Original Research Article

Molecular Variations In *Rabi* Sorghum Varieties Diversity Using SSR Markers

Abstract

Genetic diversity was assessed for 2 popular varieties and 5 promising lines of *rabi* sorghum by using11 SSR markers. The marker Xiabt312 reported 100% polymorphism rate followed by Xtxp15 (85.70%) and mSbCIR300 (85.71%). The sorghum varieties studied for this analysis showed the polymorphism information content (PIC) ranged from 0.25 to 0.87 with a mean of 0.67 indicates higher diversity within them. Clustering analysis based on the genetic dissimilarity grouped the 7 lines into 2 major and 4 sub clusters and grouping was in good agreement with pedigree. Cluster II was the largest cluster with 4 genotypes followed by cluster I with 3 genotypes. The *rabi* sorghum genotypes *viz.*, M35-1, RSV-1876 and RSLG-2422 were placed in cluster I and Phule Anuradha, RSV-2371, RSV-1910 and RSV-1988 placed in cluster II. Clustering based on SSR molecular profile of the genotypes shows that there is a distinct variability among the genotypes under this study. The selected markers have great potential in DNA fingerprinting in sorghum which in future could be integrated with DUS data descriptors for effective cultivar identification and differentiation.

Key words: *Sorghum, Molecular diversity, DNA fingerprinting, SSR markers*

INTRODUCTION

"Sorghum [*Sorghum bicolor* (L.)] is a genus with many species and subspecies, and there are several types of sorghum, including grain sorghum, grass sorghum (for pasture and hay) and sweet sorghum (for syrups). Sorghum has the high nutritional values and is grown as dietary staple food for more than 500 million people in 30 different countries" (Joshi *et al.* 2020). "Genetic diversity analysis of sorghum (*Sorghum bicolour* (L.) Moench; $2n = 2x = 20$) germplasm is fundamental for breeding and conservation strategies. Genetic advancement during selection depends on the availability of genotypes possessing favourable alleles for desired traits, which relies on the available genetic diversity. Phenotyping based on enlisted DUS (Distinctness, Uniformity and Stability) traits is a pre-requisite for varieties to be registered under PPV&FR Act, 2001. Most of these traits are quantitative in inheritance and environmentally influenced. Often, there is a risk of categorising genetically different cultivars as similar or *vice-versa* owing to subjective assessment" (Santhy and Meshram, 2015).

"The genetic diversity analysis represents the diversity of germplasm accessions which could be helpful of broadening the base of breeding program" (Joshi *et al.* 2020). "Genetic diversity analysis can be carried out using phenotypic or molecular markers. DNA-based molecular markers are more efficient to analyse a greater number of genotypes" (Reif *et al.* 2003). "Further for, molecular markers detect the presence of favourable alleles among germplasm and allow estimation of genetic diversity more reliably and efficiently than phenotypic markers, which are subject to genotype x environment interaction. Similarly, the

molecular marker technology aids conventional breeding in various aspects, such as to (1) assess of genetic diversity and establish heterotic patterns, (2) screen for useful genes, (3) accelerate backcross breeding programs *via* selection of gene(s) of interest and (4) identify and protect commercial cultivars through fingerprinting" (Xiao *et al.* 1996, Harshita and Sandal,2022). "Several DNA-based marker systems have been successfully used to assess genetic diversity in sorghum" (Nguni *et al.* 2011). "Molecular markers especially SSR are most efficient source of diversity studies in crop species" (Shehzad *et al.* 2009). "SSR markers combined with morphological traits may effectively be used for designing breeding strategy and management of biodiversity and conservation of *Maldandi* genetic resources" (Rakshit *et al* 2012). "The sorghum genome sequence project identified 71SSRs in the genome" (Paterson *et al.* 2009). "The availability of this large number of SSR markers provides a more cost-effective and rapid method for DNA profiling" (Smith *et al.* 1997).

Among the various marker systems presently available, microsatellite or simple sequence repeat(SSR) markers have become more valuable and reliable tool for genetic diversity analysis of crop varieties (Tyagi *et al.* 2014) owing to their reproducibility, co-dominant inheritance, genome-wide presence, robustness, higher polymorphism and analytical simplicity. "Molecular data generated from SSR markers has also been utilized for QTL mapping, DNA fingerprinting and genetic purity testing" (Ahmed *et al.* 2013; Ashraf *et al.*2016).Therefore, the present study was aimed to assess the molecular diversity available among the promising *rabi* sorghum genotypes by using set of SSR markers. This study intended to probe the appliance of the molecular marker in the context of DUS tests to disclose unique variety-specific fingerprints. This varietal fingerprint could be used for various varietal purity test programs of closely related sorghum cultivars and submission of fingerprint data for the crop variety registration.

MATERIALS AND METHODS

Seven sorghum genotypes included in this study were selected on the basis of grain and fodder yield performance and tolerant to abiotic and biotic stresses. Two of them are released varieties and 5 are promising genotypes. These lines have been evaluated in stress nurseries under the Sorghum Improvement Project, Mahatma Phule Krishi Vidyapeeth Rahuri over the past few years, and their reactions (susceptibility/resistance) to various biotic and abiotic stresses are known. The details of the genotypes are given in Table 1.

Plant material for DNA extraction

Fifty seeds of each genotype were grown in small plastic pots and were watered till the length of the seedlings was around 10 to 15 cm.

DNA isolation

i. Preheat (65ºC) the extraction buffer comprises of 1 M Tris- HCl (pH 8), 5 M EDTA (pH 8), 3% (w/v) CTAB, 10 µl proteinase K (20 mg/ml), and 1% (v/v) β -mercaptoethanol (added freshly) kept in water bath at 65ºC for about 15 min. Add preheated extraction buffer to each microcentrifuge tube (2 ml). ii. Fresh leaf samples (200 mg) were collected from three weeks old germinated seedlings from each line and grinded to make fine powder in liquid nitrogen with the help of pre- chilled mortar and pestle. iii. This powder was immediately transferred to centrifuge tubes (2 ml) containing pre-warmed extraction buffer and was mixed by inversion. iv. The homogenate was incubated at 65ºC for about 60 min with frequently

swirling. v. Centrifuge the tubes at 10,000 rpm for 10 min. vi. Transfer the supernatant into another autoclaved centrifuge tube (2 ml). vii. To the supernatant equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed gently by inversion for 15 min. viii. Centrifuge the tubes at 10,000 rpm for 10 min at 4ºC and collect the supernatant in centrifuge tube (1.5 ml) and using wide bored tip, carefully transfer the upper aqueous phase (supernatant), which contains DNA to a new centrifuge tube (1.5 ml). ix. Repeat the extraction steps (vii and viii). x. The supernatant then treated with RNase A, mix twice for 30 sec, and incubated at 37ºC for about 1 hour to remove any RNA present in the sample. xi. The equal volume of chloroform:isoamyl alcohol (24:1) was added to each tube, mix gently by inversion, and incubate at room temperature for about 15 min. xii. Centrifuge the tubes at 12,000 rpm for 10 min and transfer the supernatant into newly labeled autoclaved centrifuge tube (1.5 ml). xiii. To the supernatant double volume of chilled absolute alcohol was added and mixed gently by inversion. xiv. Tubes were incubated at 4ºC for overnight to precipitate the DNA. xv. The precipitated DNA was collected after centrifugation at 10,000 rpm for 10 min. xvi.DNA pellet was washed 2-3 times with 70% ethanol, air dried and dissolved in 100 µl of TE (1 M Tris-HCl, 0.5 M EDTA, pH 8) buffer. xvii. The samples were stored at -20ºC.

Genomic DNA from each of the genotypes was extracted using Cetyl Trimethyl ammonium bromide (CTAB) procedure (Gadakh *et al*., 2020). The DNA quantity of each sample was estimated and DNA concentrations were normalized at 2.5 ng/µl. The DNA quality of each sample was evaluated by running 5 µl of DNA on a 0.8 % agarose gel.

PCR and SSR assay

The parameters set for PCR amplification conditions were followed as described by Gadakh *et al.* (2020) and the PCR assay was carried out at the State Level Biotechnology Centre, M.P.K.V., Rahuri. Eleven reported SSR primers were used for genotyping the seven sorghum genotypes. PCR conditions were optimized for each of the 11 SSR markers and PCR reactions were set up in 25 µl volumes. Each PCR reaction contained µl (50 ng/*ì* l) of template DNA, 2.5 µl of 10X PCR buffer, 3.3 µl of 15 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 1 µl (10 picomol) of each primer, 0.5µl Taq DNA polymerase (1 U/µl), and 13.7µl of sterile distilled water. DNA amplification was performed in a thermal cycler (Eppendorf Master cycler, USA) by using the following thermal cycler condition: initial denaturation step at 94ºC for 5 min (1 cycle), followed by 35 cycles each at 94ºC for 1 min (denaturation), 61ºC for 1 min (annealing), 72ºC for 1 min (extension), followed by one final extension at 72ºC for 10 min. The amplified PCR product run on agarose gel electrophoresis using 1.5% agarose gel in 1 X TBE buffer for 3 hours at 80 V. The agarose gel stained with ethidium bromide (EtBr) to check PCR product quality and gel image was taken under gel documentation system (Alpha InfoTech Corporation, USA).

Cluster analyses

Data was scored for computer analysis on the basis of the presence or absence of the SSR bands. If a product was present in a genotype, it was designated as '1' and if absent; it was designated as '0'. The data was maintained in the spread sheet format for further analysis. The polymorphism percentage was calculated as per the method suggested by Blair *et al.* (1999).

Polymorphism information content (PIC) = 2Pi (1-Pi) ……(i)

Where, Pi = frequency of occurrence of polymorphic bands in different primers

$$\text{Polymorphism (\%)} = \frac{X - Y}{X} \times 100 \quad \text{.....(ii)}$$

Where X= Total number of bands

Y= Number of Monomorphic band

The data generated by SSR markers were analyzed with the software NTSYSpc version 2.02.

RESULTS AND DISCUSSION

SSR marker analysis

The genetic variability among the *rabi* sorghum genotypes and lines was analyzed using SSR molecular markers. In this study 11 SSR primers were screened (Table 2). Assessment of genetic diversity among the sorghum varieties is important to know the diverse parents for sorghum improvement. All the 7*rabi* sorghum varieties were profiled for DNA polymorphism using 11 SSR markers sourced from sorghum marker database. Present study design to estimate molecular diversity in *rabi* sorghum lines with SSR marker.

Among the 11 SSR markers 10 markers were polymorphic (Table 3) with the average percent polymorphism of 74.68%. The marker Xiabt312 and Sb5-206 reported 100% polymorphism followed by Xtxp15 (85.70%) and mSbCIR300 (85.71%). The banding pattern of polymorphic marker Xtxp15 and Xiabt437were shown in Fig. 1 and 2 respectively. *Sorghum bicolour* derived SSRs have proven to be an efficient source of markers for genetic diversity studies (Dillon *et al.* 2005).

The marker Xsnp109 had higher PIC value (r^2=0.87**) followed by Sb5-206 (0.82) and Xtxp15 (0.81).The PIC values ranged from 0.25 (Xtxp278) to 0.087 (Xsnp109) with a mean of 0.67 (Table 3). Afolayan *et al.* 2019 studied "genetic diversity among the sorghum germplasm was low with an average polymorphism information content value of 0.24". Analysis of molecular variation revealed 6% variation among germplasm and 94% within germplasm. "Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes in sorghum" (Gangurde *et al.* 2017). "PIC values depends on many factors such as breeding behaviour of the species, genetic diversity in the collection, size of the collection, sensitivity of genotyping method and location of primers in the genome used for study" (Kalivas *et al.*2011; Singh *et al.* 2013). "A low allele detection and low PIC value was observed even when a high resolution metaphor agarose/PAGE gel was employed for separating the amplicon" (Bertini *et al.* 2006). Kshirsagar *et al.* (2020)reported "polymorphic information content (PIC) ranged between 0.41 to 0.63 with the average PIC value was found 0.53 where 0.41 PIC value indicated presence of three allele per locus". When the study material was diverse such as wild accessions and hybrids, the PIC values can be more than 0.80 as observed by Zhang *et al.* (2013).PIC provides an estimate of the discriminatory power of *loci* by the number of alleles expressed and the relative frequencies of those alleles.

The NTSYSpc programme was used to calculate Jaccard's similarity coefficient. The genetic dissimilarity between *rabi* sorghum lines given in Table 4. Phule Anuradha showed maximum genetic variation to RSV-2422 (0.846) followed by RSV-1988 (0.767) and RSV-2371 (0.550). The genetic dissimilarity between lines ranges between 0.310 and 0.846 (Table 4).Sorghum lines RSV-1910 and RSV-1988exhibited less variation. 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. High PIC value observed in the present study may be the result of the higher diversity available in the study material which consisted of released varieties and promising lines of different pedigree. Madhusudhana *et al.* (2012) studied "genetic diversity in 31 sorghum parents using 413 sorghum simple sequence repeats (SSR) markers. The polymorphism information content (PIC), a measure of gene diversity, varied from 0 to 0.92 with an average of 0.53 and was significantly correlated with number of alleles". Ganapathy *et al.* (2012) demonstrated "the utility of SSR markers in classifying the hybrid parental lines of sorghum based on fertility reaction and will serve as effective tool for classifying parental lines based on heterotic groups".

Cluster analysis

The neighbourhood joining cluster analysis was carried out for 7 varieties which put them into2 distinct clusters (Fig. 3). Cluster II was the largest cluster with 4lines followed by cluster I with 3lines. The *Maldandi* blood varieties *viz.*, M35-1, RSV-1910 andRSV-1876 were placed distinctly from all the other lines. Clustering based on SSR molecular profile of the varieties match with the pedigree or source of the varieties. These agreements between varietal pedigree and molecular clustering give perfect diversity at molecular level**.** Madhusudhana *et al.* (2012) reported that, "clustering analysis based on the genetic dissimilarity grouped the 31 parents into eight clusters and grouping was in good agreement with pedigree, race and geographic origin". Ganapathy *et al.* (2012) identified "diverse set of parental lines (maintainers and restorers) based on Jaccard's similarity values will serve as effective candidates for hybrid development". "SSR markers combined with morphological traits may effectively be used for designing breeding strategy and management of biodiversity and conservation of genetic resources" (Rakshit *et al.* 2012).

Afolayan*et al.* (2019) revealed that "dendrogram clustering of three groups which indicate variations within the germplasm. The presence of private alleles and genetic variation within the germplasm indicates that the accessions are valuable resources for future breeding programs".

DNA fingerprinting of *rabi* Sorghum varieties

"One of the most important applications of molecular fingerprinting is to identify a marker or set of markers which can differentiate a particular genotype from the remaining genotypes. To decide an appropriate DNA fingerprint for the sorghum varieties, the ability of fingerprint to discriminate the given set of genotypes was assessed through probability of identical match by chance" (Choudhury *et al.* 2001). The primers of (Xtxp278 and Xiabt312) produced maximum variation among the *rabi* sorghum lines as its PIC value is maximum among all the primers under study. Sb5-206 and mSbCIR300 is widely distributed in the sorghum genome as it produced 7 alleles which are maximum among all the primers. The SSR marker Xtxp270 is uniformly distributed in all lines as it produces the DNA fragments. Some SSR markers are specific to cultivars for cultivar identification which is the primary moto of DNA fingerprinting. SSR markers used for DNA fingerprinting are given in Table

3.Molecular marker technique has proved very sensitive for fingerprinting/ characterization in sugarcane (Gadakh *et al.* 2017). Gangurde *et al.* 2017 reported that for identification of hybrid CSH-35 the Xcup02, Msbcir240, Msbcir 246 were produces a unique band (200bp) which is absent in parents. Santhiya *et al.*2020 reported that the SSR markers Xtxp024, Xtxp231, Xtxp075 produced unique alleles in CO 32 whereas Xtxp354 produced unique alleles in K12. The SSR marker Xtxp003, Xtxp201 produced unique alleles in CSV 33 MF which could serve as valid genotype-specific SSR markers in varietal purity test program. In this study, when analysis was performed with a minimal set of 11 markers, the fingerprint (Fig. 1 and 2) was able to differentiate all 7 genotypes.

CONCLUSION

The varietal-specific SSR marker will supplement the DUS test and could play a major role in varietal identification which resolving disputes during the seed certification process. The polymorphic markers identified in the present study will be of immense utility in *rabi* sorghum improvement. The DNA fingerprint developed for popular sorghum varieties of the centre will be helpful for unambiguous identification of sorghum varieties and their protection against unauthorized exploitation.

References

Afolayan,G., Deshpande,S. P., Aladele,S. E., Kolawole,A. O., Angarawai,I., Nwosu,D. J., Michael,C., Blay,E. T. and Danquah, E. Y.2019. Genetic diversity assessment of sorghum (*Sorghum bicolor* (L.) Moench) accessions using single nucleotide polymorphism markers. *Plant Genetic Resources*. 17(5): 412-420.

Ahmed, M., Guo, H., Huang, C., Zhang, X. and Lin, Z. 2013. Selection of core SSR markers for fingerprinting upland cotton cultivars and hybrids. *Aus. J. Crop Sci*. 7(12): 1912-1920.

Ashraf, J., Malik, W. U., Iqbal, M. Z., Khan, A., Qayyum, A., Noor, E., Abid, M. A., Cheema, H. M. N. and Ahmed, M. Q. 2016. Comparative analysis of genetic diversity among bt cotton genotypes using EST-SSR, ISSR and morphological markers. *J. Agr. Sci. Tech.* 18: 517-531.

Bertini, C. H. C. M., Schuster, I., Sediyama, T., Barrosi, E. G. D. and Moreira, M. A. 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. *Genet. Mol. Biol.* 29: 321-329.

Blair, R. J. R., Morris, J. S. and Frith, C. D. 1999. Dissociable neural responses to facial expressions of sadness and anger. *Brain*. 122: 883-893.

Choudhury, P. R., Kohli, S., Srinivasan, S., Mohapatra, T. and Sharma, R. P. 2001. Identification and classification of aromatic rice based on DNA fingerprinting. *Euphytica*. 118: 243.

Dillon, Sally L., Lawrence, Peter K. and Henry, Robert J. 2005. The new use of *Sorghum bicolor*-derived SSR markers to evaluate genetic diversity in 17 Australian Sorghum species. *Plant Genetic Resources.* 3(1): 19-28.

Dje, Y., Heuertz, M., Lefebvre, C. and Vekemans, X. 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. *Theoretical and Applied Genetics*. 100: 918-925.

Gadakh, S. S., Khalekar, G. D., Dalvi, U. S., Kale, A. A. and Kulwal, P.L. 2020. Simple and Efficient Method for DNA Extraction from Sweet Sorghum [*Sorghum bicolor* (L.) Moench]. *The Bioscan*. 15(3): 403-406.

Gadakh, S. S., Patel, D. U. and Diwakar Singh. 2017. Molecular characterization of Salt and Drought Tolerant Mutant and Parental Plants by Using RAPD. *International Journal of Bio-resource and Stress Management*. 8(2): 280-284.

Ganapathy, K. N., Gomashe, S. S., Rakshit, S., Prabhakar, B., Ambekar, S.S., Ghorade, R.B., Biradar, B.D., Saxena, U. and Patil, J.V. 2012. Genetic diversity revealed utility of SSR markers in classifying parental lines and elite genotypes of sorghum (*Sorghum bicolor* L. Moench). *Aus. J. Crop Sci.* 6(11): 1486-1493.

Gangurde, Sunil, Ghorade, R. B., Moharil, M. P., Ingle, K. P. and Wagh Anand. 2017. Microsatellite based DNA fingerprinting of sorghum [*sorghum bicolor* (l.) Moench] hybrid csh-35 with its 35 with its parents. *The Bioscan*. 12(1): 215-219.

Harshita,N.S. and Sandal, S.S.2022.DNA fingerprinting and its applications in crop improvement: A review, The Pharma Innovation, SP-11(5): 792-797.

Joshi, Akansha R., Kale, Sonam S. and Chavan, Narendra R. 2020. Genetic diversity among elite sorghum (*Sorghum bicolor* L.) accessions genotyped with SSR markers to enhance use of global genetic resources. *Int. J. Chemical Studies*. 8(2): 1691-1697.

Kalivas, A., Xanthopoulos, F., Kehagia, O. and Tsaftaris, A. S. 2011. Agronomic characterization, genetic diversity and association analysis of cotton cultivars using simple sequence repeat molecular markers. *Genet. Mol. Res.* 10: 208-217.

Kshirsagar, Ram C., Kale, Sonam S., Chavan, Narendra R. and Kore, Ganesh V. 2020. Analysis of Genetic Diversity in Sorghum [*Sorghum bicolor* (L.)] Accessions of Maharashtra as Estimated by Simple Sequence Repeats (SSR). *Int.J.Curr.Microbiol.App.Sci.* 9(4): 934-944

Madhusudhana, R., Balakrishna, D., Rajendrakumar, P., Seetharama, N. and Patil, J. V. 2012. Molecular characterization and assessment of genetic diversity of sorghum inbred lines. *African Journal of Biotechnology*. 11(90): 15626-15635.

Nguni, D., Geleta, M. and Bryngelsson, T. 2011. Genetic diversity in sorghum (*Sorghum bicolor* (L.) Moench) accessions of Zambia as revealed by simple sequence repeats (SSR). *Hereditas*. 148: 52-62.

Paterson, A., Bowers, J. and Bruggmann, R. *et al.* 2009.The *Sorghum bicolor* genome and the diversification of grasses. *Nature.* 457: 551–556.

Rakshit, Sujay, Gomashe, Sunil S., Ganapathy, K. N., Elangovan, M., Ratnavathi, C. V., Seetharama, N. and Patil, J. V. 2012. Morphological and molecular diversity reveal wide variability among sorghum *Maldandi* landraces from India. *Journal of Plant Biochemistry and Biotechnology*. 21: 145-156.

Reif, J. C., Melchinger, A. E., Xia, X. C., Warburton, M. L., Hoisington, D. A., Vasal, S. K., Beck, D., Bohn, M. and Frisch M. 2003. Use of SSRs for establishing heterotic groups in subtropical maize. *Theoretical and Applied Genetics*. 107: 947-957.

Santhiya, S., Kowshiga, K., Abisha, D., Sarankumar, C., Kavithamani, D., Selvi, B. and Senthil, N.2020. SSR Marker-based DNA fingerprinting and morphological characterization for varietal identification in popular sorghum varieties of Tamil Nadu. *Ele. J. Plant Breeding*. 11(2): 521-527.

Santhy, V. and Meshram, M. 2015. Widening the character base for distinctness in cotton plants. *Curr. Sci.* 109(11): 1913.

Shehzad Tariq, Okuizumi Hisato, Kawase Makoto, Okuno Kazutoshi. 2009. Development of SSR-based sorghum (*Sorghum bicolor* (L.) Moench) diversity research set of germplasm and its evaluation by morphological traits. *Genetic Resources and Crop Evolution*. 56(6): 809-827.

Singh, N., Choudhury, D. R., Singh, A. K., Kumar, S., Srinivasan, K. and Tyagi, R. K. 2013. Comparison of SSR and SNP Markers in estimation of genetic diversity and population structure of Indian rice varieties. *PLOS ONE*. 8(12): e84136.

Smith, J. S. C., Chin, E. C. L., Shu, H., Smith, O. H., Wall, S. J., Senior, M. L., Mitchell, S. E., Kresovich, S. and Ziegle, J. 1997. An evaluation of the utility of SSR *loci* as molecular makers in maize (*Zea mays* L.): comparison with data from RFLPs and pedigree. *Theoretical and Applied Genetics*. 95: 163-173.

Tyagi, P., Gore, M. A., Bowman, D. T., Campbell, B. T., Udall, J. A. and Kuraparthy, V. 2014. Genetic diversity and population structure in the US upland cotton (*Gossypium hirsutum* L.). *Theor. Appl. Genet.* 127: 283-295.

Xiao, J., Li, J., Yuan, L., McCough, S. R. and Tanks, S. 1996. Genetic diversity and its relationship to hybrid performance and heterosis as revealed by PCR based markers. *Theoretical and Applied Genetics*. 92: 637-643.

Zhang, Y. C., Kuang, M., Yang, W. H., Xu, H. X., Zhou, D. Y., Wang, Y. Q., Feng, X. A., Su, C. and Wang, F. 2013. Construction of a primary DNA fingerprint database for cotton cultivars. *Genet. Mol. Res.* 12(2): 1897- 906.

Santhy V, Meshram M, Santosh HB, Kranthi KR. Molecular diversity analysis and DNA fingerprinting of cotton varieties of India. Indian Journal of Genetics and Plant Breeding. 2019 Dec 25;79(04):719-25.

Table 1. Name and source of varieties of *rabi* Sorghum (*Sorghum bicolor* (L.) Moench) employed in the study

Sr. No.	Genotypes	Pedigree	Features
1	M35-1	Selection from local Maldandi population	Drought tolerant, widely adopted variety, straw glume colour, pearly white grain colour
2	Phule Anuradha	RSLG-559 x RSLG-1175	Early drought tolerant verity, recommended for shallow soil cultivation
3	RSLG-2422	(SPV-1830 x RSLG-2332) X SPV-1830	High grain yield, shoot fly resistant
4	RSV-1876	RSV-768 x MSV-16	Charcoal rot tolerant, non lodging, shoot fly resistant, bolder grain
5	RSV-1910	RSV-769 x MSV-16	Early, drought tolerant, shoot fly resistant, tolerance to foliar diseases, straw glume colour
6	RSV-2371	Karnataka local x AKSV-15	Non lodging, shoot fly tolerant, charcoal rot resistant, Suitable for mechanical harvesting
7	RSV-1988	RSV-1003 x RSV-458	Early, shoot fly tolerant, charcoal rot resistant,

			straw glume colour, pearly white grain

Table 2. SSR Marker sequences detail used for molecular diversity analysis

S N	SSR Marker	F - Primer	R - Primer
1	Xtxp210	CGCTTTTCTGAAAATATTAAGGAC	GATGAGCGATGGAGGAGAG
2	Xtxp270	AGCAAGAAGAAGGCAAGAAGAAGG	GCGAAATTATTTTGAAATGGAGTTGA
3	Xsnp41	GCAGCGCTTCAATAGGTTCC	TCGGCACAACAGAGCTTGAC
4	Xsnp109	ATGCAGTTGCTACTGAGCTCCG	AAACCAAACCGAACGAACTGC
5	Xiabt437	ACCACTACCTGGGGTCTGTG	GAAATTCATGAACCCCAACG
6	Xiabt178	CATGTCGTTGGTGGAGTACG	GAGACTAGGCGTCACGGAAC
7	Xiabt312	GCCAAGTCCATGGTCAAGATG	CGTCAAAGCATAAAGCACCA
8	Xtxp15	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC
9	Xtxp278	GGGTTTCAACTCTAGCCTACCGAACTTCCT	ATGCCTCATCATCATGGTTCGTTTTGCTT
10	mSbCIR300	TTGAGAGCGGCGAGGTAA	AAAAGCCCAAGTCTCAGTGCTA
11	Sb5-206	ATTCATCATCCTCATCCTCGTAGAA	AAAAACCAACCCGACCCACTC

Table 3: Analysis details of SSR marker polymorphism used for study

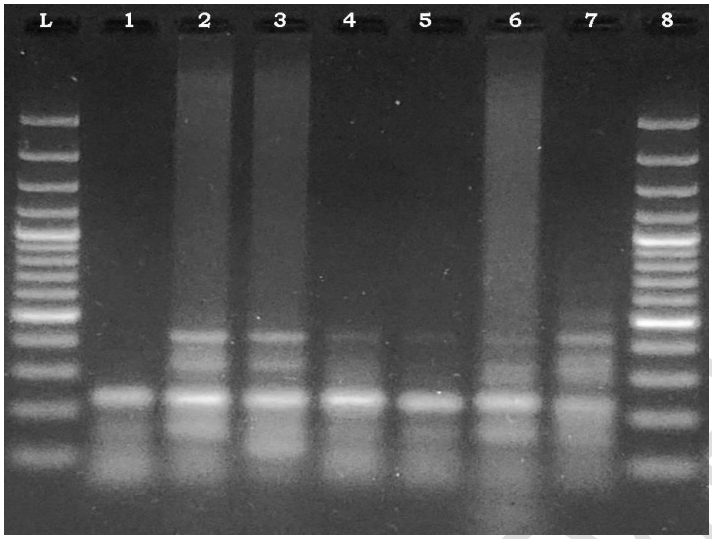
Sr. No.	Primer Name	Total Fragment	Polymorphic fragment	Monomorphic fragments	Polymorphism rate %	PIC Value
1	Xtxp210	3	2	1	66.67	0.6400
2	Xtxp270	4	0	4	0.00	0.6667
3	Xsnp41	3	2	1	66.67	0.5562
4	Xsnp109	6	4	2	66.67	0.8740
5	Xiabt437	4	3	1	75.00	0.7250
6	Xiabt178	4	3	1	75.00	0.5029
7	Xiabt312	5	5	0	100.00	0.7880
8	Xtxp15	7	6	1	85.71	0.8091
9	Xtxp278	2	2	0	100.00	0.2449
10	mSbCIR300	7	6	1	85.71	0.7469
11	Sb5-206	7	7	0	100.00	0.8210
Total		45	33	12	-	-
Mean		4.1	3	1.09	74.68	0.67043

Table 4. Genetic dissimilarity among *rabi* sorghum varieties as revealed by SSR markers

	M35-1	Phule Anuradha	RSLG-2422	RSV-1876	RSV-1910	RSV-2371	RSV-1988
M35-1	1.000						
Phule Anuradha	0.389	1.000					
RSLG-2422	0.389	0.846	1.000				
RSV-1876	0.545	0.400	0.436	1.000			
RSV-1910	0.611	0.342	0.342	0.458	1.000		
RSV-2371	0.429	0.550	0.550	0.438	0.519	1.000	

RSV-1988	0.317	0.767	0.689	0.333	0.310	0.535	1.000

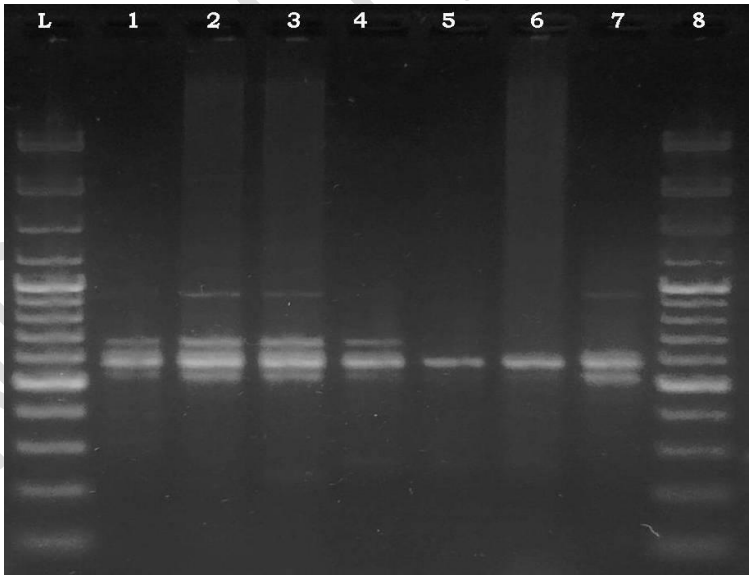
Fig. 1. Molecular profile of sorghum varieties as revealed by SSR marker Xtxp15



Where L-100 bp DNA ladder

1. M 35-1
2. Phule Anuradha
3. RSLG 2422
4. RSV 1876
5. RSV 1910
6. RSV 2371
7. RSV 1988

Fig. 2. Molecular profile of sorghum varieties as revealed by SSR marker Xiabt437



Where L-100 bp DNA ladder

1. M 35-1
2. Phule Anuradha
3. RSLG 2422
4. RSV 1876
5. RSV 1910
6. RSV 2371
7. RSV 1988

Fig. 3. Diagrammatic presentation of DNA fingerprints of 7sorghum varieties using 11SSR markers