**Original Research Article**

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

**ABSTRACT**

Pearl millet is an extensively grown, climate resilient and rainfed cereal crop cultivated on 30 million ha in the arid and semi-arid tropical regions of Asia and Africa contributing towards almost half of the global millet production. It exhibits several climate-resilient features and thus can survive in harsh climatic conditions. But, in the changing climatic scenario, its productivity is decreasing and there is a high need for developing high yielding hybrids and varieties in order to increase its productivity. Thus, understanding and analyzing the genetic diversity is very imperative and necessary to develop superior hybrids of pearl millet.In this study, a total of 24 genotypes including 19 different released hybrids and 5 varieties of pearl millet were used for diversity analysis using 156 Simple Sequence Repeat (SSR) markers. Out of these, 91 SSRs were observed 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. Cluster analysis on the basis of SSRs grouped these 24 genotypes into four major clusters viz., I, II, III, IV with the similarity coefficient varying between 0.59 to 0.78. The results revealed that adequate genetic variability subsists among the hybrids and varieties used in the present study and these 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 crop grown widely over 30 million ha and is a staple food of more than 90 million people worldwide. Majority of the crop is grown in Africa (>18 million ha) and Asia (>10million ha) contributing towards 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 utilized by humans due to its more nutritive value. It has huge yield potential and also used as both feed and fodder for livestock. . It can survive in regions with very low annual rainfall (less than 250 mm), It is a drought tolerant, highly photosynthetic proficient crop possessing high dry matter production capacity and can even prolong on less fertile soils with poor water and nutrient holding capacity. 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. It is less dependent on synthetic fertilizers and minimum vulnerability to environmental stresses and thus can survive in harsh climatic conditions. 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 substantial amount of genetic diversity owing to its abundant allocation all over the world and higher adaptability towards harsh and adverse environments, cross pollination mechanism and protogynous flowering. Heterosis studies in pearl millet have been proved useful to enhance its yield, stress tolerance, nutritional value as well as improving resilience to different diseases by recombining potential of different parental lines. It also strengths sustainable agriculture and ultimately help the farmers to adapt to different climatic changes and enhance their livelihood and food security. Heterosis breeding is quite useful for pearl millet owing to its highly cross-pollinated nature, which in turn is feasible due to protogynous flowering and availability of CMS system [6]. It has high out crossing rate of around 90% with huge diversity at both phenotypic and genotypic levels. It has enormous gene potential which can be explored to enhance its productivity. Its existing germpalsm is a very useful resource of valuable genes and alleles for resistance against different biotic and abiotic stresses and various nutritional and agronomic traits can prove useful for enriching the gene pool. Diverse alleles of different germplasms can significantly contribute to boost up genetic gain in pearl millet. Though pearl millet has a very proficient energy production system but its genetic potential needs more attention and improvement to enhance its productivity like other major cereals. Inadequate accessibility and genetic improvement of improved hybrids and varieties in addition to the 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 key targets of any crop improvement program.. 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. 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, it is highly needed to to study genetic diversity of pearl millet germplasm in order to strengthen the different breeding programs which in turn will be highly useful to conserve 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 through 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 environment and other factors and are hence not considered much reliable for 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 support and accelearte 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]. Different molecular markers have been used to evaluate genetic diversity amongst 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, various molecular makers including RFLPs, Sequence Tagged Sites (STSs), Amplified Fragment Length Polymorphism (AFLPs), genic SSRs, genomic SSRs, Single-Strand Conformation Polymorphism (SSCPS) and were developed and used for pearl millet improvement [29-30]. Afterwards, high throughput platforms suc as Diversity Arrays Technology (DArT) [31], Genotyping-by-sequencing (GBS) [18), Single Nucleotide Polymorphism (SNPs) [28, 32] were established and used for profiling genome-wide nucleotide variations in pearl millet. Though, Next Generation Sequencing (NGS)-based single-nucleotide polymorphisms have turned out to be 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, assessment of genetic distances amid populations and defining heterotic groups [21, 25, 27, 33, 35]. Thus, keeping all this in view, this study on genetic diversity analysis and molecular characterization of pearl millet hybrids and varieties was planned to explore the nature and extent of genetic variation among existing hybrids/varieties of pearl millet using SSR markers.

1. **MATERIALS AND METHODS**

**2.1 Plant material**

A total of 24 genotypes including 19 different popular released hybrids and 5 varieties of pearl millet 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 extraction and quantification**

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

**2.3 Molecular analysis using SSR primers**

A total of 156 SSRs were employed for PCR amplification and studying 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 in a volume of 10 μl having 10 mM Tris HCl (pH 8.3), 1.5 mM MgCl2,50 mM KCl, 0.4 μM 10-mer primer, 200 mM each dNTP, 1 unit Taq DNA polymerase (GeNei, India) and 10 ng of DNA. PCR amplifications were set 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. It was followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 58°C for annealing and primer extension at 72°C for 1 min Lastly, 1 cycle of final extension was carried out at 72°C for 10 mins followed by hold at 4°C [24]. The PCR products were checked on 2.5% agarose gel.

**2.4 Diversity analysis and dendrogram construction**

Gel photographs were used to score variations in the bands as absence (0) and presence (1) of bands where each band represented a genetic locus. The clear and unambiguous amplicons were only scored and their sizes were determined with 50 bp DNA ladder (HiMedia) as a standard. Cluster analysis was performed between 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 computed as, 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. Of these 156 SSRs, 128 markers amplified products of different sizes ranging between 90 to 760 bp and 91 (58.3%) were polymorphic (Fig. 1, 2) and (Table 2, 3) and 37 (23.7 %) were monomorphic (Table 3). Thus, a good number of polymorphic markers were attained which can be used for genetic diversity estimation, identification of genotypes, germplasm evaluation and further can be used in other breeding programs. A total of 284 alleles were attained in this study and the number of alleles per locus ranged between 2 to 6 with an average of 3.12 alleles. These values were found to be similar to 3.43 alleles per locus, 3.4 alleles per locus and 3.1 alleles per locus as reported in other findings [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 kind of observations may be due to diverse world collection of germplasm. Similar type of observations regarding efficiency of SSR markers in evaluating diversity have also been depicted by other investigators [21, 24, 27, 33]. Different reports were published by researchers on evaluation of genetic diversity in pearl millet lines on the basis of molecular profiling [8, 18, 19, 25, 26-28, 31]. But, in the current study, we assessed genetic diversity amongst hybrids and varieties of pearl millet which can serve as molecular 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 mostly favored for genotypic characterization due to their high polymorphism and reproducibility, co-dominant nature, simplicity and specificity, mulitiallelism, need of lesser quantity of DNA. Moreover, they provide unique allelic profiles or DNA fingerprints which can precisely and effectively ascertain genotypic identity [21]. SSRs have been widely used in different crops to evaluate crop germplasm and genetic diversity [14, 24]. They possess several benefits in contrast to SNPs in diversity analysis and thus SSR data can be more helpful in defining pedigrees as compared to SNP data. PIC values vary between 0 and 0.5 in SNPs due to their bi-allelic nature whereas it can be above 0.5 in case of SSRs owing to their mutli-allelic character. Though SNPs are getting 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 may be utilized for larger expansion of sample size without increasing the cost and they can be also easily integrated with new studies. Hence, SSRs are a good choice for small laboratories with limited budget and facilities as compared to SNPs [48].

Polymorphic information content (PIC) value determines polymorphism for a marker as it computes informativeness of markers and evaluates the diversity of alleles. It accounts the number of expressed alleles and their relative frequencies to evaluate the discriminatory power of a locus. PIC index assesses the intensity of variation of a gene and a PIC value of ≥0.5 indicates higher diversity, while PIC ≤ 0.25 describes lower diversity and PIC value between 0.25 and 0.5 is a pointer of intermediate diversity [49]. In the current study, PIC values varied from 0.31 (CTM 27) to 0.78 (IPES 0005, IPES 0024, CTM9) (Table 2) with an average of 0.58. PIC values ranging between 0.02 to 0.97 were recorded in some earlier reports [22, 24, 38, 44, 50-53]. An average PIC value of 0.58 observed in this study is comparable 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 more are considered as extremely valuable in discerning the genotypes and valuable for molecular genetic diversity studies [54]. Hhigh PIC values between 0.65 and 0.78 were recorded in 10.9 % (10 SSR) markers (Table 2). Markers IPES 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 CTM 9 are among the most informative and best markers for diversity assessment of these pearl millet genotypes followed by PSMP2072, PSMP3032, PSMP2001, PSMP3017 and PSMP2066 primers while the lowest PIC value of 0.31 was recorded for maker CTM 27 indicating that it is the least powerful marker (Table 2). High PIC values were recordeddue to the use of large number of informative markers [54]. Similar kinds of reports were observed in several other studies [8, 24-25, 27, 43-45, 55].

3.2 Gentitc diversity analysis and dendrogram construction

Pearl millet possess a significant amount of diversity at both phenotypic and genotypic 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 between the different genotypes in thispresent study were as per the available pedigree data. The cluster analysis on the basis of SSR data classified the genotypes into four major clusters viz., I, II, III, IV and similarity coefficient varied between 0.59 to 0.78 (Fig. 3, Table 4).) which was found to be alike those reported in other studies [24, 41, 44, 55- 56]. Cluster I has 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, Proagro Tejas, 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 may be further subgrouped into three sub-clusters- Ia, Ib, Ic. Sub-cluster Ia exhibited 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 MP7872 and KBH108 illustrated very close similarity with each other at a minimal genetic distance of 0.78 while CZP9802 and HHB 234 exhibited closer relationship at a genetic distance of 0.77. Sub-cluster Ib has four genotypes- Pratap, GHB1225, Proagro Tejas, GHB 719 where GHB1225, Proagro Tejas, GHB 719 are specific for A1 zone while Pratap is for B zone. Here, hybrids Pratap and GHB1225 showed closer association with each other at minimal genetic distance of 0.72 while Proagro Tejas and GHB 719 were found to show close relationship at a genetic distance of 0.70. Sub-cluster Ic exhibited three hybrids- 86M86, GHB744 and GHB 732 which are suitable for A zone and 86M86 and GHB 744 grouped together closely at a genetic distance of 0.73.

Cluster II was attained at a similarity index of 0.65 having 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 relatedness with each other at minimum genetic distances of 0.70, 0.73 and 0.66, respectively. In cluster III, four genotypes Pusa Composite 1201, NBH4903, MP7792 and GHB1129 grouped 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 demonstrated a more closer relationship at a genetic distance of 0.66. Kaveri Super Boss hybrid was entirely differentiated from all the other genotypes and included in cluster IV at a similarity index of 0.59. Thus, different genotypes clustered according to their salient features and characteristics as mentioned in other reports [15]. It has been demonstrated that SSRs are suitable and proficient tool for molecular characterization of different crop species. Similar type of 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 characterized successfully and further categorized into diverse groups which will be very helpful to estimate the evolutionary relationships with the different wild relatives. The results reveled that there is a good genetic variability amongst the different hybrids and varieties for different morphological, physiological traits and abioic/biotic stresses which will be suitable for different agrocliamtic zones of India and thus can be used in other future pearl millet improvement programs. Here, we reported a good number of polymorphic SSR primers with high PIC values indicating that SSRs can be effectively utilized for genetic diversity and genomic studies in pearl millet.These results will be also helpful in eliminating the gaps in lineage or selection history, detect differences in allele frequencies among genotypes or populations. It is anticipated that it will also prove fruitful in exploring new alleles at different loci of interest and DNA fingerprinting as well as varietal identification.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Fig. 1 Amplification profile of pearl millet hybrid/varieties on agarose gel with the primer IPES0020. Lane M-50 bp ladder, Lane 1-24 pearl millet hybrids/varieties



Fig. 2 Amplification profile of pearl millet hybrid/varieties on agarose gel with the primer IPES0045. Lane M-50 bp ladder, Lane 1-24 pearl millet hybrids/varieties

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Fig. 3 UPGMA dendrogram illustrating genetic relationships amongst 24 pearl millet genotypes on basis of Jaccard’s similarity coefficients and SSR markers.

**Table 1. Pearl millet genotypes used for molecular characterizationand 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. | Proagro Tejas | 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 Composite 1201 | 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. | PHB 2168 | 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 the 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 | ICMP 3088 | 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** |
| TTotal markers used | 156 |
|  Markers amplified markers amplified | 128 |
| Markers non-amplified  | 28 |
| Polymorphic markers | 91 |
| Monomorphic markers | 37 |
| Size of amplified products (bp) | 90-760 |
|  Percentage polymorphism  | 58.3% |
|  Number of alleles | 284 |
|  Average no. of alleles per primer | 3.12 |

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PB1852** | **Proagro****Tejas** | **BHB1602** | **Pusa Com.1201** | **NBH4903** | **KBH108** | **KaveriSBoss** | **MP7792**  | **MP7872** | **Pratap** | **86M86** | **GHB732**  | **GHB744** | **Pusa Com****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 |