

Original Research Article

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Analysis of Genetic Diversity in Sorghum [*Sorghum bicolor* (L.)] Accessions of Maharashtra as Estimated by Simple Sequence Repeats (SSR)

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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 20 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 0.41 to 0.63 with the average PIC value was found to be 0.53 where 0.41 PIC value indicated presence of three allele per locus. The SSR loci Xtxp278, msbCIR276, Xisep0310, Xtxp321, Xtxp141 and Xtxp145 were rich in allelic diversity exhibiting highest PIC value. Expected heterozygosity or Gene diversity computed according to Nei (1973) and varied from 0.46 to 0.68 with an average of 0.60.

Keywords

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Introduction

Sorghum [*Sorghum bicolor* (L.), $2n = 2x = 20$] is the fifth most important cereal crop in the world after wheat, Rice, Maize and Barley, with over 80% of the crop in Africa and Asia (FAOSTAT, 2017). 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 is also used for the production of Ethanol, Starch and Syrup in the brewing industry. It is widely adapted to harsh environmental conditions, and more specifically to arid and semi-arid regions of

the world. Sorghum is known as 'the camel amongst crops' and requires much less water than many other cereals and has a remarkable ability to produce a crop under low levels of inputs and adverse stress conditions (Zheng *et al.*, 2011). It is currently a model crop for tropical grasses that employ C4 photosynthesis because of the availability of its complete genome sequence (Billot *et al.*, 2013)

S. bicolor has three subspecies, namely *arundinaceum*, *bicolor* and *drummondii*, and the cultivated sorghums are all from *bicolor*, which has five local races, *bicolor*, *caudatum*, *durra*, *guinea* and *kafir* (Harlan, 1972). Sorghum *bicolor* contains both cultivated and wild relative races, and it provides a substantial amount of genetic diversity for traits of agronomic importance so as to develop the crop's different variety of interest for plant breeders. Sorghum has one of the largest crop germplasm collections, consisting of more than 42,000 accessions worldwide. The largest diversity of the crop germplasm provides greater opportunities for improvement regarding its environmental adaptability and acquiring better agronomic traits from the crop species.

Assessment of genetic diversity within cultivated crops is crucial for the effective utilization of crop genetic resources. It is particularly useful in characterization of accessions (Smith *et al.*, 2000), in identifying duplicates in germplasm collections (Dean *et al.*, 1999) and in the choice of parents in breeding programmes (Gupta *et al.*, 1994, 1996). The more variable genotypes or accessions can be crossed to produce better varieties that can tolerate a range of environmental changes to abiotic and biotic stresses. Therefore, a better understanding of the genetic diversity in sorghum crop species will definitely facilitate the further improvement of this cereal crop concerning its genetic architecture (Tesfaye, 2017).

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). 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).

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; Mohammadi *et al.*, 2003; Smith *et al.*, 2000; Ghebru *et al.*, 2002; Chiorato *et al.*, 2007). Several generations of DNA-based molecular markers were then used and refined the assessment.

In the early 1990s, restriction fragment length polymorphism (RFLP) markers were effectively utilized for sorghum diversity analysis, genetic mapping and comparative genome mapping. Later other marker systems were tried, including randomly amplified poly-morphic DNAs (RAPDs) (Ayana and Bekele, 2000; Prakash *et al.*, 2006), simple sequence repeats (SSRs) (Dje *et al.*, 2000), and amplified fragment length polymorphisms (AFLPs). These markers systems, independently or in combination with others, were efficiently used for sorghum genetic diversity analysis. SSRs were developed independently by several different research groups and were exploited for genetic diversity analysis.

In the present study the 20 sorghum accessions originated from Marathwada (Maharashtra) region of Maharashtra consisting of advance/improved cultivar, traditional cultivar/landrace, breeding/research material were analysed for genetic diversity in order to exploit the use of genetic resources for crop breeding and various mapping studies.

The aim of the present study is to characterize genetic structure of sorghum accessions as an initiative step in testing, the applicability of molecular markers for choosing parents, eight sorghum SSR markers evenly distributed on sorghum genome were used to analyses the genetic variation among different germplasm accessions. The objectives of this study are

To analysed the characteristics of the SSR loci and genotypic structure of sorghum accessions

To know the genetic variation within sorghum accessions.

Materials and Methods

Plant material

The 20 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 0.2g fresh leaf sample using modified CTAB protocol modified at Department of Plant Biotechnology (Kale *et al.*, 2020) (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.

SSR markers

Eight pairs of SSR primers (SSR Markers) were used in the study (Table 2). 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 sequence of the eight primer pairs were chosen from the sorghum SSR kit (http://sorghum.cirad.fr/SSR_kit) (Billot *et al.*, 2013).

PCR amplification

Eight SSR primer pairs were used to detect polymorphism among the 20 sorghum accessions. The PCR was performed in a volume of 20 μl reaction containing 2 μl (50ng) genomic DNA, 10X PCR buffer with 25mM MgCl₂ (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 94⁰C for 5 mins followed by 34 cycles at 94⁰C for 30sec, 55-65⁰C for 30 sec (depending on primer T_m) and 72⁰C for 1min, followed by a final extensions at 72⁰C for 7mins.

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 μ l of Bromophenol blue dye was added to 20 μ l of the PCR product.

Agarose gel of 3.5% was used for better resolution of PCR product. 20 μ l 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).

Results and Discussion

Characteristics of the SSR loci and genotypic structure of sorghum accessions

Analysis of genetic structure of the germplasm population is an important for the plant breeding programme and for association studies to identify the superior alleles in the accessions (Nachimuthu *et al.*, 2015). In this study attempt has been made to analyse the genetic structure of twenty sorghum accessions using evenly distributed eight SSR markers on sorghum genome that show amplification and polymorphism with different allele size (140-250bp). All the markers produce all expected SSR allele size amplicons were used for analysis of genetic diversity among the twenty accessions of elite sorghum. Thus, all these markers should represent the genetic diversity among sorghum accessions.

In total 27 alleles were detected in 20 germplasm accessions. The number of alleles per loci ranged from 3 (msbCIR276, xtxp021, xisep0310, gpsb089 and xtxp145) to 4(xtxp278, xtxp321 and xtxp141) with an average of 3.37 alleles per locus (Table 3). The fragment size of the alleles ranged from 140 bp (Xtxp141) to 250 bp (Xtxp278; Table 4). All the SSR loci show polymorphism. Similar results were reported in the previous studies were, the average of 4.4 (Nguni *et al.*, 2011), 3.44 (Billot *et al.*, 2013) alleles were observed.

Genetic variation within sorghum accessions and cluster analysis

Analysis of genetic diversity among 20 accessions revealed that the polymorphic Information Content (PIC) value represents the relative informativeness of each marker loci and in this study, the average PIC value was found to be 0.53 was comparable to past and earlier reports for sorghum and rice (Cho

et al., 2000; Jain *et al.*, 2004; Nachimuthu *et al.*, 2015). PIC values was found to be ranged from 0.41 to 0.63 where 0.41 PIC value indicated presence of three allele per locus.

Out of eight polymorphic SSR loci, six (75.0%) markers were highly informative ($PIC \geq 0.5$), two markers (25%) were reasonably informative ($PIC = 0.25-0.5$) and no one markers was found to be less informative ($PIC \leq 0.25$). No heterozygosity was found across 20 accessions which may be due to autogamous nature of sorghum. The SSR loci Xtxp278, msbCIR276, Xisep0310, Xtxp321, Xtxp141 and Xtxp145 were rich in allelic diversity exhibiting highest PIC value (Table 4).

Expected heterozygosity or Gene diversity computed according to Nei (1973) and varied from 0.46 to 0.68 with an average of 0.60. The major allele frequency across 20 accessions was ranged from 0.35 to 0.75 with the mean major allele frequency was 0.51 (Table 3). Thus, the present result revealed that 20 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.

The main focused of this study was the analysis of variation between the different races of sorghum using SSR markers. Some important findings were published by the authors on diversity analysis in sorghum accession from different origins.

The genetic diversity within small sets of Guinea-race sorghum accessions has been analyzed in the past studies using different molecular markers such as, SSR markers (Dje *et al.*, 2000), random amplified polymorphic DNA (RAPD) markers (de Oliveira *et al.*, 1996; Ayana and Bekele, 2000; Prakash *et al.*,

2006; Dahlberg *et al.*, 2002) and restriction fragment length polymorphism (RFLP) markers (Deu *et al.*, 1994).

Employing SSR markers to analyse the genetic diversity is more accurate and adventitious because of their abundance on the genome, co-dominance, reproducibility and ease of use.

The availability of complete sorghum genomic sequence information (Paterson *et al.*, 2009) on various databases, a number of genome-wide SSRs have been published and available with their sequences in sorghum and have been employed in genetic diversity and other studies.

Estimation of major allele frequency, allele number, gene diversity and PIC value with the use of evenly distributed SSR is more reliable and accurate than the use of other system. It was observed that, the PIC value obtained in this study (Mean $PIC = 0.53$) is similar to the diversity value (0.40, 0.46, 0.62, 0.58) reported by (Ali *et al.*, 2008; Schloss *et al.*, 2002; Agrama *et al.*, 2008; Smith *et al.*, 2000) respectively.

PIC provides an estimate of the discriminatory power of a locus or loci by the number of alleles expressed and the relative frequencies of those alleles. The genetic variation among five Guinea-race sorghum accessions (Dje *et al.*, 2000), revealed an observed average expected gene diversity of 0.22 which was much lesser than the gene diversity obtained in the current study (0.6) using 20 sorghum accessions originated from Marathwada region of Maharashtra, India. As observed by Chakauya *et al.*, (2006), differences in the genetic diversity could be attributed to traditional farming systems with agronomic, economic and cultural considerations that foster high levels of genetic diversity.

Also the diversity of sorghum types, varieties, and traditional cultivars/races has been related to movement of people, disruptive selection, geographic isolation, gene flow from wild to cultivated plants, and recombination of these types in different environments (Doggett, 1988; Wet and Huckabay, 1967; Harlan, 1976). Distance based approach which is based on calculating pair wise distance matrix was computed by calculating a dissimilarity matrix using a Nei distance (1973) and used for the construction of phylogenetic tree by Neighbour-Joining (NJ) method, implemented in POWERMARKER version 3.25 and the tree was viewed using MEGA V 6.0. A

neighbour-joining (NJ) tree was constructed based on genetic distance (Figure 1).

The group I and group II in NJ tree consists of the traditional landraces and breeding lines but group I was dominated by traditional landraces whereas the II group consists of mostly breeding material. Group III of NJ tree is dominated by advance/improve cultivars. Similar observations were arrived by many investigators in different crops Nachimuthu *et al.*, 2015; Agrama *et al.*, 2007; Choudhury *et al.*, 2014; Muthukumar *et al.*, 2015; Nguni *et al.*, 2011).

Table.1 Details of sorghum accessions used in the study

Sr.no.	Accession ID	Code no.	Biological status	Collection Site
1	Dukri	G1	Traditional cultivar/Landrace	Aurangabad;Aurangabad district
2	Chetapuri bhendala	G2	Traditional cultivar/Landrace	Aurangabad;Aurangabad district
3	PJ1K	G3	Advanced/Improved cultivar	Parbhani; Parbhani district
4	PJ3R	G4	Breeding/Research material	Parbhani; Parbhani district
5	SM Wani No.7	G5	Traditional cultivar/Landrace	Parbhani; Parbhani district
6	PJ14R	G6	Advanced/Improved cultivar	Parbhani; Parbhani district
7	NSL 50400	G7	Traditional cultivar/Landrace	Parbhani; Parbhani district
8	PJ36K	G8	Advanced/Improved cultivar	Parbhani; Parbhani district
9	PJ5R	G9	Advanced/Improved cultivar	Parbhani; Parbhani district
10	IC 9185	G10	Traditional cultivar/Landrace	Parbhani; Parbhani district
11	IC 9186	G11	Traditional cultivar/Landrace	Parbhani; Parbhani district
12	IC 8844	G12	Traditional cultivar/Landrace	Parbhani; Parbhani district
13	IC 8857	G13	Traditional cultivar/Landrace	Parbhani; Parbhani district
14	IC 8868	G14	Traditional cultivar/Landrace	Parbhani; Parbhani district
15	IC 8889	G15	Traditional cultivar/Landrace	Parbhani; Parbhani district
16	PJ13R	G16	Breeding/Research material	Parbhani; Parbhani district
17	PJ14R	G17	Breeding/Research material	Parbhani; Parbhani district
18	PJ15R	G18	Breeding/Research material	Parbhani; Parbhani district
19	PJ16R	G19	Breeding/Research material	Parbhani; Parbhani district
20	PJ17R	G20	Breeding/Research material	Parbhani; Parbhani district

Table.2 Characteristic of the eight SSR loci analyzed for diversity study

Sr.no	Marker Name	Forward Primer	Reverse Primer	Allele Size	Ch. No
1	Xtxp321	TAACCCAAGCCTGAGCATAAGA	CCCATTACACATGAGACGAG	192-252	SBI08
2	MsbCIR276	CCCCAATCTAACTATTTGGT	GAGGCTGAGATGCTCTGT	230-234	SBI03
3	Xtxp021	GAGCTGCCATAGATTTGGTCG	ACCTCGTCCCACCTTTGTG	169-199	SBI04
4	Xtxp141	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	135-167	SBI10
5	Xtxp278	GGGTTTCAACTCTAGCCTACCGAA CTTCCT	ATGCCTCATCATGGTTCGTTTT GCTT	243-252	SBI07
6	Xisep0310	TGCCTTGTGCCTTGTATTATCT	GGATCGATGCCTATCTCGTC	164-219	SBI02
7	gpsb089	ATCAGGTACAGCAGGTAGG	ATGCATCATGGCTGGT	165-173	SBI01
8	Xtxp145	GTTCCCTCCTGCCATTACT	CTTCCGCACATCCAC	208-244	SBI06

Table.3 Mean diversity analysis of 20 germplasm accessions using eight SSR markers

S. No.	Diversity Traits	Number	Range	
1	Total no. of alleles	27	Maximum	Minimum
2	Mean no. of alleles per locus	3.3	4	3
3	Mean major allele frequency	0.51	0.75	0.35
4	Mean Gene Diversity	0.6	0.76	0.41
5	Mean PIC	0.53	0.72	0.38

Table.4 Details of SSR loci used for genotyping in the sorghum accessions and their genetic diversity parameters using power marker V 3.25 program

Marker	Chr. no.	Minimum molecular weight	Maximum molecular weight	Major Allele Frequency	Allele No	Gene Diversity	PIC
xtp278	7	235	250	0.45	4.0	0.6850	0.6324
msbCIR276	3	230	240	0.50	3.0	0.6050	0.5270
xtp021	4	180	200	0.65	3.0	0.5150	0.4604
xisep0310	2	200	220	0.50	3.0	0.6250	0.5547
xtp321	8	195	220	0.50	4.0	0.5850	0.5009
gpsb089	1	180	190	0.70	3.0	0.4650	0.4199
xtp141	10	140	170	0.40	4.0	0.6850	0.6270
xtp145	6	220	240	0.45	3.0	0.6450	0.5720

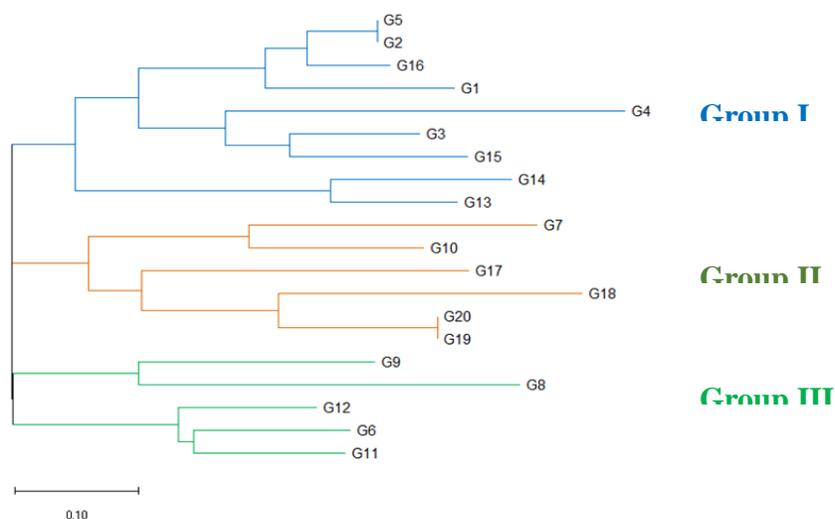


Fig.1 Neighbor-joining (NJ) tree of 20 Sorghum accessions constructed using POWERMARKER software

Cluster analyses in the collection of sorghum accessions provided important evidence to define breeding strategies and to identify superior parents for the development of new hybrid sorghum cultivars with the major focus on various biotic abiotic stresses.

Also the assessment of diversity and analysis of population structure is requiring for analysis and identification of quantitative trait loci by means of association mapping (Muthukumar *et al.*, 2015). In the present study all lines were distinctly placed in this dendrogram obtained by the molecular diversity analysis were more consistent with the some important characteristics traits and the historic background of the sorghum accessions.

Total 20 accessions were grouped into three main clusters and sub-sub groups based on the molecular marker data generated from evenly distributed SSR markers on the whole genome of sorghum. No much significant results were reported in the previous study based on the phenotypic data used to classify sorghum accessions. Limitations on use of

morphological traits, their experimental costs, effect of environment, evaluation time and genotype \times environment interactions are widely discussed (Fufa *et al.*, 2005; Chandra *et al.*, 2002). In this context, DNA-based SSR markers have become powerful application tools for illustrating and quantifying genetic differences within and among the various crop species.

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 agromorphological 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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