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Genetic diversity in Nigerian brinjal eggplant (*Solanum melongena* L.) as revealed by random amplified polymorphic DNA (RAPD) markers

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The taxonomy of *Solanum melongena* L., also known as brinjal eggplant, has remained difficult because previous studies to establish genetic relationships among taxa are mainly based on morphological features, which are insufficient to establish genetic affinities. In the present investigation, five highly polymorphic random amplified polymorphic DNA primers were used to describe the genetic similarity and diversity among its accessions in Nigeria. The results show a high level of polymorphism based on the banding patterns among the samples. This indicated a wide and diverse genetic base. Four distinct clusters were equally noticeable at a coefficient of 0.80 from the dendrogram generated. Generally, the grouping pattern clearly indicates that irrespective of variations in fruit traits among samples, they were still grouped together in different clusters with a high similarity coefficient value. This probably showed some genetic relatedness/closeness among the samples concerned. The study also revealed that there is no association between RAPD pattern and the geographic origin of accessions. These agreed with previously published data on the characterization of eggplant. The study disclosed that molecular methods coupled with morphological analyses could make proper classification of *S. melongena* and other *Solanum* species in Nigeria possible to achieve.

Key words: Eggplant, random amplified polymorphic DNA (RAPD), Data, polymorphism, taxonomy.

INTRODUCTION

Eggplant, *Solanum melongena* L., also known as aubergine or brinjal, is a member of the family Solanaceae. It is one of the few cultivated solanaceous species originating from the Old World and an important vegetable in central, southern and south-east Asia, and in a number of African countries (Kalloo, 1993). Brinjal eggplant belongs to the

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very large genus *Solanum*, as well as its largest subgenus, *Leptostemonum*, which includes many wild relatives, as well as other cultivated species, such as the Gboma eggplant (*Solanum macrocarpon* L.) and the scarlet eggplant (*Solanum aethiopicum* L.) grown mostly in Africa for their fruits and leaves. More than 200 *Solanum* species are known in Africa, with about 25 species indigenous in Nigeria (Gbile and Adesina, 1988; Burkill, 2000).

A large number of cultivars of eggplant are known and characterized by variation in morphology, physiology and biochemical features (Daunay et al., 2001a). Although India or Indo-China is considered to be the centre of eggplant diversity (Lester and Hassan, 1991; Behera et al., 2006), but the affinities of brinjal eggplant (*S. melongena*) to related species remain uncertain. The crossability and hybridization studies of *S. melongena* with its related species have been generally inconclusive and the results are often contradictory (Anis et al., 1994; Behera and Singh, 2002). Taxa that are morphologically similar to *S. melongena* are difficult to classify (Karihaloo and Gottlieb, 1995) and the relationship of the cultivated eggplant with the weedy form, *Solanum insanum* and its wild progenitor *Solanum incanum* is unclear (Lester and Hassan, 1990). The analysis of accessions derived from different geographical areas is important to study the genetic diversity. However, diverse geographic origin of two accessions cannot be considered as a parameter to describe actual genetic diversity (Skroch et al., 1998). The continuum of morphological variation, cross compatibility, and genetic distances which exist between advanced and primitive cultivars of eggplant, with weedy and wild forms and relatives provides a model system for the study of gene flow of traits affected by domestication between a crop and its spontaneous forms (Behera et al., 2006). Although an Old World taxon, *S. melongena* unexpectedly shares strong genetic similarities with New World *Solanum* species (tomato and potato).

The taxonomy of eggplant remains a challenge (Behera et al., 2006), because of species' large size, overlapping ecogeographical distribution (Levin et al., 2005), morphological plasticity, similar genomes (Okoli, 1988) and existence of swamps of natural hybrids (Oyelana and Ugborogho, 2008). The persistence of confusion in taxonomic classification of eggplant complex especially *S. melongena* is due to the fact that phylogenetic relationships established among taxa are mainly based on morphological features (Karihaloo and Rai, 1995), crossability (Hassan and Lester, 1990) and F_1 fertility (Lester and Hassan, 1991). These parameters are, however, insufficient to establish genetic affinities, because *S. melongena* can be crossed not only to putative progenitors but also to more distantly related species (Daunay et al., 1991). Moreover, because of the existence of a high level of morphological variability, morphological data can lead to ambiguous interpretations.

To overcome these problems, isozyme variation has been analysed (Lester and Hassan, 1991; Karihaloo and Gottlieb, 1995), interspecific crosses involving species of *Solanum* in Nigeria has been carried out (Oyelana and Ugborogho, 2008). Obute et al. (2006) also carried out cytogenetic studies on some brinjal eggplants of Nigerian origin. The genetic affinities at the DNA level have also been reported based on the analysis of chloroplast DNA (Sakata and Lester, 1997 ; Isshiki et al., 1998); random amplified polymorphic DNA (RAPD) (Karihaloo et al., 1995; Singh et al., 2006); amplified fragment length polymorphism (AFLP) (Mace et al., 1999; Furini and Wunder, 2004); simple sequence repeat (SSR) or sequence tagged microsatellite site (STMS) markers (Nunome et al., 2003; Behera et al., 2006) and inter-simple sequence repeat (ISSR) markers (Isshiki et al., 2008; Ali et al., 2013). But little has been done so far, to assess the genetic diversity within the Nigerian brinjal eggplant using molecular markers. The application of molecular markers offers new tools to complement morphological parameters to resolve taxonomic and phylogenetic difficulties associated with eggplant and its relatives. Resolution of these quagmires is important for conservation and genetic improvement of eggplant *Solanum* and relatives for the benefit of breeders and for its sustainable use. In the present investigation, RAPD markers were used to describe the genetic similarity as well as diversity among accessions of *S. melongena* collected from different locations in Nigeria. This was done to gain a better insight into the centre of diversity of this plant and locate the probable source in Nigeria.

MATERIALS AND METHODS

Sample collections and identification

Samples were collected from different locations within the North and South-West of Nigeria. While on the field, each sample collected was given a code for temporary identification pending the determination of their proper names. The collected samples were first compared with photographs, drawings and illustrations from existing sample collections. Details of the samples were obtained from the database using Flora of West Africa from where the specimen was collected in order to determine and authenticate the collected samples. This was achieved by the use of keys in the Flora. Voucher specimen, that is, Herbarium specimen were then prepared following the method of Ogundipe et al. (2009) and taken to expert for proper determination and identification.

Isolation of total genomic DNA

DNA was extracted from the fruit mesocarp of samples using the modified protocol of Dellaporta as described by Dellaporta et al. (1983). DNA concentration was estimated using Eppendorf BioPhotometer spectrophotometer (Eppendorf AG22331, Hamburg, Germany). The quality of the extracted DNA was then verified by electrophoresis on a 1% Agarose gel for 1 h 30 min at 60 V.

Thereafter, the gel was viewed under the ultraviolet (UV) Gel

Figure 1. Samples of *S. melongena* collected for this study and their different shapes and colours. A and B Black purple colour of samples 001 to 011; C, green colour of samples 012 to 021; D, White colour of Samples 022 to 024.

Documentation and Analysis Systems (UVdoc, GA-9000/9010 Version 12, Uvitec, Cambridge, UK).

Polymerase chain reaction (PCR)

Initial screening was done with thirty RAPD primers (Operon Technologies Inc., USA) using DNA from six accessions. Five Operon primers (P11, V04, Q07, U19 and Q03) that are highly polymorphic and gave scorable amplifications were selected and used in the analysis of all the 24 genotypes. Total reaction volume for DNA amplification was 10 µl containing 1.0 µl of 10x TAE buffer, 3 µl of 10 mg/µl sample DNA, 1.0 µl $MgCl_2$ (2.5 mM final concentration), 0.8 µl mixture of 10 mM DNTP (200 µM final concentration), 20 (5% Tween), 20 (Polyoxyethylene Sorbitan Monolaurate) with 20 ethylene oxide units, 3.6 µl of distilled water, and 0.6 µl Taq DNA polymerase (1 U final concentration). PCR was then run on the Techne TC- 412 thermal cycler (Model FTC41H2D, Barloworld Scientific Ltd, Staffordshire, UK), using the following temperature profile: Initial strand separation step of 3 mins at 94°C followed by 45 cycles each consisting of a denaturing step of 1 min at 94°C, annealing step of 1 min at 37°C and an extension step of 1 min at 72˚C. The last cycle was followed by 5 min extension at 72°C. After amplification, PCR product was stored at 4°C till electrophoresis.

PCR products (amplicon) were mixed with 2.5 µl of 10X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) preparatory to electrophoresis and spun briefly in a microcentrifuge before loading into wells formed by teeth of combs in the agarose gel (Sambrook and Maniatis, 1989). PCR products were then resolved by electrophoresis at 2% agarose gel with constant power of 75 V for 2 h followed by staining with ethidium bromide (10 mg/ml) for 1 min and then placed in distilled water to remove excess stain. After the electrophoresis the gel was visualized and photographed in the Gel Documentation and Analysis Systems (UVdoc, GA-9000/9010 Version 12).

Data analysis

Only distinct, well-resolved and unambiguous bands were scored. The amplified fragments were scored as 1 (present) and 0 (absent). From this binary matrix, similarity matrices were computed using Sequential Hierarchical and Nested (SAHN) clustering option of the NTSYS-pc 2.02j software package (Rohlf, 1996). The software generated a dendrogram (Figure 1), which grouped the test lines using unweighted pair group method with mathematic average (UPGMA) on the basis of genetic similarity and Jaccard's coefficient.

RESULTS

Thirteen fruits with purple skin colour were collected from the North (Kano State) while 11 fruits with green, white and purple-green skin colour were collected from the South-West (Ondo State) as shown in Figure 1. Table 1 shows lists of some of the qualitative morphological

Table 1. Some qualitative morphological characters observed in the 24 samples of *S. melongena* used in this study.

Fruit serial code	Fruit colour	Fruit size	Fruit shape
001	Slightly dark purple	Big	Oblong
002	Dark Purple	Slightly Big	Elongated and Bent
003	Dark Purple	Big	Base bigger than top
004	Dark Purple	Big	Flat with wide base
005	Very Dark Purple	Big	Dumb bell
006	Light Purple	Medium	Oblong with curved base
007	Lilac	Medium	Elongated
008	Light Purple	Small	Elongated with curved base
009	Light Purple	Small	Slightly Elongated
010	Very Light Purple	Small	Oval shape
011	Dark Purple	Very Small	Bent base bigger than top
012	Slightly Dark Green	Medium	Elongated and Slightly bent
013	Slightly Dark Green	Small	Elongated
014	Light Green	Small	Base bigger than top
015	Dark Green	Medium	Elongated but fleshy
016	Dark Green	Small	Top equal to base
017	Light Green	Small	Base bent bigger than top
018	Light Green	Small	Oval
019	Light Green	Very Small	Elongated
020	Dark Green	Small	Curved with bigger base
021	Light Green	Small	Base double size of top
022	White	Big	Elongated
023	White	Small	Oval
024	Green and Purple	Medium	Base equal in size to top

characters observed on the accessions.

The RAPD analysis carried out on all 24 accessions produced a large number of distinct fragments for each primer. A total of 44 bands amplified by five different oligonucleotide primers, were scored among the 24 accessions. Thirty two (32) of these bands were highly polymorphic with percentage polymorphism put at 72.7%. The numbers of amplification products obtained were in the range 8–11 with the primers OPQ-03, OPQ-07 and OPU-19 producing the minimum number of (8) bands each whereas primer OPP-11 produced the maximum number (11) of bands. Average of 8.8 bands was obtained per primer as shown in Table 2. Figure 2 shows the RAPD profile produced by OPP–11 for the 24 accessions.

Jaccard's similarity coefficient matrix was used to generate a dendrogram (Figure 3) using UPGMA clustering option of NTSYS-pc 2.02j software package (Rohlf, 1996). The scale of the dendrogram constructed from the data generated was between 0.74 and 0.94 with a mean value of 0.84 (Figure 3). Four clusters were distinguishable from the dendrogram at a truncated line of 80% (a co-efficient of 0.80). Cluster I consisted of 4 accessions (1, 5, 12, and 20). Cluster II, which happened to be the largest, consisted of 13 accessions represented by samples 6, 7, 15, 18, 22, 8, 14, 16, 13, 9, 10, 17 and 21. Cluster III consisted of samples 11, 19, 23 and 24 while Cluster IV being the smallest was made up of samples 2, 4 and 3 (Figure 3). The dendrogram showed clusters of *S. melongena* with a very high level of similarity among them. Meanwhile, in Cluster I members are morphologically different yet they were found in the same subcluster at a similarity level of 81.5%. Cluster II also consisted of members with morphological differences yet with 81.0% similarity level. Cluster III has members that were dissimilar in terms of fruit colour, size and shape yet they were found in the same cluster. Cluster IV members have a very high similarity level of 84.0%. Looking at the members, they have similar fruit colour as opposed to the fruit colour in members of Clusters I, II and III.

DISCUSSION

The molecular techniques such as RAPD and amplified fragment length polymorphism (AFLP) have been found to be useful and robust tool for detecting genetic diversity and determining genetic relationships within and among a

Table 2. Selected operon primers and their sequence as well as characteristics of amplification products in samples analysed.

Primer used	Primer sequence (5'- 3')	Number of Bands	Polymorphic bands	Percentage Polymorphic bands (%)
OPP – 11	(5'- AACGCGTCGG -3')	11	9	81.8
OPV – 04	(5'- CCCCTCACGA -3')	9	7	77.8
OPQ – 07	(5'- CCCCGATGGT -3')	8	6	75.0
OPU-19	(5'- GTCAGTGCGG -3')	8	5	62.5
OPQ – 03	(5'- GGTCACCTCA -3')	8	5	62.5
Total		44	32	72.7
Average		8.8	6.4	

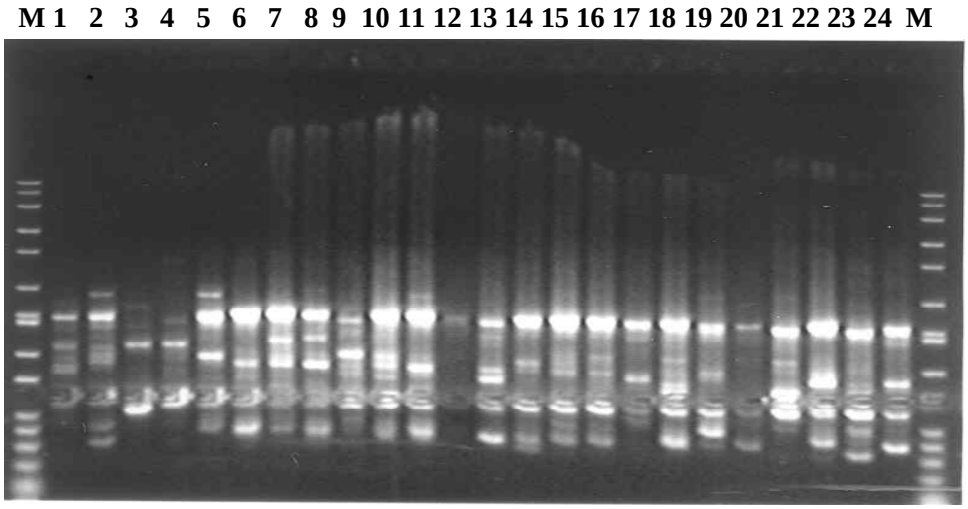

Figure 2. DNA profile produced by primer OPP-11 for samples studied. M represents the 100 bp DNA ladder which serves as the reference point; 1 to 24 corresponds to bands produced by the amplified DNA from the 24 *S. melongena* samples.

group of *Solanum* species (Mace et al., 1999; Singh et al., 2006; Toppino et al., 2008; Polignano et al., 2009; Ali et al., 2013). The present investigation, though focused on a limited number of accessions, gave useful information on the diversity of the *S. melongena*, their interrelationships and their importance in defining groupings characterized by different levels of similarity. High level of genetic diversity was observed in this study as earlier been reported among eggplant of the Indian subcontinent (Singh et al., 2006). Samuel (1996) had stated that high degree of diversity of species belonging to *Solanum* may be attributable to the fact that it is an ancient plant as well as its extraordinary rate of speciation. Singh et al. (2006) also stated that this high level of genetic variability in eggplant and related species was as a result of wide variation in the desirable genotypes/agronomy types in different regions.

The occurrence of accessions used in this study in different clusters despite the fact that they were determined to be the same species shows a genetic variability among them; hence the existence of large gene pool. Generally, the grouping pattern clearly indicates that irrespective of differences in fruit colour, shape and sizes that exist among the samples used, they were still interspersed with each other in different clusters (e.g. clusters I, II). This is an indication of some form of genetic relatedness/closeness among them despite differences in morphological features. The high value of similarity coefficient of 81.5 and 81.0% respectively is also another confirmation. This is in agreement with the findings of Singh et al. (2006).

A fairly high level of polymorphism was also observed in the present study with similarity coefficient ranging from 0.74 to 0.94, indicating a fairly wide and diverse genetic base. This observation is also in agreement with the results of some previous studies, such as that of Furini and Wunder (2004) and Polignano et al. (2009).

The explanation for the observed high degree of variation

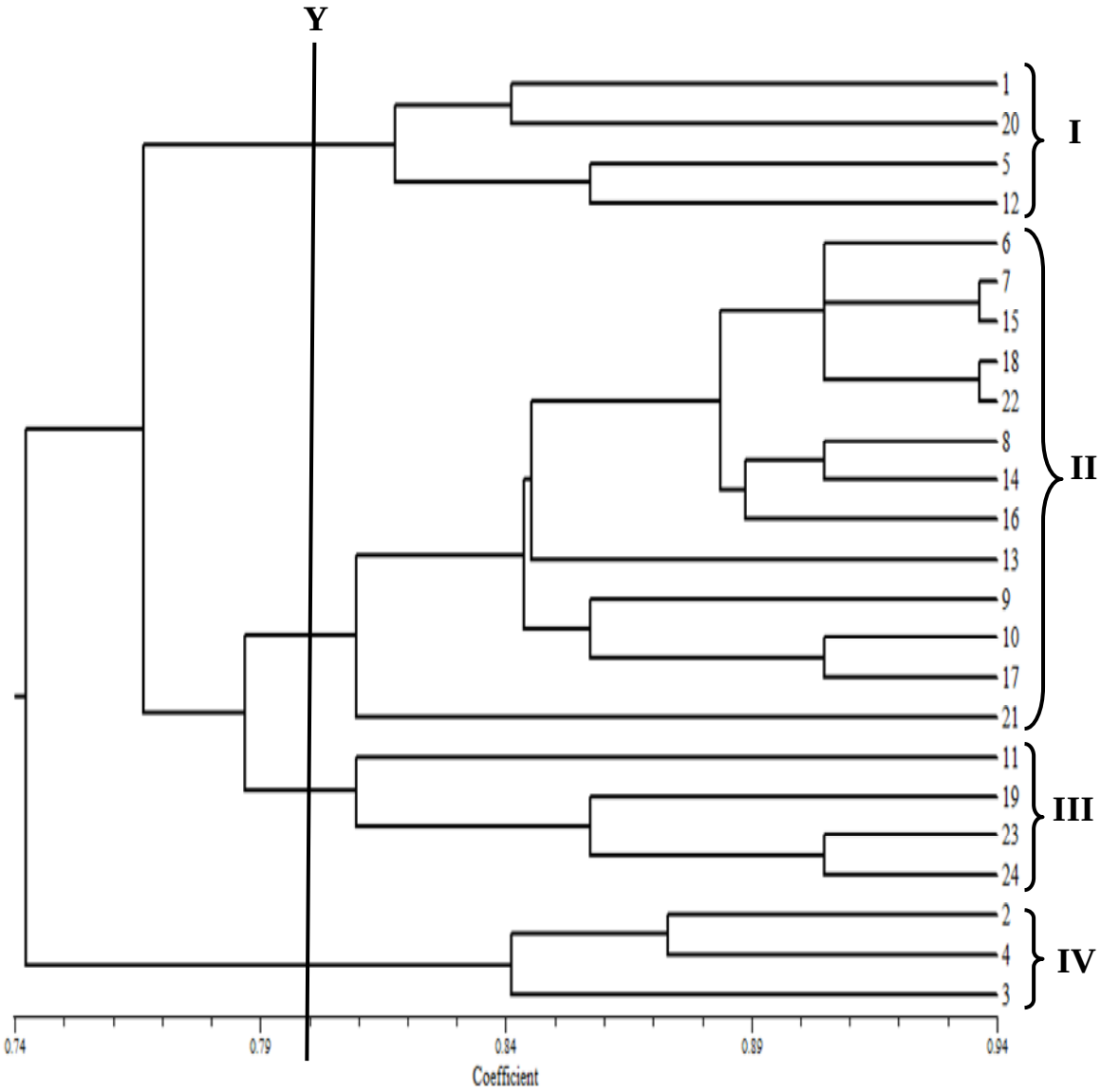

Figure 3. A dendrogram constructed by UPGMA cluster analysis from the RAPD data showing genetic relationship among accessions of *S. melongena* fruits. Y represents truncated line at a co-efficient of similarity 0.80; I to IV represents the four clusters that were distinguishable from the dendrogram at that co-efficient of similarity.

in this study could be due to the fact that the analysis of DNA variability is based on RAPD markers, which proved more informative than allozymes and morphological characters being used previously; and that the accessions of the *S. melongena* analysed in this study were collected from locations in Nigeria, where the greatest diversity has been reported (Gbile and Adesina, 1988). A high degree of variation has also been reported by using AFLP technology for *S. melongena* with weedy relative of the cultivated eggplant (Mace et al., 1999). However, this is a sharp contrast from the result of some other earlier workers who studied variation among the cultivated and weedy taxa of *S. melongena* by allozymes and RAPD analysis. Examples include the work of Karihaloo et al. (1995) and that of Karihaloo and Gottlieb (1995). These authors observed little genetic polymorphism among the genotypes studied and came up with the suggestion that very small gene pool existed from which the cultivated forms arose.

Earlier workers on indigenous *S. melongena* in Nigeria based their characterization studies on cytogenetic (Obute et al., 2006), interspecific crosses (Oyelana and Ugborogho, 2008), morphological plasticity and similarity of genomes (Okoli, 1988). This present study happened to be the pioneer work on characterization and genetic variability of eggplant especially *S. melongena* in Nigeria

using molecular markers. Interestingly, collections originnating from various parts of the country did not form well-defined distinct clusters as seen in Figure 3. They were interspersed with each other, indicating no association between RAPD pattern and the geographic origin of accessions. This finding is in agreement with the findings of Singh et al. (2006) and Polignano et al. (2009).

Conclusion

Although only a small sample of the gene pool was included in this study, within the eggplant *Solanum* the amount of variation may vary widely. Additional evaluations on the whole collection of *Solanum* species in Nigeria using different types of molecular markers (such as RAPD, AFLP, SNP, SSR, etc) as being done in other regions could give us more information on the genetic relationship among them. Detection of genetic differences and discrimination of genetic relationship between *Solanum* species could be a tool for utilization and conservation of plant genetic resources. *S. melongena* could also be a potential valuable breeding material use-ful to different users and assist in the introgression of genes. Nevertheless, the use of molecular methods cou-pled with morphological analyses could make proper classification of different *Solanum* species in Nigeria possible to achieve. Finally, our results suggest further evaluation activities to better define the eggplant diversity patterns utilizing multivariate analysis and including larger sets from the Nigerian brinjal eggplant collection.

Conflict of Interests

The author(s) have not declared any conflict of interests

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