

## Molecular Identification of Egyptian Eggplant Cultivars (*Solanum melongena* L.) Using RAPD and RFLP of 18S rRNA Gene Markers

Hesham S. Abdel-Razzak<sup>1\*</sup>, Samia A. AbdAl-Aziz<sup>2</sup>, Elsayed E. Hafez<sup>3</sup>

<sup>1</sup>Vegetable Crops Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

<sup>2</sup>Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Application, Alexandria, Egypt

<sup>3</sup>Arid Lands Research Institute, Plant Molecular Pathology Department, Borg El Arab City, Alexandria, Egypt



### ABSTRACT

Seven Eggplant (*Solanum melongena* L.) genotypes, representing the three common Egyptian local cultivars, were subjected to random amplified polymorphic DNA (RAPD) analysis using ten random primers. The ten primers generated 657 RAPD fragments, of which 77 were polymorphic with 11.72 %. The seven genotypes were discriminated with 3-11 distinct polymorphic bands generated from the primers CHI 15 (3), and RAP, EZ and NAH (11). The genetic similarity was estimated for each primer among the seven genotypes. Results of a cluster analysis showed that the genetic similarity between the seven genotypes ranged from 58.0% to 92.0%. The highest genetic similarity of 92.0% was detected between genotypes LB1 and LB2 (original population "OP" and selected population "SP"; long slender black fruits), followed by 86.0% between genotypes RB1 and RB2 (OP and SP; round or egg shaped black fruits), and 80.0% between genotypes LB2 (SP; long slender black fruits) and RB2 (SP; round or egg shaped black fruits). However, the lowest genetic similarity of 58.0% was detected between both genotypes LW1 and LW2 (OP and SP; long slender white fruits) and genotype PO (SP; purple oblong shape fruits), followed by RB1 (OP; round or egg shaped black fruits) and PO (61.0%). Restriction fragment length polymorphism (RFLP) of 18S rRNA gene was carried out using four different restriction enzymes *viz.* *EcoRI*, *TaqI*, *PstI* and *HindIII*. Only the restriction pattern of *TaqI* enzyme showed distinct polymorphic bands among the seven examined eggplant genotypes and divided them into three different groups. However, the RAPD-PCR succeeded to divide them into two major groups; group one contained only genotype PO (SP; purple oblong shape fruits) and the second group included the other genotypes.

**Keywords:** Eggplant, genetic diversity, long slender white and black fruits, RAPD and RFLP of 18S rRNA PCR techniques.

### INTRODUCTION

Aubergine, brinjal or Eggplant (*Solanum melongena* L.) is widely cultivated as one of the most important vegetable crops in both temperate and tropical areas, especially in Asia, parts of Europe and Africa (Karihaloo and Gottlieb, 1995; Sakata and Lester, 1997). In Egypt, the most widely commercial produced varieties are Balady or local cultivars (black long slender fruits, white long slender fruits and black round or egg shaped fruits), which vary in most of their vegetative characters and fruit quality.

Identification of Eggplant cultivars is mainly based on evaluation of morphological features such as plant height, fruit shape, fruit color, reproductive features, local adaptation etc. and that requires a long period survey of plant growth. Morphology-based identification is difficult because morphological parameters can vary considerably with environmental conditions and the environmental influences on morphological characteristics can cause confusion to growers and breeders (Ling *et al.*, 1997). Some eggplant cultivars are morphologically similar, particularly, during earlier stages of plant growth making their identification more difficult, such as black and white long slender fruits cultivars. Moreover, morphological characters for taxonomic delimitation in *melongena* species have been highly confusing because of large intra- and interspecific diversity, and high degree of

morphological plasticity (Karihaloo and Gottlieb, 1995; Mace *et al.*, 1999; Singh *et al.*, 2006).

Compared with traditional morphological markers, DNA-based markers such as restriction fragment length polymorphism (RFLP, Botstein *et al.*, 1980) and random amplified polymorphic DNA (RAPD, Williams *et al.*, 1990) measure genetic diversity at the DNA level, they can account for the effects of selection and possess many advantages, including their unlimited number, independence of environmental influence, heritable, availability for scoring in the early stages and lack of deleterious effects (Schulz *et al.*, 1994; Yang and Quiros, 1995).

On the other hand, information on genetic relationships among accessions within and between species has several important applications for crop improvement and breeding programs (Thormann *et al.*, 1994; Rodriguez *et al.*, 1999; Singh *et al.*, 2006). In this tendency, RFLP and RAPD markers, provide powerful tools in the assessment of genetic variation both within and between plant populations, in the elucidation of genetic relationships among accessions within a species and among genotypes of existing cultivars as well as for marker-assisted breeding (Kresovich *et al.*, 1992; Wilkie *et al.*, 1993; Thormann *et al.*, 1994; Dijkhuizen *et al.*, 1996; Hossain *et al.*, 2003; Ahmed *et al.*, 2006; Patil *et al.*, 2007).

Genetic variation and evolution within eggplant species have been investigated mainly on morphological

\* Corresponding author: heshamsaleh@hotmail.com

features (Martin and Rhodes, 1979; Deb, 1989), isozymes patterns (Isshiki *et al.*, 1994a, 1994b; Karihaloo and Gottlieb, 1995), chloroplast DNA diversity (Sakata *et al.*, 1991; Sakata and Lester, 1994 and 1997). However, to-date little studies, have been focused directly on nuclear genomic diversity by undertaking DNA-based markers i.e., RAPD, RFLP and AFLP analyses in commercial cultivated cultivars (Karihaloo *et al.*, 1995; Isshiki *et al.*, 1998; Mace *et al.*, 1999; Singh *et al.*, 2006). Moreover, greater DNA polymorphism has been reported in weedy *Solanum insanum* than in advanced cultivars of eggplant *S. melongena* (Karihaloo and Gottlieb, 1995; Singh *et al.*, 2006). Thus the potential of DNA-based markers as a tool to detect polymorphism among closely related commercial eggplant cultivars has not been determined yet. In addition, until now there is still clear lacking information concerning the use of DNA markers for genetic diversity in Egyptian eggplant cultivars. Therefore, the present study aimed to examine the relationships among the most common Egyptian eggplant local cultivars by using RAPD and RFLP of 18S rRNA and to evaluate the relative effectiveness of the two types of DNA markers in revealing variation among Egyptian eggplant cultivars.

## MATERIALS AND METHODS

### Plant material

Seven Eggplant (*Solanum melongena* L.) genotypes; representing the three common Egyptian local cultivars were assessed for RAPD-PCR and RFLP of 18S rRNA markers analyses. These genotypes were obtained from

breeding program that was, previously, conducted on eggplant local cultivars through Master study at Vegetable Crops Department, Faculty of Agriculture, Alexandria University. The used seven genotypes characters are presented in Table (1). Plants were grown and maintained during summer season of 2006 in the Experimental Station Farm (Abies region), Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

### Procedure of extraction DNA

Young leaves of the seven genotypes were collected from five field grown plants and homogenized in liquid nitrogen using the protocol of QTA gene kit.

### RAPD-PCR assay

Ten arbitrary primers were used in RAPD amplification of random DNA sequences of the seven eggplant genotypes as shown in Table (2).

The polymerase chain reaction mixture (25  $\mu$ l) consisted of; 25 pmol dNTPs, 25 pmol of random primer, 5 $\mu$ l 10x Taq DNA polymerase buffer and 20 ng of genomic DNA, 0.6 unit of Taq DNA polymerase (Fanzyme). The reaction volume was completed by sterile H<sub>2</sub>O and then incubated in a DNA Thermal Cycler (Perkin Elmer 9700) according to Ahmed *et al.* (2006). The PCR program included an initial denaturation step 95°C for 5 min followed by 35 cycles with 95°C for 1 min for DNA denaturation, annealing as mentioned with each primer (Table 2), extension at 72°C for 1 min and final extension at 72°C for 10 min, followed by cooling to 4°C. The amplified DNA samples were analyzed by electrophoresis on 2.0 agarose gel in a 0.5X TAE buffer (40 mM Tris-acetate,

**Table (1):** Eggplant genotypes characters used for DNA-based markers analyses.

Genotype	Botanical species	Population	Fruit characters	
			Fruit shape	Fruit color
LW1	<i>Solanum melongena</i> var. <i>serpentinum</i> *	Original (OP)	Long slender	White
LW2	<i>Solanum melongena</i> var. <i>serpentinum</i>	Selected (SP)	Long slender	White
LB1	<i>Solanum melongena</i> var. <i>serpentinum</i>	OP	Long slender	Black
LB2	<i>Solanum melongena</i> var. <i>serpentinum</i>	SP	Long slender	Black
RB1	<i>Solanum melongena</i> var. <i>esculenta</i> *	OP	Round or egg shaped	Black
RB2	<i>Solanum melongena</i> var. <i>esculenta</i>	SP	Round or egg shaped	Black
PO	<i>Solanum melongena</i>	SP	Oblong	Purple

\*Botanical classification according to Martin and Rhodes (1979).

**Table (2):** The sequences of the ten selected arbitrary PCR-RAPD primers and their annealing temperature

Primer name	Sequences 5' - 3'	Annealing temp. (°C)
<b>RAP</b>	GAY TTR GAT TGG GAA TAY CC	45
<b>EZ</b>	AGG AGG TGA TCC AAC CCG C	43
<b>18SF</b>	CTT CCG TCA ATT CCT TTA AG	43
<b>18SR</b>	GCA AGT CTG GTG CCA GCA GCC	42
<b>A2</b>	GAA AAC GGG TGG TGA TCG C	42
<b>NAH</b>	GTT TGC AGC TAT CAC GGC TGG GGC TTC GGC	45
<b>NS1</b>	GTA GTC ATA TGC TTG TCT C	46
<b>NS2</b>	GGC TGC TGG CAC CAG ACT TGC	46
<b>A9B7</b>	AGG AGG TGA TCC AAC CGC	37
<b>CHI 15</b>	GGY GGY TGG AAT GAR GG	38

1mM EDTA). The gel was stained with ethidium bromide (EtBr), visualized and photographed by using a gel documentation system (Alpha Imager 1220, Canada).

#### Scoring and analysis of RAPDs

RAPD banding patterns data were scored as discrete variables, using 1 to indicate presence or 0 to indicate absence of the band in the RAPD profile of the seven eggplant genotypes. The index of similarity between each two genotypes was calculated using the formula: Similarity (Bab) =  $2 Nab / [Na + Nb]$ , where Nab is the number of shared fragments between individuals a and b and Na and Nb are the total number of fragments scored in individuals a and b, respectively (Nei and Li, 1979). Cluster analysis was used to produce dendrogram showing estimates of the genetic distance values and to analyze the genetic relationships among the seven different genotypes of Egyptian eggplant. The dendrogram based on similarities was constructed using the Average Linkage between groups (Lynch, 1990).

#### PCR amplification for the 18S rRNA gene

Genomic DNA of the seven eggplant genotypes was subjected to PCR amplification using the 18S specific primers (NS1 and NS2) according to the procedure of Bruns *et al.* (1992).

The PCR amplification was consisted of 35 cycles as following; 94°C for 1 min, 54°C for 45 sec and 72°C for 1 min. The amplified product was separated on 2.0 % (w/v) agarose gel in 0.5 x TBE buffer and then the gel was stained in 0.5 µg/cm<sup>3</sup> (w/v) ethidium bromide solution and destained in deionized water. Finally the gel was visualized and photographed by using a gel documentation system.

#### Restriction fragment length polymorphism (RFLP) of 18S rRNA gene

PCR amplified product with a fragment 550 bp of 18S rRNA were digested with 3 units of four restriction enzymes, namely *EcoRI*, *TaqI*, *PstI* and *HindIII* in a total volume 30 µl containing 10 µl of the PCR product and 3 µl enzyme buffers, 2 units of the enzyme and the volume was completed by sterile H<sub>2</sub>O. Reaction mixtures were incubated at 37 °C for the three enzymes and 65°C for *TaqI* for 3 hours according to the manufacturer's instructions (Promega, Germany). The enzymes were then inactivated by heating the mixtures at 75°C for 15 min. The reaction products were separated by electrophoresis in 2.5 % (w/v) agarose gel in 0.5 x TBE buffer (Caccamo *et al.*, 2001). The gel was stained in 0.5 µg/ cm<sup>3</sup> (w/v) ethidium bromide solution and destained in deionized water. Finally the gel was visualized and photographed by using a gel documentation system.

## RESULTS

The selected ten 17-30-mer random primers amplified successfully the genomic DNAs of the different eggplant genotypes. The RAPD marker profiles of the seven eggplant genotypes yielded by the ten primers i.e., RAP, EZ, NAH, A2, 18SF, 18SR, NS1, NS2, A9B7 and CHI 15 are presented in Figures (1 and 2). The numbers of amplification products (distinct polymorphic bands) obtained ranged from 3 to 11 bands. The largest numbers of specific polymorphic bands (11) were obtained with primers RAP, EZ and NAH (Fig. 1). The primer CHI 15 produced the minimum number of polymorphic bands (3), followed by primer A9B7 (5) with an average of 7.0 polymorphic bands per primer with a range of 0.10-0.90 kb Figure (2). Of a total of 657 detected bands, 77 (11.72%) were polymorphic.

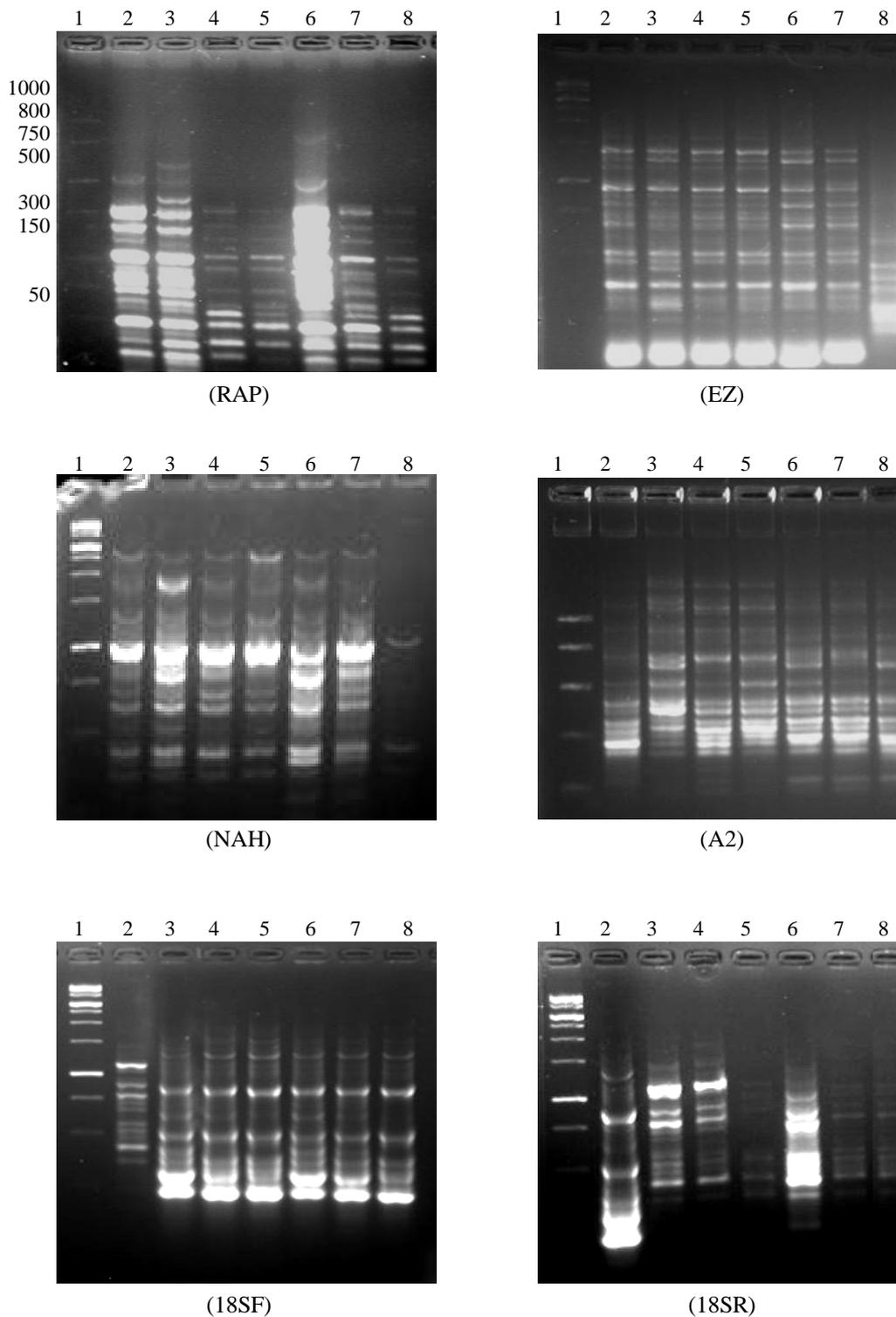
The results obtained by using RFLP amplified PCR products of 18S rRNA digested by four different restriction enzymes i.e., *EcoRI*, *TaqI*, *PstI* and *HindIII* (Fig. 3 a and b) indicated that only the restriction pattern of *Taq I* enzyme showed distinct polymorphic bands among the seven examined eggplant genotypes and divided them into three different groups. Group number one included genotypes numbers 3 (LW2, SP, long slender white fruits), 4, 5 (LB1, OP, long slender black fruits and LB2, SP, long slender black fruits) and 8 (PO, SP, purple oblong shape fruits); while, group number two contained only genotype number 6 (RB1, OP, round or egg shaped black fruits). However, group number three contained the two genotypes; numbers 2 (LW1, OP, long slender white fruits) and 7 (RB2, SP, round or egg shaped black fruits). The restriction bands were formed below 550 bp for all tested eggplant genotypes (Fig. 3b).

RFLP for 18S rRNA gene digested by other three restriction enzymes; *EcoRI*, *PstI* and *HindIII* generated only one monomorphic band and failed to detect genetic variation within eggplant genotypes used in this investigation (Fig. 3a).

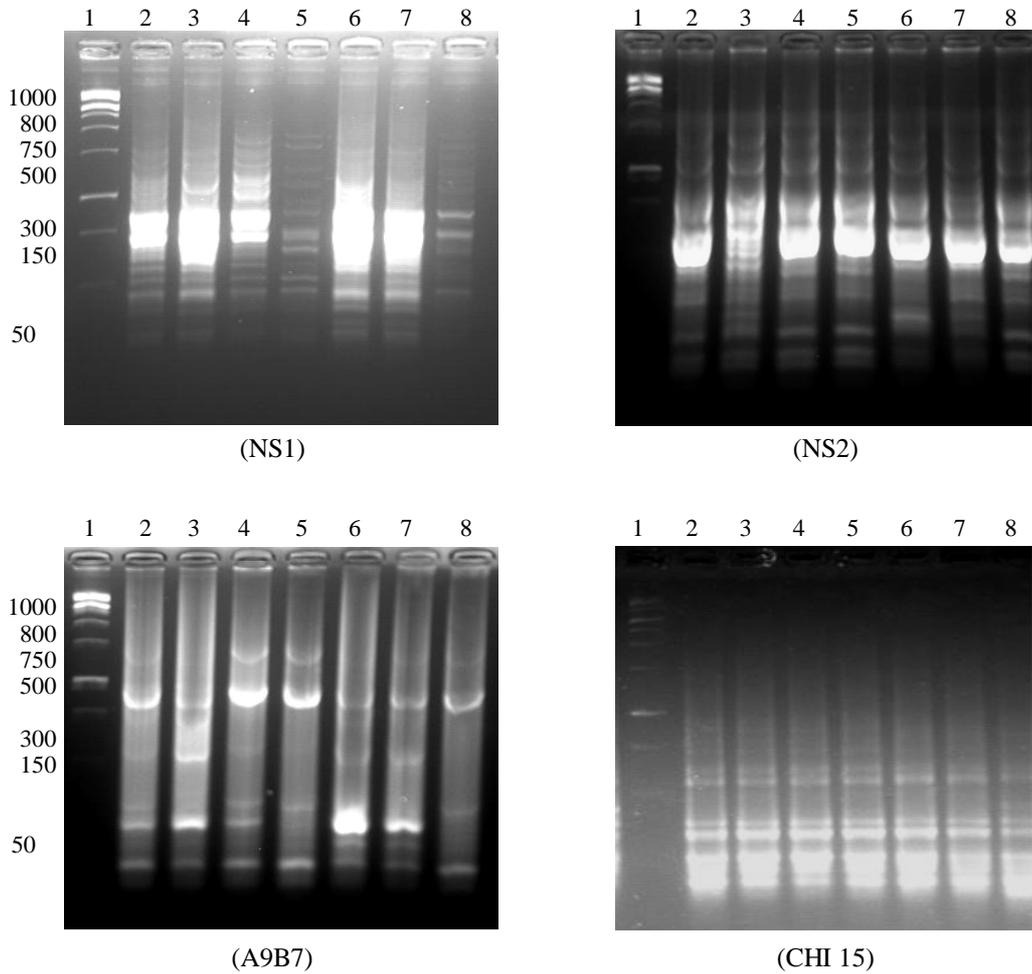
Genetic similarity percentages were estimated among the seven different eggplant genotypes as shown in Table (3).

The results in Table (3) revealed that the genetic similarity among the seven genotypes ranged from 58.0 to 92.0%. The highest percentage (92.0%) was detected between genotypes LB1 (OP; long slender black fruits) and LB2 (SP; long slender black fruits), followed by 86.0% between genotypes RB1 (OP; round or egg shaped black fruits) and RB2 (SP; round or egg shaped black fruits) and 80.0% between genotypes LB2 (SP; long slender black fruits) and RB2 (SP; round or egg shaped black fruits). The lowest genetic similarity of 58.0% was detected between both genotypes LW1 and LW2 (OP and SP; long slender white fruits) and

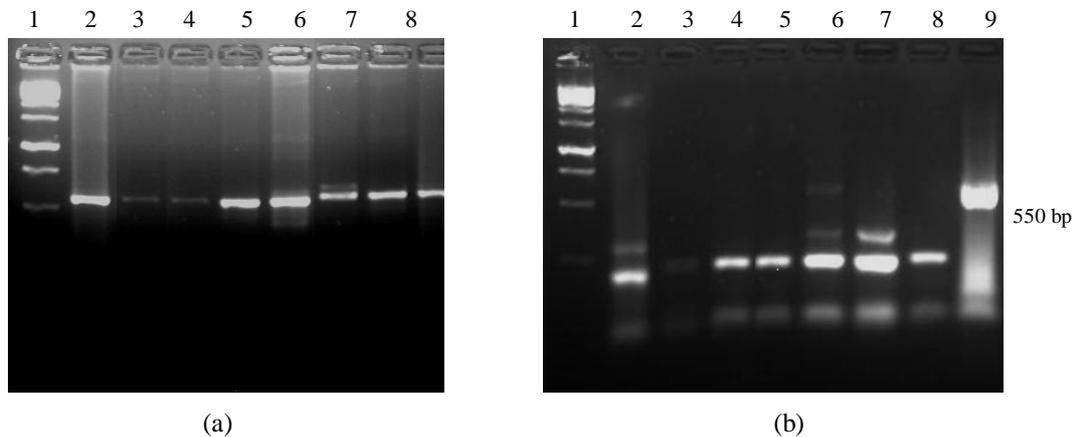
Molecular identification of Egyptian eggplant cultivars



**Figure (1):** RAPD profiles for eggplant genotypes generated by primers RAP, EZ, NAH, A2, 18SF and 18SR. Lane 1: DNA Ladder marker (1.0 kb), Lanes 2 and 3: OP and SP; long slender white fruits, Lanes 4 and 5: OP and SP; long slender black fruits, Lanes 6 and 7: OP and SP; round or egg shaped black fruits and Lane 8: SP; purple oblong shape fruits.



**Figure (2):** RAPD profiles generated from seven eggplant genotypes using NS1, NS2, A9B7 and CHI 15 primers. Lane 1: DNA Ladder marker (1.0 kb), Lanes 2 and 3: OP and SP; long slender white fruits, Lanes 4 and 5: OP and SP; long slender black fruits, Lanes 6 and 7: OP and SP; round or egg shaped black fruits and Lane 8: SP; purple oblong shape fruits.



**Figure (3):** RFLP patterns of 18S rRNA gene digested by two restriction enzymes (a) *EcoRI* and (b) *Taq I*. Lane 1: Ladder DNA marker (1.0 kb), Lanes 2 and 3: OP and SP; long slender white fruits, Lanes 4 and 5: OP and SP; long slender black fruits, Lanes 6 and 7: OP and SP; round or egg shaped black fruits, Lane 8: SP; purple oblong shape fruits and Lane 9: uncut band of 18S PCR amplicone

**Table (3):** Percentages of genetic similarity among the seven different Egyptian eggplant genotypes.

Genotype	LW1	LW2	LB1	LB2	RB1	RB2	PO
LW1	100	73	73	73	72	74	58
LW2		100	73	73	79	78	58
LB1			100	92	74	74	68
LB2				100	76	80	73
RB1					100	86	61
RB2						100	63
PO							100

LW1 and LW2 = OP and SP; long slender white fruits

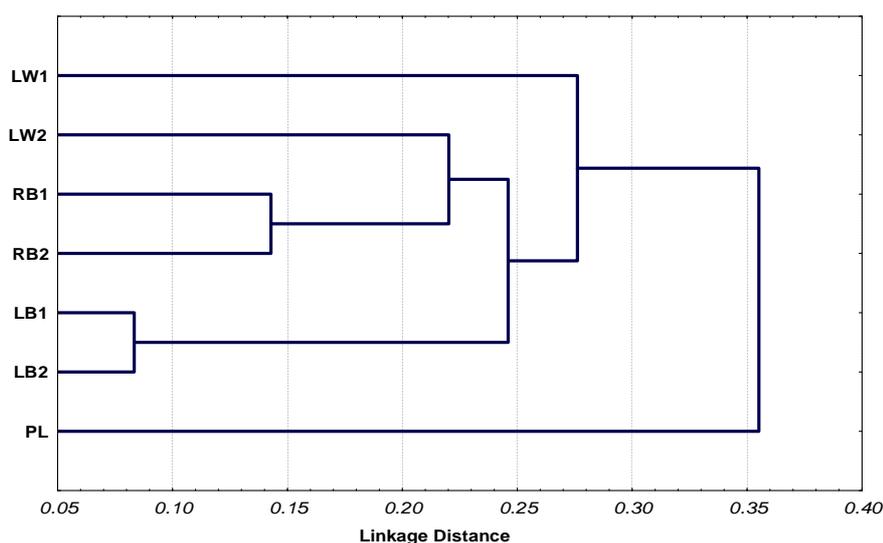
LB1 and LB2 = OP and SP; long slender black fruits

RB1 and RB2 = OP and SP; round or egg shaped black fruits

PO = SP; purple oblong shape fruits

genotype PO (SP; purple oblong shape fruits) followed by 61.0% of genotype RB1 (OP; round or egg shaped black fruits) and genotype PO (SP; purple oblong shape fruits).

A total of 657 amplified bands were used for cluster analysis (Fig. 4). A dendrogram was constructed on the basis of genetic similarity among the different seven eggplant (*Solanum melongena* L.) genotypes using the ten random RAPD primers. The results showed that the RAPD markers succeeded to categorize the seven eggplants genotypes into two major groups; the first one contained only genotype PO (SP; purple oblong shape fruits). However, group two contained two subgroups; the first subgroup contained genotype LW1 (OP; long slender white fruits), and the second subgroup included three sub-sub groups. The first sub-sub group consisted of the two genotypes LB1 and LB2 (OP and SP; long slender black fruits), the second one contained the two genotypes RB1 and RB2 (OP and SP; round or egg shaped black fruits); and, finally the third one contained genotype LW2 (SP; long slender white fruits).



**Figure (4):** Dendrogram using Average Linkage (between groups) of the eggplant genotypes obtained from the ten RAPD primers data. **LW1 and LW2** = OP and SP, long slender white fruits; **LB1 and LB2** = OP and SP, long slender black fruits; **RB1 and RB2** = OP and SP, round or egg shaped black fruits; **PO** = SP, purple oblong shape fruits.

## DISCUSSION

RAPD assay is PCR-based method of genetic typing based on genomic polymorphisms resulted from changes in DNA sequence that inhibit primer binding or interfere with amplification of a particular marker in some individuals; therefore, it can be simply generated as DNA fragments patterns and discriminated between individual genotypes or plant species (Williams *et al.*, 1990; Hossain *et al.* 2003; Semagn *et al.* 2006; and Patil *et al.*, 2007).

The low level of RAPD markers polymorphism in intraspecific Egyptian eggplant genotypes detected in the current study was similar to that existing among *S. melongena* cultivars in a previous study (Karihaloo *et al.*, 1995), indicating a narrow and small gene pool existent in the seven genotypes under consideration. This might be regarded that most of the Egyptian cultivars have been bred from local materials through simple selection. Also, all eggplant genotypes analyzed were collected from the same region (Abies region, Alexandria). However, a high degree of variation was reported for *S. melongena* species with weedy relatives of the cultivated eggplant (Mace *et al.*, 1999; and Singh *et al.*, 2006) as a result of a wide and diverse genetic base of used cultivars.

High level of monomorphic RAPD fragments similar to that existing among *S. melongena* genotypes were reported in other species of Solanaceae family, such as in tomato (Williams and St. Clair, 1993) and in pepper (Paran *et al.*, 1998; and Abdel-Razzak *et al.*, 2005). RAPD studies in a wide range of important vegetable species have showed that considerable variation found in the frequency of polymorphism was different from one species to another; for example, self-pollinating

Solanaceous species seemed to be less polymorphic, as mentioned before, compared with an outbreeder *Brassica* or *Allium* species which reflected high polymorphic patterns (Kresovich, *et al.*, 1992; Wilkie *et al.*, 1993; and Thormann *et al.*, 1994).

The results obtained by using RFLP amplified PCR products of 18S rRNA digested by restriction enzymes i.e., *EcoRI*, *TaqI*, *PstI* and *HindIII* indicated that the restriction pattern of *Taq I* enzyme showed distinct polymorphic bands among the seven examined eggplant genotypes and divided them into three different groups. This result is in accordance with Sakata and Lester (1997), who found that RFLP analysis of cpDNA by using restriction enzymes *EcoRI*, *PstI* and *HindIII* generated many fragments and displayed many polymorphisms among closely related eggplant species. However, it is in disagreement with the findings of Young-Kyu *et al.* (1999) and Abdel-Razzak *et al.* (2005), who reported that RFLP for 18S rRNA gene digested by *EcoRI* and *HindIII* did not reveal genetic variation within pepper species. In general, it is likely that RFLP amplified PCR products of 18S rRNA digested by restriction enzyme *Taq I* may be utilized as a form of DNA typing for identification Egyptian eggplant genotypes.

Based on cluster analysis results, the RAPD markers succeeded to categorize the seven eggplants genotypes into five different groups, however, RFLP of 18S rRNA succeeded to classify the seven eggplant genotypes into three groups; and that, the RAPD PCR markers were more precise. The explanation for the observed higher degree of DNA variability with RAPD marker than that of RFLP of 18S rRNA gene was due to that there were no enough detected restriction sites for the four used restriction enzymes. Moreover, to the large number of fragments that could be analyzed with a single RAPD primer and reveal high degree of polymorphism (Williams *et al.*, 1990).

The dendrogram derived from RAPD analysis demonstrated that both LB1 and LB2 (OP and SP; long slender black fruits) genotypes clustered in close proximity indicating that they had more homology at the genetic level. The other two genotypes RB1 and RB2 (OP and SP; round or egg shaped black fruits) were quite distant. On the contrary, LW1 and LW2 genotypes (OP and SP; long slender white fruits) were not clustered together, even though the origin was the same for the two genotypes. This observation implied that the genomic sequences of both genotypes varied at the genetic level.

On the other hand, it is interesting to note that both LW1 and LW2 genotypes (OP and SP; long slender white fruits) are more divergent in their DNA than the genotypes LB1 and LB2 (OP and SP; long slender black fruits), although morphologically they are more similar. This finding can confirm the results of Mace *et al.*

(1999), who emphasized that DNA often changes at a different rate from the evolutionary divergence of morphological characters.

The great degree of intraspecific variation detected in this study via the RAPD assay may well reflect differences in the pollination and breeding systems of selected Egyptian eggplant genotypes, the amount of domestication and crop improvement, the number of genotypes sampled, the number of primers screened, and/or technique optimization as declared by Kresovich *et al.* (1992) in *Brassica* species.

The obtained results supported those of Prince *et al.* (1995), who reported that both RFLP and RAPD were shown to be useful tools in delineating genetic diversity at the cultivar level within the same species. Both genotypes LB1 and LB2 (OP and SP; long slender black fruits) appeared more closely related to each other than the other two genotypes RB1 and RB2 (OP and SP; round or egg shaped black fruits), according to either RFLP or RAPD markers analyses. It is surprising and unexpected to find that the two genotypes LW1 and LW2 (OP and SP; long slender white fruits) are genetically different, although they follow the same variety *serpentinum*.

From the breeding perspective, genetic diversity is an important factor for the plant breeder because genetic variation present in a breeding program ultimately determines the potential for making gains from selection (Rodriguez *et al.*, 1999). Furthermore, it is considered as a major parameter in choosing parents for crosses (Paran *et al.*, 1998). Therefore, the study indicated that the two long slender white fruits genotypes (LW1 and LW2) could be possibly used as parents for crossing in a breeding program to yield a larger amount of variation in the progeny than using the long slender black fruits genotypes (LB1 and LB2) or the round or egg shaped black fruits genotypes (RB1 and RB2).

Although eggplant appears of highly uniform genetic architecture and has a very narrow genetic base (Karihaloo and Gottlieb, 1995), the results of RAPD markers can be used to determine the genetic relationships among different genotypes and to estimate the genetic diversity among Egyptian eggplant local cultivars. Generally, the genetic diversity displayed by RAPD or RFLP DNA-based markers ensured a high levels of differentiation of the plant materials analyzed for identification and construction of genetic linkage maps in a wide variety of auto- and allogamous vegetable crops species than those possible by using classical markers such as morphological or isozymes markers (Kresovich *et al.*, 1992; Wilkie *et al.*, 1993; Williams, and St. Clair. 1993; Thormann *et al.*, 1994; Schulz *et al.*, 1994; Yang and Quiros, 1995; Prince *et al.*, 1995; Karihaloo *et al.*, 1995; Dijkhuizen *et al.*, 1996; Isshiki, *et al.*, 1998; Paran *et al.*, 1998; Saliba-Colombani *et al.*, 2000; and Singh *et al.*, 2006).

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## التعريف الجيني لأصناف الباذنجان المصرية باستخدام التعدد العشوائى لقطع الـDNA المعظمة (RAPD) وتعدد قطع الـDNA المقصورة لجين 18S الرايبوسومى (RFLP of 18S rRNA)

<sup>1</sup> هشام عبد الرزاق، <sup>2</sup> سامية عبد العزيز، <sup>3</sup> السيد حافظ  
<sup>1</sup> قسم الخضر، كلية الزراعة، جامعة الأسكندرية، الأسكندرية، مصر  
<sup>2</sup> قسم الأحماض النووية والهندسة الوراثية وأبحاث البيوتكنولوجى، مدينة مبارك للأبحاث العلمية، الأسكندرية، مصر  
<sup>3</sup> قسم أمراض النبات الجزيئية، معهد بحوث الأراضى القاحلة، مدينة برج العرب، الأسكندرية، مصر

### الملخص العربى

تم تقييم سبعة تراكيب وراثية للباذنجان ممثلة للأصناف المصرية الثلاث المحلية التجارية لتحليل التعدد العشوائى لقطع الـDNA المعظمة (RAPD) باستخدام عشرة بادئات عشوائية. أنتجت العشرة بادئات 657 حزمة لتحليل الـRAPD من تلك الحزم 77 كانت متعددة Polymorphic بنسبة 11.72%. التراكيب الوراثة السبعة تميزت بوجود عدد من 3-11 حزمة متعددة واضحة نتجت من البادئات CHI 15 (3 حزم) و RAP و EZ و NAH (11 حزمة).

تم تقدير التماثل الجينى لكل بادئ بين التراكيب الوراثة السبعة. أظهرت نتائج Cluster analysis أن نسبة التماثل الجينى بين التراكيب الوراثة السبعة تراوحت من 58 إلى 92%. التماثل الجينى الأعلى تحدد بين التراكيب الوراثة LB1 و LB2 (العشيرة الأصلية و العشيرة المنتخبة من الباذنجان الأسود الطويل) متبوعة بـ 86% بين التراكيب الوراثة RB1 و RB2 (العشيرة الأصلية و العشيرة المنتخبة من الباذنجان الأسود الرومى) و 80% بين التراكيب الوراثة LB1 (العشيرة الأصلية من الباذنجان الأسود الطويل) و RB2 (العشيرة المنتخبة من الباذنجان الأسود الرومى). كذلك قدر التماثل الجينى الأدنى بنسبة 58% بين التراكيب LW1 (العشيرة الأصلية من الباذنجان الأبيض الطويل) و PO (العشيرة المنتخبة من الباذنجان البنفسجى المطول) متبوعاً بـ 61% بين التراكيب RB1 (العشيرة الأصلية من الباذنجان الأسود الرومى) و PO (العشيرة المنتخبة من الباذنجان البنفسجى المطول).  
تم إجراء تحليل تعدد قطع الـDNA المقصورة لجين 18S الرايبوسومى RFLP of 18S rRNA باستخدام أربعة انزيمات مختلفة للقطع هي *Hind III*, *Pst I*, *Taq I*, *Eco RI*.

وقد أظهر نموذج انزيم القطع *Taq I* عن وجود حزم متعددة بارزة بين التراكيب الوراثة السبعة موضع الدراسة وقسمهم إلى ثلاثة مجاميع مختلفة. فى حين نجح تحليل الـRAPD المعتمد على تفاعلات البلمرة المتسلسلة (PCR) في تقسيم تلك التراكيب الوراثة إلى مجموعتين رئيسيتين المجموعة الأولى تحتوى على التركيب الوراثة PO (العشيرة المنتخبة من الباذنجان البنفسجى المطول) بينما تضمنت المجموعة الثانية باقى التراكيب الوراثة.