III.2 YIELD, QUALITY, AND MOLECULAR MARKERS OF NEW LENTIL MUTANT LINES

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Abstract

The present study aimed to characterize 17 new lentil mutant lines for their seed yield, days to maturity, physical, chemical, and molecular characteristics in comparison with their mother lentil cultivar 'Giza 9' and two common cultivars 'Sinai 1' and 'Giza 51'. In addition, genetic variation and relatedness of the highest yielding eight lentil lines along with Giza 9, out of the 17 tested lines were investigated by PCR based RAPD technique. The field trials were conducted at Gemmeiza Research Station, ARC, Egypt, during 2011/2012 and 2012/2013 winter seasons, and the laboratory investigations were made in the laboratory of Seed Technology Research Department, ARC during 2013/2014. The average seed yield of the 17 mutant lines were 4.64 ardab fed.$^{-1}$, indicating their superiority over the three check cultivars. Moreover, eight mutant lines produced higher seed yield ranging from 4.85 to 5.99 ardab fed.$^{-1}$, and exceeded their mother cultivar Giza 9 by 29–59.3%. These eight lines also exceeded Giza 9 in their seed phosphorus and potassium content. Sinai 1 cultivar was the earliest in maturity, where it matured at 119 days. Giza 9 was the best in electrical conductivity test, while the hydration coefficients before (HCB) and after cooking (HCA) indicated that the 17 mutant lines have better seed quality than Giza 9. Molecular analysis of nine lentil lines showed that a total of 94 markers were amplified of which 35.1% were polymorphic. Genetic similarity among the genotypes revealed a strong relationship between mutant lines M48 and M49 (similarity index 100%), while a weak relationship was observed between line M40 and M46 (similarity index 87%). Cluster analysis divided the nine lines of lentil into three main clusters. All genotypes except lines M48 and M49 could be discriminated from one another using RAPD-PCR. Five bands were found to be useful as genotype-specific markers. Despite the observed low level of genetic diversity, genetic differences existed among Giza 9 and other eight mutant lines.

Keywords: *Lens culinaris* L., Mutant lentils, similarity index, RAPD technique.

INTRODUCTION

Lentil (*Lens culinaris* subsp. *culinaris* Medikus) is an ancient crop as it was among the earliest of humankind's plant domesticates (einkorn and emmer wheat, pea, flax and lentil). The crop is also associated with the start of the 'agricultural revolution' in the Near East (Cubero *et. al.*, 2010). Lentil has a high nutritional value, as its seed is a

rich source of protein, minerals (K, P, Fe, Zn) and vitamins for human nutrition (Grusak, 2010). Lentil straw is also a valued animal feed (Erskine *et. al.,* 1990).

In Egypt, the cultivated area of lentil has sharply decreased during the last two decades, where its area in 1993 was 19040 fed (8000 ha) and total production 15000 ton with a productivity of 4.93 ardab fed.$^{-1}$ (1.875 t ha^{-1}). A dramatic reduction has been occurred, where the cultivated area in 2013 reduced to 862 fed. only (362 ha), total production of 735 ton and 5.33 ardab fed.$^{-1}$ (2.030 t ha^{-1}) (Anonymous, 2013). lentil production in 2013 covered only 2.2% of the country requirements. The strategy Food Legume Research Section (FLRS) in Egypt is to increase lentil planted area and its productivity. Development of high yielding lentil genotypes with good seed quality is one of the important factors to improve lentil production. Recentiy, 20 high yielding lentil genotypes have been developed through mutation breeding program at FLRS, and these genotypes being evaluated for their yield potential and agronomic characters (Hamdi *et. al.,* 2013). There is a need to evaluate these genotypes for their seed quality characters and determine their molecular markers.

Seed protein content and cooking quality are the most important quality characters in lentil (Abu-Shakra and Tannous, 1981). Under rainfed conditions, a range of seed protein of 18-32.2% was found when 1853 lentil accessions of germplasm collection at ICARDA were tested by Erskine and Witcombe (1984). In smaller tested lentil collection, seed protein content in 34 lentil lines ranged from 22.1 to 26.9% (Hamdi *et. al.,* 1991). In other studies under irrigation conditions, the range of seed protein contents for 24 lentil genotypes was 17.6-24.8% (Hamdi *et. al.,* 2002a) and 22.1-28.1% for 20 lentil genotypes (Hamdi and Elemery, 1996). Several characters such as seed hydration and electrical conductivity are important aspects of seed quality in lentil (Hamdi and Elemery, 1996, and Hamdi *et. al.,* 2002a).

Among the different molecular markers, RAPD is used friendly oligomer which has the potentiality to amplify reproducible DNA fragments among different genotypes. RAPD markers provide a fast, efficient technique for variability assessment that complements methods currently being used in genetic resource management. RAPD markers have proven to be a powerful tool for molecular genetic analysis of lentil cultivars for plant breeding programs to assess genetic diversity for the development of improved cultivars able to withstand biotic and abiotic stresses. RAPD markers have been used for the identification of genetic relationship among lentil genotypes (Eujayl *et. al.,* 1997; Eujayl *et. al.,* 1998; Chowdhury *et. al.,* 2001; Tullu *et. al.,* 2003; Shaaban *et. al.,* 2009, and Megahed and Hassanein, 2010). Sharma *et. al.* (2004) suggested that RAPD markers may be an appropriate technology for the construction of genetic linkage maps between closely related *Lens* accessions.

The present study aimed to characterize 17 new lentil mutant lines for their seed yield, time to maturity, physical and chemical characters in comparison with their mother lentil cultivar 'Giza 9' and other two common cultivars 'Sinai 1' and 'Giza 51'. In addition, genetic variation and relatedness of the highest yielding eight lentil lines with Giza 9, were investigated by PCR based RAPD technique.

MATERIALS AND METHODS

Field work

A two-year field experiment was conducted to identify the yield potential and time to maturity for the new lentil mutant lines. A total of 20 lentil genotypes were used in this investigation. The genotypes included 17 mutant lines derived from the Egyptian wide spread cultivar 'Giza 9' treated with Gamma rays (Hamdi *et. al.,* 2012) in addition to the three cultivars: 'Giza 9', 'Giza 51' and the drought tolerant and very early maturing cultivar 'Sinai 1' (Hamdi *et. al.,* 2002b). The field experiments were conducted at Gemmeiza Agricultural Research Station, ARC, during 2011/2012 and 2012/2013 winter seasons. Dates of planting were 15 November 2011 and 27 November 2012. The randomized complete block experimental design with three replications was used. Each experimental plot consisted of four rows 3-m long and 0.3-m apart (experimental plot area = 3.6 m^2). Fertilizers were used at the rate of 30 kg P_2O_5 and 15 kg N $fed.^{-1}$. All other agronomic practices were applied as recommended. Days to 90% maturity and seed yield ardab $fed.^{-1}$ (ardab = 160 kg; one feddan = 4200 m^2) were recorded in each experimental plot.

Laboratory work

The laboratory works were made to identify electrical conductivity (EC), hydration coefficients before cooking (H.C.B.) and after cooking (H.C.A.) as physical characters; and seed crude storage protein, seed contents of phosphorus and potassium as chemical characters. Fresh seeds of the tested genotypes were obtained from plants of the 20 genotypes grown for evaluation in a field trial at Gemmeiza, in 2012/2013 winter season. The laboratory work for seed protein content, EC, H.C.B. and H.C.A. was made in the laboratory of Seed Technology Research Department, Field Crops Research Institute, ARC in Egypt.

For seed protein, samples of 50 g of air dried finely ground seeds of each genotype were used to estimate the seed crude protein by using the Kjeldahl method and the percentage of seed protein content was calculated by multiplying the total nitrogen by 6.25 according to AOAC (2000). For electrical conductivity (EC), four 100-seed counts from each genotype were weighed, washed and then transferred to 400

ml conical flasks containing 125 ml de-ionized water, and incubated at 25°C for 24 h. The seeds were removed from the solution with a coarse plastic sieves, and then the conductivity of the solution was determined using the electrical conductivity meter (Jenway 4010 model). A blank solution was also prepared as control, and the blank reading was subtracted from all the sample readings. The corrected conductivity readings were divided by corresponding seed dry weight of the samples. The electrical conductivity was expressed as u mhos/g/seed (ISTA, 1993).

To estimate hydration coefficient of seeds before cooking (H.C.B.), three dry seed samples (10g/each, each sample represented a replicate) for each genotype were soaked in tap water for 8h, then H.C.B. was calculated as [(weight of soaked seeds - weight of dry seeds) ÷ (weight of dry seeds) x 100]. The hydration coefficient of seeds after cooking (H.C.A.) was estimated by placing three dry seed samples, 10g/each (each sample represented a replicate) for each genotype in 9 placed glass tube (100 cm^3) containing enough water. The tubes were put in oven for 2h at 100°C. Then H.C.A. was calculated as [(weight of cooked seeds - weight of dry seeds) ÷ (weight of dry seeds) x 100] according to Fahmy *et. al.* (1996) and Hamdi *et. al.* (2002a).

For potassium analysis, seed samples were weighed and heated at 55 °C, then the ashes were dissolved with 100 ml 1 M HCL. Ash was analyzed for potassium content using the method described by AOAC (2000). Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer was used to determine potassium. Phosphorus was determined according to the method of Trough and Mayer (1939).

The statistical analysis of variance for seed yield and maturity characters was made separately for each season, and then combined analysis of variance for both seasons was performed (Gomez and Gomez, 1984). Whereas, the statistical analysis of variance for EC, H.C.B., H.C.A., and seed crude protein, potassium, and phosphorus contents was performed separately for each character (Gomez and Gomez, 1984).

Molecular markers

Genomic DNA was extracted from young, healthy leaf tissue from plants of the eight high yielding mutant lines (M10, M37, M40, M46, M47, M48, M49, M50) and Giza 9 using the DNA extraction kit (Quigen Inc., Cat. no. 69104, USA). DNA quality was tested using 1% agarose gel electrophoresis and its concentration was determined spectrophotometrically. For RAPD- PCR analysis, a total of 30 oligonucleotide primers were assayed, and nine of them which produced easily observable and repeatable fragments were used in PCR amplifications. These primers sequences are shown in Table 1.

Table 1. Name and sequence of primers used in RAPD- PCR analysis.

Primer name	Sequence
0P- A08	5′ GGTCCCTGAC 3′
0P-A11	5′ CAATCGCCGT 3′
0P-B01	5′ CAGCACCCAC 3′
0P-B07	5′ GGAGGGTGTT 3′
0P-B18	5′ CCACAGCAGT 3′
0P-C05	5′ GATGACCGCC 3′
0P-C07	5′ GTCCCGACGA 3′
0P-D09	5′ TTGGCACGGG 3′
0P-D17	5′TTTCCCACGG 3′

The reaction conditions were optimized and mixtures prepared (30 µl total volume) consisting of the following, DNTPs 2.4 µl, MgCl2 3.0 µl, 10 x buffer 3.0 µl, Primer (10 um) 2.0 µl, Taq (5u/µl) 0.2 µl, Template DNA (50 ng / µl)2.0 ul, H2O (dd) 17.4 ul. Amplification was carried out in a PTC- 200 thermal cycler (MJ Research, Watertown, USA) programmed as follows: Denaturation, 94°C for 10 minutes, followed 40 cycles. Each cycle consisted of 1 minute at 94°C, 1 minute at 37°C, 2 minutes at 72°C, followed by a final extension time of 10 minutes at 72°C and 4°C (infinitive). Gel electrophoresis was applied according to Sambrook *et. al.* (1989). RAPD products were separated on 1.2% agarose gels and bands were visualized with ethidium bromide.

To analyze the data, bands were detected on UV–transilluminator and photographed by Gel Documentation 2000; Bio- Rad. Similarity coefficients were calculated according to Dice matrix (Nei and Li, 1979). Construction of the dendrogram tree was performed using the un-weighted pair group method based on arithmetic mean (UPGMA) in the 'SPSS' program version 10.

RESULTS AND DISCUSSION

Seed yield and days to maturity

The data of seed yield fed.$^{-1}$ and days to 90% maturity are presented in Table 2. The overall mean of seed yield fed.$^{-1}$ was 4.28 ardab. Significant difference was observed between both seasons, where the first season performed higher seed yield

(4.61 ardab fed.$^{-1}$) than the second season (3.95 ardab fed.$^{-1}$). This difference may be due to the variation in climatic conditions and interactions. The average seed yield of the 17 mutant lines were 4.64 ardab fed. feddan^{-1}, indicating their superiority over the three check cultivars. Amongst, eight mutant lines (M10, M37, M40, M46, M47, M48, M49, and M50) performed higher seed yield ranged from 4.85 to 5.99 ardab fed.$^{-1}$, exceeding their mother cultivar Giza 9 by 29 – 59.3%.

Table 2. Average seed yield (ard. fed.$^{-1}$) and days to maturity of the tested lentil genotypes evaluated at Gemmeiza Research Station in 2011/12 and 2012/13 winter seasons.

No	Genotype	Seed yield			Maturity		
		Season 1	Season 2	Mean	Season 1	Season 2	Mean
1	M 7	4.29	3.54	3.92	149.0	127.7	138.3
2	M 10	5.50	4.20	4.85	156.7	128.3	142.5
3	M 13	4.45	3.75	4.10	159.7	127.0	143.3
4	M 30	3.58	3.75	3.67	151.0	124.3	137.7
5	M 33	4.03	3.60	3.82	151.7	120.3	136.0
6	M 35	4.38	3.54	3.96	154.0	126.3	140.2
7	M 37	6.04	4.50	5.27	155.3	125.7	140.5
8	M 38	2.97	2.01	2.49	155.7	124.0	139.8
9	M 40	5.48	4.34	4.91	155.0	126.7	140.8
10	M 45	4.27	3.75	4.01	155.0	128.3	141.7
11	M 46	5.13	6.84	5.99	155.0	126.3	140.7
12	M 47	5.70	5.34	5.52	155.0	127.7	141.3
13	M 48	5.45	4.50	4.98	154.7	128.0	141.3
14	M 49	6.68	4.62	5.65	155.0	125.7	140.3
15	M 50	5.08	4.98	5.03	155.0	129.0	142.0
16	M 52	4.59	3.21	3.90	155.0	126.7	140.8
17	M 55	3.69	3.06	3.78	158.3	128.3	143.3
18	Giza 9	3.97	3.54	3.76	154.3	124.7	139.5
19	Sinai 1	2.46	2.79	2.62	119.0	119.0	119.0
20	Giza 51	4.39	3.09	3.74	156.7	127.7	142.2
Mean		4.61	3.95	4.28	153.1	126.1	139.6
L.S.D. 0.05		0.57	0.79	0.34	4.17	2.67	1.73
CV%		7.42	12.12	9.72	1.65	1.28	1.52

The levels of seed yield fed.$^{-1}$ for the promising lines obtained in the present study (4.85-5.99 ard. fed.$^{-1}$) are higher than those reported in lentil by many scientists such as Ezzat *et. al.* (2005) (3.34 ard. fed.$^{-1}$); Hamdi *et. al.* (2004) (2.29 ard. fed.$^{-1}$); Hamdi *et. al.* (2011) (3.18 ard. fed.$^{-1}$) and Raslan (2011) (4.35 ard. fed.$^{-1}$), indicating

their high yield potentiality. These promising eight lines should be exploited in lentil breeding programs in Egypt.

Concerning days to maturity, the data in Table 2 show dramatic seasonal effect on days to maturity. The average days to maturity in the first season was 153.1 but it was shortened to 126.1 days in the second season. Such seasonal effects on lentil maturity were reported previously (Hamdi, 1987; Hamdi *et. al.,* 2002b; Hamdi *et. al.,* 2003a, and Hamdi *et. al.,* 2003b). That is logic since first season was warmer than second season at Gemmeiza. In this regard, Rahman *et. al.* (2010) mentioned that it is well known that in lentil (as many other crops) the rising temperatures coupled with receding soil moisture push the plants into forced maturity; thus the crop duration in hot region is shorter than cooler region. Also, Summerfield *et. al.* (1989) reported that under controlled conditions, progressively warmer post-flowering restricted vegetative growth, accelerated progress towards reproductive maturity and reduce seed yield. High temperatures associated with dry soils (and strong wind) are especially damaging.

The cultivar Sinai 1 was the earliest in maturity, where it matured at 119 days in both seasons. The earliness of Sinai 1 in flowering and maturity was previously reported by several scientists (Hamdi, 1987; Hamdi *et. al.,* 2002b; Hamdi *et. al.,* 2003a; Hamdi *et. al.,* 2003b and Hamdi *et. al.,* 2004). It should be mentioned that four out of the eight promising mutant lines (M37, M40, M46 and M49) matured at 140.3 – 140.8 days with no significant differences with Giza 9 (139.5 days). Significant genotype x season interaction has been observed in the present study for both yield and maturity traits, suggesting that varietal evaluation should be done in more than a single season and/or location.

Physical characteristics

The averages of physical characters for all tested genotypes are presented in Table 3. Regarding electrical conductivity (EC), the data showed clear differences among genotypes. The average values ranged from 19.8 for Giza 9 to 51.9 u mhos/g/seed for Sinai 1. Since 'EC' is measuring electrolytes leached from the seeds soaked in water, so low EC value indicates good seed viability (Powel, 1986).

The average 'EC' values for the 17 mutant lines ranged from 23.6 to 34.4 u mhos/g/seed. For hydration coefficients, the averages of HCB for all genotypes ranged from 89.4 for Sinai 1 to 133.2 for mutant line M10. The high value of HCB means that seeds have potential to imbibe water, and a high capacity to absorb water indicates good seed quality. Accordingly, the HCB values of the 17 mutant lines were higher than the mother cultivar Giza 9 (Table 3). Similarly, the HCA values of the 17 mutant lines were also higher than those of the mother cultivar Giza 9 (except M50, which

was lower than Giza 9 but with no significant difference), indicating their better seed quality than Giza 9. The hydration coefficients before cooking (HCB) and after cooking (HCA) indicating that the 17 mutant lines have better seed quality than Giza 9.

Chemical characteristics

The overall average of seed protein content was 25.93% (Table 4). There were consistent differences among genotypes in protein content, with a range of 20.56 – 31.13. The cultivar Giza 9 had 24.52% seed protein content. The average of seed protein content for the 17 mutant lines was 25.82%, with a range of 20.56 – 29.81%. In contrast 11 mutant lines showed significantly higher seed protein contents than Giza 9, with percentage increases ranged from 3.6 to 21.6%. Five mutant lines (M10, M46, M47, M48, and M50) out of the high yielding eight lines had higher protein content than Giza 9, and hence they combined high seed yield and protein content.

Table 3. Average values of physical characters: electrical conductivity (E.C.), hydration coefficient before cooking (H.C.B.) and after cooking (H.C.A.) for seeds of the tested lentil genotypes.

No.	Genotype	E.C.	H.C.B.	H.C.A.
1	M 7	31.899	129.546	128.084
2	M 10	25.283	133.167	107.979
3	M 13	24.782	126.330	109.602
4	M 30	30.262	127.471	103.572
5	M 33	29.829	127.527	101.582
6	M 35	24.365	122.960	110.453
7	M 37	31.624	126.275	121.709
8	M 38	25.838	126.999	111.321
9	M 40	32.317	127.641	116.582
10	M 45	28.316	128.573	126.326
11	M 46	34.376	128.427	102.508
12	M 47	30.288	127.412	121.393
13	M 48	27.354	129.304	104.167
14	M 49	29.199	125.109	129.959
15	M 50	27.128	131.321	98.608
16	M 52	29.522	131.071	102.352
17	M 55	23.582	127.469	124.083
18	Giza 9	19.771	120.706	99.632
19	Sinai 1	51.864	89.397	95.512
20	Giza 51	29.031	125.464	98.135
Mean		29.331	125.608	110.678
L.S.D. 0.05		3.699	2.348	4.393
CV%		7.57	1.13	2.40

Concerning seed phosphorus content, the data in Table 4 show that the phosphorus contents of all genotypes ranged from 3.40 g/1kg seeds for M13 to 8.47 g/1kg seeds for M7, with an overall average of 5.82 g/1kg seeds. The eight mutant genotypes (M10, M37, M40, M46, M47, M48, M49, and M50), which exceeded the seed yield of Giza 9 also had higher average seed phosphorus content of 6.45 g/1kg seeds and surpassed seed phosphorus content of Giza 9 by 13.16%. The range of seed phosphorus content in lentil reported by other authors were 2.94 – 7.25 g/1kg seeds (Andrews *et. al.,* 2001; Urbano *et. al.,* 2007 and Yadav *et. al.,* 2007), which agreed with the range found in the present study.

Table 4. Average values of seed storage protein (N%), phosphorus (P) and potassium (K) contents (g/1 kg seeds) in seeds of the tested lentil genotypes.

No.	Genotype	N%	P	K
1	M 7	20.56	8.47	20.03
2	M 10	28.44	6.00	20.17
3	M 13	29.47	3.40	17.53
4	M 30	26.50	4.83	18.30
5	M 33	26.63	4.50	9.90
6	M 35	25.47	5.80	18.87
7	M 37	23.76	8.00	22.73
8	M 38	24.96	5.50	20.03
9	M 40	24.48	5.10	21.70
10	M 45	25.40	4.20	19.37
11	M 46	26.20	6.80	21.70
12	M 47	27.23	6.73	22.87
13	M 48	26.22	6.20	21.47
14	M 49	22.11	6.80	20.97
15	M 50	29.81	6.00	18.57
16	M 52	24.42	6.90	20.17
17	M 55	27.34	6.10	18.43
18	Giza 9	24.52	5.70	10.40
19	Sinai 1	31.13	5.20	19.90
20	Giza 51	23.95	4.10	17.70
Mean		25.93	5.82	19.04
L.S.D. 0.05		0.56	0.28	0.68
CV%		1.31	2.91	2.16

Regarding seed potassium content, the overall average of potassium content was 19.04 g/1kg seeds (Table 4), with a range of 9.90 g/1 kg seeds for M33 to 22.87 g/1kg seeds for M47. Giza 9 had 10.40 g K/1kg seeds. The eight mutant genotypes (M10, M37, M40, M46, M47, M48, M49, and M50), which exceeded the seed yield and seed phosphorus content of Giza 9 also had higher average seed potassium content of 21.27 g/1kg seeds and surpassed seed potassium content of Giza 9 by 105%. The range of seed potassium content in lentil was reported to be 2.4 – 23.7 g/1kg seeds (Andrews *et. al.,* 2001; Urbano *et. al.,* 2007 and Yadav *et. al.,* 2007), which agreed with the range found in the present study.

The results of chemical characteristics indicated that there were considerable protein, phosphorus and potassium contents in lentil seeds of the tested genotypes. It is well known that lentil seeds contain several minerals that are required by humans (12 of the 17 human essential elements), although not all are necessarily required by lentil itself (Grusak, 2010). Potassium and phosphorus constitute the bulk of the mineral profile, and lentil is a very good source for phosphorus and copper and providing about half of the USA recommendation (Grusak and DellaPenna, 1999).

Molecular marker

Thirty primers were initially screened for their ability to amplify polymorphic DNA. Out of them nine primers, OPA08, OPA11, OPB01, OPB07, OPB18, OPC05, OPC07, OPD9 and OPD17 (Table 1) showed reproducible and distinct polymorphic amplified products as shown in Fig. 1. The polymorphism in Table 5 show that, a total of 94 bands were scored, 33 of which (35.1%) were polymorphic and 61 (64.9%) were monomorphic. The amplification products ranged from 140 to 2026 bp. Primer OPC07 scored the highest number of bands; whereas the lowest number of bands was produced by primer OPB01. In addition, the maximum number of polymorphic bands (80%) observed in primer OPD17, while, primer OPA08 and OPB18 generated the least (18.2%) polymorphic bands.

Despite the observed results indicated low level of genetic diversity, genetic differences among Giza 9 and other eight mutant lines existed. Similarly, Rana *et. al.* (2007) also found low level of genetic diversity in the studied lentil material using RAPD markers. Also, Sharma *et. al.* (2004) found that the level of variation detected within cultivated lentils suggests that RAPD markers may be an appropriate technology for the construction of genetic linkage maps between closely related lentil accessions.

The highest number of cultivar specific RAPD markers was scored for genotype M40, which revealed two markers at 1427, 747 bp in primer OP-A11; while, the lowest number of RAPD–PCR markers was scored for genotypes M37, M46 and M49 (1 markers) at 753, 844 and 277 bp in primers OP-B07, OPD17 and OPC07, respectively.

Table 5. Levels of polymorphism based on RAPD analysis.

Primer	TB	PB	MB	P%	Unique bands	
					Cultivar	*MS*
OP-A08	11	2	9	18.2	-	-
OP-A11	11	4	7	36.4	M40 (2)	1427, 747
OP-B01	5	1	4	20.0	-	-
OP-B07	11	4	7	36.4	M37 (1)	753
OP-B18	11	2	9	18.2	-	-
OP-C05	11	6	5	54.5	-	-
OP-C07	14	4	10	28.6	M49 (6)	277
OP-D09	10	2	8	20	-	-
OP-D17	10	8	2	80	M46 (3)	844
Total	94	33	61	35.1		

T.B: Total bands, **PB**: Polymorphic bands, **MB**: Monomorphic bands, **P%**: Polymorphism%, and **MS**: Molecular size.

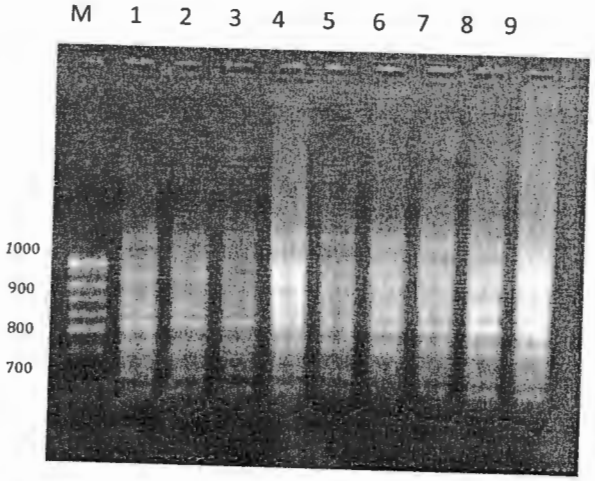
Genetic distance among genotypes

The dendrogram (Fig. 2) based on genetic distance indicates classification of the nine lines of lentil into three main clusters; M40 (no. 2), M47 (4), M48 (5), M49 (6), M50 (7), M10 (8) and Giza 9 (9) were grouped in cluster 1, while cluster 2 included line M37 (3) and cluster 3 included line M46 (6). The line M48 was closest to the line M49 with 100% similarity (Table 6) and the highest genetic distance was found between M40 and M46 with 87% similarity. These results were in agreement with Hoque and Hasan1(2012) who found that RAPD markers have proven to be a powerful tool for molecular genetic analysis of lentil cultivars for plant breeding programs to assess genetic diversity for the development of improved cultivars.

Table 6. Similarity matrix among the nine lentil genotypes based on RAPD analysis.

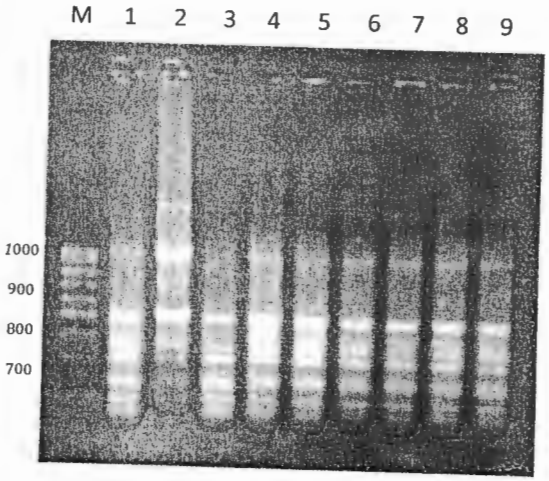
Line	M37	M40	M46	M47	M48	M49	M50	M10
M37	-							
M40	0.89							
M46	0.88	0.87						
M47	0.89	0.95	0.89					
M48	0.89	0.96	0.88	0.97				
M49	0.89	0.96	0.88	0.97	1.00			
M50	0.88	0.97	0.89	0.97	0.99	0.99		
M10	0.89	0.97	0.89	0.98	0.99	0.99	0.99	
Giza 9	0.89	0.95	0.90	0.96	0.96	0.96	0.97	0.97

OP-A08



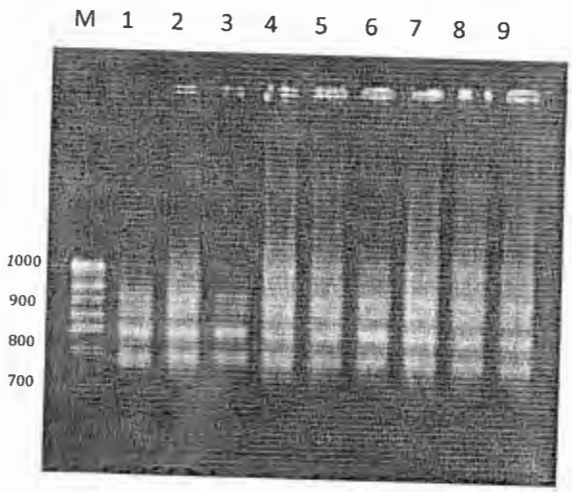
B1

OP-A11



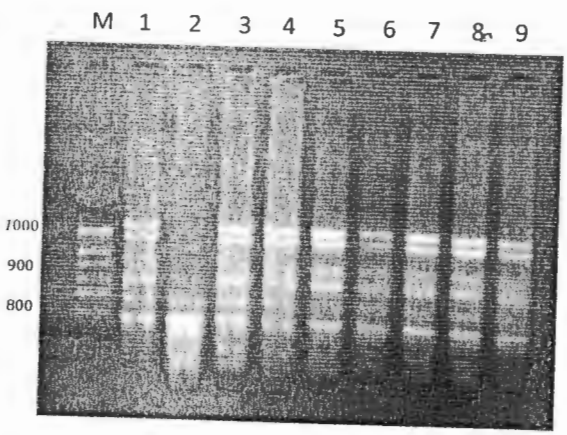
B7

OP-B01

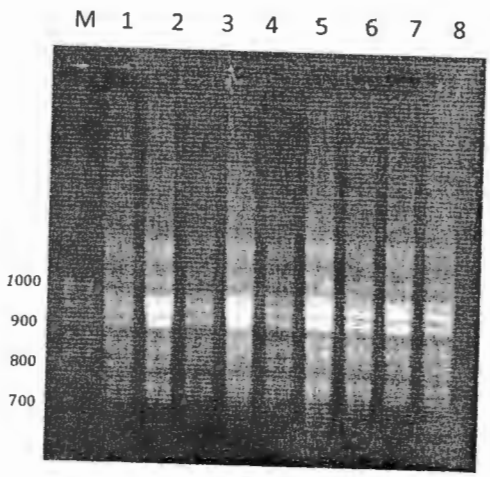


OP-B18

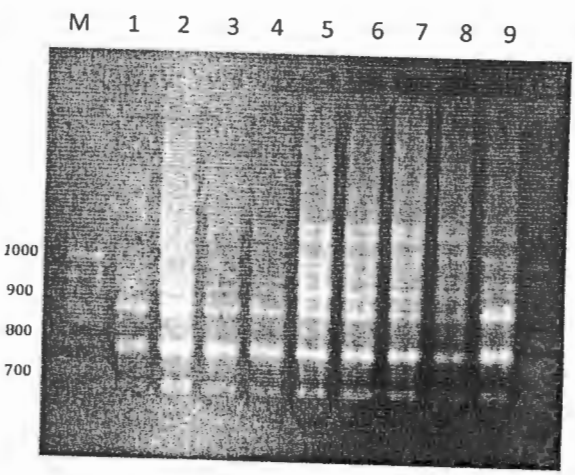
OP-B07



OP-C05



C7



D9

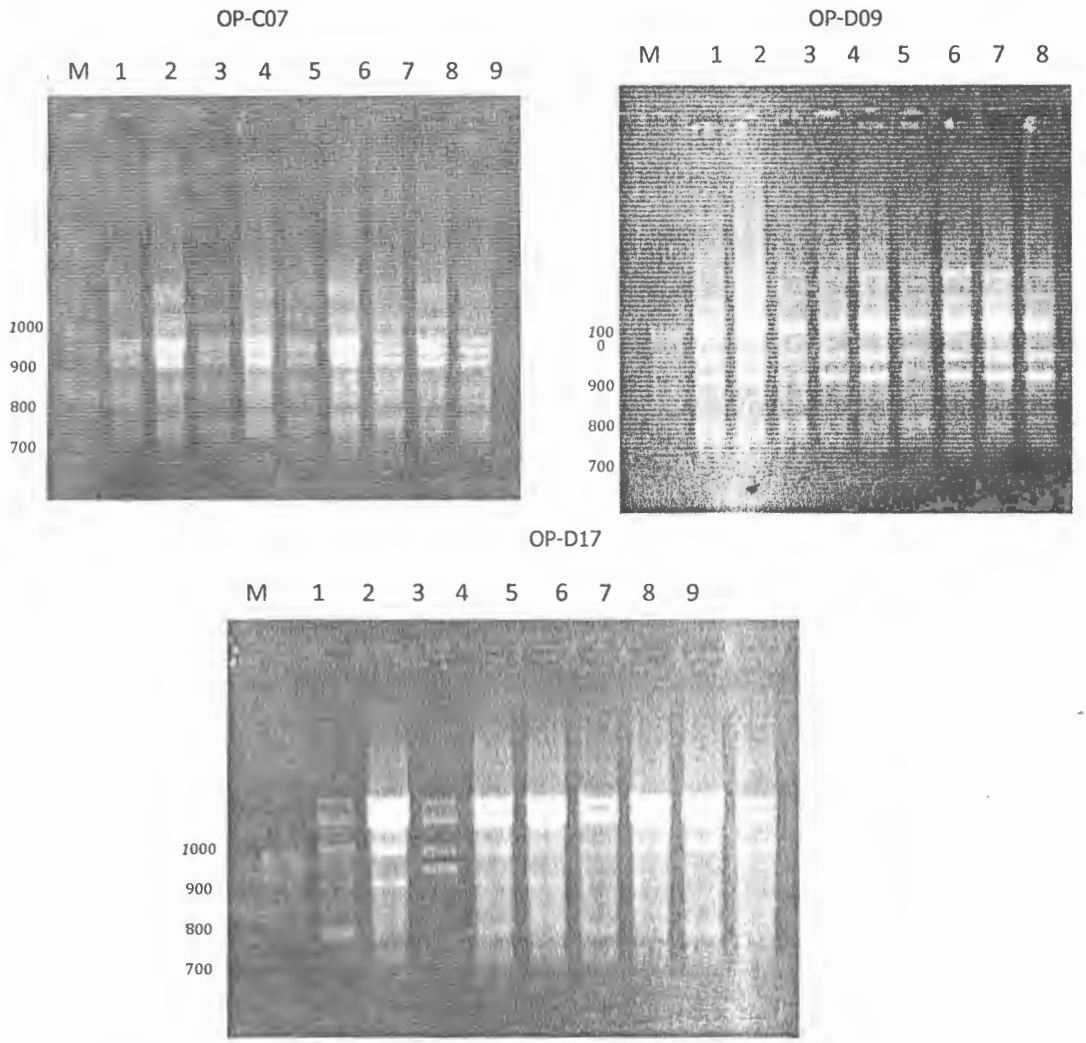


Figure 1. RAPD fingerprinting of the nine lentil genotypes from left to right: M= Marker, (1) M37, (2) M40, (3) M46, (4) M47, (5) I

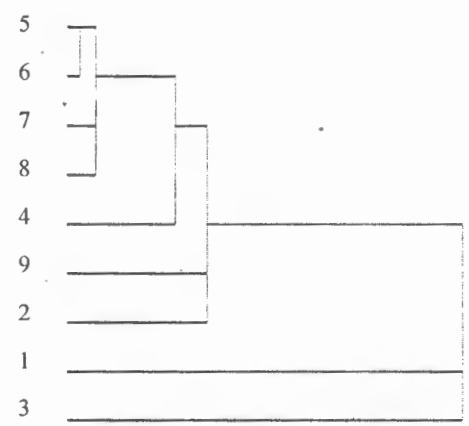


Fig. 2. Dendrogram of the genetic distances among the nine lines of lentil based on RAPD analysis. (1) M37, (2) M40, (3) M46, (4) M47, (5) M48, (6) M49, (7) M50, (8) M10, and (9) Giza 9.

REFERENCES

1. Abu-Shakra, S. and R.I. Tannous. 1981. Nutritional value and quality of lentil. In: Webb, C. and G. Hawtin (eds.), Lentils, pp. 191-202. Commonwealth Agricultural Bureaux.
2. Andrews, M., B.A. Mckenzie, A. Joyce and M.E. Andrews. 2001. The potential of lentil, *Lens culinaris*, as a grain legume crop in the UK: an assessment based on a crop growth model. Annals of Applied Biology, 139:293-300.
3. Anonymous 2013. Statistical report of lentil crop in Egypt. Ministry of Agriculture, Egypt.
4. AOAC. 2000. Official Methods of Analysis of the Associated of Official Analytical Agricultural Chemist, 17th ed., published by AOAC, USA.
5. Chowdhury, M.A., C.P. Andrahennadi, A.E. slinkard and A. Vandenberg. 2001. RAPD and SCAR markers for resistance to ascochyta blight in lentil. Euphytica, 118:331-337.
6. Cubero, M., P.De. Vega and R. Fratini. 2010. Origin, Phylogeny, Domestication and Spread. In: Erskine, W.; F.J. Muehlbauer; A. Sarker and B. Sharma (eds.), The Lentil, Botany, Production and Uses, pp. 13-33. CABI, UK. (ISBN-13: 978-1-84593-487-3).
7. Erskine, W., S. Rihawe and B.S. Capper. 1990. Variation in lentil straw quality. Animal Feed Sci. and Technology, 28:61-69.
8. Erskine, W. and J.R. Witcombe. 1984. Lentil germplasm catalog. International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.
9. Eujayl, I., M. Baum, W. Erskine, E. Pehu and F.J. Muehlbauer. 1997. The use of RAPD markers for lentil genetic mapping and the evaluation of distorted F_2 segregation. Euphytica, 96:405-412.
10. Eujayl, I., M. Baum, W. Powell, W. Erskine and E. Pehu. 1998. A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines. Theoretical and Applied Genetics, 97:83-89.
11. Ezzat, M. Zakia, M. Shaaban and A. Hamdi. 2005. Effect of plant density on the performance of three new released lentil varieties. Egyptian J. Agric. Res. 83 (1):167-176.

12. Fahmy, H.M., S. Srivastava and M.A. Uebersax. 1996. Physical and textural characteristics of soaked and cooked American Common Beans. Egypt. J. Food Sci., 24(2):1-3.

13. Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd edition, John Wiley & Sons, New York.

14. Grusak, M.A. 2010. Nutritional and Health- beneficial Quality. In: Erskine, W.; F.J. Muehlbauer; A. Sarker and B. Sharma (eds.), The Lentil, Botany, Production and Uses, pp. 368-390. CABI, UK. (ISBN-13: 978-1-84593-487-3).

15. Grusak, M.A. and D. DellaPenna. 1999. Improving the nutrient composition of plants to enhance human nutrient and health. Annual Review of plant Physiology and Plant Molecular Biology, 50:133-161.

16. Hamdi, A. 1987. Variation in lentil (*Lens culinaris* Medik.) in response to irrigation. Ph. D. Thesis, Faculty of Science, Botany Dept., Durham Univ., UK.

17. Hamdi. A., Mona M.A. Ali, M. Shaaban and Zakia M. Ezzat. 2012. Agronomic, seed protein and quality characters of the most promising lentil genotypes in Egypt. World Appl. Sci. J., 20(1):70-79.

18. Hamdi, A. and M.I. Elemery. 1996. Evaluation of some seed quality characters in the promising lentil genotypes in Egypt. Annals Agric., Ain Shams Univ., 41(2):725-738.

19. Hamdi, A., A.A. El-Ghareib, S.A. Shafey and M.A.M. Ibrahim. 2003a. Genetic variability and expected genetic advance for earliness and seed yield from selection in lentil. Egypt. J. of Agric. Res., 81(2):125-138.

20. Hamdi, A., R.E.E. El-Lathy, M. Shaaban and Zakia M. Ezzat. 2003b. Performance of some lentil genotypes in the new reclaimed lands at Abu-Simble. Egyptian J. Agric Res., 81(3):1117-1135.

21. Hamdi, A., W. Erskine and P. Gates. 1991. Relationships among economic characters in lentil. Euphytica, 57:109-116.

22. Hamdi. A., Zakia M. Ezzat, M. Shaaban, T.A.A. Selim, M.A. Ibrahim, E.M. Rabeia, M.A.M. Ibrahim, Salwa A.A. Hassanen, E.K. Gendy and H.T. Abd El-Aal. 2013. Improving productivity of the lentil cultivar Giza 9 through mutation breeding. Egypt. J. Appl. Sci., 28(1):29-46.

23. Hamdi, A., Zakia M. Ezzat, M. Shaaban, F.H. Shalaby, M.S. Said, R. El-Lathy, M.S.M. Eisa, M. Abdel-Mohsen, A.M.A. Rizk, K.M.M. Morsy and Saidea S. Abd El-

Rahman. 2002b. A new early-maturing lentil cultivar: Sinai 1. J. Agric. Sci., Mansoura Univ., 27(6):3631-3645.

24. Hamdi, A., M.A. Raslan and M.A. Bakheit. 2011. Performance of some lentil genotypes under two irrigation systems in South Egypt. Egypt. J. of Appl. Sci., 26(1):17-31.

25. Hamdi, A., M. Shaaban and El. El. Abbas. 2004. Yield potential of some lentil genotypes under salinity conditions in North Egypt. Egypt. J. Agric. Res., 82(2):685-696.

26. Hamdi, A., Somaya M. Morsy and E.M. El-Ghareib. 2002a. Genetic and environmental variation in seed yield and its components, protein and cooking quality of lentil. Egypt. J. Agric. Res., 80(2):737-752.

27. Hoque M.E. and M.M. Hasan[1]. 2012. Molecular Diversity Analysis of Lentil (*Lens culinaris* Medik.) through RAPD Markers. Plant Tissue Cult. & Biotech, 22(1):51-58.

28. ISTA 1993. International rules for seed testing. Seed Sci. and Technology, 2 supplement:25-254.

29. Megahed, M.A.H. and Aziza M. Hassanein 2010. Molecular and bio-chemical characterization of some lentil genotypes. Egypt. J. Agric. Res., 88(1):229-242.

30. Nei, M. and W.H. Li. 1979. Mathematical model of studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

31. Powel, A.A. 1986. Cell membranes and seed leashing conductivity in relation to the quality of seed for sowing. J. of Seed Technology, 10:81-100.

32. Rahman, M. M., A. Sarkar, S. Kumar, A. Ali, N.K. Yadav and M.L. Rahman. 2010. Breeding for short season environments. In: Erskine, W.; F.J. Muehlbauer; A. Sarker and B. Sharma (eds.) The Lentil: Botany, Production and Uses. pp. 121-136. CABI, UK. (ISBN-13: 978-1-84593-487-3).

33. Rana, M. K., K. Ratna, S. Sonika and K.V. Bhat. 2007. Genetic analysis of Indian lentil (*Lens culinaris* Medikus) cultivars and landraces using RAPD and STMS markers. Journal of Plant Biochemistry and Biotechnology. Vol. 16, Issue 1.

34. Raslan, M.A. 2011. Evaluation of lentil (*Lens culinaris* Medikus) genotypes under Upper Egypt condition. Egypt. J. Appl. Sci., 26(12):790-804.

35. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular Cloning. A Laboratory manual, second edition, Vol.1. Cold Spring Harbor, New York.

36. Shaaban, M., E.K. Gendy and A. Hamdi. 2009. Morphological and molecular identification for ten lentil genotypes. Egypt. J. of Plant Breeding, 13:319-331.

37. Sharma S.K., I.K. Dawson and R. Waugh. 2004. Relationships among cultivated and wild lentils revealed by RAPD analysis. TAG Theoretical and Applied Genetics, 91(4):647-654.

38. Summerfield, R.J., F.J. Muehlbauer and R.W. Short. 1989. Controlled environments as an adjunct to field research on lentil (*Lens culinaris*). V. Cultivar responses to above- and below-average temperatures during the reproductive period. Experimental Agricultural, 25:327-341.

39. Trough, E. and A.H. Mayer. 1939. Improvement in the denies calorimetric method for phosphorus and arsenic. Indian Eng. Chem. Annual Ed., 1:136-139.

40. Tullu, A., L. Buchwaldt, T. Warkentin, B. Taran and A. Vandenberg. 2003. Genetics of resistance to anthracnose and identification of AELP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theoretical and Applied Genetics, 106:428-434.

41. Urbano, G., J.M. Porres, J. Frias and V.V. Concepeio. 2007. Nutritional value. In: Yadav, S.S.; D.L. McNeil and P.C. Stevenson (eds.), Lentil: an Ancient Crop for Modern Times. Springer, Heidelberg, pp. 47-93.

42. Yadav, S.S., P.C. Stevenson, A.H. Rizvi, M. Manohar, S. Gailing and G. Matelyan. 2007. Uses and Consumption. In: Yadav, S.S.; D.L. McNeil and P.C. Stevenson (eds.), Lentil: an Ancient Crop for Modern Times. Springer, Heidelberg, pp. 33-46.

٣-٢ المحصول وصفات الجودة والمعلمات الجزيئية لسلالات جديدة مطفرة من العدس

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الملخص العربى

تهدف هذه الدراسة إلى تقييم ١٧ سلالة عدس جديدة مستنبطة من طفرات لصفات محصول البذور، ميعاد النضج، والصفات الطبيعية والكيميائية مقارنة مع الصنف الأم جيزة٩، وصنفين آخرين شائعين وهما سيناء ١ و جيزة ٥١. وبالإضافة لهذه الصفات فقد تم أيضاً دراسة التباين الوراثى والتعرف على مدى التقارب الوراثي بين ثماني سلالات عالية المحصول من بين تلك السلالات السبعة عشر والصنف جيزه ٩ باستخدام تقنية الـ RAPD-PCR (التكبير العشوائى لجزيئات الحأمض النووى DNA). وقد أقيمت التجارب الحقلية للتقييم فى محطة بحوث الجميزة بوسط الدلتا والتابعة لمركز البحوث الزراعية، وذلك فى موسمي شتاء ٢٠١٢/٢٠١١ و ٢٠١٣/٢٠١٢. أما التجارب المعملية فقد تمت فى المعامل التابعة لقسم بحوث تكنولوجيا البذور بالجيزة والذى يتبع أيضاً معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية. وقد أظهرت النتائج أن متوسط محصول البذور للسلالات المستنبطة بواسطة الطفرات بلغ ٤,٦٤ أردب/فدان محققة بذلك تفوقاً على محصول البذور لأصناف المقارنة الثلاثة. وقد تفوقت ثماني سلالات من ضمن السلالات السبعة عشر على صنف الأم جيزة ٩ بنسب تراوحت من ٢٩ إلى ٥٩,٣%، كما تفوقت أيضاً على جيزة ٩ فى محتوى بذورها من الفوسفور والبوتاسيوم. وكان صنف المقارنة سيناء ١ هو أبكر الأصناف نضجاً، حيث تم نضجه عند ١١٩ يوماً من الزراعة، بينما كان الصنف جيزة ٩ هو الأفضل فى إختبار التوصيل الكهربائي. وقد أظهرت تقديرات مُعامل امتصاص البذور للماء قبل وبعد عملية الطهى تفوق السلالات السبعة عشر على جيزة ٩. ومن ناحية أخرى، أشارت نتائج التحليل الجزيئى للسلالات الثمانى المتفوقة فى المحصول مع جيزة ٩ إلى وجود ٩٤ معلمة جزيئية (marker) حيث كان ٣٥,١% منهم فى صورة متعددة الأشكال (polymorphic). وقد أوضح تحليل التشابه الوراثى بين سلالات العدس المطفرة وجيزة ٩ وجود تشابه وراثي بدرجة ١٠٠% بين السلالتين M49،M48، بينما كان التشابه الوراثي بين السلالتين M40, M46 أقل وبلغ ٨٧%. وقد أظهر التحليل العنقودى (cluster analysis) سلالات العدس الثمانية وجيزة ٩ وجود ثلاث مجموعات رئيسية. وأكدت هذه الدراسة إمكانية التمييز وراثياً بين هذه السلالات باستخدام تقنية الـ RAPD-PCR (ما عدا السلالتين M48, M49). وقد وجد أيضاً خمس حزم (bands) يمكن إستخدامها كمعلمات وراثية واضحة للتفرقة بين السلالات. وبالرغم من أن النتائج أشارت إلى انخفاض التباين الوراثي بين السلالات المستنبطة بالطفرات وجيزة ٩، إلا أن الاختلافات الوراثية بين جيزة ٩ من جهة والسلالات الثمانية المطفرة من جهة أخرى كانت موجودة وواضحة.