**Original Research Article**

**Genetic diversity analysis and molecular characterization of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] hybrids/varieties**

**ABSTRACT**

Pearl millet is a widely grown, climate resilient rainfed cereal crop cultivated on 30 million ha in the arid and semi-arid tropical regions of Asia and Africa accounting for almost half of the global millet production. It has deep root system and exhibit climate-resilient features including adaptation to a wide range of ecological conditions, less irrigational requirements, better growth and productivity in low nutrient input conditions, less dependent on synthetic fertilizers and minimum vulnerability to environmental stresses and thus can survive in harsh climatic conditions and less fertile soil under water scarcity.But, there is a high need to develop high yielding hybrids and varieties in order to increase the productivity of pearl millet and this has been always the priority of the breeders. Thus, understanding of genetic diversity is very imperative and necessary to develop superior hybrids in any crop improvement program. In this study, 24 different released hybrids and varieties of pearl millet were used for diversity analysis using 156 Simple Sequence Repeat (SSR) markers. Out of these, 91 SSRs were found to be polymorphic giving 284 alleles. The number of alleles per locus varied from 2 to 6 with an average of 3.12 alleles.Polymorphic Information Content (PIC) values ranged from 0.31 to 0.78 with an average of 0.58 PIC value. The cluster analysis on the basis of these SSRsgroupedthe 24 genotypes into four major clusters viz., I, II, III, IV with the similarity coefficient varying from 0.59 to 0.78. The results revealed that adequate genetic variability subsists among the different hybrids and varieties used in the present study which can be further used in the pearl millet improvement programs. The findings also divulge that SSR markers are very dexterous and can be used proficiently for genetic diversity evaluation in pearl millet. It is also foreseen that results of the current study may be used further for varietal identification and DNA fingerprinting.

**KEYWORDS:** Dendrogram, genetic diversity, molecular characterization, pearl millet, simple sequence repeats

1. **INTRODUCTION**

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a climate-resilient, rainfed, nutritious cereal cropgrown widelyover 30 million ha and is a staple food of more than 90 million people. Majority of the crop isgrown in Africa (>18 million ha) and Asia (>10million ha) accounting for around half of the global millet production with 60% cultivation area in Africa and 35% in Asia [1].It is being cultivated over thousands of years and has always been a part of the traditional farming system.It is highly rich in several nutrients in comparison to other cereals and is being consumed by humans due to its more nutritive value. It has huge yield potential and also used as both feed and fodder for livestock.It is a drought tolerant, highly photosynthetic efficient crop with high dry matter production capacity and can even sustain on less fertile soils having poor water and nutrient holding capacity. Due to these features, it can prove very useful for farmers under changing climatic scenario [2]. It is highly rich in several nutrients with high levels of energy and protein and a more balanced amino acid profile [3-5]. Recognizing its value, Government of India declared Year 2018 as the ‘‘National Year of Millets”and FAO Committee on Agriculture (COAG) forum declared Year 2023 as “International Year of Millets”.It has considerable amount of genetic diversity owing to its abundant distribution all over the world and higher adaptability towards harsh environments, cross pollination mechanism and protogynous flowering [6]. Though pearl millet has a very proficient energy production system but its genetic improvement needs more attention to enhance its productivity like other major cereals. Inadequate accessibility and genetic improvement of improved hybrids and varieties besides agronomic and socioeconomic production constraints are among some of the reasons for its less productivity [7-8].Genetic diversity assessment and identification of superior genotypes are the major targets of any crop improvement programme. Numerous studies have been carried out in different crops to estimate genetic diversity for enhancing the genetic base of parental lines in order to develop superior cultivars [9-14]. Accessibility, evaluation and utilization of genetic diversity are very useful and have been used in pearl millet also for developing new cultivars and heterotic groups. Pearl millet cultivars have been bred from a narrow gene pool and as a result, there is a high need to study genetic diversity of pearl millet germplasm in order to strengthen the different breeding programs which in turn will be highly useful toconserve genetic resources, develop and improve pearl millet hybrids and varieties and speed up genetic enhancement of pearl millet for its different agronomical and nutritional traits. Hybrid breeding is a very effective strategy and has played a crucial role in pearl millet improvement [7]. Several superior, climate-smart and improved pearl millet cultivars have been developed using innovative breeding strategies to enhance its productivity[15].

Morphological characterization based on different phenotypic traits has been mainly used by researchers to estimate the genetic diversity in pearl millet [6, 13, 14, 16-18]. But, these data are more influenced by the environmentand other factors and are hence not considered much reliablefor traits exhibiting lower heritability.Thus, molecular markers are considered more steadfast over morphological markers for estimation of genetic diversity and characterization of various germplasm accessions. They are more useful for characterizing and estimating genetic distances among different groups of genotypes and thus can increase the power of conventional plant breeding and genetics [19].They have been widely used to estimate the extent of genetic diversity among various crops like sorghum, maize, soybean, rice, cumin, cluster bean, mustard etc. [9, 11, 12, 20-23].Various molecular markers have been used to evaluate genetic diversity among different cultivars of pearl millet also since past many years [8, 24-28]. Restriction Fragment Length Polymorphism (RFLP) markers were used first of all in pearl millet to construct the first genetic linkage map. Subsequently, several molecular makers including RFLPs, Sequence Tagged Sites (STSs), Amplified Fragment Length Polymorphism (AFLPs), genomic SSRs, Single-Strand Conformation Polymorphism (SSCPS) and genic SSRs were developed and applied in pearl millet improvement studies [29-30].Afterwards, high throughput platforms like Diversity Arrays Technology (DArT) [31], Genotyping-by-sequencing (GBS) [18), Single Nucleotide Polymorphism (SNPs) [28, 32] were developed and used for profiling genome-wide nucleotide variations in pearl millet. Though, Next Generation Sequencing (NGS)-based single-nucleotide polymorphisms have become the marker of choice nowadays, but still SSRs appear to be more consistent and considered as markers of choice due to different features like co-dominant inheritance, multi-allelism, high polymorphism, low cost, even distribution throughout the genome, automation, simple methodology and easy detection etc. [24, 33-34].Due to these useful features, they are considered highly useful for assessing diversity, DNA fingerprinting, germplasm characterization, estimation of genetic distances among populations and defining heterotic groups[21, 25, 27, 33, 35]. Thus, keeping all this in view, the present study was planned toexplore the nature and extent of genetic variation among hybrids/varieties of pearl millet using SSR markers.

1. **MATERIALS AND METHODS**

**2.1 Plant material**

A total of 24 popular released pearl millet hybrids and varieties developed under Indian Council of Agricultural Research-All India Coordinated Research Program on Pearl Millet, Jodhpur, India were used in this study.

**2.2 Genomic DNA isolation and quantification**

DNA was extracted from fresh and young leaves of 12 days old plants of 24 genotypes following CTAB method along with some modifications without using liquid nitrogen as described in our previous study [36] and quantified on 0.8 % agarose gel.

**2.3 Molecular analysis using SSR primers**

A total of 156 SSR primers were used for PCR amplification and study of molecular diversity among 24 pearl millet genotypes (Table 1). The sequences of these primers were obtained from the previous studies [24, 37-40]. For carrying out PCR reaction, DNA was diluted to a final concentration of 10 ng/μl and amplification reactions were performed ina volume of 10 μl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 mM each dNTP, 0.4 μM 10-mer primer, 1 unit Taq DNA polymerase (GeNei, India) and 10 ng of DNA. Amplifications were carried out in a 96-well thermal cycler(Agilent Technologies)which was programmed to 1 cycle of 5 mins at 94°C for initial strand separation. This was followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 58°C for annealing and 1 min at 72°C for primer extension. Finally, 1 cycle of 10 mins at 72°C was carried out for final extension followed by hold at 4°C[24]. The PCR products were analyzed on 2.5% agarose gel.

**2.4 Diversity analysis and dendrogram construction**

Gel photographs were used to score variations in the bands as presence (1) and absence (0) of bands where each band represented a genetic locus. Only, the clear, unambiguous amplicons were scored and their sizes were estimated using 50bp DNA ladder (HiMedia) as standard. Cluster analysis was performed among the genotypes based on the basis of Jaccard’s similarity coefficients, UPGMA and SAHN-clustering algorithms of NTSYS-PC (Numerical Taxonomy System, Version 2.02e NTSYS-pc, version 2.02e (Applied Biostatistics) software. Polymorphism Information Content (PIC) was calculated as, Polymorphism Information Content (PIC) =Σ (1-P2i)/n, where n is the number of band positions analyzed in the set of accessions and P2i is the frequency of ith allele.

1. **RESULTS AND DISCUSSION**

**3.1 Molecular characterization and SSR marker analysis**

In this study, a total of 156 SSRs were used for molecular characterization of 24 popular pearl millet hybrids/ varieties. Out of 156 SSRs, 128 primers amplified products of varying sizes ranging from 90 to 760 bp and 91 (58.3%) were polymorphic (Table 2,3) and (Fig. 1, 2) and37 (23.7 %) were monomorphic (Table 3). Thus, a good amount of polymorphic markers were attained which can be used for genetic diversity estimation, identification of genotypes, germplasm evaluation and further used in breeding programs.A total of 284 alleles were obtained in this study and the number of alleles per locus varied between 2 to 6 with an average of 3.12 alleles. These values are similar to 3.43 alleles per locus, 3.4 alleles per locus and 3.1 alleles per locus as observed by other researchers[8, 41-42]. But, they were comparatively lower than 2-18 alleles (6.8 alleles per locus), (5.5 alleles per locus) 4.62 alleles per primer and 12.5 alleles per locus as reported by different researchers in their studies [25, 43-45].Such observations may be due to diverse world collection of germplasm. Such kind of results regarding efficiency of SSR markers in evaluating diversity have also been depicted by other investigators [21, 24, 27, 33].Differentreports have been published by researchers on estimation of genetic diversity in pearl millet lines on the basis of molecular profiling [8, 18, 19, 25, 26-28, 31].But, in the present study, we assessed genetic diversity among hybrids and varieties of pearl millet which can serve asmolecular database for the existing hybrids and varieties and can be helpful towards genomic studies and DNA fingerprinting of pearl millet hybrids and varieties. Molecular analysis is very helpful in evaluation and management of genetic resources, characterization and identification of new genotypes, divulging genetic relationships among breeds/varieties, using marker and trait association and analysis of population structure[8, 24, 32, 46- 47].SSR markers are mostlyfavored for genotypic characterization due to their high polymorphism and reproducibility, co-dominant nature, simplicity and specificity, mulitiallelism, requirement of lesser quantity of DNA. Moreover, they provide unique allelic profiles or DNA fingerprints which can precisely and effectively establish genotypic identity [21].SSRs have been widely used in different cropsto evaluatecrop germplasm and genetic diversity[14, 24]. Theypossess severaladvantages in comparison to SNPs in diversity analysis and thus SSR data can be more useful in defining pedigrees than SNP data. PIC values vary between 0 and 0.5 of in SNPs due to their bi-allelic nature whereas it can be above 0.5 in case of SSRs owingto their mutli-allelic nature. ThoughSNPs are gaining more popularity and are believed to be markers of choice in the current context of genomics era, but SSR markers will continue to be desirable and preferable due to various advantages [20, 34]. In some studies, in-depth genotyping divulged by SNPs is not desired and thus in such cases, SSRs are a better choice as they can be utilized for larger expansion of sample size without increasing the cost and they can be also easily integrated with new studies. Hence, SSRsare a good choice for small scale laboratories with limited facilities and budget as compared to SNPs[48].

Polymorphic information content (PIC) value determines polymorphism for a marker locus as it computes informativeness of markers and evaluates the diversity of alleles. It accounts the number of expressed alleles as well as their relative frequencies to evaluate the discriminatory power of a locus. PIC index assesses the intensity of gene variation and a PIC value of ≥0.5 indicates higher diversity, while PIC ≤ 0.25 depicts lower diversity and PIC value between 0.25 and 0.5 is indicator of intermediate diversity [49]. In the current study, PIC values ranged from varied from 0.31 (CTM 27) to 0.78 (IPES0005, IPES0024, CTM9) (Table 2)with an average of 0.58. PIC values ranging between 0.02 to 0.97 were reported in some earlier reports[22, 24, 38, 44, 50-53]. An average PIC value of 0.58 observed in this study is similar to 0.56 to 0.59 reported by other researchers [44, 25, 27, 39-40]. Conversely, it was lesser in comparison to average PIC value of 0.671 recorded by [51]whereas higher than 0.37 to 0.43 PIC values as reported in other studies[8, 24, 52]. Out of 91 markers, 48 markers (52.7%) had PIC value > 0.5 signifying that these were highly informative and the most useful markers for discriminating these hybrids and varieties. Markers having PIC values of 0.5 or above are considered as extremely valuable in discerning the genotypes and useful for molecular genetic diversity studies [54]. A high PIC value between 0.65 and 0.78wererecorded in 10.9 % (10 SSR) markers (Table 2). MarkersIPES 0005, IPES 0024 and CTM9 exhibited the highest PIC value (0.78) followed by IPES0007, IPES0043, CTM 03, ICMP3006, ICMP3020, Xcump006 (0.77) and IPES0009, IPES0012, IPES0022, ICMP4010, CTM 08, CTM 10, Xcump009 (0.76) indicating that IPES 0005, IPES 0024 and CTM9are among the most informative and best markers for diversity estimation of these pearl millet genotypes followed by PSMP2072, PSMP3032, PSMP2001, PSMP3017 and PSMP2066 markers while the lowest PIC value of0.31 was recorded for maker CTM 27 indicating that is the least powerful marker (Table 2). High PIC values may be observed due to the use of large number of informative markers [54].Similar reports were observed in several other studies [8, 24-25, 27, 43-45,55].

3.2 Diversity analysis and dendrogram construction

Pearl millet has a significant amount of diversity at both genotypic and phenotypic levels. Genetic variation is very important and assessment of diversity and germplasm characterization can play crucial role in development of commercial hybrids and the crop improvement programs (Yadav et al., 2013). The genetic relationships established among the different genotypes in the present study were according to the available pedigree data. The cluster analysis based on SSR data classified the genotypes into four main clusters viz., I, II, III, IV and similarity coefficient varied between 0.59 to 0.78 (Fig. 3, Table 4).) which are similar to those reported in other studies [24, 41, 44, 55- 56].Cluster I contained thirteen genotypes and grouped together at similarity index of 0.69. In this cluster, early maturing pearl millet hybrids/varieties viz. CZP9802, HHB 234, BHB1602, ProagroTejas, GHB 719, GHB1225 are clustered together while PB1852, Pratap, GHB744, GHB 732 are medium maturing and grouped together. In addition, KBH108 and MP7872, 86M86 are late maturing and grouped together. This cluster can be further subdivided into three sub-clusters- Ia, Ib, Ic. Sub-cluster Ia included six hybrids/varieties viz. PB1852, KBH108, MP7872, CZP9802, HHB 234, BHB1602 where CZP9802, HHB 234, BHB1602 are specific for A1 zone and grouped together indicating that they can be used for developing drought tolerant pearl millet hybrids for drier parts of Rajasthan. On the other hand, hybrids PB1852, KBH108, MP7872 are specific for A zone which are clustered together in separate group. Here, hybrids KBH108, and MP7872 showed very close similarity with each other at minimal genetic distance of 0.78 while CZP9802 and HHB 234 exhibited closer relationship at a genetic distance of 0.77. Sub-cluster Ib contained four genotypes- Pratap, GHB1225, ProagroTejas, GHB 719 where GHB1225, ProagroTejas, GHB 719 are specific for A1 zone while Pratap is for B zone. Here, hybrids Pratap and GHB1225 showed closer relationship with each other at minimal genetic distance of 0.72 while ProagroTejas and GHB 719 were found to show close similarity at a genetic distance of 0.70. Sub-cluster Ic contained three hybrids- 86M86, GHB744 and GHB 732 which are suitable for A zone and 86M86 and GHB 744 grouped closely at a genetic distance of 0.73.

Cluster II was obtained at a similarity index of 0.65 containing six genotypes namely RHB177, CZPIC923, PHB2168, Raj 171, Pusa Composite 443 and RHB173. In this cluster, RHB177, PHB2168 and Pusa Composite 443 are from early maturity group and specific for A1 zone while CZPIC923, Raj 171 and RHB173 are specific for A zone with medium maturity group. Further, in this cluster, RHB177 and CZPIC923; PHB2168 and Raj 171; Pusa Composite 443 and RHB173 showed closer relationship with each other at minimal genetic distances of 0.70, 0.73 and 0.66 respectively. In cluster III, four genotypes Pusa Composite 1201, NBH4903, MP7792 and GHB1129 clustered close to each other at a similarity index of 0.60 where NBH4903 and GHB1129 are specific for A1 zone while Pusa Composite 1201 and MP7792 are for A zone. Here, Pusa Composite 1201 and NBH4903 showed a more close similarity at a genetic distance of 0.66. Hybrid Kaveri Super Boss was entirely separated from all the genotypes and included in cluster IV at a similarity index of 0.59. Thus, different genotypes grouped according to their characteristics and salient features as described in other reports [15]. It has been proved that SSRs can be suitable and efficient tool for molecular characterization of different crop species. Similarly, clustering between genotypes of pearl millet was also recorded in other studies [18-19, 24, 44, 57-60].

1. **CONCLUSION**

In this study, **t**he pearl millet hybrids/varieties have been successfully characterized and categorized into diverse groups which will be useful to assess the evolutionary relationships with the wild relatives. The results reveled thatthere is a good genetic variability among the different hybrids and varieties which can be used in future pearl millet improvement programs. Here, we reported a good amount of polymorphic SSR markers with high PIC values indicating that SSRs can be effectively used for genetic diversity and genomicstudies in pearl millet. These results will be also helpful in eliminating the gaps in lineage or selection history, detecting differences in allelic frequencies within genotypes or populations. It is anticipated that it will be also fruitful to explore new alleles at various loci of interest and DNA fingerprinting and varietal identification.

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Fig. 1 Agarose gel showing amplification profiles of pearl millet hybrid/varieties using the primer IPES0013. Lane M-50 bp ladder, Lane 1-24 pearl millet hybrids/varieties



Fig. 2 Agarose gel showing amplification profiles of pearl millet hybrid/varieties using the primer IPES0012. Lane M-50 bp ladder, Lane 1-24 pearl millet hybrids/varieties

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Fig. 3 UPGMA dendrogram illustrating genetic relationshipsamong 24pearl millet genotypesbased onJaccard’s similarity coefficients and SSR markers data

**Table 1. Pearl millet genotypes used for molecular characterization and genetic diversity analysis**

| **S. No.** | **Name of Hybrid/Variety** | **Year** | **Organization** | **Salient features** |
| --- | --- | --- | --- | --- |
| 1. | PB1852 | 2019 | Bayer Bio Science, Pvt. Ltd., Hyderabad | Medium maturing, grey colour grain with bold size, lodging tolerant, responsive to fertilizers, resistant to downy mildew and blast, tolerant to moisture stress |
| 2. | ProagroTejas | 2016 | Bayer Bio Science, Hyderabad | Early maturing, medium height, candle ear heads, grey seeds, resistant to downy mildew |
| 3. | BHB1602 | 2020 | ICAR-AICRP on Pearl millet, SKRAU, Bikaner | Early maturing, compact, conical ear heads, grey brown, globular grains, highly resistant to downy mildew, blast, insect pests and resistant to smut |
| 4. | Pusa Composite1201 | 2018 | ICAR-IARI, New Delhi | Medium maturing, yellow anthers, cylindrical panicles, stay green trait, highly resistant to downy mildew, smut and rust, highly resistant to pests, highly responsive to fertilizers |
| 5. | NBH 4903 | 2018 | Nuziveedu Seeds Pvt. Ltd., Hyderabad | Late maturing, medium plant height with long exerted compact panicles, medium bold grains, non lodging, non shattering, resistant to drought  |
| 6. | KBH 108 | 2014 | Krishna Seeds (P) Ltd., Agra | Late maturing, tall plant height, purple anther colour, cylindrical very compact ear heads, obovate grey seed, resistant to downy mildew, blast and smut |
| 7. | Kaveri S. Boss | 2012 | Kaveri Seeds Co. Ltd., Secunderabad | Late maturing, tall height, long compact cylindrical earheads, purple anther colour, globular grey colored seed |
| 8. | MP 7792  | 2012 | Metahelix Life Science Ltd., Bangalore | Late maturing, medium height, yellow anther colour, cylindrical earheads, grey coloured globular seed |
| 9. | MP 7872 | 2012 | Metahelix Life Science Ltd., Bangalore | Late maturing, medium height, yellow anther colour, spindle earheads, grey coloured globular seed |
| 10. | Pratap (MH 1642) | 2012 | Nuziveedu Seeds Pvt. Ltd., Hyderabad | Medium maturity, medium plant height, cylindrical semi compact earheads, globular grey seed |
| 11. | 86M86 | 2012 | Pioneer Overseas Corp., Hyderabad | Late maturing, Medium to tall plant height, conical very compact earheads, purple anther colour, grey hexagonal seeds |
| 12. | GHB732  | 2008 | AICRP on Pearl millet, MRS, Jamnagar | Medium maturity, medium tall, compact lanceolate earheads, purple anthers, globular grey brown bold grains |
| 13. | GHB744 | 2008 | AICRP on Pearl millet, MRS, Jamnagar | Medium maturity, medium tall, medium thick stem with basal pigmentation, compact cylindrical shaped panicles with yellow anthers, globular grey brown grains |
| 14. | Pusa Composite 443 | 2011 | ICAR-IARI, New Delhi | Early maturity, medium tall, rod shaped earheads with bold grain |
| 15. | GHB719 | 2007 | AICRP on Pearl Millet, MRS, Jamnagar | 70-75 days maturity, fully exerted conical shaped, compact and bristled earheads, globular, medium in size, grey coloured grains, tolerant to drought |
| 16. | RHB173 | 2011 | AICRP on Pearl Millet, RARI, Jaipur | Medium maturity, medium to tall plant height, compact cylindrical ear heads, resistant to downy mildew |
| 17. | CZP9802 | 2003 | ICAR-CAZRI, Jodhpur | 70-72 days, medium tall, good tillering, thin stem, narrow leaves, thin candle-shaped earheads, yellowish grains of medium size, drought tolerant, very high stover of good quality |
| 18. | HHB234 | 2013 | AICPMIP,CCS HAU, Hisar | Early maturing, candle shaped earheads with small bristles, medium seed size and tolerant to downy mildew |
| 19. | GHB1225 | 2020 | Gujarat | Late maturing, resistant to downy mildew, blast, smut, rust and ergor, salt and water stress tolerant, good quality stover |
| 20. | GHB1129 | 2020 | Gujarat | Suitable for Kharif and summer seasons, Medium maturing, resistant to downy mildew and lodging, salt and water stress tolerant, good quality stover |
| 21. | RHB177 | 2011 | AICPMIP, RARI, Durgapura, Jaipur | Early maturing, medium tall, cylindrical bristled earheads, resistant to downy mildew, light yellow anthers |
| 22. | CZPIC 923 | 1997 | CAZRI, Jodhpur | 72-80 days, tall, thick stem, long oblanceolate thick panicle, light yellow to brown anthers and light grey seed with yellow base |
| 23. | PHB2168 | 2008 | PAU, Ludhiana | Early maturity, medium tall, compact cylindrical shaped panicles with yellow anthers, obovate grey grains |
| 24. | Raj171 | 1992 | AICPMIP, ARS, Durgapura, Jaipur and ICRISAT, Hyderabad | 82-85 days, tall, medium thick stem, long cylindrical semi compact to compact ear heads, obovate grey brown grains, resistance to downy mildew, bred from inter varietal composite |

**Table 2. List of polymorphic SSR markers used for genetic diversity analysis among pearl millet genotypes**

| **S. No.** | **Oligo Name** | **Forward primer sequence (5’ – 3’)** | **Reverse primer sequence (5’ – 3’)** | **Product range(bp)** | **No. of alleles** **amplified** | **PIC** |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | PGIRD43 | GTTCATGCAGCTTGGTTTCC | AGTGACCTGGGGTACAGACG | 110-115 | 2 | 0.38 |
| 2 | PGIRD44 | TCTCTCTCGGATCGCTGTG | GCTGGTTGGTAGAGGCTGAC | 90-110 | 2 | 0.40 |
| 3 | PGIRD46 | GAACAATTGCTTCTGTAATATTGCTT | GCCGACCAAGAACTTCATACA | 100-120 | 2 | 0.35 |
| 4 | PGIRD49 | AGCTCCTCGACGGAGAAAGT | GACGGTGTCGACGAAGATG | 200-350 | 2 | 0.50 |
| 5 | PGIRD54 | GCCTGGGATGTGTTTCTTCT | GCCTTTCATTTCCACCATGA | 130-140 | 3 | 0.64 |
| 6 | PGIRD56 | ATCACTCCTCGATCGGTCAC | ACCAGACACACGTGCCAGT | 140-350 | 2 | 0.50 |
| 7 | PGIRD57 | GGCCCCAAGTAACTTCCCTA | TCAAGCTAGGGCCAATGTCT | 130-150 | 3 | 0.66 |
| 8 | IPES0004 | GTGCGTTCTTCCTTGCCTAC | TCATCACACAGGGCTAGCTG | 140-300 | 2 | 0.50 |
| 9 | IPES0005 | CTTCTTCCCTTCAGTGGCTG | AAATGAAGAAATGCACAAGCAA | 100-500 | 5 | 0.78 |
| 10 | IPES0007 | ACACCTCGCTGCACCTCTA | GCAACACAGATGAGACTGGC | 110-550 | 6 | 0.77 |
| 11 | IPES0008 | CAGAATCAACCAGAAGGGGA | CGGTGCTACATGTGCGTTAT | 350-600 | 5 | 0.74 |
| 12 | IPES0009 | TTGATCGATCGTCTACGGTT | TATACTCACTCACGGCAGCG | 180-700 | 6 | 0.76 |
| 13 | IPES0010 | AAAGACAACGAACGCGAAGT | GCTTCAGTTCCCATCGTAGG | 190-600 | 5 | 0.75 |
| 14 | IPES0011 | TGGAGAAAGGGAAGCTCAGA | TGCTGCATCATCAACCCTTA | 100-200 | 4 | 0.74 |
| 15 | IPES0012 | TCAAATGCACGCCTAAGAAA | TCACCCGAAATGTCACAAGA | 190-410 | 5 | 0.76 |
| 16 | IPES0013 | CCTCTGGCAGTGGTCGTAGT | GAACTGAGGTAGAACCCCGC | 120-600 | 4 | 0.70 |
| 17 | IPES0014 | GCACAATCAAAATGACAGCG | TTTCTTGCTTCCTTGCTGGT | 160-170 | 2 | 0.46 |
| 18 | IPES0016 | CCGTTTGACCCTCAACATCT | GAGCACATTGGTTCCCAACT | 100-300 | 4 | 0.68 |
| 19 | IPES0017 | CCTATGGCGGCAGAGTAGTG | TTCCGGCACAATTACTTTCA | 100-120 | 2 | 0.35 |
| 20 | IPES0019 | ATTGCTCTTCCAACGAGGTG | TGCTATAGGCAGACTTTGAGAAA | 110-120 | 3 | 0.63 |
| 21 | IPES0020 | TTTACAGCCCGGATATCGTC | TCCACGCCACAGATAACAAC | 100-150 | 2 | 0.38 |
| 22 | IPES0021 | TTTTCCCTCTTCTTGGCTCTT | CGATCTTCTGGCTCAACTCC | 100-140 | 3 | 0.62 |
| 23 | IPES0022 | GGAACACATACGGAGTGACAGA | TGTGTCTTACCCCTTGCTGA | 90-500 | 6 | 0.76 |
| 24 | IPES0024 | TCATCACCATCACCATCACC | TTGTTTGGGTTTCAGTGGCT | 100-450 | 5 | 0.78 |
| 25 | IPES0026 | AGTATCCGCGTATTGGGTTG | GTACACCCAGCCAGCCTAAG | 110-120 | 2 | 0.48 |
| 26 | IPES0028 | TGCCATGACCCCTGTATATG | TCAATTCCAGTCGTGTGATGA | 110-120 | 2 | 0.41 |
| 27 | IPES0029 | AAACTCTGTTGCTGCTGCTG | ATCATCTGGGAAGCCTTTGA | 100-110 | 2 | 0.52 |
| 28 | IPES0030 | GCGTCATGGCGTCTTAATCT | TCGACTCCTGAACTCAAGCA | 195-350 | 4 | 0.72 |
| 29 | IPES0032 | GCTTTTCCGTGGTAGCTCAG | AAATGCTGCTTGCGTCTTCT | 100-200 | 2 | 0.46 |
| 30 | IPES0034 | CCACAGGAGGAAAGAACACC | AGCACCGTGAACACAACAAC | 170-300 | 3 | 0.66 |
| 31 | IPES0036 | GACTGCCGGTGAGTTTGATT | TTCTTTTCAGATCCACTAGCTGC | 140-400 | 4 | 0.67 |
| 32 | IPES0038 | GAGAAGGGTCAGGAGGGAAC | AAACGTCCGGTCTTCATGTC | 235-300 | 3 | 0.67 |
| 33 | IPES0040 | GGTAGACCTAAAACTGAGAGGCA | ACCTGTCTGTCAAAGCGTCC | 90-120 | 2 | 0.50 |
| 34 | IPES0043 | TGGATTGACGACTGGAATTG | GACTGACCAGGCACACCTTT | 150-500 | 5 | 0.77 |
| 35 | IPES0044 | AGGAAGAGTCGGACTGCAAA | GAGGCTTTGTTGCATTGACC | 125-130 | 2 | 0.46 |
| 36 | IPES0045 | CAGCACCATTAGTGGCAAAA | CGTAACTTTGGTCAGGCATACA | 150-200 | 2 | 0.38 |
| 37 | IPES0047 | GAATCTTCCCGACAAATGGA | CCTTGGCTAGCGTCATCTTC | 100-120 | 2 | 0.33 |
| 38 | ICMP 3080 | CAAACAGCATCAAGCAGGAG | GCGTAGACGGCGTAGATGAT | 250-750 | 3 | 0.66 |
| 39 | ICMP 3086 | ACCAAACGTCCAAACCAGAG | ATATCTCTTCGCTGCGGTGT | 200-600 | 2 | 0.49 |
| 40 | ICMP3018 | ACGAGGACAAGCTCTTGGAA | ACGGCGCATACTCGATCATA | 140-210 | 3 | 0.65 |
| 41 | ICMP3056 | ACGGAGCTACGGTTGGAATA | CACAAGGGACCCCACGATA | 150-170 | 2 | 0.54 |
| 42 | ICMP3068 | CTGGCAAAGTTGTAGCGTGA | ATGTCGCTCTCTGCCAAGAT | 200-230 | 2 | 0.43 |
| 43 | ICMP3078 | TCCAGACAGTTCAGCAGGTG | CCACACGAGACAGAGCACAC | 260-280 | 2 | 0.45 |
| 44 | ICMP3088 | TCAGGTGGAGATCGATGTTG | TTACGGGAGGATGAGGATG | 160-305 | 3 | 0.61 |
| 45 | ICMP3091 | AACAAGGACCTGCGATTCAC | CATGACAGCAACGACGAATC | 100-500 | 5 | 0.68 |
| 46 | ICMP3093 | AGTTTCCAATCCCACCCTCT | GTTGGAGATGAGGTCGAGGT | 110-250 | 2 | 0.50 |
| 47 | ICMP3095 | GGGAGGCCACGATTTAAAGA | ACAATGTGCACGCAAGGA | 250-300 | 2 | 0.48 |
| 48 | ICMP3096 | CTGCATTGCAACATCCTCAC | AACCTGCAGTGGAAGCAATC | 250-450 | 3 | 0.67 |
| 49 | ICMP4006 | TGAGGACCGAGAAGAAGCAT | CAACACCCAACAGAAACTGAA | 130-310 | 4 | 0.75 |
| 50 | ICMP4010 | ATCCCCTACAGCATCAGCAC | CGGCGGAGAGATCTTATTCA | 175-600 | 5 | 0.76 |
| 51 | ICMP3017 | CACCAAACAGCATCAAGCAG | AGGTAGCCGAGGAAGGTGAG | 200-210 | 2 | 0.39 |
| 52 | CTM 03 | GTCCATCGTCGCCGACGAA | GGATTTGCTAGTTGTGGGCT | 200-450 | 4 | 0.77 |
| 53 | CTM 08 | GCTGCATCGGAGATAGGGAA | CTCAGCAAGCACGCTGCTCT | 120-260 | 5 | 0.76 |
| 54 | CTM9 | GCCTCCTCTTGATACCATATT | TAGCCTTGGCTGCTATATTC | 100-500 | 5 | 0.78 |
| 55 | CTM 10 | GAGGCAAAAGTGGAAGACAG | TTGATTCCCGGTTCTATCGA | 200-760 | 5 | 0.76 |
| 56 | CTM12 | GTTGCAAGCAGGAGTAGATCGA | CGCTCTGTAGGTTGAACTCCTT | 320-400 | 2 | 0.50 |
| 57 | CTM26 | GCAAGTGATCCATGACATTACGA | GCGAAGTAGAACACCGCGCT | 90-350 | 3 | 0.67 |
| 58 | CTM 27 | GTTGCAAGCAGGAGTAGATCGA | CGCTCTGTAGGTTGAACTCCTT | 330-360 | 2 | 0.31 |
| 59 | CTM21 | ATGCCTCCCACCCCACGTCG | CGTCGCACTAGCCACAGTCA | 300-350 | 2 | 0.49 |
| 60 | CTM25 | GCGAAGTAGAACACCGCGCT | GCACTTCCTCCTCGCCGT | 150-200 | 2 | 0.47 |
| 61 | ICMP3038 | CTCTCGGTTTGACGGTTTGT | GGGGAAAACAAAGTTGCTCA | 170-250 | 3 | 0.62 |
| 62 | ICMP3035 | GCCAAGGAGGTCAAGATCG | ACACGACTCGACTCAGACCA | 190-200 | 2 | 0.44 |
| 63 | ICMP3033 | GAGGGCCAGCTCTCCTAGAT | CCCTAACCACAGAGGGACAC | 180-200 | 2 | 0.32 |
| 64 | ICMP3029 | ACCAGCAACAGCAGCAGAG | ACACACTGCGACAAGTGGAG | 180-700 | 4 | 0.56 |
| 65 | ICMP3028 | ACGATTCTTCGTCGTTCCAG | ATACGATACGCGCGAGCTAC | 160-400 | 5 | 0.75 |
| 66 | ICMP3019 | GCGCACCACCTGTGTCTAT | CATGCAGAGAAAAATCAAGCA | 200-210 | 2 | 0.35 |
| 67 | ICMP3016 | TTGTGGCTGAAGAAGAGATCC | AATGTGGGGAGAGACACACG | 100-450 | 4 | 0.68 |
| 68 | ICMP3014 | TGCTTCACAGCCTCTCCATA | CCACCATGCAACAGCAATAA | 200-240 | 2 | 0.35 |
| 69 | ICMP3013 | TGTGGGAGAGAGGAGAGTCC | CGCGAGATGATGTGTGGT | 250-280 | 2 | 0.45 |
| 70 | ICMP3009 | CTGTACCATGTGCGCTGATT | GCGCATATATGTGGGTGTGT | 200-230 | 2 | 0.36 |
| 71 | ICMP3006 | AAATCGGTCGTGGTGAAGTT | GAGAATGTGGGAGACACACG | 130-500 | 6 | 0.77 |
| 72 | Xcump017 | ATAGCTGGGTGTTGTCTGGC | CCCTGGCGCTTAATTGTAAA | 110-170 | 2 | 0.50 |
| 73 | Xcump018 | TGCTTTCTTCCCAACCAGTGG | TGCTGAGTGGGGTGCTGCT | 450-500 | 2 | 0.47 |
| 74 | Xcump003 | CATGCGACGTGGTCTATCTG | GAGAGAGAACCAGCAGCACC | 250-410 | 3 | 0.66 |
| 75 | Xcump007 | GAGGGATTCCAGGCGGTTC | GCGAGGAGCACATTCGATGAA | 110-410 | 2 | 0.48 |
| 76 | Xcump009 | ATCTGATCGTGAGGCCTCAAC | GCCGACCAAGAACTTCATACAAT | 160-700 | 6 | 0.76 |
| 77 | ICMP3025 | GTTGCAGATGAGCGATCGTA | CGCCGACCAAGAACTTCATA | 240-250 | 2 | 0.42 |
| 78 | ICMP3026 | GTGAGGCCTCGAACAAACAC | GCCGACCAAGAACTTCATACA | 150-170 | 2 | 0.48 |
| 79 | ICMP3020 | GTTCCATGGAGCTGGAAGTC | GCTAGAACAGGGCCGTTACA | 190-610 | 6 | 0.77 |
| 80 | ICMP3021 | GCCGACAGGAAGATTACGAT | AGCAAAACGCAGAACAACAG | 400-450 | 2 | 0.46 |
| 81 | ICMP3022 | CTGGAAGTCCTTCTCGGTTG | CTGCTCCGCTCTGAATCTG | 150-160 | 2 | 0.50 |
| 82 | ICMP3002 | AAAGTTACCGGGAGGGTAAAAA | TCGCCTAAAAACTGGAGGAA | 200-350 | 2 | 0.43 |
| 83 | Xcump001 | GCACGAGGCTTATCTGTGTTTC | CAACTCTTGCCTTTCTTGGCCT | 150-200 | 2 | 0.48 |
| 84 | Xcump004 | CACGAGGCTCACTAGGGTTT | ACCCGGGTCTGGTTAGACTT | 110-550 | 4 | 0.75 |
| 85 | Xcump005 | GCACGAGGGCCAGATTCTAGAA | CACGGTGATGACACGACATGGT | 159-160 | 2 | 0.64 |
| 86 | Xcump006 | GAAATCGGCAGAGGGCAT | CAATGAGTATGTGCACGCTGCA | 90-500 | 6 | 0.77 |
| 87 | Xcump010 | GCTGAACTATTCTGTAAACTTAAC | TATCGAAACGGTACTAAAATCATG | 150-160 | 2 | 0.44 |
| 88 | Xcump012 | TGTGATCTGTGGTCTCAGGC | CGTGAAAGCTCTCCAGGACT | 100-120 | 2 | 0.48 |
| 89 | Xcump013 | ACCGACAGCAACAAATCCTCC | GCTCTTGTGTGTAGTTGTGCTT | 140-250 | 3 | 0.62 |
| 90 | Xcump014 | CTGACCTCTCCTCTCCTTCG | GAGCAGATCCTTGGCCTTCTTG | 110-350 | 3 | 0.66 |
| 91 | Xcump016 | CATTTCTCTCGCCAGTGCTC | ATCTCCAGAACCGAGCGCA | 110-600 | 5 | 0.74 |

**Table 3. Summary of SSR primers used for molecular characterization**

|  |  |
| --- | --- |
| **Markers** |  **No. of markers** |
| Number of markers used | 156 |
| Number of amplified markers  | 128 |
| Number of non-amplified markers  | 28 |
| Number of polymorphic markers | 91 |
| Number of monomorphic markers | 37 |
| Size of amplified products (bp) | 90-760 |
|  Polymorphism percentage | 58.3% |
|  Total number of alleles | 284 |
|  Average no. of alleles per primer | 3.12 |

**Table 4. Summary of Nei’s (1972) genetic distances among 24 pearl millet genotypes**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PB1852** | **Proagro****Tejas** | **BHB1602** | **PusaCom.1201** | **NBH4903** | **KBH108** | **KaveriSBoss** | **MP7792**  | **MP7872** | **Pratap** | **86M86** | **GHB732**  | **GHB744** | **PusaCom****443** | **GHB719** | **RHB173** | **CZP9802** | **HHB****234** | **GHB1225** | **GHB1129** | **RHB177** | **CZPIC923** | **PHB2168** | **Raj171** |
| **PB1852** | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **ProagroTejas** | 0.73 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **BHB1602** | 0.72 | 0.70 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **PusaCom1201** | 0.62 | 0.60 | 0.62 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **NBH4903** | 0.67 | 0.66 | 0.69 | 0.66 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **KBH108** | 0.75 | 0.69 | 0.75 | 0.62 | 0.70 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **KaveriS Boss** | 0.61 | 0.59 | 0.60 | 0.59 | 0.59 | 0.61 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **MP7792**  | 0.63 | 0.60 | 0.62 | 0.59 | 0.64 | 0.64 | 0.59 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **MP7872** | 0.75 | 0.71 | 0.75 | 0.61 | 0.70 | 0.78 | 0.62 | 0.66 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Pratap** | 0.67 | 0.67 | 0.68 | 0.59 | 0.64 | 0.68 | 0.59 | 0.59 | 0.74 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **86M86** | 0.72 | 0.68 | 0.65 | 0.62 | 0.61 | 0.67 | 0.61 | 0.62 | 0.71 | 0.71 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **GHB732**  | 0.70 | 0.67 | 0.68 | 0.59 | 0.66 | 0.69 | 0.60 | 0.62 | 0.70 | 0.71 | 0.68 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |
| **GHB744** | 0.73 | 0.68 | 0.69 | 0.61 | 0.64 | 0.62 | 0.60 | 0.63 | 0.72 | 0.70 | 0.73 | 0.71 | 1 |   |   |   |   |   |   |   |   |   |   |   |
| **PusaCom443** | 0.63 | 0.64 | 0.67 | 0.59 | 0.62 | 0.64 | 0.60 | 0.60 | 0.68 | 0.68 | 0.65 | 0.65 | 0.66 | 1 |   |   |   |   |   |   |   |   |   |   |
| **GHB719** | 0.70 | 0.70 | 0.68 | 0.61 | 0.67 | 0.68 | 0.60 | 0.62 | 0.71 | 0.69 | 0.67 | 0.66 | 0.62 | 0.67 | 1 |   |   |   |   |   |   |   |   |   |
| **RHB173** | 0.64 | 0.61 | 0.61 | 0.60 | 0.63 | 0.67 | 0.63 | 0.65 | 0.67 | 0.68 | 0.62 | 0.59 | 0.68 | 0.66 | 0.66 | 1 |   |   |   |   |   |   |   |   |
| **CZP9802** | 0.74 | 0.67 | 0.75 | 0.62 | 0.68 | 0.74 | 0.59 | 0.66 | 0.78 | 0.68 | 0.65 | 0.67 | 0.71 | 0.63 | 0.70 | 0.65 | 1 |   |   |   |   |   |   |   |
| **HHB234** | 0.73 | 0.67 | 0.69 | 0.59 | 0.64 | 0.73 | 0.59 | 0.64 | 0.73 | 0.67 | 0.64 | 0.63 | 0.68 | 0.62 | 0.67 | 0.69 | 0.77 | 1 |   |   |   |   |   |   |
| **GHB1225** | 0.71 | 0.68 | 0.67 | 0.59 | 0.61 | 0.72 | 0.60 | 0.60 | 0.76 | 0.72 | 0.67 | 0.68 | 0.69 | 0.64 | 0.65 | 0.66 | 0.72 | 0.72 | 1 |   |   |   |   |   |
| **GHB1129** | 0.59 | 0.59 | 0.60 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.60 | 0.61 | 0.59 | 0.63 | 0.59 | 0.59 | 0.62 | 0.62 | 0.62 | 0.60 | 1 |   |   |   |   |
| **RHB177** | 0.67 | 0.61 | 0.70 | 0.59 | 0.59 | 0.69 | 0.59 | 0.59 | 0.68 | 0.66 | 0.63 | 0.63 | 0.65 | 0.62 | 0.65 | 0.65 | 0.66 | 0.62 | 0.63 | 0.65 | 1 |   |   |   |
| **CZPIC923** | 0.68 | 0.60 | 0.60 | 0.59 | 0.59 | 0.66 | 0.59 | 0.59 | 0.67 | 0.65 | 0.62 | 0.63 | 0.67 | 0.59 | 0.63 | 0.61 | 0.66 | 0.63 | 0.61 | 0.66 | 0.70 | 1 |   |   |
| **PHB2168** | 0.68 | 0.66 | 0.70 | 0.62 | 0.65 | 0.69 | 0.59 | 0.61 | 0.70 | 0.66 | 0.63 | 0.66 | 0.64 | 0.62 | 0.68 | 0.60 | 0.68 | 0.66 | 0.65 | 0.61 | 0.66 | 0.69 | 1 |   |
| **Raj171** | 0.69 | 0.66 | 0.67 | 0.59 | 0.59 | 0.67 | 0.60 | 0.59 | 0.70 | 0.68 | 0.64 | 0.63 | 0.71 | 0.61 | 0.65 | 0.67 | 0.71 | 0.70 | 0.67 | 0.68 | 0.66 | 0.71 | 0.73 | 1 |