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Evaluation of a New Thornless Strain of Mexican Lime (*Citrus aurantifolia*, Swingle) Under Kaferelsheikh Governorate Conditions, Egypt

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ABSTRACT

This work has been carried out to evaluate a new thornless strain of lime trees as compared with Mexican lime (local or Balady cultivar) planted at 5x5m and grown in clay soil at a private orchard at Kaferelsheikh Governorate during 2015 and 2016 seasons. The evaluation involved growth, flowering, seeds, yield and fruit parameters. The new strain revealed morphological characteristics distinct from the local cultivars but it had a small size of canopy which may be reflected in increasing the number of planted trees/ feddan. Fruits of the selected strain is distinguished by high percentage of juice acidity (7.2-8.2%), juice weight % (47.8-48.9%) over the local cultivar. Also, it was characterized as thornless trees with less seed number /fruit (2.06-2.23). DNA fingerprint was performed using RAPD technique for the molecular characterization. RAPD analyses exhibited a total of 42 bands with an average of 10.4 bands / primer when six primers were used. The polymorphism percentage (18%) was observed between the new strain and Mexican lime. The new lime strain is considered as a valuable genetic material for Egyptian citriculture industry.

Keywords: lime; citrus; thornless strain; evaluation; PCR

INTRODUCTION

Mexican lime (*Citrus aurantifolia*, Swingle) is one of the most popular fruit among citrus cultivars in Egypt due to its fresh consumption throughout the year, aromatic flavor and high fruit quality. It is a rich source of vitamin C which is used as juice and in salad preparations. Moreover, it has medicinal properties as antimicrobial and oxidiates and used for the inhibition of various diseases (Dhillon and Randhawa, 1993). In Egypt, Mexican lime is known as Egyptian lime, Balady or Banzaheir lime. The cultivated area in Egypt reached 55797.47 feddan (feddan = 4200 m^2) which represented about 10.3% of total area of citrus (541723 feddan) according to Ministry of Agriculture and Land Reclamation statistics (2015).

Limes are commonly propagated through seeds in Egypt. So, there is a variation in one or more of some characters such as growth, yield, and fruit quality, seasonality in flowering, harvesting time and disease resistance among trees. To achieve significant improvements in quality and productivity of limes, selection can play a vital role in this respect (Sapkota, 2006 and Vinu *et al.,* 2013). Several authors studied the different selections of citrus trees in order to increase the number of genotypes involved in breeding programs and release new varieties. It is difficult to distinguish among citrus cultivars by using morphological traits because citrus trees usually do not bear fruits until three to four years after planting. So apart from morphological characterization, it is desirable to develop alternative methods which are rapid, reliable and more or less not influenced by environment (Paithankar, *et al.,* 2018 and Yadlod, *et al.,* 2018). During the past few years, new strategies based on molecular markers have been proposed by numerous investigators to decrease time and power (Laxman ,2009).

Thornless trees could replace the thorny ones; it can help the development of lime industry because it can reduce harvesting time and costs, decreasing fruit damage and facilitate fruit harvesting, fruits transportation, also storability of the produced fruits can be longer because fruit damaging is low, however the propagation is easier than thorny lime and it has a genetically source in breeding programs (Shokrollah and Abdullah, 2012).

Seedless fruits are desirable product for consumers, and have been produced by traditional farming and breeding methods for several times, citrus fruits that have less than 5 seeds are seedless fruits. (Grosser, 1998).

Now, molecular markers application have been increasingly adopted to address the problems in citrus taxonomy compared to morphological data. Moreover, molecular tools provide abundant information, highly efficient and are insensitive to environmental factors Also, molecular markers have provided ideal means for identifying genotypes, estimation of relatedness between different accessions and following inheritance of economically important characters. (Ahmed *et al*., 2017). Various Polymerase Chain Reaction (PCR) - based molecular marker tools for example Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) has emerged as powerful tools for screening biodiversity. These techniques have been usually used to study the genetic diversity, taxonomy, cultivar identification and the construction of genetic linkage maps (Novelli *et al*., 2000 , Sanker and Moore, 2001) in different Citrus spp.

The objective of the present study was to assess a new thornless strain of Mexican lime (Balady lime) trees based on growth and yield performance, fruit characteristics and develop RAPD marker depending on DNA fingerprinting for identification of the selected strain.

MATERIALS AND METHODS

The present study was implemented during 2015 and 2016 seasons on seedlings trees of Mexican lime (*Citrus aurantifolia*, Swingle) as well as the selected new strain. All trees were (3 replications of old cultivar and one tree of the selected strain) 15 years –old, planted at 5x5m and grown in clay soil at a private orchard of Kaferelsheikh Governorate, Egypt. Four branches were tagged in four directions of the local trees and nine branches were also selected and tagged in various directions around the tree of the new strain (these branches were considered replicates) to determine the main characteristics as follows:

Morphological Characterization

Tree characteristics

The measurements of tree height (H) and diameter (D) were recorded and tree canopy volume (CV) was calculated according to the following equation:

$$CV = 0.528 \times H \times D^2.$$

Whereas,

H = tree height, D = tree diameter (Castle, 1983).

Leaf characteristics

Thirty mature leaves (7 months old) per each selection from one year old shoot were sampled in the first week of September and used for measuring leaf length and width then leaf length/width ratio was calculated. Consequently, leaf area (cm^2) was estimated using formula: Leaf area = 2/3 x length x width which reported by Chou (1966). Petiole length and number of leaves per one meter length of shoot were recorded.

Thorn characteristics

Number of thorns per one 10 cm of shoot length (thorn density) and thorn length were recorded.

1. Flower characteristics

A Sample of 150 flowers was taken to assess flower characters i.e. pedicel length (cm), number of stamens per flower, number of petals, sepals per flower and petal length were recorded.

2. Yield and its components

At harvest time (the second week of September) yield of each tree was determined as number of fruits/tree, fruit weight (g) and yield kg/ tree.

3. Fruit quality

Physical fruit quality

At harvest time 50 fruits from each tree under study were chosen to define the following parameters: fruit weight (g), volume (cm^3), length (cm), diameter (cm), and shape index (length /diameter ratio). Rind thickness (mm) and fruit juice % as well as calculated according to the following equation:

$$\text{Juice weight \%} = \frac{\text{Juice weight}}{\text{Fruit weight}} x\,100$$

Chemical fruit quality

Twenty fruit samples were taken at random from old cultivar and new strain to determine chemical fruit quality as follow: Total soluble solids (TSS %) was determined by hand refractometer, total acidity as citric acid and ascorbic acid (vitamin c) were determined according to (A.O.A.C., 1990).

4. Seed characteristics

Seed characters i.e. seed number per fruit, seed width and length, weight of 10 seeds and number of normal and aborted seeds / fruit were recorded. The number of embryos per seed was calculated as follows: 50 seeds were collected and washed with tap water, then disinfected in two steps, initially; seeds were exposed to 70% ethanol for 1 min, washed with distilled water then immersed in 2.5% sodium hypochlorite solution for 30 min. After disinfection seed testa were removed and placed on sterile petri dishes which were kept in dark at 27 ^{0}C. After 72 h the number of embryos was counted.

5. Molecular characterization

Healthy young and fresh leaves samples were collected from the Mexcan lime (Mexican lime) and the new strain, saved in ice box and quickly transferred to laboratory. Plant tissues were ground to a fine powder in the presence of liquid nitrogen thin DNA was extracted from fresh leaves by Cetyltrimethyl Ammonium Bromide (CTAB) according to (Doyle and Doyle, 1990). RAPD was performed using 6 random decamer primers (Table 1). Polymerase Chain Reaction (PCR) was carried out in presence of 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2), 100 µM dNTPs, 5 picomole single random primer, 25 ng template DNA, 0.5 unit of Taq DNA polymerase in a total volume of 25 µl. PCR amplification was performed in automated thermal cycler (MJ-Mini, Bio Rad) programmed as follow, 95C for 4 min followed by 40 cycles of 1 min for denaturation at 94C, 30 sec for annealing at 37C and 1.30 min for polymerization at 72C, followed by a final extension step at 72C for 7 min. The amplification products were resolved by electrophoresis in 1.5% agarose gels in 0.5 X TBE buffer using O'GeneRuler™ 100 bp Plus DNA Ladder, gels were documented on Gel Documentation UVITEC, UK. Each reaction was repeated twice and only reproducible bands were considered for analysis.

Table 1. List of primer names and their nucleotide sequences used in this study.

No.	Primer name	Primer sequence 5⁻ ---------3⁻
1	OPA-8	GAACACTGGG
2	OPA-12	TCGGCGATAG
3	OPA-13	CAGCACCCAC
4	OPA-14	TCTGTGCTGG
5	CA-1	AGGTCACTGA
6	CA-2	AAGGATCAGA

Statistical analysis

The obtained data were subjected to analysis of variance using T test to compare between the means of Mexcan lime and new strain.

RESULTS AND DISCUSSION

1. Morphological Characterization

Tree characters:

Results in Table (2) show that the differences in tree size between the Mexican lime and the new strain were significant in both seasons. The highest values of tree height, diameter of tree canopy and canopy volume obtained with Mexican lime comparing with new strain which recorded the least values in the two successive seasons.

Similar findings for the variation in growth parameters were reported by Singh *et al* (2010), Paithankar *et al.,* (2018) and, Yadlod *et al.,* (2018) who observed similar variation in tree size (tree height and canopy volume) among twenty eight strains of Kagzi lime.

Table 2. Comparison of tree characters between Mexican lime and new strain during 2015 and 2016 seasons .

Characters	Season 2015		
Genotypes	Tree height (m)	Diameter of tree canopy (m)	Canopy volume (m^3)
New strain lime	4.01	3.50	25.74
Mexican lime	4.75	4.85	58.48
Difference	0.02 *	0.005 *	0.65 *
	Season 2016		
New strain lime Mexican lime	4.35 5.07	3.65 5.57	30.42 82.61
Difference	0.0015 *	0.019 *	7.18 *

NS: Not Significant and * : Significant at 0.05 level.

Leaf characters

Table (3) and Fig.1 show the variations in leaf characters between Mexican lime and the new strain. It is clear that the leaves of Mexican lime were significantly larger than the leaves of the new strain. The differences were significant regarding leaf length, leaf width, leaf length/width and petiole length in the two successive seasons, whereas the variation was not statistically significant for leaf area in the two seasons under study. The observed variation in the leaf area was mainly due to the remarkable differences in the leaf length more than those in leaf width. The petiole length of new strain leaves reached an average (1.90 and 1.63 mm) compared to the local cultivar (4.3 and 5.33 mm) in the two successive seasons, respectively. Also, leaves of the new strain were thicker than those of the local cultivar. Similar results were obtained by El-Barkouky and Khalil (1993) who found highly significant variations between the two types of striking lime selections As for leaf characters, Singh *et al*., (2010) found similar differences in six strains of Nagpur lime for leaf lamina length, leaf lamina width, and leaf length: width ratio and thorn length. Variation among kinnow mandarin and some mutants for leaf lamina thickness were observed and thorn density was low in some mutants and their parent Kinnow mandarins but no thorns were found on other genotypes(Kaur, 2016).

Thorn characters

Results in Table (3) and Fig.1 indicate that the differences between Mexican lime and the new strain were significantly as for number of thorns /10cm of shoot and thorn length in the two successive seasons. The new strain had no thorns in 10 cm per shoot compared to the Balady cultivar which had an average 7 thorns per 10 cm in shoot and the length of thorn reached (6.8 and 7.2 mm) in both seasons, respectively. Thornless trees are very imperative because they have fewer problems in speed of the harvesting, transporting, and marketing. The harvested fruits from thorny trees are commonly injured, because the fruits contact to the thorn on the trees so these fruit could not transported for long time. Moreover, some fungi may attack the injured fruits accordingly they will be infected after short time. However, if thornless trees could replace to thorny lime, it can helps the development of lime industry because it can reduce harvesting time and costs, decreasing fruit damage and facilitate fruit harvesting, fruits transportation and storability of thornless lime can be longer because fruit damaging is low. (Shokrollah and Abdullah, 2012).

Table 3. Comparison of leaf and thorn characters between Mexican lime and new strain during 2015 and 2016 seasons.

Characters / Genotypes	Leaf length (cm)	Leaf width (cm)	Leaf L/W Ratio	Leaf area (cm^2)	Leaf Thickness (cm)	Petiole length (mm)	Leaf number/ 1 meter of shoot	Thorn number / 10 cm of shoot	Thorn length (mm)
	Season 2015								
New strain	5.63	4.20	1.34	15.842	0.03	1.90	67.0	0.0	0.0
Mexican lime	7.10	3.33	1.99	15.840	0.03	4.33	52.6	7.0	6.84
Difference	0.014*	0.0144*	0.02*	0.097NS	0.0251NS	0.0005*	8.1111*	-	-
	Season 2016								
New strain	5.63	4.45	1.543	16.78	0.03	1.63	72.6	0.0	0.0
Mexican lime	6.93	3.26	2.130	15.14	0.02	5.33	48.6	10.0	7.2
Difference	0.0300*	0.0233*	0.011*	1.62NS	0.0005*	0.0010*	12.333*	-	-

NS: Not Significant and * : Significant at 0.05 level.

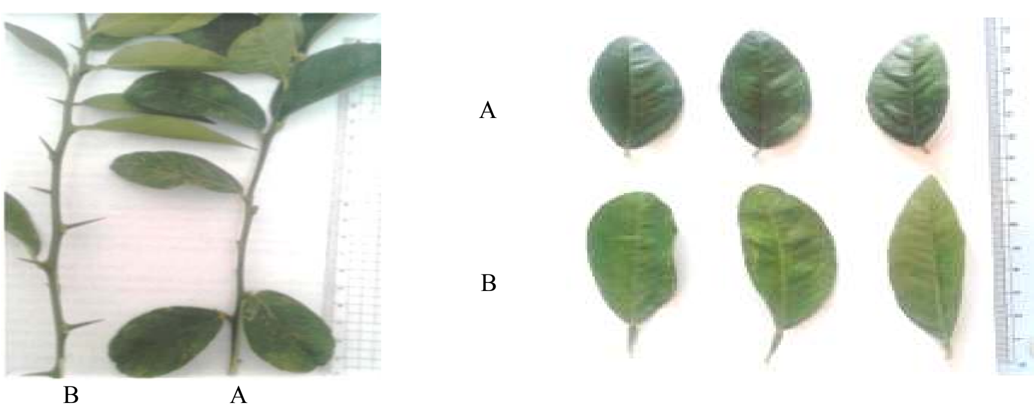


Fig. 1. Variability in leaf characteristics and thorns between Mexican lime and new strain; Where, A. New strain, B. Mexican lime.

2. Flower characters

There were significant differences between the new strain and Mexican lime for petal length, petal width, flower pedicel length and number of stamens for the both seasons under study (Table 4 and Fig.2). The new strain shows the highest petal length which ranged from 0.96 to 1.02 cm, however in local Mexican lime it ranges from 0.74 to 0.68cm. The highest flower pedicel length 0.33 and 0.35 cm observed with the new strain while the lowest length of flower pedicel 0.247 and 0.270 cm obtained with local Mexican lime in both seasons. Also, the highest stamen number obtained by new strain lime which ranges from 22.0 to 21.75 compared with local Mexican lime which ranges from 18.5 to19.5 .The abovementioned results are in agreement with the results obtained by El-Barkouky and Khalil (1993) on lime, Dorji and Yapwattanaphun (2011). and Kinley and Chinawat (2011)on mandarin trees.

Table 4. Comparison of flower characters between Mexican lime and new strain during 2015 and 2016 seasons.

Characters / Genotypes	Petal length (cm)	Petal number	Petal width (cm)	Flower pedicel length(cm)	Stamen number
Season 2015					
New strain lime	0.963	4.5	0.430	0.330	22.000
Mexican lime	0.740	4.48	0.433	0.247	18.500
Difference	0.0004 *	.0005 NS	0.0002 NS	0.0001 *	0.2500 *
Season 2016					
New strain lime	1.017	4.5	0.400	0.357	21.750
Mexican lime	0.683	4.49	0.333	0.270	19.500
Difference	0.0053 *	0.0004 NS	0.0002 *	0.0003 *	0.187 *

NS: Not Significant and * : Significant at 0.05 level.

A B

Fig. 2. Variability in flower characteristics between Mexican lime and new strain; Where, A. New strain, B. Mexican lime.

3. Yield and its components

Results in Table (5) show that there were significant variation in fruit weight in the first season while, they were not statistically significant in the second one. Mexican lime gave a higher fruit weight (35g) compared with the lowest fruit weight (31g) obtained by the new strain. The new strain produced 81.04 - 79.83- kg/tree while Mexican lime yield ranged between 81.93- 80.49 - kg/tree in the two seasons under study, respectively. These results are in accordance with the findings of El-Barkouky and Khalil (1993) on lime, El-Agamy *et al.,* (2000) on orange and mandarin, Khalil *et al., (*2009) on Balady orange and Sayed and Adawy (2009) on Navel orange trees.

Table 5. Yield and its components of Mexican lime comparing with the new strain during 2015 and 2016 seasons.

Characters / Genotypes	Av. fruit weight (g)	Number of fruits/ tree	Yield (kg/tree)
Season 2015			
New strain lime	31	2614.45	81.04
Mexican lime	35	2341.14	81.93
Difference	0.175 *	0.0001 NS	0.0001 NS
Season 2016			
New strain lime	31	2575.42	79.83
Mexican lime	34	2367.51	80.49
Difference	0.670 NS	0.000 NS	0.0001 NS

NS: Not significant and * : Significant at 0.05 level.

4. Fruit quality

Physical fruit quality

Results in Table (6) and Fig.(3) show that differences between the new strain and Mexican lime in most parameters of physical fruit quality were not significant except for fruit length and volume in the second season. The fruit length and volume ranged from 3.74 to 3.76cm and 30.03 to 30.06 cm^3, respectively in the new strain lime while, 3.96 to 4.17cm and 31 to 39.68cm^3, respectively in local Mexican lime. Regarding peel thickness, the highest values was obtained from Mexican lime as compared with the new strain. Similar observations were found by Kahn and Bier (2000) on five strains of navel orange, singh *et al*., (2010) on some Rangpur lime strains and Zandkarimi *et al.,* (2011) on some lime and lemon genotypes.

Table 6. Physical fruit quality of Mexican lime comparing with the new strain during 2015 and 2016 seasons.

Characters / Genotypes	Fruit length (cm)	Fruit diameter (cm)	Fruit shape L/D	Fruit volume (cm^3)	Peel thickness (mm)
Season 2015					
New strain lime	3.747	3.837	1.013	30.030	1.433
Mexican lime	3.963	3.887	1.010	31.000	1.667
Difference	0.0080 NS	0.0020 NS	0.0009 NS	0.3504 NS	0.0006 *
Season 2016					
New strain lime	3.760	3.717	1.087	30.060	1.723
Mexican lime	4.170	4.133	0.990	39.683	1.687
Difference	0.0070 *	0.0871 NS	0.0136 NS	0.1171 *	0.0053 NS

NS: Not Significant and * : Significant at 0.05 level.

A

B

Fig 3.Variability in fruits characteristics between Mexican lime and the new strain; Where, A. New strain, B. Mexican lime.

b- Chemical fruit quality

The differences between the Mexican lime and the new strain were significant as for juice acidity and juice weight percentage in both seasons under study (Table 7). Fruits of the new strain had higher acidity (8.2 and 7.20%) and juice weight % (48.9 and 47.8%) compared to the Mexican lime which recorded 7.2 and 6.45% of acidity and 47.2 and 40.73% of juice weight percentage. While, the differences were not significant as for TSS%, TSS/Acidity and vitamin C during the two seasons under study. These results are in conformity with the earlier findings of Tirthakar *et al.,* (2004) on 48 acid lime genotypes and Srinivas *et al.,* (2006) on seedling strains of Kagzi lime. Also, similar results were found by singh *et al*. (2010) on six strains of Rangpur lime. They found variability on TSS %, Acidity, TSS: acid ratio and rind thickness among the strains. Also, Abhilash *et al.,* (2017) evaluate the quality parameters of the elite strains kagzi lime variety including acid lime in major growing parts of Vijayapura district. They indicated that among the selected trees 'KLS-23'strain had the highest acidity (, vitamin C and total soluble solids.

Table 7. Chemical fruit quality of Mexican lime comparing with the new strain during 2015 and 2016 seasons.

Characters / Genotypes	T.S.S. %	Acidity (%)	T.S.S/acidity ratio	Juice weight %	Vitamin C mg/100 ml juice
Season 2015					
New strain lime	8.2	8.2	1.14	48.900	48.267
Mexican lime	8.0	7.2	1.15	47.277	48.433
Difference	0.0400 NS	0.0375 *	0.0271 NS	0.0039 *	0.4011 NS
Season 2016					
New strain lime	7.827	7.200	1.157	47.800	48.267
Mexican lime	7.793	6.450	1.133	40.730	48.567
Difference	0.0678 NS	0.0108 *	0.0143 NS	0.0148 *	1.7033 NS

NS: Not Significant and * : Significant at 0.05 level.

5. Seed characters

Physical characters

Regarding to the seed characters, results in Table (8) and Fig.4 declare that significant variations were noticed between the new strain and local cultivar in the total number of seeds and the normal seeds while the differences were not significant in most seed characters in both seasons. The new strain had lower seed number / fruit (2.06and 2.2) compared with the highest seed number (10.25and 9.46 seeds/ fruit) recorded with the local cultivar during 2015 and 2016 seasons, respectively. One of the important qualities in lime and other citrus cultivars is seedless. The fruit with less than 1-4 seeds are seedless, 4-8-is little seeds and more than 8 is seedy (Khan, 2007). Differences in seed number / fruit were also observed by Fatima *et al.,* (2010)and singh *et al*., (2010) found significant variation among six strains of Rangpur lime for the seed number / fruit, seed length and seed width.

Number of embryos per seed

Fig (5) illustrated that the seeds of new strain are mono-embryonic and seed has one embryo but the seeds of old cultivar are poly-embryonic (the seed has nucellar embryos beside the zygotic embryo).

Mono-embryonic seeds are very important in citrus breeding programs because seed will be hybrid not true to type Also, most poly-embryonic cultivars produce only a small number of crosses and , it is often difficult to distinguish nucellar and zygotic seedlings at an early stage, however, discrimination of nucellar seedlings at early stage is essential to avoid the 5-10 years of expense that accompany the unwarranted growth and maintenance of nucellar seedlings that are genetically identical to seed parents .Also, nucellar embryony, which occurs commonly in citrus species, creates a serious problem for cross breeding studies as it produces a large number of asexual embryos, greatly limiting the genetic variability obtained by controlled pollination. Moreover, the seedlings from nucellar embryos are preferred by nursery growers for the production of rootstocks. However, the zygotic embryo is the objective in breeding programs for the selection of superior genotypes and variability achievement. (Rao *et al*., 2008; Yildiz *et al.,* 2013; Kashyap *et al.,* 2018).

Table 8. Comparison of seed characters between Mexican lime and new strain during 2015 and 2016 seasons.

Characters / Genotypes	Average number of seeds/fruit	Average number of sound Seeds	Empty seeds	Weight of 10 seeds (g)	Seed length (mm)	Seed width (mm)	Seed l/w
	Season 2015						
New strain	2.064	1.737	0.327	1.433	1.853	0.520	2.047
Mexican lime	10.253	8.250	2.003	1.200	2.137	0.440	1.867
Difference	0.0878 *	0.7890 *	0.0125 *	0.0078 NS	0.1310 NS	0.0061 NS	0.0325 NS
	Season 2016						
New strain	2.233	1.517	0.713	1.400	0.867	0.423	1.543
Mexican lime	9.467	6.200	3.267	1.267	0.890	0.487	2.130
Difference	0.0744 *	0.163 *	0.446 NS	0.0011 NS	0.0001 NS	0.0021 NS	0.011 *

NS: Not significant and *: Significant at 0.05 level.

A
B

A
B


Fig. 4. Cross section of the new strain and the Mexican lime fruit Where, A. New strain and B. Mexican lime.

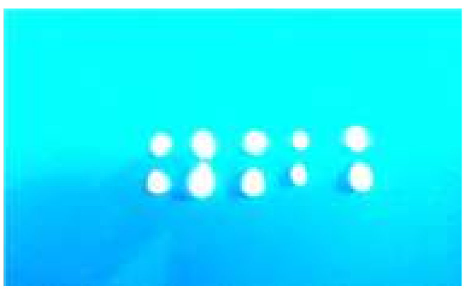
A

B


Fig. 5. Number of embryos per seed of the new strain and the Mexican lime. Where, A. New strain (monoembryonic seed) and B. Mexican lime(polyembryonic seed) .

6. Molecular characterization

Assessment of morphological diversity between Mexican lime and the new strain by cluster analysis

Results in Fig.6 show that the cluster analysis based on morphological variables from tree, leaves, seeds, yield and fruits divided the two cultivars into two main cluster, the first cluster included the new strain where the second one involved the local cultivar of Mexican lime.

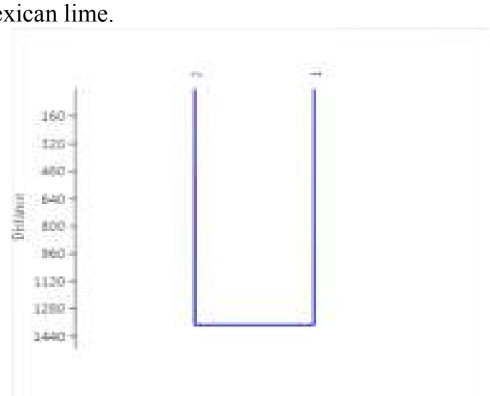


Fig. 6. UPGMA dendrogram based on morphological variables of trees, leaves, seeds, yield and fruit quality. Where, 1. New strain „2. Mexican lime

Molecular analysis:

Fingerprint Detected by Randomly Amplified Polymorphic DNA (RAPD)

In the present study six primers were screened with the DNA of Mexican lime and the new strain only four primers were found. These primers generated reproducible and easily scorable RAPD profiles (Fig.7) .The total number of Bands results from the six primers was 42 with an average of 10.4 bands/ primer. In this line, Munankarmi *et al.* (2014) reported that in acid lime (*Citrus aurantifolia,* Swingle) the average number of bands were 8.8. The amplified bands ranged from 9 to 12 (Table 9). Polymorphic bands ranges from 0 to 3. Primer OPA12 and OPA13 produced the highest number of bands (12). While the lowest number of bands was produced by OPA-8, OPA-14 (9). The highest number of polymorphic bands (3) produced by OPA-8. Also, the highest (33%) percentage of polymorphism produced by OPA-8. The average number of polymorphic fragments was 11.10. The number of DNA fragments amplified ranged from 7 to 11 which is quite consistent with Number reported by other authors such as 3 to 15 (Nicolosi *et al.,* 2000) and 5 to 14 (Baig *et al.,* 2009).

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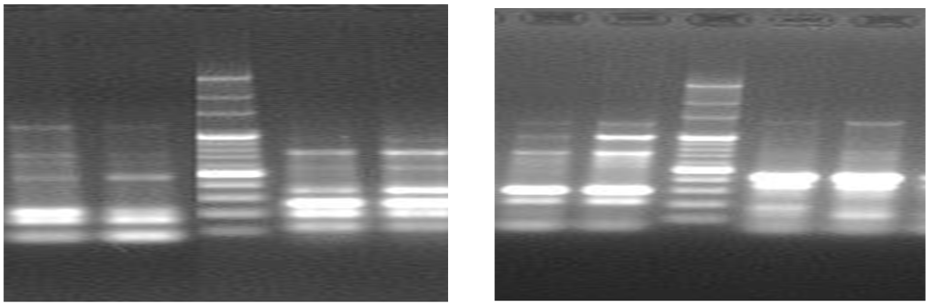

OPA-8 OPA-12 OPA-13 OPA-14

Fig. 7. The electrophotograh of DNA amplified fragments using OPA-8, OPA-12, OPA-13 and OPA-14 for selected cultivars .M, 50 bp DNA ladder ; where ;1 Balady cultivar , 2 New strain.

Table 9. Total number of bands, monomrphic bands, polymorphic bands and percentage of polymorphism revealed by RAPD markers

primer	sequences	Total number of Bands	Monomorphic Bands	Polymorphic Bands	Percentage polymorphism
OPA-8	GAACACTGGG	9	6	3	33.3
OPA-12	TCGGCGATAG	12	12	-	0.00
OPA-13	CAGCACCCAC	12	12	-	0.00
OPA-14	TCTGTGCTGG	9	8	1	11.11
Total		42	38	4	44.41
Mean		10.5	9.5	1	11.10

Genetic similarity and the phylogenic tree

The data representing the similarity index are shown in Table (10). The data showed the existence of considerable amount of molecular diversity between the tested genotypes. The similarity percentage (82%) was observed between Mexican lime and the new strain.

Table 10. Genetic similarity index between the new strain and the Mexican lime cultivar.

Genotypes	Mexican lime	New strain
Mexican lime	100	0.82
New strain	0.82	100

Based on Nei and lei (1979) coefficient of similarity, cluster analysis was performed and a dendrogram illustrating the phylogenic relationships among the tested genotypes were obtained (Fig. 8). The phylogenic tree explaining the relationships cleared two main clusters. The first main cluster contained Mexican lime cultivar, while the second main cluster contained the new strain. RAPD fingerprinting has a number of potential application including the determination of cultivars purity, efficient use and management of genetic resources collection, particularly in identification of mislabeled accessions (Ahmed, 1999).

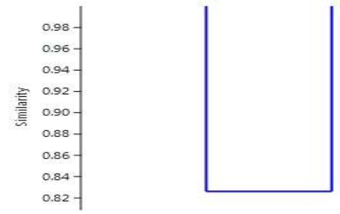

Fig. 8. UPGMA phenogram derived from similarity matrix of Jaccard's coefficient, demonstrating the genetic relationships between Mexican lime and the new strain. Where: 1; Mexican lime; 2; new strain.

CONCLUSION

This study was conducted to evaluate a new selected strain with respect to Mexican lime. It can be concluded that new strain is superior to Mexican lime due to produced seedless fruits with higher acidity and its juice content. It is also considered as thornless trees which make the harvesting more easily and eventually decreasing fruit damage, in addition, less vigorous and wider leaves with shorter petiole. The new strain recorded a genetic polymorphism with the Mexican lime cultivar and considered as a valuable genetic material for Egyptian citriculture industry.

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تقييم سلالة جديدة من الليمون البنزهيرتحت ظروف محافظة كفر الشيخ-مصر

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اجريت هذة الدراسة لتقييم سلالة جديدة من الليمون مقارنة بالليمون البنزهير (الصنف المحلي) حيث تم دراسة صفات النمو الخضرى ، والازهار ، والبذور ، والمحصول ، وجودة الثمار. وقد لوحظ ان السلالة الجديدة لها مواصفات مورفولوجية مختلفة عن الصنف المحلي (الليمون المكسيكي) مثل صغر حجم الشجرة مما يتيح الفرصة لزيادة عدد الأشجار فى الفدان. وجد أن السلالة الجديدة تفوقت على الصنف المحلي في زيادة نسبة حموضة العصير (7,2-8,2٪) والنسبة المئوية وزن العصير/ ثمرة (47,8-48,9 ٪) أيضا عدم وجود الاشواك و أقل عدد من البذور لكل ثمرة (2,06-2,23). تم تعريف والتمييز مابين السلالة الجديدة والصنف المحلى عن طريق استخدام البصمة الوراثية على المستوى الجزيئى من خلال تقنية RAPD-PCRحيث تم استخدام ستة من المعلمات الجزيئة وتم الحصول على42 حزمة (شظية) بمتوسط 10.5 حزمة للبادئ الواحد وسجلت السلالة الجديدة درجة قرابة وراثية بنسبة 82% مع الصنف المحلى وهذة الاختلافات الوراثية يمكن الاستفادة منها فى برامج تربية الموالح .