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# GENETIC ANALYSIS OF SORGHUM CULTIVARS FROM USA USING SSR MARKERS

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## Abstract

The goal of this study was to evaluate the diversity and the genetic relationships among ten the varieties of sorghum (Sorghum bicolor) using SSR markers. A field experiment was conducted at a greenhouse in Texas A&M University–Soil and Crop Science. Simple sequence repeat (SSR) markers reveals a high polymorphism ratio among sorghum cultivars, where polymorphism values ranged from (90%), (25.32%). The highest number of polymorphic bands (15) was given with primer (4), while the lowest band (6) gave with primer (26). The size of sequence primer ranged between (370) Pb with primer (21) and (370) Pb with primer (38). The genetic distance values ranged between (0.25) and (0.43), where the lowest was between sorghum cultivars (G7) and (G6), while the highest genetic distance was between (G10). In this study SSR markers were found to be powerful tools for identifying genetic diversity in sorghum populations. Markers are given high polymorphic values among of sorghum genotypes under study. Cluster analysis grouped the ten sorghum cultivars into nine groups based on genetic and morphological traits. We conclude that the results of SSR–PCR are extremely helpful in identify the genetic distance among sorghum genotypes. This leads to selection sorghum genotypes, and SSR may be used for producing commercial hybrids among them.

***Key words :*** sorghum bicolor, genetic diversity, simple sequence repeats (SSR).

## Introduction

Sorghum is an important cereal crop in the world, especially in semi –arid areas. Its staple food for people in Asia and Africa and the fifth most important cereal crop after wheat, barely, rice and maize (FAO, 2004). This crop has chromosome number (2n=20), a nuclear DNA approximately (2.0 pg.) and genomic size 735 MPb (Dillon *et al.,* 2007). In some countries in the south of Africa, sorghum is mainly used as food, and stalk of sweet sorghum consumed like sugar cane because content high level of sugar concentration in stalks. Sorghum is used as a biofuel crop in several countries in the world because sorghum crops based biomass production lies in its photosynthetic capacity, where biomass is increased by increasing the amount of the nutrients in the soil and $CO_2$ concentration in the air as well as exposure to sunlight, and then converting into sugar and biofuel based on plant product (Vermerris *et al.,* 2007; Zhao *et al.,* 2009). In other places like America and Asia, sorghum is commonly used to feed animals. In addition, sorghum forge can used as green forage, silage, and hay (Mothaodi *et al.,* 2014). There are four groups of sorghum cultivated in many different regions in the world (durra, bicolor, guina and kefir). This collection of sorghum groups was assembled to study their genetic diversity in order to design appropriate of cultivars with suitable traits for utilization in programs for crop improvements and genetic resources conservation in gene banks (Kumar *et al.,* 2011). Sorghum species have wide adaption to harsh environmental conditions and are more suitable than other crops in arid and semi-arid regions of the world because their cells and their tissues survive dehydration by several mechanisms, so their complete of genome sequence (Casa *et al.,* 2008; Upadhyaya *et al.,* 2009). Genetic markers are robust technology for assaying genetic diversity between and among genotypes (Westman and kresovich, 1997). DNA markers provide information that improves understanding of the genetic rules and selection

parameters in plant breeding.It is helpful in selecting parents of hybrids, and provides an efficient way to identify favorable genes related with desirable traits in germplasms (Agrama *et al.*, 2002). The objective of this study is to compare among ten sorghum genotypes and the utility of SSR markers in genetic diversity analysis.

## Materials and Methods

All material including 10 sorghum genotypes collected from USA (Texas) were used in this study (Table 1). Seeds of these genotypes were planted in greenhouse and DNA was extracted from each genotypes according to Saghi –Maroof *et al.*, (1984). DNA concentration was 170-230 ng µl-1. The markers was provided by Erufuns company to the Texas sorghum research lab of Soil and Crop Science Department - A&M university table 2 and distributed widely of sorghum genome (1-10 chromosome). The reaction of PCR consisted of 2 µl of DNA solution, 1.2 ASB Buffer, 1.1 µl of 25 mM of MgCl2, 0.5 dNTP,1.1 µl from forward primer and 1.1 µl reverse primer, 0.2 Taq enzyme and 5.5 µl of double distilled water. The amplification reaction were carried out in a SSR as follows:

The reaction mixture was denatured at 95°C for min initially, then subjected to 40 cycles 96°C (1 min), 50°C (1min) and 72°C (1 min), and final extension at $72^0$C for 5 min, prior to cooling at 4°C. The PCR products was separated by electrophoresis 40 agrolmid gel With TBE 10 ml, APS with 1400 µl and EMET 40 µl. The amplified bands were recorded by "quantity one" system, then binary coded were (1) represented the presence band and (0) represented with not band in each genotypes. The polymorphism of each SSR was determinated and described in Assar *et al.*, (2005). The POPGEN software was used to calculate the genetic distance, genetic similarity and cluster analysis by weir (1996).

## Results and Discussion

### Result of SSR –PCR technique

SSR markers are used to determinate DNA fingerprinting studies because they display high level of polymorphism. The total number of main primers was (950) bands; this include monomorphic and polymorphic bands. The total number of bands was higher than that found by the studies by (Agrama and Tuinstra, 2003) which assessed twenty- two of genotypes of sorghum by using (28) primers, while another study on genetic diversity among eighty-two genotypes of sorghum by using 48 SSR primers ( Canapathy *et al.*, 2012)

### Genetic distance

Genetic distance is a quantitative parameter of genetic variation and can be evaluated through of the study of sequence or the allelic frequency that calculated between cultivars, inbred, populations or species (Mutegi *et al.*, 2011, EL-sahookie *et al.*, 2010). The genetic distance values range as shown in (Table 3). The lowest value of genetic distance (0) was obtained with several genotypes, while the highest value of genetic distance (0.43) came from genotype (G10) and genotype (G2). Overall, the genetic distance results revealed high similarity in genetic material between genotype (G9) and genotype (G6), which was (0.4), these results were similarity to those of studies such as (Bowditch *et al.*, 1993; Shehzed *et al.*, 2011). On the other hand, the lowest similarity in genetic materials between genotype (G8) and genotype (G10): the purpose of analysis of the genetic variation in sorghum germplasm is the utilization of these cultivars to plan appropriate conservation of this germplasm. Molecular markers were used to show the utility of this technology in evaluating genetic distance, as well as establishing the relationship between cultivars and their structures with the ultimate goal of determining

**Table 1:** Characteristics and place of collection sorghum progenies used in experiment.

| No. of genotypes (G) | Name of genotypes | Characteristics | Place of collection | Grain weight (mg) | Grain yield per plant (gm) |
|---|---|---|---|---|---|
| 1 | SOB1-HA/53BOR#4 | Tall (stay green) | Texas USA | 111.2 | 32.03 |
| 2 | SOB1-HA/1754#4 | Dwarf (non -stay green) | Texas USA | 109.46 | 26.27 |
| 3 | AT×623/8-20 | Tall (non -stay green) | Texas USA | 101.32 | 29.180 |
| 4 | 41-Keller (SOB1) | Dwarf (non -stay green) | Texas USA | 94.32 | 25.32 |
| 5 | Stg 1 | Dwarf (stay green) | Texas USA | 97.2 | 26.244 |
| 6 | Stg 2 | Dwarf (stay green) | Texas USA | 108.66 | 29.33 |
| 7 | Stg 3 | Dwarf (stay green) | Texas USA | 110.86 | 30.20 |
| 8 | Stg 4 | Dwarf (stay green) | Texas USA | 115.30 | 31.13 |
| 9 | SOB1-HA/8BF2 | Dwarf (stay green) | Texas USA | 103.55 | 29.82 |
| 10 | SOB-HA/2t-bm | Dwarf (stay green) | Texas USA | 93.70 | 25.299 |

**Table 2:** SSR Markers Used to diverse 10 genotypes of sorghum cultivars.

| No. | Forward Primers | Primers Reverse | Tm (°C) | Repeats | Product size (pb) | Number of main bands | No. of polymorphic bands | Polymorphism % |
|---|---|---|---|---|---|---|---|---|
| 1 | Xsbarslbk1.04F | Xsbarslbk1.04R | ATA (25) | 59 | 340 | 15 | 8 | 44.4 |
| 2 | Xsbarslbk1.09F | Xsbarslbk1.09R | AAAT (32) | 59 | 360 | 13 | 10 | 76.9 |
| 3 | Xsbarslbk1.43F | Xsbarslbk1.43R | TAA (72) | 58 | 366 | 19 | 13 | 68.42 |
| 4 | Xsbarslbk1.68F | Xsbarslbk1.68R | TTA (42) | 55 | 350 | 20 | 15 | 75.00 |
| 5 | Xsbarslbk2.04F | Xsbarslbk2.04R | ATA (64) | 55 | 360 | 19 | 12 | 63.15 |
| 6 | Xsbarslbk2.26F | Xsbarslbk2.26R | TGG (12) | 55 | 360 | 12 | 8 | 66.6 |
| 7 | Xsbarslbk2.43F | Xsbarslbk2.43R | TAGA (15) | 55 | 360 | 13 | 11 | 84.6 |
| 8 | Xsbarslbk2.72F | Xsbarslbk2.72R | GGA (12) | 55 | 352 | 16 | 12 | 75 |
| 9 | Xsbarslbk3.03F | Xsbarslbk3.03R | TAA (28) | 55 | 361 | 14 | 6 | 42.85 |
| 10 | Xsbarslbk3.21F | Xsbarslbk3.21R | TAT (16) | 55 | 367 | 12 | 8 | 66.66 |
| 11 | Xsbarslbk3.70F | Xsbarslbk3.70R | TAA (25) | 54 | 360 | 16 | 8 | 50 |
| 12 | Xsbarslbk4.05F | Xsbarslbk4.05R | TAGA (20) | 55 | 354 | 17 | 9 | 52.9 |
| 13 | Xsbarslbk4.48F | Xsbarslbk4.48R | TAA (22) | 55 | 350 | 18 | 9 | 50.00 |
| 14 | Xsbarslbk4.68F | Xsbarslbk4.68R | GAA (30) | 55 | 358 | 16 | 8 | 50.0 |
| 15 | Xsbarslbk5.02F | Xsbarslbk5.02R | TAA (25) | 55 | 370 | 17 | 9 | 52.9 |
| 16 | Xsbarslbk5.14F | Xsbarslbk5.14R | TAA (34) | 54 | 354 | 10 | 8 | 80.0 |
| 17 | Xsbarslbk5.37F | Xsbarslbk5.37R | TCT (12) | 54 | 363 | 10 | 7 | 70.0 |
| 18 | Xsbarslbk5.56F | Xsbarslbk5.56R | TTA (18) | 54 | 366 | 8 | 7 | 87.5 |
| 19 | Xsbarslbk6.00F | Xsbarslbk6.00R | TTA (50) | 53 | 343 | 17 | 8 | 47.05 |
| 20 | Xsbarslbk6.28F | Xsbarslbk6.28R | TAT (12) | 54 | 354 | 15 | 8 | 53.33 |
| 21 | Xsbarslbk6.36F | Xsbarslbk6.36R | ATA (26) | 54 | 300 | 16 | 9 | 56.25 |
| 22 | Xsbarslbk7.00F | Xsbarslbk7.00R | AGC (12) | 54 | 315 | 11 | 7 | 63.36 |
| 23 | Xsbarslbk7.07F | Xsbarslbk7.07R | TTA (38) | 54 | 316 | 10 | 9 | 90.0 |
| 24 | Xsbarslbk7.08F | Xsbarslbk7.08R | TTC (25) | 54 | 355 | 12 | 10 | 83.33 |
| 25 | Xsbarslbk7.18F | Xsbarslbk7.18R | TAAA (12) | 55 | 365 | 14 | 9 | 64.28 |
| 26 | Xsbarslbk8.00F | Xsbarslbk8.00R | CAA (20) | 55 | 358 | 18 | 6 | 33.33 |
| 27 | Xsbarslbk8.06F | Xsbarslbk8.06R | TAA (26) | 53 | 361 | 16 | 9 | 56.25 |
| 28 | Xsbarslbk8.09F | Xsbarslbk8.09R | TGGA (10) | 54 | 367 | 15 | 9 | 60 |
| 29 | Xsbarslbk8.11F | Xsbarslbk8.11R | TCT (12) | 54 | 356 | 17 | 8 | 47.05 |
| 30 | Xsbarslbk9.00F | Xsbarslbk9.00R | TACA (42) | 54 | 369 | 16 | 7 | 43.75 |
| 31 | Xsbarslbk9.03F | Xsbarslbk9.03R | TTTC (12) | 54 | 357 | 13 | 7 | 53.84 |
| 32 | Xsbarslbk9.09F | Xsbarslbk9.09R | TTG (15) | 55 | 352 | 13 | 6 | 46.15 |
| 33 | Xsbarslbk9.13F | Xsbarslbk9.13R | AAAT (12) | 54 | 366 | 16 | 8 | 50.0 |
| 34 | Xsbarslbk10.02F | Xsbarslbk10.02R | CTGC (15) | 54 | 306 | 16 | 9 | 56.25 |
| 35 | Xsbarslbk10.08F | Xsbarslbk10.08R | TTA (20) | 53 | 369 | 10 | 9 | 90 |
| 36 | Xsbarslbk10.46F | Xsbarslbk10.46R | GAA (15) | 54 | 328 | 18 | 8 | 44.44 |
| 37 | Xsbarslbk10.54F | Xsbarslbk10.54R | TAGA (30) | 54 | 310 | 14 | 12 | 85.71 |
| 38 | Xsbarslbk3.52F | Xsbarslbk3.52R | TAA (32) | 50 | 370 | 12 | 9 | 75 |
| 39 | Xsbarslbk4.00F | Xsbarslbk4.00R | TAA (52) | 50 | 361 | 18 | 10 | 55.55 |
| 40 | Xsbarslbk6.52F | Xsbarslbk6.52R | TAA (94) | 48 | 366 | 15 | 12 | 80.0 |

the genetic variation and distribution among all genotypes under study (Glaszmann *et al.*, 2010; Kumar *et al.*, 2011; Ziyad *et al.*, 2018). DNA markers have revealed advantage on morphological markers in germplasms descriptors since they are not affected by environments condition (Folkertsms *et al.*, 2005; Barnaud *et al.*, 2007; Ng -uni *et al.*, 2011; Ziyad,2011)

**Cluster analysis**

Using the method of genetic measurement Nei and

**Table 3:** Values of genetic distance calculated according (data matrix) among ten sorghum genotypes.

| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 |
|---|---|---|---|---|---|---|---|---|---|---|
| G1 | 0 | | | | | | | | | |
| G2 | 0.33 | 0 | | | | | | | | |
| G3 | 0.37 | 0.30 | 0 | | | | | | | |
| G4 | 0.38 | 0.36 | 0.36 | 0 | | | | | | |
| G5 | 0.35 | 0.38 | 0.35 | 0.34 | 0 | | | | | |
| G6 | 0.38 | 0.40 | 0.37 | 0.35 | 0.35 | 0 | | | | |
| G7 | 0.38 | 0.38 | 0.34 | 0.37 | 0.29 | 0.25 | 0 | | | |
| G8 | 0.35 | 0.36 | 0.33 | 0.33 | 0.28 | 0.28 | 0.30 | 0 | | |
| G9 | 0.36 | 0.40 | 0.41 | 0.38 | 0.38 | 0.40 | 0.33 | 0.34 | 0 | |
| G10 | 0.4 | 0.43 | 0.40 | 0.38 | 0.33 | 0.26 | 0.35 | 0.26 | 0.36 | 0 |

Li (1979) and using by the Past program for Cluster analysis, the genetic relationship among ten genotypes of sorghum was constructed. The dendrogram shown four major clusters (Fig. 1). The first cluster was G9 and G4 that occurred as a separate cluster from other genotypes of sorghum in this study because they have large value of genetic diversity. The second cluster (G10 and G6) occurred within this cluster, as sub cluster, also (G10 and G8) and (G6 and G7) were also sub-cluster within G9 and G7 cluster. The third was composed of G1 and G9; but G2 and G3 gave similarity with morphological traits with yield per plant (29.33) and (30.86 gm) respectively. Where these results were similarity with cluster analysis. The results were shown that sorghum genotypes G9 and G4 had large distance values from other genotypes and constructed separate clusters, so they can be used in hybridization and selection programs to invest heterotic phenomena. On the other hand, the genetic distance between (G6 and G7), (G8 and G10), and (G2 and G3) were especially low; and there is no predictable hybrid vigor predictable between them.

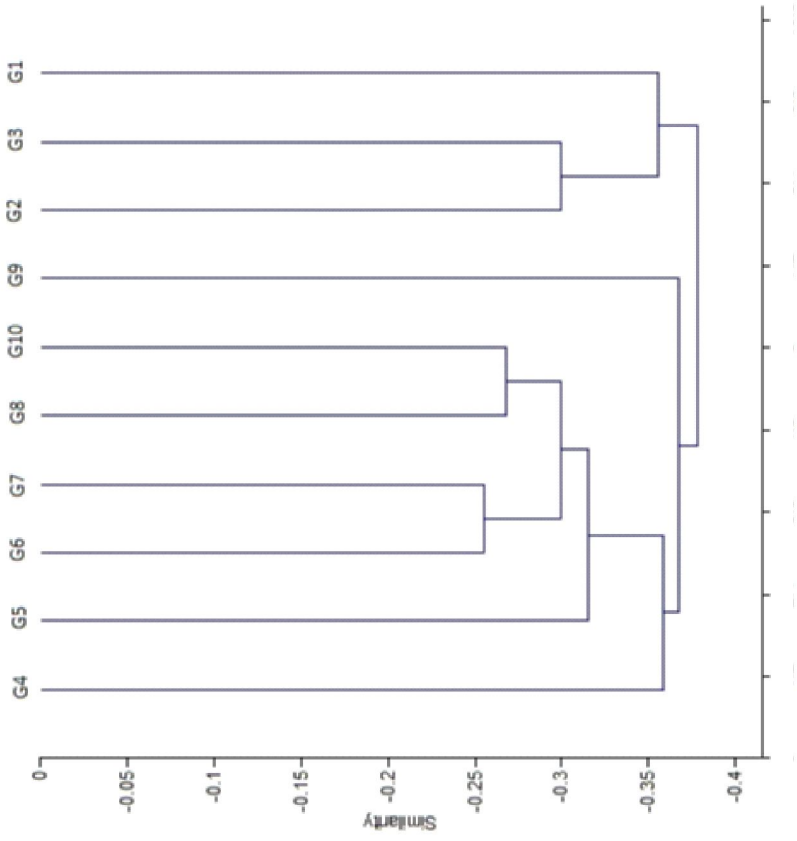


**Fig. 1:** Dendogram of ten sorghum genotypes producing by Past cluster analysis program deepened on genetic distance matrix.

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