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RESEARCH ARTICLE

Efficiency of SNP and SSR-based Analysis of Genetic Diversity, Population Structure, and Relationships among Cowpea (*Vigna unguiculata* (L.) Walp.) Germplasm from East Africa and IITA Inbred Lines

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Abstract

The extent of genetic diversity and relatedness of cowpea germplasm from East Africa are poorly understood. A set of 13 microsatellites (SSR) and 151 single nucleotide polymorphisms (SNPs) markers were applied to assess the levels of genetic diversity in a sample of 95 accessions of local cowpea germplasm and inbred lines of *Vigna unguiculata*. The average genetic diversity (D), as quantified by the expected heterozygosity, was higher for SSR loci (0.52) than for SNPs (0.34). The polymorphic information content was 0.48 for SSR and 0.28 for SNP while the fixation index was 0.095 for SSR and 0.15 for SNPs showing moderate differentiation and high gene flow among cowpea accessions from East African countries. The results of data analysis of both SSR and SNP markers showed similar clustering patterns suggesting a substantial degree of association between origin and genotype. Principal coordinate analysis (PCoA) with SSR and SNP markers showed that accessions were grouped into two and three broad groups across the first two axes, respectively. Our study found that SNP markers were more effective than SSR in determining the genetic relationship among East African local cowpea accessions and IITA inbred lines. Based on this analysis, five local cowpea accessions Tvu-13490, Tvu-6378, Tvu-13448, Tvu-16073, and 2305675 were identified to be tightly clustered sharing several common alleles with the drought tolerant variety Danila when analyzed with SSR and SNP markers. The findings will assist and contribute to future genetic diversity studies aimed at the genetic improvement of local Eastern Africa cowpea accessions for improved overall agronomic performance in general and breeding for drought tolerant in particular.

Key words : Cowpea, genetic diversity, drought tolerant, principal coordinate analysis, germplasm

Introduction

Cowpea [Vigna unguiculata(L.) Walp.] is one of the most important food legumes in the tropical and sub-tropical regions, where drought is a major production constraint due to low and/or erratic rainfall (Singh et al. 1997). Cowpea grows in a wide range of environments covering 400N to 300S (Richie 1985), and it has considerable ability to adapt to high temperatures and drought compared to most crop species (Ehlers and Hall 1997). Cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops and is a valuable and dependable commodity that produces income for farmers and traders (Langyintuo et al. 2003). Like other grain legumes, the protein found in cowpeas is rich in the essential amino acids lysine and tryptophan (Timko and Singh 2008). Cowpea is a multi-purpose crop, as it provides both human food and

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animal feed. The crop is a source of income to both small-scale farmers (especially women who farm) and larger scale grain traders (Singh 2005; Timko and Singh 2008).

Cowpea breeding and genetic improvement programs around the world are mainly focused on combining desirable agronomic characteristics, e.g., early maturity, photoperiod insensitivity, plant type, and seed quality with resistance to major diseases, insect pests or parasites, which afflict cowpea cultivars (Timko et al. 2007; Timko and Singh 2008). The conventional methods for estimating genetic diversity have been based on the use of morphological markers. However, the low number of useful morphological markers, the lack of knowledge about how genes are controlled, and the environmental influence on phenotypic expression at different stages of growth have been the major limitations for using these markers as reliable tools in diversity studies (Dikshit et al. 2007).

Molecular genetics techniques based on DNA polymorphism have been increasingly used to characterize and identify novel germplasm within the available collections for uses in crop breeding process (O'Neill et al. 2003). Diversity studies in wild and cultivated cowpea germplasm employ a variety of approaches, such as analyzing morphological and physiological traits (Ehlers and Hall 1996), using allozymes (Pasquet 1993, 1999, 2000), seed storage proteins (Fotso et al. 1994), chloroplast DNA polymorphism (Vaillancourt and Weeden 1992), restriction fragment length polymorphisms (RFLP) (Fatokun et al.1993), amplified fragment length polymorphisms (AFLP) (Fang et al. 2007), DNA amplification fingerprinting (DAF) (Simonet et al. 2007), random amplified polymorphic DNA (RAPDs) (Ba et al. 2004; Diouf and Hilu 2005; Nkongolo 2003; Xavier et al. 2005; Zannou et al. 2008), simple sequence repeats (SSRs) (Ogunkanmi et al. 2008; Uma et al. 2009; Wang et al. 2008; Xu et al. 2010) and sequence tagged microsatellites (STMS) (Abe et al. 2003; Choumane et al. 2000; He et al. 2003; Li et al. 2001). Of these techniques, the use of SSRs has proven extremely useful (Asare et al. 2010; Ogunkanmi et al. 2008; Uma et al. 2009; Wang et al. 2008; Xu et al. 2010;). In addition to being abundant and distributed throughout eukaryotic genomes, SSRs are highly polymorphic, inherited co-dominantly and they are easily reproducible and traceable with simple screening requirements (Wang et al. 2008). Li et al. (2001), in which 27 SSR primers were tested, conducted the earliest cowpea SSR research. Subsequently, a number SSR-based studies of cowpea from different areas, mainly Africa and Asia, has been carried out. Ogunkanmi et al. (2008) demonstrated that Africa to be the center of diversity of wild cowpea, using SSR marker analyses.

The advent of new sequencing technologies has dramatically changed the landscape for detecting and monitoring genome-wide polymorphism (Craig et al. 2008; Metzker 2005; Schuster 2008). Today, single nucleotide polymorphisms (SNPs) are rapidly replacing simple sequence repeats (SSRs) as the DNA marker of choice for applications in plant breeding and genetics because they are more abundant, stable, amenable to automation, efficient, and increasingly cost-effective (Duran et al. 2009; Edwards and Batley 2010; Rafalski 2002). However, the review by Tan et al. (2012), indicated that SSR was the most frequently used molecular marker, whereas the use of SNP markers for genetic diversity study of cowpea was not common. A very large number of SNP markers are now available for detailed analysis of genome structure, genome-wide association studies, and precision breeding, especially for those animals and plants for which high-density genotyping arrays are commercially produced (Ganal et al. 2011; Ramos et al. 2009). However, this activity has largely bypassed "orphan crops" such as cowpea which are crops of relevance to food security and income for subsistence farmers in developing countries (Delmer 2005). Nowadays, SNP markers are available with genotyping service providers to quickly and affordably assay lines for diversity analysis.

Knowledge of the genetic diversity available within the local and regional germplasm collections of cowpea can enhance the utilization of this germplasm ineffective cowpea improvement programs (Hegde and Mishra 2009). Whereas some studies were carried out on local cowpea accessions of East African countries such as Kenya (Kuruma et al. 2008), Tanzania using SSR markers (Sariah et al. 2010) and Ethiopia using SSR (Desalegne et al. 2016), the diversity and relatedness of cowpea germplasm in Ethiopia, Kenya, Somalia and Sudan are poorly understood. Hence, it is essential to study the genetic diversity of East African cowpea genotypes using these highly informative DNA markers. The aim of present study was to determine the genetic diversity and relationships between some local East African (Ethiopia, Kenya, Somalia, and Sudan) cowpea germplasm and inbred lines obtained from the International Institute of Tropical Agriculture (IITA), Nigeria, based on fluorescent SSR and SNP markers, facilitating breeding for improved cowpea varieties in the face of changing abiotic factors such as drought affecting production and productivity, as well as to compare and determine the efficiency of results obtained from SSR with SNP markers analysis.

Materials and Methods

Plant materials

Ninety-five cowpea accessions consisting of 26 from Ethiopia, 29 from Kenya, 15 from Somalia, 9 from Sudan, and 16 inbred lines from IITA were used in the present study (Table 1). The TVu lines were obtained from the genetic resources centre of IITA, Ibadan, Nigeria but collected originally from the different countries as stated in Table 1. Leaf samples for DNA isolation were collected from 15-day-old seedlings of each accession grown in pots in a screen house at IITA, Ibadan, Nigeria (7°23′16″ N and 3°53′47″ E). A standard check of drought-resistant variety, Danila, and drought susceptible variety, Tvu-7778 varieties were included in our study to see their grouping patterns with the East

Table 1. List of cowpea accessions evaluated in this study.

No.	Accession name	Accession type	Genus/species	Geographical Origin	Developer*
1	IT-99K-1122	Inbred line	*V. unguiculata*	Nigeria	IITA
2	IT-97K-356-1	Inbred line	*V. unguiculata*	Nigeria	IITA
3	IT-99K-1060	Inbred line	*V. unguiculata*	Nigeria	IITA
4	IT-96K-719	Inbred line	*V. unguiculata*	Nigeria	IITA
5	IT-93K-556-7	Inbred line	*V. unguiculata*	Nigeria	IITA
6	IT-98K-1111-1	Inbred line	*V. unguiculata*	Nigeria	IITA
7	IT-93K-452-1	Inbred line	*V. unguiculata*	Nigeria	IITA
8	IT-95K-207-22	Inbred line	*V. unguiculata*	Nigeria	IITA
9	IT-93K-428-3	Inbred line	*V. unguiculata*	Nigeria	IITA
10	IT-95K-268-1-4	Inbred line	*V. unguiculata*	Nigeria	IITA
11	IT-97K-569-9	Inbred line	*V. unguiculata*	Nigeria	IITA
12	IT-97K-569-9	Inbred line	*V. unguiculata*	Nigeria	IITA
13	IT-99K-1245	Inbred line	*V. unguiculata*	Nigeria	IITA
14	IT97K-449-38	Inbred line	*V. unguiculata*	Nigeria	IITA
15	TVu 7778	Drought susceptible	*V. unguiculata*	Nigeria	IITA
16	Danilla	Drought resistance	*V. unguiculata*	Nigeria	IITA
17	82D-889(CH)	Released variety	*V. unguiculata*	Ethiopia	EIAR
18	BOLE (CH)	Released variety	*V. unguiculata*	Ethiopia	EIAR
19	208776	Local accession	*V. unguiculata*	Ethiopia	Land race
20	211443	Local accession	*V. unguiculata*	Ethiopia	Land race
21	211435	Local accession	*V. unguiculata*	Ethiopia	Land race
22	211446	Local accession	*V. unguiculata*	Ethiopia	Land race
23	211491	Local accession	*V. unguiculata*	Ethiopia	Land race
24	211444	Local accession	*V. unguiculata*	Ethiopia	Land race
25	211557	Local accession	*V. unguiculata*	Ethiopia	Land race
26	211490	Local accession	*V. unguiculata*	Ethiopia	Land race
27	211436	Local accession	*V. unguiculata*	Ethiopia	Land race
28	211430	Local accession	*V. unguiculata*	Ethiopia	Land race
29	241761	Local accession	*V. unguiculata*	Ethiopia	Land race
30	211429	Local accession	*V. unguiculata*	Ethiopia	Land race
31	211385	Local accession	*V. unguiculata*	Ethiopia	Land race
32	211441	Local accession	*V. unguiculata*	Ethiopia	Land race
33	211433	Local accession	*V. unguiculata*	Ethiopia	Land race
34	230575	Local accession	*V. unguiculata*	Ethiopia	Land race
35	230044	Local accession	*V. unguiculata*	Ethiopia	Land race
36	221727	Local accession	*V. unguiculata*	Ethiopia	Land race
37	223403	Local accession	*V. unguiculata*	Ethiopia	Land race
38	223402	Local accession	*V. unguiculata*	Ethiopia	Land race
39	TVU-1977	Local accession	*V. unguiculata*	Ethiopia	Land race
40	VWT	Local accession	*V. unguiculata*	Ethiopia	Land race
41	Black eye bean	Local accession	*V. unguiculata*	Ethiopia	EIAR
42	Assebot	Local accession	*V. unguiculata*	Ethiopia	EIAR
43	TVu-433	Local accession	*V. unguiculata*	Kenya	Land race
44	TVu-114	Local accession	*V. unguiculata*	Kenya	Land race
45	TVu-115	Local accession	*V. unguiculata*	Kenya	Land race

Table 1. List of cowpea accessions evaluated in this study. (continued)

No.	Accession name	Accession type	Genus/species	Geographical Origin	Developer*
46	TVu-139	Local accession	*V. unguiculata*	Kenya	Land race
47	TVu-552	Local accession	*V. unguiculata*	Kenya	Land race
48	TVu-1190	Local accession	*V. unguiculata*	Kenya	Land race
49	TVu-2651	Local accession	*V. unguiculata*	Kenya	Land race
50	TVu-6378	Local accession	*V. unguiculata*	Kenya	Land race
51	TVu-8450	Local accession	*V. unguiculata*	Kenya	Land race
52	TVu-8767	Local accession	*V. unguiculata*	Kenya	Land race
53	TVu-11414	Local accession	*V. unguiculata*	Kenya	Land race
54	TVu-11419	Local accession	*V. unguiculata*	Kenya	Land race
55	TVu-11422	Local accession	*V. unguiculata*	Kenya	Land race
56	TVu-11431	Local accession	*V. unguiculata*	Kenya	Land race
57	TVu-13448	Local accession	*V. unguiculata*	Kenya	Land race
58	TVu-13454	Local accession	*V. unguiculata*	Kenya	Land race
59	TVu-13457	Local accession	*V. unguiculata*	Kenya	Land race
60	TVu-13467	Local accession	*V. unguiculata*	Kenya	Land race
61	TVu-13469	Local accession	*V. unguiculata*	Kenya	Land race
62	TVu-13470	Local accession	*V. unguiculata*	Kenya	Land race
63	TVu-13473	Local accession	*V. unguiculata*	Kenya	Land race
64	TVu-13475	Local accession	*V. unguiculata*	Kenya	Land race
65	TVu-13485	Local accession	*V. unguiculata*	Kenya	Land race
66	TVu-13490	Local accession	*V. unguiculata*	Kenya	Land race
67	TVu-13501	Local accession	*V. unguiculata*	Kenya	Land race
68	TVu-13511	Local accession	*V. unguiculata*	Kenya	Land race
69	TVu-13516	Local accession	*V. unguiculata*	Kenya	Land race
70	TVu-14160	Local accession	*V. unguiculata*	Kenya	Land race
71	TVu-16410	Local accession	*V. unguiculata*	Kenya	Land race
72	TVu-16031	Local accession	*V. unguiculata*	Somalia	Land race
73	TVu-16038	Local accession	*V. unguiculata*	Somalia	Land race
74	TVu-16041	Local accession	*V. unguiculata*	Somalia	Land race
75	TVu-16043	Local accession	*V. unguiculata*	Somalia	Land race
76	TVu-16044	Local accession	*V. unguiculata*	Somalia	Land race
77	TVu-16050	Local accession	*V. unguiculata*	Somalia	Land race
78	TVu-16053	Local accession	*V. unguiculata*	Somalia	Land race
79	TVu-16054	Local accession	*V. unguiculata*	Somalia	Land race
80	TVu-16061	Local accession	*V. unguiculata*	Somalia	Land race
81	TVu-16073	Local accession	*V. unguiculata*	Somalia	Land race
82	TVu-16078	Local accession	*V. unguiculata*	Somalia	Land race
83	TVu-16083	Local accession	*V. unguiculata*	Somalia	Land race
84	TVu-16086	Local accession	*V. unguiculata*	Somalia	Land race
85	TVu-16174	Local accession	*V. unguiculata*	Somalia	Land race
86	TVu-16176	Local accession	*V. unguiculata*	Somalia	Land race
87	TVu-11955	Local accession	*V. unguiculata*	Sudan	Land race
88	TVu-11957	Local accession	*V. unguiculata*	Sudan	Land race
89	TVu-11978	Local accession	*V. unguiculata*	Sudan	Land race
90	TVu-11979	Local accession	*V. unguiculata*	Sudan	Land race

Table 1. List of cowpea accessions evaluated in this study. (continued)

No.	Accession name	Accession type	Genus/species	Geographical Origin	Developer*
91	TVu-11982	Local accession	*V. unguiculata*	Sudan	Land race
92	TVu-11983	Local accession	*V. unguiculata*	Sudan	Land race
93	TVu-11984	Local accession	*V. unguiculata*	Sudan	Land race
94	TVu-11986	Local accession	*V. unguiculata*	Sudan	Land race
95	TVu-11987	Local accession	*V. unguiculata*	Sudan	Land race

*IITA-International Institute of Tropical Agriculture, EIAR-Ethiopian Institute of Agricultural Research

Table 2. List of fluorescent microsatellite (SSR) markers with forward/reverse nucleotide sequence, dye color, product size, and amount used in this study.

No	Fluorescent primer	Primer Sequence	Product Size (bp)	color	Amount (µl)
1	Vm39	5′ GAT GGTTGTAATGGGAGAGTC-3′ 5′ AAAAGGATGAAATTAGGA GAG CA-3′	212	yellow	2
2	Vm40	5′ TAT TAC GAG AGG CTA TTT ATT GCA-3′ 5′ CTC TAA CAC CTC AAG TTA GTG ATC-3′	200	Blue	3
3	Vm53	5'-GAG TTC CGT TCG TTG TGA GTA GAG-3' 5'-ACA GAG GAG GAA AAG GAA GTA TGC-3'	288	Red	4
4	Vm35	5′GGT CAA TAG AATAATGGAAAGTGT-3′ 5′ ATGGCTGAAATAGGTGTCTGA-3′	127	Green	2
5	Vm9	5′ ACCGCA CCC GAT TTATTT CAT-3′ 5′ ATCAGCAGA CAG GCAAGACCA-3′	271	Red	1
6	Vm70	5'-AAA ATC GGG GAA GGA AAC C-3' (AG) 5'-GAA GGC AAA ATA CAT GGA GTC AC-3'	186	Green	1.6
7	Vm94	5'-TCG AAC TTT GGC TTG AGG-3' 5'-TGT CGT TTT GTC CCC CAT TA-3'	253	Blue	3
8	Bmd2	5′-AGCGACAGCAAGAGAACCTC-3′ 5′-CAACAAACGGTGATTGACCA-3′	106	Blue	2.6
9	Bmd17	5′-GTTAGATCCCGCCCAATAGTC-3′ 5′-AGATAGGAAGGGCGTGGTTT-3′	98	Yellow	3
10	Vm31	5'-CGC TCT TCG TTG ATG GTT ATG-3' 5'-GTG TTC TAG AGG GTG TGA TGG TA-3′	200	Blue	1.4
11	Vm37	5′ TGTCCGCGTTCTATAAAT CAG C-3′ 5′ CGAGGATGAAGTAACAGATGATC-3′	289	Red	2.2
12	Vm51	5'-CAT TGC CAC TGG TTT CAC TTA-3' 5'-GAG GCT CAG CAT TTT GTT TCT AT-3	256	Yellow	4
13	Vm74	5′ CTGCTACACCTTCCATCATTC-3′ 5′ CCTTTGCTGTGTGGTGGTTT-3′	135	Green	1.2

Note: Markers were PCR amplified with Yellow (NED), Blue (6-FAM), Red (PET) and Green (Vic/hex) forward primers and unlabeled reverse primers.

African cowpea accessions and IITA inbred lines.

DNA isolation and quantification

Total genomic DNA was extracted following the CTAB modified protocol of Dellaporta et al. (1983) at International Institute of Tropical Agriculture (IITA) Bioscience Laboratory, Ibadan, Nigeria. Approximately 200 mg leaf samples were harvested in 1.5 ml Eppendorf tube from each accession. The samples were kept on ice during collection and transportation to the lab. Samples were ground into fine powder with liquid nitrogen using autoclaved konte pestles. The quality of extracted DNA samples was checked by running 2 ul samples on 1% agarose gel and the concentrations of genomic DNA samples were estimated using NanoDrop1000 spectrophotometer (Thermo Scientific, Wilmington, DE 19810 USA).

PCR amplification

PCR amplification was carried out in 10.05 µl final volume mixture containing 1 µl 10X PCR buffer, 1.25 µM of each primer, 0.8 mM of each dNTP, 0.06 ul (0.3U) Taq DNA polymerase, and 2 µl of 20 ng DNA and 3.34 µl double distilled water on Eppendorf Mastercycler Gradient Thermocycler (Foster City, California 94404, USA). The PCR cycle was programmed for initial denaturation at 94°C for 1 min followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C, and a final extension of 10 min at 72°C. PCR products were resolved on 2% agarose.

Fragment analysis using ABI sequencer

Fragment analyses of SSR primers that yielded polymorphisms in the PCR analyses were carried out using 13 of 16

fluorescent primers. Three primers gave monomorphic amplicons or were highly inconsistent in their output, and were hence removed from the analysis. The primers were run in multiplexes, based on their fluorescence dye and allele size using BIONEER ACCUPOWER® Multiplex PCR Premix Kits (Table 2). PCR products were run on an ABI PRISM 3730xl fragment analyzer (Applied Biosystems, Foster City, CA, USA) at the IITA Bioscience Laboratory, Ibadan, Nigeria and allele peaks were sized and called using the Genemapper v. 3.7 software. The observed allele size was then adjusted for the discrete allele size using AlleloBin software (http://test1.icrisat.org/gt-bt/download_allelobin.htm).

Single Nucleotide Polymorphism (SNP) Genotyping

One hundred and sixty-four SNP markers were selected out of 1,122 used to construct a linkage map for cowpea at the University of California, Riverside (Lucas et al. 2011; Muchero et al. 2009) (Supplementary). The 164 SNP markers were selected in such a way that they would cover evenly each of the 11 chromosomes of cowpea as represented by the linkage groups. Among the 164 SNPs markers, 151 SNPs were polymorphic. Seven SNPs primers were monomorphic, and therefore, were excluded from the analysis. In addition, six SNPs failed to produce a genotype call because of poor signals (Table 3), hence were excluded from the analysis.

Table 3. Polymorphism of 164 SNPs based on 95 cowpea accessions.

Class	No. of Marker	Percentage (%)
Polymorphic	151	92.10%
Monomorphic	7	4.30%
No amplicons (banding)	6	3.60%
Total	164	100.00%

The 95 DNA samples were prepared on ABI plate for shipping per the requirement of SNP analysis service provider, LGC Genomics (http://www.lgcgroup.com/) (formerly KBioscience), UK. After genotyping and filtering, 151 SNP markers were used for final analysis (Table 4).

Statistical analysis

Allele frequency, genetic diversity, and polymorphism information content (PIC) were determined for each of the 151 SNP and 13 SSR markers using PowerMarker Version: V3.25 software (Liu and Muse 2005). Neighbor-Joining dendrogram was generated by the software program, DARwin 5, Version: 5.0 (http://darwin.cirad.fr/darwin) (Perrier et al. 2003). In addition, the genetic structure of the accessions was investigated by Analysis of Molecular Variance (AMOVA), where fixation indices were used to elucidate the resulting genetic structure and Principal Coordinate Analysis (PCoA) was performed to identify genetic variation patterns among the cowpea accessions using GenAlex version 6.5b3 (http://biology.anu.edu.au/GenAlEx/) software (Peakall and Smouse 2006).

Results

SSR polymorphism

A set of 13 polymorphic SSR primers were used to analyze the genetic diversity of 95 cowpea lines, which consisted of local accessions, and inbred lines obtained mainly from some East African countries and IITA. The number of alleles varied from three for Vm51 and BMD17 and 15 for Vm70, with an average of 6.38 (Table 5). The SSR marker Vm35 gave the highest allele frequency of 0.81 while Vm70 had the lowest at 0.21 with a mean of 0.61 among the tested cowpea accessions. Genetic diversity (D) revealed by the markers varied from 0.32 for SSR Vm35 to 0.87 for Vm70 with the mean value of 0.56. The polymorphic information content (PIC) representing the allele diversity for a specific locus varied from 0.28 for primer Vm35 to 0.86 for primer Vm70 with a mean of 0.51 (Table 5).

Genetic variation among accessions based on SSR markers

The Neighbor-Joining (NJ) tree generated using the 13 SSR markers separated the 95 *V. unguiculata* accessions into three main clusters (Fig 1 and Table 6). A clear genetic structure was observed among the 95-cowpea accessions from East African countries and IITA inbred lines. The total number of main clusters is different from the five-total number of the regions (Ethiopia, Kenya, Somali, Sudan, and IITA inbred lines); nonetheless, the geographic distance and the genetic background of the accessions were clearly reflected in the neighbor-joining tree grouping. Accessions from the five sources of germplasm used in this study were distributed into the three main clusters but with varying numbers per cluster. Thirty-six of 95 (38%), 40 of 95 (42%), and 19 of 95 (20%) cowpea accessions were placed in the three main clusters I, II, and III respectively, showing most of the cowpea accessions (80%) grouped in the main clusters I and II. Almost half of the main cluster I is made up of accessions from Kenya (14). There are nine accessions from Somalia and six accessions each from Sudan and four accessions from Ethiopia and only one inbred line from IITA is in this cluster. Main cluster II contains mostly accessions from Ethiopia (22), 11 from Kenya, six inbred lines from IITA, two accessions from Sudan, and only one from Somalia. Main cluster III contains mostly inbred lines from IITA (9), four accessions from Kenya, three from Somalia, two from Ethiopia, and only one from the Sudan. The six released cowpea varieties from Ethiopia distributed and clustered with all the three main clusters, Asebot and 82D-889(CH) tightly clustered with two local Ethiopian accessions (211444 and 211490) of sub-cluster I. Similarly, BLACK EYED BEANS, WWT, TVU1977 all tightly clustered with 18 local Ethiopian accessions of sub-cluster II, whereas the released varieties Bole (CH) is in a

Table 4. List of selected genetic linkage map of cowpea SNPs in this study.

No.	SNP IDs Marker	Chromosome No.
1	1107_518	1
2	12526_795	1
3	12882_709	1
4	12929_463	1
5	13294_282	1
6	14619_471	1
7	18_107	1
8	25_592	1
9	2820_248	1
10	3787_812	1
11	5735_110	1
12	9432_1340	1
13	9815_2051	1
14	10480_616	2
15	1297_783	2
16	14497_540	2
17	16946_421	2
18	2046_754	2
19	3427_925	2
20	3838_830	2
21	4200_155	2
22	4273_342	2
23	6580_67	2
24	708_159	2
25	8044_1006	2
26	8253_397	2
27	8395_1157	2
28	8947_802	2
29	9739_495	2
30	10378_737	3
31	10650_1563	3
32	1165_701	3
33	12505_1312	3
34	12905_686	3
35	13022_1425	3
36	14056_564	3
37	15129_553	3
38	15183_436	3
39	16139_2530	3
40	16566_353	3
41	16655_1561	3
42	2_341	3
43	2453_65	3
44	2591_569	3
45	2974_1109	3
46	7068_60	3
47	7087_1100	3
48	1202_1215	4
49	12854_535	4
50	13269_270	4
51	13386_815	4
52	13873_544	4
53	4702_954	4
54	5268_412	4
55	5503_54	4
56	5652_704	4
57	6867_337	4

Table 4. List of selected genetic linkage map of cowpea SNPs in this study. (continued)

No.	SNP IDs Marker	Chromosome No.
58	8166_564	4
59	897_240	4
60	9114_900	4
61	1004_587	5
62	11613_1075	5
63	11920_1704	5
64	1441_128	5
65	14814_511	5
66	1980_886	5
67	5058_372	5
68	534_355	5
69	6046_661	5
70	6663_368	5
71	7344_500	5
72	7967_1210	5
73	8121_1880	5
74	8905_1569	5
75	10738_1400	6
76	10974_245	6
77	12568_234	6
78	14654_1071	6
79	14784_1653	6
80	15305_818	6
81	279_179	6
82	3900_562	6
83	437_590	6
84	4692_429	6
85	4749_1972	6
86	5270_452	6
87	5356_124	6
88	7233_543	6
89	7383_1042	6
90	8438_669	6
91	9134_1559	6
92	11558_901	7
93	11585_1881	7
94	12349_535	7
95	13586_1058	7
96	13872_1420	7
97	15113_1068	7
98	17196_517	7
99	17450_1553	7
100	17513_514	7
101	234_249	7
102	4131_472	7
103	4778_497	7
104	5692_1408	7
105	1936_545	8
106	1281_790	8
107	14702_888	8
108	15637_1357	8
109	15875_801	8
110	311_1536	8
111	3803_763	8
112	5135_477	8
113	6378_514	8
114	7248_578	8

Table 4. List of selected genetic linkage map of cowpea SNPs in this study. (continued)

No.	SNP IDs Marker	Chromosome No.
115	9607_1753	8
116	1060_220	9
117	12126_561	9
118	122_468	9
119	14034_820	9
120	15764_405	9
121	15773_423	9
122	1989_448	9
123	5137_1051	9
124	5656_680	9
125	658_460	9
126	7548_1327	9
127	7565_739	9
128	9779_613	9
129	12029_2782	10
130	1283_371	10
131	1653_181	10
132	2245_530	10
133	2870_790	10
134	4237_650	10
135	4306_482	10
136	4800_500	10
137	5993_278	10
138	6205_632	10
139	8877_1528	10
140	10277_636	11
141	11599_1036	11
142	14825_288	11
143	16413_395	11
144	3494_143	11
145	4712_832	11
146	5449_242	11
147	5756_456	11
148	7184_257	11
149	734_340	11
150	8150_1237	11
151	8842_943	11

separate group with only 230567 from Ethiopia.

Analysis of Molecular Variance (AMOVA) using SSR markers

The Analysis of molecular variance (AMOVA) showed that all sources of variation were highly significant ($P < 0.001$) and approximately 10% of the overall variation was attributed to genetic differentiation among regions; 84% was explained by differences among populations within regions and 6% was attributed to genetic differentiation among individuals and within populations (Table 7). This indicated that the accessions in this study possess wide diversity among populations and within regions. Previous investigators have reported that fixation index (Fst) values in a range of 0–0.05 generally indicate little differentiation, whereas values in the 0.05-0.15 range suggest moderate differentiation, 0.15-0.25 large differentiation, and above 0.25 very large differentiation (De Vicente et al. 2004; Kiambi et al. 2005;

Table 5. Allele Frequency, Allele Number, Genetic Diversity, and Polymorphism Information Content (PIC) of the simple sequence repeat (SSR) markers analyzed.

Marker	Allele Frequency	Allele Number	Genetic Diversity	PIC
Vm39	0.45	4	0.64	0.56
Vm40	0.51	7	0.68	0.64
Vm53	0.75	7	0.42	0.39
Vm35	0.81	4	0.32	0.28
Vm9	0.73	4	0.42	0.38
BMD17	0.48	3	0.59	0.49
BMD2	0.66	8	0.52	0.48
Vm70	0.21	15	0.87	0.86
Vm94	0.69	5	0.47	0.42
Vm31	0.39	8	0.71	0.66
Vm37	0.63	6	0.54	0.48
Vm51	0.75	3	0.39	0.36
Vm74	0.4	9	0.71	0.67
Mean	0.57	6.38	0.56	0.51

Table 6. Neighbor-Joining (NJ) tree analysis identified three sub-clusters among 95 Cowpea accessions.

No	Cluster	I				II				III		
	Main group	36				40				19		
	Sub-group	I (22)		II (14)		I (25)		II (15)		I (9)		II (10)
	Sub-sub-group	I (14)	II (8)	I (7)	II (7)	I (15)	II (10)	I (8)	II (7)	I (8)	II (1)	I (5)
1	Ethiopia (26)	4	-	-	-	15	1	1	3	2	-	-
2	Kenya (29)	8	3	-	3	-	3	6	2	2	-	-
3	IITA inbred lines (16)	-	-	1	-	-	5	1	-	2	1	5
4	Somalia (15)	2	5	-	4	-	1	-	-	1	-	-
5	Sudan (9)	-	-	6	-	-	-	-	2	1	-	-

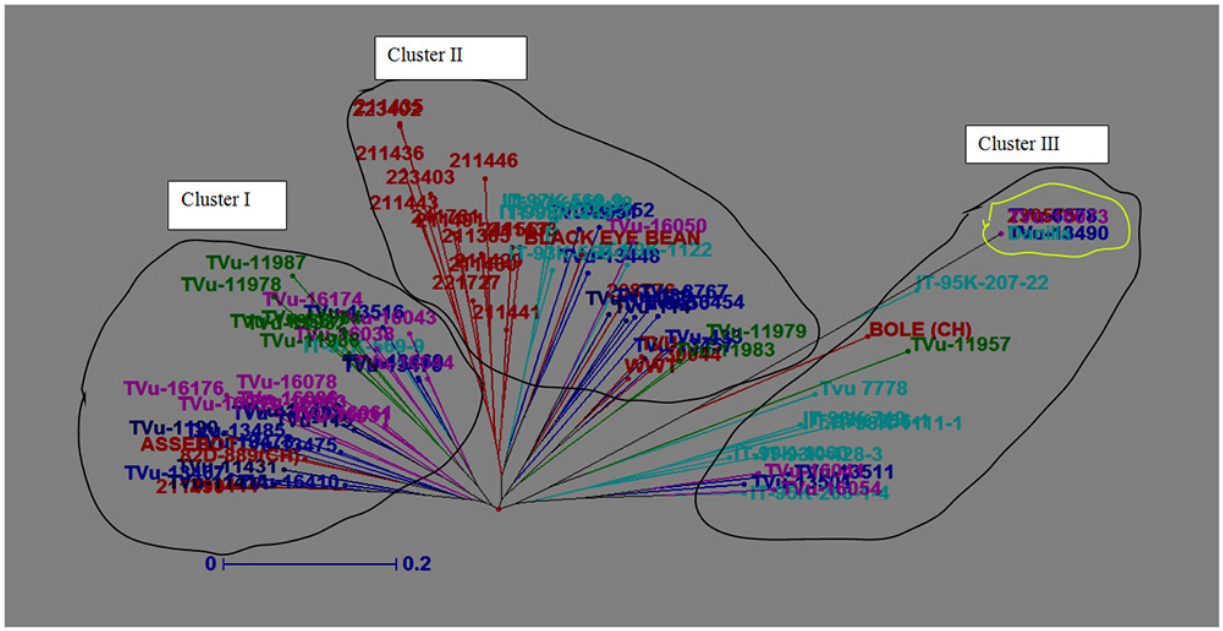

Fig. 1. Neighbor-Joining (NJ) tree constructed for SSR data based on countries Ethiopia (Red), Kenya (Blue), Somalia (Pink), Sudan (Green), and IITA inbred lines (Light blue). Drought tolerance accessions (Yellow).

Table 7. Analysis of molecular variance (AMOVA) among regions.

Source of variation	DF	Sum of squares	Var components	Variation (%)	F statistics (Fst)
Among regions	4	89.540	0.408	10**	0.095
Among populations within regions	90	672.002	3.589	84**	
Among individuals Within populations	95	27.500	0.289	6**	
Total	189	789.042	4.286	-	

***$P < 0.001$, Nm (gene flow) =2.38

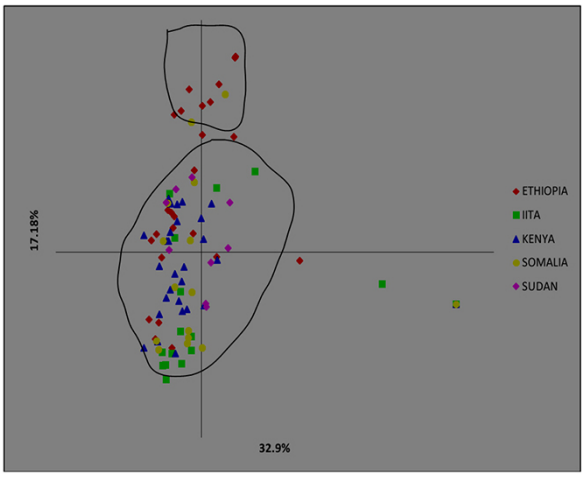

Fig. 2. Principal coordinates analysis (PcoA) of 95 cowpea accessions based on SSR data.

Wright 1984). In this study, the observed the fixation index (Fst) was 0.095 which showed moderate differentiation between accessions based on country of collection and the value recorded for the number of migrants' gene (gene flow between and within countries) between countries was moderate (Nm) 2.38; illustrating the existence of relatively moderate germplasm exchange among cowpea accessions of East African origin and with IITA inbred lines.

Genetic differentiation between regions groups based on SSR markers

The accessions were getting grouped into two broad groups across the first two axes. The first three PCoA vectors explain a total of 65.87% of the genotypic variability by the spatial separation of the genotypes (Fig. 2), in which PC1, PC2, and PC3 explained 32.9, 17.18, and 16.54% of the total molecular variance, respectively. Along the PC1 axis, the accessions were more dispersed in their distribution, hence the PC1 is effective as compared with the PC1 in separating each region into the left and right side of the plot. In PCoA all accessions were labeled with different colors based on their different regions to indicate their region specificity, there was intermixing of color across the coordinates.

SNP polymorphism

All possible SNP types were found in the cowpea lines

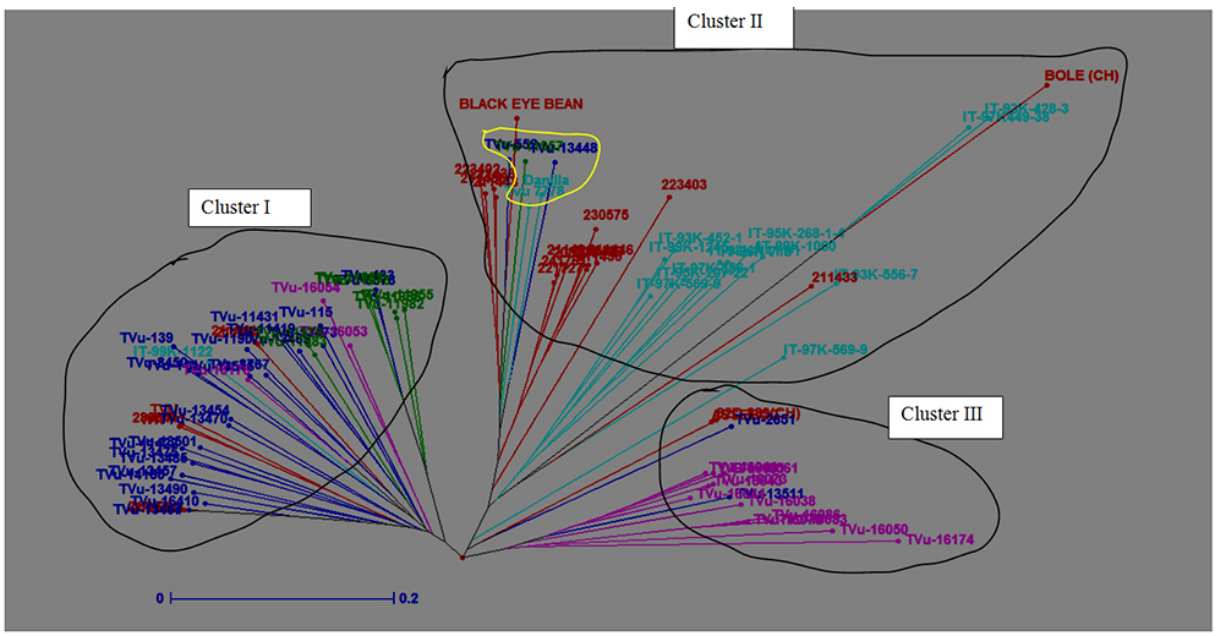

Fig. 3. Neighbor-Joining (NJ) tree constructed for SNP data based on countries Ethiopia (Red), Kenya (Blue), Somalia (Pink), Sudan (Green), and IITA inbred lines (Light blue). Drought tolerance accessions (Yellow).

Table 8. Allele frequency, Allele type, genetic diversity, and polymorphism information content (PIC) of the SNPs markers analyzed.

No.	Marker	Allele frequency	Genetic diversity	SNP type	PIC
1	1936_545	0.76	0.37	G/A	0.30
2	1004_587	0.85	0.26	G/A	0.22
3	10277_636	0.78	0.35	G/C	0.29
4	10378_737	0.61	0.48	G/A	0.36
5	10480_616	0.65	0.45	G/A	0.35
6	1060_220	0.83	0.28	C/T	0.24
7	10650_1563	0.57	0.49	C/A	0.37
8	10738_1400	0.59	0.48	G/A	0.37
9	10974_245	0.54	0.50	G/A	0.37
10	1107_518	0.97	0.05	G/A	0.05
11	11558_901	0.66	0.45	C/T	0.35
12	11585_1881	0.87	0.23	C/T	0.20
13	11599_1036	0.95	0.10	T/A	0.10
14	11613_1075	0.67	0.44	G/A	0.35
15	1165_701	1.00	0.46	G/C	0.35
16	11920_1704	0.65	0.27	G/A	0.23
17	1202_1215	0.84	0.50	G/A	0.37
18	12029_2782	0.54	0.40	G/T	0.32
19	12126_561	0.73	0.06	T/A	0.06
20	122_468	0.97	0.03	C/A	0.03
21	12349_535	0.98	0.17	T/A	0.16
22	12505_1312	0.90	0.50	G/A	0.37
23	12526_795	0.52	0.41	C/T	0.32
24	12568_234	0.72	0.18	G/T	0.16
25	1281_790	0.90	0.37	C/T	0.30
26	1283_371	0.75	0.47	C/T	0.36
27	12854_535	0.62	0.43	T/A	0.34
28	12882_709	0.69	0.43	G/A	0.34
29	12905_686	0.69	0.27	C/T	0.23
30	12929_463	0.84	0.30	G/A	0.26
31	1297_783	0.82	0.48	T/A	0.37
32	13022_1425	0.59	0.39	G/A	0.31
33	13269_270	0.74	0.43	C/T	0.34
34	13294_282	0.69	0.45	C/T	0.35
35	13386_815	0.65	0.19	G/A	0.18
36	13586_1058	0.89	0.43	C/T	0.34
37	13872_1420	0.69	0.29	G/A	0.25
38	13873_544	0.82	0.44	G/A	0.34
39	14034_820	0.68	0.49	C/T	0.37
40	14056_564	0.58	0.40	G/T	0.32
41	1441_128	0.73	0.10	G/A	0.10
42	14497_540	0.95	0.46	C/T	0.35
43	14619_471	0.65	0.50	C/T	0.37
44	14654_1071	0.52	0.49	C/T	0.37
45	14702_888	0.58	0.50	G/C	0.37

Table 8. Allele frequency, Allele type, genetic diversity, and polymorphism information content (PIC) of the SNPs markers analyzed (continued).

No.	Marker	Allele frequency	Genetic diversity	SNP type	PIC
46	14784_1653	0.52	0.42	G/C	0.33
47	14814_511	0.70	0.20	C/T	0.18
48	14825_288	0.89	0.50	C/A	0.37
49	15113_1068	0.53	0.44	T/A	0.35
50	15129_553	1.00	0.33	G/A	0.28
51	15183_436	0.67	0.44	C/T	0.34
52	15305_818	0.79	0.43	G/A	0.34
53	15637_1357	0.68	0.29	G/A	0.25
54	15764_405	0.68	0.36	G/A	0.30
55	15773_423	1.00	0.33	C/T	0.27
56	15875_801	0.82	0.32	G/A	0.27
57	16139_2530	0.76	0.49	G/C	0.37
58	16413_395	0.79	0.50	G/C	0.37
59	1653_181	0.80	0.45	G/A	0.35
60	16566_353	0.58	0.37	C/T	0.30
61	16655_1561	0.53	0.12	G/A	0.11
62	16946_421	0.65	0.38	C/T	0.31
63	17196_517	0.76	0.41	G/C	0.33
64	17450_1553	0.93	0.45	C/A	0.35
65	17513_514	0.74	0.14	C/A	0.13
66	18_107	0.71	0.02	C/T	0.02
67	1980_886	1.00	0.12	T/A	0.11
68	1989_448	1.00	0.28	C/T	0.24
69	2_341	0.65	0.22	C/T	0.20
70	2046_754	0.92	0.47	C/T	0.36
71	2245_530	0.99	0.24	G/C	0.21
72	234_249	0.94	0.49	C/T	0.37
73	2453_65	0.84	0.33	C/T	0.27
74	25_592	0.87	0.43	T/A	0.34
75	2591_569	0.62	0.46	C/T	0.35
76	279_179	1.00	0.40	G/A	0.32
77	2820_248	0.90	0.17	T/A	0.16
78	2870_790	0.50	0.50	C/A	0.38
79	2974_1109	0.79	0.33	G/A	0.28
80	311_1536	0.82	0.30	G/T	0.26
81	3427_925	0.91	0.17	G/A	0.15
82	3494_143	0.80	0.32	C/T	0.27
83	3787_812	0.71	0.41	C/T	0.33
84	3803_763	0.71	0.41	G/A	0.32
85	3838_830	0.86	0.25	G/A	0.22
86	3900_562	0.70	0.42	C/T	0.33
87	4131_472	0.90	0.19	G/A	0.17
88	4200_155	0.60	0.48	C/T	0.37
89	4237_650	0.54	0.50	T/A	0.37
90	4273_342	0.77	0.36	G/A	0.29

Table 8. Allele frequency, Allele type, genetic diversity, and polymorphism information content (PIC) of the SNPs markers analyzed (continued).

No.	Marker	Allele frequency	Genetic diversity	SNP type	PIC
91	4306_482	0.71	0.41	G/A	0.33
92	437_590	0.71	0.41	T/A	0.33
93	4692_429	0.82	0.30	C/T	0.25
94	4702_954	0.85	0.26	C/T	0.22
95	4712_832	0.54	0.50	C/T	0.37
96	4749_1972	0.60	0.48	C/T	0.37
97	4778_497	0.70	0.42	C/T	0.33
98	4800_500	0.93	0.13	C/T	0.12
99	5058_372	0.74	0.38	G/C	0.31
100	5135_477	0.78	0.35	G/A	0.29
101	5137_1051	0.76	0.36	C/T	0.30
102	5268_412	0.77	0.36	G/A	0.29
103	5270_452	0.80	0.32	G/T	0.27
104	534_355	0.71	0.41	G/A	0.33
105	5356_124	0.53	0.50	C/A	0.37
106	5449_242	0.73	0.39	C/T	0.32
107	5503_54	0.61	0.47	G/C	0.36
108	5652_704	0.76	0.36	C/T	0.30
109	5656_680	0.97	0.06	G/T	0.06
110	5692_1408	0.65	0.46	G/A	0.35
111	5735_110	0.79	0.33	G/A	0.28
112	5756_456	0.73	0.40	T/A	0.32
113	5993_278	0.90	0.18	C/T	0.17
114	6046_661	0.73	0.40	T/A	0.32
115	6205_632	0.83	0.28	C/A	0.24
116	6378_514	0.96	0.08	G/T	0.08
117	658_460	0.93	0.13	G/T	0.12
118	6580_67	0.51	0.50	C/T	0.37
119	6663_368	0.83	0.28	C/A	0.24
120	6867_337	0.85	0.26	G/T	0.23
121	7068_60	0.64	0.46	G/C	0.35
122	708_159	0.83	0.28	G/C	0.24
123	7087_1100	0.90	0.18	C/T	0.16
124	7184_257	0.98	0.04	G/A	0.04
125	7233_543	0.86	0.23	C/T	0.21
126	7248_578	0.92	0.14	C/T	0.13
127	734_340	0.88	0.21	C/T	0.19
128	7344_500	0.86	0.24	C/T	0.21
129	7383_1042	0.56	0.49	C/A	0.37
130	7548_1327	0.72	0.40	G/T	0.32
131	7565_739	0.84	0.27	C/T	0.24
132	7967_1210	0.93	0.13	C/T	0.12
133	8044_1006	0.66	0.45	G/C	0.35
134	8121_1880	0.54	0.50	C/T	0.37
135	8150_1237	0.84	0.27	T/A	0.24

Table 8. Allele frequency, Allele type, genetic diversity, and polymorphism information content (PIC) of the SNPs markers analyzed (continued).

No.	Marker	Allele frequency	Genetic diversity	SNP type	PIC
136	8166_564	0.60	0.48	G/C	0.37
137	8253_397	0.66	0.45	G/A	0.35
138	8395_1157	0.85	0.25	T/A	0.22
139	8438_669	0.66	0.45	G/C	0.35
140	8842_943	0.95	0.10	C/T	0.09
141	8877_1528	0.56	0.49	C/A	0.37
142	8905_1569	0.72	0.40	G/A	0.32
143	8947_802	0.59	0.49	G/T	0.37
144	897_240	0.72	0.40	G/C	0.32
145	9114_900	0.97	0.05	C/T	0.05
146	9134_1559	0.53	0.50	G/A	0.37
147	9432_1340	0.94	0.12	C/T	0.11
148	9607_1753	0.94	0.11	G/C	0.10
149	9739_495	0.78	0.34	G/A	0.28
150	9779_613	0.67	0.44	G/A	0.35
151	9815_2051	0.62	0.47	C/T	0.36
	Mean	0.76	0.34		0.28

tested. The majority of SNPs included A/G (or T/C) followed by small but equal SNP types of G/C and T/A, and very small for C/A followed by G/T. The polymorphic information content (PIC) representing the allele diversity for a specific locus varied from 0.02 to 0.38 with a mean of 0.28. Gene diversity (D) was 0.34 on average and ranged from 0.02 to 0.5. SNP marker 6580_67 exhibited highest gene diversity (D) with 0.5 while the least was 18_107 detecting 0.02. Almost all of the SNP markers had allele frequency greater than 0.5. SNP 122_468 detected the highest level of allele frequency 1.0, whereas SNP 2870_790 detected the lowest allele frequency of 0.5 (Table 8).

Genetic variation among accessions

The Neighbor-Joining (NJ) tree obtained from the SNP primers' data delineated cowpea accessions into three main clusters (Fig. 3 and Table 9). We observed a clear grouping in the 95 cowpea accessions analyzed from East African countries and IITA inbred lines. The total number of main clusters is different from the total number of the regions; nonetheless, the geographic distance and the genetic background of the accessions were almost clearly reflected in the genetic group relation of dendrogram tree construction. Forty-five out of 95 (47.4%), 37 out of 95 (39%), and 13 out of 95 (13.6%) cowpea accessions were detected in the main cluster group of I, II, III respectively, showing most of the cowpea accessions grouped in the main cluster I. More than half of the main cluster group I contains accessions from Kenya (25 out of 45), followed by 7 accessions from Ethiopia and three accessions each from Sudan and Somalia but only one accession from IITA inbred lines (IT-99K-1122). The main cluster group II contains half of the accessions (19 out of 37) from Ethiopia, almost all the accessions (15 out of 16) from IITA inbred lines, only three accessions (TVu-265, Tvu-13448, and TVu-552) were from Kenya; however, Somalia and Sudan were not represented in this group. Main cluster group III contains all its accessions from Somalia 12 out of 13 (92%), only one accession (TVu-13511) from Kenya was included. The six released cowpea varieties from Ethiopia distributed and clustered in main clusters II and I. The released varieties Bole (CH), Black eye bean, Asebot, and 82D-889(CH) closely clustered with the local Ethiopian accession of the biggest sub-cluster II. Similarly, the released varieties WWT and Tvu tightly clustered together to each other but loosely clustered with four local Ethiopian accessions (211557, 211490, 211444, and 211441) of sub-cluster I. Both the drought-resistant (Danila) and drought-susceptible (TVu-7778) closely clustered in main cluster II along with the local accession TVu-13448 from Kenya.

Genetic differentiation between regions groups based on SNP markers

The accessions were getting grouped into three broad groups across the first two axes. The first three PCoA vectors explain a total of 68.42% of the genotypic variability by the spatial separation of the genotypes (Fig. 4), in which the three principal coordinates (PC1, PC2, and PC3) explained 32.9, 20.38, and 15.14% of the total molecular variance, respectively. Along the PC2 axis, the accessions were more dispersed in their distribution, hence the PC2 is effective as compared with the PC1 in separating each region into the left and right sides of the plot. In PCoA, all accessions were labeled with different colors based on their different regions to indicate their region specificity; there was intermixing of

Table 9. Neighbour-Joining (NJ) tree analysis identified three main clusters among 95 Cowpea accessions.

No.	Cluster	I			II			III
	Main group	45			37			13
	Sub group	I (37)		II (8)	I (33)		II (4)	I (8)
	Sub-sub-group	I (35)	II (2)	-	I (19)	II (14)	I (4)	-
1	Ethiopia (26)	7	-	-	15	2	2	-
2	Kenya (29)	23	-	2	2	-	1	1
3	IITA inbred lines (16)	1	-	-	2	12	1	-
4	Somalia (15)	1	2	-	-	-	-	7
5	Sudan (9)	3	-	6	-	-	-	-

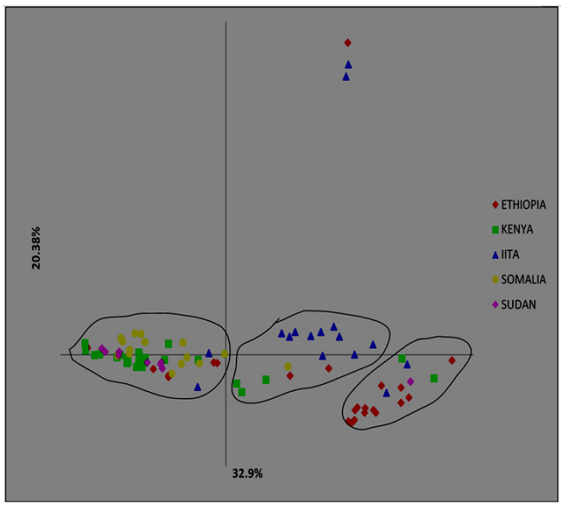

Fig. 4. Principal coordinate analysis (PcoA) of 95 cowpea accessions based on SNP data.

color across the coordinates.

Analysis of Molecular Variance (AMOVA) using SNP markers

The Analysis of molecular variance (AMOVA) showed that all sources of variation were highly significant ($P < 0.001$) and approximately 17% of the overall variation was attributed to genetic differentiation among regions; 77% was explained by differences among populations within regions, and 6% was attributed to genetic differentiation among individuals and within populations (Table 10). These indicate that the accessions in this study possess wide diversity among populations and within regions. Previous investigators have reported that fixation index (Fst) values in a range of 0-0.05 generally indicate little differentiation, whereas values in the 0.05-0.15 range suggest moderate differentiation, 0.15-0.25 large differentiation, and above 0.25 very large differentiation (De Vicente et al. 2004; Kiambi et al. 2005; Wright 1984). In this study, the observed Fst value was 0.15 which showed moderate differentiation between accessions on the basis of country of collection and the value recorded for the number of migrants gene (gene flow) between countries was lower (Nm) 1.2; illustrating the existence of relatively lower germplasm exchange among cowpea accessions of East African origin and with IITA inbred lines.

Evaluation of resistance to drought tolerance within East African germplasm

Cowpea suffers considerable damage due to frequent drought in the Savanna and Sahel sub-region. Genetic enhancement of cowpea for drought tolerance at different stages such as early, intermediate and terminal drought stress is important for ensuring sustainable and improved crop yield in a variable and changing climate. A standard check of drought-resistant variety, Danila and drought susceptible variety, Tvu-7778 varieties were included in our study to see their grouping patterns with the East African cowpea accessions and IITA inbred lines. Our analysis showed that both the drought-resistant (Danila) and drought-susceptible (TVu-7778) closely clustered in main cluster group III. Local accessions TVu-13490 and TVu-6378 from Kenya, local accessions 2305675 from Ethiopia, and Tvu-16073 Somalia and the inbred line Danila from IITA were all tightly clustered and shared a common allele when analyzed with SSR markers. Similarly, both the drought-resistant

Table 10. Analysis of Molecular Variance (AMOVA) among regions.

Source of variation	DF	Sum of squares	Var components	Variation (%)	F statistics (Fst)
Among regions	4	949.6	5.17	17***	0.15
Among populations within regions	90	4350.1	23.31	77***	
Among individuals Within populations	95	163.0	1.72	6***	
Total	189	5462.7	30.2	-	

***$P < 0.001$, Nm (gene flow) =1.2

Table 11. Summary statistics of SSRs and SNPs.

Accession origin	Marker	Diversity parameter				
		Nb	R	D	PIC	Fst
Ethiopia (n=26)	SSR (n=12)	4	2 to 10	0.48	0.44	0.055
	SNP (n=151)	1.88	2	0.29	0.23	0.037
Kenya (n=29)	SSR (n=13)	3.7	2 to 9	0.47	0.43	0.012
	SNP (n=151)	1.905	1 to 2	0.28	0.22	0.067
Somalia (n=15)	SSR (n=10)	3	2 to 6	0.48	0.41	0.036
	SNP (n=151)	1.69	1 to 2	0.23	0.18	0.059
Sudan (n=9)	SSR (n=11)	3.5	2 to 6	0.54	0.48	0.01
	SNP (n=151)	1.65	0 to 2	0.22	0.18	0.2
IITA Breeding lines (n=16)	SSR (n=13)	3.8	2 to 9	0.51	0.45	0.28
	SNP (n=151)	1.86	1 to 2	0.28	0.23	0.039
All (n=95)	SSR (n=13)	6.3	3 to 15	0.56	0.5s1	0.095
	SNP (n=151)	2	2	0.34	0.27	0.15

Mean number of alleles per locus (Nb), Range of number of alleles per locus (R), Gene diversity (D), Polymorphism Information Content (PIC) and Fst

(Danila) and drought-susceptible (TVu-7778) closely clustered in main cluster group II along with the local accession TVu-13448 from Kenya, when analyzed against SNP markers. However, no inbred lines from IITA shared common alleles with Danila and Tvu-7778 when screened with both SSR and SNP markers. Therefore, whether these accessions are indeed potential sources of drought tolerance remains to be confirmed in phenotypic trials under drought conditions. Furthermore, an additional molecular trial with SSR and SNP markers which tags drought tolerance is required.

Comparisons of results obtained following SSR and SNP marker analyses

The number of alleles per locus ranged from 3 to 15 for SSR markers and the number of alleles registered for SNPs was 2 across all the tested cowpea lines; the average number of alleles per locus was 6.3 for the SSR and 2 for the SNP markers. Considering each group of accessions based on their origin separately, the average number of SSR alleles per locus was 3.8 for the IITA inbred lines, 3.5 for those from Sudan, 3.0 for Somalia, 3.7 for Kenya, and 4.0 for Ethiopia, whereas the average number of SNP alleles per locus was 1.86, 1.65, 1.69, 1.9, and 1.88 for the IITA inbred lines, Sudanese, Somali, Kenyan, and Ethiopian accessions, respectively (Table 11).

The total gene diversity (D) was 0.56 for the SSRs and 0.34 for the SNPs. D estimates of each region ranged from 0.44 (Kenya) to 0.54 (Sudan) for the SSRs and from 0.22 (Sudan) to 0.29 (Ethiopia) for the SNPs. The genetic diversity values were higher in samples from Sudan (D=0.54) than in samples from IITA (D=0.51), Somalia (D=0.48), and Ethiopia (D=0.47), while lower D values were obtained for Kenya (D=0.44) when screened with SSR markers. Different and lower D values were observed when screened with SNP markers; higher D values for Ethiopia (D=0.29), IITA inbred lines (D=0.28), Kenya (D=0.28), although lower D values were observed for Somalia (0.23) and the Sudan (0.22). The PIC value for SSR was 0.51 and 0.27 for SNP. The PIC values for each region ranged from 0.39 (Kenya) to 0.48 (Sudan) for the SSRs and from 0.18 (Sudan and Somalia) to 0.23 (inbred lines) for the SNPs; in each of the region, the average PIC value registered for SNP markers were half of SSR markers.

The overall fixation index (Fst) was 0.09 and 0.15 for the SSR and SNP markers, respectively (Table 11). In both cases, there was moderate differentiation but different gene flow between regions. Both SSR and SNP analysis of molecular variance (AMOVA) showed higher among populations and within regions variation. The PCoA plot analysis of cowpea accessions using SSR vs SNP generated grouping into two broad groups across the first two axes in case of SSR markers, whereas it generated grouping into three broad groups in case of SNP markers. The proportion of variance explained by first three coordinates in case of SNP (68.42%) was higher than the SSR (65.87%) (Figs. 2 and 4).

Mean number of alleles per locus (Nb), Range of number of alleles per locus (R), Gene diversity (D), Polymorphism Information Content (PIC) and Fst

Discussion

One hundred fifty-one polymorphic SNP and 13 SSR markers were used to genotype ninety-five cowpea accessions of local cultivars from Ethiopia, Kenya, Somalia, Sudan, and inbred lines from IITA. The SNP loci had lower PIC values than the SSRs; we found mean polymorphic information content (PIC) 0.28 for SNP and 0.51 for SSR. A study of Desalegne et al. (2016) observed PIC ranging from 0.04 to 0.68 with a mean 0.4 using cowpea accessions collected from Ethiopia. Fatokun et al. (2008) observed PIC ranging

from 0.29 to 0.87 with a mean of 0.68 among 48 wild cowpea lines using SSR markers. Study of Li et al. (2001) reported a PIC ranging from 0.02 to 0.73 with a mean of 0.47 among cultivated cowpea with a different set of SSR markers. Badiane et al. (2012) reported PIC values which varied from 0.08 to 0.33 with a mean of 0.23 using cowpea, collected from the Senegalese national germplasm collection. Asare et al. (2010) reported PIC, which varied from 0.07 to 0.66 with an average of 0.38 using cowpea collected from the Ghanaian germplasm collection. Ogunkanmi et al. (2014) assessed 48 accessions of cultivated cowpea, the PIC from West African accessions was 0.369, South African had 0.329 while North East and Central Africa with 0.332, another evidence confirming that West Africa contains greater diversity. Kuruma et al. (2008) assessed the genetic diversity of 81 Kenyan cowpea accessions and reported PIC value of varying from 0.09 to 0.82 with a mean of 0.34. The low level of polymorphism detected in our study agrees with previous studies and may be the result of a bottleneck induced by a single domestication event in this crop (Badiane et al. 2004; Diouf and Hilu 2005; Li et al. 2001; Tosti and Negri 2002) in addition to its inherent self-pollinated reproduction mechanism. Individual SNP markers, being

biallelic, have lower information content than SSRs, but as they occur at much higher density throughout the genome, are amenable to high-throughput methods such as genotyping arrays, and have lower genotyping error rates (Kennedy et al. 2003; Morin et al. 2004; Rafalski 2002; Schlotterer 2004). Almost all the SNP markers had allele frequencies above 0.5. A total of 83 alleles were detected for SSR markers with an average of six alleles per locus. The study of Desalegne et al. (2016) observed the number of alleles ranging from 1 to 5 with a mean 3 using cowpea accessions collected from Ethiopia. Similarly, Asare et al. (2010) reported 4 to 13 alleles in cowpea collected from Ghana, while Sawadogo et al. (2010) reported 5 to 12 alleles in cowpea collected from Burkina Faso using cross-species SSRs from Medicago. Badiane et al. (2012) reported 1 to 16 alleles in cowpea collected from Senegalese national germplasms. Diouf and Hilu (2005) reported 1 to 9 of alleles for different germplasm which ranged from 1 to 9 and Li et al, (2001) reported that 27 cowpea microsatellite primers detected between 2 and 7 alleles among 91 cowpea inbred lines. According to Asare et al. (2010) the number of alleles detected per primer pair varied from a minimum of 1 to a maximum of 6 with an average of 3.8 using Ghanaian germplasm. Ogunkanmi et al. (2014), reported a total of 37 alleles with mean alleles of 3.1 when they used 48 cultivated cowpea accessions with 12 microsatellite markers. Kuruma et al. (2008) reported allele numbers ranging from 2 to 14 and with a mean allele of 4.5 using 81 cowpea accessions collected from Kenya. The average number of alleles per SSR locus was considerably higher than that for the SNPs; this is because the SNPs are usually biallelic (Vignal et al. 2002), whereas SSRs are multi-allelic markers. This multiallelism has established SSRs as the effective marker platform in the current crop diversity studies (Gupta and Varshney 2000).

The averaging gene diversity (D) in our study was 0.56 for SSR and 0.3 for SNP marker. Desalegne et al. (2016) observed the gene diversity ranging from 0.06 to 0.68 with a mean 0.4 using cowpea accessions collected from Ethiopia. Asare et al. (2010) reported the genetic diversity (D) which ranged from 0.12 to 0.68 with an average of 0.44 using Ghanian germplasms. On the other hand, the genetic diversity value reported by Badiane et al. (2012) varied from 0.08 to 0.42 with an average of 0.28 using the cowpea collected from Senegalese national germplasms. The high gene diversity (D) values of SSR markers were supported by the results of large number of alleles per locus observed in this study. Despite the difference in D estimates calculated for SSRs and SNPs, we observed the same trend for both markers when gene diversity (D) was studied across the countries. According to Delphine et al. 2010, the theoretical considerations show that the maximum gene diversity D observable with biallelic markers is 0.5, whereas for multi-allelic markers such as SSRs the maximum can approach 1. Another factor, which contributes to the observed difference in the D estimates of SSRs and SNPs, is the selection history of the two marker types, SSRs were selected over years with respect to their PIC value in various sets of cowpea accessions, whereas the SNPs have not undergone such a selection procedure. Thus, this property of SNPs explains together with the definition of gene diversity D that, D values found for SNPs are lower than those for SSRs (Jones et al. 2007). Hence, the two theoretical considerations would be more applicable for cowpea diversity studies as compared to other crops because cowpea is ''an orphan crop'' has not been a subject of study using the new sequencing technologies (Delmer 2005). Therefore, it is expected that in the future the D estimates of the SNPs increase towards the above mentioned theoretical maximum of 0.5 (Delphine et al. 2010). Due to the abovementioned reasons, SSR markers reported being the most frequently used marker than SNP markers in cowpea diversity study (Tan et al. 2012).

In both SSR and SNP analysis, we have clearly observed the grouping of accessions from different countries and IITA inbred lines in the same cluster group. The grouping within the main clusters identified a substantial degree of association between provenance and genotype. Almost all of the accessions from each country and inbred lines from IITA were tightly clustered together with each other within the three main cluster groups identified. Therefore, the neighbour-joining tree construction tend to show the geographical distance of the counties and the genetic background of our cowpea material; even though we have observed the grouping of accessions from different countries, this clearly showed the presence of germplasm exchange between East African countries and IITA centre. However, a clearer grouping based on their geographical origin was obtained using SNP markers than SSR markers. The released varieties from Ethiopia tightly clustered with some of the local accession of Ethiopia, hence the result showed the existence of a high

neighbour-joining relation between the released and the local cowpea accession of Ethiopia. Danilla, tightly clustered and shared a common allele with the local cowpea accessions from Kenya, Ethiopia, and Somalia; similarly, Tvu-7778 was tightly clustered and shared a common allele with the local cowpea accessions from (Somalia, Kenya) when analyzed with (SSR, SNP) markers, respectively. Hence, whether these accessions are indeed potential sources of drought tolerance remains to be confirmed in phenotypic trials under drought conditions and additional molecular trial with SSR and SNP markers which tags drought tolerance is required. The broad pattern of distribution of accessions in the PCoA plots was similar with both the markers, but a closer look revealed three major groups for cowpea accessions in the case of SNP markers, such grouping was two using SSR markers. In both markers, there was intermixing of color across the coordinates, further support the neighbor-joining tree with SSR and SNP marker that, there is no location-specific grouping.

The results of AMOVA analysis showed moderate fixation index (Fst) value in both SSR (0.095) and SNP (0.15) markers, which showed moderate differentiation between regions and there was relatively moderate gene flow (Nm) between countries 2.38 for SSR and 1.2 for SNP; illustrating the existence of germplasm exchange among East African countries and with IITA. Most variations were found among countries and within regions; demonstrating the presence of sufficient time for genetic differentiation among the East African countries cowpea accessions to ultimately form isolated cluster groups. Kuruma et al. (2008) assessed 81 Kenyan cowpeas, the total accessions among the geographical regions revealed low fixation index value 0.04, indicating low differentiation among Kenyan cultivated cowpea accessions, a similar low Fst value of 0.012 was obtained in our study with 29 cowpea accessions collected from Kenya. A study of Sariah et al. (2010) reported a low fixation index value of 0.033 using 312 Tanzanian cowpea accessions, showing low differentiation among Tanzanian cowpea accessions.

In conclusion, in both SSR and SNP analysis, the clustering identified a substantial degree of association between provenance and genotype. However, the geographic grouping was not reflected in the genetic structural grouping; i.e., accessions originating from different countries clustered together, showing the existence of germplasm exchange among East African countries and with IITA. The geographic distance and the genetic background of the accessions were clearly reflected in the grouping of within cluster of neighbour-joining tree construction and principal component analysis when analysed using both SSR and SNP markers. The neighbour-joining analysis of both SSR and SNP markers consistently showed that most of the accessions from each country tend to cluster together and shared common alleles with each other. This result was further supported by the moderate fixation index (Fst) demonstrating moderate differentiation and moderate gene flow between countries. Our study found that SNP markers are found to be more effective than SSR in determining the relationship among cowpea accessions and varieties. SSR turned out to be well suited as it has high PIC values but for future cowpea genetic diversity study SNP markers with high PIC values should be incorporated. To improve the genetic information of cowpea SNP markers, many SNP markers should be utilized as compared with the number of SSR markers. High-throughput next generation sequencing technologies based genotyping approach such as genotyping by sequencing (GBS) (Elshire et al. 2011) can be used to generate many SNP markers across cowpea genome. The five local cowpea accessions, which all tightly clustered and shared common alleles with the drought tolerant variety Danila and with the drought susceptible variety TVu-7778, should be further checked for their drought tolerance characteristics using well-characterized marker which is associated with drought tolerance. Future investigations need to include a wider number of East African germplasm and perhaps additional informative SSR and SNP markers to assess the genetic relationship among accessions for a rational exploitation in inbred improved varieties.

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