Horticultural And Molecular Genetic Evaluation Of Some Peach Selected Strains Cultivated Under Kalubiah Governorate Conditions

Fathi, M.A.; SH. M. Hussein and S.Y. Mohamed

Horticulture. Research. Institute. Agriculture. Research. Center, Giza, Egypt

ABSTRACT: The present investigation was carried out for two successive years (2010 and 2011) in Kalubia Governorate to evaluate the percentage of vegetative and floweral growth as well as fruit set, date of full bloom, petal fall, fruit set and harvesting time. At harvest time, number of fruits/tree were counted, also fruit yield/tree and /fedd. were estimated as well as fruit characters. Molecular genetic evaluation included protein, isozymes, RAPD and ISSR of ten selected peach strains viz: Early Sinai (C9)- Dakahlia (K3)- Mawy (K21)- Sultany1 (K25) – Sultany2 (K40) – Kulaby (K55) – Late Sinai1(L1) - Late Sinai2(2L) - Late Sinai3 (X2) and Late Sinai 4(X4), were studied also. The results revealed that, 2L, K55 and X2 peach strains produced the highest fruit yield/tree and /fedd. also X4, X2 and K55 strains have the highest number of fruits/tree. The strain Mawy (K21) was the earliest strain of flowering, fruit set and harvesting time. On the other hand, K3, K40 and L1 were the latest strains of flowering, fruit set and harvesting time while K55 was earlier of fruit set and harvesting time. Moreover K21 and K55 have trace peak, while C9, K25, L1,2L and X2 have medium peak as well as X2 and 2L strains which have larger fruits, the strains L1 and 2L have higher T.S.S. While, L1, X4 and X2 have lower acidity than others. The studied peach strains have white or creamy flesh, while (except L1 strain which has orange skin and flesh), the red color occupied 40-60% on the skin color of K3, K55 and L1. In this study we attempted to characterize molecular genetic markers between the ten peach selected strains and to detect the genetic relationship between the selected peach strains as scions and Okinawa rootstock. Peach selected strains and Okinawa discriminated by their leaves for protein, isozymes (Peroxidase and Poly Phenyl Oxidase) on biochemical level and RAPD and ISSR based on PCR techniques on molecular level. Protein banding patterns revealed a total number of bands 17 with 23.5% polymorphic bands. While, each of peroxidase and Poly Phenyl Oxidase exhibited present and absent of bands and differences in density of bands between peach strains and Okinawa Rootstock. Five 10-mer arbitrary primers of each RAPD and ISSR had successfully generated reproducible polymorphic products. The generated profiles revealed high levels of polymorphism among the studied strains. Data of these primers recorded a sum of 146 bands. These bands were identified as 53 polymorphic bands and 93 monomorphic ones in all strains under study. The polymorphic bands were scored as 25 unique bands. These unique bands were used to discriminate between the studied selected peach strains as scions and Okinawa rootstock. In addition, the results generated from Protein, Isozymes, RAPD and ISSR profiles were pooled together to elucidate the genetic relationships among the examined peach selected strains. The constructed dendrogram tree divided the studied strains into two major groups. The first main group included Dakahlia and Early Sinai, while the second main group divided into two subgroups, the first subgroup included each of Late Sinai3 and Late Snai1 Sinai4 only and the other subgroup included all other strains (Sultany1, Sultany2, Kulaby, Late Sinai1, Late Sina2 and Mawy). On the other hand, genetic relationship between Okinawa rootstock and peach selected strains from Protein, Isozymes, RAPD and ISSR profiles were pooled together to elucidate the genetic relationships represented the constructed dendrogram tree divide into two major groups. The first main group included Okinawa only, while the second main group divided them into two subgroups, the first subgroup included Early Sinai and Dakahlia only and the other subgroup included all other strains (Sultany1, Sultany2, Kulaby, Late Sinai1, Sina2, Mawy, Late Sinai3 and Late Sinai4). From the foregoing results, it can be recommended to spread these selected strains especially strains: Kulaby (K55), Late Sinai2(2L), Late Sinai3(X2), Mawy (K21) and Dakahlia(K3) in desert land conditions and more studies are very important to evaluate it from nematode resistance and it is ability to success under different environmental conditions.

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1. INTRODUCTION

Peach [Prunus persica (L.) Batsch] belongs to the genus Prunus, and is a member of the family Rosaceae. Flower bud of peach is solitary, without leaves and always in lateral rather than terminal position (Westwood, 1988). Peach is a diploid plant with n = 8 (Jelenkovic and Harrington 1972), and has a

comparatively small genome; 5.9 x 108 bp or 0.61 pg / diploid nucleus, with a haploid size of 300 Mb (Baird et al., 1994). Peach is a popular fruit and considered one of the important fruits in the world. In Egypt, peach cultivated area is about 80609 Feddans with fruit production of about 273256 tons (statistics data of Ministry of Agriculture in 2010). More than 80% of the production is located in north Sinai which depends on rain and in the newly reclaimed land mainly in west Nobareia.

Local peach strains of Dakahlia have attractive fruits with special taste and aroma. Production of superior quality of peaches is highly demanded for consumption and exportations (Mehanna et al., 1982; Mansour and Shaltout,1986;Mohamed, 1995 and EL-Said et al., 1997). Early peach of Sinai are vigorous, good shaped, resistant to drought, high fruit quality and big adaptability for handling and storage ability (Mansour et al., 1998). So, there was a recommendation to peach growers to establish some superior Dakahlia and Sinai peach strains (Mansour et al., 1999 and Eliwa,2005).

Molecular markers are interest to plant geneticists and breeders as a source of new genetic information on plant genomes and for use in trait selection. Randomly Amplified Polymorphic DNA analysis (RAPD) can be used to identify many useful polymorphisms quickly and efficiently, and as such, it has tremendous potential for use in cultivar identification. RAPD analysis has been used to study genetic relationships in a number of fruit trees including almond (Bartolozzi et al., 1998), plum varieties (Ortize et al., 1997), peach varieties (Chaparro et al., 1994; Warburton and Bliss, 1996), peach rootstocks (Lu et al., 1996) and RAPD markers have been used in peach genetics and breeding programs (Rajapakse et al., 1995).

The protein banding patterns of the selected Peach lines of north Sinai comprise five major bands as well as a number of minor bands. The major bands were common among all the different leaf Peach of the selected trees, which confirmed the close relationships among them. These major bands exhibited pronounced variation in their intensities and densities among all the examined peach samples. Javeri and Wicker (1991) and Mansour et al., (1998). On the other hand, Mansour et al., (1999) and Hassan et al., (2002) fingerprinted eleven peach (Prunus persica L.) selections representing six local strains growing at different locations by SDS-proteins. The strains are Kulaby,Sultani, Hegazy, Shamy, Fark, Mawy and Neely. Low level of polymorphisms was scored in the protein profiles of the studied peach selections.

The study aimed to deduce the reflection of the morphological and fruit characteristics at the molecular level, beside to evaluate their characters in Kalubia governorate and establish mother plants farm (germplasm collection). Additional; objective of this study was to obtain DNA fingerprints of those strains using RAPD, and ISSR-PCR techniques.

2. MATERIALS AND METHODS

During 2010 and 2011 seasons a wide survey was made on some peach strains well common in Dakahlia governorate: Dakahlia (K3), Sultany1(K25), Sultany2(K40), Mawy (K21) and Kulaby(K55) as well as in north Sinai: early Sinai(C9), late Sinai1 (L1), late Sinai2 (2L), late Sinai3 (X2) and late Sinai4 (X4). These strains were 8- years- old, planted in Kalubia governorate (El-Kanater Res. Station) at 3x4 m apart on clay loamy soil.

The present survey included date of full bloom, petal fall, fruit set and harvest time. Also, percentages of vegetative growth, flower growth and fruit set were calculated. Yield parameters (fruit yield/tree and feddan, as well as, number of fruits/tree) were estimated at picking date. Fruit quality as: fruit shape, peak, flesh dimensions (cm), fruit flesh and stone weight (gm), flesh thickness (cm), fruit firmness(Lb/inch2), fruit juice T.S.S (%) and acidity (%) were assisted on ten fruit sample/tree (According to A.O.A.C,1985).

2.1.Materials:

2.1.1.Samples for Protein extraction

Samples of squash leaves were taken from ten peach selected strains.

2.1.2.Samples for RAPD-PCR Analysis

Yong and freshly excised leaves were collected separately for each peach selected strain. Then DNA extraction was performed as described by Dellaporta et al. (1983).

2.1.3.Primers:

Table (1) lists the base sequences of these DNA primers that produced informative polymorphic bands. Only five primers succeeded to generate reproducible polymorphic DNA products. Table (2) lists the base sequences of these DNA primers that produced informative polymorphic bands.

2.2.Methods:

2.2.1. **Protein extraction**

Total soluble protein were extracted by grounding 0.25g of each sample in 0.9 ml extraction buffer (10ml 0.5MTris pH6.8, 16ml 10% SDS, 30ml D.W) with shaking thoroughly. The extracts were transferred to Eppendorf tubes and centrifuged for 10 min. at 10000 rpm under cooling. Supernatant were

transferred by fresh tubes and used for SDS-PAGE analysis and extraction of isozymes was used as described by Jonathan and Weeden, (1990).

Table (1): List of the used RAPD primers, names and their nucleotides sequences.

No.	Name	Sequence	No.	Name	Sequence
1	OP-A10	5' TCGGCCATAG 3'	4	OP-L12	5' GGG CGG TAC T 3'
2	OP-A19	5' CCTTGACGCA 3'	5	OP-L16	5' GGACCCAACC 3'
3	OP-D07	5' GTGACCCCTC 3'			

Table (2): List of the used ISSR primers, names and their nucleotides sequences.

No.	Primer	Sequence	No.	Primer	Sequence
1	HB10	5' GTGTGTGTGTGTGG 3'	4	HB13	5' GAGGAGGAGGC 3'
2	HB11	5' GAGAGAGAGAGACC 3'	5	HB15	5' CTCCTCCTCGC 3'
3	HB12	5' CACCACCACGC 3'			

Protein related index:

Fractionation electrophoresis was performed under identical conditions on sodium dedocyl sulphate polyacrylamide gel (SDS-PAGE) (12%W/V) vertical slab using BIORAD Techware 1.5 mm according to the method of Laemmli (1970) as modified by Studier (1973). The molecular weights of proteins were estimated relative to marker, a wide range molecular weight protein (Fermentas com.).

***Isozymes electrophoresis*:**

Native–polacrylamide gel electrophoresis (Native-PAGE) was performed in 12% (W/V) slab gel (Davis, 1964). The gel was stained after run according to Tankesley and Rick (1980) for Poly Phenyl Oxidase (PPO) isozymes and Graham et al .(1964) peroxidase isozymes. The staining gel was incubated at 37 °C in dark for complete staining after adding the appropriate substrates and staining solutions.

Gel documentation:

Gels were photographed scanned, analyzed using Gel Doc Vilber Lourmat system to capture the image and to calculate band intensities.

2.2.2. RAPD-PCR Analysis

- DNA Extraction

About 0.1 gm (fresh weight) of plant tissues was ground to fine powder in liquid N2 in a mortar. Before the tissue thawed, 1 ml extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA and 0.5 M NaCl) and 0.2 ml 20% SDS were added. The mixture was incubated at 65 ◌C in water bath for 20 minutes. Then 1 ml of phenol, chloroform and isoamyl alcohol (25: 24: 1) was added. Centrifugation was performed at 10,000 rpm for 10 minutes. The supernatants of each sample were transferred separately to new tubes, and then 1 ml of chloroform and isoamyl (24: 1) was added. Centrifugation was performed at 10,000 rpm for 10 minutes. The supernatants of each sample were transferred separately to a new tube, then 1 ml of isopropanol was added and then kept overnight in a freezer. Centrifugation was performed at 10,000 rpm for 10 minutes. The resulted pellets containing DNA were re-suspended in 1 ml ethanol. Centrifugation was performed at 10,000 rpm for 2 minutes. The DNA pellets were re-suspended in 200 (l TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) buffer. DNA was quantities by quantitatively determined and gel electrophoresis.

a-RAPD -PCR Analysis

Polymerase Chain Reaction (PCR).

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, MgCl2 and Taq polymerase. A total of twenty random DNA oligonucleotide primers were independently used according to Williams et al. (1990) in the PCR reaction. Only five primers succeeded to generate reproducible polymorphic DNA products.

The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 96 ºC for 30 seconds, 37 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 5 minutes. PCR products were run at 100 V for one hour on 1.4 % agarose gels to detect polymorphism between peach strains under study. After electrophoresis, the RAPD patterns were visualized with UV transilluminator. RAPD markers were scored from the gels as DNA fragments present or absent in all lanes. Gels were photographed using a Polaroid camera.

b-ISSR-PCR Analysis

Polymerase Chain Reaction (PCR).

ISSR-PCR reactions were conducted using five primers. Amplification was

conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl Mgcl2 (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of Primer (10 pmol), 3.0 µl of template DNA (25 ng/ µl), 1 µl of Taq polymerase (1U/ µl) and 12.5 µl of sterile dd H2O. the PCRs were programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94 ºC, 1 min at 57 ºC, and 2 min at 72 ºC the reaction was finally stored at 72 ºC for 10 min. the PCR products were separated on a 1.5 % agarose gels and fragments sizes were estimated with the 100bp ladder marker.

2.2.3. Statistical analysis:

A randomized complete block design was adopted for the present trial data and statistically analysed by the factorial method according to Snedecor and Cochran (1990) where L.S.D test was used for comparison between means. The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the studied peach strains . Calculation was achieved using Dice similarity coefficients (Dice, 1945) as implemented in the computer program SPSS-10.

3. RESULS AND DISCUSSION

3.1.Growing Manners:

Data in Tables (3 and 4) revealed that, K21 strain had earlier full bloom (5-6 Feb.) and petal fall (5-6 Feb.) (13-14 Feb.) in both studied seasons. The strains K21, K25, X2 and X4 had earlier fruit set (21-23 Feb.). While, K40 and L1 were the latest (6-7 Mar.). However, harvesting time was 23-25 Apr. for K21 and K55 while was 1-3 May for C9,K3,K25, K40,L1, 2L,X2 and X4 strains. Also, the highest fruit set (Table 4) has recorded to K25 (40.27- 45.8%). While, the opposite was for L1 and K21 (28.0-28.33). Moreover, higher vegetative growth was noticed with K21(23.83-28.17) but the lower vegetative growth was with C9 and K40 strains. However,C9, K40 and X2 have higher flower percentage while, K3 and K21 have lower percentage (13.4-15.83%).

3.2. Fruit Quality Attributes:

All studied peach strains have round fruit shape (Tables 5 and 6) with trace peak (K21 and K55), small (K3, X4 and K40) or medium (C9, K25, L1, 2L and X2). The skin color was yellowish green (K40 and K55), light green with red color (K21) or greenish yellow (C9,K3,K25, X2 and X4). Moreover, fruits of L1 strain have orange color, while 2L strain were yellow red color. Also, the flesh color was white (K21, K25, K40, K55 and X4) or creamy (C9, K3,2L and X2)or orange (L1). However, the red color percentage on fruit skin was 20-40% (K25), 30-40% (C9, K21 and K40), 40-50% (X2) or 40-60% (K3, K55 and L1). In addition to the mean fruit weight was 67.67 – 99.8g, where 2L and X2 recorded heavier weight (96.47 – 101.1g) in 2010 and (96.17 – 99.8g) in 2011 seasons respectively. At harvest time the fruit firmness was 16.05 Ib/inch2 (L1) in the 1st season as well as was from 16.36 (C9) Ib/ inch2 for the 2nd season. The strains L1, 2L and K40 recorded the highest T.S.S. (12.41, 11.67 and 11.3) in the 1st season and (12.27,11.23 and 11.08) for L1,K40 and 2L respectively in the 2nd season. While, K25 recorded the latest T.S.S. (10.17%) in the 2010. Acidity percentage in the fruit juice was less in K21, X2, X4 and L1 but was higher in K25 and K55.

3.3.Yield Components:

This component includes number of fruits/tree, fruit yield/tree and/feddan (Table 7). However, number of fruits on K55,X2, X4 and K21 trees were more than the other studied strains while, L1,C9 and K40 recorded the least fruits (220-230), fruit yield was superior on 2L, K55 and X2 (28.55 – 32.25Kg/tree).

The present results revealed that K21 strain (Mawy) privileged with earlier full bloom, petal fall, fruit set and early harvesting time (23 Apr.). It also, has superior vegetative growth percentage, more fruits on the tree and ideal round fruit shape with trace peak. The fruits of K21 strain was light green with 30-40% red color with white flesh which has less acidity percentage (0.767%).

The strain K3 (Dakahli) is preferred with late full bloom, petal fall, fruit set and late harvesting time (2-17May). The fruits of K3 have round shape with small peak. The fruits have greenish yellow skin and creamy flesh with 40-60% red color on the skin. At harvest time K3 strin has firm fruits with higher T.S.S. and less acidity percentages.

Late Sinai1 strain (L1) considered an excellent Late peach strain with orange skin and flesh color as well as with higher T.S.S. and lower acidity. Moreover, 2L, K55 and X2 strains (late Sinai2, Kulaby and late Sinai3) have highest yield and number of fruits on the tree. The fruits are yellow red, yellowish green or greenish yellow, round with trace peak or medium. These strains have heavier and medium fruits and the flesh is white or creamy with high, medium and lower acidity percentages respectively.

However, there was a recommendation to peach growers to establish some superior Dakahlia and Sinai peach strains where they have attractive fruits with special taste and aroma, as well as, big adaptability for handling and storageability with vigorous trees and resistant to drought specially on Almond rootstock (Mehana et al., 1982; Stino et al., 1982;Mansour and Shaltut, 1986; Messquer et al., 1987; Mohamed,1995;El-Said et al., 1997; Fahmy and Abou El-Nasr, 1998; Mansour et al., 1998; Hassan et al.,2002, Mansour et al.,1999; and Eliwa, 2005.

Table (3): Different stages of flowering and Harvesting time date for ten peach strains in 2010 and 2011 seasons.

	Season 2010			
Strains Character	Full Bloom	Petal Fall	Fruit Set	Harvesting Time
C9	11Feb – 18 Feb	18 Feb – 26 Feb	1Mar – 10 Mar	1 May – 17 May
K3	19Feb – 24 Feb	24 Feb – 3 Mar	3Mar – 8 Mar	2 May – 17 May
K21	6Feb – 13 Feb	13 Feb – 21 Feb	21 Feb – 1 Mar	23 Apr – 9 May
K25	12Feb – 17 Feb	17 Feb – 20 Feb	21 Feb – 1 Mar	1 May – 16 May
K40	19Feb – 25 Feb	25 Feb – 4 Mar	6Mar – 9 Mar	1 May – 15 May
K55	14Feb – 22 Feb	22 Feb – 28 Feb	1Mar – 10 Mar	25 Apr –10 May
L1	21Feb – 28 Feb	28 Feb – 6 Mar	7Mar – 15 Mar	3 May – 17 May
2L	18Feb – 24 Feb	24 Feb – 28 Feb	1Mar – 10 Mar	2 May – 17 May
X2	13Feb – 19 Feb	19 Feb – 21 Feb	22Feb – 28Feb	1 May – 16 May
X4	14Feb – 19 Feb	19 Feb – 22 Feb	22Feb – 1 Mar	1 May – 16 May
	Season 2011			
	Full Bloom	Petal Fall	Fruit Set	Harvesting Time
C9	10Feb – 17 Feb	17 Feb – 26 Feb	28 Feb – 9 Mar	1 May – 17 May
K3	17Feb – 23 Feb	23 Feb – 3 Mar	3Mar – 8 Mar	2 May – 17 May
K21	5Feb – 13 Feb	14 Feb – 21 Feb	21Feb – 1 Mar	23 Apr – 9 May
K25	11Feb – 17 Feb	17 Feb – 21 Feb	22 Feb – 1 Mar	1 May – 16 May
K40	18Feb – 25 Feb	25 Feb – 4 Mar	5Mar – 9 Mar	1 May – 15 May
K55	15Feb – 22 Feb	22 Feb – 28 Feb	1Mar – 10 Mar	25 Apr –10 May
L1	20Feb – 27 Feb	27 Feb – 5 Mar	6Mar – 15 Mar	2 May – 17May
2L	17Feb – 23 Feb	23 Feb – 28 Feb	1Mar – 9 Mar	1 May – 18 May
X2	13Feb – 18 Feb	18 Feb – 22 Feb	21 Feb – 28 Feb	3 May – 15 May
X4	13 Feb- 19 Feb	19Feb- 23 Feb	22 Feb- 1Mar	1May – 15 May

Table (4): Percentage of fruit set, vegetative growth and flower growth for ten peach strains in 2010 and 2011 seasons.

Peach Strains	Percentage of Flower Growth %		Percentage of Vegetative Growth %		Percentage of Fruit Set %	
	2010	2011	2010	2011	2010	2011
C9	22.9 BC	22.93 A	11.53 D	12.87 D	37.0 B	37.6 AB
K3	13.4 G	14.93 D	18.2 C	20.33 BC	32.0 CDE	38.0 AB
K21	14.73 G	15.83 D	28.17 A	23.83 A	28.33 EF	30.33 EF
K25	17.53 F	18.17 C	18.77 C	19.0 BC	45.8 A	40.27 A
K40	25.97 A	24.47 A	11.03 D	12.0 D	31.35 DE	34.87 BC
K55	21.3 CD	23.83 A	16.73 C	17.67 C	34.17 BCD	37.77 AB
L1	21.9 C	23.9 A	20.57 BC	19.0 BC	28.0 EF	31.57 CDEF
2L	18.97 EF	18.97 BC	23.9 AB	23.67 A	28.57 E	35.5 BC
X2	24.07 AB	25.07 A	20.57 BC	21.0 AB	30.17 DE	32.97 CDE
X4	19.8 DE	20.63 B	21.37 BC	21.0 AB	35.93 BC	34.67 BCD

Table (5): Fruit Characters for ten peach strains in 2010 season.

Characters	Fruit Shape	Peak	Skin Color	Flesh Color	Red Color in Skin (%)	Fruit diameter (cm)	Fruit Length(cm)	Fruit Weight (g)	Flesh Weight (g)	Flesh Thickness (cm)	Stone Weight (g)	Firmness (lb/inch)2	T.S.S (%)	Acidity (%)
C9	Round	Medium	Greenish Yellow	Creamy	30-40	5.3 CDE	5.633 DE	83.47 C	78.58 C	1.7 AB	4.883 DE	15.87 A	11.27 BCD	0.847 BC
K3	Round	Small	Greenish Yellow	Creamy	40-60	5.0 E	5.1 G	81.1 C	76.97 C	1.6 D	4.127 G	14.98 AB	10.83 DE	0.813 CD
K21	Round	Trace	Light green with red color	White	30-40	5.217 DE	5.517 EF	83.43 C	79.17 C	1.617 CD	4.267 FG	13.63 BC	10.77 E	0.767 DEF
K25	Round	Medium	Greenish Yellow	White	20-40	5.097 E	5.25 FG	84.17 C	79.87 C	1.6 D	4.3 FG	9.987 E	10.17 F	0.953 A
K40	Round	Small	Greenish Yellow	White	30-40	5.3 CDE	5.883 CD	85..0 C	79.62 C	1.683 ABC	5.383 BC	11.77 D	11.3 BCD	0.88 B
K55	Round	Trace	Greenish Yellow	White	40-60	5.55 BC	5.5 EF	83.73 C	79.3 C	1.717 AB	4.433 EFG	12.26 CD	11.02 DE	0.907 AB
L1	Round	Medium	Orange	Orange	40-60	5.133 E	5.233 FG	70.03 D	65.37 D	1.617 CD	4.667 EF	16.05 A	12.41 A	0.703 G
2L	Round	Medium	Yellow Red color	Creamy	50-60	5.6 BC	6.067 BC	96.47 B	89.92 B	1.7 AB	6.55 A	14.92 AB	11.67 B	0.81 CDE
X2	Round	Medium	Greenish Yellow	Creamy	40-50	5.8 AB	6.217 AB	101.1 A	95.4 A	1.767 A	5.733 B	8.767 E	11.1 CDE	0.75 EFG
X4	Round	Very Small	Greenish Yellow	White	20-30	5.467 CD	5.817 CDE	87.4 C	82.22 C	1.667 BCD	5.183 CD	10.27 E	10.83 DE	0.733 FG

Table (6): Fruit Characters for ten peach strains in 2011 season.

Characters	Fruit Shape	Peak	Skin Color	Flesh Color	Red Color in Skin (%)	Fruit diameter (cm)	Fruit Length(cm)	Fruit Weight (g)	Flesh Weight (g)	Flesh Thickness (cm)	Stone Weight (g)	Firmness (lb/inch)2	T.S.S (%)	Acidity (%)
C9	Round	Medium	Greenish Yellow	Creamy	20-40	5.3 DE	5.58 CD	81.87 C	76.98 D	1.7 AB	4.89 DEF	16.36 A	10.83 CD	0.843 BC
K3	Round	Small	Greenish Yellow	Creamy	40-50	5.0 E	5.08 D	79.8 C	75.69 D	1.6 C	4.12 G	15.16 AB	10.83 CD	0.833 CD
K21	Round	Trace	Light green with red color	White	30-40	5.32 DE	5.5 CD	83.27 C	79.07 D	1.6 C	4.2 G	14.17 B	10.23 F	0.767 CDE
K25	Round	Medium	Greenish Yellow	White	20-30	5.07 E	5.23 D	85.17 C	80.9 D	1.63 C	4.2 G	10.46 D	10.5 EF	0.937 A
K40	Round	Small	Greenish Yellow	White	30-40	5.3 DE	5.87 BC	84..1 C	78.77 D	1.65 BC	5.35 CD	11.91 CD	11.23 B	0.853 BC
K55	Round	Trace	Greenish Yellow	White	40-50	5.55 BC	5.47 CD	83.2 C	78.74 D	1.7 AB	4.46 FG	12.4 C	10.68 DE	0.92 AB
L1	Round	Medium	Orange	Orange	40-60	5.98 E	5.23 D	67.67 D	62.99 E	1.6 C	4.68 EFG	16.58 A	12.27 A	0.75 DE
2L	Round	Medium	Yellow Red color	Creamy	45-55	5.6 BC	6.0 ABC	96.17 B	89.58 B	1.7 AB	6.59 A	15.09 AB	11.08 BC	0.85 BC
X2	Round	Medium	Greenish Yellow	Creamy	40-50	5.8 A	6.18 A	99.8 A	94.02 A	1.75 A	5.78 B	8.75 E	10.33 F	0.75 DE
X4	Round	Very Small	Greenish Yellow	White	20-30	5.40 DE	5.8 BC	86.17 C	80.98 C	1.65 BC	5.19 DE	10.46 D	9.83 G	0.767 CDE

Table (7): Fruit yield per tree, number of fruits/tree and fruit yield ton/feddan for ten peach strains in 2010 and 2011 seasons.

Peach Strains	Yield/tree (Kg)		No. of Fruits/ tree		Fruit Yield Ton/Fed.	
	2010	2011	2010	2011	2010	2011
C9	19.2DE	20.47FG	230E	250D	6.72DE	7.16FG
K3	21.0CDE	21.15EF	260CDE	265CD	7.35CDE	7.4EF
K21	26.65BCD	27.20DEF	286.7BCD	326.7AB	9.33BCD	9.52CD
K25	21.04CDE	21.77DEF	250DE	255CD	7.36CDE	7.62DEF
K40	19.36CDE	19.79FG	230E	235D	6.78DE	6.92FG
K55	29.19AB	29.33AB	350A	353.3A	10.22ABC	10.26BC
L1	15.32E	15.27G	220E	226.7D	5.36E	5.34G
2L	28.55B	28.54BC	296.7Bc	296.7DC	9.99AB	9.99C
X2	32.25A	31.62A	320AB	316.7AB	11.29A	11.06A
X4	26.79BC	26.70CDE	306.7AB	310AB	9.38BCD	9.35CDE

3.4. SDS-Protein electrophoresis

Seed protein electrophoresis as well as leaf proteins provide valuable evidence for taxonomic and evolutionary relationships of plant species (Yates et al., 1990). It is worthy to note that leaf protein profiles are often species – specific, highly stable and unlikely to be not influenced by environmental conditions and seasonal fluctuations.

The electrophoretic banding pattern of proteins extracted from leaves of peach strains are shown in Figure (1) and their denistrometric analysis are illustrated in Table (8) the presence and absence of band were assessed with (1) and (0), respectively.

Results of leaves SDS-PAGE revealed a total number of 17 bands with molecular weight (MW) ranging from about 10.0 to 120.0 KDa. Analysis of data showed thirteen common bands while, the remaining four bands were polymorphic with 23.5 % polymorphism.

3.5.Peroxidase banding patterns:

Figures (2 and 4) represent peroxidase electrophoresis banding patterns among examined fresh leaves of the peach strains.

Data being represented explain that, total of five bands were characterized for the studied samples, all of them (monomorphic) were present in all treatments with differences in banding patterns density and they could be considered as common bands.

3.6.Poly phenyl Oxidase banding patterns:

Figures (3 and 5) demonstrated Poly Phenyl Oxidase (PPO) banding patterns among examined leaves of the peach strains. Obtained patterns exhibited three bands, all of them were present in all cultivars (monomorphic) with differences in banding patterns density which could be considered as common band for all peach strains.

3.7.Molecular genetic identification

Randomly amplified polymorphic DNA (RAPD) markers

Data of the amplified fragments using those five 10-mer arbitrary primers for the Okinawa rootstock and ten selected peach succeeded in amplifying DNA fragments Table (10) and plate (1). Polymorphism levels differed from one primer to the other. Primer (OP-C18) exhibited low polymorphism (35.7%). On the other hand, primers OP-A07 (47.0%) and OP-C05 (50.0 %) exhibited moderate levels polymorphism. While, primer, OP-B01 (64.2%) exhibited high levels polymorphism.

Primer OP-A07 resulted in seventeen bands with molecular sizes from 240 to 13500bp (plate 1). Eight bands were polymorphic (47.0%) and the other nine bands were present in all samples which considered as common bands, Primer OP-B01 indicated the amplification of fourteen bands with molecular size range from 180-1050bp , nine bands of them were polymorphic (64.2 %) while, the other five bands were present in all cultivars which considered as common bands. Primer OP-C05 indicated the amplification of fourteen bands with molecular weight size range from 220 -1250bp, seven bands were polymorphic (50.0 %) and the other seven bands were present in all strains which considered as common bands, primer OP-C18 resulted in fourteen DNA fragments with molecular weight ranging in 120-1340bp, five bands were polymorphic (35.7 %) and the other nine bands were present in all cultivars which considered as common bands. Primer OP-D07 resulted in

twelve DNA fragments with molecular weight ranging in 170-1335bp, seven bands were polymorphic (42.8 %) and the other five bands were present in all strains which considered as common bands.

Inter Simple Sequence Repeats (ISSRs)

Data of the amplified fragments using those five ISSR primers for the Okinawa rootstock and ten selected peach succeeded in amplifying DNA fragments (plate 2) Polymorphism levels differed from one primer to the other. Three primers HB-10 (46.6%), HB-11 (50.0%) and HB-12 (50.0%) exhibited moderate polymorphism, while, primer HB-13 (58.8%) exhibited high level of polymorphism and primer HB-15 (27.2 %) exhibited low level of polymorphism.

There were some specific fragments which discriminated each strain from the others as follows:-

Primer HB-10 showed fifteen DNA fragments with molecular weight ranging in 170-1460bp, seven bands were polymorphic (46.6 %). Primer HB-11 showed sixteen DNA fragments with molecular weight ranging in 95-1360bp, eight bands were polymorphic (50.0 %). Primer HB-12 showed fourteen DNA fragments with molecular weight ranging in 60-1010bp, seven bands were polymorphic (50.0 %). Primer HB-13 showed seventeen DNA fragments with molecular weight ranging in 115-890bp, ten bands were polymorphic (58.8 %). Primer HB-15 showed eleven DNA fragments with molecular weight ranging in 210-102bp, three bands were polymorphic (27.2 %) and the other seven bands were present in all strains which considered as common bands.

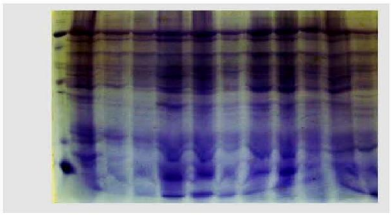

Fig. (1): SDS-protein banding patterns of leaf proteins from Okinawa rootstock and ten selected peach strains.

Table (8) : Densitometric analysis for SDS leaf proteins from Okinawa rootstock and ten selected peach strains.

Bnad No.	M.W KDa	OK	Peach Strains									
			1	2	3	4	5	6	7	8	9	10
1	120	1	1	1	1	1	1	1	1	1	1	1
2	116	1	1	1	1	1	1	1	1	1	1	1
3	96	1	1	1	1	1	1	1	1	1	1	1
4	85	1	1	1	1	1	1	1	1	1	1	1
5	70	1	1	1	1	1	1	1	1	1	1	1
6	65	1	0	0	1	1	0	0	1	1	1	0
7	60	1	1	1	1	1	1	1	1	1	1	1
8	55	1	1	1	0	1	1	1	0	1	1	1
9	40	1	1	1	1	1	1	1	1	1	1	1
10	35	1	1	1	1	1	1	1	1	1	1	1
11	30	1	0	0	1	1	1	1	1	1	1	1
12	28	1	1	1	1	1	1	1	1	1	1	1
13	25	1	1	1	1	1	1	1	1	1	1	1
14	20	1	1	1	1	1	1	1	1	1	1	1
15	16	1	1	1	1	1	1	1	1	1	1	1
16	12	1	1	1	1	1	1	1	1	1	1	1
17	10	1	1	1	1	1	1	1	1	1	1	1
Total		17	15	15	16	17	16	16	16	17	17	16

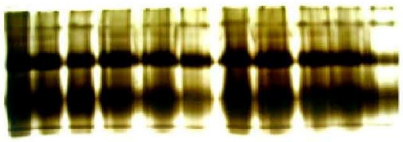

Fig. (2): Peroxidase isozyme banding patterns of leaf from Okinawa rootstock and ten selected peach strains.

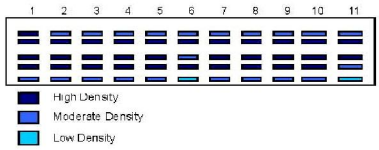

Fig. (4): Edeogram analysis for leaf Peroxidase isozyme banding patterns from Okinawa rootstock and ten selected peach strains.

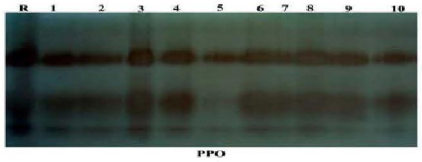

Fig. (3): Poly phenyl Oxidase isozyme banding patterns of leaf from Okinawa rootstock and ten selected peach strains.

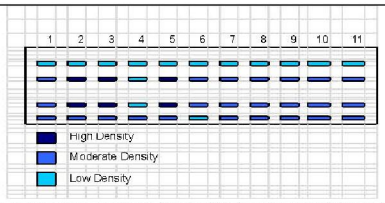

Fig. (5): Edeogram analysis for leaf Poly phenyl Oxidase isozyme banding patterns from Okinawa rootstock and ten selected peach strains.

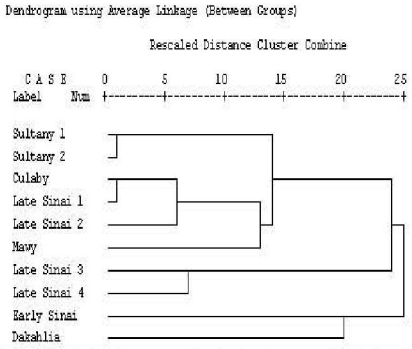

Fig. (6): A dendrogram illustrates the genetic distance between ten selected peach strains based on protein isozymes , RAPD and ISSRdata.

Table (9): Similarity index (Pairwise comparison) of ten selected peac based on protein isozymes , RAPD and ISSR data..

	1	2	3	4	5	6	7	8	9
1	-								
2	0.5								
3	0.5	0.3							
4	0.9	0.7	0.2						
5	0.9	0.9	0.5	0.0					
6	0.9	0.5	0.4	0.2	0.3				
7	0.6	0.4	0.1	0.1	0.2	0.0			
8	0.6	0.3	0.3	0.5	0.5	0.1	0.1		
9	0.8	0.6	0.5	0.8	0.8	0.6	0.4	0.2	
10	0.7	0.5	0.6	0.9	1.0	0.6	0.5	0.5	0.1

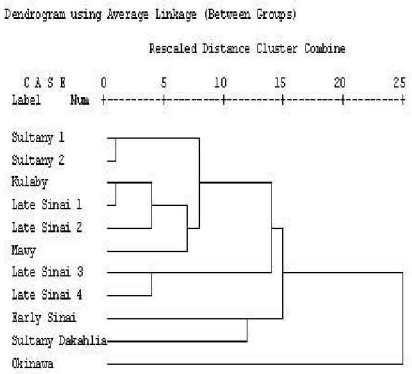

Fig. (7): A dendrogram illustrates the genetic distance between Okinawa rootstock and ten selected peach strains based on protein isozymes , RAPD and ISSR data

Table (10): Similarity index (Pairwise comparison) of Okinawa rootstock and ten selected peach based on protein isozymes , RAPD and ISSR data.

	Okin	1	2	3	4	5	6	7	8	9
1	0.9									
2	0.8	0.3								
3	0.8	0.4	0.2							
4	0.9	0.6	0.5	0.1						
5	1.0	0.7	0.7	0.3	0.0					
6	0.7	0.7	0.4	0.3	0.1	0.2				
7	0.6	0.4	0.3	0.1	0.1	0.1	0.0			
8	1.0	0.5	0.2	0.2	0.2	0.4	0.1	0.0		
9	0.9	0.5	0.4	0.3	0.6	0.6	0.4	0.3	0.1	
10	0.3	0.5	0.4	0.4	0.6	0.7	0.4	0.3	0.3	0.3

Overall genetic relationship analysis based on protein, Isozymes, RAPD and ISSR markers between Peach strains.

The Overall genetic relationship based on protein, isozymes, RAPD and ISSR data were used to estimate the genetic similarity among the ten selected peach strains by using UPGMA computer analysis (Table 9). The highest similarity index was recorded (1.0), between Late Sinai4 and Sultany2.While, the lowest similarity index was recorded (0.0) between Kulaby and Late Sinai1 and also between Sultany 1 and Sultany2.

A dendrogram for the genetic relationship among the ten selected peach strains was carried out as shown in Fig. (6) which separated them into two major groups. The first main group included Dakahlia and Early Sinai, while the second main group divided into two sub groups, the first sub group included each of Late Sinai3 and Late Sinai4 only and the other sub group included all other strains (Sultany1, Sultany2, Kulaby, Late1 Late Sina2 and Mawy.

Overall genetic relationship based on protein, Isozymes, RAPD and ISSR markers between Okinawa roostock and Peach strains.

The Overall genetic relationship based on protein, isozymes, RAPD and ISSR data were used to estimate the genetic similarity among Okinawa rootstock and the ten selected peach strains by using UPGMA computer analysis (Table 10). The highest similarity index was recorded (1.0), between Okinawa and Each of Sultany2 and Late Sinai2 strains. While, the lowest similarity index was recorded (0.0) between Sultany 1 and Sultany2 and also between Kulaby and Late Sinai1.

A dendrogram for the genetic relationship among the ten selected peach strains was carried out as shown in Fig. (7) which separated them into two major groups. The first main group included Okinawa only,

while the second main group divided into two sub groups, the first subgroup included Early Sinai and Dakahlia only and the other sub group included all other strains (Sultany1, Sultany2, Kulaby, Late Sinai1, Late Sina2, Mawy, Late Sinai3 and Late Sinai4). Our studies indicated that protein and isozymes, RAPD and ISSR techniques are useful in the establishment of the genetic fingerprinting and estimation of relationships among Okinawa rootstock and ten selected peach strains. Also, these techniques could detect enough polymorphism in the Okinawa rootstock and ten selected peach strains to distinguish each strain from the others by at least band or group of combined banding pattern. Furthermore, the use of these data in the future is important for Rootstock and ten selected peach germplasm management, improvement as well as for the selection strategies of parental lines that facilitate the prediction of crosses in order to produce hybrids with higher performance (Mansour et al., 1998; Mansour et al., 1999; Hassan et al., 2002). In general the overall results indicated the possible use of protein, isozymes, RAPD and ISSR analysis to detect species - specific and characteristic specific markers for the Okinawa rootstock and ten selected peach strains that can be used to discriminate among the strains to detect genetic relationships among these strains which can be used in breeding programs. The molecular genetic studies are efficient tools for the characterization of these strains and also, more studies are very important to evaluate it from nematode resistance and it is ability to success under different environmental conditions.

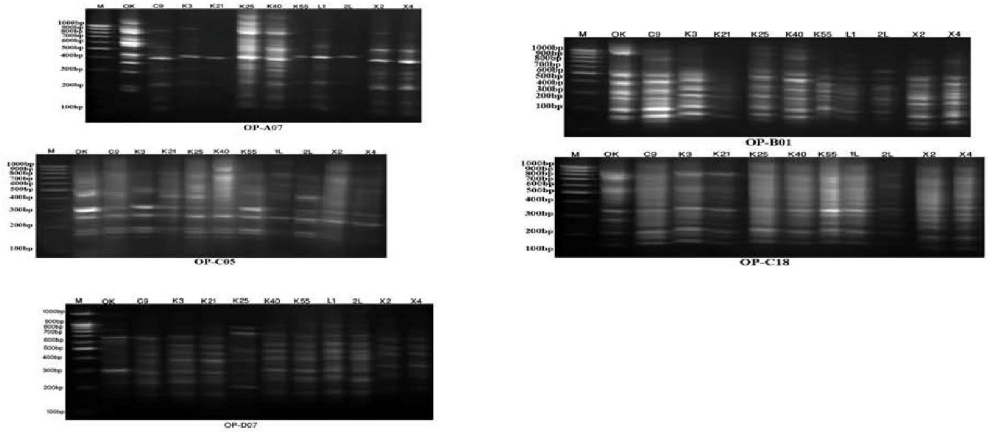

Plate (1): RAPD profiles of Okinawa rootstock and ten selected peach strains. amplified with five primers.

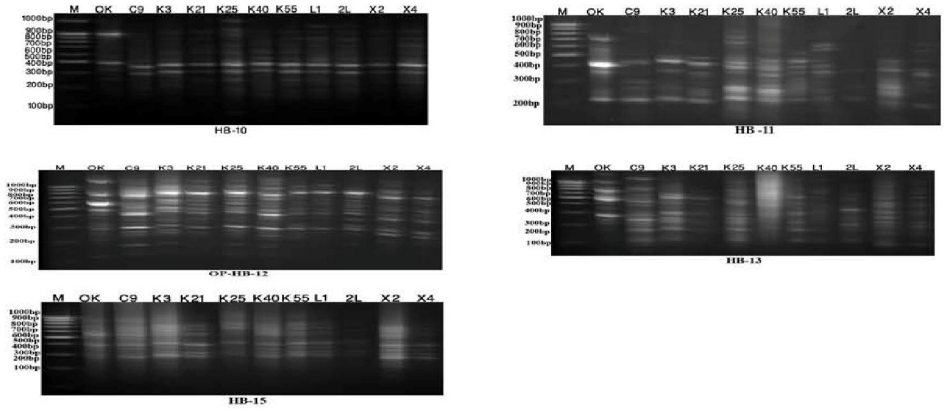

Plate (2): ISSR profiles of Okinawa rootstock and ten selected peach strains. amplified with five primers.

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