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Partitioning and distribution of random amplified polymorphic DNA (RAPD) variation among eggplant *Solanum* L. in Southwest Nigeria

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***Solanum* L., the largest genus of the Solanaceae family, vary morphologically, is diverse in number and is ecogeographically distributed. In Nigeria, previous studies had focused mainly on chromosome morphology, genome description and medicinal values, which are insufficient for genetic affinities. This study used four highly polymorphic random amplified polymorphic DNA primers to describe both the genetic relatedness and variability among 25 accessions of eggplant from Southwestern Nigeria. At a truncated line of 65%, five clusters and two ungrouped samples are distinguishable from the dendrogram. The data reveals that *Solanum dasyphyllum* Schum. & Thonn. is more closely related to *Solanum macrocarpon* L. than to *Solanum melongena* L. The relatedness between *Solanum incanum* L. and *Solanum melongena*, a probability of being progenitors from a common ancestral lineage was also shown. Occurrence of *Solanum scabrum* L. and *Solanum nigrum* L. in the same clusters different from *S. melongena*, is an indication of distant relatedness to *S. melongena* but close relatedness between them. High level of polymorphism was observed in this study going by the coefficient of variation which exhibited a good separation from a conserved region of the genome. This study, therefore, reveals a wide and diverse genetic base in Nigerian eggplant *Solanum*.**

Key words: Eggplant, genome, synonymy, polymorphism, phylogenetic.

INTRODUCTION

Solanum L., a complex and large genus of the family Solanaceae has an unresolved proper delineation of the species. The genus contains roughly between 1,500 and 2,000 species (Bohs, 2001). They are morphologically varied, numerically diversed and vastly ecogeographically distributed. Several species of vegetable *Solanum* important for human diet and health are referred to as eggplant (Daunay et al., 2001). Examples include *Solanum melongena, Solanum aethiopicum, Solanum macrocarpon, Solanum quitoense* Lam., *Solanum sessiliflorum* Dunal and related species. The taxonomy of eggplant *Solanum* has remained challenging due to species' large size, overlapping ecogeographical distribution (Levin et al., 2005), morphological plasticity, similar genomes (Okoli, 1988) and existence of swamps of natural hybrids (Obute et al., 2006; Oyelana and Ugborogho, 2008). The inconsistencies and misconceptions generated by these factors have made past attempts at taxonomically resolving the complexities associated with the genus difficult. The taxonomic

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uncertainties still persist in this genus largely because previous studies to address the taxonomic problem of vegetable *Solanum* have focused mainly on morphology (Karihaloo and Rai, 1995; Kumar et al., 2013), crossability and F1 fertility (Baksh, 1979; Hassan and Lester, 1990a; Lester and Hassan, 1991; Furini and Wunder, 2004) and anatomy (Hassan and Lester, 1990b). Establishing genetic affinities on such parameters are insufficient, as *Solanum* makes successful crosses with putative progenitors as well as distantly related species.

The advent of molecular biology has revolutionized the field of plant systematics and has been used successfully in phylogenetic relationships at all taxonomic levels (Bohs, 2005) as well as in DNA fingerprinting of plant genomes (Cervera et al., 1998) and in genetic diversity studies (Isshiki et al., 2008; Fory et al., 2010). The use of molecular techniques in genetic diversity studies is supported by the finding that evolutionary forces such as natural selection and genetic drift produce divergent phylogenetic branching which can be recognized because the molecular sequences, on which they are based, share a common ancestor (Singh et al., 2006). Random amplified polymorphic DNA (RAPD), when compared with other molecular markers, is more effective in this regard as it is simple, rapid, requires only a small quantity of DNA and it is well adapted for nonradioactive DNA fingerprinting of genotypes (Cao et al., 1999). It is also able to generate numerous polymorphisms (Williams et al., 1990). Karihaloo et al. (1995) focused directly on nuclear genomic diversity of *Solanum* by undertaking RAPD analysis. Karihaloo and Gottlieb (1995) also reported that greater DNA polymorphism exists in weedy *Solanum insanum* than in advanced cultivars of eggplants. RAPD data were used in several other studies such as Miller and Spooner (1999), Stedje and Bukenya-Ziraba (2003) and Singh et al. (2006) to clarify phylogenetic relationships. Other molecular markers have also been previously used to study the variability as well as relatedness among eggplant *Solanum* species. For instance, Nunome et al. (2003a) and Ge et al. (2013) both employed microsatellite markers or simple sequence repeat (SSR) markers, Behera et al. (2006) used STMS markers, Fory et al. (2010) worked on Colombian collection of *Solanum* using amplified fragment length polymorphism (AFLP), and more recently, Ali et al. (2013) studied the diversity among samples of Chinese *Solanum* by comparing results of RAPD and SSR markers.

In Nigeria, not many works have been done on the nature of genetic diversity and characterization of vegetable *Solanum*, especially using molecular methods. Many vegetable *Solanum* species that occur in Nigeria are sources of food and of medicinal importance (Gbile and Adesina, 1988). Taxonomic studies on the vegetable *Solanum* species in Nigeria have been based on chromosome morphology (Oyelana and Ugborogho, 2008), genome description (Okoli, 1988), medicinal and food values (Gbile and Adesina, 1988). These have not resolved the problems of synonymy and taxa misidentification common to the genus. As a result, this study attempts to resolve to a larger extent the taxonomic difficulties associated with vegetable *Solanum* especially among the species found in Southwestern Nigeria using RAPD molecular marker.

MATERIALS AND METHODS

Sample collection and identification

Fresh leaves (young and matured), fruits and seeds of eggplant *Solanum* samples of different species were collected from different locations in Southwestern Nigeria (Longitude 3° 20′E - 5° 10′E and Latitude 6° 15′N - 9° 00′N) especially in areas known for eggplant diversity. Each sample was labelled accordingly. The fresh leaves were prepared for molecular analysis while mature leaves were prepared for herbarium. A total of 25 samples were collected and analyzed in this study. Their authenticated names and places of collection are shown in Table 1. The breakdown showed that the collections consists of 10 different *Solanum* species made of 2 samples of *Solanum dasyphyllum*, 2 of *Solanum nigrum*, 3 *Solanum macrocarpon*, 2 *Solanum torvum*, 1 *Solanum erianthum*, 3 *Solanum melongena*, 7 *Solanum gilo*, 2 *Solanum scabrum*, 2 *Solanum aethiopicum* and 1 of *Solanum incanum*. Figure 1 shows some of the samples with variations in shapes and colours.

Voucher specimens were prepared from the samples following the method of Ogundipe et al. (2009) and sent to Forestry Herbarium Ibadan (FHI) where they were authenticated by taxonomists. These specimens were then deposited at both the University of Lagos Herbarium (LUH) and Forestry Herbarium Ibadan (FHI) for reference purposes.

Total genomic DNA extraction

Total genomic DNA extraction was carried out on young fresh leaves of each sample (Dellaporta et al., 1983). This was followed by additional purification in a silica-column inserted into vacuum manifold connected to a vacuum pump using QIAquick purification kit (Promega). Verification of the quality of the purified DNA samples was achieved by electrophoresis on a 1% Agarose gel.

Polymerase chain reaction (PCR)

Twenty seven (27) Operon primers (Operon Technologies Inc., USA) were screened based on higher GC content (between 60 - 70%) and their previous workability. Only four (4) that are highly polymorphic and gave reproducible bands were selected and used in the analysis of all the 25 genotypes. Total reaction volume for PCR was 10 µl containing 1.0 µl of 10x TAE buffer, 2 µl of 10 mg/µl sample DNA, 1.0 µl $MgCl_2$, 0.8 µl mixture of 10 mM dNTP, 20 (5% Tween), 20 polyoxyethylene sorbitan monolaurate with 20 ethylene oxide units, 4.6 µl of distilled water, and 5 U Taq DNA polymerase (1 U final conc.). Amplification was accomplished 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 min at 94°C followed by 40 cycles each consisting of a denaturing step of 20 s at 94°C, annealing step of 40 s at 35°C and an extension step of 1 min at 72°C. The last cycle was followed by 5 min extension at 72°C to allow complete extension of the PCR products with a final hold at 4°C till electrophoresis. The reaction was repeated two times for each

Table 1. Eggplant *Solanum* samples and places of collection.

Sample I.D no.	Identification Name	Place of collection	State of collection
OG02	*Solanum dasyphyllum*	Wasinmi	Ogun
OG03	*S. nigrum*	Wasinmi	Ogun
OG04	*S. dasyphyllum*	Joga orile	Ogun
OG05	*S. nigrum*	Joga orile	Ogun
OG06	*S. macrocarpon* (White fruit)	Abulemaria	Ogun
OG07	*S. macrocarpon* (Green fruit)	Abulemaria	Ogun
OG08	*S. torvum*	Wasimi-Imasai	Ogun
OG09	*S. erianthum*	Wasimi-Imasai	Ogun
OG10	*S. melongena* (Green fruit)	Wasinmi-Imasai	Ogun
OY11	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY12	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY13	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY14	*S. gilo* Raddi (White fruit)	Igboho	Oyo
OY15	*S. incanum* L. (Green small fruit)	Igboho	Oyo
OY16	*S. scabrum*	Igboho	Oyo
OY17	*S. aethiopicum*	Igboho	Oyo
OY18	*S. scabrum*	Igboho	Oyo
OY19	*S. melongena* (White fruit)	Igbope	Oyo
OY20	*S. aethiopicum*	Igboho	Oyo
OS21	*S. torvum*	Iwo	Osun
OG22	*S. melongena* (Green fruit)	J3 Camp, Ijebu Ode	Ogun
LA23	*S. gilo* Raddi (Green egg-shaped fruit)	Bariga, Lagos	Lagos
LA24	*S. gilo* Raddi (Green round fruit)	Agbowa-Ikosi	Lagos
LA25	*S. gilo* Raddi (Green round fruit with greenish purple stem)	Agbowa-Ikosi	Lagos
LA26	*S. macrocarpon* (Green fruit)	Agbowa-Ikosi	Lagos

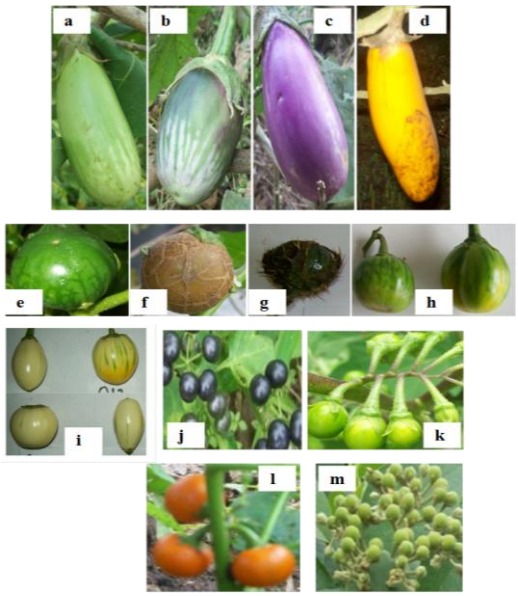

Figure 1. Variability in fruit colour and shape of some eggplant samples studied. Legend: (a - d) *S. melongena*; (e and f) *S. macrocarpon*; (g) *S. dasyphyllum*; (h and i) *S. gilo*; (j) *S. scabrum*; (k) *S. incanum*; (l) *S. aethiopicum*; (m) *S. erianthum.*

Table 2. Operon primers selected with their nucleotide sequence and their characteristic number of bands at amplification in samples analyzed.

Primer used	Primer sequence (5'- 3')	Total number of bands	Number of polymorphic bands	Percentage polymorphic bands (%)
V-19	(5'- GGGTGTGCAG -3')	12	10	83.3
B-18	(5'- CCACAGCAGT -3')	12	10	83.3
OPU-13	(5'- GGCTGGTTCC -3')	13	9	69.2
OPU-15	(5'- ACGGGCCAGT -3')	15	13	86.7
Total		52	42	80.8
Average		13	10.5	

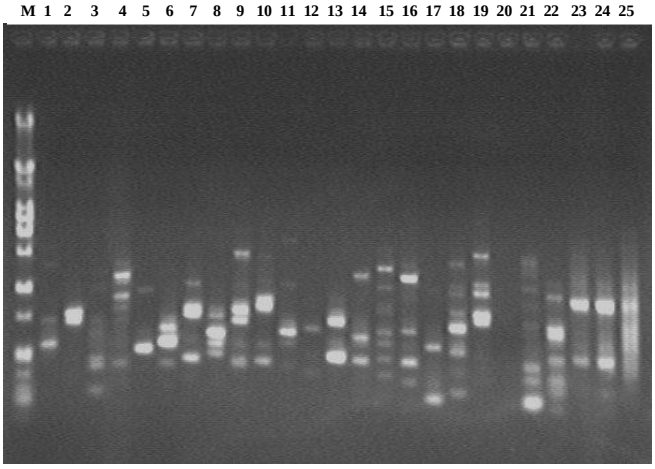

Figure 2. RAPD profiles generated by primer B-18 for *Solanum* samples studied. Legend: M represents the 100 bp DNA Ladder which serves as the reference point; 1 to 25 corresponds to bands produced by the amplified DNA from the 25 samples.

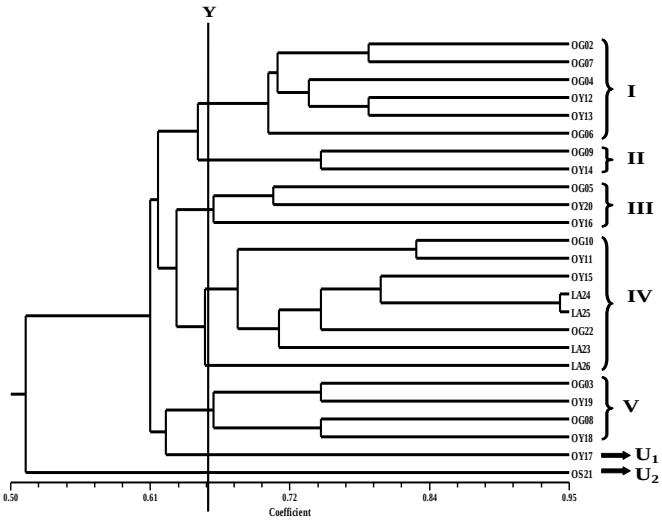

Figure 3. A UPGMA dendrogram showing genetic relationship among accessions of eggplants studied. Legend: Y represents truncated line at a co-efficient of similarity 0.65; I to V represent the five clusters that were distinguishable from the dendrogram while U_1 and U_2 represent ungrouped samples at that co-efficient of similarity.

selected primer to make the result more reliable. 5 µl of each of PCR product (amplicon) were mixed with 3 µl of 10X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a micro centrifuge before loading on a1.5% agarose gel which has been previously stained with safe view. This was run for 1 h 30 min at 110 V/cm. Thereafter, the gel was viewed (with the aid of eye protector) and photographed in the Gel Documentation and Analysis Systems (UVdoc, GA-9000/9010 Version 12).

Data analysis

For each sample, only distinct, well-resolved and unambiguous bands were scored. Faint bands were discarded. The amplified fragments were scored as 1 (present) and 0 (absent) to generate binary matrices. From this 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, 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

The RAPD analysis of the 25 samples revealed a total of fifty two (52) bands, amplified by four (4) different oligonucleotide primers namely OPU-13, OPU-15, B-18 and V-19 (Table 2). Forty two (42) of these bands were highly polymorphic with percentage polymorphism put at 80.8% (Table 2). The numbers of amplification products obtained were in the range 12-15. Primers V-19 and B-18 produced the minimum number of (12) bands each, OPU-13 produced 13 bands and primer OPU-15 produced the maximum number of (15) bands. Average of 13 bands was also obtained per primer as shown in Table 2. Figure 2 shows the RAPD profile produced by B-18 Operon primer for the 25 samples.

Jaccard's similarity coefficient matrix generated a dendrogram (Figure 3) based on polymorphism obtained with all the selected four primers using UPGMA clustering option of NTSYS-pc 2.02j software package (Rohlf, 1996). The scale of the dendrogram constructed from the data was between 0.50 and 0.95 with a mean value of 0.73 (Figure 3). At a truncated line of 65% (a similarity co-efficient of 0.65), five clusters (I - V) and two ungrouped

samples (U_1 and U_2) are distinguishable from the dendrogram. Cluster IV is the largest consisting of 8 samples while Cluster II being the smallest is made up of 2 samples (Figure 3). All the samples of *S. dasyphyllum* occur in Cluster I together with 2 samples (out of 3) of *S. macrocarpon* and 2 of *S. gilo*. Cluster IV contains most samples of *S. gilo* together with 2 of *S. melongena* and 1 of *S. incanum* and *S. macrocarpon*, respectively. One sample each of *S*. *nigrum*, *S. aethiopicum* and *S. scabrum* grouped together in Cluster III; so also, Cluster V contains one sample each of *S. melongena*, *S. nigrum*, *S. torvum* and *S. scabrum,* respectively. The only sample of *S. erianthum* occurs with one *S. gilo* in Cluster II while the remaining samples of *S. aethiopicum* and *S. torvum* remained ungrouped U_1 and U_2 respectively. It is worthy of notice that just as the selected primers were able to detect inter-specific polymorphism, they equally did so intra-specifically. This accounted for the occurrence of samples of the same species in different clusters e.g. one sample each of *S. nigrum* and *S. scabrum* occurring in both clusters III and V.

DISCUSSION

Hammond (1979) stated that biosystematics and evolutionary studies have for long time, and for the most part, considered the morphological features of the mature organism. This observation is evident in earlier works on *Solanum* taxonomy such as that of Isshiki et al. (2008), Karihaloo and Rai (1995), Karihaloo and Gottlieb (1995) and Oyelana and Ugborogho (2008). Unfortunately, these and many other studies based on morphological features have not totally resolved the difficulties associated with *Solanum* taxonomy. Discontinuous markers such as random fragment length polymorphism (RFLP), RAPD, AFLP and Single Nucleotide Polymorphism (SNP) have been useful in providing a measure of genetic distances to establish both the taxonomy and phylogenetic relationships among *Solanum* taxa (Karihaloo et al., 1995; Rodriguez et al., 1999; Poczai et al., 2008; Polignano et al., 2009).

The dendrogram constructed based on RAPD data obtained from all the four primers used reflected the morphological variation observed on the samples of eggplant and related species during their collections. It is evident from the dendrogram that collections originating from various parts of the study area did not form well-defined distinct clusters. They were interspersed with each other, indicating no association between RAPD pattern and the area of collection of accessions. This however, contrasted with the finding of Ge et al. (2013) who used SSR markers to obtain clusters among Chinese eggplant accessions that resulted in clades corresponding to the geographic divisions.

The present data revealed that *S. dasyphyllum* is more closely related to *S. macrocarpon* than to *S. melongena* as evident in cluster I. This observation is in agreement with the findings of Mace et al. (1999), and Isshiki et al. (2008). These workers used AFLP markers to determine the taxonomic position of *S. dasyphyllum* and *S. macrocarpon* both of series Macrocarpa outside section Melongena which comprises *S. melongena*. According to Mace et al. (1999), this close relationship between *S. macrocarpon* and *S. dasyphyllum* is also supported by earlier findings of Jaeger (1986) who considered *S. macrocarpon* to be a domesticated modification of the wild plants known as *S. dasyphyllum*. Mace et al. (1999) stated further that Jaeger (1986) then assigned the wild form of a subspecies status under *S. macrocarpon*, the earlier name.

The occurrence of most samples of *S. gilo* and two samples (out of three) of *S. melongena* in cluster IV is an indication of close relatedness and possibility of having a common ancestor. Occurrence of *S. incanum* together with *S. melongena* still in cluster IV also indicates relatedness and probably progenitors from a common ancestral lineage. This observation of closeness between *S. incanum* and *S. melongena* supports the earlier finding of Sakata and Lester (1994) that used chloroplast DNA, Karihaloo et al. (1995), Furini and Wunder (2004) and Singh et al. (2006). In fact, Karihaloo et al. (1995) had earlier observed that wild forms of *S. incanum* are regarded as belonging to the same species as *S. melongena*. Singh et al. (2006) also stated that at the species level the cultivable type of *S. melongena* is more closely related to *S. incanum* followed by *S. viarum* whereas *S. surattence* and *S. nigrum* showed a closer association among themselves in comparison with the cultivated *S. melongena. S. scabrum* and *S. nigrum* occur together in both Clusters III and V, an indication of similarity between the two. The implication of this is that they are only distantly related to *S. melongena* and are more closely related to each other.

The level of polymorphism observed in the present study was high going by the coefficient of variation. The correlation coefficient 0.95 for the highest similarity between genotypes and the least 0.50 exhibited a good separation from a conserved region of the genome. This is an indication that eggplant *Solanum* has a wide and diverse genetic base.

These results agreed with those obtained by previous workers on *Solanum* e.g. Furini and Wunder (2004), Singh et al. (2006) and Levin et al. (2006). However, these are not in agreement with some earlier workers; for instance, Karihaloo and Gottlieb, (1995) studied variation among the cultivated and weedy taxa of *S. melongena* by allozymes and RAPD analyses; also Ge et al. (2013) examined the genetic diversity and relationships among eggplant accessions collected from seven areas in China using SSR markers. These authors observed little or moderate amount of genetic polymer-phism among the genotypes studied; even Karihaloo and Gottlieb (1995) suggested the existence of a very small gene pool from

which the cultivated forms of *S. melongena* arose.

However, RAPD has some disadvantages which may affect the reliability of these results. For example, it is non-reproducible; they are dominant thereby making it impossible to distinguish between homozygosity and heterozygosity, and also RAPD results can be difficult to interpret. To overcome these, Ali et al. (2013) for example, analyzed the diversity of Chinese eggplant using inter-simple sequence repeat (ISSR) and RAPD procedures. The results showed that ISSR markers were more effective than RAPD markers for detecting genetic diversity.

Notwithstanding, the overall results of the present study were satisfactory enough in terms of their statistical values and concordance with previously published data. However, the accuracy of the clustering result may be increased by increasing the data and sample numbers of eggplant accessions as well as employing other better markers such as SSR, AFLP, ISSR, etc, in the analysis.

Conclusion

The study provides species database of the vegetable, *Solanum* and related species in Southwestern Nigeria and by extension in the country as a whole with emphasis on variation patterns which is a major contribution to global biodiversity information system. From the study also, it is evident that RAPD and other discontinuous markers can be made use of as a means of genetic distances to establish *Solanum* taxonomy as well as phylogenetic relationships among taxa. Detection of genetic differences and discrimination of genetic relationship between *Solanum* species are for sustainable utilization and conservation of plant genetic resources.

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