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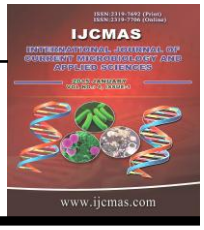

Original Research Article

Genetic diversity of Nigerian Sesame cultivars (*Sesamum indicum L*) based on simple sequence repeat (SSR) markers and its relationship with phytochemicals

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A B S T R A C T

Genetic characterization of Nigerian sesame genotype (*Sesamum indicum* L.) and its relationship with phytochemical composition was investigated using simple sequence repeats markers as well as standard and spectrophotometric methods of the Association of Analytical Chemists (AOAC). High genetic variability was observed among the genotypes with the repeat motifs (TC12-TC25) generated from ten highly informative primer pairs. The 30 accessions were divided into seven main groups and two cultivars not belonging to any group on the basis of an un-weighted pair- group method using arithmetic average (UPGMA) cluster analysis. The largest group consists of seven sesame genotypes (group IV). The average allele per microsatellite locus was 4.6 alleles and the genetic distances among the accessions ranged from 0 to 0.12. The phytochemical screening showed tannins with 1.72±0.35 mg/100g; flavonoids 1.14±0.37 mg/100g; saponins 2.40±0.66 mg/100g; glycosides 0.42±0.14 mg/1 00g and alkaloids 5.89±0.58 mg/1 00g. The result of phytochemical analysis of sesame seeds with respect to genetic association showed the occurrence of cultivar groupings by microsatellite polymorphisms on the basis of the phytochemical composition of the seeds. Significant variation occurred in phytochemical composition in the cultivars clustered on the basis of microsatellite polymorphisms. Groups V and VI cultivars constitute a highly heterogeneous group in relationship to the content of the analyzed phytochemicals. The results indicate that the seeds of sesame contain phytochemicals that may be useful in nutrition and health. The broad genetic base observed in the Nigerian sesame germplasm would provide the basis for the selection of genotypes for future plant breeding programs.

Keywords

Sesame, Genetic Characterization, SSRs-Markers, Phytochemicals

Introduction

Sesame seeds (*Sesamum* or benniseed) are the seeds of the tropical annual crop *Sesamum indicum.* Sesame is grown in many parts of the world on over 5 million

hectares (20,000 km^2). Seventy percent of the world's sesame crop is grown in Asia, with Africa growing 26% (Kinman and Martin, 1999). Commercial production of sesame seeds has been reported in the United States of America in the 1950s (Bruce, 1953) with Nigeria a major producer of sesame contributing significantly to the global sesame export market (Laurentin and Karlovsky, 2006).

Sesame meal is an excellent feed for poultry and livestock (Oplinger *et al*., 1997). Many recipes contain sesame seeds as an ingredient such as sesame seed sprouts, sesame spread, tangerine and sesame, sesame seed cookies, hummus, sesame seed bagels, sesame granola, sesame broccoli rice, sesame mustard sauce, ginger sesame chicken, sesame pastry, sesame seed sauce, and sesame green beans (Home Cooking, 1998). Further, *Sesamum indicum* has medicinal properties used by traditional Chinese and Japanese medical practitioners in their Ayurvedic preparations (Smith and Salerno, 2001). Cancer-protective phytochemicals have also been identified and. these beneficial substances inhibit various hormone actions and metabolic pathways associated with the development of cancer (Osagie *et al*., 1986).

Many studies have revealed that a regular consumption of some seeds provides a significant protection against breast, colon and other types of cancer. The risk of cancer is typically reduced by about 50 percent or more in those regularly eating seeds (Temple *et al*., 1991). Flavonoids act as antioxidants; protect cholesterol from oxidation to the unsafe cholesterol oxides; inhibit the formation of blood clots and have anti-inflammatory and anti-tumor action (Furkuda *et al*., 1986). Saponins in seeds have been known to posses both beneficial and deleterious properties depending on its concentration in sample (Oakenful and Sidihu, 1989).

Characterization of the variability in phytochemical composition among the sesame plant genetic resources in Nigeria is an important step towards sesame genetic improvement. Deoxyribonucleic acid (DNA)-based marker systems have been effectively used as a tool for the analysis of genetic variation (Lee, 1995). Despite high economic value of sesame, only few reports are available on the use of molecular markers such as isozyme (Isshiki and Umezaki, 1997), random amplified polymorphism DNA (RAPD) (Bhat *et al*.,1999) and inter-simple sequence repeats (ISSR) (Kim *et al*., 2002) in genetic diversity analysis of sesame for its improvement. Among these DNA-based marker systems, microsatellites or simple sequence repeats (SSRs) have proven to be the most powerful polymerase chain reaction (PCR)-based DNA markers in plant diversity analysis due to their relative abundance, high variability, co-dominant nature, ease of scoring and high reproducibility (Ellegren, 2004).

However, there is no report relating SSR polymorphisms and phytochemical composition in sesame. In this report, we used microsatellite marker technology for the characterization of the genetic diversity of 30 Nigerian sesame cultivars and the elucidation of the relationship in the microsatellite polymorphisms and the variation in the phytochemical composition.

Materials and Methods Plant material

Plant material

Thirty (30) different accessions, breeding lines, experimental lines and local varieties of sesame were collected from the National

Cereals Research Institute (NCRI) Badeggi, Niger State, Nigeria. Plants were grown from seeds in the field at the crossing block of the Biotechnology Research and Development Center of Ebonyi State University, Abakaliki, Nigeria and also in a temperature controlled greenhouse at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Field/greenhouse plant growth

All plants were grown in the field during the rainy season in the year 2008 and 2009. The experiment was conducted on clay-loam soil at an altitude of 1300–1500 m above sea level and an average daily temperature of 29.8^{o}C. Annual precipitation ranged from 1650 to 2000 mm distributed in two rainy seasons. The field layout was a Randomized Complete Block Design with three replications. Plot size was 1.5 m by1.0 m and seeds were sown at a spacing of 0.25 and 0.50 m within and between rows, respectively. Diammonium phosphate (DAP) fertilizer was applied at a rate of 50 kg/ha during planting. Experimental plots were weeded twice and diseases and pests controlled by spraying pesticide. Seeds were harvested at maturity and used for phytochemical analysis.

Sesame seeds were raised in the greenhouse of FABI using a mixture of vermiculite and polystyrene growth media at temperature of 25-37oC, pH of 5-8 and water treatment of 50-70cm^{3}. Plant growth was maintained using Hoagland's nutrient solution for fertilization.

Phytochemical screening

Phytochemical screening of sesame seeds for saponin, tannins, glycosides, alkaloids and flavonoids was carried out according to Standard methods. Titrimetric methods of Harbone (1983) were used for quantitative determination of tannins and glycosides, while spectrophotometric methods of Association of Analytical Chemists (AOAC, 1989, Odebiyi and Sofowora, 2007) were used for determination of saponins, alkaloids and flavoniods.

DNA Isolation

DNA was extracted from leaves based on the protocol of Voss *et al*. (2007) with minor modifications using the Zymo Research plant/seed DNA KIT^{TM} D6020 using the supplier's instruction. The only modifications to the method were (shortly mention any modifications to the method) The eluted DNA was finally filtered through a Zymo-spinTM IV-H RC spin filter (C1 010- 50) and collected in a clean 1.5 ml micro- centrifuge tube and centrifuged for 1 minute at 8,000 g. DNA concentration and purity was determined using the nanodrop technique (ND-1000 Spectrophotometer). The integrity and concentration of the DNA was confirmed by separation of DNA on a 2% agarose gel where DNA bands were visualized under UV light after ethidium bromide staining.

PCR analysis of SSRs

The polymerase chain reations (PCR) for SSR detection were carried out in a PTC100 thermocycler (MJ Research). Each PCR (20 µL) contained 20 ng of genomic DNA, 2 µl of 10×PCR buffer, 1.2 µl of 25 mM MgCl2, 1.0 µl of 10 mM dNTP, 0.5 µl of 25uM of forward and reverse primer and 0.2 µl of 5 units *Taq* polymerase (NeoTherm). PCR profile was one cycle at 94°C for 3 min (pre-heating) followed by 35 cycles at 94°C for 30sec (denaturing), 55–60°C for 45 sec (annealing), 72°C for 30 sec with a final extension at 72°C for 10 min. and stored at

4°C. Amplified products were resolved on 2% agarose gel, visualized under UV light after ethidium bromide staining and then photographed.

SSR PCR products were purified using the QIAquick purification kit and a direct sequencing PCR reaction (10 µl) was performed using 2 µl Big dye, 4 µl DNA sample, 1 µl forward or reverse primer, 1 µl Big dye buffer and 2 µl of water. The sequencing reaction (PCR) consisted of 35 cycles at 96^{o}C for 10 sec, 57^{o}C for 5 sec, 60^{o}C for 4 min, 72^{o}C for10 min. Product fragments were cleaned using a Millipore sephacryl S-500 spin column and transferred into 0.5 ml tube and dried in a vacuum drier at 60^{o}C for 25min. Microsatellite alleles were resolved on ABI Prism 3010 DNA sequencer (Applied Biosystems) using GENESCAN 3.7 software.

Molecular data analysis

The repeat numbers were physical counted from the DNA sequences. Number of allele per primer, polymorphism information content (PIC) and gene diversity was determined as described by Weir (1996) using the formula PIC = 1-ΣPi^2. Gene diversity values based on allele frequencies were calculated for each SSRs locus using the Nei's unbiased statistics (2007). A dendrogram was constructed by the UPGMA clustering method from the SSRs repeats sequences using the CLC Bio-sequence analysis software version 6.4 to show the genetic relationship among sesame accessions at the selected microsatellites loci.

Results and Discussion

Genomic DNA of sesame leaves

Table 1 shows the result of the determined DNA amount and the A260/280 ratio sesame leaf DNA. DNA yields ranged from 43.48 ng/g tissue in yobe gadaka white to 146.70 g/g tissue in otobi. The 260/280 ratio, an indication for DNA purity, was 1.66 to2.08 among the samples analyzed indicating isolation of clean DNA. Results further indicated that the yield and purity of isolated sesame DNA was sufficient for measuring SSRs using PCR analysis.

The number of alleles per microsatellite locus determined ranged from 3 to 6 with an average of 4.6 alleles. The fragment size varied from 150 bp to 307 bp. Expected heterozygosities (*H*E) and polymorphism information contents (PICs) using the POPGENE version 1.32 (Yeh and Boyle, 1997) for analysis ranged from 0.437 to 0.858 and 0.34 to 0.80, respectively (Table 2), This indicated a high informative nature of microsatellites.

Genetic distances among the accessions ranged from 0 to 0.120. A dendrogram of the 30 sesame cultivars was constructed using bootstrapping and the Unweighted paired Group Method using Arithmetic Average (UPGMA) on the basis of genetic similarity, distinguished the tested accessions into 6 main clusters and into 2 cultivars not belonging to any cluster (Figure 1).

Phytochemicals determined in sesame seeds were tannins (1 .72±0.35mg/1 00g), flavonoids (1.1 4±0.37mg/1 00g), saponins (2.40±0.66mg/1 00g), cynogenic glycosides (0.42±0.14 mg/100g) and alkaloids (5.89±0.58mg/100g) (Table 3). Significant variability was found in these phytochemicals among the tested cultivars ($p < 0.001$) (Table 4). The most abundant phytochemicals in sesame seeds were alkaloids (5.85±0.58) and the least available were cynogenic glycosides (0.42±0.14).

Table.1 Nanometer values of sesame genomic DNA

Genotypes	ng/ µl	A_{260}	A_{280}	260/280	260/230
ABBS	82.74	1.97	1.09	1.81	1.95
Alaide	52.40	0.25	0.14	1.70	1.89
Adaukiari	103.98	0.14	0.09	1.73	1.64
Cameronu white	86.93	0.15	0.11	1.71	1.90
Chimkwale	71.69	0.14	0.07	1.66	1.89
Chimkwale yellow	58.29	0.29	0.19	1.81	1.79
Ciano 16	142.16	0.16	0.09	1.77	1.73
Ciano 27	138.75	0.50	0.31	1.69	1.35
Cross 95	51.32	0.35	0.20	1.89	1.70
Domu	115.11	0.15	0.07	1.74	1.68
E.8	107.72	0.10	0.07	1.68	1.07
Eva	48.11	0.40	0.23	1.72	1.21
Jigaw	78.33	0.21	0.14	1.80	1.24
Kachia	80.44	0.14	0.07	1.80	2.20
Kwander	82.53	0.16	0.10	1.83	1.55
Incriben 0.1m	46.20	0.16	0.11	2.08	1.62
Incriben0.2m	105.77	0.19	0.10	1.70	1.42
Incriben0.3l	72.65	0.25	0.15	1.68	1.08
Otobi	146.70	0.36	0.21	1.67	1.77
Pachequeno	102.48	0.27	0.16	1.88	1.00
Yobe gadaka brown	88.34	0.13	0.07	1.75	1.21
Yobe gadaka white	43.48	0.25	0.15	1.80	1.07
Yobe machine	118.00	0.15	0.10	1.72	1.16
Yorri	93.43	0.20	0.13	2.08	1.95
Zuru	45.12	0.21	0.15	2.00	1.66
34-4-1	52.58	0.05	0.05	1.66	1.64
43-9-1	100.71	0.13	0.13	2.05	1.67
69-1-1	46.16	0.17	0.13	1.40	1.18
69-882	49.45	0.06	0.03	1.84	1.77
NCRI (Iwo)	57.31	0.27	0.17	1.66	1.97

Table.2 Characteristics and sequence information of 10 simple sequence repeats (SSR) markers used on 30 sesame accessions

Table 2 Characteristics and sequence information	GenBank Accession no.	Primer Sequence	Repeat motif	*T*a (□ C)	*N*a	Size range of alleles (bp)	*HO*	*HE*	PIC
GBssr-sa-05	AY838904	F: 5-TCATATATAAAAGGAGCCCAAC-3 R: 5-GTCATCGCTTCTCTCTTCTTC-3	(CT)13	55	6	158–172	0.10	0.847	0.800
GBssr-sa-08	AY838905	F: 5-GGAGAAATTTTCAGAGAGAAAAA-3 R: 5-ATTGCTCTGCCTACAAATAAAA-3	(AG)17	58	5	150–164	0.40	0.679	0.720
Sesame-09	AY838907	F: 5-CCCAACTCTTCGTCTATCTC-3 R: 5-TAGAGGTAATTGTGGGGGA-3	(CT)18	58	6	217–231	0.10	0.858	0.800
GBssr-sa-33	AY838909	F: 5-TTTTCCTGAATGGCATAGTT-3 R: 5-GCCCAATTTGTCTATCTCCT-3	(AG)24	54	5	263–275	0.00	0.779	0.740
GBssr-sa-72	Y838913	F: 5-GCAGCAGTTCCGTTCTTG-3 R: 5-AGTGCTGAATTTAGTCTGCATAG-3	(CT)9	61	3	289–307	0.10	0.437	0.340
GBssr-sa-108	AY838915	F: 5-CCACTCAAAATTTTCACTAAGAA-3 R: 5-TCGTCTTCCTCTCTCCCC-3	(GA)7, (GA)15	61	4	204–218	0.00	0.654	0.617
GBssr-sa-123	AY838916	F: 5-GCAAACACATGCATCCCT-3 R: 5-GCCCTGATGATAAAGCCA-3	(TC)21, (TC)15	61	4	272–282	0.00	0.695	0.660
GBssr-sa-173	AY838919	F: 5-TTTCTTCCTCGTTGCTCG-3 R: 5-CCTAACCAACCACCCTCC-3	[(G)5CTAGT (G)3(A)2]2	55	3	218–245	0.00	0.484	0.460
GBssr-sa-182	AY838921	F: 5-CCATTGAAAACTGCACACAA-3 R: 5-TCCACACACAGAGAGCCC-3	(AT)11, (TC)18, (TG)12	55	6	221–259	0.00	0.734	0.700
GBssr-sa-184	AY838922	F: 5-TCTTGCAATGGGGATCAG-3 R: 5-CGAACTATAGATAATCACTTGGAA-3	(TC)20	55	4	179–193	0.20	0.774	0.740

*T*a, annealing temperature; *N*a, number of alleles; *HO*, observed heterozygosity; *HE*, expected heterozygosity;
PIC, polymorphism information content.

Table.3 Genetic diversity and phytochemical concentration in sesame cultivar seeds

	Genotypes	Repeats	Tannins mg/100g	Flavoniods mg/100g	Saponins mg/100g	Cyanogens mg/100g	Alkaloids mg/100g
Group I	Ciano 27	TC12	1.68 L	0.28 L	1.26 L	0.59 H	4.75 L
Group II	Kwander	TC20	1.69 L	1.91 H	1.93 H	0.21 L	4.24 L
	Incriben0.2m	TC20	1.30 L	2.01 H	2.83 H	0.28 L	6.13 H
	Pachequeno	TC20	1.81 L	0.81 L	3.76 H	0.39 L	5.84 H
	43-9-1	TC20	1.29 L	1.30 H	2.79 H	0.24 L	5.60 L
	Yobe gadaka white	TC20	1.25 L	0.81 L	3.77 H	0.44 L	7.09 H
Group III	Ciano 16	TC19	2.32 H	0.79 L	2.78 H	0.30 L	4.18 L
	69-1-1	TC19	1.61 L	1.20 L	2.78 H	0.80 H	5.37 L
Group IV	Adaukiari	TC22	1.68 L	2.02 H	2.38 H	0.32 L	5.86 H
	Chimkwale	TC22	2.20 H	1.62 H	1.57 L	0.18 L	6.87 H
	Chimkwale yellow	TC22	1.10 L	1.76 H	2.06 L	0.21 L	6.14 H
	E.8	TC22	1.27 L	0.91 L	1.19 L	0.21 L	6.19 H
	Eva	TC22	1.27 L	0.97 L	2.38 H	0.78 H	4.11 L
	Zuru	TC22	2.51 H	0.77 L	2.21 L	0.31 L	6.27 H
	34-4-1	TC22	2.07 H	1.12 H	3.04 H	0.60 H	5.71 H
Group V	Alaide	TC21	2.42 H	1.24 L	2.04 L	0.76 H	6.06 H
	Cameronu white	TC21	2.50 H	1.77 H	2.83 H	0.46 L	5.18 L
	Domu	TC21	1.39 L	0.84 L	2.70 H	0.44 L	6.74 H
	Otobi	TC21	1.62 L	0.76 L	3.68 H	0.28 L	6.47 H
	Yorri	TC21	2.20 H	0.78 L	3.00 H	0.46 L	6.87 H
Group VI	ABBS	TC25	2.20 H	0.75 L	3.79 H	0.60 H	5.18 L
	Incriben0.3L	TC25	1.35 L	0.81 L	2.04 L	0.46 L	6.44 H
	Cross 95	TC25	2.59 H	0.82 L	2.21 L	0.31 L	4.82 L
Group VII	Jigawa	TC18	1.26 L	2.16 H	0.90 L	0.60 H	6.68 H
	Kachia	TC18	1.10 L	1.90 H	1.24 L	0.32 L	5.84 H
	Incriben 0.1m	TC18	2.99 H	0.78 L	1.24 L	0.17 L	6.16 H
	NCRI (Iwo)	TC18	1.82 L	0.79 L	2.08 L	0.72 H	5.57 L
	Yobe gadaka Brown	TC17	1.61 L	0.58 L	3.68 H	0.24 L	6.05 H
	69-882	TC17	1.69 L	0.91 L	3.12 H	0.31 L	6.33 H
Group VIII	Yobe machine	TC15	1.24 L	0.83 L	3.8 H	0.72 H	6.86 H
	Mean ± SE	_	1.72± 0.35	1.14±0.37	2.40± 0.66	0.42± 0.14	5.89± 0.58
	LSD	_	0.37	0.06	0.05	0.03	0.04
	P – Values	-	<.0001	<.0001	<.0001	<.0001	<.0001

N/B Where the mean difference is higher than the LSD values then the cultivar is significantly different from each other in the mean phytochemical content. Similarly, where $P < 0.05$ then it is statistically different in phytochemicals composition among genotypes. (1) Tannins; low (L) concentration = 1.10-2.03 mg/100g; high (H) concentration = 2.04-2.99 mg/100g (2) Flavoniods; low (L) = 0.28-1.23 mg/100g; high = (H) 1.24-2.16 mg/100g (3) Saponins; low = 0.90-2.35 mg/100g; high = 2.36-3.8 mg/100g (4) Glycosides; low = 0.17-0.54 mg/100g; high = 0.55-0.80 mg/100g (5) Alkaloids; low = 4.11-5.61 mg/100g; high = 5.62-7.09 mg/100g.

Table.4 Descriptive statistics of phytochemicals in sesame cultivars (mg/100g)

Phytochemicals	N	Mean	Std Dev	Sum	Minimum	Maximum
T annins	60	1.72	0.35	106.84	1.10	2.99
Flavoniods	60	1.14	0.37	70.69	0.28	2.16
Saponins	60	2.40	0.66	148.99	0.90	3.80
Glycoside cyanogens	60	0.41	0.14	25.97	0.17	0.80
Alkaloids	60	5.88	0.58	365.00	4.11	7.09

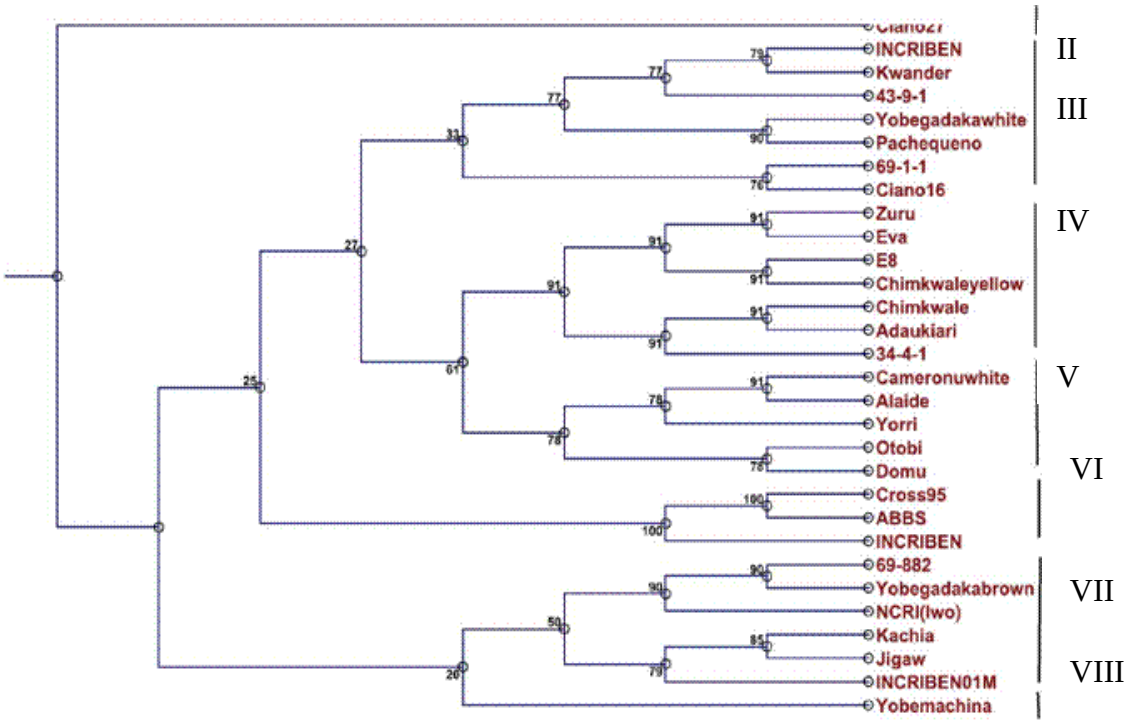
N- Number of variables; Alkaloids showed the highest value of 7.09mg/100g, followed by saponins 3.8mg/100 g, while glycoside cyanogens had the lowest value of 0.17mg/100g followed by flavonoids 0.28 mg/100 g.

Table.5 Correlation matrix among phytochemical composition of sesame seeds

Factor	Tannins	Flavoniods	Saponins	Glycoside cyanogens	Alkaloids
Tannins	1.00000				
Flavoniods	-0.08688 0.5019	1.00000			
Saponins	-0.02033 0.8753	-0.28597** 0.0242	1.00000		
Glycoside cyanogens	-0.22773 0.0750	-0.18250 0.1557	0.30391* 0.0163	1.00000	
Alkaloids	-0.19512 0.1286	-0.00883 0.9457	0.12762 0.3229	-0.09952 0.4416	1.00000

*Positively correlated and statistically significant at $p < 0.05$
** Negatively correlated and statistically significant at $P < 0.05$

Figure.1 Dendrogram based on SSRs data of 30 Sesame cultivars using boot strapping and UPGMA cluster analysis



Significantly negative correlation (r = -0.2859, P < 0.0242) was found between saponins and flavonoids, while a positive correlation (r = 0.30391, p < 0.0163) was observed between saponins and cyanogens glycosides (Table 5).

The difference in concentrations of these phytochemicals found among genotypes might possibly be attributed to genetic differences among members of a highly diverse germplasm collection (Jimoh and Oladiji, 2005).

This study further revealed that accessions formed clusters and sub-clusters in for tannins, for concentrations of phytochemicals. The single cultivar "Ciano" in Group I, with a $_{TC12,}$ was low in tannins, flavonoids, saponins, and alkaloids, but high in cyanogens glycosides. In contrast, "Yobe machina" was at the other end of the dendrogram (Figure 1) comprising of Group VIII (TC15). This cultivar contains a relatively low concentration of tannins and flavonoids, but has higher concentrations of saponins, glycoside cyanogens and alkaloids (Table 3). Group II consisted of five cultivars $_{(TC20}$ sequence repeat) which were all low in tannins and glycoside cyanogens, but high in saponins, and were either high or low in flavonoids or alkaloids. Cultivars in Group III (TC19) slightly differed in their phytochemical amounts with flavonoids, saponins and alkaloids amounts almost the same. Group IV consists of seven cultivars, with a $_{TC22}$ polymorphic repeat sequence. They all had high alkaloid amounts but varied slightly in the amounts of other phytochemicals. Groups V and VI are seemingly heterogeneous groups of cultivars with no consistent association of an SSR to phytochemicals determined. Group VII cultivars, consisting of six cultivars with TC18 ^{and} $_{TC17}$ sequence repeats, greatly

differed in their phytochemicals amount.

Saponins and glycosides compounds often referred to as natural detergent because of their foamy nature (Seigler, 1998). They posses both beneficial and deleterious properties depending on the concentration in the seed (Oakenful and Sidihu, 1989). Seigler (1998) further reported that saponins have anti-carcinogenic properties, immune modulation activities and regulate cell proliferation as well as providing health benefits such as inhibition of cancer cell growth and lowering cholesterol. Flavonoids exert multiple biological effects including antibacterial, antiviral, antitoxic and anti-inflammatory activities (Cook and Samman, 1996). Many of these alleged effects have been linked to known functions as strong antioxidants, free radical scavengers and metal chelators (Torel *et al*., 1986; Nakayama *et al*., 1993). Alkaloids and tannins boost the immune system, helping the body to fight infection and protecting it against degenerative diseases like cancer. (Musa *et al*., 2000). Tannins in sesame seeds might therefore be of immense health benefit to consumers.

Overall, theis study has investigated for the first time any possible relationship between phytochemicals and SSRs in sesame. This study will provide the basis for further detailed studies better characterizing the Nigerian sesame germplasm collection on the molecular level.

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