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In vitro Screening of Molecular Diversity among Sorghums (*Sorghum bicolor* (L.) Landraces in the Marathwada Region by Molecular Markers

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ABSTRACT

Allelic variation is a valuable tool for displaying high levels of polymorphism within species and is closely correlated with crop productivity. In Marathawada, there is a significant amount of phenotypic heterogeneity among sorghum landraces. However, molecular variability needs to be reevaluated to identify any potential barriers that can interfere with current improvement initiatives. In the current work, we used 5 SSR markers to categorize 20 genotypes of the elite (Sorghum bicolor L Moench) accessions from the Marathwada region, including obicolourdard cultivars from various agro-economic zones. According to the results of this study, 14 alleles were found among the 20 genotypes, with a PIC value that ranged from 0.37 to 0.70 and a mean of 0.44 per locus. Each locus had anything from 1 (gpsb089) and 5, (mSbCIR223) with an average of 2.80 alleles per locus. A neighbor-joining tree was constructed and showed clustering of genotypes into two groups, which indicates that there is considerable diversity in genotypes compared with advanced cultivars for the desired genotype (IS1042) by using SSR markers. The results show that the most diverse cultivars were IS-4564, IS18357, and IS-18381, and significant variation was also reported in IS4566 and IS18379.

Keywords: Molecular diversity, Temperature stress, Sorghum, SSR, Neighbor-joining tree

INTRODUCTION

Sorghum (*Sorghum bicolor L. Moench*) is the third most important grain crop in India after rice, wheat, maize, and millet [1]. Maharashtra is the leading producer of sorghum, ranking third after wheat and rice, even though before the agricultural revolution, sorghum was ranked second in India in terms of area and production [2]. Globally, it is the fifth most important cereal after rice, wheat, maize, and barley. In alcohol production, sorghum is used to produce ethanol, starch, and syrups [3].

Sorghum belongs to the *Poaceae* family, the genus *Andropogoneae*, and the species *Sorghastrum*. Sorghum originated from the Greek word "sorgo," which means to supersede, as in having grown taller than in other crops in the field. The Sorghum family has been arranged into two all-around emblazoned areas, specifically Eu-sorghum and Para-sorghum [4]. We divided the category Sorghum into six subgenera, namely, *Eu-sorghum, Chaetero-sorghum, Heterosorghum, Para-sorghum, Sorghastrum,* and *Stipo-sorghum,* and officially assigned the specific name Sorghum imlgare to the detailed outline and its cross-viable wild and weed species members [5]. However, James Price assumed the title *Sorghum bicolor (L.)* Moench was correct, and this idea has indeed been endorsed by WenQian Kong, *Sorghum bicolor (L.)* Moench varies yearly with 10 sets of chromosomes (2n=20), which includes all advanced grain sorghum and Sorghum-sudan grass crossovers, as well as their cross-viable wild and weedy relatives [6,7].

The primary focus is on genetic analyses of sorghum for its excellent quality. [*Sorghum bicolor (L.*), 2n = 2x = 20] is the nation's fifth most important food crop after wheat, rice, maize, and barley, with over 80% of the crop grown in Africa and Asia [8]. The ICRISAT sorghum collection represents the major sorghum diversity centers. The National Plant Genetic Resources Centers house a wide range of sorghum landraces. The ICRISAT gene bank is a major source of diversity for subtropical crops [9].

SSR markers (Simple Sequence Repeats) are suitable to study genetic variability. Typical landraces, optimized cultivars, and breeder cultivars are among the accessions. A further important feature of SSR markers is their convenience of use [10]. DNA markers are typically nucleotide sequences that conform to a physical in a genome as well as their polymorphisms. SSR fingerprints are often extremely specific, and they are frequently used to differentiate various individuals and identities, as well as to expose polymorphisms [11,12].

Simple Sequence Repeat (SSR) markers, also called microsatellites, instantly became the DNA markers of choice for plant and animal genomes[13]. SSRs are tandem repeats of di-, tri-, tetra-, penta- or hexanucleotide units in the DNA of plants [12]. A good number of microsatellite markers have been developed for sorghum that allows a high rate of sorghum genotype assessment [14].

The analysis of genetic diversity in 20 sorghum accessions from ICRISAT genebank Hyderabad India, Both agro-morphological character and SSR markers. Protein and mineral composition vary considerably within and between 20 sorghum accessions from the ICRISAT genebank in Hyderabad, India [15]. The accessibility of such markers for evaluating genetic variation is a simple way for conventional breeding to offer integrated genetic traits for breeding programs. Predefined kinds of molecular markers used for sorghum diversity evaluations became relevant, and they differ in complexity, reliability, and information-generating capacity [16]. When genetic diversity was assessed using microsatellite (SSR) markers, a $p<0.05$ of genetic variation was evidenced among 20 sorghum accessions from different agroecological regions in Marathwada (70% between many accessions and 30 percent within accessions) [17].

Molecular markers and their value have indeed been demonstrated in studies of genetic variation, breeding systems, pollination biology, and pollination ecology. Crop diversification has increased the production of crops such as wheat via domestication, yet this has diminished crop genetic diversity. As a consequence, a strategy to enhance the advancement of a crop is necessary. Advanced species are determined by genetic diversity. For the protection and use of germplasm of particular species diversity, genetic diversity is essential.

To broaden the genetic base for crop improvement, it is essential to reveal genetic variation. The employment of markers serves the purpose of revealing genetic diversity. Variety understanding also assists in the development of methods for integrating relevant varieties into breeding plans. The use of markers to describe crops reveals both similarities (shared alleles) and diversity (typical alleles) across cultivars of the same crop. Additionally, it aids in determining the genetic pool or provenance of the selected crop. The genetic basis should be extended to develop elite cultivars. Species whose genetic diversity has indeed been discovered utilizing markers are utilized to generate superior hybrids via germ-line exchanges. Markers are used to characterize and construct a genetic profile. Such crop DNA profiles are used to maintain a crop's gene pool in the gene bank.

MATERIALS AND METHODS

The twenty seed material accessions for the current experiment were obtained from the ICRISAT gene bank, originally obtained from various locations of Maharashtra, with passport data for seeds listed in Table 1. These cultivars were selected because they had 90% to 100% germination rates and are cultivated in the semiarid regions of Maharashtra, India. Selected seeds were allowed to germinate by the blotter paper method under controlled and optimum conditions on Petri dishes lined with 75% humidity by Whatman No. 1 filter papers and incubated at 30°C. After germination, 16-day-old leaf tissues were harvested from 20 different sorghum accessions and stored at -20°C for DNA extraction.

Table 1. List of germplasm accessions along with their biological status and geographical location

Sr.no	Code no.	Accession I.D	Code no.	Biological Status	Collection Site
1	AC. 1	IS1042	AC. 1	Advanced/Improved cultivar	Parbhani; Parbhani district
2	AC. 2	IS 4516	AC. 2	Traditional cultivar/Landrace	Parbhani; Parbhani district
3	AC. 3	IS 4517	AC. 3	Traditional cultivar/Landrace	Parbhani; Parbhani district
4	AC. 4	IS 4518	AC. 4	Traditional cultivar/Landrace	Parbhani; Parbhani district
5	AC. 5	IS 4561	AC. 5	Traditional cultivar/Landrace	Parbhani; Parbhani district
6	AC. 6	IS 4562	AC. 6	Traditional cultivar/Landrace	Parbhani; Parbhani district
7	AC. 7	IS 4564	AC. 7	Traditional cultivar/Landrace	Parbhani; Parbhani district
8	AC. 8	IS 4565	AC. 8	Traditional cultivar/Landrace	Parbhani; Parbhani district
9	AC. 9	IS 4566	AC. 9	Traditional cultivar/Landrace	Parbhani; Parbhani district
10	AC. 10	IS 18356	AC. 10	Breeding/Research material	Parbhani; Parbhani district
11	AC. 11	IS 18357	AC. 11	Breeding/Research material	Parbhani; Parbhani district
12	AC. 12	IS 18358	AC. 12	Breeding/Research material	Parbhani; Parbhani district
13	AC. 13	IS 18359	AC. 13	Breeding/Research material	Parbhani; Parbhani district
14	AC. 14	IS 18360	AC. 14	Breeding/Research material	Parbhani; Parbhani district
15	AC. 15	IS 18361	AC. 15	Breeding/Research material	Parbhani; Parbhani district
16	AC. 16	IS 18378	AC. 16	Breeding/Research material	Parbhani; Parbhani district
17	AC. 17	IS 18379	AC. 17	Breeding/Research material	Parbhani; Parbhani district
18	AC. 18	IS 18380	AC. 18	Breeding/Research material	Parbhani; Parbhani district
19	AC. 19	IS 18381	AC. 19	Breeding/Research material	Parbhani; Parbhani district
20	AC. 20	IS 40778	AC. 20	Traditional cultivar/Landrace	Parbhani; Parbhani district

DNA Extraction

Genomic DNA was extracted from 4-day-old seedlings by the CTAB extraction method modified by [18]. Three to four seedlings from each sample were selected randomly, and roots and shoots were both sampled and used for DNA isolation. Genomic DNA was purified by RNAse treatment. The concentration of purified DNA was measured spectrophotometrically by using a UV visible spectrophotometer at 260 nm and 280 nm, and purity was also confirmed by gel electrophoresis in an electrophoresis system in a 0.8% (w/v) agarose gel containing 0.5 µl/ml ethidium bromide at 6 V/Cm in 1X TBE buffer. Approximately 20 ng to 100 ng of total DNA was selected for the next experiment [19] (Table 2).

Table 2. PCR Master mixture for the individual reaction of SSR analysis

Component	Concentration	Volume
Template DNA	10 ng	5.5 µl
$MgCl_2$	1.5 mM	0.50 µl
dNTP	0.1 mM	1.5 µl
Taq polymerase	0.2 U	0.25 µl
D/W	-	9.75 µl
Exon 2 Primer pair	25 µM	2.5 µl
Final Volume		20 µl

Polymerase Chain Reaction (PCR) amplification of SSR

The PCR program was set in a thermal cycler amplification reaction mixture prepared in 0.2 ml thin-walled flat cap PCR tubes containing the following components. The total volume of each reaction mixture was 20 µl, and the details of the PCR Master Mix are given in Table 2. Five pairs of SSR primers were chosen for the current experiment for the analysis of diversity among the selected accessions. kit29, which provides reasonable coverage across the sorghum nuclear genome. Details of the primer sequences and type of microsatellite repeats are depicted.

In Table 3, PCR The PCR program consisted of an initial denaturation for 30 seconds at 94°C and then 30 cycles of denaturation for 30 seconds at 94°C, annealing at 57°C (55°C or 60°C) for 60 seconds, depending on the annealing temperature for the primer, and extension at 72°C for 60 seconds. The last PCR cycle was followed by a 5 minutes extension at 72°C and then put on hold at 10°C, at infinity (∞). The amplified products were stored at -20°C until they were needed to run gels.

Table 3. Details of SSR Primer Sequence and microsatellite repeats

Sr. No.	Marker name	Forwards Primer	Reverse Primer	Allele Size	Allele No.
1	gpsb089	ATCAGGTACAGCAGGTAGG	ATGCATCATGGCTGGT	165-177	4
2	msbCIR223	CGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	108-118	5
3	msbCIR276	CCCCAATCTAACTATTTGGT	GAGGCTGAGATGCTCTGT	230-234	4
4	Xtxp321	TAACCCAAGCCTGAGCATAAGA	CCCATTCACACATGAGACGAG	192-252	9
5	Xtxp057	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	223-257	8

Gel electrophoresis of PCR products

After amplification, the amplified product was first separated by gel electrophoresis, and the gel was run using a 2.5% agarose gel. The gel contained 6.25 gm of agarose, weighed using a Mettler Toledo Electronic Balance, dissolved in 250 ml TAE in a conical flask (volume of flask is required), and the mouth was covered with cotton wool. The mixture was then heated in a microwave to dissolve and allowed to cool to approximately 60°C, and 5 µl of ethidium bromide was added [20,21]. This was swirled gently to avoid bubbling, and when cooled, the solution was poured into a mold with a comb placed on a level surface and allowed to cool and solidify. The comb was gently removed, and the gel was transferred into an electrophoretic tank filled with 1X TAE buffer. PCR products of 10 µl were loaded into each well and run at 90 V for 45 minutes. The gel was then observed under a UV trans illuminator and Gel Doc. Each set of PCR product data obtained from each accession was scored [22].

Scoring SSR bands

Products from any of the eight primers with different dye labels were pooled into groups based on their respective agarose band length and resolution capacity of dye labels. One microliter from each coloaded group was added to 8 µl of a solution containing 0.108 µl of GSLIZ500 internal size standards to 8 µl master mix and vortexed at 900 RCF for solution mixing. Vortexed samples were denatured and immediately placed on ice for 3 minutes. Each fragment analysis was performed separately to estimate the particular fragment size and allele size, and homozygous and heterozygous alleles were detected with gene mapper software. The amplified fragments were visualized using the GEL DOCUMENTARY unit. The size of the DNA fragments was determined using Pe, which could spontaneously recognize the lane and band and measure the fragment size. Genotypes of individual plants were indicated by the allele size at all SSR loci, and the pairwise different method was determined for each haplotype pair using the parameters. Polymorphic Information Content (PIC) values for each primer set were calculated using the algorithm described by Smith , R. K. Varshney and J. A. Davila, 2004[23,24].

$$PIC = 1 - \sum_{i=0}^{n} f_i^2 \quad i = 1$$

where f_i^2 is the frequency of the i[th] allele. PIC is the estimated polymorphism of the locus by taking into consideration not only the number of alleles that are expressed but also the estimated frequency of alleles. The PIC value ranges from 0 (monomorphic) to 1 (polymorphic). Statistical analysis of data was performed using POWER-MARKER Ver 3.25 to estimate genetic distance, locus, heterozygosity, PIC content, allele size, length, range, and abundance within chromosomes. It is also reported for allele size of loci and the product of SSR was used for genotyping [25]. The genetic diversity of the landraces was measured at different levels of population structure. The genetic variation of each locus shows the utility and general information of each SSR tool [26] (Table 4).

RESULTS AND DISCUSSION

Five SSR primers located in variable regions of chromosomes that we selected for the study of *In vitro* variability among sorghum accessions were highly polymorphic and therefore provided powerful data for the evaluation of molecular diversity within sorghum landraces. These results are also closely related to those of, those who concluded that wild sorghum and landraces possess greater allelic diversity than cultivars and might contain alleles not present in cultivars, which can strengthen further conservation and utilization [27]. This means that a higher level of polymorphism is directly proportional to unique replication slippage and mutations responsible for the generation of SSR allelic diversity [11,15]. As the results indicate that 5 markers were involved in this study, an average of 14 alleles were observed, with 2.5 alleles per locus, which is a direct and indirect consequence of artificial selection for traits that improve agronomic qualities. The favorable alleles and variation allow the fixation of traits among populations during the introduction and acclimatization of species. Similar results were found for Murray SC and Ritter KB [28,29]. The number of microsatellites in SSR markers is correlated with allele number and the PIC (polymorphic information content) ratio, which was found to be 0.37-0.70 with an average mean of 0.44. SSR markers with di-, tri-, and tetra-repeats show a higher PIC ratio than mono-repeats, which also means that the number of repeats is associated with a higher number of microsatellites and a higher PIC number. Similar results were found by G. Afolayan S. P. who also added that the PIC cannot be more than 0.50, which is contrary to what is obtainable for multiallelic markers, but according to Smita Shingane, the PIC value can give better and more information on variability, such as SSRs, in which the maximum number of repeats [15,22]. This further confirms the fact that this method detected more alleles than RAPD markers [4]. Similar PIC values found for J. A. Davila and G. Afolayan S. P. were significant with Joshi Akansha R and El-Awadybut significantly on par with J. A. Davila [13,15,24,26]. Thus, the above shows that significant variation is observed in the RAPD, SSR and RFLP marker findings, confirming the effective application of SSR markers over others in evaluating allele frequency and PIC content (Table 5). Average gene diversity is high, and according to Onyango, This strong allelic variation is due to di-nucleotide repeats containing SSRs as a result of high mutation rates among such repeats [25]. Earlier studies evaluating the genetic relation between the cultivars and landraces of wild relatives were successfully identified by H.A. Ajeigbe and Maugham [8]. Based on their studies, 20 sorghum accessions can be divided into three different groups; hence, SSR marker analysis is an effective tool for the quantification of genetic diversity.

Table 4. Analysis of genetic diversity across the sorghum accession

S. No.	Diversity Traits	Number	Range	
1	Total no. of alleles	14	Maximum	Minimum
2	Mean no. of alleles per locus	2.8	5	1
3	Mean major allele frequency	0.53	1	0.3
4	Mean Gene Diversity	0.51	0.7	0
5	Mean PIC	0.44	0.7	0

Table 5. Diversity indicators revealed by 5 SSR primer combination used in the study

Marker	PIC	Range	Alleles			Repeat length	H	GD	MGD	PIC	MPIC
		(mw)	No	%	freq.						
GPSB089	1	165-173	2	36	1	2	0.45	0.61	0.44	0.55	0.51
msbCIR223	2	108-118	5	41	5	2	0.42	0.74	0.44	0.7	0.51
msbCIR276	3	230-234	3	52	3	2	0.78	0.65	0.44	0.58	0.51
XTTXP321	8	192-252	1	78	2	2	0.55	0.49	0.44	0.37	0.51
XTSEP057	9	223-257	3	65	3	2	0.4	0.65	0.44	0.58	0.51

Of the five SSR markers, MsbCIR223 and XTSEP SSRs usually had more SSR loci in 20 accessions than in the other accessions, which may be due to differences in allele number and position in the chromosome. XTSEP markers are reported to be extracted from small tandem repeat libraries [30]. These loci were therefore more likely to include noncoding regions than the other SSR primers. A Neighbor-Joining (NJ) tree was constructed based on genetic distance (Figure 1). Group I in the NJ tree consists of most of the traditional landraces, whereas group II consists of mostly breeding material. Furthermore, in groups 1 and 2, the accessions were classified into sub-sub groups based on their origin and types. Similar observations have been made by many investigators in different crops [31,32].

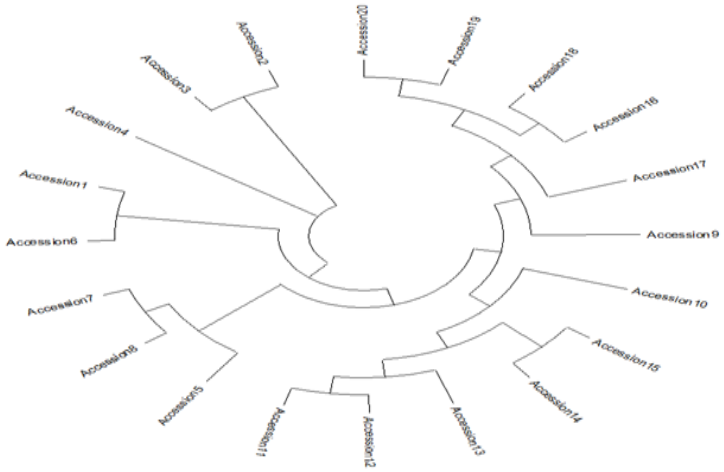

Figure 1. Neighbor-joining (NJ) tree of 20 Sorghum accessions constructed using POWER MARKER software

CONCLUSION

Our results suggest that sorghum accessions from different geographic origins or institutions could come from the same gene pool and may not be useful for continual sorghum improvements. Therefore, when planning for a new germplasm collection and conservation, it is imperative to characterize the available germplasm before commencing a breeding program. Conclusively, our study revealed high genetic diversity within the sorghum germplasm. Hence, these sorghum accessions will be protected from genetic vulnerability to biotic and abiotic stress in the environment, and breeders will guide how to utilize and conserve germplasm.

REFERENCES

1. Zabala, E.C., DeJesus, N.G., and Battad, Z.M., Acceptability of Food Products from Sweet Sorghum Sorghum bicolor L. Moench Grain Developed at Pampanga Agricultural College, Philippines. *International Journal on Advanced Science, Engineering and Information Technology*, **2015**.5(4):p. 286-290.
2. Shaik, S., Dayakar, B., and Janaiah, A., Indian State-Level Rice Productivity And Its Impact On Poverty Alleviation. *In2004 Annual meeting, August 1-4, Denver, CO 2004 (No. 20228). American Agricultural Economics Association (New Name 2008: Agricultural and Applied Economics Association)*,**2004**.
3. Nayak, A.K., et al., Carbon and water footprint of rice, wheat & maize crop productions in India. *Pedosphere*, **2022**.
4. Hariprasanna, K., and Patil, J.V., Sorghum: origin, classification, biology and improvement. *Sorghum molecular breeding*, **2015**:p. 3-20.
5. Ma, K.H., et al., Development of SSR markers for studies of diversity in the genus Fagopyrum. *Theoretical and Applied Genetics*, **2009**.119:p. 1247-1254.
6. Price, H.J., et al., Genome evolution in the genus Sorghum (Poaceae). *Annals of Botany*, **2005**. 95(1):p. 219-227.
7. Kong, W., et al., Genotyping by sequencing of 393 Sorghum bicolor BTx623× IS3620C recombinant inbred lines improves sensitivity and resolution of QTL detection. *G3: Genes, Genomes, Genetics*, **2018**. 8(8):p. 2563-2572.
8. Ajeigbe, H.A., et al., Handbook on Improved Agronomic Practices for Sorghum Production in North East Nigeria, **2020**.
9. Kimber, C.T., Dahlberg, J.A., and Kresovich, S., The gene pool of Sorghum bicolor and its improvement. *Genomics of the Saccharinae*, **2013**:p. 23-41.
10. Min, W., et al., Assessing the genetic diversity of cultivars and wild soybeans using SSR markers. *African Journal of Biotechnology*, **2010**.9(31):p. 4857-4866.
11. Agarwal, M., Shrivastava, N., and Padh, H., Advances in molecular marker techniques and their applications in plant sciences. *Plant cell reports*, **2008**.27:p. 617-631.
12. Kalia, R.K., et al., Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, **2011**. 177(3):p. 309-334. [Goolge Scholar]
13. El-Awady, M., et al., Genetic diversity among Sorghum bicolor genotypes using simple sequence repeats (SSRs) markers. *Arab Journal of Biotechnology*, **2008**.11(2):p. 181-92.
14. Ng'uni, D., et al., Comparative genetic diversity and nutritional quality variation among some important Southern African sorghum accessions ['Sorghum bicolor'(L.) Moench]. *Australian Journal of Crop Science*, **2012** .6(1):p. 56-64.

15. Afolayan, G., et al., Genetic diversity assessment of sorghum (Sorghum bicolor (L.) Moench) accessions using single nucleotide polymorphism markers. *Plant Genetic Resources*, **2019**. 17(5):p. 412-420.

16. Cavagnaro, P.F., et al., Microsatellite isolation and marker development in carrot-genomic distribution, linkage mapping, genetic diversity analysis and marker transferability across Apiaceae. *BMC genomics*, **2011**:p. 1-20.

17. Geleta, N., Labuschagne, M.T., and Viljoen, C.D., Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. *Biodiversity & Conservation*, **2006**.15:p. 3251-3265.

18. Khan, I.A., et al., A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. *Plant Molecular Biology Reporter*, **2004**.22:p. 89.

19. Stein, N., Herren, G., and Keller, B., A new DNA extraction method for high-throughput marker analysis in a large-genome species such as Triticum aestivum. *Plant breeding*, **2001**. 120(4):p. 354-356.

20. Stirling, D., Multiplex amplification refractory mutation system for the detection of prothrombotic polymorphisms. *PCR protocols*, **2003**:p. 323-325.

21. Dubey, S.C., et al., Diversity of Rhizoctonia solani associated with pulse crops in different agro-ecological regions of India. *World Journal of Microbiology and Biotechnology*, **2014**. 30:p. 1699-1715.

22. Shingane, S., et al., Assessing genetic diversity among Foxtail millet (Setaria italica (L.) P. Beauv.) accessions using RAPD and ISSR markers. *International Journal of Bio-resource and Stress Management*, **2018**.9(1):p. 1-6.

23. Varshney, R.K., et al., A high density barley microsatellite consensus map with 775 SSR loci. *Theoretical and Applied Genetics*, **2007**.114:p. 1091-1103.

24. . Davila, J.A., et al., Comparison of RAMP and SSR markers for the study of wild barley genetic diversity. *Hereditas*,**1999**.131(1):p. 5-13.

25. Ngugi, K., and Onyango, C.M., Analysis of the molecular diversity of Kenyan sorghum germplasm using microsatellites. *Journal of Crop Science and Biotechnology*, **2012**.15:p. 189-194.

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

27. Mondini, L., Noorani, A., and Pagnotta, M.A., Assessing plant genetic diversity by molecular tools. *Diversity*, **2009**. 1(1):p. 19-35.

28. Murray, S.C., et al., Sweet sorghum genetic diversity and association mapping for brix and height. *The plant genome*, **2009**. 2(1):p. 48-62.

29. Ritter, K.B., et al., An assessment of the genetic relationship between sweet and grain sorghums, within Sorghum bicolor ssp. bicolor (L.) Moench, using AFLP markers. *Euphytica*, **2007**.157:p. 161-176.

30. Murray, A.E., Hollibaugh, J.T., and Orrego, C., Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Applied and Environmental Microbiology*, **1996** .62(7):p. 2676-2680.

31. Huo, Y., et al., An integrated strategy for target SSR genotyping with toleration of nucleotide variations in the SSRs and flanking regions. *BMC bioinformatics*, **2021**:p. 1-4.

32. Milkman, R., Horizontal transfer, genomic diversity, and genomic differentiation. *Microbial Evolution: Gene Establishment, Survival, and Exchange*, **2004**:p. 295-318.