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# Genetic diversity of African sorghum (*Sorghum bicolor* L. Moench) accessions based on microsatellite markers

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**Abstract**

Genetic diversity plays an important role in selection of parental stocks in plant breeding. Sorghum [*Sorghum bicolor* (L.) Moench] breeding initiatives have been constrained by lack of information on genetic diversity of cultivated accessions which would otherwise guide in the choice of heterotic parents for hybridization. This study was carried out to determine genetic diversity of sorghum accessions collected from Africa and ICRISAT using simple sequence repeats (SSR) of microsatellite marker. Thirty sorghum SSR markers were used to assess the genetic diversity of 134 sorghum accessions. The number of alleles per microsatellite locus in the 134 sorghum accessions ranged between 2 to 22, with a total of 259 different alleles having been amplified. The greatest number of alleles was found at the locus 3590e705f67911e0b58c0010185a4b14 with 22 alleles. The average Polymorphism Information Content (PIC) for all the assayed sorghum accessions was 0.55. Expected heterozygosity of population ranged between 2.91 for Sudan and 1.58 for Central Africa. Pairwise population comparisons for genetic identity were derived based on *Nei's*. Genetic identity of the populations ranged from 0.36 for Central Africa and Northern Africa to 0.93 between Eastern Africa and Rwanda. Analysis of Molecular Variance (AMOVA) revealed that 75% of the molecular variation in sorghum accessions was due to within individual populations while 25% of the total variation was partitioned among populations. There was low population differentiation due to either continuous exchange of genes between sorghum populations largely by germplasm exchange or no intense selection processes. The diversity observed within sorghum accession from Sudan and East African region could be useful in improvement of sorghum for various traits.

**Keywords**: genetic diversity, identity, sorghum.
**Abbreviations**: CTAB-Cetyl trimethylammonium bromide; SSR-Simple Sequence Repeats, AMOVA-Analysis of Molecular Variation, PCA-Principal Component Analysis, PIC-Polymorphism Information Content.

## Introduction

Genetic diversity is important in sorghum [*Sorghum bicolor* (L.) Moench] breeding because it plays an important role in determining heterotic groups. Sorghum is one of the traditional crops grown in Africa with potential to provide food security in low potential areas (de Vries and Toenniessen 2001; Mehmood et al., 2008). Besides being a food cereal, sorghum is used for feed manufacture, distillation of ethanol, industrial malting and brewing purposes (Taylor, 2005; Chakauya et al., 2006; Antonopoulou et al., 2008; Etuk et al., 2012). The renewed impetus for sorghum production for use in brewing, feed and baking industries has stimulated increased production of the crop in the East African region. However, among the challenges hindering response to the industrial demand for sorghum is availing suitable cultivars for the desired grain and stalk uses. The success in this venture largely depends on the germplasm collection and evaluation for subsequent development to meet the desired industrial needs, which calls for both genotyping and phenotyping. Genotyping offers basic information on genetic diversity of the materials under consideration. Although sorghum originated from North-Eastern Africa, it is being cultivated successfully in most parts of the world with diverse environmental conditions for different uses. The major cereals have experienced significant reduction in genetic diversity due to improvements of various agronomic traits (Petrovic and Dimitrijevic, 2012). The impact of such genetic reduction has occasionally been witnessed when calamities strike such as the current ug99 virus that is threatening wheat production globally following breakdown in resistance to stem rust (Joshi et al., 2008). Information on genetic diversity coupled with a deliberate conservation of genetic material can forestall an otherwise devastating situation especially when dealing with food crops. The farmers' techniques of saving seed and managing varietal diversity in response to their demands and ecological distinctiveness has lead to interrelated diversity outcome. The landraces are adapted to a particular region in which they have evolved and their yield potential varies depending on genotype (de Boef et al., 1996; Barnaud et al., 2007). The genetic diversity of sorghum has been studied extensively using agro-morphological characteristics (Grenier et al., 2000). However; this information has its own limitation and is usually influenced by environmental factors (Alamnza-Pinzon et al., 2003; Fufa et al., 2005). Consequently, use of molecular markers in estimating genetic diversity is the most effective method that plays a key role in success of any

breeding program (Ali et al., 2007). In this study the genetic diversity of sorghum genotypes collected from Africa and ICRISAT India was determined using SRR markers.

**Results**

***Mean allelic analysis across the nine populations***

Among the sorghum accessions analysed for gentic diversity, the Kenyan and Indian accessions showed the highest Shannon diversity index of $I$ = 1.07 and $I$ = 1.05, respectively (Table 3), while Central Africa (CA) accessions exhibited the lowest index of 0.39. However an evaluation of diversity based on expected heterozygosity demonstrated that the highest population diversity existed among the sorghum accessions from Sudan (SUD), followed by INDIA> Kenya > ICRISAT-K> South Africa> North Africa> East Africa> Rwanda and Central Africa in that order (Table 3). The proportion of rare alleles within regions ranged from 0.03 in Central Africa to 0.69 in Kenya with the latter exhibiting the highest number of rare allele of 0.67 ( Table 3). The analysis of allelic patterns across the 9 sorghum accessions revealed that accessions from Kenya had the largest number of different Alleles (*Na*) of 5.17 and highest number of effective alleles *Ne* (2.98) (Table 3, figure 1). The number of different alleles in the populations from Central Africa, East Africa, ICRISAT Kenya, Northern Africa, Rwanda, India, Southern Africa and Sudan were 1.63, 2.43, 4.60, 4.37, 1.97, 3.40, 2.50 and 3.37, respectively. The lowest number of effective allele was observed in the Central African accessions (1.576). The mean expected heterozygosity estimate (*He*) for polymorphic markers ranged from 0.26 to 0.56, with the overall mean expected heterozygozity estimate (*He*) for all the accessions was 0.41.

***Population diversity***

The Principal Coordinate Analysis (PCoA) based on a correlation matrix of the SSR marker showed two clearly significant components, which explained 44% and 32% of the total variance (Figure 2). This analysis showed well-defined distribution patterns of the accessions, according to the genetic distances and the relationships among them. In the PCoA plot, the accessions were divided into three main groups with group (A) was consist of sorghum from Rwanda, East Africa, and ICRISAT–Kenya accessions. The second group (B) included accessions from Kenya, India and ICRISAT-Kenya. The third group (C) included mainly ICRISAT-Kenya and few accessions from Southern Africa, Kenya and India. The sorghum accessions from Sudan (group D) and Northern Africa (group E) were clearly classified separately from the first three groups.

***Genetic identity across the nine populations***

The level of relatedness between the nine populations was established through a genetic identity matrix derived from the proportion of shared (common) loci (Nei, 1978). Pairwise comparison of Nei's unbiased genetic identity among the nine populations ranged from a low of 0.36 between population from Central Africa and Northern Africa, to a high of 0.93, between the Eastern Africa and Rwanda populations (Table 4).

***Principal co-ordinate analysis***

A principal coordinate analysis plot of the first two coordinates was derived and used to display the multidimensional relationship of 134 accessions grouped into nine regions of origins in this study (fig 3). The two principal coordinate accounted for 66% of the total variance. Significant overlap was noted for most of the populations with accessions from ICR-K and Kenya being dispersed widely in the plot.

***Analysis of Molecular Variance (AMOVA)***

Population diversity components were partitioned using Analysis of Molecular Variance (AMOVA) (Table 5). The estimation of the variance components among and within populations using nested analysis of molecular variance (AMOVA) was significant ($P<0.01$). The AMOVA indicated that most (75%) of the molecular variation in sorghum accessions was partitioned within populations, with lesser amounts (25%) partitioned among populations. A value of ΦPT of 0.251 was observed showing the extent of differentiation among the populations.

**Discussion**

Sorghum diversity based on expected heterozygosity (*He*) revealed that genetic diversity decreased in the order Sudan>India>Kenya>ICRISAT-Kenya>SA>NA>EA>RWD >CA (Table 1). The observed trend could be attributed to the origin and domestication of sorghum. It has been observed that the race bicolor is widely distributed in Africa and Asia (de Wet and Price, 1976). The cultivated sorghum in India and most parts of the world initially came as a result of domestication of wild races in Africa. It is believed that domestication of wild races of African sorghum was then re-introduced to the African continent (Haaland, 1995) which explains the decrease of heterozygosity reflected in the populations' trend above. It is therefore prudent to conserve these landraces for the breeding program, although very poor in terms of yield but a good source of genetic variability. Partitioning of the revealed genetic diversity by analysis of molecular variances (AMOVA) revealed that most of the molecular variations in the sorghum accessions existed within individual populations (75%). Sorghum is predominantly a self-pollinating crop, with significant levels of natural out crossing occurring (House 1985). As a result of a combined self-pollination and out crossing, most of the farmer-cultivated landraces of sorghum are mixtures. Though the populations in this study are distant from each other the low divergence among the populations could be explained by the occurrence of gene flow among populations, through exchange of germplasm. As observed by Chakauya et al., (2006), the differences in the genetic diversity could be attributed to traditional farming systems with agronomic, economic and cultural considerations that foster high levels of genetic diversity. In most cases, traditional farmers maintain more than one distinct landrace as varieties which are carried over from one generation to the next. The driving forces behind the practice of maintenance of sorghum landraces by farmers is adaptability to local environmental conditions and potential for multiple end-uses. In regards to adaptability to environmental conditions preference is given to drought tolerance and early maturity (Adeline et al., 2007). While considering sorghum for multiple end-uses, priority is given to grain sorghum for food and feed with cultivar preference selected on the basis of sweet grain or their sweet stalks (Gepts, 2004). The weak differentiation among populations of sorghum from different regions as noted in this study could be attributed to transit of sorghum via human migration coupled with seed trade between regions. The regional

**Table1.** Sorghum accessions from different regions used for molecular, characterization using SSRs markers. The accessions came from South Africa, Zimbabwe, Kenya, Rwanda, DR Congo, Sudan, Ethiopia and ICRISAT.

| *ACC./Variety | Region | ACC./Variety | Region | ACC./Variety | Origin |
|---|---|---|---|---|---|
| NYANGEZI | DR Congo | SP 993515 | ICRISAT Kenya | Siaya # 2-3 | Kenya Siaya |
| Kabamba | DR Congo | IESV 92028 DL | ICRISAT Kenya | Siaya # 41-2 | Kenya Siaya |
| IS 11162 | Ethiopia | IESV23013 DL | ICRISAT Kenya | Ainamoi #1 | Kenya Siaya |
| IS 11909 | Ethiopia | SP 993520-1 | ICRISAT Kenya | Siaya # 42 | Kenya Siaya |
| E 36-1 | Ethiopia | IESV 94025 SH | ICRISAT Kenya | Siaya # 6-1 | Kenya Siaya |
| ICSV 700 | ICRISAT INDIA | IESV 23017 DL | ICRISAT Kenya | Siaya # 81-2 | Kenya Siaya |
| ICSB 324 | ICRISAT INDIA | IESV 23010 DL | ICRISAT Kenya | Siaya # 29-1 | Kenya Siaya |
| ICSV 654 | ICRISAT INDIA | IESV 92037 SH | ICRISAT Kenya | Teso # 5 | Kenya Teso |
| ICSR 161 | ICRISAT INDIA | IESV 92041 SH | ICRISAT Kenya | Teso # 11-2 | Kenya Teso |
| S 35 | ICRISAT INDIA | IESV 94079 SH | ICRISAT Kenya | Uasin Gishu #1 | Kenya Uasin Gishu |
| ICSV 89094 | ICRISAT INDIA | IESV 92033 SH | ICRISAT Kenya | Uasin Gishu #2 | Kenya Uasin Gishu |
| SP 993442-1 | ICRISAT INDIA | IESV 94121 SH | ICRISAT Kenya | NYUNDO | Rwanda |
| 104GRD | ICRISAT INDIA | IESV 23007 DL | ICRISAT Kenya | MB 23 | Rwanda |
| SPV 422 | ICRISAT INDIA | IESV 23011 DL | ICRISAT Kenya | MB 27 | Rwanda |
| SPV 1411 | ICRISAT INDIA | IESV 92170 DL | ICRISAT Kenya | S 79 | Rwanda |
| ICSR 93034 | ICRISAT INDIA | IESV 92041/1 SH | ICRISAT Kenya | MB 39 | Rwanda |
| ICSV 93046 | ICRISAT INDIA | SDSL 90167 | ICRISAT Zimbambwe | IS 25562 | Rwanda |
| SP 993442-1 | ICRISAT INDIA | Ent# 64DTN | ICRISAT Zimbambwe | ABALESHYA | Rwanda |
| SP 993532 | ICRISAT INDIA | NTJ 2 | INDIA | IMBUNDI | Rwanda |
| ICSV 93041 | ICRISAT INDIA | IS 8193 | Kenya | S 87 | Rwanda |
| ICSV 500 | ICRISAT INDIA | Siaya # 6-2 | Kenya | IS 25557 | Rwanda |
| IESV 92008 DL | ICRISAT Kenya | Kari Mtama 1 | Kenya | GATARAGA | Rwanda |
| IESV 91131 DL | ICRISAT Kenya | IS 8884 | Kenya | IS 9203 | Rwanda |
| IESV 93042 SH | ICRISAT Kenya | AINAMOI #2 | Kenya Ainamoi | GICAMUNKONI | Rwanda |
| IESV 92001 DL | ICRISAT Kenya | Busia_21 | Kenya Busia | BM 16 | Rwanda |
| IESV 91018 LT | ICRISAT Kenya | Busia # 3-3 | Kenya Busia | IS 25558 | Rwanda |
| IESV 92165 DL | ICRISAT Kenya | Busia # 30-2 | Kenya Busia | CYIHURE | Rwanda |
| IESV 92038/2 SH | ICRISAT Kenya | Kiboko local 2 | Kenya Kibiko | NDAMOGA | Rwanda |
| IESV 92043 DL | ICRISAT Kenya | Kipkelion # 1 | Kenya Kipkelion | BM 27 | Rwanda |
| IESV 91069 LT | ICRISAT Kenya | Kisanana | Kenya Kisanana | MB 30 | Rwanda |
| IESV 23008 DL | ICRISAT Kenya | E 1291 | Kenya Lanet | Nyiragikori | Rwanda |
| IESV 23004 DL | ICRISAT Kenya | LONDIANI | Kenya Londiani | MB 29 | Rwanda |
| IESV 91104 DL | ICRISAT Kenya | Siaya # 50-3 | Kenya Siaya | BM 18 | Rwanda |
| IESV 92021 DL | ICRISAT Kenya | Siaya # 24-2 | Kenya Siaya | URUKARAZA | Rwanda |
| IESV 23006 DL | ICRISAT Kenya | Siaya # 62-1 | Kenya Siaya | MUHIMPUNDU | Rwanda |
| IESV 92036 SH | ICRISAT Kenya | Kipkelion # 2 | Kenya Siaya | IS 25546 | Rwanda |
| IESV 23005 DL | ICRISAT Kenya | Siaya #81-4 | Kenya Siaya | IS 25545 | Rwanda |
| IESV_91111_DL | ICRISAT Kenya | Siaya #27-3 | Kenya Siaya | BM 32 | Rwanda |
| IESV 23016 DL | ICRISAT Kenya | Siaya # 46-1 | Kenya Siaya | N 2 | Rwanda |
| Sila | Zimbambwe SADC | Siaya # 93-1 | Kenya Siaya | BM 21 | Rwanda |
| IS 2331 | South Sudan | IS 2331 | South Sudan | BM 29 | Rwanda |
| Nyan-Jang | Sudan | IS 9201 | Uganda | Tegemeo | Tanzania |
| Nyondok | Sudan | ZSV 3 | Zimbambwe | IS 25547 | Uganda |
| Gadam Hamam | Sudan | Macia | Zimbambwe | IS 25563 | Uganda |
| Kech | Sudan | Sima | Zimbambwe SADC | IS 25561 | Uganda |
| Mulual-agoot | Sudan | | | | |

*ACC: Accessions

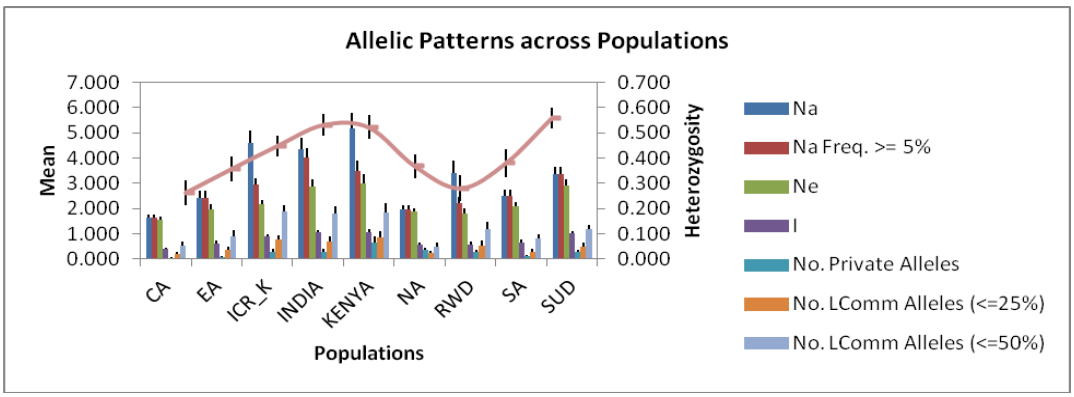


**Fig 1.** Comparative estimate of the genetic diversity parameters in allelic patterns across the nine populations. Na, number of different alleles; Ne, number of effective alleles; I, Shannons information index; No. Privates Alleles, number of unique to a single population, No. LComm Alleles, number of locally common alleles.

**Table 2.** Characteristics of the 30 sorghum SSR markers indicating major allele, number of alleles, polymorphism information content *(PIC)* and expected heterozygosity ($H_e$)

| SSR Marker | Major allele frequency | Number of alleles | $H_e$ | *PIC* |
|---|---|---|---|---|
| *gpsb*067 | 0.74 | 6 | 0.42 | 0.38 |
| *gpsb*123 | 0.65 | 5 | 0.54 | 0.50 |
| *Sep*107 | 0.95 | 2 | 0.10 | 0.09 |
| *Isep*310 | 0.99 | 2 | 0.012 | 0.02 |
| *msb*CIR223 | 0.53 | 4 | 0.55 | 0.46 |
| *msb*CIR240 | 0.47 | 6 | 0.58 | 0.50 |
| *msbCIR*246 | 0.75 | 5 | 0.41 | 0.37 |
| *msb*CIR248 | 0.53 | 3 | 0.61 | 0.54 |
| *msb*CIR262 | 0.53 | 5 | 0.55 | 0.45 |
| *msb*CIR276 | 0.88 | 3 | 0.21 | 0.20 |
| *msb*CIR283 | 0.31 | 8 | 0.76 | 0.73 |
| *msb*CIR286 | 0.41 | 7 | 0.67 | 0.61 |
| *msb*CIR300 | 0.75 | 4 | 0.41 | 0.37 |
| *msb*CIR306 | 0.50 | 4 | 0.55 | 0.45 |
| *msb*CIR329 | 0.40 | 5 | 0.71 | 0.66 |
| 3590e705f67911e0b58c0010185a4b14 | 0.29 | 22 | 0.86 | 0.85 |
| *Xcup*02 | 0.58 | 6 | 0.54 | 0.46 |
| *Xcup*53 | 0.95 | 3 | 0.09 | 0.09 |
| *Xgap*72 | 0.43 | 8 | 0.64 | 0.58 |
| *Xgap*84 | 0.25 | 18 | 0.86 | 0.84 |
| *Xgap*206 | 0.26 | 21 | 0.90 | 0.89 |
| *Xtxp*012 | 0.37 | 21 | 0.82 | 0.80 |
| *Xtxp*015 | 0.47 | 9 | 0.71 | 0.68 |
| *Xtxp*021 | 0.43 | 14 | 0.75 | 0.72 |
| *Xtxp*057 | 0.25 | 10 | 0.82 | 0.80 |
| *Xtxp*136 | 0.58 | 2 | 0.49 | 0.37 |
| *Xtxp*141 | 0.43 | 12 | 0.74 | 0.72 |
| *Xtxp*265 | 0.30 | 16 | 0.83 | 0.81 |
| *Xtxp*320 | 0.29 | 11 | 0.81 | 0.78 |
| *Xtxp*321 | 0.23 | 17 | 0.89 | 0.88 |
| **Mean** | **0.51** | **8.63** | **0.59** | **0.55** |

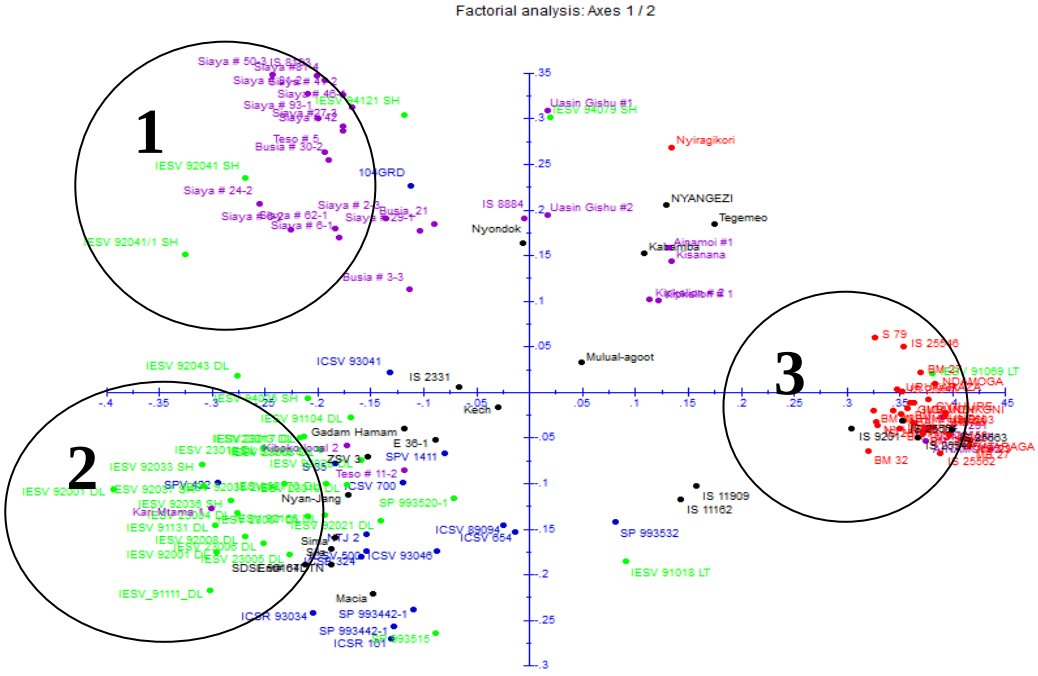


**Fig 2.** Biplot of axis 1 and 2 of the principle coordinate analysis based on the dissimilarity of 30 SSR markers for 134 sorghum accessions where 3 main groups were delineated. Group 1 mainly composed of Siaya, IESV and Teso accessions mainly from Kenya; Group 2 composed of IESV, SP and ICSV accessions mainly from India, ICRISAT Kenya and Sudan; Group 3 composed of accessions with prefix IS, S and BM mainly from Rwanda.

**Table 3.** Mean number of different loci ($N_a$), number of effective loci ($N_e$) Expected heterozygosity ($H_e$), shannnon index ($I$) across the nine populations. The populations are CA, Central Africa; EA, Eastern Africa; ICR-K, ICRISAT Kenya; India; Kenya; NA, Northern Africa; RWD, Rwanda; SA, Southern Africa; and SUD, Sudan.

| population | CA | EA | ICR-K | INDIA | KENYA | NA | RWD | SA | SUD |
|---|---|---|---|---|---|---|---|---|---|
| $N_a$ | 1.63 | 2.43 | 4.60 | 4.37 | 5.17 | 1.97 | 3.40 | 2.50 | 3.37 |
| $N_e$ | 1.58 | 1.97 | 2.17 | 2.85 | 2.98 | 1.87 | 1.80 | 2.07 | 2.91 |
| I | 0.39 | 0.62 | 0.90 | 1.05 | 1.07 | 0.57 | 0.57 | 0.67 | 1.01 |
| Rare allele | 0.03 | 0.07 | 0.30 | 0.30 | 0.67 | 0.37 | 0.27 | 0.10 | 0.30 |
| $H_e$ | 0.26 | 0.36 | 0.45 | 0.53 | 0.52 | 0.37 | 0.28 | 0.38 | 0.56 |

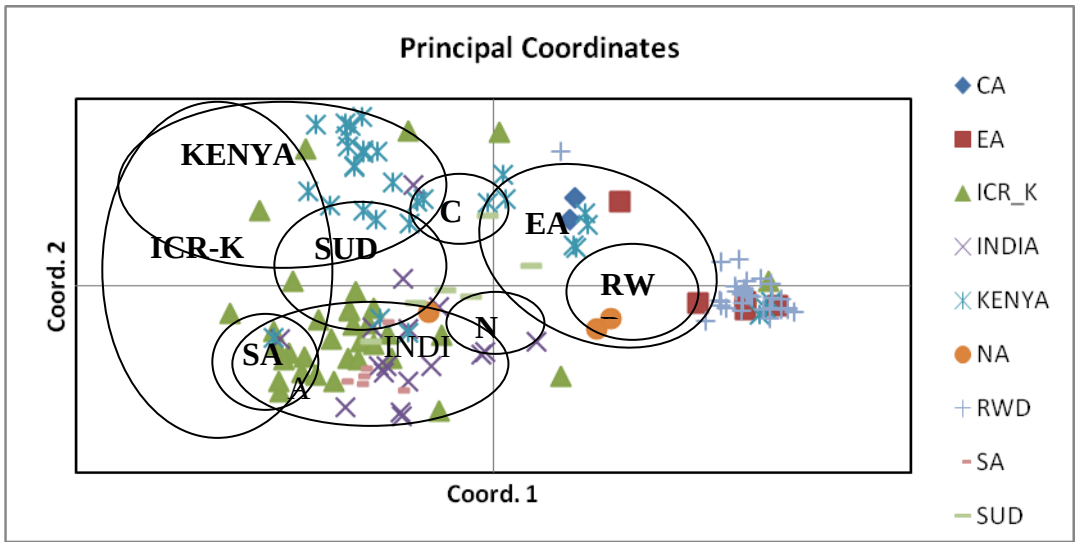


**Fig 3.** PCoA scatter plot showing the clustering pattern of nine sorghum populations identified and are represented by different colours and symbols. The nine populations are CA, Central Africa; EA, East Africa; ICR-K, ICRISAT-Kenya; India; Kenya; NA, Northern Africa; RWD, Rwanda; SA, Southern Africa; and SUD, Sudan.

differentiation revealed in our study was similar to what has been reported in other studies; for instance, Eritrean sorghum landraces revealed 23% of the total variation among populations and 77% within populations (Ghebru et al., 2002). Given the low population variance and partitioning of most of the diversity into within population components, collection strategies for conserving of sorghum should focus on sampling a few populations with the surety of covering as wide geographic amplitude of the targeted population as is possible. Based on the current study the Sudan and Kenya population should be targeted for collection owing to their high expected heterozygosity and Kenya unique alleles. Highest population diversity exhibited particular between Sudan (SUD) and CA populations can be used together with others to expand the genetic base of sorghum breeding programs.

## Material and methods

### *Plant materials*

Nine populations of sorghum based on the origin of the accession and diversity analysis were used for this study. A total of 136 sorghum accessions were obtained from different African countries, India, the national Gene Bank of Kenya and ICRISAT Kenya and India were used for this study (Table 1). Seeds were grown in a green house at Egerton University and about 5g leaves for each accession was harvested after 3 weeks after planting and transported in dry ice to the laboratory.

### *DNA extraction*

The leaf samples were groung in a Geno Grinder 2000 (Spex Certi Prep Inc, USA) until fine texture was achieved. The isolation of DNA was done using cetyl trimethyl ammonium bromide (CTAB) according to the modified method from Risterucci et al., (2000)… CTAB buffer [3% (w/v) CTAB, 1.4m NaCl, 20mM hydrodymethyl aminomethane hydrochloride (Tris- HCl), 20mM ethylenediaminetetraacetic acid (EDTA) pH 8.0, 0.17% (v/v) β-mercaptoethanol] corresponding to 450µL per sample was dispensed into a glass bottle and incubated in awater bath maintained at 65 $^{o}$C. The DNA was then purified using chloroform: isoamylcohol (24:1). About an equal volume of Iso-propanol was added to the aliquot precipitate the DNA. The DNA pellet was washed with 70% ethanol, air dried at room temperature over night then dissolved in 100µL 1X Tris-EDTA.. A volume of 3 $\mu L$ RNAse (10 mg/ml) was added to DNA sample before incubating in an oven at 37°C for one hour. The quality and quantity of the isolated DNA was confirmed by loading 2 $\mu L$ of DNA and 0.5 $\mu L$ of loading dye (bromophenol blue) in 0.8% agarose gel (Sigma, UK) in 1x TBE buffer (89.2 mM Tris, 89.0 mM Boric acid, 1.25 mM EDTA pH 8.0) and electrophoresed at 100 volts/hour. The concentration and quality was further determined spectrophotometrically by using Nanodrop ND8000 (Thermo Scientific NanoDropCHEM-CF-8 (1) at 260 nm and 280 nm. The concentrations were used to determine the dilution factors for each sample in order to achieve a concentration of 50 ng/$\mu L$.

**Table 4.** Nei's genetic identity matrix for 9 populations of Sorghum bicolor (L.) Moench.

| CA | EA | ICR-K | INDIA | KENYA | NA | RWD | SA | SUD | |
|---|---|---|---|---|---|---|---|---|---|
| 1.00 | | | | | | | | | CA |
| 0.55 | 1.00 | | | | | | | | EA |
| 0.49 | 0.51 | 1.00 | | | | | | | ICR-K |
| 0.48 | 0.56 | 0.86 | 1.00 | | | | | | INDIA |
| 0.63 | 0.63 | 0.79 | 0.75 | 1.00 | | | | | KENYA |
| 0.36 | 0.49 | 0.45 | 0.56 | 0.48 | 1.00 | | | | NA |
| 0.54 | 0.93 | 0.51 | 0.55 | 0.62 | 0.47 | 1.00 | | | RWD |
| 0.39 | 0.49 | 0.87 | 0.88 | 0.70 | 0.53 | 0.48 | 1.00 | | NA |
| 0.60 | 0.61 | 0.77 | 0.82 | 0.80 | 0.55 | 0.58 | 0.76 | 1.00 | SUD |

CA -Central Africa, EA-Eastern Africa, ICR-K-ICRISAT-Kenya, India, Kenya, NA-Northern Africa, RWD-Rwanda, SA-Southern Africa and SUD-Sudan.

**Table 5.** Nested AMOVA for 9 populations of sorghum and partitioning of the total diversity into population components.

| Variance Components | df | Ss | Ms | Ev | %Tv | p | PhiPT(ΦPT) |
|---|---|---|---|---|---|---|---|
| Among population | 8 | 1235.150 | 154.394 | 9.336 | 25 | 0.010 | 0.251 |
| Within population | 125 | 3474.738 | 27.798 | 27.798 | 75 | <0.01 | |
| Total | 133 | 4708.888 | | | 100 | | |

df- degree of freedom, Ss- Sum of squares, Ev-Estimate variances, %Tv-Percentage total variances .

***PCR and primers optimization***

Polymerase chain reaction (PCR) optimization were carried out using five randomly selected DNA samples by varying the concentration of the template DNA, *Taq* DNA polymerase, annealing temperature, number of cycles and the $Mg^{2+}$ salt concentration. Each optimized 10 µL PCR contained 1 µL of 10XPCR buffer (10 mM Tris-HCl pH 8.3), 0.8 µL of 10 mM $MgCl_2$, 0.8 µL of 2.5 mM dNTPs (dATP, dCTP, dGTP and dTTP), 0.075 µL 0.2 units of Amplitaq Gold TaqDNA polymerase (AppliedBiosystems), 1.0 *µL* of 2.0 pmoles/ µL of the forward and 1.0 µL of 10 pmoles/*µL* reverse primers, 1 *µL* of sample DNA at a concentration of 50 ng/µL and 4.325 µl of $dH_2O$. A reaction lacking genomic DNA was also loaded in each PCR run to serve as a negative control (Table 2).

***Amplification of DNA fragments***

PCR (thermocycler) amplification was carried out using simple sequence repeat (SSR) markers well dispersed on the sorghum genetic map. In this study, a total of 30 microsatellites markers were used to screen 134 sorghum accessions (Table 2). The forward primer for each of the SSR markers was labeled at the 5' end of the oligonucleotide using fluorescent dyes to enable detection by the ABI 3730 sequencer (Applied Biosystems). The fluorescent labels used were 6-FAM™ (Blue), VIC™ (Green), NED™ (Yellow), PET™ (Red) (Applied Biosystems Inc., USA). DNA fragments were amplified in a gradient thermal cycler (ABI 9700, Applied Biosystems Inc., USA) using the following program: initial denaturing at 95°C for 3 min followed by 35 cycles of 94°C for 30 seconds, 56°C annealing temperature (which was set to vary with the primer as shown on Table 2) for 1 min and 72°C for 30 s and a further primer extension at 72° for 2 min before holding at 4°C.

***Data analysis***

The microsatellite allele data obtained were analyzed using GeneMapper software version 4.0 (Applied Biosystems Inc., USA). A single or pair of peaks was detected as expected for the co-dominant markers such as SSRs. Fragment sizes were automatically calculated with reference to the internal lane size standard GeneScan-500 LIZ ranging from 35 to 500 bp. Allelic size data for each SSR locus was used to estimate percentage of polymorphic loci, Shannon's information index (*I*), Nei's gene diversity, observed (*Ho*) and expected (*He*) heterozygosities using power marker version 3.25 (Liu and Muse, 2005). Genetic variation within and among populations was estimated through analysis of molecular variance (AMOVA) (Schneider et al., 2000) using GenAlEX6.2 (Peakall and Smouse, 2006). Cluster analysis based on Unweighted Pair Group Method with Arithmetic Average (UPGMA) method within sequential agglomerative hierarchical nested (SAHN) and principal co-ordinate analysis were performed based on Nei's distance matrix (Mathew et al., 2000). The observed heterozygosity (*Ho*) was calculated for each locus as the number of heterozygous individuals over the total number of individuals analysed. Polymorphism information content (PIC) or expected heterozygosity scores for each SSR marker was calculated.

**Conclusion**

Sorghum accessions generally clustered based on their geographical regions. Genetic diversity of sorghum was highest in the Sudan, Indian and Kenyan populations and least in Central Africa and Rwanda. Rare alleles were common in the Kenyan sorghum population and least common in Central Africa. The genetic identity between populations was highest between Rwanda and the East African populations. Most of the diversity resided in individuals within a population. The results from this study suggest that collection strategies of accessions for conservation should focus on the Sudan population because of its high population diversity and Kenyan population because of the high levels of unique alleles.

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