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# Genetic diversity analysis in sorghum [*Sorghum bicolor* (L.)] genotypes by using SSR markers

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**Abstract**
*Sorghum bicolor* is most important cereal crops around the world where the availability of water is less. Study of genetic variability among the sorghum accessions will enable accurate results in breeding programme and in mapping studies. By using SSR markers evenly distributed on sorghum genome revealed that, all the 23 accessions originated from Marathwada (Maharashtra) region were grouped into three main classes and it show correlation with the biological status and other characteristics features of accessions. The gene diversity measured in term of polymorphic information content (PIC) was ranged between 0.00 to 0.24 with the average PIC value was found to be 0.067 where 0.24 PIC value indicated presence of two allele per locus. Expected heterozygosity or Gene diversity computed according to Nei (1973) and varied from 0.00 to 0.28 with an average of 0.07. A total of 9 SSR marker alleles were detected, with an average of 1.5 alleles per marker. Numbers of alleles per marker ranged from 1(msbCIR329, Xtxp021 and Xtxp278) to 2(Xtxp321, msbCIR306 and Xisep0310). The fragment size of allele ranged from 109(msbCIR329) to 252(Xtxp321 and Xtxp278).

**Keywords:** Sorghum, SSR, diversity, polymorphic information content

## Introduction
Sorghum is one of the most important crops worldwide after wheat, rice, maize and barley, providing food, fodder and bioenergy feedstock. It is considered as the king of millets and extensively grown in Africa, China, USA, Mexico and India). Sorghum is popularly known as "jowar" in Bangladesh and India. It is used as a staple food and fodder crop and has gained the status of a "failsafe" crop in global agro- ecosystems. Sorghum in India is cultivated in rainy and post-rainy seasons with cultivars specifically adapted to each seasons. Sorghum is the second cereal after rice for which complete genome sequencing is available. The world sorghum collection assembled at ICRISAT represents the major diversity centers of sorghum. At present, ICRISAT is a major repository for world Sorghum germplasm with a total of 37,904 accessions from 91 countries. The collection is estimated to represent about 80% of the variability present in Sorghum. India has contributed the highest 6229 varieties of Sorghum to the germplasm collection. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), headquartered in India, serves as a world center for Sorghum germplasm conservation and breeding. Numerous studies have demonstrated that Sorghum is a very diverse crop, with cultivated Sorghums exhibiting great phenotypic variability. Sorghum is an important target for plant genomics due to its adaptations to harsh environments, diverse germplasm collection and relatively small genome size (Menz *et al.*, 2002) [14]. The Sorghum genome contains 750 Mb of DNA, which is 3–4-fold smaller than that of maize (2,400 Mb). Several reports indicate that Sorghum (2n=2x=20) can be classified into two groups, the wild and the cultivated Sorghums (Smith *et al.*, 2000; Ayana *et al.*, 2002) [21, 2].
Assessment of genetic diversity is of paramount importance for current and future breeding programmes (Mohammadi and Prasanna, 2003) [16]. Genetic advancement during selection depends on the availability of genotypes possessing favourable alleles for desired traits, which relies on the available genetic diversity. Genetic diversity is valuable for selections of varieties that may serve as future parents for hybrids and other new varieties (Geleta and Labuschagne, 2005) [9]. Genetic diversity among accessions provides opportunities for improvement of agronomic and nutritional quality traits in crops (Huang, 2004) [11]. It aids plant breeders to characterize and classify accessions into heterotic groups (Menz *et al.*, 2004) [15]. Diverse landraces, breeding stocks and wild relatives are useful in broadening genetic base for greater genetic gains. Such studies are also helpful in choosing parents to create and maintain genetic diversity, develop mapping populations for detecting quantitative trait loci (QTL)s/genes

(Varshney, 2011) and to categorize lines into heterotic groups for hybrid crop breeding (Menz *et al.,* 2004) [15]. Genetic variation consists of sequence variation and structure alteration. Sequence variation normally is manifested by SNPs, short sequence insertions and deletions (indels), microsatellites or simple sequence repeats, and transposable elements. Many molecular marker technologies have been developed and applied for studying patterns of genetic diversity in sorghum (Ghebru *et al*., 2002; Folkertsma *et al*., 2005; Ali *et al*., 2008; Bhosale *et al*., 2011) [10, 7, 1, 3] Simple sequence repeat (SSR) are the markers of choice in the study as they are polymerase chain reaction (PCR)-based, easy to use, locus-specific, co-dominant, highly reproducible, hypervariable and informative (Powell *et al*., 1996) [19]. Biochemical genetic markers provided the first assessment of neutral genetic variation and enabled demarcation of groups by race and origin. Several strategies have been used for the analysis of diversity which uses morphological, geographical, biochemical and molecular differences among the accessions (Franco *et al*., 2001; Chiorato *et al*., 2007) [8, 5].

However, problems with the reproducibility in amplification of RAPD markers and with data scoring have been reported. Many molecular marker technologies have been developed and applied for studying patterns of genetic diversity in Sorghum (Ghebru *et al.,* 2002; Bhosale *et al.,* 2011) [10, 3]. Simple sequence repeat (SSR) markers were markers of choice as they are polymerase chain reaction (PCR)-based, easy to use, co-dominant, locus-specific, highly reproducible, hypervariable and informative (Powell *et al.,* 1996) [19]. Simple sequence repeat (SSR) markers are generally highly discriminative and are often used to distinguish varieties, or even individuals, and reveal parentage and identity (Karp *et al.,* 1996) [13]. The use of SSR markers has been proved as a powerful technique for studying the diversity of Sorghum (Dje *et al.,* 2000; Ghebru *et al.,* 2002; Smith, *et al.,* 2000) [6, 10, 21]; and genetic redundancy among Sorghum germplasm. The Sorghum genome sequence project identified 71 000 SSRs in the genome (Paterson *et al.* 2009) [18]. The availability of this large number of SSR markers provides a more cost-effective and rapid method for DNA profiling (Smith *et al.* 1997) [20]. A better understanding of genetic diversity in Sorghum will facilitate crop improvement. Therefore there is a need to evaluate the available accessions for genetic diversity. In the present study, an attempt has been made to determine the extent of diversity among twenty three Sorghum accessions using the quantitative traits.

## Materials and Methods

### Plant Material

The 23 Sorghum accessions used in the present investigation were procured from "International Crop Research Institute for Semi-Arid and Tropics" (ICRISAT) gene bank and were evaluated for genetic diversity study. The Sorghum accessions were originated from Marathwada region in Maharashtra state where Sorghum is an important cereal crop because of its adaptation to environmental conditions. The biological status and the line grouping of the Sorghum accessions are listed in the table 1.

### DNA Extraction

Sorghum seeds of 20 accessions were sown in pots under polyhouse at MGM College of Agricultural Biotechnology, Gandheli, Aurangabad during Rabi-December 2019. The seedlings were allowed to grow for 20-22 days. The leaf samples were collected from all 20 accessions and store at -20 °C until use. Genomic DNA was extracted from 0.2 g fresh leaf sample using modified CTAB protocol modified at Department of Plant Biotechnology (Kale *et al*., 2020) [12] (CABT, Aurangabad). The yield of genomic DNA obtained was 1700-3700 ng/µl. DNA of all the extracts diluted to 50 ng/µl was used for the PCR amplification.

**Table 1:** List of 23 Sorghum germplasm accession used in this study with their biological status

| Sr. No. | Accession No. | Biological status |
|---|---|---|
| 1 | IS 4503 | Advanced/Improved cultivar |
| 2 | IS 4504 | Advanced/Improved cultivar |
| 3 | IS 4580 | Traditional cultivar/Landrace |
| 4 | IS 4582 | Traditional cultivar/Landrace |
| 5 | IS 4583 | Traditional cultivar/Landrace |
| 6 | IS 4584 | Traditional cultivar/Landrace |
| 7 | IS 4585 | Traditional cultivar/Landrace |
| 8 | IS 4586 | Traditional cultivar/Landrace |
| 9 | IS 4587 | Traditional cultivar/Landrace |
| 10 | IS 4588 | Traditional cultivar/Landrace |
| 11 | IS 4589 | Traditional cultivar/Landrace |
| 12 | IS 4591 | Traditional cultivar/Landrace |
| 13 | IS 4592 | Traditional cultivar/Landrace |
| 14 | IS 4593 | Traditional cultivar/Landrace |
| 15 | IS 4595 | Traditional cultivar/Landrace |
| 16 | IS 4596 | Traditional cultivar/Landrace |
| 17 | IS 4597 | Traditional cultivar/Landrace |
| 18 | IS 18404 | Breeding/Research material |
| 19 | IS 18405 | Breeding/Research material |
| 20 | IS 18406 | Breeding/Research material |
| 21 | IS 18407 | Breeding/Research material |
| 22 | IS 18792 | Traditional cultivar/Landrace |
| 23 | IS 18793 | Traditional cultivar/Landrace |

**PCR Amplification**

Six SSR primer pairs (Table 2) were used to detect polymorphism among the 23 sorghum accessions. The PCR was performed in a volume of 20 µl reaction containing 2 µl (50 ng) genomic DNA, 10X PCR buffer with 25 mM $MgCl_2$ (2.0 µl), 2mM dNTPs (2.0 µl), 10µM forward and reverse primer (2.0µl), 1µl Taq DNA Polymerase (1U/µl) and Nanopore water. For the maximum yield and specificity, annealing temperature and cycling duration was rearranged for each primer pair. Amplification was performed in an APPLIED BIOSYSTEM thermal cycler with an initial denaturation at 94 °C for 5 mins followed by 34 cycles at 94 °C for 30 sec, 55-65 °C for 30 sec (depending on primer Tm) and 72 °C for 1 min, followed by a final extensions at 72 °C for 7 mins.

**Table 2:** Primer sets used for amplification of SSR loci in Sorghum bicolor in the present study (Billot *et al*., 2013) [4].

| S. No. | Name of Marker | Forward Primer | Reverse Primer | Ta | Size Range | Chr. No. |
|---|---|---|---|---|---|---|
| 1 | Xtxp321 | TAACCCAAGCCTGAGCATAAGA | CCCATTCACACATGAGACGAG | 55 | 192-252 | SBI08 |
| 2 | msbCIR329 | GCAGAACATCACTCAAAGAA | TACCTAAGGCAGGGATTG | 55 | 109-117 | SBI05 |
| 3 | msbCIR306 | ATACTCTCGTACTCGGCTCA | GCCACTCTTTACTTTTCTTCTG | 56 | 120-124 | SBI01 |
| 4 | Xisep0310 | TGCCTTGTGCCTTGTTTATCT | GGATCGATGCCTATCTCGTC | 60 | 164-219 | SBI02 |
| 5 | Xtxp021 | GAGCTGCCATAGATTTGGTCG | ACCTCGTCCCACCTTTGTTG | 60 | 169-199 | SBI04 |
| 6 | Xtxp278 | GGGTTTCAACTCTAGCCTACCGAACTTCCT | ATGCCTCATCATGGTTCGTTTTGCTT | 50 | 243-252 | SBI07 |

**Gel Electrophoresis and Detection**

The amplified PCR products for each marker were resolved on agarose gel for detection of amplified product. For the visualization of the DNA fragments and to identify small polymorphic differences between them, 5-6 µl of Bromophenol blue dye was added to 20 µl of the PCR product. Agarose gel of 3.5% was used for better resolution of PCR product. 20 µl of the sample was loaded into the wells in gel with 1X TAE buffer with 100 bp DNA ladder (Genei) and the electrophoresis unit was operated at 200 volts. The gel was visualized under UV transilluminator and Gel documentation unit and the fragment sizes for each marker were determined. The size of the fragments was determined using the 100 bp DNA ladder and the scoring of markers was performed for each fragment.

**Statistical Analysis**

For the estimation of genetic diversity for each primer, parameters such as number of alleles per locus, heterozygosity, major allele frequency, gene diversity and polymorphic information content (PIC) for each marker were estimated using POWERMARKER version 3.25 (Liu *et al*., 2005) Allele frequency represents the frequency of particular allele for each marker. Heterozygosity is a proportion of heterozygous individual in the population. Polymorphic information content (PIC) represents the amount of polymorphism within a population. Nei's distance (Nei *et al*., 1973) was calculated and used for the construction of phylogenetic tree and unrooted phylogeny using neighbour-joining (NJ) method, bootstrapping of the data (10,000 permutations) as implemented in POWERMARKER, and MEGA 6.0 was used to visualize the Tree (Tamura *et al*., 2007) [22].

**Result and Discussion**

**Characteristics of the SSR loci and genotypic structure of sorghum accessions**

The genotypic data was generated in term of base pairs (bp) from the gel images of six SSR markers and used for genetic diversity study. The PCR amplification was carried out for total six SSRs in a reaction of 20 ul and then electrophoresis was performs for each marker. Six pairs of SSR primers (SSR Markers) were used in the study. Primer pairs were chosen on the basis of their properties of detecting single loci, their broad coverage of the genome and their high levels of polymorphism when applied to a broad range of Sorghum germplasm. The gel was visualized using gel documentation unit and images were capture to generate the genotypic data. The 100 bp DNA ladder was used to determine the band size of PCR product amplified in each Sorghum accession.

**Polymorphism and allelic diversity of SSR markers**

In the present study the six SSR markers (http://sorghum.cirad.fr/SSR_kit) were used for diversity study in twenty three accessions of sorghum show amplification and polymorphism. All the markers produce all expected SSR allele size amplicons were used for analysis of genetic diversity among the twenty three accessions of elite sorghum.

Polymorphism among the 23 sorghum genotypes was investigated with 6 SSR markers. The 6 SSR primers, which cover the 6 linkage groups (i.e., A, B, D, E, H and J), were able to uniquely fingerprint each of the 23 genotypes. A total of 9 SSR marker alleles were detected, with an average of 1.5 alleles per marker. Numbers of alleles per marker ranged from 1(msbCIR329, Xtxp021 and Xtxp278) to 2(Xtxp321, msbCIR306 and Xisep0310). The fragment size of allele ranged from 109(msbCIR329) to 252(Xtxp321 and Xtxp278). A mean gene diversity of 0.07 was observed across the sorghum accessions, with values ranging from 0.28 (Xisep0310) to 0.08 (Xtxp321 and msbCIR306) for individual markers (Table 3 and 4). Even though the observed heterozygosity at each locus over all accessions (Ho) was 0. Therefore, no heterozygosity was found across 23 accessions which may be due to autogamous nature of sorghum. The PIC values for SSR loci ranged from 0.07 for Xtxp321 to 0.24 for Xisep 0310, with a mean value of 0.06.

**Genetic relationships among sorghum genotypes and cluster analysis**

Cluster analysis based on the simple allele matching and unweighted neighbor-joining was performed on the 6 SSR markers for 23 genotypes. Out of six polymorphic SSR loci, 1 (16.6%) markers were highly informative (PIC =0.24), two markers (33.2%) were reasonably informative (PIC = 0.07%) and three markers was found to be less informative (PIC = 00) (Table 4).

Two main clusters were recognized which corresponded well with the pedigree information, soil type, environmental conditions and geographic origin of the genotypes (Figure 1).

The cluster I had 4 genotypes which were mostly of Traditional cultivar or landraces. Among them accession 6 is different from remaining 3 genotypes. In cluster II, there are 19 genotypes. From which the accession 3, 4 9, 10, 11, 12, 13, 14, 15, 16, 17, 22 and 23 are traditional cultivar or landraces, then accession 1 and accession 2 are the advanced or improved cultivar. The remaining genotypes i.e., accession 18, 19, 20 and 21 are the breeding or research material. Among these genotypes accession 16 is traditional cultivar but it is different from remaining genotypes may be due to its soil type, environmental conditions or ecology. Although SSR primers amplify PCR products from only one locus per assay, an average of 1.5 alleles per locus was detected in this study, indicating a large degree of genetic diversity among accessions.

The major allele frequency across 23 accessions was ranged from 0.82 to 1.00 with the mean major allele frequency was 0.95 (Table 4). Thus, the present result revealed that 23 sorghum accessions used in this study have wide genetic diversity and are good candidates for candidate gene specific association studies of complex trait such as yield and for using parents in plant breeding programme.

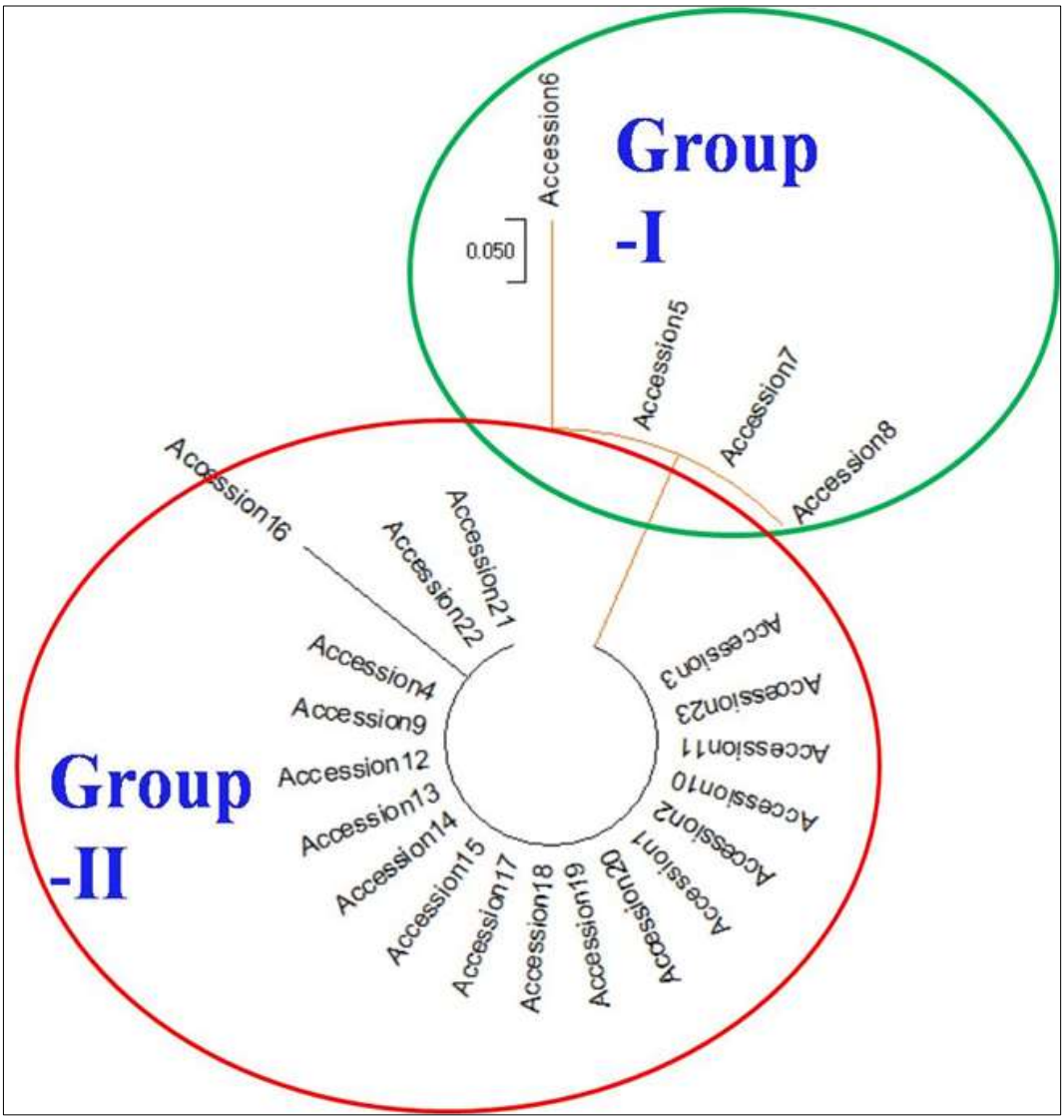


**Fig 1:** Neighbor joining (NJ) tree of 23 Sorghum accessions constructed using POWERMARKER Software

**Table 3:** Details of SSR loci used for genotyping in the sorghum accessions and their genetic diversity parameters using power marker V 3.25 program

| Locus | LG | Chr. No. | Min.MW | Max.MW | MAF | GN | No. of obs. | Na | He | Ho | PIC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Xtxp321 | 8(H) | SBI08 | 195 | 252 | 0.9565 | 2.0000 | 23.0000 | 2.0000 | 0.0832 | 0.0000 | 0.0797 |
| msbCIR329 | 10(J) | SBI05 | 109 | 117 | 1.0000 | 1.0000 | 23.0000 | 1.0000 | 0.0000 | 0.0000 | 0.0000 |
| msbCIR306 | 1(A) | SBI01 | 120 | 124 | 0.9565 | 2.0000 | 23.0000 | 2.0000 | 0.0832 | 0.0000 | 0.0797 |
| Xisep0310 | 2(B) | SBI02 | 164 | 219 | 0.8261 | 2.0000 | 23.0000 | 2.0000 | 0.2873 | 0.0000 | 0.2461 |
| Xtxp021 | 4(D) | SBI04 | 169 | 199 | 1.0000 | 1.0000 | 23.0000 | 1.0000 | 0.0000 | 0.0000 | 0.0000 |
| Xtxp278 | 5(E) | SBI07 | 243 | 252 | 1.0000 | 1.0000 | 23.0000 | 1.0000 | 0.0000 | 0.0000 | 0.0000 |

**Table 4:** Mean diversity analysis of 20 germplasm accessions using eight SSR markers

| S. No. | Diversity Traits | Number | Range | |
|---|---|---|---|---|
| 1 | Total no. of alleles | 9 | Maximum | Minimum |
| 2 | Mean no. of alleles per locus | 1.5 | 2 | 1 |
| 3 | Mean major allele frequency | 0.95 | 1 | 0.82 |
| 4 | Mean Gene Diversity | 0.07 | 0.28 | 0 |
| 5 | Mean PIC | 0.067 | 0.24 | 0 |

The study examined the genetic diversity present in 23 Sorghum genotypes from the Marathwada Region. The SSR markers were useful in discriminating all the genotypes and revealed wide genetic diversity among the Sorghum genotypes studied. A Neighbor-joining tree was constructed (Nei 1973) and the analyses formed two major distinct clusters. This indicated the genetic distinctness of germplasm collection of Marathwada region. Cluster analysis based on the simple allele matching and unweighted neighbor-joining was performed on the 6 SSR markers for 23 genotypes. Out of six polymorphic SSR loci, 1 (16.6%) markers were highly informative (PIC =0.24), twomarkers (33.2%) were reasonably informative (PIC = 0.07%) and three markers was found to be less informative (PIC = 00).The selected lines with high genetic diversity could serve as important sources of novel alleles for breeding and genetic conservation. Furthermore, phenotypic evaluations are needed to select suitable agronomic traits associated with the genetic markers for breeding and conservation strategies. The construction of dendogram revealed that, the cluster I had 4 genotypes which were mostly of Traditional cultivar or landraces. Among them accession 6 is different from remaining 3 genotypes. In cluster II, there are 19 genotypes. From which the accession 3, 4 9, 10, 11, 12, 13, 14, 15, 16, 17, 22 and 23 are traditional cultivar or landraces, then accession 1 and accession 2 are the advanced or improved cultivar.

## Conclusion

The SSR marker analysis revealed genetic variation among the sorghum genotypes. Compare with other marker system present study used the SSR markers that proven the utility of sorghum SSR markers in understanding the genetic similarities and discrimination among a set of sorghum genotypes. Based on previous studies analysis, it is more accurate to find out the genetic structure of any germplasm collection at molecular level than that using agro-morphological characteristic of accessions. Abundant genetic diversity exists in the germplasm accessions at genetic level and it is easy to find out. The results of statistical analysis reflects the broad genetic variability was existed across the germplasm accessions that provided the scope to identify the allelic variants (superior alleles) through candidate gene based association mapping approach in sorghum breeding program.

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