

Short Research Article

Assessment of genetic diversity in improved and local accessions of okra (*Abelmoschus esculentus* and *Abelmoschus callei*) using RAPD markers.

ABSTRACT

Aims: The work was conducted to ascertain the level of genetic diversity in some local and improved accessions of okra in Ibadan. This is to contribute to the conservation of plant genetic resources efforts by breeders.

Place and Duration of Study: Improved accessions of okra were collected from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan; National Institute of Horticulture, (NIHORT), Ibadan; and Agronomy Department, University of Ibadan, Ibadan; while local accessions were collected from local farmers in Ibadan between January and June, 2012.

Methodology: Nine accessions of okra (*Abelmoschus esculentus* and *Abelmoschus callei*), which cut across improved and local accessions, were assessed for genetic diversity. Genomic DNA was extracted from the 9 okra accessions, and thirteen (13) RAPD markers in the OPB, OPT and OPH series; which exhibited high level of DNA polymorphism, were used to PCR amplify it (DNA). The PCR products were checked on 1% agarose gel and the banding pattern visualized under UV-light and then photographed. Presence and absence of bands on the gel were scored '1' and '0' respectively to generate a binary data matrix. Using NTSYS-pc, a pair wise distance matrices was calculated and a dendrogram generated by UPGMA cluster analysis

Results: All the 13 primers used showed polymorphism across the 9 accessions. The genetic similarity of the studied accessions ranged from 76% to 88%. At 88% similarity level 55.6% of the accessions were distinct while 44.4% displayed minimum genetic variation. Maximum genetic variation was observed between *A. callei* UI accession (an improved accession) and Igala (a local accession).

Conclusion: The accessions displayed genetic diversity. The maximum genetic diversity observed between an improved and local accession, and the minimum genetic diversity observed between two local accessions and two improved accessions shows RAPD markers are still very much useful for genetic diversity study.

Keywords: [Genetic diversity, RAPD markers, *Abelmoschus esculentus*, *Abelmoschus callei*]

15 1. INTRODUCTION

16
17 The word okra is of West African origin and is cognate with ọkwurụ in the Igbo
18 language spoken in Nigeria (1). Okra, also known as "lady's fingers" is one of the vegetable
19 crops grown in Southwestern Nigeria. In 2016, the world production of okra was estimated at
20 8.9 million tons, Nigeria producing 1.98 million tons as the largest African producer and
21 second largest producer in the world, next to India which produced 5.51 million tons in 2016
22 (2).

23 Okra, with numerous accessions which include *Abelmoschus esculentus* and
24 *Abelmoschus callei*, has a lot of economic importance. It is known for its high antioxidants
25 content (3), series of culinary uses and industrial applications in the food and pharmaceutical
26 industries (4). Besides these, Okra has even been reported to be a good source of biofuel
27 (5). It is therefore an economic crop which is yet to be fully tapped though greatly cultivated
28 in Nigeria.

29 Different accessions of this crop are cultivated in various locality in Nigeria, and are
30 identified with the name of the locality where they are predominant with respect to their
31 cultivation. While some of these local accessions can be morphologically distinguished
32 easily, there are some which morphological differences are not distinct. It is essential to
33 know whether these are duplication of the same accession or unique accessions, and
34 genetic diversity study is the best tool for this. Genetic diversity studies help in distinct
35 identification of genotypes thereby eliminating, to a reasonable extent, the confusion
36 associated with morphological characterization which include multiple cultivar registration.
37 (6)

38 Besides, considering the economic potential of this crop; which is yet to be fully
39 tapped, there is need for conservation of the plant besides its genetic improvement to suit
40 specific purpose and various humans' needs. For this to happen genetic diversity studies are
41 inevitable. This study aims at identifying the dissimilarities and similarities between some

42 accessions of local and improved accessions of okra in Ibadan. This will help plant breeders
43 in the genetic improvement of the crop.

44 Over the years RAPD markers have proven very useful in genetic diversity studies
45 and has been reported as a great tool for sequence polymorphism (7). There are many
46 recent studies which have successfully used RAPD markers for genetic diversity studies (6,
47 8, 9, 10)

48 This study was conducted using five (5) local accessions of okra and four (4)
49 improved accessions of okra gotten from research institutes in Ibadan, Nigeria.

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51 **2. MATERIAL AND METHODS**

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53 **2.1 Plant material**

54 Dried viable seeds of improved accessions of okra were collected from the
55 department of Agronomy, University of Ibadan, Ibadan; National Institute of Horticulture,
56 Ibadan and National Centre for Genetic Resources and Biotechnology, Ibadan. For the local
57 accessions, dried pods were collected from some farmers in Ibadan. Three seeds of each of
58 the okra accessions were then planted in a well labelled perforated pots, filled with top soil,
59 and watered once every forty-eight hours. The seeds germinated between five to seven days
60 after planting. Three weeks after germination, fresh apical portion of the plants were
61 harvested. The harvested leaf samples were put in a well labelled polythene bags inserted
62 into an ice pack and quickly taken to the laboratory for DNA extraction. The accessions
63 used for this study and their sources are as stated in Table [1] while some of their pictures
64 are presented in Figures [1].

65

66 **2.2 DNA Extraction and PCR Amplification**

67 0.5g of the harvested leaf samples was grinded in liquid nitrogen and DNA was
68 extracted using CTAB method (8) with little modifications. To the ground leaf was added
69 600µl of 2xCTAB buffer (50mM Tris HCl pH 8.0, 500mM NaCl, 10mM EDTA, 2%
70 hexadecyltrimethylammonium bromide, 2% w/v PVP and 0.1% 2-mercaptoethanol). This

71 was incubated for 20 minutes at 65°C with occasional swirling. The samples were then
72 cooled to room temperature and 600ml solution of Phenol:Chloroform:Isoamylalcohol
73 (25:24:1) was added to the sample and mixed gently for 5 minutes. The sample was
74 then centrifuged for 8 minutes at 9,000rpm. The supernatant was then transferred to a
75 new labelled Eppendorf tube. PCI was added again and centrifuged, after which an
76 equal volume of isopropanol was added and the solution incubated at -20°C overnight.
77 DNA was then pelleted at 4°C by centrifuging at maximum speed for 15minutes. The
78 supernatant was decanted and the DNA pellet washed with 70% ice-cold ethanol and
79 then centrifuged for 10 minutes at maximum speed. The DNA pellet was then air dried
80 and dissolved in 50ml TE.

81 Following the Williams *et al's* (9) protocol with slight modifications, the DNA was pcr
82 amplified in a 25µl reaction volume that contains: 2.5µl PCR 10x buffer; 1.2µl MgCl₂
83 (50mM); 2.0µl dNTPs (2.5mM); 1.0µl DMSO; 1.0µl Primer (10 µM); 2.0µl template DNA;
84 0.2µl Taq polymerase and 15.1µl ultra-pure water. The pcr was conducted using
85 MyGene™ Series Thermal Cycler with the programme set at 94°C for 3 minutes
86 initialization, 45 cycles of 94°C for 20 seconds denaturation, 38°C for 40 seconds
87 annealing, and 72°C for 1 minute extension; and concluded by 72°C for 7 minutes final
88 extention then 4°C forever. Using 1% agarose gel in 1 X TBE buffer, gel electrophoresis
89 was conducted with 3µl loading dye and 8µl amplified product. The gel was then
90 visualized under UV light and photographed. The gel is shown in Figure [3] while the
91 primers used are stated in Table [2]

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93 Table 1. The accessions of okra used and their sources

S/No	Ascension	Source
1	Oyo	Local Farm, Ibadan
2	Iroko	Local Farm, Ibadan
3	Iwo	Local Farm, Ibadan
4	Igala	Local Farm, Ibadan
5	<i>Abelmoscos Callei</i> NGAE – 96 - 0064	NACGRAB, Ibadan.
6	<i>Abelmoscos esculentus</i> 47 - 4	NIHORT, Ibadan.
7	<i>Abelmoscos Callei</i> NGAE – 96 - 011	NACGRAB, Ibadan.
8	<i>Abelmoscos esculentus</i> 8 -11	Agronomy Dept, UI
9	<i>Abelmoscus callei</i> UI accession	Agronomy Dept, UI

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Oyo (a local accession)



Iroko (a local accession)



Iwo (a local accession)



A. esculentus 8 – 11
(an improved accession)

97 Figure 1. Dried pods of some of the accessions of okra used for the work.
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2.3 Data Analysis

The bands seen on the gel were transformed into a binary data matrix by scoring positions with bands “1” and positions without bands “0”. Using the NTSYS-pc, the transformed character matrix data were first transferred into the software data collection module from which a pair-wise distance matrices was calculated (10). Genetic similarity was calculated using the Jaccard coefficient of similarity (11) given as $J = A/(N - D)$, where A = number of positive matches; D = number of negative matches; and N = the sample size. Using the output data and the graphical model of the software, a dendrogram was generated by the Unweighted Pair-Group Method of Arithmetic Average cluster analysis (UPGMA) (12). The dendrogram generated is displayed in Figure [4].

3. RESULTS AND DISCUSSION

As shown in Table [2], thirteen out of the twenty five primers used showed polymorphisms. None of these thirteen showed low polymorphisms rather five of them representing 38.46% showed average polymorphisms while eight of them representing 61.54% showed high polymorphisms across the nine accessions. Variations in the banding patterns for each of the RAPD primers used indicates polymorphisms in the accessions being studied and in fact each primer was unique in the binding pattern revealed. This is consequent of the fact that the base sequence in the primers is different and this agrees with the report of Ogunbayo *et al.* (13)

From the dendrogram as presented in Figure [4], at 76% similarity the accessions clustered expectedly into two groups of improved accessions (Z) and local accessions (X) with the exception of one improved accession (Y), *A. callei* NGAE -96-0064, which clustered with the local accessions. This suggests that the local accessions were all closely linked, same with the improved accessions. Maximum genetic diversity was observed between *A. callei* – UI accession (improved accession) and Igala (a local accession) while minimum genetic diversity was observed between Iroko and Oyo (local accessions) and between *A.*

129 *callei* NGAE-096-011 (improved accession from NACGRAB) and *A. esculentus* 8 – 11
 130 (improved accession from Agronomy department, University of Ibadan).

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132 Table 2. RAPD markers used with the PIC and alleles.

S/No	Primer	Sequence	Total No of Alleles	Percentage Alleles (%)	PIC	Percentage PIC (%)
1	OPB-01	GTTTCGCTCG	14	2.85	0.57	6.5
2	OPT-05	GGGTTTGGCA	18	3.66	0.67	7.6
3	OPH-05	AGTCGTCCCC	10	2.03	0.32	3.6
4	OPH-06	ACGCATCGCA	10	2.03	0.56	6.4
5	OPH-03	AGACGTCCAC	20	4.07	0.50	5.7
6	OPH-04	GGAAGTCGCC	22	4.47	0.71	8.1
7	OPT-04	CACAGAGGGA	8	1.63	0.38	4.3
8	OPT-14	AATGCCGCAG	16	3.25	0.66	7.5
9	OPT-06	CAAGGGCAGA	110	22.36	0.91	10.4
10	OPT-10	CCTTCGGAAG	66	13.41	0.93	10.6
11	OPB-05	TGCGCCCTTC	74	15.04	0.89	10.1
12	OPH-07	CTGCATCGTG	78	15.85	0.87	9.8
13	OPB-04	GGACTGGAGT	46	9.35	0.83	9.4
Total			492	100	8.79	100.0
Mean			37.85	7.69	0.68	7.7

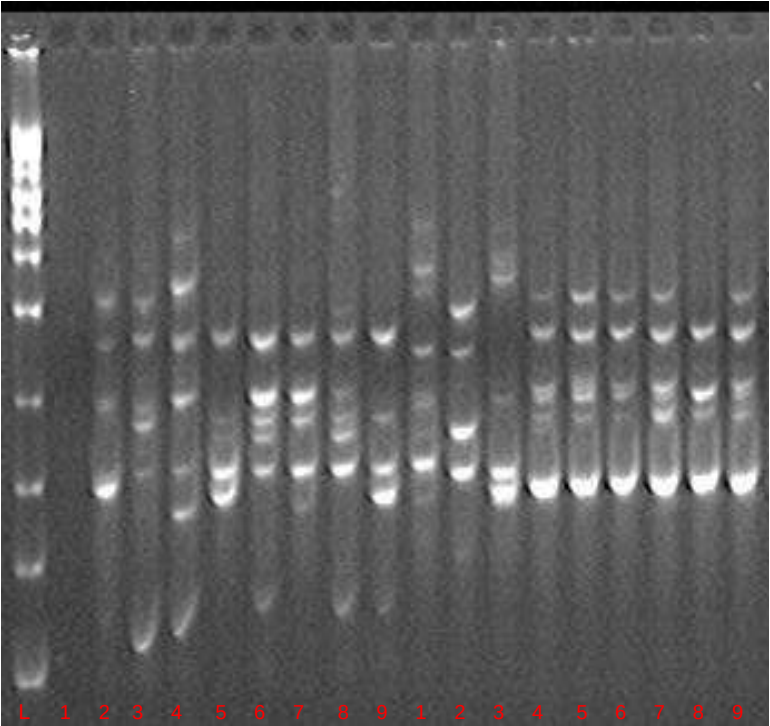
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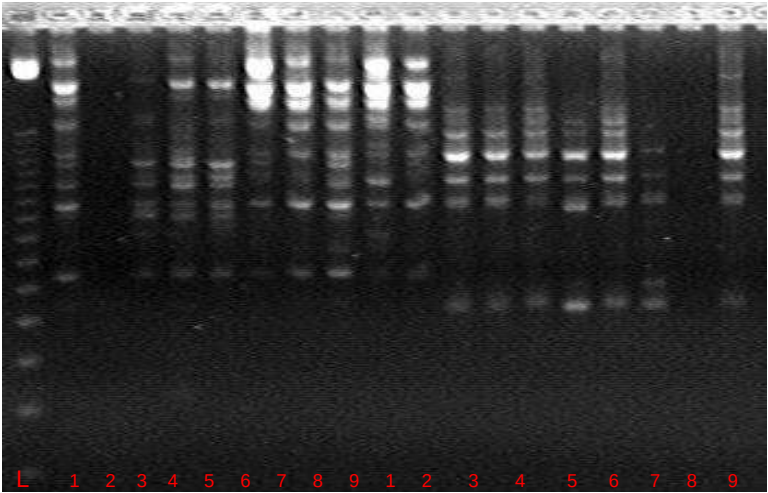
135 However, *A. callei* NGAE 96-0064 widely diverged from other improved accessions
 136 and clustered together with iwo (a local accession) till 86% similarity. It is likely *A. callei*
 137 NGAE 96-0064 is from iwo accession hence their clustering together till 86% similarity. It
 138 would have been expected that improved *A. esculentus* accessions would be more

139 genetically related, likewise the improved *A. callei* accessions. This was observed until
140 unexpectedly at 81%

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OPB_05 OPH_7



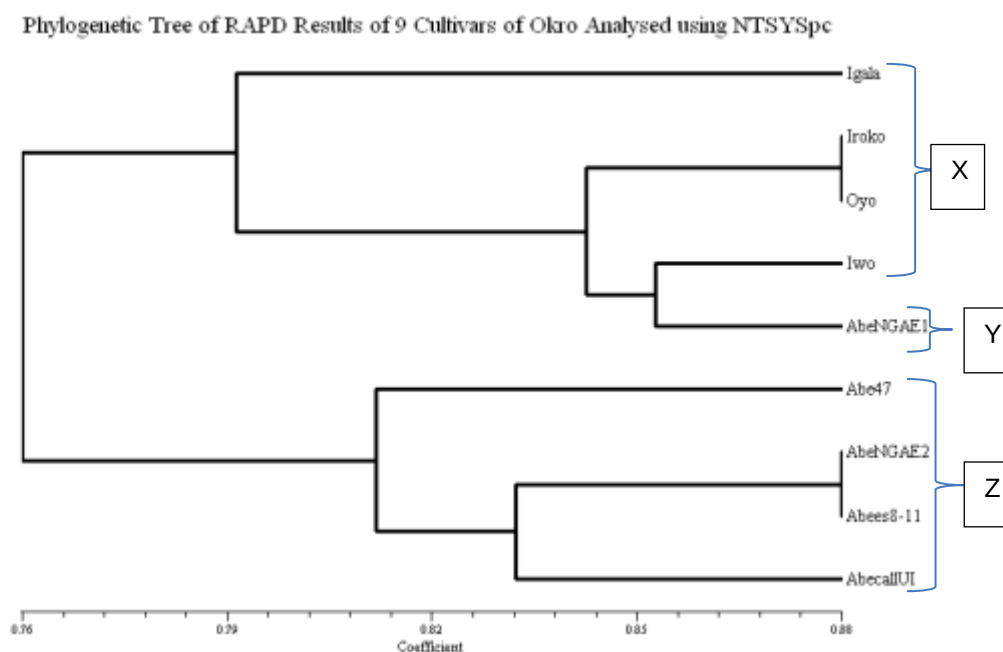
OPT_06 OPT_10

187 Figure 3: RAPD Profile of 9 okra accessions UP: For primers OPB 05 and OPH 07. **Down:**
188 For primers OPT 05 and OPT 10. Lanes L are for the DNA ladder while lane 1 – 9 are for
189 various okra accessions in the order presented in Table 1 above.

190 similarity when *A. esculentus* 8-11 diverged away from *A. esculentus* 47-4 to cluster with two
 191 *A. callei* accessions.

192 Again, rather than be the first to diverge from the *A. callei* accessions, *A. esculentus*
 193 8-11 clustered with *A. callei* NGAE-96-011 at 83% similarity. This implies that *A. callei*
 194 NGAE-96-011 is more related to *A. esculentus* 8-11 than the former is to *A. callei* (UI
 195 accession). At the level of University of Ibadan, it can be acceptably said that at 83%
 196 similarity, University of Ibadan *A. esculentus* accessions diverged from University of Ibadan
 197 *A. callei* accessions. But when compared with accessions from other institutions, it is safe to
 198 say that there might be a miss up in University of Ibadan accessions. The *A. esculentus* (UI
 199 accession) might actually be *A. callei* and vice versa. This reinforces the need for DNA
 200 barcoding for classification of closely related species (14) which will eliminate miss up in
 201 genetic material storage and conservation.

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205 Figure 4: Dendrogram showing genetic diversity in the nine (9) accessions of okra used for
 206 the study.

207 X – local accessions cluster; Y – an improved accession which clustered with the local
 208 accessions; Z - improved accessions cluster.

209 AbeNGAE1: *Abelmoschus callei* NGAE 96-0064

210 AbeNGAE2: *Abelmoschus callei* NGAE 96-011

211 Abe47: *Abelmoschus esculentus* 47 - 4
 212 Abees8-11: *Abelmoschus esculentus* UI accession
 213 AbecallUI: *Abelmoschus callei* UI accession

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217 4. CONCLUSION

218 According to Torkpo *et al* (15), information on genetic relatedness among genetic resources
 219 of crop plants is useful not only for breeding purpose but also for the conservation of
 220 germplasm. The ability of 80% of the improved accessions to cluster together and 100% of
 221 the local accessions clustered together proves RAPD markers useful in genetic diversity
 222 studies (6). There are therefore very useful for breeding programmes. RAPD markers can
 223 also contribute to solving morphological characterization problems not limited to multiple
 224 cultivar registration. (6). The high similarity (76% - 88%) between the studied accessions
 225 reveal there are very similar one to the order hence crosses between them might not give a
 226 good offspring

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281 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

282	UPGMA:	Unweighted Pair-Group Method of Arithmetic Average
283	PIC:	Polymorphic Information Content
284	PCR:	Polymerase Chain Reaction
285	UI:	University of Ibadan
286	TE:	Tris EDTA Buffer
287	EDTA:	Ehtylene Diamine Tetra Acetic Acid
288	PVP:	Poly Vinyl Pyrrolidione
289	CTAB:	Hexadecyl Trimethyl Ammonium Bromide
290	PCI:	Phenol : Chloroform : Isoamylalcohol

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294 APPENDIX

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APPENDIX I

297 INFORMATION ON PHYLOGENETIC TREE GENERATION:

298 **Software used:** NTSYSpc 2.02, (C) 1986-1999, Applied Biostatistics Inc.

299 **Clustering method:** UPGMA

300 **Matrix type =** 3

301 **Size =** 9 by 9,

302 **Missing value code** ="none" (similarity)

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APPENDIX II

306 FIT CRITERION using DARwin

307 Edge length sum: 1.437

308 Mean error: 0.002

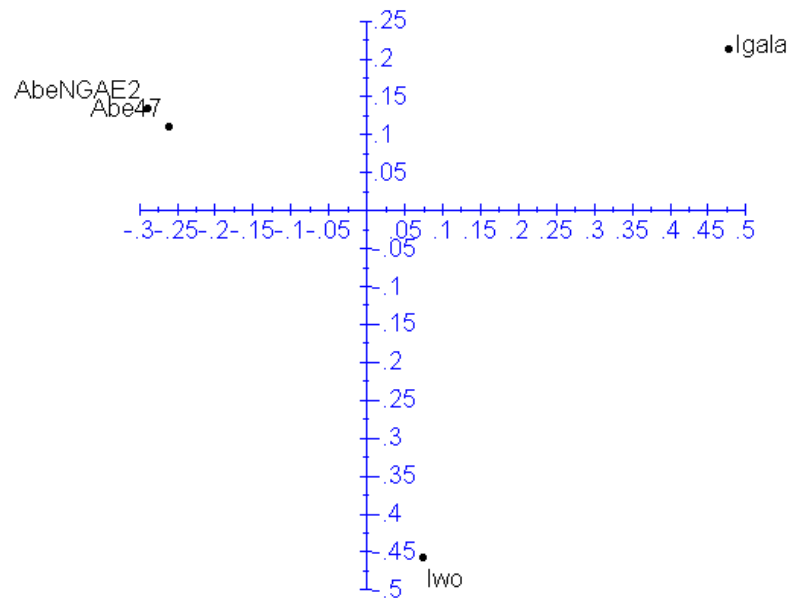
309 Mean absolute error: 0.0089

310 Maximum absolute error: 0.0192

311 Mean square error: 0.0001

312 Cophenetic ratio: 0.9965

Factorial analysis: Axes 1 / 2



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314 **Figure 3.3.1: Factorial analysis of the okra accessions**

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317 File Comments:
318 Factorial coordinates calculated from dissimilarity: Okro.dis

319	Axis	Eigen value	Inertia%
320	1	0.09634	46.83
321	2	0.07128	34.65
322	3	0.03809	18.52
323	4	0	0

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325 Coordinate are calculated for the 3 finrst axes (with positive eigenvalue)
326 Dissimilarity calculated from data file: Okro.var (type:'single')
327 User selection Units: 10/10 and Variables: 146/146
328 Dissimilarity index: Presence / Absence - Jaccard
329 Missing data options:
330 Integer code for missing data = 9
331 Complete unit deletion - Remove all units with missing data
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333 **LEAST-SQUARES EDGE LENGTH RE-ESTIMATION**

334 Fit criterion for initial tree:Okro.arb and dissimilarity: Okro.dis
335 Edge length sum: 1.437
336 Mean error: 0.002
337 Mean absolute error: 0.0089
338 Maximum absolute error: 0.0192
339 Mean square error: 0.0001
340 Cophenetic r: 0.9965
341 Fit criterion for adjusted tree:
342 Edge length sum: 1.4387
343 Mean error: -0.0005
344 Mean absolute error: 0.001
345 Maximum absolute error: 0.0029
346 Mean square error: 0
347 Cophenetic r: 1
348 The degree of confidence in association of the studied accessions is very good as revealed by the
349 cophenetic correlation of 0.9965.