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DNA Fingerprinting of pearl millet hybrids [*Pennisetum glaucum* (L.) R. Br.] Using SSR markers

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Abstract
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major cereal crop grown mainly in the arid and semi-arid regions of Asia and Africa. DNA fingerprinting is mandatory for registration of newly developed hybrids and varieties with National Bureau of Plant Genetic Resources (NBPGR), New Delhi and Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). In the present study, two advanced hybrid entries of pearl millet of third year testing *viz*. MH 2423 (NBH5929) and MH 2439 (86M80) were analyzed for DNA fingerprinting along with two checks (KBH108 and 86M86) using 36 SSR markers. Out of the 36 primers used, 26 SSR primers (72.2%) were found polymorphic with MH 2423 (NBH5929) and 28 SSR primers (77.8%) were found polymorphic with MH 2439 (86M80). The SSR profiles were analyzed based on pattern of bands and a unique profile was obtained for both the hybrids with amplicon size ranging between 100 to 500 bp. Thus, the SSRs very well differentiated the two hybrids generating a unique DNA fingerprinting profile for both the hybrids. The identified makers can prove useful further for genetic purity testing, utilization for identification of the diverse germplasm and future DNA fingerprinting studies.

Keywords: DNA fingerprinting, SSR markers, pearl millet, germplasm characterization, hybrids and varieties

Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the most widely cultivated cereal crop in Asia and Africa accounting for almost half of the global millet production. In India, pearl millet is the 4th most widely grown food crop after rice, wheat and maize. It is a dual purpose crop as it can be used as both feed and fodder for livestock. It has excellent properties like high photosynthetic efficiency, high dry matter production capacity, early maturity, drought tolerance, requirement of minimal purchase inputs and mostly free from biotic and abiotic stresses. It can withstand the most adverse agro-climatic conditions where other crops like sorghum and maize fail to produce economic yields (Satyavathi *et al*., 2021b) [17]. It also possess high nutritional values and is a rich source of energy, carbohydrate, fat (5-7%), ash, dietary fibre (1.2g/100g), α-amylase activity, quality protein (9-13%), antioxidants such as ferulic acid and coumaric acids, vitamins and micronutrients like iron, zinc, calcium, magnesium, copper, manganese, phosphorous. 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 (Satyavathi *et al*., 2021a) [16]. Till date, a total of 185 hybrids and 62 varieties of pearl millet have been identified and released for cultivation in different agro ecological zones of India through ICAR-All India Coordinated Research Project on Pearl millet (Satyavathi, 2021) [18].
Nowadays, identification of crop varieties is one of the major areas of interest for different scientists including plant breeders, plant pathologists, seed technologists and plant biochemists. Varietal identification is an important criterion for registration of the newly developed variety by a plant breeder in order to ensure for availability of pure parent and hybrid development program. Conventional approaches are generally used to identify and characterize the released hybrids/varieties which are mainly based on specific morphological and agronomic data. But, these traditional approaches are time-consuming, inefficient, expensive, restricted to a few characteristics, influenced by environmental effects (Kumar *et al*., 1995) [9]. With the increase in development and identification of new hybrids and varieties, it is becoming difficult for seed analysts to differentiate varieties only on the basis of conventional approaches (Gowda, 2001) [6]. Hence, rapid laboratory techniques have been developed for varietal identification in order to overcome the limitations of traditional

approaches. These rapid chemical and biochemical tests are very simple to perform and do not require more sophisticated and costly equipments except gel electrophoresis, restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), micro satellites (SSRs) markers (Rajendra and Shankar, 2001) [15]. Molecular marker assisted identification with high power genetic resolution has gained importance as a robust technique for identity profiling, cultivar fingerprinting, analyzing and comparing genetic similarity and variety protection. Molecular markers have been used for DNA fingerprinting in different crops (Williams *et al*., 1990; Smith and Helentjaris, 1996; Hoffman and Hang, 2003; Hasehmi *et al*., 2009; Dongre *et al*., 2012; Shaikh, 2015, Waghmode *et al*., 2018; Natesan *et al*., 2020a, b) [23, 20, 8, 7, 5, 19, 21, 13, 14]. DNA fingerprinting plays a vital role in protecting the novelty of a newly evolved plant variety which is submitted to NBPGR and PPV&FRA for notification and its registration. Germplasm registration in NBPGR needs DNA fingerprint to show the uniqueness of germplasm in comparison to existing varieties. It is a better option to identify unique markers to differentiate the varieties as the DNA fingerprints. They are more accurate as they differentiate the individuals based on marker information which is further used for estimating genetic diversity, marker-assisted selection in plant breeding (Weising *et al*., 2005) [22].

Pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic level in India but the use of DNA based markers in fingerprinting of pearl millet is limited. A few studies have been reported on the use of DNA markers on identification of cultivars in some of the millets (Avadh *et al*., 2013; Kumar *et al*., 2015, Nagawade *et al*., 2016, Natesan *et al*., 2020a,b, Waghmode *et al*., 2018) [3, 10, 12, 13, 14, 21]. The fingerprinting of pearl millet hybrids and identification of their genetic relationships are very important for plant improvement, seed purity testing, variety registration system, DUS (distinctness, uniformity and stability) testing and the protection of plant variety and breeders' rights. Clear-cut identification of elite crop varieties and hybrids is crucial for protection and prevention of unauthorized commercial use. Entries promoted for third year testing will be later identified for release and need DNA profile for submission of proposal. Hence, the present study was designed to develop specific fingerprints of entries of third year testing and identified along with the newly released variety for varietal identification and germplasm registration.

Materials and Methods

Plant Material

The two advanced hybrid entries of third year testing *viz*. MH 2423 (NBH5929) and MH 2439 (86M80) along with two checks C1 (KBH108) and C2 (86M86) tested under Indian Council of Agricultural Research-All India Coordinated Research Program on Pearl Millet, Jodhpur, India were used for DNA fingerprinting in this study. Twelve days old, tender and healthy leaves of plants grown in field conditions were collected in labeled self-sealing polyethylene bags and stored at -20°C until use.

Genomic DNA isolation and quantification

Genomic DNA was isolated from young and fresh leaves of 12 days old plantlets using Cetyl trimethyl ammonium bromide (CTAB) method along with some modifications as suggested by Ambawat *et al*. (2020b) [2] and was analyzed on 0.8% agarose gel. Further, the extracted DNA was diluted to a final concentration of 10 ng/µl for PCR reactions.

DNA profiling using SSRs

PCR reaction was carried out in a 96-well thermal cycler (Agilent Technologies) using a set of 36 SSR primers already reported in literature (Table 1). The PCR master mix consisted of 10 µl reaction mixture having 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM each dNTP, 0.4 µM 10-mer primer, 1 unit Taq DNA polymerase (Bangalore GeNei, India) and 10 ng of DNA. This reaction mixture was allowed to undergo initial denaturation at 94°C for 5 min followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 55°C for annealing and 1 min at 72°C for primer extension as described by Ambawat *et al*. (2020 a) [1]. Lastly, 1 cycle of final extension was carried out for 10 mins at 72°C followed by hold at 4°C. The amplified products were analyzed on 3.5% agarose gel and the DNA bands were visualized on a Gel Doc system and documented. Finally, scoring was done based on the presence or absence of the allele.

Table 1: List of SSRs markers used for DNA fingerprinting

S. No.	Primer name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
1	IPES0007	ACACCTCGCTGCACCTCTA	GCAACACAGATGAGACTGGC
2	IPES0019	ATTGCTCTTCCAACGAGGTG	TGCTATAGGCAGACTTTGAGAAA
3	IPES0021	TTTTCCCTCTTCTTGGCTCTT	CGATCTTCTGGCTCAACTCC
4	IPES0034	CCACAGGAGGAAAGAACACC	AGCACCGTGAACACAACAAC
5	IPES0013	CCTCTGGCAGTGGTCGTAGT	GAACTGAGGTAGAACCCCGC
6	IPES0030	GCGTCATGGCGTCTTAATCT	TCGACTCCTGAACTCAAGCA
7	IPES0016	CCGTTTGACCCTCAACATCT	GAGCACATTGGTTCCCAACT
8	IPES0012	TCAAATGCACGCCTAAGAAA	TCACCCGAAATGTCACAAGA
9	IPES0203	CCCTCGAAGAGATCGAAGTG	CTGAAACAACAGCCTGCAAA
10	Xctm 27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
11	Xctm3	GTCCATCGTCGCCGACGAA	GGATTTGCTAGTTGTGGGCT
12	ICMP3013	TGTGGGAGAGAGGAGAGTCC	CGCGAGATGATGTGTGGT
13	ICMP3014	TGCTTCACAGCCTCTCCATA	CCACCATGCAACAGCAATAA
14	ICMP3028	ACGATTCTTCGTCGTTCCAG	ATACGATACGCGCGAGCTAC
15	ICMP3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA
16	PGIRD5	CAACCCAACCCATTATACTTATCTG	GCAACTCTTGCCTTTCTTGG
17	PGIRD21	GCTATTGCCACTGCTTCACA	CCACCATGCAACAGCAATAA
18	PGIRD54	GCCTGGGATGTGTTTCTTCT	GCCTTTCATTTCCACCATGA
19	PGIRD57	GGCCCCAAGTAACTTCCCTA	TCAAGCTAGGGCCAATGTCT
20	PSMP2210	CAATGATGACCGTAATCTGGGTG	GGGCAAGATATGTGAAATCAAG

21	PSMP2203	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT
22	PSMP2076	GGAATAGTATATTGGCAAAATGTG	ATACTACACACTGTAAGCATTGTC
23	PSMP2070	ACAGAAAAAGAGAGGCACAGGAGA	GCCACTCGATGGAAATGTGAAA
24	PSMP2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC
25	PSMP2275	CCAGTGCCTGCATTCTTGGC	GCATCGAATACTTCATCTCA
26	PSMP2218	CTCTGTAAGTTCCTGGTGCTCAA	TCAGGCCAGTAACACATCTCAA
27	XPSMP3032	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA
28	PSMP2043	TCATATTCTCCTGTCTAAAACGTC	ACAAATCGTACAAGTTCCACTC
29	PSMP2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA
30	PSMP2064	ACCGAATTAAAGTCATGGATCG	TTGATTCTTCTGACACAAATGAG
31	PSMP2072	GAAATCTACACAAGGGTCTCCA	GTACGGCAGAATGACATCTGAA
32	PSMP2077	GCCAATATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT
33	PSMP2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA
34	PSMP2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT
35	PSMP2019	TGTGCCACAGCTTGTTCCTC	CAAGCAGCCAGTTCCTCATC
36	PSMP2206	AGAAGAAGAGGGGGTAAGAAGGAG	AGCAACATCCGTAGAGGTAGAAG

Results and Discussion

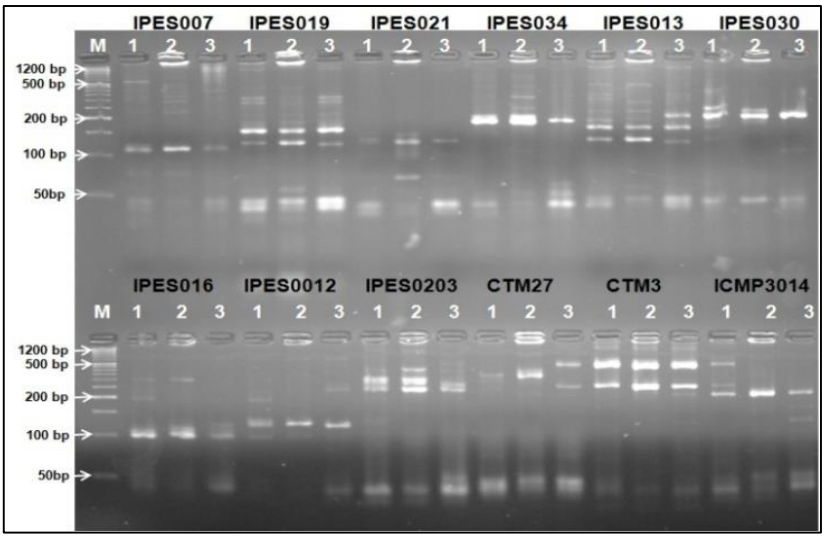
DNA fingerprinting using SSRs

Good quality genomic DNA having sharp band was successfully isolated from fresh and young leaves of two advanced hybrid entries - MH 2423 (NBH5929) and MH 2439 (86M80) and two checks - C1 (KBH108) and C2 (86M86). A total of 36 SSR primers were used for PCR amplification and differentiating the newly identified hybrids from the existing one and establish the DNA profiling of advanced hybrid entries of pearl millet. All the 36 primers amplified and analysis of hybrids revealed a good level of genetic polymorphism which allowed unique banding pattern. The SSR profiles were analyzed based on pattern of bands and they showed unique amplicons between 100 to 500 bp. Analysis of MH 2423 (NBH5929) using 36 primers displayed a unique profile for this genotype (Fig. 1 a, b, c). Among the 36 primers screened, 26 SSR primers (72.2%) namely IPES0019, IPES0021, IPES0034, IPES0013, IPES0030, IPES0016, IPES0012, IPES0203, XCTM27, XCTM3, ICMP3014, ICMP3013, PGIRD5, PGIRD21, PGIRD57, PSMP2203, PSMP2070, PSMP2027, PSMP2275, PSMP2218, XPSMP3032, PSMP2064, PSMP2072, PSMP2077, PSMP2087, PSMP2206 were found polymorphic between the three genotypes - MH 2423 (NBH5929), C1 (KBH108) and C2 (86M86) and the amplicon size varied from 100 to 450 bp (Table 2). Similarly, analysis of MH2439 (86M80) using the same 36 primers displayed a unique profile for this genotype (Fig. 2 a, b, c). Out of the 36 primers used, 28 SSR primers (77.8%) namely IPES0019, IPES0021, IPES0034, IPES0030, IPES0012, IPES0203, XCTM 27, ICMP3014, ICMP3028, PGIRD5, PGIRD54, PGIRD57, PSMP2210, PSMP2203, PSMP2076, PSMP2070, PSMP2027, PSMP2275, PSMP2218, XPSMP3032, PSMP2043, PSMP2064, PSMP2072, PSMP2077, PSMP2080, PSMP2087, PSMP2019, PSMP2206 were found polymorphic between the test entry- MH 2423 (NBH5929) and two checks - C1 (KBH108) and C2 (86M86) and the amplicon size varied from 100 to 500 bp (Table 3). Similar kind of reports of DNA fingerprinting in millets using molecular markers were also reported by many other researchers (Avadh *et al*., 2013; Kumar *et al*., 2015, Nagawade *et al*., 2016, Natesan *et al*., 2020a,b, Waghmode *et al*., 2018) [3, 10, 12, 13, 14, 21] which can further be used for varietal identification purpose. The set of SSR markers used in this study can be further used and may prove helpful for positive assessment of the ability of SSRs to produce unique DNA profiles in pearl millet. We used SSRs for DNA profiling because microsatellites are considered appropriate for varietal identification due to their high accuracy and efficiency to detect large numbers of discrete alleles (Danquah *et al*., 2002)[4]. Simple Sequence Repeats are the tandem repeat of around six nucleotides in both the coding as well as non-coding regions. The SSRs have become a marker of choice in genotyping because of their abundance, high level of allelic variation, co-dominant inheritance and analytical simplicity (Miah *et al*., 2013) [11]. Moreover, microsatellite markers can't be effectively applied to differentiate phylogenetically related species consistent with their conserved sequences, which could be useful to study the genetic constituents of the related species (Ambawat *et al*., 2020a, Natesan *et al*., 2020 a,b) [1, 13, 14]. SSR fingerprints are generally highly discriminative and are generally used to differentiate varieties or individuals and know their parentage and identity. The entries promoted for third year testing will be later identified for release and thus need DNA profile for submission of proposal. The DNA fingerprint data is given along with plant variety notification proposal which would provide great help to the plant breeders for registration of germplasm in NBPGR and it can be also used to validate the findings of DUS testing through DNA markers.

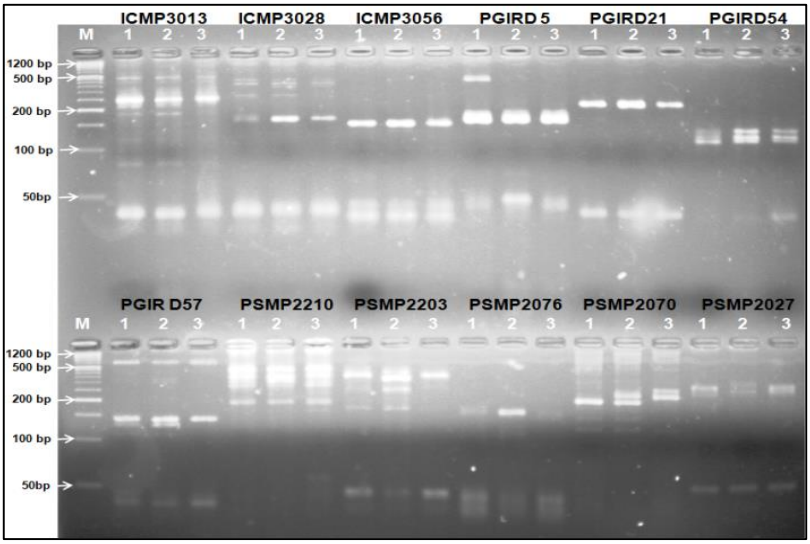
Table 2: Allelic profile of MH 2423 (NBH5929) using 36 SSR primers along with two checks- C1 (KBH108) and C2 (86M86)

S. No.	SSR primer	Observed band size (bp)			Polymorphism
		C1 (KBH108)	C2 (86M86)	MH2423(NBH5929)	
1	IPES0007	110	110	110	No
2	IPES0019	140/120	140/120	140/115	Yes
3	IPES0021	110	115/110	110	Yes
4	IPES0034	190/180	190/180	180	Yes
5	IPES0013	140/110	140/110	190/140/110	Yes
6	IPES0030	210/200	210/200	200	Yes
7	IPES0016	100	100	110/100	Yes
8	IPES0012	120/100	120/100	120	Yes
9	IPES0203	300/225	400/300/225	290/225	Yes
10	XCTM 27	380	380	450/280	Yes

11	XCTM 3	450/250	450/240	450/250/220	Yes
12	ICMP3014	450/240/200	210	210	Yes
13	ICMP3013	250/240/190	250/240/190	250	Yes
14	ICMP3028	160	160	160	No
15	ICMP3056	150	150	150	No
16	PGIRD5	500/170/160	170/160	170/160	Yes
17	PGIRD21	200	205/200	200	Yes
18	PGIRD54	150/140/130	150/140/130	150/140/130	No
19	PGIRD57	140/130	135/130	135	Yes
20	PSMP2210	450/400/300/190	450/400/300/190	450/400/300/190	No
21	PSMP2203	400/190	350/250/190	400	Yes
22	PSMP2076	150	150	150	No
23	PSMP2070	200	210/200	220/210	Yes
24	PSMP2027	250/240	260/250	250/240	Yes
25	PSMP2275	300/290	290	310/290	Yes
26	PSMP2218	260/250	250/240	250	Yes
27	XPSMP3032	215	215/210	215	Yes
28	PSMP2043	220	220	220	No
29	PSMP2059	140	140	140	No
30	PSMP2064	190/100	240	240	Yes
31	PSMP2072	150	150	140	Yes
32	PSMP2077	150	140	150	Yes
33	PSMP2080	190	190	190	No
34	PSMP2087	120	125	120	Yes
35	PSMP2019	250/200	250/200	250/200	No
36	PSMP2206	240/220	240/220	220	Yes



(a)



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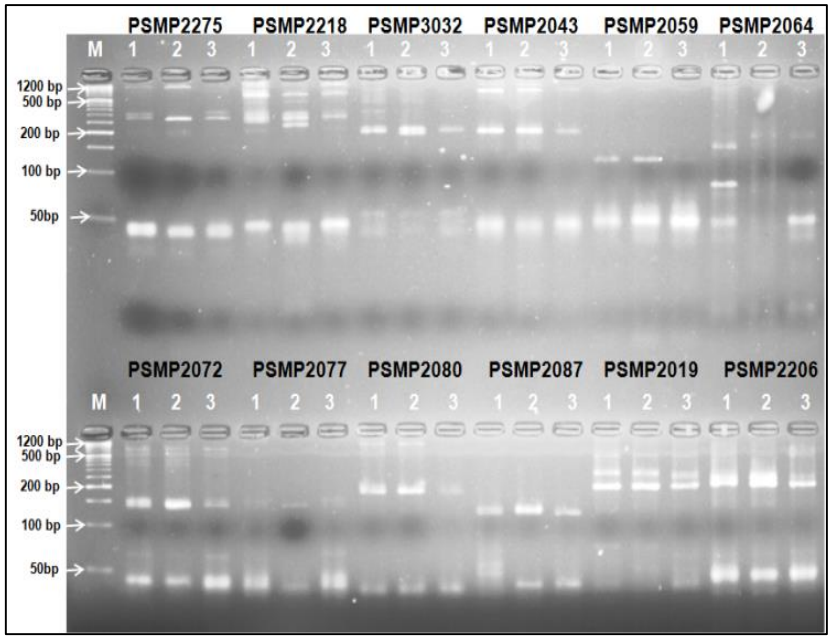
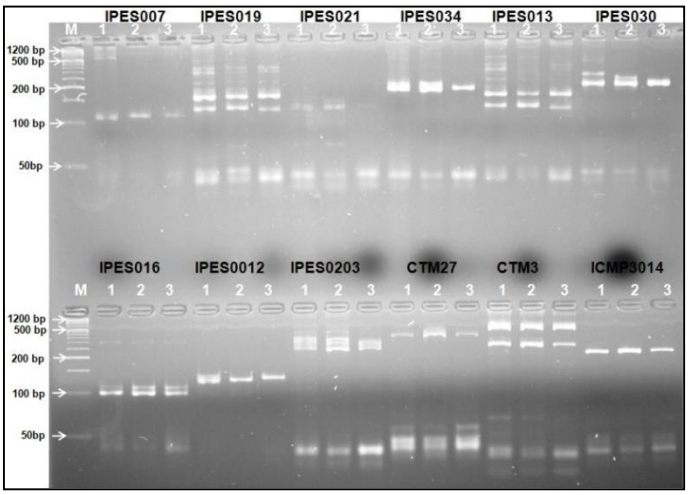


(c)

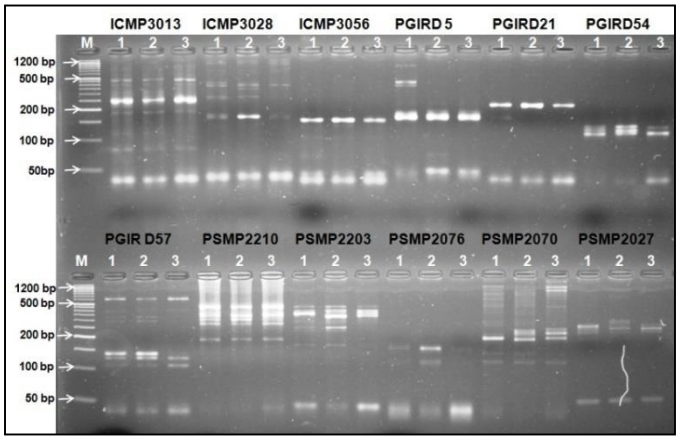
Fig 1(a, b, c): Gel photograph showing SSR allelic profile of MH2423 (NBH5929) with 36 SSRs. M-50 bp DNA ladder; Lane 1- KBH108 (Check), 2- 86M86 (Check), 3- MH2423 (NBH5929)

Table 3: Allelic profile of MH 2439 (86M80) using 36 SSR primers along with two checks- C1 (KBH108) and C2 (86M86)

S. No.	SSR primer	Observed band size (bp)			Polymorphism
		C1(KBH108)	C2 (86M86)	MH 2439 (86M80)	
1	IPES0007	115	115	115	No
2	IPES0019	150/125	150/125	155/120	Yes
3	IPES0021	120	125/120	120	Yes
4	IPES0034	180/175	180/175	175	Yes
5	IPES0013	150/125	150/125	150/125	No
6	IPES0030	300/250	260/250	250	Yes
7	IPES0016	110/100	110/100	110/100	No
8	IPES0012	140/120	130	130	Yes
9	IPES0203	300/280/240	300/280/230	290/280/230	Yes
10	XCTM 27	400	410/400	400	Yes
11	XCTM 3	500/260	500/260	500/260	No
12	ICMP3014	215	215	210	Yes
13	ICMP3013	450/250	450/250	450/250	No
14	ICMP3028	450/440/250/190	500/450/250/190	500/190	Yes
15	ICMP3056	150	150	150	No
16	PGIRD5	450/440/160	160	160	Yes
17	PGIRD21	200	200	200	No
18	PGIRD54	140/125	150/130	140/125	Yes
19	PGIRD57	600/140/130/120	600/140/130/120	600/130/120	Yes
20	PSMP2210	450/350/300/275/ 190	450/350/300/275/ 190	450/350/275/190	Yes
21	PSMP2203	450/400/250/180	450/430/400/250/ 180	430/400	Yes
22	PSMP2076	150/110	150/110	150	Yes
23	PSMP2070	200	210/200	220/210/200	Yes
24	PSMP2027	250/240	260/250	250/240	Yes
25	PSMP2275	300	300/200	300	Yes
26	PSMP2218	500/300/275	500/300/275/225	500/275	Yes
27	XPSMP3032	200	200	300/200	Yes
28	PSMP2043	200	200	220/200	Yes
29	PSMP2059	140	140	-	No
30	PSMP2064	200/100	210/200/140/100	150	Yes
31	PSMP2072	150/140	150/140	150	Yes
32	PSMP2077	150	140	140	Yes
33	PSMP2080	190	190	200	Yes
34	PSMP2087	150	150	140	Yes
35	PSMP2019	250/190	250/190	240/190	Yes
36	PSMP2206	220/200	230/190	220/200	Yes



(a)



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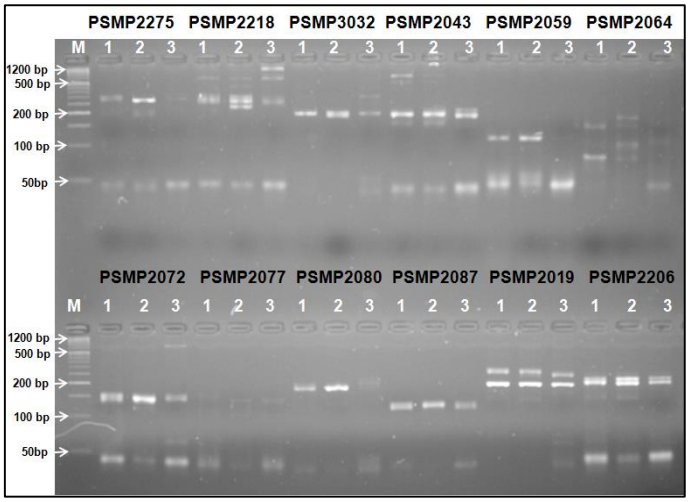

(c)

Fig 2(a, b, c): Gel photograph showing SSR allelic profile of MH2439 (86M80) with 36 SSRs. M-50 bp DNA ladder; Lane 1- KBH108 (Check), 2- 86M86 (Check), 3- MH2439

Conclusion

This study will be helpful for validity of identification proposal and can meet the basic requirements of proposal submission. Further, it was also revealed that the PCR based molecular markers especially SSRs are probably most promising for identification, registration and protection of commercial sample and will gain more and more influence on plant breeding in future and will speed up breeding procedure significantly. The primers used here can be used for screening new cultivars and could be utilized for identification of the diverse germplasms and also for fingerprinting in the future.

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Conflict of Interest: The authors declare that they have no competing interest.

References

1. 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 Appl Sci Tech 2020a;39(31):92-104.
2. Ambawat S, Singh S, Satyavathi CT, Meena R, Meena RC, Khandelwal V. Development of a high quality, rapid, efficient and economical DNA extraction protocol from climate resilient pearl millet crop without liquid nitrogen. International Journal of Environment and Climate Change 2020b;10(12):85-94.
3. Avadh S, Talati JG, Shah H. Varietal identification of pearl millet using markers. LAP Lambert Academic Publishing 2013, 138.
4. Danquah EY, Hanley SJ, Brookes RC, Aldam C, Karp A. Isolation and characterisation of microsatellites in *Echinochloa* (L.) beauv. spp. Molecular Ecology 2002;2:54-56.
5. Dongre AB, Raut MP, Paikrao VM, Pande SS. Genetic purity testing of cotton F_1 hybrid DHH-11 and parents revealed by molecular markers. Int Res J Biotech 2012;3(3):32-36.
6. Gowda R. Testing of varietal identity and purity: Development and prospectus: in a compendium of lectures on innovative techniques and their applications in varietal purity testing, Ed. Gowda, RP, Uni. Of Agfi. Sci., Bangalore 2001, 75-85.
7. Hashemi SH, Mirmohammadi SAM, Nematzadeh GA, Arzani A. Identification of rice hybrids using microsatellite and RAPD markers. African J. of Biotechnology 2009;8(10):2094-2101.
8. Hoffman D, Hang AN. The conversion of an RAPD marker to an STS marker for barley variety identification. Plant Mol Biol Reporter. 2003;21:81-91.
9. Kumar A, Chowdhury RK, Kapoor RL, Dahiya OS. Identification of pearl millet hybrids and their parental lines using seed and seedling characters, chemical tests and gel electrophoresis. Seed Sci. & Technol 1995;23:21-32.
10. Kumar S, Rachel S, Jacob, Satyavathi CT, Dadlani M, Kumar MBA. Utility of SSR marker in assessing the purity and identity of pearl millet hybrids, Journal of Plant Biochemistry and Biotechnology 2015, 1-4.
11. Miah RMY, Ismail MR, Puteh AB, Rahim HA, Islam K, Latif MA. A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. Int J Molecular Sciences 2013;14:22499-22528.
12. Nagawade DR, Markad NR, Patil HT. Hybridity and Genetic Purity Testing of Pearl Millet [*Pennisetum glaucum* (L.) R. BR.] Hybrid Shraddha. Int J Advances in Sci Engineering and Technology 2016;4(3):131-135.
13. Natesan S, Murugesan G, Murugan N, Chandran S, Angamuthu N. DNA fingerprinting of foxtail millet (*Setaria italica* L.) variety ATL 1 using SSR and RAPD markers along with morphological descriptors. Tropical Plant Research 2020a;7(3):587-593.
14. Natesan S, Venugopalan G, Selvamani SB, Krishnamoorthy M, Chandran S, Angamuthu N. Characterization of little millet (*Panicum sumatrense*) varieties using morphological descriptors and SSR based DNA fingerprinting Journal of Phytology 2020b;12:29-34
15. Rajendra P, Shankar AN. Variety identification by rapid chemical test: In a compendium of lectures on innovative techniques and their applications in varietal purity testing, Ed. Gowda, R.P., Uni. of Agri. Sci., Bangalore 2001, 107-123.
16. Satyavathi CT, Ambawat S, Khandelwal V, Srivastava RK. Pearl millet: A climate-resilient nutria-cereal for mitigating hidden hunger and provide nutritional security. Front. Plant Sci. 2021a;12:659938.doi: 10.3389/fpls.2021.659938
17. Satyavathi CT, Ambawat S, Sehgal D, Lata C, Tiwari S, Srivastava RK *et al*. Genomic designing for abiotic stress tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. In: Kole C. (eds) Genomic designing for abiotic stress resistant cereal crops. Springer, Cham. 2021b, 223-253. https://doi.org/10.1007/978-3-030-75875-2_6
18. Satyavathi CT. Project Coordinator's Review. ICAR-All India Coordinated Research Program on Pearl millet, Jodhpur, Rajasthan, India 2021, 1-18.
19. Shaikh TR. Assessment of hybrid purity in sorghum by using molecular markers. Submitted to Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani-431402 (M.S.) India 2015, 150.
20. Smith S, Helentjaris T. DNA fingerprinting and plant variety protection. Genome mapping in plants 1996;10:95-110.
21. Waghmode BT, Sharma KM, Pawar GS, Salunke VD, Kale SP. Genetic Diversity and Hybrid Purity Analysis of Pearl Millet Using Molecular Markers. Int J Curr Microbiol App Sci 2018;6:772-779
22. Weising K, Nybom H, Pfenninger M, Wolff K, Kahl G. DNA fingerprinting in plants: principles, methods, and applications, 2nd edition. CRC press. Boca Raton, Florida 2005.
23. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 1990;18:6531-6535.