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Research Article

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ABSTRACT

95 sorghum accessions (1,425 individuals) sampled represented most of crop- cultivated areas in Sudan. The genetic diversity and population structure was assessed using a panel of 39 SSRs marker, which covered the sorghum genome. Genotypic data was generated using the ABI 3730 genetic analyzer. The alleles were called and sized using GeneMapper software version 3.7. The molecular data analysis software's PowerMarker v3.25, DARwin 5, and GenAlEx 6.5x were used to calculate the different diversity indices within and between populations. A total of 332 alleles were detected, with an average of 8.5 per marker pair. The gene diversity averaged at 0.6671. The Polymorphism Information Content (PIC) values averaged of 0.68 showing the highly polymorphic and discriminatory nature of the selected markers. The accessions showed lower mean of observed heterozygosity (H_o = 0.187) than the expected heterozygosity (H_e = 0.547). AMOVA calculated low variants among populations (1%), and moderate variants within individuals (20%). However, variants among individuals were relatively high within population (79%). The fixation indexes showed little genetic differentiation among populations (F_{ST} = 0.008, P = 0.012). However, in the total population high level of inbreeding (F_{IS} = 0.802, P = 0.001) was exhibited with deviation from Hardy-Weinberg proportions (F_{IT} = 0.804, P = 0.001). Neighbor joining rooted phylogeny tree based on genetic similarity coefficient revealed three distinct groups independent of their geographic origins clustering close to each other; groups also have sub-groups. The study estimated genetic diversity and structure of Sudanese sorghum accessions.

Keywords: sorghum land races and accessions, SSRs markers, genetic diversity, analysis of molecular variants (AMOVA), cluster analysis, Sudan.

INTRODUCTION

Sorghum (Sorghum bicolor. L. Moench), is an important crop ranking fifth worldwide after wheat (Triticumspp), rice (Oryzaspp), maize (Zea maize L.) and barley (Hordeum vulgdre) (FAO, 1995). It is considered to be the most important, stable, and preferred food crop for majority of population in Sudan. Moreover, high percent of entire cropped area in the country is sorghum planted (Mohamed, 2005).It was first domesticated in the region of North East Africa (Doggett, 1988);an area bordering Sudan and the neighboring Ethiopia is considered to be the center of origin of sorghum.

Taxonomically, sorghum has five main races:viz; bicolor, caudatum, kafir, durra and guinea type, as well as 10 intermediates (Harlan and de Wet, 1972). Also, previous studies have succeeded to recognize three S. bicolor subspecies: the cultivated types (ssp. bicolor), the widely distributed and extremely diverse wild sorghumtypes (ssp. verticilliflorum) and the weedy types generated through the dispersal of pollen grains and hybridization between the domesticated and wild sorghums (ssp. drummondii) (De Wet, 1978).

One of the major goals of plant breeders is to create genetically diverse individuals, then select the valuable gene of interest for subsequent breeding programs. Even though the phenotypic characteristics of the hybrids are clearly visible, heterosis is not fully understood. It is well documented that crosses between unrelated individuals and between genetically distant parents, show greater hybrid vigor due to an enhanced degree of heterozygosity than crosses between closely related parents (Stuber, 1994; Hallauer, 1999; Menz et al., 2004).

Efforts have been devoted to understand the precise genetic variability of sorghum accessions using the phenotypic and molecular markers (Dean et al., 1999; Dje et al., 2000; Ghebru et al., 2002). The advantage of using molecular markers characterization over the morphological is the possibility to identify the duplication, assist the cataloging of germplasm, the assessment of genetic structure and initiated well defined system of

germplasm collections (Bretting and Widrlechner, 1995). Moreover the developed molecular markers for genetic analysis expanded the circle of knowledge of genetics and understanding of the structure and behavior of organism genomes (Korzun, 2005).

Recently, many molecular marker techniques have been developed and using for studying plant genetic variability and germplasm assessment as simple sequence repeats (SSRs), random amplified polymorphic DNAs (RAPDs), amplified length polymorphisms (AFLPS), etc.The importance of studying the extent and structure of genetic diversity in germplasm accessions through characterization is essential for better understanding of the evolutionary trends and also will help in gene bank management and strategies for the collection and conservation of the germplasm.

The objective of this study is to characterize and determine the genetic diversity of Sudanese sorghum accessions collected from the different agricultural regions.

MATERIALS AND METHODS

Germplasm

95 sorghum accessions (Table1) brought from Agricultural Research Corporation (ARC) gene bank unit had been collected from Central, Central Eastern (Gadaref area), Eastern, Southern, Blue Nile, and Western regions of Sudan.

Germplasm germination

The 95 accessions were planted in Petri dishes and incubated for 48 hours to germinate, then, 15 plant tissues from each accession were bulked and advanced for further DNA extraction and genotyping with 39 SSRs markers (Table.2).

DNA extraction and marker analysis

The genotyping laboratory work was carried out at International Live Stock Research Institute (ILRI), Biosciences Eastern and Central Africa (BecA) facilities. Modified CTAB protocol (mace et al., 2004) was used for DNA extraction from two week old sorghum roots. 15 roots per plant per accession were bulked. 450 µl of preheated CTAB (65℃) extraction buffer was added to facilitate grinding the root particles using steel balls in the genogrinder 2000 in 96 well plates. The macerated material substance was incubated for 20 minutes at 65℃ in a water bath with occasional mixing. The supernatant harvested after spinning at 14,000 rpm for 30 min was mixed with 450 µl chloroform: isoamylalcohol (24:1). Then the 96 well plate was centrifuged at 12,000 rpm for 10 minutes and a fixed volume of 400 µl was transferred to a fresh 96 well plate. 280 µl of iso-propanol was added (stored at -20C˚) to the supernatant and inverted several times to mix. The plate was centrifuged at 12,000 rpm for 15 minutes and the supernatant drawn off and pellet exposed to air drying for 30 min. The pellets were washed twice with 200 µl of ice-cold 70% ethanol. Each pellet was re-suspended in low salt buffer (200µl) with 3 µlRNAse A (10mg/ml) stored at -4C˚.

DNA qualification and quantification

The extracted DNA then qualified using agarose gel electrophoresis and quantified using nanodrop 1000spectrophotometer.

Genotyping

The DNA has been normalized to 5ng/µl as working stock for PCR.39 directly-labeled markers were used for genotyping Table. 1.

The master mix calculated using the concentration of 0.2 µM of forward and of reverse SSR primers, 2unit Taq polymerase (5 u/µl), 0.2 µMdNTPsmix, 2mM $MgSO_4$, 1Xthermopol reaction buffer [10 mMKCl, 20 mMTris-HCl, 10 mM $(NH_4)_2$ SO_4] PCR buffer. Then, each prepared primer mix was added to PCR plate put in ice. The components mixed; $MgSO_4$,dNTPs, reaction buffers, DNA, Taq polymerase ddH_2O and the primer. After loading the normalized DNA and primers, then the PCR plate transferred to the PCR machine. The final volume of the reaction mixture was made up to 5 µl with doubled distilled water and 1µl of normalized DNA.

The PCR program was used as described by Folkertsma et al. (2005): initial denaturation at 94℃ for 15 min followed by 10 cycles of 94℃ for 15 s, annealing for 20 s at touchdown temperatures declining from 61℃ to 51℃ (annealing temperature of each cycle was reduced by 1℃), and extension at 72℃ for 30 s; this was followed by denaturation at 94℃ for 10 s, and annealing at 54℃ for 20 s, with extension at 72℃ for 30 s for 34 cycles; and a final extension of 20 min at 72℃.Then the PCR products were prepared for a 384 well plate, with Hidi formamide/Liz 500 cocktail (1,000µlof Hidi formamide to 12µlof Liz 500). A volume 0.7 µl of the amplified

DNA were co-loaded four labeled markers as, Fam, Vic, Ned, and Pet, (each marker has certain florescence), then 8µl of Liz and Hidi was added to the mixture. Then the prepared 384 well plate transferred to the ABI for DNA separation. Scoring of peaks achieved using gene-mapper v.4.0 software program (Fig. 1).

Statistical analysis

Genotyping data analysis

The genotyping data manipulation and interpretation were done using PowerMarker v3.25, DARwin 5.0, and GenAlEx6.5b3.

Table (1): Plant material and geographic region

No	Genotype	region	no	Genotype	Region	No	Genotype	region
1	PI55555121	C	33	TSS1	G	65	PI569260	C
2	PI569028	C	34	Abu Shai	G	66	PI562923	E
3	PI569300	C	35	PI563324	C	67	PI569898	C
4	Safra	G	36	PI569861	C	68	PI217841	N
5	MiloYellow	G	37	PI568267	B. Nile	69	PI569808	E
6	PI563327	C	38	Arfa	G	70	PI563321	C
7	PI563298	C	39	PI570948	S	71	HSD5120	N
8	PI563320	C	40	PI570506	B. Nile	72	PI569408	C
9	PI568996	S	41	Wad Akar	G	73	SRN	G
10	PI569299	W	42	PI568990	S	74	PI217760	C
11	Gadamblia	G	43	PI569878	C	75	PI569854	C
12	HSD2779	N	44	Serina	G	76	PI570518	W
13	AjebSeido	G	45	PI563323	E	77	PI569811	E
14	PI570947	S	46	HSD3456	W	78	HSD5276	E
15	PI569911	C	47	PI569390	C	79	PI217797	W
16	PI570505	S	48	PI569344	C	80	PI217674	W
17	PI568990	S	49	HSD1529	W	81	Dabar	G
18	Eriana	G	50	PI217784	W	82	PI563297	C
19	PI569164	C	51	PI563314	C	83	PI569805	E
20	PI563304	C	52	PI568994	C	84	PI569879	C
21	PI563312	C	53	Regan	G	85	SD1	G
22	PI569394	C	54	PI569071	C	86	PI563309	C
23	HSD5275	E	55	PI570453	B. Nile	87	PI569201	C
24	PI217822	W	56	Dabar	G	88	PI563322	C
25	Red Mugod	G	57	PI569925	C	89	PI563302	C
26	PI569339	C	58	PI568989	C	90	PI569007	E
27	PI217798	C	59	PI568276	C	91	PI569867	C
28	PI569891	C	60	PI563311	C	92	PI569451	S
29	GEW-37-13	G	61	PI563308	W	93	PI569863	C
30	PI569907	C	62	PI563313	C	94	PI568998	E
31	Tall Milo	G	63	PI569858	C	95	Feterita	G
32	PI569280	C	64	PI563306	C			

Key: C; Central, G; Gadaref (Central East), E; East, B. Nile; Blue Nile(South East), S; South, W; West

Table (2): GCP Global marker set

Set1	Fam	Xtxp040	154.03	Set6	Fam	gpsb067	197.27
	Vic	Xtxp145	255.95		Vic	gpsb123	313.92
	Ned	mSbCIR286	127.5		Ned	SbAGB02	112.75
	Pet	Xcup02	218.08		Pet	Xisep0310	223.63
Set2	Fam	Xtxp010	162.81	Set7	Fam	mSbCIR240	123.51
	Vic	mSbCIR329	132.41		Vic	Xgap84	200.9
	Ned	Xtxp114	251.76		Ned	Xtxp015	239.34
	Pet	mSbCIR238	90.55		Pet	Xcup63	166.57
Set3	Fam	Xtxp141	175.21	Set8	Fam	mSbCIR306	139.19

	Vic	Xtxp012	213.22		Vic	Xcup53	213.28
	Ned	mSbCIR223	132.07		Ned	mSbCIR248	109.48
	Pet	Xtxp273	242.7		Pet	mSbCIR262	234.04
Set4	Fam	mSbCIR276	245.32	Set9	Fam	Xgap72	209.45
	Vic	Xtxp320	292.11		Vic	mSbCIR246	118.9
****	Ned	Xcup61	214.49		Ned	Xtxp265	227.22
	Pet	Xtxp021	199.35		Pet	Xtxp136	260.55
Set5	Fam	Xtxp278	266.91	Set10	Fam	Xcup14	227.51
	Vic	mSbCIR283	163.42		Vic	Xtxp057	268.68
	Ned	Xtxp321			Ned	Xgap206	135.63
	Pet	mSbCIR300	123.85				

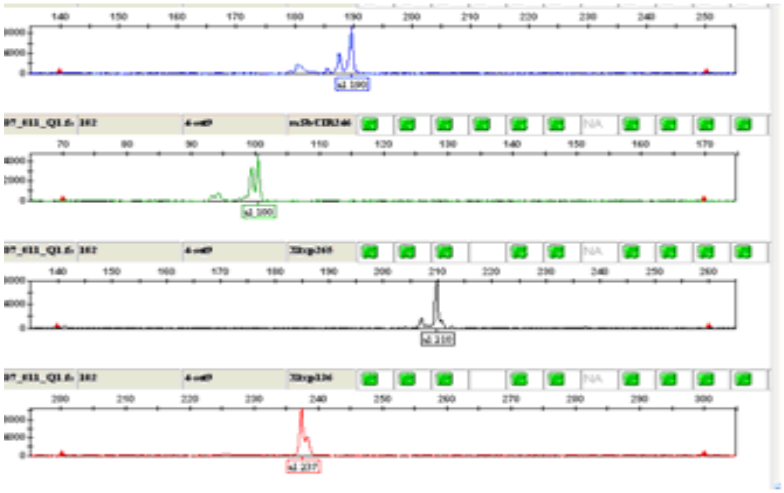

Figure (1): gene-mapper results (co-loaded samples)

RESULTS AND DISCUSSIONS

Performance of SSRs markers

A total of 39 SSR markers covered sorghum genome with at least two markers per chromosome were optimized and used to genotype 95 sorghum accessions with the line BTx623 used as the out-group control. The number of alleles detected in each of the 39 SSR markers were highly variable and ranged from 2 (Xcup61) to 23 (Xtxp206). A total of 332 alleles were identified among the 95 Sudanese sorghum accessions (Table 3).The average number of alleles per SSR marker was 8.5, ranging from 2alleles (Xcup61) to 23 alleles (Xtxp206) (Table 3).Considering only alleles with frequencies ≥5% or defined alleles with frequencies <5% as rare, the average number of common alleles per SSR marker was reduced to 6.1, ranging from 2 alleles (Xcup61) to 15 (Xtxp145 and SbAGB02).

The PIC value over the 39 SSR markers averaged 0.63, ranging from 0.36(Xtxp278)to 0.88(Xtxp206). Allelic patterns across populations showed high number of different alleles in Gadaref population followed by East, Central, West, South, Blue Nile and north (Figure 2). Also, number of effective alleles showed high values in Gadaref and Central followed by East, West, South, North and Blue Nile. However, the number of private alleles was almost similar between the West, South, Central, Gadaref and East, and slightly different in North and Blue Nile populations (Figure2).

Pattern of genetic diversity among 95 Sudan sorghum accessions

The 39 SSR primer pairs used in this study were able to structure the 95 sorghum accessions. The observed heterozygosity (H_o) was 0 0.187 when the expected heterozygosity (H_e) was 0.547.

The genetic diversity parameters calculated from 39 SSR markers data at the population level for seven regions in Sudan shows that there is considerable diversity within each population (Table 4) . Central , Gadref ,

East, south and West showed the highest number of polymorphic loci, with 100% of their loci polymorphic (allelic frequencies > 5%). 89.7% of the loci in Blue Nile showed polymorphism (allelic frequencies >5%).

The Rooted phylogeny tree cluster results (Fig.3), the clusters were described as follows: first group; including 31 accessions which 45% of them from Central also the first group sub-divided into subgroups and sub sub-group. The second group includes 16 accessions which 63% of them from the Gadaref and Central also sub-divided into subgroups and sub sub-groups. The third group 49 accessions most of them from Central and sub-divided into 10 subgroups.

Population structure and relationship

Analysis of molecular variance (AMOVA) showed low percentage of variations among populations and within individuals, 1% and 20% respectively. The results also showed high percentages of variation among individuals within population 79% (Table2).

The fixation indexes showed high level of inbreeding (F_{IS}), and deviation from Hardy-Weinberg proportions in the total population (F_{IT}) which estimated 0.80. Also, the result showed low variance among sub populations (F_{ST}) 0.008 (Table).

Population specific F_{IS} indices showed high percentage of F_{IS} value for all populations which rated between 71% to 84%(Table3). However, the expected heterozygosity (63% to 70%) was greater than the observed heterozygosity (7% to 23%) in all populations (Table4).

Unbiased Pairwise genetic distance between population showed low genetic distance in all populations (Table5), with a range from between 0.032 to 0.297. The lowest genetic distance showed between West and Central0.032), and highest between North and Blue Nile(0.297).

DISCUSSIONS

A total of 39 SSR markers were used to discriminate 95 sorghum accessions and the line BTx623 was used as an internal control accession. The selected SSRs markers covered the 10 chromosomes of sorghum genome. A total of 332 alleles were identified among the 95 Sudanese sorghum accessions (Table 1).This is comparable to Uptmooret.al. (2003) who detected 217 alleles using 25 SSR loci. The average number of alleles detected was 8.5; this value is considering high when compared with average 5.8 obtained in other studies (Folkertsma et.al., (2005), Menz et al., 2004; Uptmoor et al., 2003; Dje et al., 2000; Grenier et al., 2000). The average of the major allele frequency detected in this study was 0.47 and is used to calculate differences within individuals, level of gene flow and inbreeding. Polymorphic information content (PIC) of the selected primers averaged at 0.62 (Table1); generally a PIC > 0.5 is considered high. In this study, the value is comparable to those from Agrama H. A. and Tuinstra M. R, (2003), and considered very high when compared to studies by Folkertsmaet.al., (2005), Anas and Yoshinda, (2004) as well as Brown et al. (1996).This signifies that the selected panel of markers used in this study is highly informative and capable to separate sorghum accessions from each other.

Allelic patterns across populations showed high number of different alleles in Gadaref population followed by East, Central, West, South, Blue Nile and north (Fig.6). The genetic diversity parameters calculated from 39 SSR markers data at the population level for seven regions in Sudan shows that there is considerable diversity within each population (Table 4). Central, Gadref, East, south and West showed the highest number of polymorphic loci, with 100% of their loci polymorphic (allelic frequencies > 5 %) while 89.7% of the loci in Blue Nile showed polymorphism (allelic frequencies >5%).This result correspond to the fact that Sudanese regions bordering Ethiopia (the center of origin of sorghum) show greater genetic variability with different types of families and species (Vavilov 1926; Doggett, 1965).

The 39 SSR primer pairs used in this study were able to separate the 95 sorghum accessions. The cluster analysis based on genetic similarity in general PCoA and un-weighted neighbor joining tree showed three main clusters close to each other, these groups also have sub-groups Fig. 6, and 7). The populations detected are close to each other and slight structured in different groups, the detected differences are result of inbreeding and drift, because principal inbreeding can be defined as the mating between individuals related by a shared ancestry (Falconer and Mackay 1996. this correspond with the assumption of material flow from Gadref area (which considered as part of center of origin of sorghum), to other parts of country.

Analysis of molecular variance (AMOVA) showed low percentage of variations among populations and within individuals, 1% and 20% respectively. The low variations among populations this result correspond with sorghum accessions flow from Gadref to other parts of sorghum planting areas in Sudan. This is depicted in the results showing high percentages of variation among individuals within population 79% (Table2). The high percent of variation among individuals comes as a result of inbreeding and drift. Also, the variations among individuals could be due to drift in small populations, which farmers initially selecting panicles from field to be used as seeds for the coming seasons. The results showed high level of inbreeding (F_{IS}), and deviation from Hardy-Weinberg proportions in the total population (F_{IT}) which estimated 0.80. Similar values of inbreeding coefficient (F_{IS} = 0.70) were obtained using both alloenzyme and microsatellite markers in cultivated sorghum

sampled *in situ* in North-Western Morocco (Dje et al., 1999). The coefficients obtained were higher than those of Dje et al. (2000).

However, the result showed F_{ST}= 0.008(Table3), moderate variance among demes also means low percentage of gene flow. Wright (1978) cited by Kiambi et al., (2005); Semagn et al. (2001) and Hartl (1987), suggested that an F_{ST} range of 0-0.05 indicates little differentiation, 0.05-0.15 moderate and 0.15-0.25 large differentiation and above 0.25 indicates very large differentiation.

Also investigators stated that F_{ST} can be used for identifying loci under selection (Casa, et.al, 2005). The F_{ST} value obtained was very lower than in other sorghum population's genetics studies. Dje et al. (2000) reported (F_{ST}= 0.68) for landraces on the basis of only three different SSR loci. Also, Ghebru B.et. al., (2002) calculated F_{ST}=0.50 when studying diversity of 28 Eritrean sorghum accessions using 15 SSRs markers.

Population specific F-Statistic indices showed high percentage of F_{IS} value for all populations which rated between 0.71% to 0.84(Table4). This result means high values of inbreeding which confirming farmers are keeping seeds from their farms.

The result showed that H_o ranged from 0.07 to 0.023 was estimated less than the H_e (0.63 to 0.70) in all populations (Table4) meaning that the populations are isolated and there is low external gene flow. This result corresponds with the fact that farmers are keeping seeds from fields for planting in next seasons. This result corresponds with Folkertsma et.al., (2005) observed heterozygosity rated between 0.2 to 0.10 less than he expected heterozygosity rated between 0.13 to 0.45 when measuring the diversity within eleven countries from five eco-geographical regions based on 21 SSRs markers.

Nei's unbiased Pairwise genetic distance between population showed low genetic distance in all populations (Table5), which estimated rating between 0.032 and 0.297. The lowest genetic distance showed between West and Central (0.032), and a high value between North and Blue Nile (0.297). Different levels of genetic diversity among countries may be due to several factors including mating systems, rate of mutation, migration and dispersal mechanisms, biotic and a biotic selection intensities which are determined by geographic location, climate and soil (Kiambi*et al*., 2005).

Table(3): Summary evaluation of SSR markers

Marker	Major. Allele Frequency	Genotype No	Allele No	Gene Diversity	Heterozy -gosity	PIC	Repeat motif
Xtxp40	0.5879	7.0000	4.0000	0.5628	0.0659	0.4971	$(GGA)_7$
Xtxp145	0.5000	18.0000	15.0000	0.7081	0.1758	0.6861	$(AG)_{22}$
CIR286	0.4415	14.0000	8.0000	0.7171	0.1702	0.6785	(AC)9
Xcup02	0.2849	12.0000	7.0000	0.7484	0.1183	0.7035	(GCA)6
Xtxp010	0.4202	16.0000	8.0000	0.7423	0.1383	0.7097	$(CT)_{14}$
CIR329	0.5526	6.0000	5.0000	0.6434	0.0105	0.6102	(AC)8.5
Xtxp114	0.2609	9.0000	6.0000	0.7564	0.9348	0.7122	$(AGG)_8$
CIR238	0.3090	20.0000	13.0000	0.8335	0.0899	0.8164	(AC)26
Xtxp141	0.5272	23.0000	14.0000	0.6886	0.2283	0.6691	$(GA)_{23}$
Xtxp012	0.2419	28.0000	20.0000	0.8729	0.1398	0.8618	$(CT)_{22}$
CIR223	0.5761	7.0000	6.0000	0.5950	0.0217	0.5442	(AC)6
Xtxp273	0.5000	9.0000	6.0000	0.6755	0.0778	0.6346	$(TTG)_{20}$
CIR276	0.5538	7.0000	4.0000	0.6072	0.0968	0.5519	(AC)9
Xtxp320	0.2247	19.0000	11.0000	0.8537	0.1236	0.8372	$(AAG)_{20}$
Xcup61	0.7043	3.0000	2.0000	0.4165	0.1183	0.3298	$(CAG)_7$
Xtxp021	0.3596	16.0000	10.0000	0.7731	0.0674	0.7427	$(AG)_{18}$
Xtxp278	0.7742	6.0000	5.0000	0.3825	0.0430	0.3605	$(TTG)_{12}$
CIR283	0.3495	22.0000	12.0000	0.7453	0.3333	0.7094	(CT)8 (GT)8.5
Xtxp321	0.2784	27.0000	18.0000	0.8461	0.1818	0.8303	$(GT)_4$ + $(AT)_6$ + $(CT)_{21}$
CIR300	0.6813	5.0000	3.0000	0.4812	0.0220	0.4293	(GT)9
gpsb067	0.6474	13.0000	8.0000	0.5541	0.1158	0.5312	(GT)10
gpsb123	0.3636	11.0000	5.0000	0.7173	0.1136	0.6661	(CA)7+(GA)5
SbAGB02	0.6330	20.0000	15.0000	0.5813	0.1170	0.5665	$(AG)_{35}$
Xisep0310	0.6170	6.0000	4.0000	0.5591	0.0213	0.5118	(CCAAT)4

CIR240	0.4205	6.0000	3.0000	0.6408	0.0341	0.5645	(TG)9
Xgap84	0.4080	24.0000	17.0000	0.7784	0.1724	0.7579	(AG)14
Xtxp015	0.2604	27.0000	14.0000	0.8518	0.1771	0.8368	$(TC)_{16}$
Xcup63	0.5833	4.0000	3.0000	0.5036	0.0333	0.3973	$(GGATGC)_4$
CIR306	0.4368	7.0000	4.0000	0.6688	0.0632	0.6073	(GT)7
Xcup53	0.5707	6.0000	4.0000	0.5774	0.0543	0.5112	$(TTTA)_5$
CIR248	0.6092	7.0000	5.0000	0.5556	0.0805	0.4993	(GT)7.5
CIR262	0.5460	8.0000	4.0000	0.5997	0.0805	0.5352	(CATG)3.25
Xgap72	0.6059	10.0000	7.0000	0.5875	0.1059	0.5532	(AG)16
CIR246	0.7312	8.0000	5.0000	0.4394	0.0968	0.4113	(CA)7.5
Xtxp265	0.3077	26.0000	14.0000	0.8211	0.1667	0.8014	$(GAA)_{19}$
Xtxp136	0.6280	4.0000	3.0000	0.5265	0.5732	0.4625	$(GCA)_5$
Xcup14	0.3966	12.0000	6.0000	0.7015	0.1379	0.6469	$(AG)_{10}$
Xtxp057	0.3608	22.0000	11.0000	0.8113	0.2152	0.7933	$(GT)_{21}$
Xtxp206	0.2278	36.0000	23.0000	0.8914	0.2556	0.8829	(AC)13/(AG)20
Mean	0.4739	13.6154	8.5128	0.6671	0.1480	0.6270	-

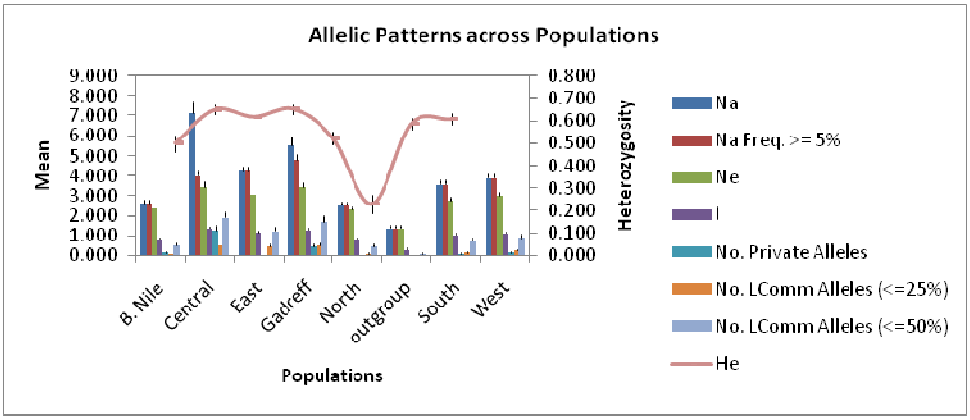

Figure 2. Allelic patterns across populations

Table4. AMOVA calculated according to Weir, B.S. 1996

Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Pops	6	164.157	27.360	0.116	1%
Among Indiv	92	2259.575	24.561	10.932	79%
Within Indiv	99	267.000	2.697	2.697	20%
Total	197	2690.732		13.745	100%

Table5. F-Statistics fixation indices and heterozygosity in all populations

Index	Value	**P(rand >= data**
FIS	0.802	0.020
FST	0.008	0.010
FIT	0.804	0.010
H_o	0.187	
H_e	0.547	

FIT is the deviation from Hardy-Weinberg proportions in the total population.
FIS is the average deviation from Hardy-Weinberg proportions in subpopulations. It is most often due to inbreeding.
FST is Wright's standardized variance. It is due to the variance among demes.
H_{ob} Observed heterozygosity
H_e Expected heterozygosity

Table6. Population specific FIS indices and heterozygosity:

Pop	Name	FIS	FIS Obs	H_{ob}	H_e	Po.
1	Blue Nil	0.71277	0.061584	0.2307	0.6889	89.74%
2	Central	0.80654	0.000	0.1345	0.6888	100%
3	East	0.78066	0.000	0.1595	0.6939	100.00%
4	Gadaref	0.78611	0.000	0.1539	0.70398	100.00%
5	North	0.8444	0.062561	0.1197	0.6393	94.87%
6	South	0.89034	0.000	0.0769	0.6534	100.00%
7	West	0.79242	0.000	0.1435	0.6628	100%

Table7. Pairwise Population Matrix of Nei Unbiased Genetic Distance

	B. Nile	Central	East	Gadreff	North	outgroup	South	West
B. Nile	0.000							
Central	0.113	0.000						
East	0.172	0.081	0.000					
Gadreff	0.120	0.051	0.059	0.000				
North	0.297	0.114	0.130	0.095	0.000			
outgroup	0.326	0.317	0.302	0.279	0.486	0.000		
South	0.173	0.026	0.091	0.061	0.135	0.336	0.000	
West	0.178	0.032	0.078	0.037	0.087	0.240	0.040	0.000

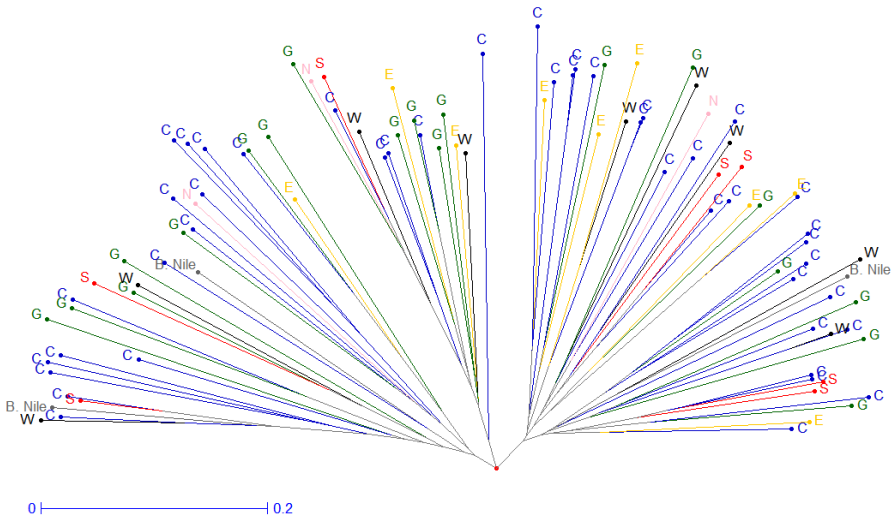

Figure (3): Rooted phylogeny tree of selected material:

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