken into a tube. These tubes were incubated for 60 min at 65 °C for 60 min. The tubes were mixed for every 10 min of interval. After which the tubes were mixed by every 10 min of interval to homogenize the samples. The tubes were removed and allowed to cool, centrifuged at 10,000 rpm for 10 min at room temperature and the supernatant was then transferred into freshly labelled tubes. Then equal volume of phenol: chloroform: isoamylalcohol (25:24:1) was added. These were then again centrifuged at 10,000 rpm for 10 min at room temperature. The centrifugation was repeated once again using phenol: chloroform: isoamylalcohol (25:24:1) treatment. Aqueous layer was transferred into freshly labelled tubes. An equal volume of ice-cold isopropanol was added. The contents were mixed gently and were incubated 4 h for overnight to precipitate the DNA. It was then centrifuged at 10,000 rpm at 15 min at 4 °C. The supernatant was decanted and added 500 µl of 70 % ethanol to wash the DNA pellet, then centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was decanted and the pellet was air dried until the ethanol smell disappeared. 20 µl of molecular graded water was added to resuspend the DNA. 2 µl of RNase was added and incubated at 37 °C for 45 min. In order to check the DNA quality and removal of RNA, 0.8 % gel was prepared by boiling 0.8 gms of agarose in 100 ml of 1X TAE and allowed to cool at 55 °C. Then 0.2 µl of EtBr was added and gently mixed. The solution was poured in the casting tray before it polymerized. 2 µl of DNA and 2 µl of loading dye were mixed together. 4 µl of the mixture was loaded onto the 0.8 % agarose gel. The gel was run at 70 V for 45 min and the gel image was saved under the gel documentation system. When the protein and RNA was completely removed it was then proceeded to the Biospectrometer.

2.3 SSR amplification

A total of 20 primers were used in PCR amplification table no.1. SSR in this study were selected from the study of [11]. PCR amplification was carried out in 10 µl reaction volume containing 1.5 µl of template DNA (50ng), 1.6 µl Taq PCR buffer with MgCl₂ (17.5 mM), 0.3 µl of Taq polymerase (1 U/µl), 1.5 µl dNTPs (2.5 mM) and 1 µl primer (10 pmol/µl). SSR PCR was performed at an initial denaturation 94 °C for 2.30 mins, 36 cycles of 94 °C for 30 sec, 55-60 °C for 1.30 min and 72 °C for 50 sec and final extension 72 °C for 7 mins.

Table no.1 List of SSR primers

Sr. No	Primer Name	Sequence (forward and Reverse)	Annealing
1.	PSMP 2276	F-GGAATAGTATATTGGCAAAATGTG R-ATACTACACACTGTAAGCATTGTC	59 °C

2.	PGIR D25	F- CGGAGCTCCTATCATTCCAA R- GCAAGCCACAAGCCTATCTC	55□
3.	SSR-06	F-GCCTCGAGCATCATCATCAG R- CAACCTGCACTTGCCTGG	58□
4.	PSMP 2070	F-ACAGAAAAAGAGAGGCACAGG R-GCCACTCGATGGAAATGTGAAA	55□
5.	Xpsmp-2070	F-ACAGAAAAAGAGAGGCACAGGAG R-GCCACTCGATGGAAATGTGAAA	59□
6.	Xpsmp-2084	F-AATCTAGTGATCTAGTGTGCTTCC R-GGTTAGTTTGTGGAGCAAATGA	58□
7.	Xpsmp-2204	F- GAACTTGAYGAYGCCACTAGC R- TTGTGTAGGGAGCAACCTTGAT	59□
8.	Xpsmp-2001	F-CATGAAGCCAATTAGGTCTC R-ACCATCTGACTTGTCTTATCC	55□
9.	PSMP-2018	F-CTCTGTAAGTTCCTGGTGCTCAA R-TCAGGCCAGTAACACATCTCAA	59□
10.	UGEP-53	F-TGCCACAACCTGTCAACAAAAG R- CCTCGATGGCCATTATCAAG	60□

2.4 Agarose gel electrophoresis

Amplified products of SSR were analyzed in 3.5 % Agarose in 1X TAE buffer and the bands were visualized on gel a Gel Doc system and documented.

2.5 Data analysis and scoring

Amplification profile of all cultivars compared with each other and bands of DNA fragments scored manually as (1) or (0) depending on the presence or absence of a particular band respectively. The data was analyzed using Numerical Taxonomy System of Multivariate Statistical Programme (NTSYS) software package. The dendrogram was constructed using Unweight Pair Group Method of Arithmetic Averages (UPGMA).

To compare the efficiency of primer, polymorphic information content (PIC) was calculated by using the formula

$$PIC = 1 - \sum_{i=1}^k P_i^2$$

Where P_i is frequency of allele for SSR marker.

3. Results and Discussion

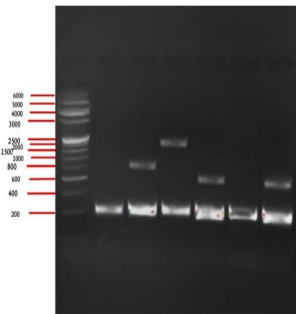
SSR Analysis

In present study, six genotypes were studied with SSR marker. For SSR analysis among 20 primers tested, 10 primers generated 111 bands of these 77 bands were polymorphic with an average polymorphism of 67 %. Out of 10 primers, 4 primer (PGIRD 25, PSMP 2070, Xpsmp 2001 and UGEP 53) showed 100 % polymorphism, similar results were reported by [1]. The molecular size of the amplified SSR products ranged from 200 bp (PSMP 2070) to 800 bp (PSMP 2076). The total number of amplicons varied from 1 to 6 with an average of 3.5 per primer. The number of polymorphic amplicons ranged from 7 (PSMP 2018) to 19 (PSMP 2076) with an average of 13 bands per primer. A similar study was reported by [15]. PSMP 2076 showed the highest PIC (0.69), while it was lowest for PSMP 2018 (0.37) with an average of 0.44 as mentioned in table no. 2.

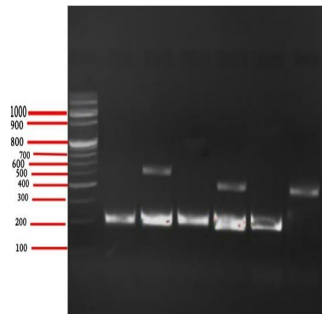
Table no.2 Sequence of SSR Primer used for analysis of genetic divergence in pearl millet

Sr.No.	Primers	Total no. of bands	Total no. of Polymorphic bands	Total no. of Monomorphic bands	Polymorphism (%)	PIC value
1.	PSMP 2076	21	19	2	90%	0.69
2.	PGIRD 25	8	8	0	100%	0.36

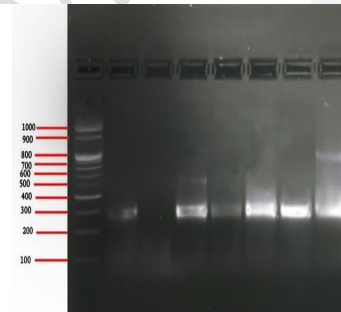
3.	PSMP 2070	12	12	0	100%	0.62
4.	Xpsmp 2204	10	9	1	90%	0.53
5.	Xpsmp 2001	14	14	0	100%	0.63
6.	PSMP 2018	8	7	1	87.5 %	0.37
7.	UGE53	8	8	0	100%	0.47
8.	Xpsmp 2070	6	0	6	0	0
9.	SSR06	12	0	12	0	0.37
10.	Xpsmp 2084	12	0	12	0	0.37
Total		111	77	34	67%	0.44



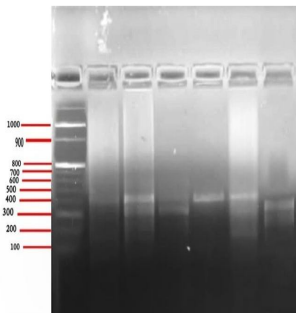
Result of Xpsmp 2204 primer



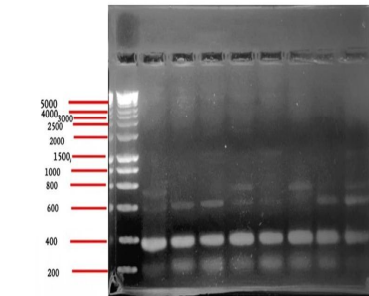
Result of UGE53 primer



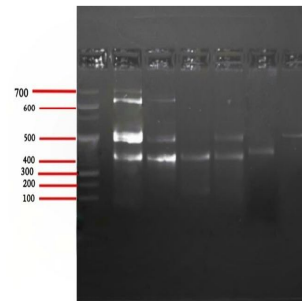
Result of Xpsmp 2070 primer



Result of PGIRD 25 primer

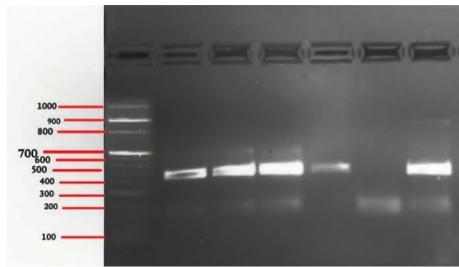


Result of PSMP 2076 primer

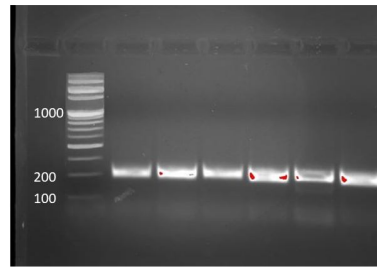


Result of PSMP 2070 primer

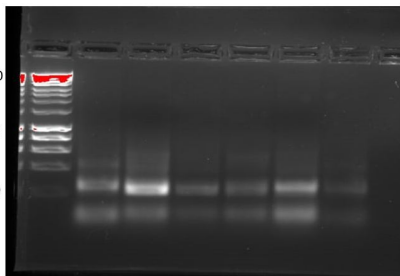
Plate no. 1 Gel Photograph showing SSR allelic profile of Pearl millet genotypes.



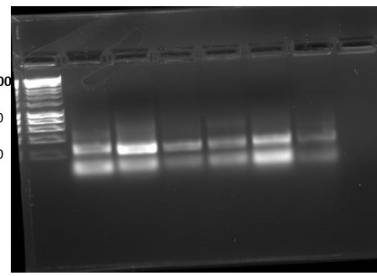
Result of Xpsmp 2001 primer



Result of SSR 06 primer



Result of PSMP 2018 primer



Result of Xpsmp 2084 primer

Plate no. 2 Gel Photograph showing SSR allelic profile of Pearl millet genotypes.

SSR-based cluster analysis

Jaccard's similarity coefficients based on SSR markers among the all pairwise combinations of genotypes ranged from 0.38 [Between Dhanshakti and HBB 197] to 0.90 [Between Sampada and HBB 67] with an average value of 0.72 as mentioned in table no. 3. The dendrogram grouped into two main clusters on the basis of the reference line drawn at a similarity coefficient of 0.62 which consisted all the six genotypes of Pearl millet. Cluster-1 consisted of C1- HBB 67, V6-ICMV-155, C2- HBB 197 and C4- Sampada. Cluster-2 comprised C3-Dhanshakti and V5-PC 612 as an out group. C2- HBB 197 and C4- Sampada genotypes have highest similarity coefficient 0.76. Among all the genotypes, C1- HBB 67 and V5- PC 612 were found most diverse as they were separated from all other genotypes at a very low similarity coefficient of 0.6 as mentioned in table no. 3. A similar study was reported by [15]. A similar type of report of this DNA fingerprinting of pearl millet using molecular markers has also been reported by many other researchers [1] [15] and can be further used for cultivar identification purposes. The set of SSR markers used in this study could be used further and prove useful in positively assessing the ability of SSR to create unique DNA profiles in pearl millet. In the present investigation we used SSRs for DNA profiling because microsatellites were considered suitable for cultivar identification due to their high accuracy and efficiency for detecting a large number of individual alleles [1][4]. Simple sequence repeats are tandem repeats of approximately 6 nucleotides in both coding and noncoding regions. SSR has become the marker of choice for genotyping because of its frequency, high allelic variation, codominant inheritance, and ease of analysis [2] [11]. Furthermore, microsatellite markers cannot be effectively applied to distinguish phylogenetically related species according to conserved sequences and may be useful in studying the genetic components of related species. SSR fingerprints are generally highly discriminatory and are commonly used to identify breeds and individuals and to know their pedigree and identity [1]. A DNA profile is required for submission of proposals, as entries promoted for third year consideration will be identified for clearance later. DNA fingerprinting data will be provided with the proposed plant variety registry. This will be of great help to a plant breeder when registering germplasm in his NBPGR. It can also be used to report the results of the DUS test with DNA markers.

Table no. 3 Similarity index for the six pearl millet genotypes using SSR banding profile

Variety	HHB 67	HHB 197	Dhanshakti	Sampada	PC612	ICMV-155
HHB 67	1.0000					
HHB 197	0.62	1.00				
Dhanshakti	0.71	0.71	1.00			
Sampada	0.71	0.71	0.62	1.00		
PC612	0.71	0.71	0.71	0.90	1.00	
ICMV-155	0.66	0.38	0.48	0.48	0.48	1.00

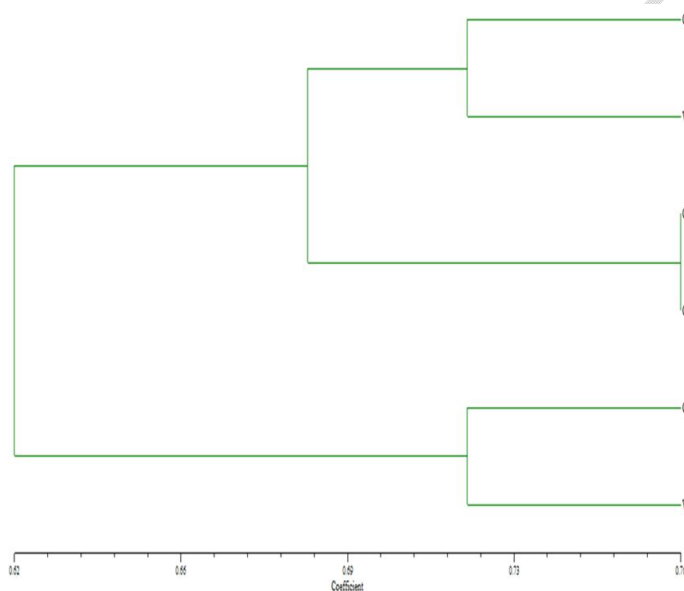


Fig no. 1 Dendrogram of SSR marker

Conclusion

The identification of primers that can apparently generate species specific profiles is significant for further phylogenetic studies in *Pennisetum glaucum*. SSR techniques indicated that it is useful in estimation of polymorphism and phylogenetic relationships among *Pennisetum glaucum* morphotypes. The data generated will be used for further breeding and characterization of the species. These findings not only highlight the capacity of the SSR technique but also helps in the selection of diverse Pearl millet landraces for conservation and crop improvement.

REFERENCES

- Ambawat S, Satyavathi CT, Meena RC, Meena R, Khandelwal V, Singh S and Geela R. DNA fingerprinting of pearl millet hybrids [*Pennisetum glaucum* (L.) R. Br. using SSR markers. *J.Pharma.Innova.* 2021;10(11):07-13
- Ambawat S, Satyavathi CT, Meena R, Khandelwal V, Meena RC. Molecular characterization and genetic diversity analysis of released hybrids and varieties of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Curr. J. of Appl.* 2020;39(31): 92-104.

3. Adeoti K, Djedatin G, Ewedje E, Beulé T, Santoni S. Assessment of genetic diversity among cultivated pearl millet (*Pennisetum glaucum*, Poaceae) accessions from Benin, West Africa. *Afr. J. Biotechnol.* 2017;16 (15) 782-790.
4. Bougma LA, Ouedraogo MH, Ouoba A, Zoure AA, Sawadogo N and Sawadogo M. Genetic differentiation for gene diversity among pearl millet (*Pennisetum glaucum* (L.) R. Br.) landraces as revealed by SSR markers. *Int. J. Agron.* 2021;10.1155.
5. Doyle JJ. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 1990;12, 13-15.
6. Diack O, Kane NA, Berthouly-Salazar C, Gueye MC, Diop BM, Fofana A and Barnaud A. New genetic insights into pearl millet diversity as revealed by characterization of early- and late-flowering landraces from Senegal. *Front. Plant Sci.* 2017; 8, 818.
7. Hemender, Yashveer S, Sangwan VPS., Sharma S, Mor VS. Molecular Diversity analysis based on microsatellite markers in pearl millet hybrids [*Pennisetum glaucum* (L.) R. Br. and their parental lines. *Indian J. Ecol.* 2018;45(1):146-151.
8. Kapila RK, Yadav RS, Plaha P, Rai KN, Yadav OP, Hash CT and Howarth CJ. Genetic diversity among pearl millet maintainers using microsatellite markers. *Plant Breeding.* 2008;127(1), 33-37.
9. Kannan B, Senapathy S, Bhasker Raj AG, Chandra S, Muthiah A, Dhanapal AP, and Hash CT. 2014. Association analysis of SSR markers with phenology, grain, and stover-yield related traits in pearl millet (*Pennisetum glaucum* (L.) R. Br.). *Sci. World J.* 2014. [Page numbers](#)
10. Nehra M, Kumar M, Vart D, Kaushik J, Sharma RK. Molecular characterization of pearl millet [*Pennisetum glaucum* (L.) R. Br.] inbreds using microsatellite markers. *J. Appl. Nat Sci* 2017;9 (1): 357 - 363.
11. Ponnaiah G, Gupta SK, Blümmel M, Marappa M, Pichaikannu S, Das RR and Rathore A. Utilization of molecular marker based genetic diversity patterns in hybrid parents to develop better forage quality multi-cut hybrids in pearl millet. *Agriculture*, 2019;9(5), 97.
12. Ramya AR, Ahamed ML, Satyavathi CT, Rathore A, Katiyar P, Raj AB and Srivastava RK. Towards defining heterotic gene pools in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Front. Plant Sci* 2018;8, 1934.
13. Ramya AR, Ahamed M L, and Srivastava RK. Genetic diversity analysis among inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] based on grain yield and yield component characters. *Int. J. Curr. Microbiol. App. Sci* 2017;6(6): 2240-2250.
14. Satyavathi CT, Tiwari S, Bharadwaj C, Rao A, Bhat J and Singh S. Genetic diversity analysis in novel set of restorer lines of pearl millet *Pennisetum glaucum* using SSR markers. 2013;26(1), 72-82.
15. Tanwar H, Yashveer S, Sangwan VP, Sharma S, Sangwan S, and Mor VS. Genetic diversity in pearl millet restorers based on seed vigour traits and SSR markers. *Bangladesh J. Bot.* 2020;49(2), 357-365.
16. Waghmode BT, Sharma KM, Pawar GS, Salunke VD and Kale SP. Genetic diversity and hybrid purity analysis of pearl millet using molecular markers. *Int J Curr Microbiol Appl Sci*, 2018;6, 772-779.
17. Zidani S, Ferchichi A, and Chaieb M. Genomic DNA extraction method from pearl millet (*Pennisetum glaucum*) leaves. *A. J. B.* 2005;4(8), 862-866

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