African Journal of Biotechnology Vol. 7 (10), pp. 1426-1431, 16 May, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Genetic relationships among West African okra (*Abelmoschus caillei*) and Asian genotypes *(Abelmoschus esculentus)* using RAPD

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Accepted 24 March, 2008

Ninety-three accessions of okra which comprises of 50 West African genotypes (*Abelmoschus caillei*) and 43 Asian genotypes (*A. esculentus*) were assessed for genetic distinctiveness and relationships using random amplified polymorphic DNA (RAPD). The molecular analysis showed that all the thirteen primers used revealed clear distinction between the two genotypes. There were more diversity among the Asian genotypes; this might be due to the fact that they were originally collected from six different countries in the region. Six duplicates accessions were discovered while accession TOT7444 distinguished itself from the other two okra species, an indication which suggests that it might belong to a different species.

Key words: West African okra, genetic relationship, *Abelmoschus caillei*, *Abelmoschus esculentus*.

INTRODUCTION

Okra is an important vegetable crop in India, West Africa, South-East, Asia, U.S.A, Brazil, Australia and Turkey. In some regions, the leaves are also used for human consumption. This vegetable provides an important input of vitamins and mineral salts, including calcium; which are often lacking in diet of developing countries (IBPGR, 1990).

The value of a germplasm collection depends not only on the number of accessions it contains, but also upon the diversity present in those accessions (Ren et al., 1995). Knowledge of genetic diversity and relationships among okra germplasm may play significant role in breeding programmes to biotic and abiotic stress of okra. Within species variation among 30 African genotypes was found to be considerably large based on phenotypic assessment (Ariyo, 1993).

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of plant genetic resources. Diversity based on phenotypic and morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification. Currently, the rapid development of biotechnology allows easy analysis of large number of loci distributed throughout the genome of the plants. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006).

Omonhinmin and Osawaru (2005) reported that high degree of wide morphological variation exist among accessions of okra, especially in West African type; which requires further evidence using molecular marker to clarify. To conserve and use these plant genetic resources effectively, it is essential to develop markers that not only distinguish individuals and accessions, but also reflect the inherent diversity and relationships among collection holdings (Kresovich and McFerson, 1992).

Random amplified polymorphism DNA (RAPD) markers have been used to characterize identities and relationships of various crops (Tingey and Deltufo, 1993; Lima et al., 2002). Kresovich et al. (1992) showed that these markers could be of great value in genetic resources management as a quick, cost-effective and reliable method for identification, measurement of variation, and

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determination of similarity at the intra-specific level.

Molecular markers for classification of genotypes are abundant, but unlike morphological traits, markers are not affected by environment (Staub et al., 1997). Martinello et al. (2001) demonstrated that genetic distance based on molecular data (RAPD), average distance from the morphological data and descriptors generated by quantitative data has similar dendrograms pattern in *Abelmoschus* spp. Barazani et al. (2003) however reported the importance of combining molecular markers with morphological data by researchers.

Unfortunately, most of the tropical vegetables such as okra lacked detailed data at biochemical level (Hamon and Noirot, 1991). Molecular markers can be used to identify unique genotypes and associated agronomic traits. Studies using molecular markers in okra are lagging behind the other major crop species; this include the reports of Martinello et al. (2001) using random amplified polymorphic DNA (RAPD) marker and sequence related amplified polymorphism (SRAP) marker by Gulsen et al. (2007). Collecting DNA marker data to determine whether phenotypically similar cultivars are genetically similar would be of great interest in okra breeding programme (Duzyaman, 2005). The present investigation was conducted to assess the genetic distinctiveness and relationships among 50 accessions of West African okra and 43 accessions of South East Asian genotypes using RAPD.

MATERIALS AND METHODS

Fifty accessions of West African Okra (*A. caillei)* collected from the National Centre for Genetic Resources and Biotechnology, Nigeria and 43 accessions of Asian origin (*A. esculentus)* collected from the Genebank of Asian Vegetable Research and Development Centre (AVRDC) - The World Vegetable Centre, Taiwan were used for this study (Table 1). Seeds were raised in plastic pot filled with soil sample for 2 weeks in the controlled green house at an average minimum and maximum temperature of 24 and 31°C, respectively, at AVRDC.

Young leaves from 10 days old plants harvested from 4 randomly selected seedlings of each accession and placed in a well labeled eppendorf tube with tight cover and immediately inserted into an ice pack, but later transferred and kept at – 80°C overnight. Leaf samples were freeze-dried for another 3 days at -51°C with Labconco Lyophilizer.

DNA extraction

DNA was extracted from 25 mg of lyophilized leaf tissue from each of the 93 samples using a modified protocol of Echevarria-Machado et al. (2005). The dry leaves were clipped as finely as possible into 6 ml Eppendorf tubes, 6 to 8 glass beads were added and the tubes were mechanically shaken for 30 s to produce a fine powder (Biospect Product Inc. USA). Each tube was filled with 75 ml of warm extraction buffer [1 M Tris-HCL (pH 7.5), 0.5 M EDTA (pH 7.5), 5 M NaCl, 20% SDS] and heated at 65°C in water bath with occasional agitation mechanically by the water bath. Other reagents added later include 5 M potassium acetate, cold isoproanol, 0.5% sodium metabisulfite solution and 2% PVP 40 and the homogenate was centrifuge at 13200 rpm for 20 min to remove cell debris. Supernatant containing DNA was transferred into a new 2 ml U – shape eppendorf tube and 0.5 ml silica solution was mixed manually inside a shaker for 5 min at 65°C. The samples were loaded in the centrifuge at 13200 rpm again for 2 min to spin down and DNA pellet were washed with 70% ethanol and centrifuge again at 8,000 rpm for 1 min. The pellets were dried using vacuum dryer with heat for 20 min; and each tube re-suspended in water and centrifuge at 13200 for 3 min. The DNA suspension was later transferred into a 1.5 ml V–shape eppendorf tube, treated with RNase and incubated for 2 h at 37°C.

DNA quantification

DNA was quantified by running on a 1.5% agarose gel, and also by Rotor Gene 6000 (www.corbettlifescience.com).

PCR reaction and screening of primers

Each PCR reaction utilized 2 µl template of DNA, H_2O (15 µl), 10X buffer (2.5 µl), dNTP (1 µl), Primer (1 µl), MgCl2 (3.0 µl) and Taq (0.1 - 0.375 µl). PCR programmme used for the RAPD was as follows: 94°C for 3 min; 2 cycles of 94°C for 1 min, 40°C for 1 min and 72°C for 2 min; and ycles of 94°C for 30 s, 40°C for 30 s and 72°C for 1 min. An extension at 72°C for 2 min was included after the amplification.

All PCR products were separated by electrophoresis in a 1.5% agarose gel in 1 X TBE for 2 h. Gels was stained with ethidium bromide and photographed on a digital image documentation computer system (INNOTECH Corporation).

Data analysis

RAPD fragments bands were scored as present (1) or absent (0) to produce a binary data matrix. A fragment was considered polymorphic if it is present in at least one clone and absent in the others. Genetic similarity values between pairs of clones were calculated using the simple matching coefficients from the NTSYS-pc software package (Rohlf, 2005). The genetic similarity matrix was used to generate a dendrogram using the Unweighted Pair-Group method of Arithmetic averages (UPGMA) and the Neighbour Joining (NJ) method.

RESULTS AND DISCUSSION

Reproducible results can be obtained in RAPD if an experimental protocol is properly tested and care is taken to avoid alteration of experimental parameters (Newbury and Ford-Lloyd, 1993). In an attempt to optimize RAPD amplification in okra, several cycling conditions and reaction components were tested. Therefore 2 µl DNA and 0.125 µl Taq polymerase were used for the PCR reaction.

The thirteen primers generated a total of 125 RAPD bands most of which were polymorphic across accessions because they were able to differentiate at least any two of the 93 okra accessions at a time. The number of amplification products per primer varied from eight to twelve with a mean of 9.7. Primers were able to produce fragments that varied from 180 to 2000 bp in size (Table 2). One of the advantages of the RAPD method is that the arbitrarily designed primers can potentially anneal to homologous sequences in the entire genome, providing

Table 1. List of accessions of two species of *Abelmoschus* family and countries of collection.

S/N	Accession	Species	Country of Collection	Stem pubescence
1	CEN 010	*Abelmoschus caillei*	Nigeria	Glaborous
2	NGAE – 96-002	*Abelmoschus caillei*	Nigeria	Glaborous
3	NGAE –96-012-1	*Abelmoschus caillei*	Nigeria	Glaborous
4	NGAE –96-012-2	*Abelmoschus caillei*	Nigeria	Pricky
5	NGAE –96-012-3	*Abelmoschus caillei*	Nigeria	Glaborous
6	CEN 016	*Abelmoschus caillei*	Nigeria	Slightly Pricky
7	CEN 012	*Abelmoschus caillei*	Nigeria	Pricky
8	CEN 007	*Abelmoschus caillei*	Nigeria	Glaborous
9	NGAE –96-04	*Abelmoschus caillei*	Nigeria	Glaborous
10	CEN 015	*Abelmoschus caillei*	Nigeria	Glaborous
11	OAA 96/175-5328	*Abelmoschus caillei*	Nigeria	Slightly Pricky
12	AGA 97/066-5780	*Abelmoschus caillei*	Nigeria	Glaborous
13	ADO-EKITI-1	*Abelmoschus caillei*	Nigeria	Glaborous
14	CEN 001	*Abelmoschus caillei*	Nigeria	Glaborous
15	CEN 009	*Abelmoschus caillei*	Nigeria	Glaborous
16	NGAE –96-0062-1	*Abelmoschus caillei*	Nigeria	Slightly Pricky
17	NGAE –96-0062-2	*Abelmoschus caillei*	Nigeria	Glaborous
18	NGAE –96-0066	*Abelmoschus caillei*	Nigeria	Glaborous
19	NGAE –96-0061	*Abelmoschus caillei*	Nigeria	Glaborous
20	NGAE –96-0060	*Abelmoschus caillei*	Nigeria	Glaborous
21	NGAE –96-0067	*Abelmoschus caillei*	Nigeria	Glaborous
22	NGAE –96-0064	*Abelmoschus caillei*	Nigeria	Slightly Pricky
23	CEN 006A	*Abelmoschus caillei*	Nigeria	Glaborous
24	NGAE –96-0063	*Abelmoschus caillei*	Nigeria	Slightly Pricky
25	NGAE –96-011	*Abelmoschus caillei*	Nigeria	Glaborous
26	CEN 005	*Abelmoschus caillei*	Nigeria	Glaborous
27	NGAE –96-0068	*Abelmoschus caillei*	Nigeria	Glaborous
28	NGAE –96-0065	*Abelmoschus caillei*	Nigeria	Glaborous
29	ABC-1	*Abelmoschus caillei*	Nigeria	Glaborous
30	NCRI- 02	*Abelmoschus caillei*	Nigeria	Glaborous
31	NCRI- 05	*Abelmoschus caillei*	Nigeria	Glaborous
32	NGAE –96-0069	*Abelmoschus caillei*	Nigeria	Glaborous
33	OJAOBA- 1	*Abelmoschus caillei*	Nigeria	Glaborous
34	OJAOBA- 2	*Abelmoschus caillei*	Nigeria	Glaborous
35	OJAOBA- 3	*Abelmoschus caillei*	Nigeria	Glaborous
36	OJAOBA- 4	*Abelmoschus caillei*	Nigeria	Glaborous
37	ADO-EKITI- 2	*Abelmoschus caillei*	Nigeria	Glaborous
38	ADO-EKITI- 3	*Abelmoschus caillei*	Nigeria	Glaborous
39	ADO-EKITI- 5	*Abelmoschus caillei*	Nigeria	Glaborous
40	IFE –1	*Abelmoschus caillei*	Nigeria	Glaborous
41	IFE –2	*Abelmoschus caillei*	Nigeria	Glaborous
42	AKURE –2-2	*Abelmoschus caillei*	Nigeria	Glaborous
43	AKURE –2-9	*Abelmoschus caillei*	Nigeria	Glaborous
44	AKURE –1-1	*Abelmoschus caillei*	Nigeria	Glaborous
45	AKURE –2-4	*Abelmoschus caillei*	Nigeria	Glaborous
46	OWODE- 1	*Abelmoschus caillei*	Nigeria	Glaborous
49	OWODE-4	*Abelmoschus caillei*	Nigeria	Slightly Pricky
50	OWODE- 5	*Abelmoschus caillei*	Nigeria	Slightly Pricky
51	TOT0543	*Abelmoschus esculentus*	LAO P. Dem. Rep	Pricky
52	TOT0545	*Abelmoschus esculentus*	LAO P. Dem. Rep	Pricky
53	TOT0546	*Abelmoschus esculentus*	Malaysia	Pricky

Table 1. Contd.

54	TOT0547	*Abelmoschus esculentus*	Malaysia	Pricky
55	TOT0548	*Abelmoschus esculentus*	Malaysia	Pricky
56	TOT0549	*Abelmoschus esculentus*	Malaysia	Pricky
57	TOT0550	*Abelmoschus esculentus*	Malaysia	Pricky
58	TOT0552	*Abelmoschus esculentus*	Malaysia	Pricky
59	TOT0555	*Abelmoschus esculentus*	Malaysia	Pricky
60	TOT0556	*Abelmoschus esculentus*	Philippines	Pricky
61	TOT0557	*Abelmoschus esculentus*	Philippines	Pricky
62	TOT1214	*Abelmoschus esculentus*	Philippines	Pricky
63	TOT2418	*Abelmoschus esculentus*	India	Pricky
64	TOT2419	*Abelmoschus esculentus*	India	Pricky
65	TOT2422	*Abelmoschus esculentus*	India	Pricky
66	TOT2423	*Abelmoschus esculentus*	India	Pricky
67	TOT2424	*Abelmoschus esculentus*	Cambodia	Pricky
68	TOT2425	*Abelmoschus esculentus*	India	Pricky
69	TOT2742	*Abelmoschus esculentus*	Bangladesh	Pricky
70	TOT2743	*Abelmoschus esculentus*	Bangladesh	Pricky
71	TOT2767	*Abelmoschus esculentus*	Bangladesh	Pricky
72	TOT2768	*Abelmoschus esculentus*	Bangladesh	Pricky
73	TOT2769	*Abelmoschus esculentus*	Bangladesh	Pricky
74	TOT2770	*Abelmoschus esculentus*	Bangladesh	Pricky
75	TOT2771	*Abelmoschus esculentus*	Bangladesh	Pricky
76	TOT2772	*Abelmoschus esculentus*	Cambodia	Pricky
77	TOT3146	*Abelmoschus esculentus*	Cambodia	Pricky
78	TOT3148	*Abelmoschus esculentus*	Cambodia	Pricky
79	TOT5863	*Abelmoschus esculentus*	Philippines	Pricky
80	TOT5864	*Abelmoschus esculentus*	Philippines	Pricky
81	TOT5865	*Abelmoschus esculentus*	Philippines	Pricky
82	TOT5866	*Abelmoschus esculentus*	Philippines	Pricky
83	TOT5867	*Abelmoschus esculentus*	Philippines	Pricky
84	TOT7219	*Abelmoschus esculentus*	Philippines	Pricky
85	TOT7444	*Abelmoschus esculentus*	Thailand	Pricky
86	TOT7637	*Abelmoschus esculentus*	Thailand	Pricky
87	TOT7697	*Abelmoschus esculentus*	Thailand	Pricky
88	TOT7957	*Abelmoschus esculentus*	Thailand	Pricky
89	TOT7958	*Abelmoschus esculentus*	Thailand	Pricky
90	TOT7959	*Abelmoschus esculentus*	Thailand	Pricky
91	TOT7960	*Abelmoschus esculentus*	Thailand	Pricky
92	TOT7961	*Abelmoschus esculentus*	Thailand	Pricky
93	TOT7962	*Abelmoschus esculentus*	Thailand	Pricky

greater opportunities to uncover regions (Williams et al., 1990). Since most of the RAPD primers were able to amplify more than one band per genotype, residual heterogeneity within the lines is suspected (Ogunbayo et al., 2005). Figure 1 shows the similarity dendrogram of the 93 accessions and proved clearly that *A. caillei* are very distinct from *A. esculentus*. Surprisingly, one of the accessions from AVRDC already classified as *A. esculentus* shows a different pattern from both *A. esculentus* and *A. caillei*. The result of this study suggests that TOT7444 might belong to a different species in the *Abelmoschus* family. At 89% level of similarity, *A. esculentus* and *A. caillei* formed nine and eight clusters respectively. The RAPD dendrogram result placed three pairs of TOT2771 and TOT2772, CEN015 and NGAE96004; and Akure29 and Owode1 at 100% similarity. This might suggest that the pairs were the same genotypes but collected at different locations and time. The *esculentus* seems to be more diverse than the *caillei*, this might be due to the fact that they were originally col-

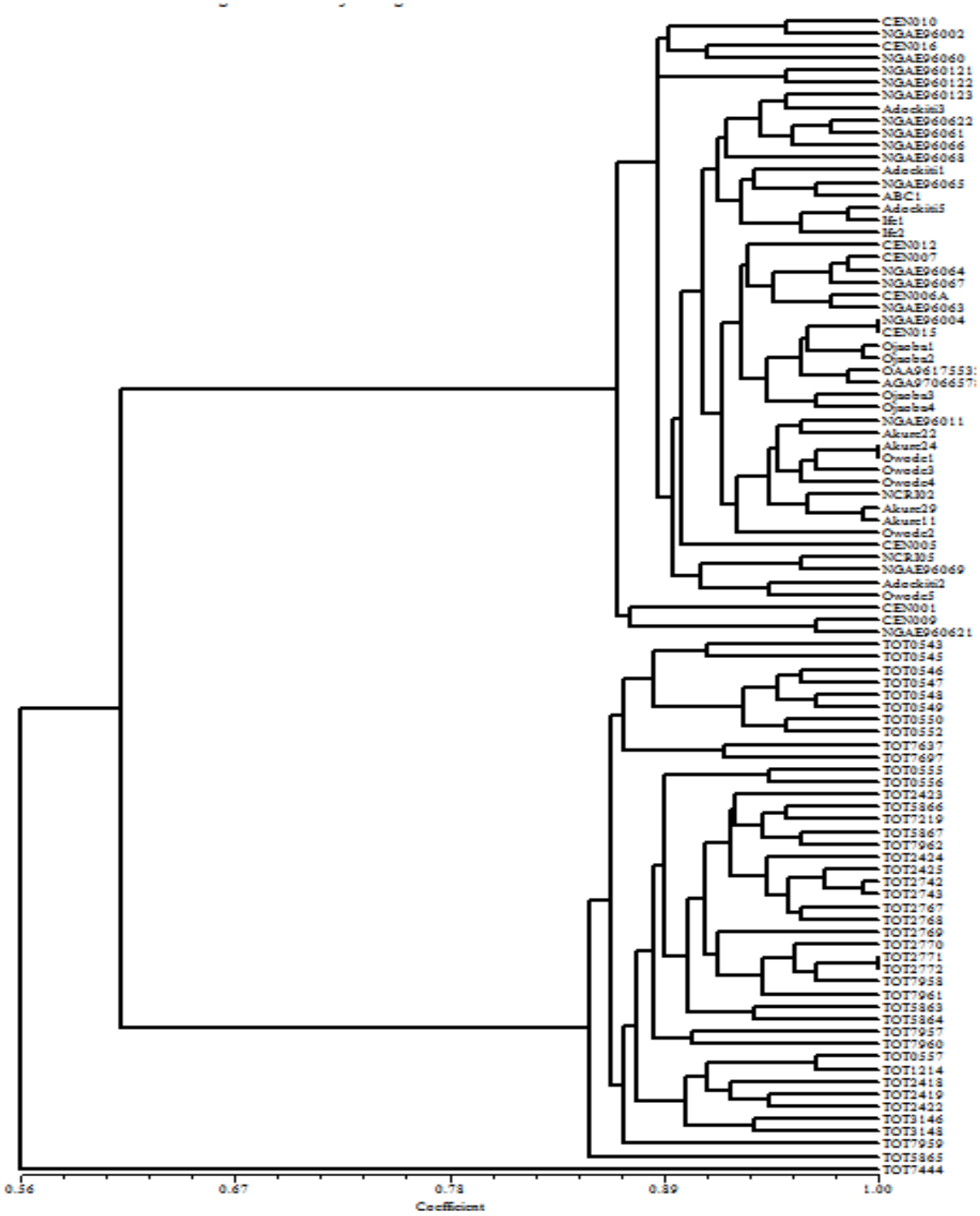

Figure 1. Molecular dendrogram showing the genetic relationships among 93 okra accessions revealed by UPGMA cluster analysis based on RAPDs

lected from seven different countries while all the *caillei* were collected from Nigeria. CEN 001 and CEN 005 formed a single entry clusters among the 50 accessions of West African species *(A. caillei)* while TOT7959 and TOT9865 equally formed single entry clusters among the (*A. esculentus)*.

Cluster I of the Asian genotypes consists of the 2 accessions from LAO Peoples Democratic Republic,

Table 2. Nucleotide sequence of selected primers with the number of amplified products and fragment size range (base pair).

Primer	Sequence 5' to 3'	No of polymorphic bands	Fragment size range (bp)
UBC 54	GTC CCA GAG C	8	180 – 1700
UBC 123	GTC TTT CAG G	10	350 – 1500
UBC 210	GCA CCG AGA G	12	380 – 1550
UBC 292	AAA CAG CCC G	9	350 – 1450
UBC 465	GGT CAG GGC T	11	350 – 2000
UBC 509	ACA GAG ACT G	10	350 – 1250
UBC 514	CGG TTA GAC G	10	300 – 1900
UBC 620	TTG CGC CCG G	10	350 – 1900
OPAN 01	ACT CCA CGT C	8	300 – 1600
OPAE 03	CAT AGA GCG G	8	580 – 1400
OPAE 15	TGC CTG GAC C	9	450 – 1350
OPX 17	GAC ACG GAC C	9	380 – 1200
OPX 18	GAC TAG GTG G	11	300 – 1700

cluster II comprises of 6 accessions from Malaysia while TOT0556 from Philippines and TOT0556 from Malaysia formed cluster III. Seventeen accessions which cut across five countries are clustered in Group IV. TOT5863 and TOT5864 from Philippines formed cluster V. Cluster VI are 2 accessions from Thailand, seven accessions from India, Philippines and Cambodia belong to cluster VII; while TOT7959 from Thailand and TOT5865 from Philippines formed single accession cluster VIII and XI respectively.

ACKNOWLEDGEMENT

I am grateful to Dr. de Lapena, the Head of Biotechnology laboratory for accepting us to carry out this work in AVRDC-World Vegetable Centre and the Federal Ministry of science and Technology, Nigeria for providing the fund.

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