*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6880

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **6 (5):** 150-160 (2018)

*Research Article*

International Journal of Pure & Applied Bioscience

OPEN ACCESS

# Genetic Variability Analysis of Sorghum (*Sorghum bicolor* L. Moench) Genotypes by Using SSR Markers

**Zinzala Sumita, Davda B. K., Modha K. G., Vadodariya G. D. and Rathod Snehal**

Department of Genetics and Plant Breeding,

N. M. College of Agriculture, NAU, Navsari-396450, Gujarat

*Corresponding Author E-mail: sumitazinzala@gmail.com

Received: 14.07.2018 | Revised: 21.08.2018 | Accepted: 28.08.2018

**ABSTRACT**

*DNA markers are being increasingly utilized in cultivar development, seed production quality control, measurement of genetic diversity for conservation, management, varietal identity and to tackle issues related to intellectual property rights. The aim of this study was to assess genetic variability among 24 genotypes of sorghum by simple sequence repeats (SSRs) technique using 22 primers set. The total number of amplified PCR bands was 460, of which 436 (94.78%) were polymorphic displaying PIC values ranging from 0.11 to 0.77 with an average of 0.52. Genetic coefficient of similarity among the genotypes ranged from 0.25 to 1.00. The use of simple sequence repeats (SSRs) for variety profiling can provide high discrimination with excellent reproducibility at low cost.*

***Key word:*** *Sorghum, variability, SSR marker.*

## INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the major cereal crops of the semi-arid tropics. Sorghum comes fifth after wheat, rice, maize and barley at world level both in area and production. Cultivated sorghum is grouped into five races based on panicle morphology *viz.*, bicolor, kafir, guinea, caudatum and durra. Cultivated sorghum is diploid (2n=2x=20) species. Genome size of sorghum is 730 Mb[10]. Sorghum is a multi-purpose crop, yielding food in the form of grain, fuel in the form of ethanol from its stem juice, and fodder from its leaves and bagasses. It is the major source of food for millions of people in tropics and semi-arid tropics. The stems and foliage are used as green fodder, hay, silage and pasture.

Genetic diversity analysis of sorghum germplasm is fundamental for breeding and conservation strategies.

**Cite this article:** Sumita, Z., Davda, B.K., Modha, K.G., Vadodariya, G.D. and Snehal, R., Genetic Variability Analysis of Sorghum (*Sorghum bicolor* L. Moench) Genotypes by Using SSR Markers, *Int. J. Pure App. Biosci.* **6(5):** 150-160 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6880

Genetic advancement during selection depends on the availability of genotypes possessing favorable alleles for desired traits, which relies on the available genetic diversity. Genetic diversity analysis can be carried out using morphological observation or molecular markers. DNA-based molecular markers are more efficient to analyze a greater number of genotypes[11]. Furthermore, monogenic nature of molecular markers detect the presence of favorable alleles among germplasm and allow estimation of genetic diversity more reliably and efficiently than complex phenotypic markers, which are subjected to continuous variation as well as genotype by environment interaction.

Simple sequence repeats (SSRs) are marker of choice for diversity analysis because of their ability to produce informative multiallelic loci and greater genotypic differentiations. SSRs are highly polymorphic[4] and provide wider genome coverage than amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. The sorghum genome sequence project identified 71000 SSRs in the genome[10]. The availability of this large number of SSR markers provides a more cost-effective and rapid method for DNA profiling[12].

## MATERIAL AND METHODS

### Plant material

Twenty four genotypes of sorghum shown in the table 1 were used in the current study. Seedlings of genotypes were grown at greenhouse, Department of Genetics and Plant Breeding, NMCA, NAU, Navsari, Gujarat in polythene covers and fresh young leaves of seedlings were collected for DNA extraction.

### DNA Extraction

Total genomic DNA was extracted from the 30 days mature leaves by Cetyl Trimethyl Ammonium Bromide (CTAB) method[17] with some modifications.

**Table 1: List of 24 sorghum genotypes used in the experiment**

| Sr. No | Name of Genotypes | Sr. No | Name of Genotypes |
|---|---|---|---|
| 1 | GJ-36 | 13 | SR-2958 |
| 2 | GJ-41 | 14 | GJ-38 |
| 3 | NIZER GOTI | 15 | SR-2812 |
| 4 | GJ-35 | 16 | SR-2949 |
| 5 | SR-2970 | 17 | SR-2972 |
| 6 | SR-2973 | 18 | SR-2960 |
| 7 | SR-2914 | 19 | GJ-39 |
| 8 | SR-2957 | 20 | SR-2975 |
| 9 | GJ-40 | 21 | ICSR-13008 |
| 10 | SR-2987 | 22 | BP-53 |
| 11 | SURAT LOCAL | 23 | RS-627 |
| 12 | GJ-42 | 24 | SR-2872 |

**Simple sequence repeats (SSR) marker analysis**

The PCR reactions for SSR were carried out according to method given by Yadav *et al.*[14] with some modifications.

**PCR Components**

The PCR amplification reactions were performed in a final volume of 25 µl, consisting of 1x PCR reaction buffer, 1 µl Forward primer, 1 µl Reverse primer, 2 µl dNTP mix, 2.5 µl Taq buffer (10X) with MgCl2, 0.3 µl Taq polymerase, 16.7 µl sterile distilled water and about 1.5 µl of genomic DNA. The amplifications were performed using a Thermocycler (BIO-RAD, USA). The amplifications were programmed for 5 min at 94°C for initial denaturation, followed by 35 cycles of 30 sec at 94°C; 30 sec at respective annealing temperature for annealing and 2 min at 72°C for extension, using the fastest possible transition times between each temperature. A final extension was programmed for 7 minutes at 72°C and this was followed by halt at 4°C until samples were collected. Amplification products were resolved by 2% agarose gels run in 1x TAE (10 mM Tris-HC1 and 1 mM EDTA) buffer, pH 8.0 for 2 hr at 80 volt. The gel was stained with ethidium bromide (10 mg/ml) and the DNA fragments (bands) were detected by UV trans-illumination using gel documentation system. A band was scored as present (1) or absent (0).

**Data analysis**

Polymorphic products were used to generate Jaccard's similarity coefficient by NT-SYS-pc version 2.02e software. A dendrogram was constructed based on Jaccard's similarity coefficient using the markers data for all genotypes. Polymorphism information content (PIC) values were calculated as in Anderson *et al.*[5], who assumed homologous alleles. PIC for a locus is calculated as:

$PIC = 1- \Sigma P^2_{ij}$

Where,

Pij is the relative frequency of the jth allele of the ith locus, summed over all the alleles for individual marker locus over all lines. A marker with a PIC value of more than 0.5 is considered as highly informative, between 0.25 and 0.5 as informative and less than 0.25 as slightly informative[6]. The genetic diversity was estimated by similarity indices calculated from sharing data of each pair of DNA fingerprints.

## RESULTS AND DISCUSSION

The study was aimed at determining the genetic variability among sorghum genotypes. Twenty two primer pairs were used to amplify the repeated regions in the sorghum samples presented in table 2. Eighteen of the twenty two primers tested produced informative, polymorphic products resolvable by agarose gel electrophoresis. Plates 1-18 show the PCR products of the amplified fragments. The markers produced 460 bands, 436 of them (94.78%) were polymorphic displaying PIC values ranging from 0.11 to 0.77 with an average of 0.52 (Table 2). The result of the present study was in agreement with finding of Wang *et al.*[13], Madhusudhana *et al.*[8] Abraha *et al.*[1], Adugna[2], Mofokeng *et al.*[9] and Disasa *et al.*[7].

**Table 2: Total number of bands, polymorphic bands and percentage of polymorphisms using eighteen pair of SSR primers in 24 sorghum genotypes**

| Primer | Annealing temperature in $^0$C | Total number of bands | Number of polymorphic bands | Percentage of polymorphisms | PIC |
|---|---|---|---|---|---|
| Xtxp18 | 55 | 24 | 24 | 100 | 0.57 |
| SB4558 | 55 | 24 | 24 | 100 | 0.57 |
| Xtxp400 | 61.4 | 24 | 24 | 100 | 0.53 |
| Xisep1236 | 61.4 | 24 | 24 | 100 | 0.53 |
| Xtxp406 | 54.5 | 24 | 24 | 100 | 0.57 |
| SB4560 | 61.4 | 45 | 45 | 100 | 0.11 |
| Xtxp211 | 59 | 24 | 24 | 100 | 0.57 |
| Xtxp50 | 57.7 | 26 | 26 | 100 | 0.49 |
| SB1028 | 57.7 | 23 | 23 | 100 | 0.61 |
| Xtxp616 | 57.7 | 25 | 25 | 100 | 0.60 |
| Xtxp65 | 55 | 24 | 24 | 100 | 0.57 |
| Xnhsbm1102 | 59 | 24 | 24 | 100 | 0.57 |
| Xnhsbm1103 | 55 | 24 | 24 | 100 | 0.53 |
| SB05-3693459 | 57.7 | 24 | 24 | 100 | 0.68 |
| SB2385 | 57.7 | 23 | 23 | 100 | 0.61 |
| SB2386 | 59 | 30 | 30 | 100 | 0.77 |
| SB2387 | 57.7 | 24 | 0 | 0 | 0 |
| SB2388 | 61.4 | 24 | 24 | 100 | 0.53 |
| Total | | 460 | 436 | 94.78 | - |
| Average | | 25.55 | 24.22 | 94.78 | 0.52 |

**Table 3: Jaccard's similarity coefficient for 24 genotypes of sorghum based on SSR marker**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 0.80 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.68 | 0.65 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.90 | 0.89 | 0.73 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| 5 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | | | | | | | | | | | | | | | | | | | |
| 6 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | | | | | | | | | | | | | | | | | | |
| 7 | 0.81 | 0.80 | 0.66 | 0.90 | 0.90 | 0.90 | 1.00 | | | | | | | | | | | | | | | | | |
| 8 | 0.90 | 0.72 | 0.61 | 0.81 | 0.81 | 0.81 | 0.90 | 1.00 | | | | | | | | | | | | | | | | |
| 9 | 0.81 | 0.80 | 0.66 | 0.90 | 0.90 | 0.90 | 0.80 | 0.73 | 1.00 | | | | | | | | | | | | | | | |
| 10 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | 0.90 | 0.81 | 0.90 | 1.00 | | | | | | | | | | | | | | |
| 11 | 0.46 | 0.37 | 0.46 | 0.44 | 0.44 | 0.44 | 0.39 | 0.41 | 0.39 | 0.44 | 1.00 | | | | | | | | | | | | | |
| 12 | 0.81 | 0.80 | 0.66 | 0.90 | 0.90 | 0.90 | 0.80 | 0.73 | 0.80 | 0.90 | 0.50 | 1.00 | | | | | | | | | | | | |
| 13 | 0.80 | 0.78 | 0.72 | 0.89 | 0.89 | 0.89 | 0.80 | 0.72 | 0.80 | 0.89 | 0.37 | 0.80 | 1.00 | | | | | | | | | | | |
| 14 | 0.81 | 0.80 | 0.66 | 0.90 | 0.90 | 0.90 | 0.80 | 0.73 | 0.80 | 0.90 | 0.50 | 0.80 | 0.80 | 1.00 | | | | | | | | | | |
| 15 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | 0.90 | 0.81 | 0.90 | 1.00 | 0.44 | 0.90 | 0.89 | 0.90 | 1.00 | | | | | | | | | |
| 16 | 0.95 | 0.85 | 0.70 | 0.95 | 0.95 | 0.95 | 0.85 | 0.86 | 0.85 | 0.95 | 0.48 | 0.85 | 0.85 | 0.85 | 0.95 | 1.00 | | | | | | | | |
| 17 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | 0.90 | 0.81 | 0.90 | 1.00 | 0.44 | 0.90 | 0.89 | 0.90 | 1.00 | 0.95 | 1.00 | | | | | | | |
| 18 | 0.73 | 0.71 | 0.73 | 0.80 | 0.80 | 0.80 | 0.72 | 0.66 | 0.72 | 0.80 | 0.44 | 0.72 | 0.71 | 0.72 | 0.80 | 0.77 | 0.80 | 1.00 | | | | | | |
| 19 | 0.81 | 0.80 | 0.81 | 0.90 | 0.90 | 0.90 | 0.80 | 0.73 | 0.80 | 0.90 | 0.50 | 0.80 | 0.80 | 0.80 | 0.90 | 0.85 | 0.90 | 0.90 | 1.00 | | | | | |
| 20 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | 0.90 | 0.81 | 0.90 | 1.00 | 0.44 | 0.90 | 0.89 | 0.90 | 1.00 | 0.95 | 1.00 | 0.80 | 0.90 | 1.00 | | | | |
| 21 | 0.42 | 0.44 | 0.48 | 0.46 | 0.46 | 0.46 | 0.40 | 0.37 | 0.40 | 0.46 | 0.39 | 0.40 | 0.44 | 0.40 | 0.46 | 0.44 | 0.46 | 0.46 | 0.52 | 0.46 | 1.00 | | | |
| 22 | 0.37 | 0.44 | 0.60 | 0.40 | 0.40 | 0.40 | 0.35 | 0.33 | 0.35 | 0.40 | 0.25 | 0.35 | 0.44 | 0.35 | 0.40 | 0.39 | 0.40 | 0.40 | 0.46 | 0.40 | 0.72 | 1.00 | | |
| 23 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | 0.90 | 0.81 | 0.90 | 1.00 | 0.44 | 0.90 | 0.89 | 0.90 | 1.00 | 0.95 | 1.00 | 0.80 | 0.90 | 1.00 | 0.46 | 0.40 | 1.00 | |
| 24 | 0.90 | 0.89 | 0.73 | 1.00 | 1.00 | 1.00 | 0.90 | 0.81 | 0.90 | 1.00 | 0.44 | 0.90 | 0.89 | 0.90 | 1.00 | 0.95 | 1.00 | 0.80 | 0.90 | 1.00 | 0.46 | 0.40 | 1.00 | 1.00 |

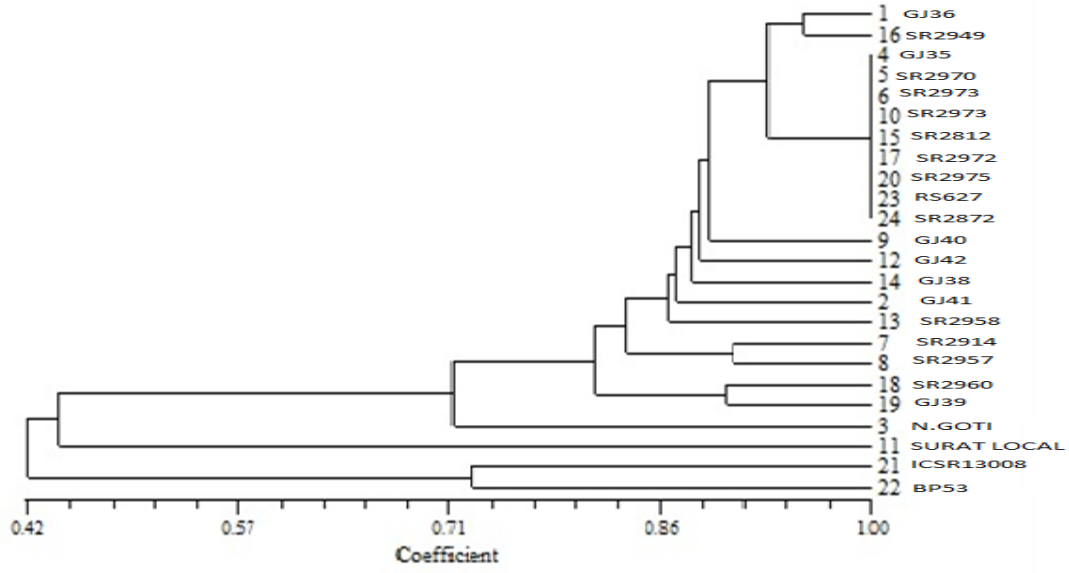


**Fig. 1: Dendrogram generated for twenty four sorghum genotypes using UPGMA cluster analysis based on jaccard's similarity coefficient using SSR marker**

Similarity indices based on the polymorphic data obtained were used to estimate the genetic relatedness among the sorghum genotype. Results (Table 3) indicate that the genetic similarity coefficients for all genotypes based on SSR markers ranged from 0.25 to 1.00. Present study is in confirmation with results obtained by Ali *et al.*[3], Yonemaru *et al.*[15] and Zhan *et al.*[16]. It is also evident that genotypes GJ-35, SR 2970, SR 2973, SR 2987, SR 2912, SR 2972, SR 2975, RS 627 and SR 2872 are the most closely related genotypes as they showed the highest similarity index (1.00), while the genotypes SURAT LOCAL and BP-53 with the lowest index (0.25), are the most distantly related.

The dendrogram was constructed from the binary data deduced from the SSR profiles of the samples. The first major cluster contained two sub-clusters shown in Fig.-1. Sub cluster I was the largest with 21 genotypes categorized in ten groups followed by cluster II with 2 genotypes. Group one consisted GJ-36 and SR 2949 with a genetic similarity of 95 per cent. Group two comprised of GJ-35 SR 2970, SR 2973, SR 2987, SR 2972, SR 2812, SR 2975, RS 627 and SR 2872 with a highest genetic similarity of 100 per cent.

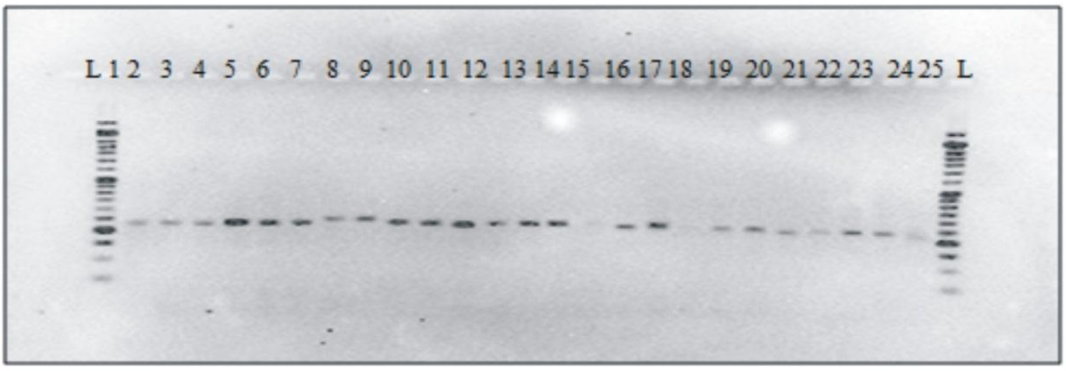


Plate-1: SSR gel profiling of sorghum genotypes for Xtxp18 primer

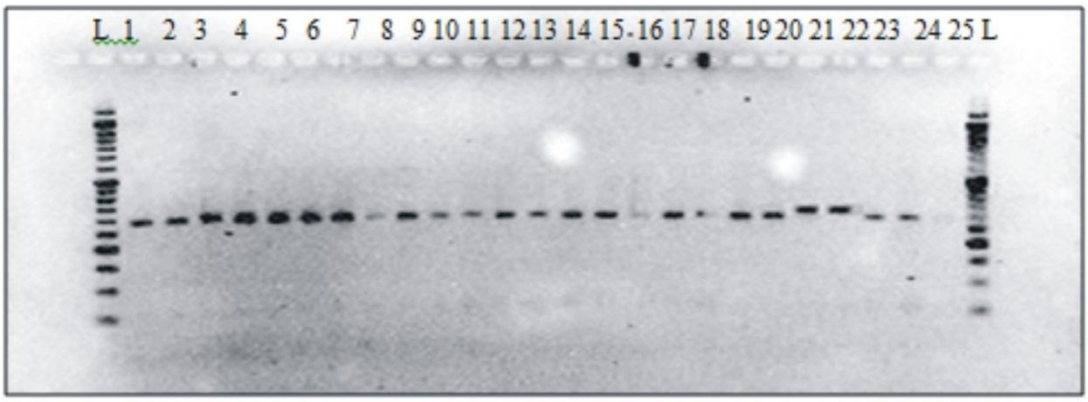


Plate-2: SSR gel profiling of sorghum genotypes for SB4558 primer

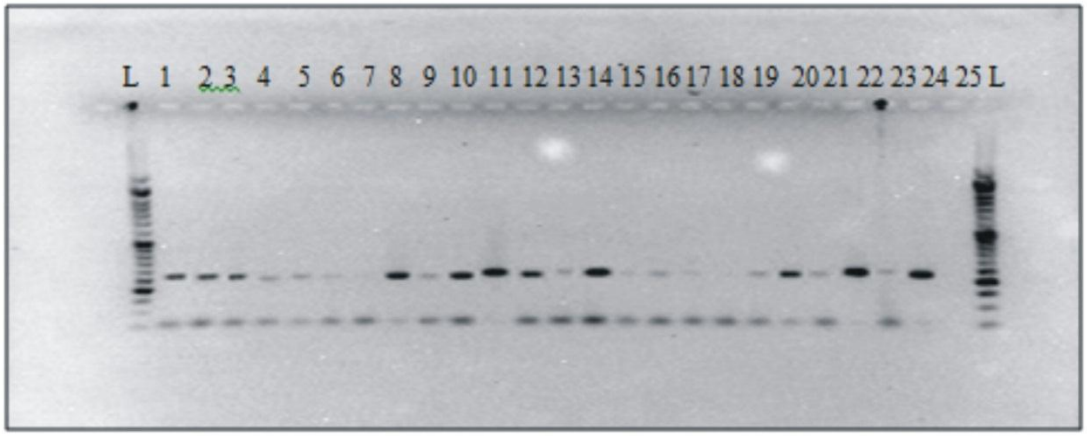


Plate-3: SSR gel profiling of sorghum genotypes for Xtxp400 primer
L- 50 bp DNA ladder

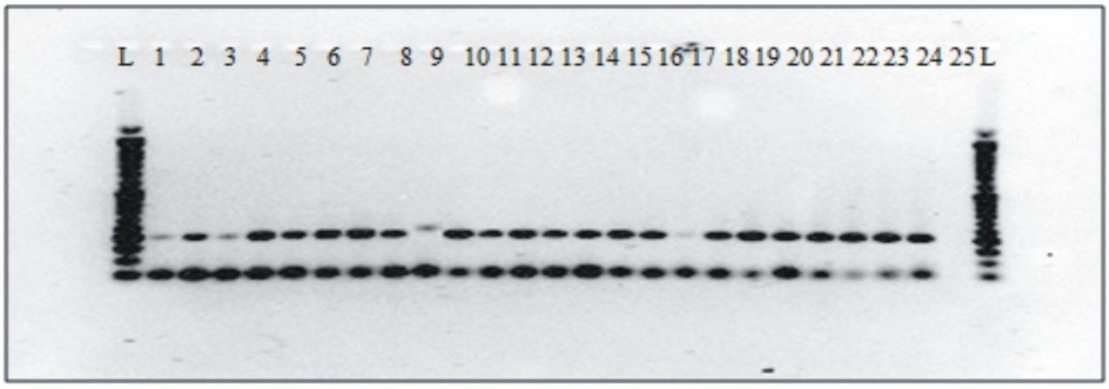


Plate-4: SSR gel profiling of sorghum genotypes for Xisep1236 primer

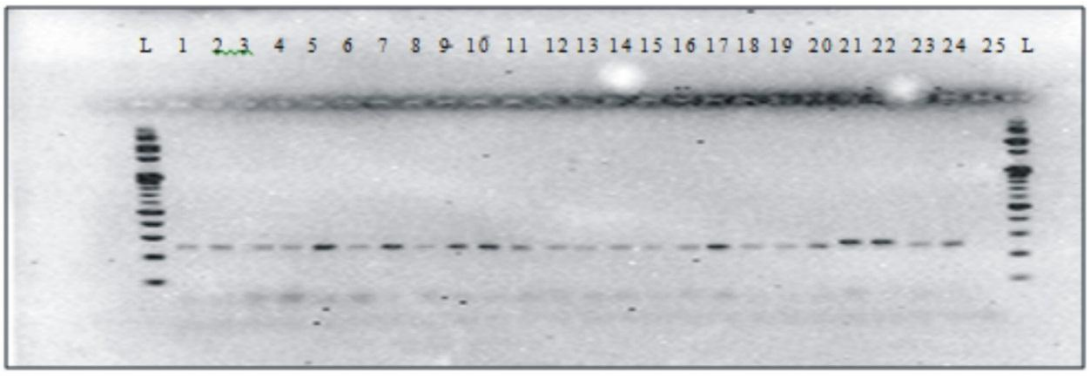


Plate-5: SSR gel profiling of sorghum genotypes for Xtxp406 primer

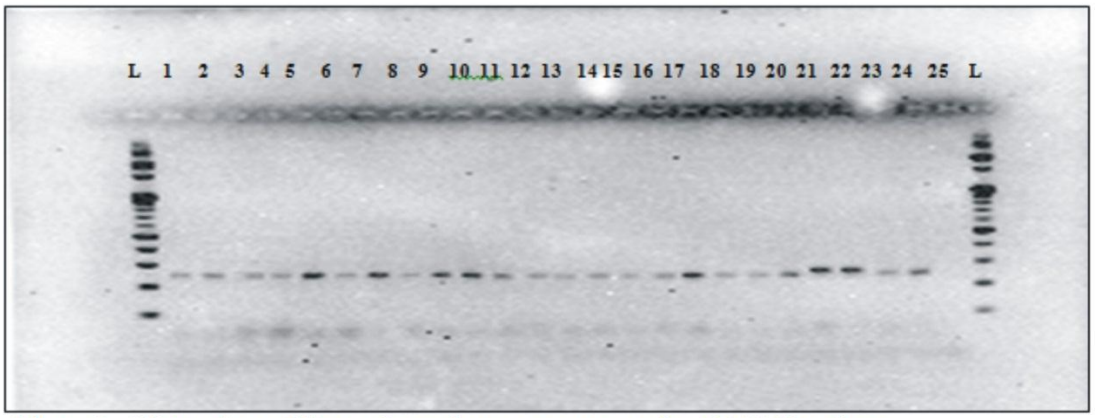


Plate-6: SSR gel profiling of sorghum genotypes for SB4560 primer
L- 100 bp DNA ladder

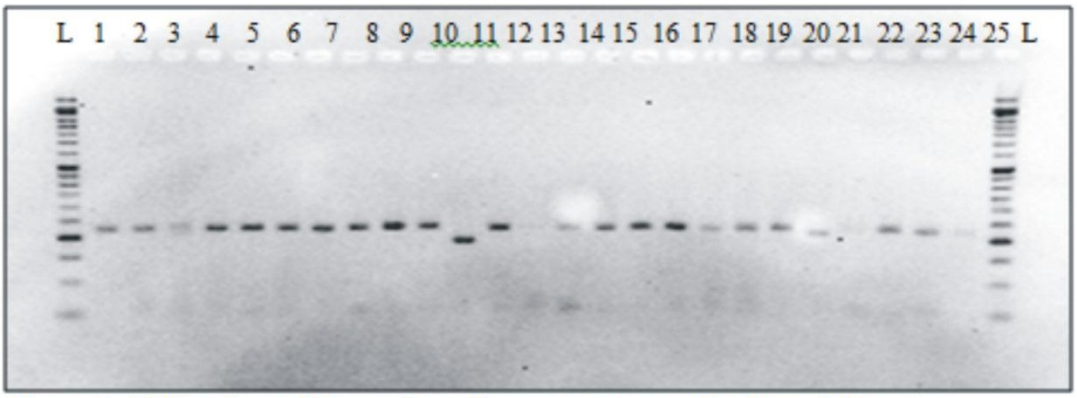


Plate-7: SSR gel profiling of sorghum genotypes for Xtxp211 primer
L-50 bp DNA ladder

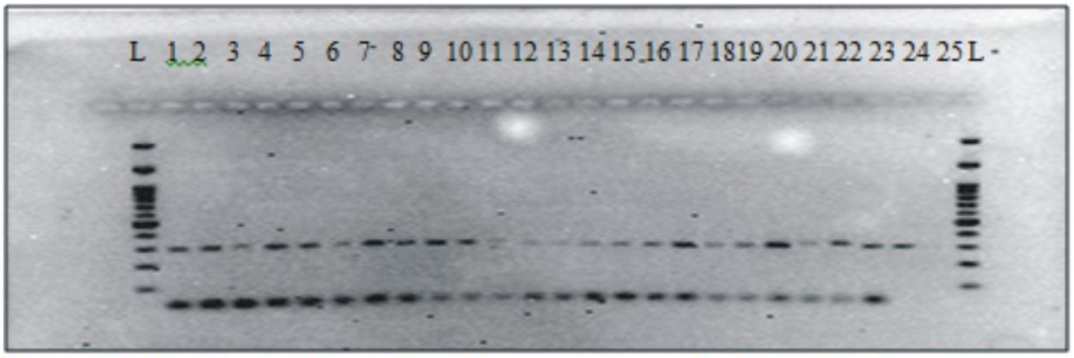


Plate-8: SSR gel profiling of sorghum genotypes for Xtxp50 primer

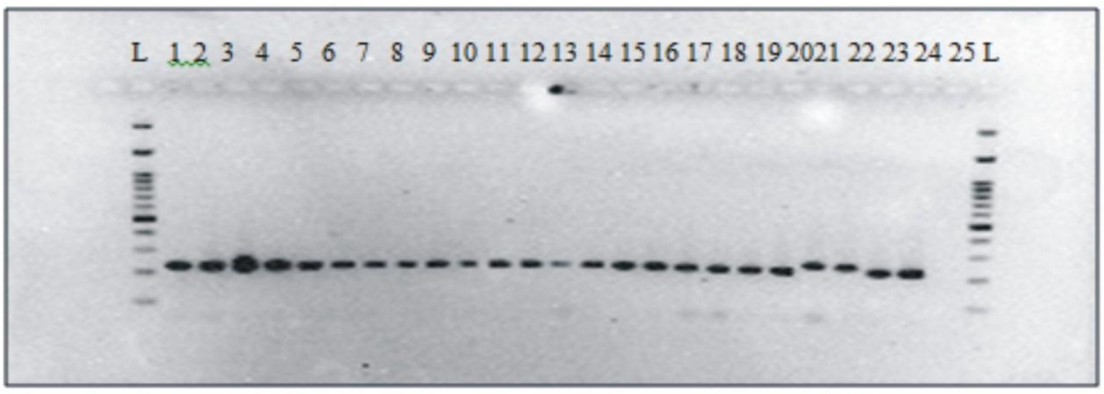


Plate-9: SSR gel profiling of sorghum genotypes for SB1028 primer
L- 100 bp DNA ladder

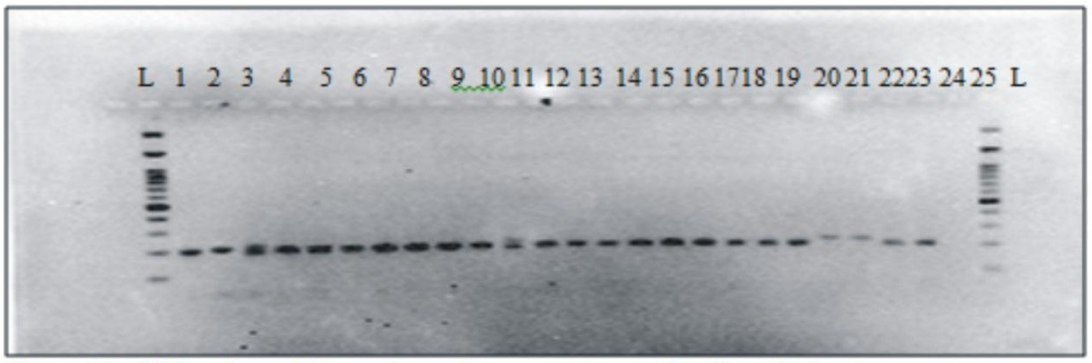


Plate-10: SSR gel profiling of sorghum genotypes for Xtxp616 primer

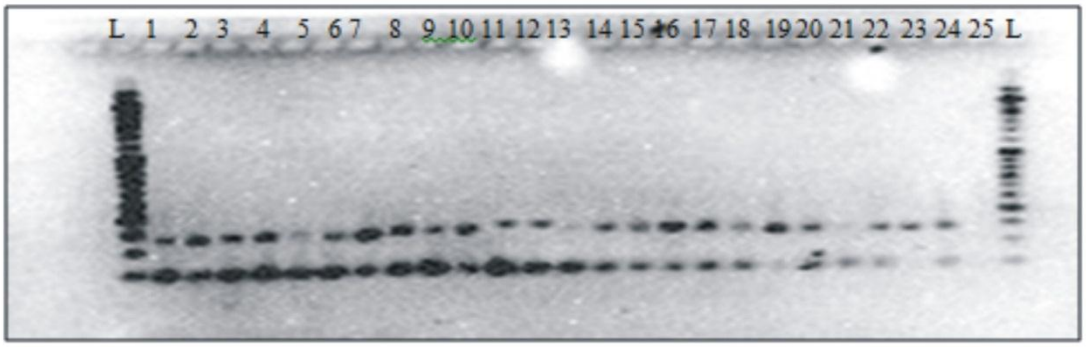


Plate-11: SSR gel profiling of sorghum genotypes for Xtxp65 primer
L- 100 bp DNA ladder

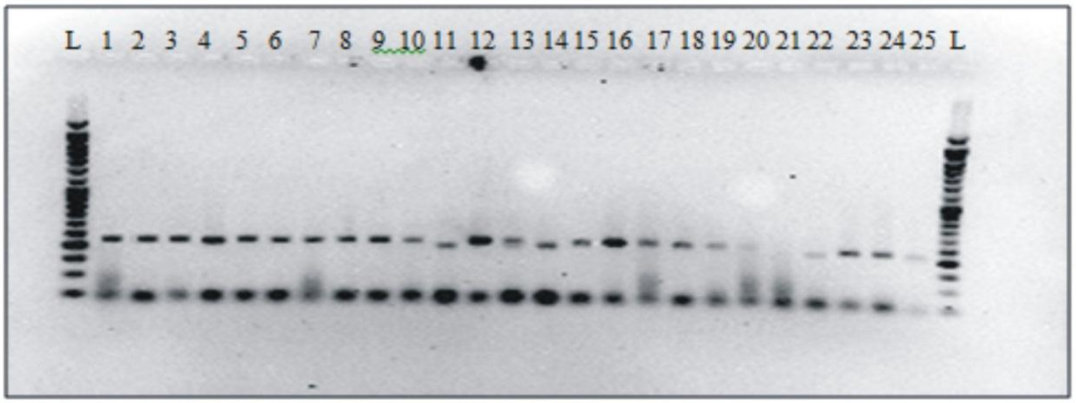


Plate-12: SSR gel profiling of sorghum genotypes for Xnhsbm1102 primer
L- 50 bp DNA ladder

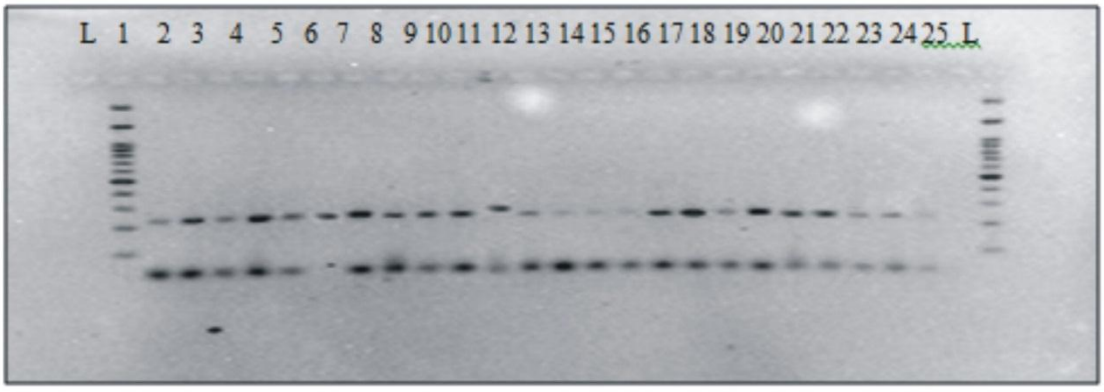


Plate-13: SSR gel profiling of sorghum genotypes for Xnhsbm1103 primer

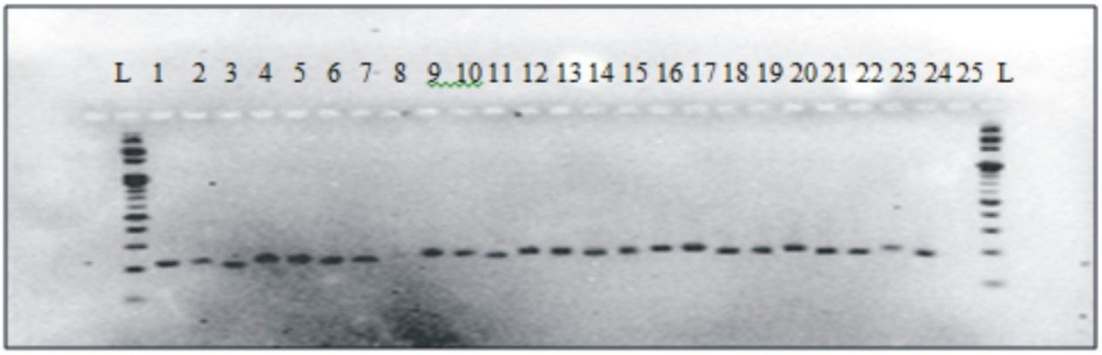


Plate-14: SSR gel profiling of sorghum genotypes for SB05-3693459 primer

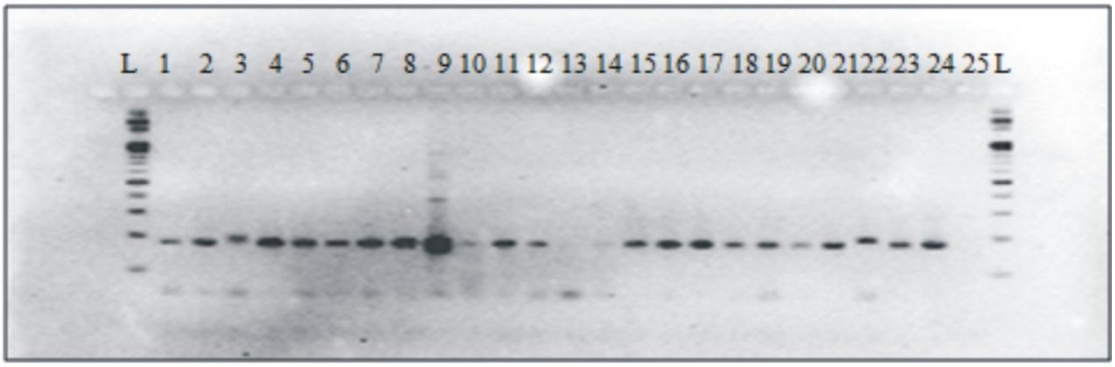


Plate-15: SSR gel profiling of sorghum genotypes for SB2385 primer
L- 100 bp DNA ladder

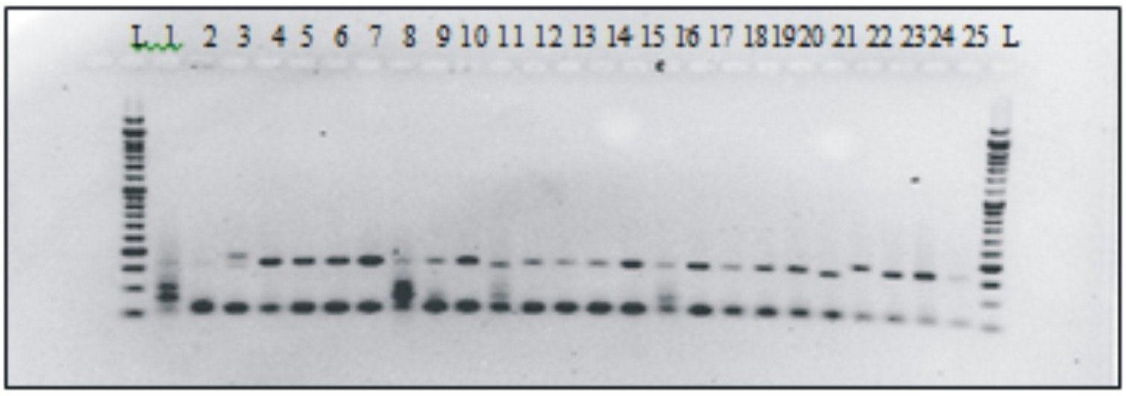


Plate-16: SSR gel profiling of sorghum genotypes for SB2386 primer

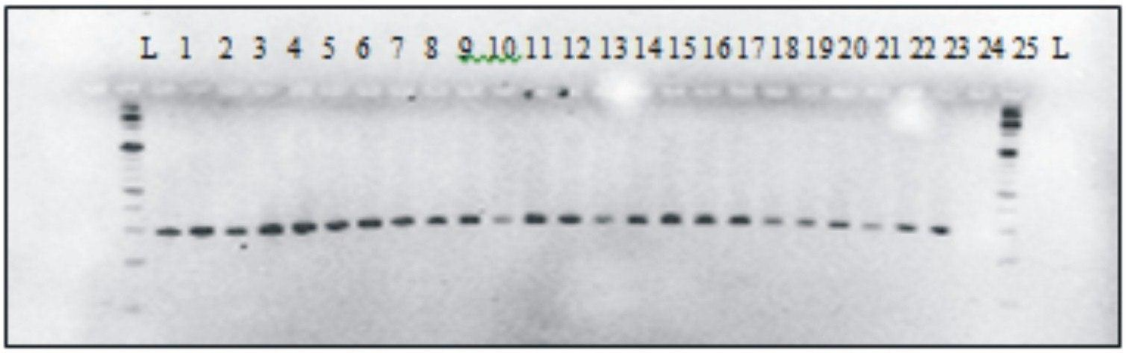


Plate-17: SSR gel profiling of sorghum genotypes for SB2387 primer

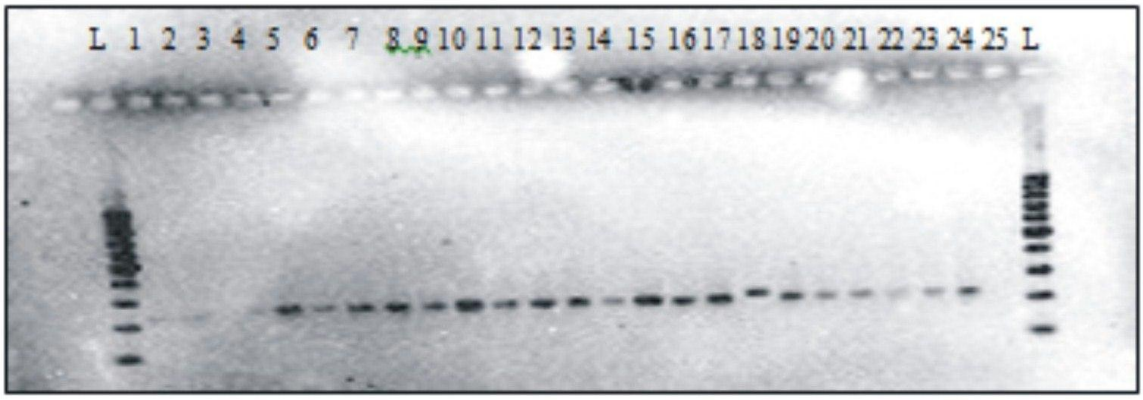


Plate-18: SSR gel profiling of sorghum genotypes for SB2388 primer
L - 100 bp DNA ladder

Group three included only one genotype GJ-40 with a genetic similarity of 89.5% with group two. Group four contained the genotypes GJ-42 with a genetic similarity of 89% with group three. Group five contained only one genotype GJ-38 with a high genetic similarity of 88% to group four. Group six contained only GJ-41 with a genetic similarity of 87% to group five. Group seven comprised the genotype SR 2958 with a genetic similarity of 86% to group six. Group eight consisted of SR 2914 and SR 2957 which had a genetic similarity of 91 per cent. Group nine included two genotypes SR 2960 and GJ-39 with a genetic similarity of 91 per cent. Group ten contained only one genotype NIZER GOTI with a genetic similarity of 71% to group nine.

Sub cluster II consisted only one genotype SURAT LOCAL with a genetic similarity of 49% to sub cluster I. Cluster II included two genotypes ICSR 13008 and BP 53 with a genetic similarity of 72 per cent.

In conclusion, this study identified great genetic diversity and heterogeneity in sorghum genotypes. The genetic diversity and relationship information from this study will benefit sorghum breeding program to get the maximum heterogeneities. This may enable breeders to exploit the potential of transgressive segregation and for strategic genetic conservation.

## REFERENCES

1. Abraha, T., Githiri, S. M., Kasili, R. W., Skilton, R. A., Solomon, M. and Nyende, A. B., Genetic Diversity Analysis of Eritrean Sorghum [*Sorghum bicolor* (L.) Moench] Germplasm using SSR Markers. *Molecular Plant Breeding*, **5(13):** 1-12 (2014).
2. Adugna, A., Analysis of in situ diversity and population structure in Ethiopian cultivated *Sorghum bicolor* L. landraces using phenotypic traits and SSR markers. *Adugna SpringerPlus*, **3(212):** 1-14 (2014).
3. Ali, M. L., Rajewski, J. F., Baenziger, P. S., Gill, K. S., Eskridge, K. M. and Dweikat, I., Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Mol Breeding*, **21:** 497–509 (2008).
4. Anas, T. and Yoshida, T., Genetic diversity among Japanese cultivated sorghum assessed with simple sequence repeat markers. *Plant Production Science*, **7:** 217–223 (2004).
5. Anderson, J. A., Churchill, G. A., Autrique, J. E., Sorells, M. E. and

Tanksley, S. D., Optimizing parental selection for genetic linkage maps. *Genome* **36:** 181-186 (1993).

6. Botstein, D., White, R. L., Skolnick, M. and Davis, R. W., Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American J. Human Genet,* **32:** 314-331 (1990).
7. Disasa, T., Feyissa, T., Admassu1, B., Paliwal, R., Villiers, S. M. D. and Odeny, D. A., Molecular evaluation of Ethiopian sweet sorghum germplasm and their contribution to regional breeding programs. *AJCS*, **10(4):** 520-527 (2016).
8. Madhusudhana, R., Balakrishna, D., Rajendrakumar, P., Seetharama, N. and Patil, J. V., Molecular characterization and assessment of genetic diversity of sorghum inbred lines. *African Journal of Biotechnology*, **11(90):** 15626-15635 (2012).
9. Mofokeng, A., Shimelis, H., Tongoona, P. and Laing, M., A genetic diversity analysis of South African sorghum genotypes using SSR markers. *South African Journal of Plant and Soil*, **31(3):** 145–152 (2014).
10. Paterson, A. H., Bowers, J. E., Maher, C. A., Narechania, A., Zhang, L., Ware, D., Messing, J. and Rokhsar, D. S., The *Sorghum bicolor* genome and the diversification of grasses. *Nature*, **457:** 551–556 (2009).
11. 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., Use of SSRs for establishing heterotic groups in subtropical maize. *Theoretical and Applied Genetics*, **107:** 947–957 (2003).
12. 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., 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 (1997).
13. Wang, M. L., Zhu, C., Barkley, N. A., Chen, Z., John E. E., Murray, S. C., Tuinstra, M. R., Tesso, T., Pederson, G. A. and Yu, J., Genetic diversity and population structure analysis of accessions in the US historic sweet sorghum collection. *Theor Appl Genet,* 5-6 (2009).
14. Yadav, O. P., Mitchell, S. E. and Zamora, A., Development of new simple sequence repeats markers for pearl millet. *SAT e journal*, **3:** (2007).
15. Yonemaru Ando, T., Mizubayashi, T., Kasuga, S., Matsumoto, T. and Yano, M., Development of genome-wide simple sequence repeat markers using whole-genome shotgun sequences of sorghum *(Sorghum bicolor* (L.) Moench). *DNA Research,* **16:** 187–193 (2009).
16. Zhan, Q., Zhou, L., Bi, N., Wu, H., Li, J., Lu, J., Lu, J. and Lin, P., A Comprehensive analysis of genetic diversity and relationships of 13 sweet sorghum varieties as energy sources. *Journal of Sustainable Bioenergy Systems*, **2:** 86-91 (2012).
17. Zidani, S., Ferchichi, A. and Chaieb, M., Genomic DNA extraction method from Pearl millet (*Pennisetum glaucum*) leaves. *Afr. J. of Biotechnol*, **4(8):** 862-866 (2005).