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MORPHOLOGICAL AND MOLECULAR EVALUATION OF SOME MANDARIN CULTIVARS

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ABSTRACT

This work was carried out to evaluate ten new mandarin cultivars (*Citrus reticulata* Blanco) with respect to the local cultivar (Baladi mandarin). The investigation was executed during the two successive seasons of 2011 to 2013 at the Horticultural Research Station South El Tahrier, Beheira Governorate. The evaluation involved both the growth and productivity which included yield traits and fruit physical and chemical characteristics. The ten cultivars showed significant variations when compared with the local mandarin cultivar for leaf characteristics, tree canopy volume, and fruit weight and fruit chemical properties. DNA fingerprint was performed using RAPD technique for characterization the studied cultivars. RAPD analyses exhibited a total of 14 bands among them, 13 bands were polymorphic of about 92.85%. Those bands were used to distinguish among cultivars. Both morphological and molecular analyses showed a high degree of variation among the new cultivars, indicating that they have an important source of genetic diversity which would be used in future citrus in breeding programs.
Keywords: Mandarin, Morphological evaluation, genetic diversity, fingerprint

INTRODUCTION

Citrus belongs to the sub family Auarantioideae of the family Rutaceae .It is one of the most important commercially cultivated fruit crops in the world (Swingle & Reece, 1967). Mandarin group is comprised of numerous species as well as inter- Generic and interspecific hybrids which made them the most phenotypically heterogeneous of the genus Citrus. Mandarin (*C.reticulata*) together with the grapefruit (*C.maxima*) and citron (*C.medica*) are the three basic species of the subgenus Citrus.

Traditionally, morphological characters have been used to identify and characterize Citrus. However, there is a high level of genetic variability which, would sometimes, make an accurate identification for each variety. Although, there is a large amount of variability within the Citrus genus , the breeder would utilize this variability in breeding programs for selection of desired characters. Morphological characterization in combination with molecular markers would be more rewarding in terms of accurate identification and characterization of most closely related cultivar at intra-specific level. Presently, molecular marker techniques are routinely used for proper characterization, management and conservation of germplasm collections of horticultural species (Karp *et al*.1997).

Morphological analysis was used in citrus to study the variations between kinnow mandarin and rough lemon (Altaf & Khan, 2008). In Himalayan citrus, morphological marker was used to study the genetic diversity (Sharma *et al.,* 2004). The morphological marker is known for its

coverage in the studies of agronomic traits. Further more the technique is relatively cheaper and easier to conduct. Molecular and morphological diversity are independent and rather complementary to genetic diversity in citrus *(*Campos *et al.,* 2005)

Several molecular markers such as random amplified polymorphic DNA (RAPD) ; inter-simple sequence repeat (ISSR), simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP),coding and non-coding regions of chloroplast DNA, internal transcribed spacer (ITS) region etc. have been used for the analyses of genetic diversity , relationships, cultivars identification, linkage mapping, and molecular phylogeny in Citrus (Jena *et al* 2009).Among the molecular markers, RAPD marker has been extensively used to study genetic diversity and relationships in Citrus species (Digvender *et al*., 2013).

The main objective of this study was to characterize some mandarin cultivars using morphological and molecular markers and to assess the genetic diversity and relationships among them.

MATERIALS AND METHODS

This study has been carried out during 2011/2012 and 2012/2013 seasons on about 10 years old of ten mandarin cultivars (*Citrus reticulata* Blanco) budded on volkamer lemon rootstock (*Citrus volkameriana*) at Horticultural Research Station South El Tahrir, Beheira Governorate. The studied cultivars in Table1 were : Fina, Avana apireno, Seedless mandarin, Fedele, Clementine, Spinoso, Thorny Clementine and Thornless Clementine imported from Italy via Horticulture Research Institut*e* while Balady mandarin was used as a local cultivar .Trees were planted at 5x5 meter apart. Normal agriculture practices were applied in this orchard as recommended by the Ministry of agriculture.

Table 1: The scientific names, parentages and origin of the mandarin cultivars in this study.

Cultivar	Scientific name	Parentage	Origin
Avana apireno	*C. deliciosa* TAN.	a selection (bud sport) of Mediterranean	Italy
Clementine	*C.clementina* HORT.ex.TAN.	a hybrid between orange and mandarin	Algeria
Nour	*Citrus reticulata*	a mutation of 'Cadoux'	Moroccan
Fina	*C. clementina* HORT. ex. TAN.	The original Clementine cultivar	imported from Algeria into Spain in 1925
Seedless clementine	*Citrus reticulata*	Unknown	Italy
Fedele	*C.clementina* HORT.ex.TAN.	Spontaneous mutation from C. reticulata commune	Italy
Spinoso	*C.clementina* HORT.ex. TAN.	C.clementina commune bud mutation	Italy
Thorny clementine	*Citrus reticulata*	Unknown	Moroccan
Thornless clementine	*C. clementina* HORT.ex.TAN.	Unknown	Italy

Morphological Characterization:

In this investigation, seven morphological and fruit traits were studied .These traits were:

1-Leaf characteristics:

Leaf and width were measured, then leaf length/width was calculated where

Leaf area was calculated according to the equation presented by Chou (1966).

Leaf area (cm^2) =2/3[leaf length x leaf width].

Tree Canopy volume (m^3) was calculated according to the equation presented by Turrel (1946), Tree Canopy volume (m^3) = Plant height (m) x Plant spread (m) x 0.524

2-Fruit weight and Yield

Thirty fruits from each cultivar were used to estimate average fruit weight and yield was calculated as Kg/tree by multiplication number of fruits per tree with an average fruit weight.

3-Number of mature seeds, aborted seeds and segments per fruit was recorded.

4-Soluble Solids Content: (SSC %) :

It was measured by using a hand refractometer.

5-Total acidity %

Titrable acidity was determined according by **A.O.A.C (1980)** by titrating 10 ml juice with (0.1N) NaOH using phenolphthalein as indicator . Acidity was expressed as citric acid percentage

6-SSC /acid ratio:

This ratio was calculated by diving of SSC% on titrable acidity % to be used as a criterion for maturity determination

7- Ascorbic acid (Vitamin C) :

Ascorbic acid or vitamin C was determined by using 2, 6-dichlorophenol indophenol method described by **A.O.A.C (1980).** Vitamin C content was calculated as mg/100 ml juice

Statistical analysis:

The experimental design was used a complete randomized block with three replications . The obtained data were statistically analyzed according to Little & Hills (1972), means separations according to Duncan, (1955).

Molecular characterization

Plant materials: Healthy young and fresh leaves samples were collected from the mandarin cultivars,saved in ice box and quickly transferred to laboratory .Plant tissues were ground to afine powder in the presence of liquid nitrogen .The DNA extraction was performed using DNeasy plant Mini Kit(QIAGEN).

DNA Isolation*:* DNA of the 6 selected cultivars was isolated using CTAB (Cetyl-tetramethyl ammonium bromide) method, (Murray & Thompson, 1988).

For DNA isolation, one hundred mg of fresh leaves were homogenized in a chilled pestle and mortar using liquid nitrogen. 700 µl of 2X CTAB extraction buffer were added and homogenized well. The samples were transferred to Eppendorf tubes and incubated at 65 °C for 30-60 min with

occasional gentile swirling. 700 µl of Chloroform Isoamyle alcohol (24:1) were add and mixed by inverting the tube several times. Sample was centrifuged at 15000 rpm for 15 min at 4°C. The aqueous was transferred to a fresh centrifuge tube with a wide bore tips to avoid DNA shearing. Then, 0.6 volume of chilled isopropanol was added and followed by quick and gentle inversion and incubated at -20°C for 30 minutes. DNA pellet was precipitated at 10000 rpm for 10 min at 4 °C. Pellet was washed three times with 70 % ethanol, well dried and dissolved in 100 µl TE buffer. After some cycles of dilutions, the concentration of DNA was approximately adjusted to 15 ng*/ µl,* and this concentration is suitable for PCR reaction.

RAPD PCR Reactions:

Polymerase chain reaction (PCR) was conducted using two primers.The nucleotide sequences of these primers are as presented in Table 2

Table 2: List of RAPD primers and their nucleotide sequences

No.	Primer	Sequence
2	ISJ-5	5'-CAG GGT CCC ACC TGC-3'
3	ISJ-9	5'-AGG TGA CCG ACC TGC A-3'

PCR reactions ,were conducted according to El-Moghazy (2007): Amplification condition was carried out with the following specification : initial denaturation at 94°C for 3 min, 45 cycles of amplification under the following parameters; template denaturation at 94°C for 1 min, primer annealing at 48°C for 1 min and extension at 72°C for 2.30 min by the end of the 45th cycle, final extension at 72°C for 7 min followed by storage at 4**°C**.

Electrophoresis, staining and analysis

DNA amplified fragments were loaded onto 1.5 % agarose gel containing ethidium promide (2 µl/100 ml). The 0.5X TAE was used as a running buffer and 50 and 100 bp DNA ladders (0.5 µg / *µl*, fermentas) as molecular weight markers. Electrophoresis was conducted at 70 V, 50 mA for 3 hours. Then, gels were photographed and analyzed using BioDoc Analysis software (Biometra, Germany).

Phylogenetic tree construction

The presence/absence matrix for amplified DNA fragments of the two RAPD markers was used to study the phylogenic relationships among the studied genotypes. The statistical software NTSYS pc2.0 (Rohlf, 2000) was used to estimate the genetic relationships among the tested genotypes. Employing the computer package NTSYS pc2.0, Nei and Lei's similarity coefficients (**Nei and Le**i, **1979**) were calculated and used to establish genetic relationships among the genotypes based on un-weighted pair group method with arithmetic means (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering**.**

RESULTS AND DISCUSSION

Morphological characterization

Data presented in Table 3 indicated that all cultivars have significant variation for leaf length , leaf width , leaf length/width and leaf area in the first season but there were no significant variation for leaf length and leaf length/width in the second one where cultivars were highly significant in second season for leaf width and leaf area.

Concerning the variation among cultivars, Nour cultivar had the longest leaf length, leaf width and leaf length/width ratio followed by Clementine but Clementine had the highest leaf area in both seasons under study On the contrary, Balady mandarin cultivar had the shortest leaf length and seedless mandarin had the narrowest leaf width in both seasons of study.

Tree Canopy volume:

Fig.1 showed that Nour cultivar had the biggest canopy volume in both seasons of study**.** On the contrary, Fina cultivar had the smallest canopy volume for first and second season , These significant results in line with those obtained by Nicotra (2011) on Spinoso and Fedele cultivars.

Mandarin (*Citrus reticulata* Blanco.) is considered as highly heterogeneous specie among three true citrus (Campos *et al.*, 2005). The cultivars are varied in leaves, flowers and fruits characters. The phenotypically differences in cultivar individuals could be attributed to mutations, and cross pollination. Almost all the scion and roots stocks of citrus have emerged spontaneously as chance seedlings. The bud sport mutations were different from its original habitat might be the factors that added to variation. Further, the lack of reproductive barrier both within the genus and species might have continually added to it variation and heterogeneity.

Table 3: Vegetative growth characters of the studied mandarin cultivars in 2011 and 2012 seasons.

cultivar	Leaf length (cm)		Leaf width (cm)		Leaf length / width		Leaf area (cm^2)	
	2011	2012	2011	2012	2011	2012	2011	2012
Fina	6.8 ab	6.33	2.2b c	2.33bc	3.0	2.84	10.3 bc	9.6bc
Avana apireno	5.2 c	5.33	1.9b c	2.06cd	2.6	2.63	6.8 c	7.37cd
Nour	7. 6 a	8.16	2.5b c	3.30 a	2.9	2.47	13.0 ab	17.7a
Seedless mandarin	5.4b c	6.78	1.8 c	1.60 d	2.9	3.18	6.6 c	7.1 d
Fedele	7.1 ab	6.74	2.0 bc	1.90cd	3.5	3.57	9.5 bc	8.7cd
Clementine mandarin	7.3 a	7.93	3. 2 a	3.06 a	2.2	2.0	16.0a	16.2 a
Spinoso	7.5 a	7.63	2.3 bc	2.0 cd	3.2	3.34	12.2ab	10.1 bc
Thorny Clementine	7.1 ab	6.63	2.6 b	2.9 ab	2.5	2.37	12.1 ab	12.6 b
Thornless Clementine	6.4 ab	7.3	2.1 bc	2.2 cd	2.9	3.33	9.3 bc	10.7 bc
Balady mandarin	5.6 bc	5.9	2.1bc	2.0 cd	2.7	2.95	8.2 bc	8.0 cd
F test	*	NS	*	**	NS	NS	*	**

NS = Non significant * = significant ** = highly significant

Means followed by the same letter are not significantly different at 5% level by DMRT

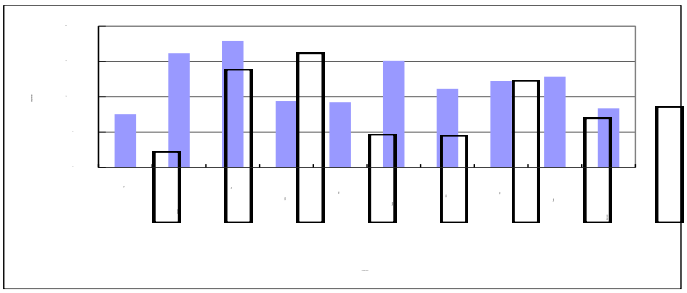

Fig.1: Mean values for tree canopy volume in the two seasons of study

Fruit weight, number of fruits and yield showed highly significant differences among cultivar in both seasons of the study (Table 4).

Avana apireno cultivar gave the highest number of fruits and yield in both seasons of study. Yield varied among cultivars because of the different characteristics of each cultivar. The obtained data are in agreement with those obtained by Sayed *et al.* (2004) who reported that vegetative growth and fruit production is a continuing process. Fruit weight and volume are heritage attributes of scions, and they varied among cultivars because of different characteristics among citrus cultivars (Fotouhi & moghadam 2010) .Crop load is likely the main cause of the alternate bearing behavior of many citrus species and varieties Valiente & Albrigo (2004).These significant results in line with those obtained by Nicotra (2011) on Spinoso and Fedele cultivars, also Our results of high variations among the cultivars are in line with (Sayed *et al.,*2010) and confirmed the published information from the originated area where those cultivars were introduced.

Table 4: Fruit weight and yield of the studied mandarin cultivars in 2011 and 2012 seasons.

cultivar	Fruit weight		Fruits number/tree		Yield/tree(kg)	
	2011	2012	2011	2012	2011	2012
Fina	100.0de	106.d	171.6c	488.3 d	17.2ef	46.5 c
Avana apireno	124.6 c	116.8cd	614.1a	890.3 a	76.1 a	79.36 a
Nour	111.6 d	130.1 c	317.6b	619.5 b	35.1bc	54.36 b
Seedless mandarin	103.4de	119.3cd	230.0bc	392.0 e	23.7de	32.44 e
Fedele	83.1 f	145.3 b	206.6bc	386.0e	17.0 ef	33.4e
Clementine	68.4 g	129.3 c	191.6bc	223.6f	12.9 f	17.6fg
Spinoso	79.0 fg	118.0cd	210.0bc	533.6 c	10.6 f	43.56 d
Thorny clementine	159.6a	161.1 a	196.3bc	192.0 g	31.3cd	19.65 f
Thornless clementine	142.4 b	145.1 b	294.0bc	485.6d	41.9 b	48.8c
Balady mandarin	95.8 e	156.0ab	225.3bc	156.0 h	12.9 f	14.82g
F. test	**	**	**	**	**	**

NS = Non significant * = significant ** = highly significant

Means followed by the same letter are not significantly different at 5% level by DMRT

Number of seeds per fruit

Data presented in Table 5 indicated that cultivars showed highly significant variation in number of seeds per fruit and the aborted seeds in both seasons of the study .However, regarding segments number significant differences were found in the second season only.

Regarding cultivars, Balady mandarin had the greater number of seeds per fruit in both seasons. while, Nour and Fedele cultivars gave the lowest seeds per fruit for second season.

These significant results are in line with those obtained by Nicotra (2011) on Spinoso and Fedele cultivars. He reported that the number of seeds was significantly influenced by the rootstocks in Nova and Robinson fruits. However, seed number did not reach commercially unacceptable levels. Georgiou (2000) reported similar results for seed numbers. Pollination and pollination efficiency are the most important factors for number of seeds per fruit (Ferraro *et al*., 2006). The fact that the pollinators for both species were similar in this study may have contributed to similar numbers of seeds obtained from experimental plots.

Table 5: Number of seeds, aborted seeds and segments number of the studied mandarin cultivars in 2011 and 2012 seasons.

cultivar	number of Seeds/fruit		Aborted seeds/fruit		Segments number	
	2011	2012	2011	2012	2011	2012
Fina	1.0de	2.6 cd	3.0b	2.3 c	10.0	10.0ab
Avana apireno	3.5bc	4.26 bc	4.6a	5.6 a	11.0	11.0ab
Nour	0.83de	0.0 d	0.0d	0.0 e	10.0	10.33ab
Seedless mandarin	5.5 b	4.53 bc	4.3a	4.6b	10.0	10.0ab
Fedele	2.6 cd	0.0 d	0.0d	0.0 e	11.0	10.66ab
Clementine	2.1 de	2.80 bc	1.0cd	1.0d	8.0	9.0 c
Spinoso	0.66 e	0.0 d	0.0 d	0.0 e	7.3	10.0 ab
Thorny Clementine	3.1 bc	2.93 bc	1.6 c	1.0d	11.3	11.33 a
Thornless Clementine	5.1 bc	5.66b	1.6 c	0.93d	9.6	9.66 bc
Balady mandarin	23.8 a	19.60 a	0.0 d	0.0 e	11.0	11.0 ab
F. test	**	**	**	**	NS	*

NS = Non significant * = significant ** = highly significant

Means followed by the same letter are not significantly different at 5% level by DMRT

Fruit Chemical characters

Data in table 6 clearly show that, there are variations in the fruit chemical parameters of fruit quality among cultivars. Clementine had the highest SSC% and SSC/acidity where Balady mandarin and Spinoso had the highest value for acidity and vitamin C, respectively.

The differences in chemical composition of juices can be attributed to the genetic influence occurring among different cultivars and physiological factors (Sharma *et al*., 2006**).**

Table 6: Fruit chemical properties of ten mandarin cultivars in 2011 and 2012 seasons.

cultivar	SSC%		Acidity %		SSC/Acidity		Vitamin C (mg/100ml)	
	2011	2012	2011	2012	2011	2012	2011	2012
Fina	10.53	10.46 b	1.1ab	1.12b	9.32 cd	9.4ab	63.03 b	49.7bc
Avana apireno	9.83	8.13 c	1.18ab	1.18 ab	8.32 d	6.87 d	36.50cd	36.3 de
Nour	11.0	10.66 b	1.01bc	1.02 bc	10.88 b	10.66ab	62.60 b	60.80 a
Seedless mandarin	10.06	10.66 b	1.20 a	0.96 c	8.39 d	11.08 a	36.73cd	41.43cd
Fedele	10.13	10.66b	1.10ab	1.06 bc	9.18 cd	10.14ab	41.95 c	42.33cd
Clementine	11.33	12.33ab	0.88 c	1.18 ab	12.89 a	10.58ab	40.60 c	39.49 d
Spinoso	11.66	10.33 b	1.16ab	1.20 ab	10.09bc	8.58 ab	73.46 a	55.70ab
Thorny Clementine	10.33	10.46 b	1.16ab	1.35 a	8.87 cd	7.77 cd	31.83 de	36.66de
Thornless Clementine	10.66	9.66 b	1.19a	1.25 ab	9.06 cd	7.77 cd	40.16 c	43.53cd
Balady mandarin	10.85	11.20ab	1.21 a	1.36 a	8.98cd	8.21 bc	30.40e	29.80 e
F. test	NS	**	**	*	**	*	**	**

NS = Non significant * = significant **=highly significant
Means followed by the same letter are not significantly different at 5% level by DMRT

Assessment of morphological diversity for mandarin cultivars by cluster analysis

Fig. 2 showed clearly that the cluster analysis based on morphological variables from tree, leaves, seeds , yield and fruits divided the mandarin cultivars into two main clusters (I and II) at a distance of 0.91 the first cluster included nine cultivars, distributors two sub-cluster at a distance of 0.96 almost, the first sub-cluster involved Avana apireno and seedless mandarin cultivars while the second sub-cluster involved (Fina , Fedele , Nour ,Clementine , Thorny ,Thornless and Spinoso) cultivars

On the other hand, the second cluster also incorporates of six cultivars, distributors two sub-cluster at a distance of 0.95 almost, one of them contains cultivar Avana apireno while the other the sub-cluster sub-divided into two groups at a distance of 0.98 almost, the first group involved Fedele and seedless mandarin, while the second group two sub group the first containing Fina cultivar where the second containing Nour and Spinoso cultivars .Over all, the morphological qualitative parameters for the cultivars diverged at similarity coefficient of 0.98 for 1.0 approximately.

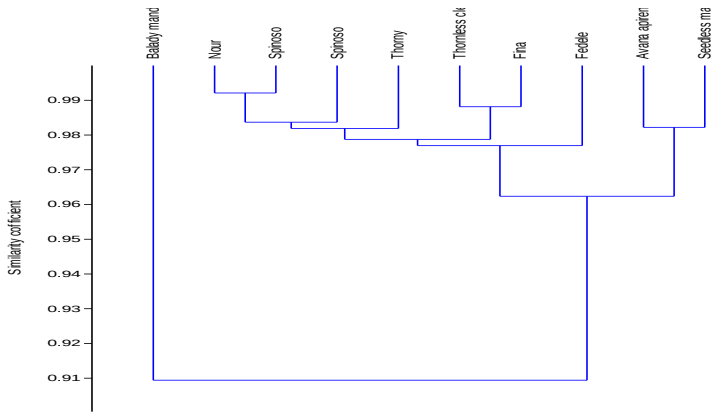

Fig.2: UPGMA dendrogram based on morphological variables of trees, leaves, ,seeds, yield and fruits quality.

Molecular analysis

1. ISJ-5 primer

For ISJ-5 primer, the electrophotograph for the amplified DNA fragments is presented in Figure 3. The presence/absence matrix and the estimated molecular weights for the amplified fragments using this primer are presented in Table 7.

As shown in Figure 3, a total of ten amplified DNA fragments were generated in the selected cultivars. The molecular weights of the amplified fragments ranged from 1466 to 491 bp. All the ten fragments were polymorphic revealing polymorphism % of 100%.

Data in Table 7 showed that Nour and spinoso cultivars have the same patterns of bands, as Fina and Thornless. The fragments 4,7,10 were present in fedele cultivar but they were absent in the other cultivars.

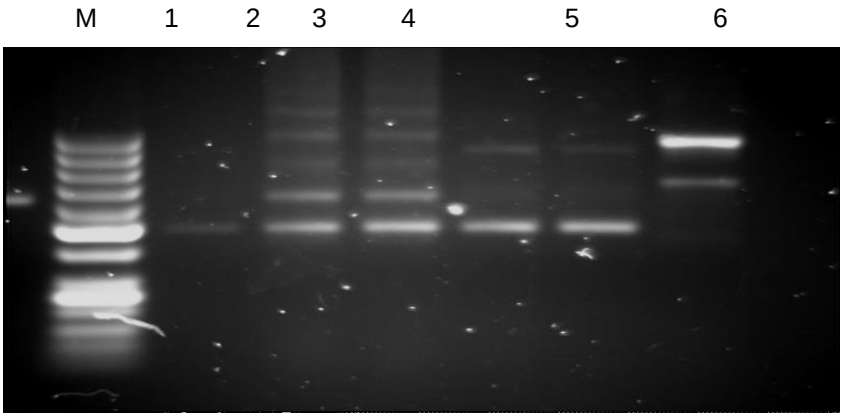

Figure 3: The electrophotogram of DNA amplified fragments using ISJ-5 for selected cultivars. M, 50 bp DNA ladder; 1, (Where: 1; Fedele; 2, Nour ; 3, Spinoso; 4, Fina ; 5, Thornless Clementine; 6, Clementine.

Table 7: The presence (+), absence (-) matrix for ISJ-5 amplified fragments for the studied cultivars.

Genotypes / Fragments	1	2	3	4	5	6	M.W. (bp)
ISJ-5.1	-	-	-	+	+	-	1466
ISJ-5.2	-	-	-	+	+	-	1286
ISJ-5.3	-	-	-	+	+	-	1108
ISJ-5.4	+	-	-	-	-	-	1054
ISJ-5.5	-	+	+	-	-	-	1003
ISJ-5.6	-	-	-	+	+	-	900
ISJ-5.7	+	-	-	-	-	-	784
ISJ-5.8	-	-	-	+	+	-	708
ISJ-5.9	-	+	+	+	+	+	540
ISJ-5.10	+	-	-	-	-	-	491

Where: -, absent; +, present; 1,; Fedele; 2, Nour ; 3, Spinoso; 4, Fina ; 5, Thornless Clementine; 6, Clementine

2. ISJ 9 primer

For ISJ-9 primer, the electrophotogram for the amplified DNA fragments is presented in Figure 4.. The presence/absence matrix and the estimated molecular weights for the amplified fragments using this primer are presented in Table 8.

As shown in Figure 4, a total of two amplified DNA fragments were generated in the selected cultivars. The molecular weights of the amplified fragments ranged from 930 to 665 bp . One band was monomorphic (665 bp) while the other fragment was polymorphic that revealed polymorphic ratio of 50 %.

Data in Table 8 showed that Fedele, Nour , spinoso and Clementine cultivars have the same patterns of bands, also Fina and thornless.

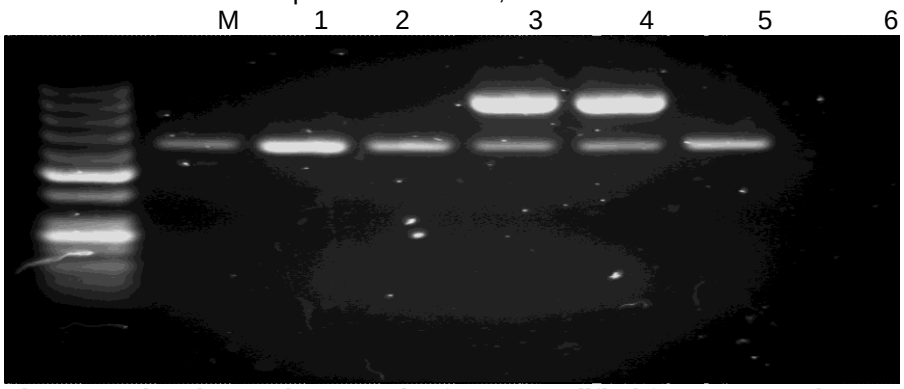

Figure 4: The electrophotograph of DNA amplified fragments using ISJ-9 for selected cultivars. M, 50 bp DNA ladder; 1, (Where: 1; Fedele; 2, Nour ; 3, Spinoso; 4, Fina ; 5, Thornless Clementine; 6, Clementine

Table 8: The presence (+), absence (-) matrix for ISJ-5 amplified fragments for the studied cultivars

Genotypes	1	2	3	4	5	6	MW
ISJ-9.1	-	-	-	+	+	-	930
ISJ-9.2	+	+	+	+	+	+	665

Where: -, absent; +, present; 1,; Fedele; 2, Nour ; 3, Spinoso; 4, Fina ; 5, Thornless Clementine; 6, Clementine

Mandarin Cultivars Genetic Characterization Based on RAPD Products: The amplification of 6 DNA samples of Citrus cultivars using Two RAPD primers produced 14 fragment out of them 13 bands were polymorphic (92.857%s. RAPD Markers showed polymorphism (100%) in ISJ 5 primer and 50% in ISJ9 primer among cultivars as shown in Table 5 and 6.

Genetic similarity and phylogenic tree

The data representing the similarity index are shown in Table 9. The data clearly showed the existence of considerable amount of molecular diversity among the tested genotypes. The lowest similarity percentage (0 %) was present between the cultivar (Fedele) and (Nour, Spinoso, Fina, Thornless and Clementine) cultivars, while, the highest similarity percentage (50%) was observed between the cultivars (Fina, thornless) and (Nour, Spinoso) cultivars.

Table 9: Genetic similarity index among all pairs of the studied cultivars

Gen.	Fedele	Nour	Spinoso	Fina	Thornless	Clementine
Fedele		0	0	0	0	0
Nour			1	0.14	0.14	0.5
Spinoso				0.14	0.14	0.5
Fina					0.14	0.16
Thornless						0.16
Clementine						

Based on Nei and Lei's coefficient of similarity, cluster analysis was performed and a dendrogram illustrating the phylogenic relationships among the tested genotypes were obtained. The phylogenic tree Fig.5 explaining the relationships cleared two main clusters , the first main cluster contained cultivar (Fedele), while the second main cluster split to two sub –clusters , the first sub cluster consisted of (Fina and Thornless Clementine cultivars). The second sub cluster included two groups; the first groups contain cultivar (Clementine), while the second group contained two cultivars (Nour and Spinoso).

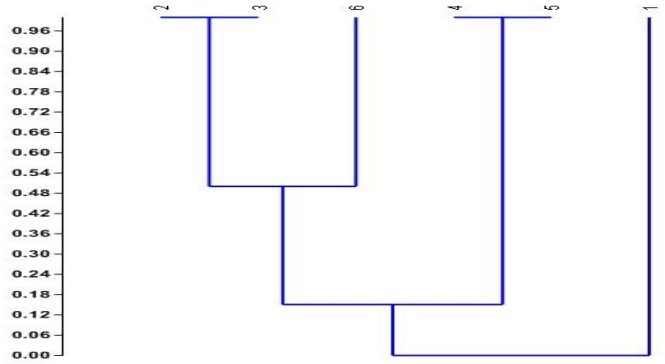

Figure 5: Dendogram derived from UPGMA cluster analysis of six mandarin cultivars based on Nei and Lei (1979)similarity coefficient using ISJ 5 primer. Where 1**,; Fedele;** 2**, Nour ;** 3**, Spinoso;** 4**, Fina ;** 5**,**

Thornless Clementine; 6**, Clementine.**

While data in Fig. 6 cleared two main clusters, the first main cluster contained cultivars (Fina and Thornless Clementine), while the second main cluster contained cultivars (Fedele, Nour,spinoso and Clementine)

Table 10 :Genetic similarity index between all pairs of the selected cultivars

Cultivar	Fedele	Nour	Spinoso	fina	Thornless	clementine
Fedele		1	1	0.5	0.5	1
Nour			1	0.5	0.5	1
Spinoso				0.5	0.5	1
Fina					1	0.5
Thornless						0.5
Clementine						

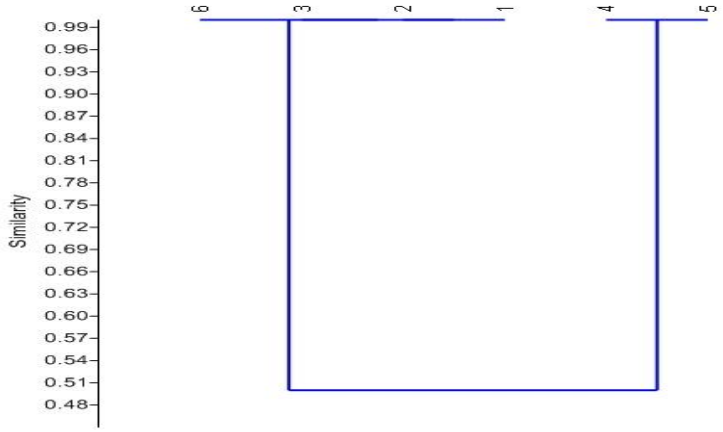

Figure 6:Dendogram derived from UPGMA cluster analysis of six mandarin cultivars

It is well known that morphological plasticity in study is a major weak point in assessment of phenotypic diversity. However, several combined studies in mandarin, both morphological and molecular markers in the past had shown to be independent of genetic diversity (Campos *et al*., 2005). Further, the study on inheritance of agronomic traits of citrus reports them to be controlled by multiple genes which can be assessed only through morphological assessment (Liu and Deng, 2007).

Mandarin (*Citrus reticulata* Blanco.) is considered as highly a heterogeneous species among three true citrus (Campos *et al*.,2005). A study on the diversity of Himalayan citrus both through morphological and Random Amplified Polymorphic DNA (RAPD) analysis revealed the existence of huge diversity (Das *et al*., 2005).It is known that citrus cultivars are origin from hybridization between the three true species or as mutation from them .so, the genetic similarity among different cultivars may be explaining its origin (Barkely *et al*.,2006)

Generally, both morphological and molecular markers showed a high degree of variation among the selected mandarin cultivars. Also, the results revealed that the tested cultivars were promising in terms of vegetative growth and yield .they can be cultivated in areas similar to the experimental climate conditions. in addition, they provide a wide range of diversity to citrus varieties collection. Moreover, climate changes should be taken into consideration when introducing new cultivars .However, these cultivars need more precise investigations to evaluate some other morphological traits.

REFERENCES

A.O.A.C.(1980).Association of Official Analytical Chemist.14th Ed Published by the A.O.A.C., USA.

Altaf, N. and A.R. Khan (2008). Variation Within Kinnow (*Citrus reticulata*) and Rough Lemon (*Citrus Jambhiri*). Pak. J. Bot., 40 (2): 589-598.

Barkely, N.I.; M.L. Roose; R. R. Kruegar and C.T. Federici, (2006). Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor. Appl. Genet. ,112: 1519-1531.

Campos, E. T.; M. A. G. Espinosa ; M. L. Warburton ; A. S. Varela and Á.V. Monter (2005). Characterization of mandarin (*Citrus SPP*.) using morphological and AFLP markers. Formato Documento Electrónico (ISO) 30(11): 687-693.

Chou,G.J.(1966). A new method of measuring the leaf area of citrus trees. Acta Hort.,5:17

Das, A. ; B. Mondal; J. Sarkar and S. Chaudhuri (2005). Genetic resource survey of mandarin orange (Citrus reticulata Blanco) in the northeastern Himalayan region of India. PGR News letter 2004 (143): 35-39

Digvender, P. ; S. K. Malik ; S. Kumar ; R. Choudhary ; K.C.Sharma and R. Chaudhur(2013). Genetic Variability and Relationship Studies of Mandarin(*Citrus reticulata* Blanco) Using Morphological and Molecular Markers. Agric. Res.2(3):236–245.

Duncan, D.B. (1955). Multiple Range and Multiple F. test. Biometrics, 11: 1-42.

El-Moghazy, A.M. (2007). Genetical and Molecular Breeding For Drought Tolerance in Rice. Ph.D. Thesis, Dept. of Genetic, Fac. of Agric. Kafr El-Sheikh Univ.

Ferraro, A.E.; R.M. Pio and F.A. Azevedo (2006). Pollination influence of sweet orange varieties on Nova tangelo seeds production. Rev. Bras. Frutic. 20 (2).

Fotouhi, G. R. and J .F. Moghadam (2010). Citrus growing in Iran. University of Guilan Press Iran.

Georgiou, A. (2000). Performance of 'Nova' mandarin on eleven rootstocks in Cyprus. Sci. Hort., 84: 115-126.

Jena , S.N.; S. Kumar and N.K. Nair (2009). Molecular phylogeny in Indian Citrus L. (Rutaceae) inferred through PCR-RFL P and trnL -trn F sequence data of chloroplast DNA. Sci Hort.119:403–416.

Karp, A. ; S. Kresovich ; V. BhatK ; W. G. Ayad and T.Hodgkin (1997). Molecular tools in plant genetic resources conservation: A guide to the technology. IPGRI Bulletin 2: 47.

Little, T.M. and F.J. Hills (1972). Statistical methods in Agricultural Research. Universty of Califonia, Davis, 242P.

Liu, Y. Z. and X. Deng (2007). Citrus Breeding and Genetics in China. The Asian and Australian Journal of Plant Science and Biotechnology: 23-28.

Murray, A. A. and W.F. Thompson (1988). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. 8: 4321-4325.

Nei, M. and W.H. Lei (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nati. Acad. Sci. USA. 76:5269-5273.

Nicotra, A. (2011). Mandarin-like Hybrids of Recent interest for Fresh Consumption. Problems and Ways of Control. China/FAO Citrus Symposium.

Rohlf, J. (2000). Numerical taxonomy and multivarieties analysis system NTSYS.PC. version 2Exeter software, New York.

Sayed, R.A. and R.A. Abdel-Aziz (2010). Performance of some new citrus varieties under south El-Tahrier District conditions.1(2):291-300.

Sayed, R.A.; A.Z. Sabah and A.I. Ibrahim (2004). Effect of calcium and Gibberillic acid sprays on yield, quality and abscessing of grapefruit trees. J. Agric. Sci. Mansoura Univ.,29(3): 1239-1255.

Sharma, B.D.; D.K. Hore and S.G. Gupta (2004). "Genetic resources of Citrus of north-eastern India and their potential use." Genet Resour Crop Evol., 51: 411-418.

Sharma, R.R.; R. Singh and S.K. Saxena (2006). Characteristics of citrus fruits in relation to granulation. Sci. Hortic. 111: 91-96.

Swingle, W.T. and P.C. Reece (1967).The botany of Citrus and its wild relatives. In: Reuther W, Batchelor LD, Webber HJ (eds) The Citrus Industry. University of California Press, Berkeley, pp190–340.

Turrel, F.M.(1946).Tables of surfaces and volumes of spheres and of prolate and oblate spheroids and spheroidal coefficient. Uni. of California press, Berkeley.

Valiente, J.I. and L.G. Albrigo (2004). Flower bud induction of sweet orange trees [Citrus sinensis (L.) Osbeck]: effect of low temperatures, crop load, and bud age. J. Am. Soc. Hort. Sci., 129:158-164.

التقييم الجزيئي والمورفولوجي لبعض أصناف اليوسفي٩*

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أجريت هذه الدراسة لتقييم تسعة أصناف من اليوسفي هي)فينا-نور -سبينوزا -فيداليا-كليمانتين-اليوسفي عديم البذور-افانابرينا-اليوسفي عديم الأشواك-اليوسفي ذو الأشواك (مقارنة بالصنف المحلى)اليوسفي البلدي (وتهدف هذه الدراسة التي أجريت بمزرعة محطة بحوث البساتين بجنوب التحرير عام 2011-2013إلى قياس درجة الاختلافات الوراثية والمورفولوجية بين هذه الأصناف.وكان الانتخاب على أساس الإنتاجية وصفات الثمار الفيزيائية والكيمائية.وقد أظهرت النتائج وجود اختلافات معنوية بين الأصناف موضع الدراسة واليوسفي البلدي بالنسبة لصفات الأوراق والمجموع الخضري وصفات الثمار وكان أفضلهم الصنف)يوسفي نور (يليه الصنف كليمانتين .تم تعريف لهذه الأصناف والتمييز بينهم من خلال تقنية الحامض النووي DNA))والتي تعتمد على تفاعل البلمرة المتسلسل PCR))وهى دنا العشوائي متعدد المظاهر (RAPD)حيث استخدم اثنين من المعلمات الجزيئية وتم الحصول على 14شظية من الحمض النووي (DNA) منهم شظية متشابهة و 13شظية مختلفة أعطوا تنوع وراثي بنسبة 92.89%

أوضحت الدراسة أن هناك تباينا بين الأصناف يمكن أن يحدد كل صنف عن الأخر وذلك من خلال 14شظية تم حصرها موضحة التباينات بين الأصناف .ومن خلال تلك الشظيات (Bands) الوراثية تم تحديد درجة القرابة الوراثية والمسافات الوراثية من خلال Dendogrameوهذه الاختلافات المورفولوجية والوراثية يمكن الاستفادة منها في برامج تربية الموالح.

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