PHYLOGENETIC RELATIONSHIPS BETWEEN SOME *Prunus* SPECIES USING MORPHOLOGICAL AND MOLECULAR MARKERS

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ABSTRACT

Morphological studies of Leaf characteristics (leaf blade area, Leaf blade length, Leaf blade width, Leaf blade length/ blade width, Leaf petiole length). Meanwhile, fruit characteristics (fruit weight. flesh weight the flesh/stone ratio stone weight polar length, cross length, polar diameter and cross diameter) and Chemical properties (TSS, juice ascorbic acid) have been studied on mature tree of 17 accessions for Prunus species were studied during the two successive seasons of 2004 and 2005. However such characteristics give no clear differentiation between these accessions. On the other hand some DNA bands were found to be unique for some accessions. (primer OPG-05/0.88 showed correlation with high TSS content, low anthocynin and carotene in Canino and Amar apricots. The phylogenetical data, based on three biochemical markers (total soluble proteins, peroxidase and esterase isozyme variants) and nucleic acid-based (RAPD) indicated that the examined Prunus accessions, can be clasyfied into five main taxonomical groups: the first, included (TropicSnow, Desertred and Sweeling peches); the second included 3 accessions (Nectarine 1GC/100, Nectarine 966 and Nectarine 24/90); the third included 2 accessions (Canino and Amar apricots); the fourth group included 3 accessions (Early Sweet, Santa Rosa and Beauty plums); and the fifth group included 6 accessions (Flordaprince, Flordastar, Flordaglo, Florda 834, Early Grande and Yellow peach).

Key words:, Prunus species, Morphological characteristics, Chemical properties, Molecular, Biochemical, Phylogenetica.

INTRODUCTION

The family *Rosaceae* includes many species of a great economical importance, among them the genus *Prunus,* that include, many important species such as peaches, apricots, ... etc, which are thrive well in temperate as well as warm regions to some extend. The genus *Prunus* (*Rosaceae*, subfamily *Prunoidaeae)* comprises five subgenera, *Prunophora, Amygdalus, Cerasus, Padus,* and *Laurocerasus.* Redher (1954) recognized 77 species, although Sauer (1993) suggest that as many as 150 species may exist. Classification is generally based on morphology, although chromosome counts are available for many of the species.

The total acreage of stone fruits in Egypt was estimated as 116710 feddans producing 490602 tons. The total acreage of peach is about 76784 feddans, producing 339266 tons , the total acreage of plum is about 3603 feddans, produced about 19763 tons and the total apricot area reach 20091 feddans, with a production of 103070 tons of fresh fruit (Agriculture Statistic, Ministry of Agriculture, Part II July, 2003)

The pomology sector in Egypt has experienced a number of problem lead to decline in the production of fruit crops. One of these handicaps is the identifying between species and cultivars that causes low production and quality. In addition, diseases, which attack these cultivars are out of control. Some of these diseases relate to soil or fruit and vegetative growth.. This problem is considered as the first obstacle that faces the commercial yield of high quality fruits.

There are many available traditional curing methods for identifying or distinguishing between species and cultivars that affects fruit trees production which causes low production and quality. However such methods give no clear differentiation between species and cultivars. The possible answer is the use of biotechnological methods for the identification and differentiation between various species and cultivars.

Molecular biology technology has provided new approach for piologists to identify molecular markers linked to economically important traits to be used in crop improvement especially in long – lived perennial species. Several types of molecular markers may be used, protein, isozyme (Liou *et al.,* 1996). and the RAPD technique which provides an innovative technology for DNA maping and fingerprinting.

The present investigation aimed to evaluate physical characteristics and chemical constituents of both fruit and leaf in the genus *Prunus*. Establishment the phylogenetic relationships between some *Prunus* species using the molecular markers (Nucleic acid-based markers (RAPD) and Biochemical markers (isozyme and total soluble proteins).

MATERIALS AND METHODS

This present investigation was carried out during two successive growing seasons of 2004 and 2005 to evaluate nine peach cultivars (*Prunus persica L.*) namely yellow peach, Early Grande(Earligrande), Flordaprince, Flordastar, Flordaglo, Florda 834, TropicSnow, Desertred and Swelling. In addition, three Nectarine cultivars (*Prunus persica* var. *nectarina*, Maxim) namely nectraine1GC/100, nectraine 966 and nectraine 24/90. Furthermore, three plum cultivars (*Prunus salicina* Lindl) namely Early

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Sweet, Santa Rosa and Beauty and two apricot cultivars (*Prunus armeniaca* L.) namely Canino and Amar.

The trees were five year-old, grown in sandy soil budded on Nemaguard rootstock and spaced at 3.5 x 5 meter apart in Pico company, El-Behera Governorate

A- Morphological studies

Leaf characteristics

Twenty fully mature leaves from the middle of tagged shoots (spring growth cycle) were taken at randomly. The blade area, blade length, petiole length, blade width and blade length/ blade width ratio were measured. For leaf area determination samples were taken from the fourth to sixth leaves from the top of the selected shoots

Fruit characteristics

At harvest time for each species, sample of 20 mature fruit were taken at randomly for studying the following physical and chemical properties.

- Physical properties

Physical properties determination depend on picking period, flesh colour, stone freeness , presence or absence of peak, fruit, flesh and stone weight , fruit firmness , fruit dimension (length and diameter)

- Chemical properties

Total Soluble Solid percentage (TSS%), acidity Vitamin C (Ascorbic acid), and Carotene content were determined according to the (A.O.A.C, 1990) and expressed as milligrams ascorbic acid/100 millimeter juice. Anthocyanin content was determined (mg/100 g fresh weight) according to Rabino *et al.*, (1977).

B- Molecular marker studies

Biochemical markers

SDS Polyacrylamide gel electrophoresis (SDS–PAGE) was performed on water soluble protein fractions according to the method of Laemmli (1970) as modified by Studier (1973) .Proxidase and esterase were identified by native –PAGE system according to Stegmann *et al.* (1985).

Nucleic acid – based markers

PCR analysis was carried out using genomic DNA from some *Prunus* species. DNA was isolated from fresh young leaves of terminal shoots for cultivars (accessions) using a Wizard Genomic DNA Purification Kit (Promega, Comp.USA). __Eight primers (Table 1), from Pharmacia Biotech (Amersham Pharmacia Biotech UK Limited, England HP79NA), were tested in this Experiment to amplify the templated DNA.

Table (1) : Primer used in the present study.

Primer	M.W	%GC	Nucleotide Sequence 5′ to 3′
OPG-05/0.88	3157	60	CTGAGACGGA
OPV-08/1.05	3164	70	GGACGGCGTT
OPS-19/1.34	3157	60	GAGTCAGCAG
OPK-01/0.86	3068	60	CATTCGAGCC
OPZ-12/0.47	3117	60	TCAACGGGAC
OPT-07/0.54	3164	70	GGCAGGCTGT
OPM-11/1.24	3099	60	GTCCACTGTG
OPK-11/1.02	3077	60	AATGCCCCAG

Amplification reaction volumes were 25 µl, each containing 1xPCR buffer with $MgCl_2$ (50 mMKCl, 10mM Tris-HCl (pH=9.0) 2mM $MgCl_2$ and 1% triton x-100), 200 µM each of dATP, dCTP, dGTP and dTTP, 50 ng template DNA and 1.5 µ of taq polymerase. Reaction mixtures were overlaid with 15 µl mineral oil and exposed to the following conditions: 94°C for 3 min, followed by 45 cycles of 1 min at , 1 min. at 36 °C, 2 min. at 72 °C, and a final 7 min. extension at 72 °C. Amplification products were visualized with DNA marker (100 bp DNA lader marker.

C- Statistical analysis

Data collected through out the course of the present study were statistically analyzed according to Snedecor and Cochran (1990) and L.S.D test was used for comparison between accessions (varieties). PAST software (Hammer et *al.*, 2003) was used to generate a dendogram for the 17 accessions of *Prunus* species.

RESULTS AND DISCUSSION

Morphological studies:

Leaf characteristics

The data representing the leaf blade area, Leaf blade length, Leaf blade width, Leaf blade length/ blade width, Leaf petiole length of the studied accessions in both 2004 and 2005 seasons are listed in Table (2). the Desert Red peach had significantly higher leaf blade area than other accessions, while Santa Rosa and Beauty plum had higher than the lowest ones. Nectarine 1GC/100 had higher leaf blade length than the other accessions, whereas Early Sweet

Table (2): Average of leaf characteristics, fruit physical characteristics and fruit chemical properties of studied cultivars (taxa) in 2004 and 2005 seasons.

Traits	Leaf blade area (cm^2)	Leaf blade length (cm)	Leaf blade width (cm)	Leaf blade length /blade width	Leaf petiole length (cm)	Fruit weight (gm)	Flesh weight (gm)	Stone weight (gm)	Flesh/ stone ratio	Firmness $Lb/inch^2$	Juice volume (ml)	polar length (cm)	cross length (cm)	polar diameter (cm)	cross diameter (cm)	Polar length / polar diame -ter ratio	Cross length/cross diame-ter ratio
Yellow peach	40.47	14.17	4.07	3.63	1.03	57.20	109.06	1.68	65.03	4.75	53.87	4.75	5.26	5.91	5.54	0.80	0.95
Early Grande	41.81	15.64	3.96	3.95	0.94	121.80	118.55	3.26	37.00	6.63	56.71	5.56	5.86	6.11	5.96	0.91	0.98
Flordaprince	40.94	15.31	3.82	4.0	1.13	121.03	118.76	3.27	35.94	7.34	43.12	5.16	5.53	5.72	5.84	0.93	0.91
Flordastar	40.36	15.10	3.50	4.29	0.93	110.09	107.16	2.94	36.31	6.41	45.75	5.17	5.29	5.50	5.59	0.92	0.95
Flordaglo	46.96	16.60	3.96	4.14	0.93	133.24	125.96	3.79	34.25	7.43	52.5	5.20	5.50	6.10	5.86	0.86	0.94
Florda 834	35.03	14.70	3.82	3.97	0.86	128.02	124.36	3.83	32.50	9.94	46.97	5.31	5.40	6.02	5.94	0.86	0.91
TropicSnow	32.21	13.15	3.72	3.69	1.23	147.03	143.02	3.72	38.37	12.25	79.12	5.56	5.80	6.40	6.25	0.85	0.92
Desertred	40.40	16.65	4.24	3.93	0.84	138.09	133.31	4.83	27.82	11.69	84.62	5.53	6.22	6.35	6.54	0.91	0.95
Swelling	32.11	13.36	2.83	4.65	0.85	129.60	124.89	4.53	28.26	12.12	34.15	5.48	5.63	5.74	5.85	0.93	0.95
Nectarine 1GC/100	50.10	16.57	4.71	3.51	0.83	94.60	90.80	3.78	24.11	11.06	39.75	5.58	5.76	5.34	5.26	1.04	1.09
Nectarine 966	49.74	16.19	4.49	3.61	0.89	90.60	87.64	3.00	29.20	8.85	33.75	5.23	5.85	5.24	5.15	1.05	1.08
Nectarine 24/90	38.82	14.65	3.53	4 17	0.83	96.10	92.87	3.33	28.09	9.26	43.12	5.47	5.30	5.54	5.43	0.92	0.97
Early Sweet	21.54	6.38	4.33	1.48	0.86	22.72	22.17	0.56	39.95	2.92	2.25	3.43	2.86	3.31	3.11	0.84	0.98
Santa Rosa	19.11	6.67	4.10	1.64	1.24	54.60	53.28	1.39	38.43	4.61	28.75	4.06	4.34	4.34	4.23	0.95	1.03
Beauty	19.73	8.16	3.55	2.31	1.6	46.83	43.30	1.03	42.11	5.07	27.55	4.16	4.09	4.08	3.96	1.00	1.03
Canino	42.42	7.10	7.15	0.99	2.99	70.22	68.23	2.04	33.54	6.16	24.87	4.28	4.27	4.49	4.45	0.96	1.00
Amar	25.96	6.22	5.93	1.05	2.93	31.01	29.48	1.52	19.37	4.56	15.87	3.15	3.41	3.07	3.17	1.02	1.06
$LSD_{0.05}$	2.5	0.70	0.29	0.25	0.13	13.46	13.55	0.42	6.07	0.61	4.33	0.36	0.33	0.30	0.30	0.06	0.06

plum and Amar apricot had the lowest ones. Canino apricot had the highest leaf blade width, while Swelling peach had the lowest one. The differences among *Prunns* species , in respect with leaf blade area, were previously reported by Khalil and Abd-Alla (2002) and Eissa (2003) who mentioned that leaf blade area of *Prunus* species varied from one species to another. Swelling peach had higher blade length/blade width than the other accessions whereas Canino and Amar apricot had the lowest ones. In accordance of these results are those previously reported by KyeongHo and Chung (1999) who revealed that the blade width were useful characters for plum identification and understanding of taxonomical relationships .Canino and Amar apricots had higher petiole length than the other accessions, while Flordastar, Flordaglo, Early Garnde, Florda 834 peaches, Early Sweet plum, Nectarine 1GC/100, Nectarine 966, Nectarine 24/90, Swelling and Desertred peaches had the lowest ones. These results are in agreement with those obtained by KyeongHo and Chung (1999) on *Prunus cerasife*ra , *P domestica* and *P salicina.*

Fruit characteristics

The present results indicated that in both seasons, TropicSnow and Desertred fruit were significantly heavier than fruit of other accessions, whereas those of Amar and Early

Sweet showed significantly the lowest average fruit weight. Generally these results are in line with the previous study of (Khalil and Abd-Alla, 2002) which indicated that fruit weight of Amar apricot cultivars was 29.68 and 29.76 gm in 1999 and 2000 respectively.

TropicSnow peach fruit had higher flesh weight than the other accessions, while Early Sweet plum and Amar apricot had the lowest ones. These results are in agreement with those reported by Haikal (2005) on Desertred and Ibrahim *et al.,* (2005) on Amar and Canino apricot cultivars.

The data concerning the flesh/stone ratio of the studied accessions in both 2004 and 2005 On the other hand, the obtained results showed that flesh/stone ratio of Early Sweet, Beauty, TropicSnow, Early Grande, Santa Rosa Flordaprince and Flordastar had significantly higher than the rest accessions. It was also noticed that there were no significant differences found between flesh/stone ratio of Flordastar and Flordaglo and also, between Nectarine 1GC/100 and each of Swelling, Desertred, Nectarine 966 and Nectarine 24/90.

No consistent relationship could be concluded between flesh to stone ratio. The improvement in flesh to stone ratio is a desired quality attribute that is related to fruit size, weight and flesh weight. These characteristics could be improved by increasing the rate of cell division during stage I of fruit growth curve and by increasing cell size during stage III or the final swell stage of fruit growth Scorza *et al.,* (1991).

Desertred peach had the highest stone weight, while Early Sweet plum had the lowest one.Yellow peach had the highest flesh/stone ratio as compared with the other accessions, while Nectarine 1GC/100 and Amar apricot had the lowest values. The obtained results were in agreement with findings of Shama, Hyam (2000) who studied stone weight of Flordaprince, Early Grande and Destertred. Since the duration of stage I of the double sigmoid curve is similar for the plums, peaches and apricots and about 70-80 % of the mature size of the endocarp is attained in this stage I, there are not much expected differences in stone weight, moreover, seeds grow rapidly at the end of this phase I , Nucells and integument reach nearly their maximum size . This might explain the small differences in stone weight (Zucconi , 1986).

Desertred peach fruit was bigger (polar length, cross length, polar diameter and cross diameter) than the other accessions, while Early Sweet plum had the shortest fruits. There results are in agreement with data obtained by Fathi *et al.*, (2002) who showed that polar and cross diameter of Swelling peach were 6.0 and 6.3 in 2001 respectively. Nectarine 1GC/100, Nectarine 966 and Amar apricot had higher polar length/polar diameter and cross length/cross diameter ratio as compared with the other accessions, while Yellow peach, TropicSnow peach and Early sweet plum had the lowest values. The mentioned above results agreed with those previously reported by Ibrahim *et al.*, (2005) and Haikal (2005) . TropicSnow peach had higher fruit firmness and juice volume than the other accessions. Variations in fruit firmness and other quality traits were attributed to genetic factors but very environmentally influenced (Smole, 1992).

Chemical properties

Canino apricot had higher TSS than the other accessions, while Flordaglo peach, Early Sweet plum and Nectarine 1GC/100 had the lowest ones. Khalil *et al.,* (2002) noted in Amal and Canino apricot cultivars. TSS was being around 17.0 %. In addition, Shaltout (2003) who mentioned that TSS% of Flordaprince, Desertred, TropicSnow and Swelling were 10.9, 9.5, 11.0 and 14.0 % respectively.Santa Rosa plum had higher juice acidity than the other accessions, while Swelling peach had the lowest percent. On the contrary, Swelling had higher juice TSS/acid ratio than the other accessions, while Santa Rosa plum had the lowest one. In accordance with those results those previously reported by Shakweer (2004), and Haikal (2005).

In addition, Santa Rosa plum had higher juice ascorbic acid than the other accessions, while Amar apricot had the lowest one. The results were in agreement with those obtained by El- Beacy (2001) who mentioned that ascorbic acid content for

Flordaprince were 13.8 and 10.8 mg/100 gm fresh weight during 1998 and 1999 seasons respectively. On the contrary, the data in this study were in disagreement with those found by Haikal (2005) who stated that the ascorbic acid was ranged from 0.52 to 1.25 mg /100 ml Juice for 12 peach cultivars. Santa Rosa plum had higher anthocyanin content than the other accessions, while Canino and Amar apricots the lowest ones. In accordance with these results are these previously reported by Eliwa (2005). On the contrary the data in this study were in disagreement with this found by Haikal (2005). In addition Yellow peach had higher carotene content than the other accessions, Amar apricot had the lowest one. These results were in line with those obtained by Munzuroglu *et al.*, (2003) and Ibrahim *et al.*, (2005).

Molecular marker studies:

Total soluble proteins

PAGE- SDS electrophoretic protein patterns are shown in Figures (1,a). In total, these patterns manifested a maximum of 19 bands,
which were not necessarily presented in all accessions used. Nectarine 24/90, Nectarine 966 and Nectarine 1GC/100 showed the similarity of 5 bands, in addition Amar and Canino showed the similarity of 9 bands as well as Early Sweet and Beauty that showed the similarity of 3 bands. On the other hand high similarity between TropicSnow and Swelling, concerning their banding patterns, except for the absence of one band for Swelling as well as Flordaprince and Flordaglo shared in one band. Early Grande and Yellow peach were highly similar, concerning their banding patterns, except for the absence 5 bands for Yellow peach. Furthermore, Flordaprince and Flordastar were high similar, concerning their banding, except for the absence of 4 bands for Flordaprince .

Isozymes

The polyacrylamide gel electrophortic for peroxidase and esterase isozymes speration of the present accessions revealed five peroxidase isozymes Prx. In all accessions (species), locus Prx1 was homozygous. In addition locus Prx2 was heterozygous in all species (showing both the fast (F) and the slow (S) bands). Desertred was the only species showing the activity of Prx4 and Prx5 loci among the present accessions. Loci Prx3 was present only in Florda 834, Desertred, Swelling, Nectarine 24/90, Nectarine 966 and Nectarine 1GC/100. Figure (1, b and c) showed esterase isozyme patterns for the 17 accessions examined in the present study. Similar results were obtained on peach by Sandhya et al (2001), whereas Durham, *et al* (1987) found single loci for peroxidase that should Mendelian inheritance.

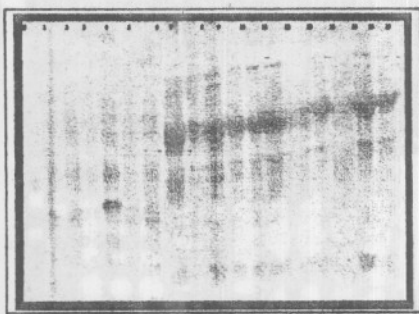

Figure (1,a) : SDS protein profiles for the studied accessions

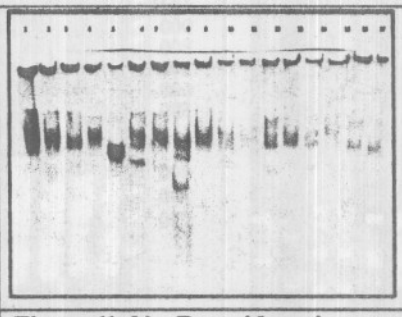

Figure (1, b) : Peroxidase isozyme profiles for the studied accessions

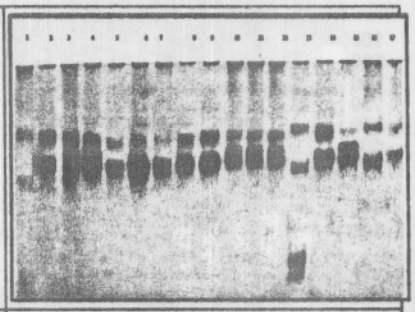

Figure (1,c) : Esterase isozyme profiles for the studied accessions

1-Yellow peach	2-Early Grande	3-Flordaprince	4-Flordastar	5-Flordaglo	6- Florda834
7-TropicSnow	8-Desertred	9-Swelling	10-Nectarine1GC/100	11-Nectarine 966	12- Nectarine 24/90
13- Early Sweet	14- Santa Rosa	15- Beauty	16- Canino	17-Amar	

In general, four Loci were detected all were polymorphic except for locus Est1 which was monomorphic, showing allelic variation among accessions. Locus Est1 was homozygous in all species. Est3 was present only in Yellow peach. In addition Locus Est4 was present in Yellow peach which were homozygous, showing the fast band (FF) while heterozygous (showing both the fast(F) and the slow (S) bands) in Early Sweet. Furthermore, Est2 was heterozygous in Flordaprince, Flordastar, Nectarine 1GC/100, Nectarine 966, Nectarine 24/90, Early Sweet and Beauty while homozygous in Early Grande,

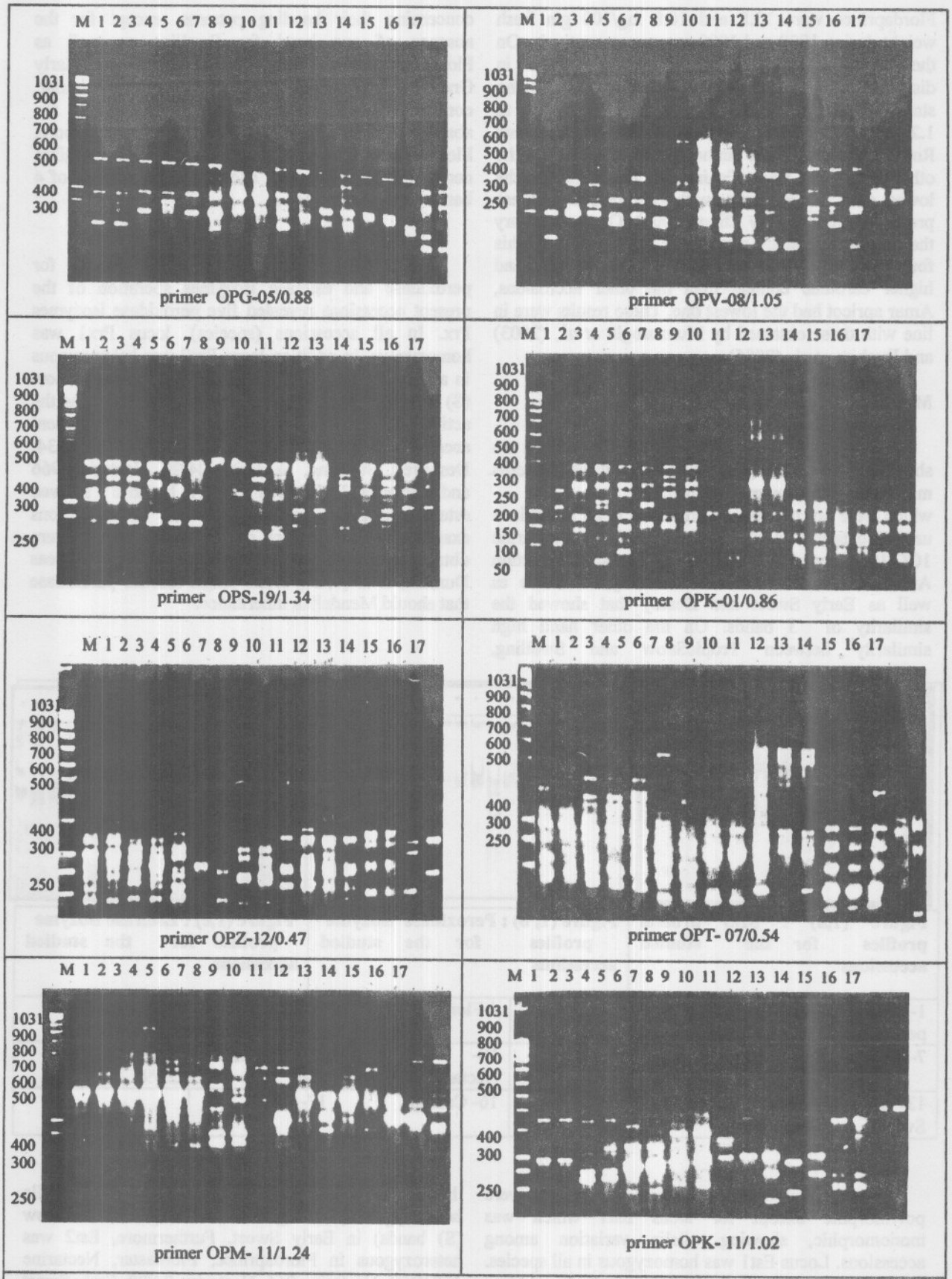

Figure (2): RAPD fragments amplified from genomic DNA extracted from 17studied accessions by eight primers

Flordaglo, TropicSnow, Desertred, Swelling, Canino and Amar (showing the slow band (SS)). In addition, Est2 was homozygous in Santa Rosa (showing the fast band). Other studies showed similar results of peroxidase isozymes for example, similar polymorphism was detected in *Prunus* species by Blenda *et al.* (1999).

Random Amplified Polymorphism DNA (RAPD).

Eight primers of arbitrary nucleotide sequence (Table1) were used to amplify the genomic DNA of the 17 studied accessions for *Prunus* species. The number of bands per primer varied from 56 bands in primer OPG-05/0.88 to 115 in primer OPM-11/1.24. The Eight primers amplified a total of 704 DNA fragments (bands) as shown in Table (3), most of them were polymorphic. This results are in ageeement with those obtained by (Zhang *et al.,2004)* who used random primers and the genomic DNA of 42 cultivar forms of *P. mume* pink Double as template for PCR to develop a random amplified polymorphic DNA (RAPD) fingerprints for the species. They screened 120 primers, 21 primers can generate 96 fragments, 70.8 % of which were polymorphic. The comparison between the studied accessions showed differences in the number and size (molecular weight) of the amplified fragments produced by each studied accessions. All DNA fragments with various size ranging from 20 to 700 bp. The number of bands in cultivars varied from one to nine bands. Primer OPV-08/1.05 resulted in highest number of bands (14) with various size ranging from 50bp to 500bp, while OPK-11/1.02, showed the lowest number of bands (10). Similar stuidies was done by Zheng *et al. (2002)* who pointed out the genetic relationship among peach (*Prunus persica)* and its wild relatives using RAPD technique. Forteen decanucleotide arbitrary primers and 181 DNA fragments were generated, of which 143 were polymorphic.

Table (3): Number of amplified, polymorphic products, the percentage of polymorphic and total number of bands .

Primer	No. of amplified products (a)	No. of polymorphic products (b)	(%) Polymorphism b/a	Total number of bands
OPG-05/0.88	13	13	100	91
OPV-08/1.05	14	14	100	91
OPS-19/1.34	11	11	100	76
OPK-01/0.86	15	15	100	97
OPZ-12/0.47	11	11	100	82
OPT-07/0.54	12	11	92	96
OPM-11/1.24	13	12	92	115
OPK-11/1.02	10	10	100	56
Total				704

Some bands were common among all accessions while others were considered specific for some cultivars,Table (4) shows some RAPD fragments which were represented in specific accessions and correlated with some economic traits. Haikal (2005) conducted a comparison between the twelve tested peach cultivars and showed differences in the number and size (MW), some bands were common among all tested cultivars, while others were considered specific for cultivars. Jun *et al.* (2004) constructed a linkage map of peach using amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and random amplified polymorphic DNA(RAPD) markers in 109 F2 population derived from the cross of Nectarine cultivars Okubo and Xingjini. The selected primers including 36 AFLP, 3 SSR and 2RAPD with rich polymorphism and steady bands were tested in the progenies.

Table (4) : Summary of DNA fragments that showed association to some traits .

Primer	Accessions	No.of Bands	Characteristics
OPG-05/0.88	Canino & Amar apricots	13	High TSS and low in Anthocyanin & carotene
OPG-05/0.88	Eary Sweet , Santa Rosa & Beauty plums	12	High in Anthocyanin
OPV-08/1.05	Desertred peach	1	High stone weight , fruit dimension and juice volume
OPS-19/1.34	Early sweet plum	5	Low in fruit, flesh & stone weight , firmness and juice volume
OPK-01/0.86	Nectarine 1GC/100& Nectarine 966	1,2,3	None
OPZ-12/0.47	Yellow peach & Early Grande peach	2	High in Carotene
OPZ-12/0.47	Early sweet plum	10	Low in fruit, flesh & stone weight , firmness and juice volume
OPT-07/0.54	Nectarine 1GC/100, Nectarine 966 and Nectarine 24/90	1	None
OPM-11/1.24	Flordaprince & Flordastar peach	1	None
OPK-11/1.02	Canino & Amar apricots	1	High TSS and low in Anthocyanin & carotene
OPK-11/1.02	TropicSnow peach	4	High in fruit weight and fresh weight

Biochemical and Molecular marker analysis (Cluster analysis).

The dendrogram resulting from the banding patterns of total soluble proteins , isozymes and RAPD were used to assort the relationships among the present accessions. Figure (4) showed that the phylogenetic tree segregated into two main clusters (I and II) at a relatively lower similarity level 0.21. Cluster I was separated into two subclusters (1 and 2) at a lower similarity level 0.25. Subcluster 1 was divided into two subsubclusters (a and b) at lower similarity level 0.30. Subsubcluster a was separated into two groups (I and II) at similarity level 0. 50. Group I included branch 1 which represents TropicSnow, while group 2 separated two branches (2 and 3) at higher similarity level 0.54. Branch 2 included Dsertred, whereas branch 3 included Swelling. Subsubcluster b was separated into two groups (III and IV) at lower similarity level 0.31, Group III was separated into two subgroups (1 and 2) at higher similarity level 0.68. Subgroup 1 included branch 4 which represents Nectarine 1GC/100, while subgroup 2 was separated into two branches (5 and 6) at higher similarity level 0.85. Branch included Nectarine 966 while branch 6 included Nectarine 24/90. Group IV was separated into two brances (7 and 8), branch 7 included Canino, while branch 8 included Amar. Subcluster 2 was divided into two subsubclusters (c and d) at higher similarity level 0.54. Subsubcluster c included branch 9 which represents Early Sweet, while subsubcluster d was separated into two branches (10 and 11). Branch 10 included Santa Rosa, while branch 11 included Beauty.

At about 0.33 similarity level cluster II was separated into two subclusters (3 and 4). Subcluster 3 included branch 12 which represents Florda 834, while subcluster 4 was divided into two subsubclusters (e and f) at lower similarity level 0.41. Subsubcluster e was separated into two groups (V and VI) at higher similarity level 0.54. Group V was separated two branches (13 and 14), branch 13 included Flordaprince, while branch 14 included Flordastar. Group VI included branch 15 which represents Flordaglo. Subsubcluster f was separated into two branches (16 and 17) at higher similarity level 0.65. Branch 16 included Early Grande, while branch 17 included Yellow peach. Many authers studied the relationships among different cultivars of peach (ZhongPing *et al.*, 2002) who studied 19 accessions of Nectarine peach (*Amygdalus persica var. nectarina (Prunus persica)*, Bianchi *et al.* (2003) studied 17 plum cultivars using 12 RAPD markers and Haikal (2005) who tested twelve cultivars and classified them.

In conclusion, the present data shows that there is a genetic relationship among the17 accessions. Total protein profile in combination with other data sets of molecular proparities are the main determinants to distinguish between these cultivars Some DNA bands showed a potential to be employed in marker – assisted identification or selection for some economic traits which is very valuable in breeding of *Prunus* species.

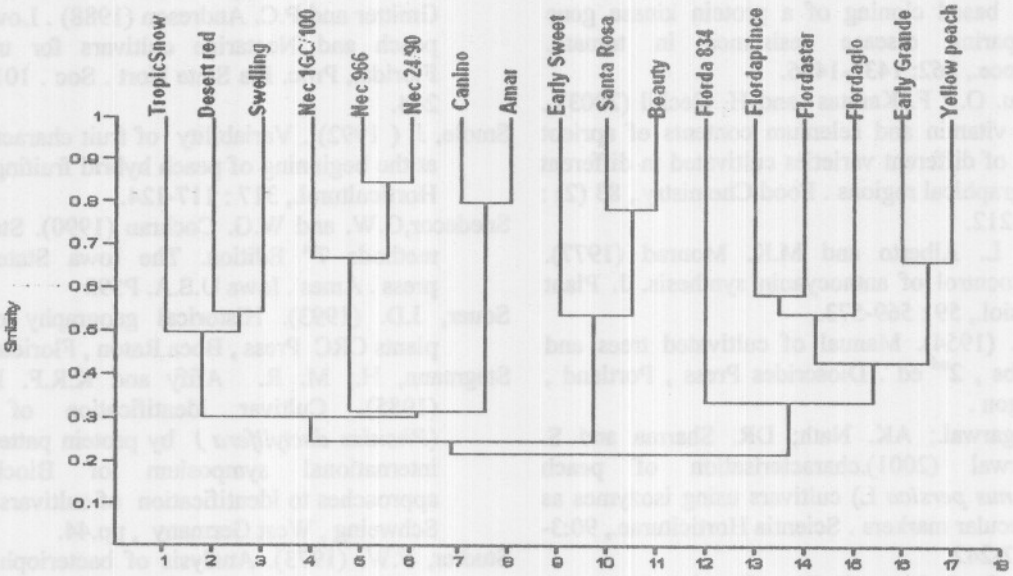

Figure (3): Dendrogram of correlation similarity obtained by PAST software showing the relation ship among the17 accessions of *Prunus* species based on isozyme, protein and RAPD results .

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الملخص العربى

العلاقات التقسيمية لبعض الفواكة ذات النواة الحجرية باستعمال الواسيمات المورفولوجية والجزيئية

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أجريت الدراسة خلال عامى ٢٠٠٤ ، ٢٠٠٥ فى كلية الزراعة جامعة الإسكندرية على اشجار تامة النمو وهى:٩ أصناف خوخ (خوخ مشمشى ، ديزت رد ، فلوردابرنس ، فلورداستار ، فلورداجلو ، فلوردا ٨٣٤ ، سويلنج ، تروبيكسنو ، إيرلى جراند)و ٣ أصناف نكتارين (نكتارين ٩٦٦ ، نكتارين ٩٠/٢٤ ، نكتارين ١ جى سى/١٠٠)و٣ أصناف برقوق (سنتاروزا ، بيوتى ، إيرلى سويت)و٢ صنف مشمش هى (كاتينو ، عمار). هذة الأصناف مطعومة على اصل نيماجارد ونامية في مزرعة بيكو – محافظة البحيرة ولقد أظهرت نتايج دراسة الصفات المورفولوجية للأوراق (مساحة نصل – طول نصل – عرض نصل- طول عنق) وصفات الثمار(وزن الثمرة- فى وزن اللب – وزن البذرة – قطر الثمرة- طول/ قطر الثمرة) وكذا الصفات الكيماوية (حجم العصير- نسبة المواد الصلبة الذائبة الكلية – نسبة الحموضة – نسبة المواد الصلبة الذائبة الكلية / الحموضة) على العينات قيد الدراسة الخاصة بالفواكة ذات النواة الحجرية أنه لا يمكن الاعتماد على هذه الصفات فقط للتعرق والتمييز بين تلك الأصناف نظرا للاختلافات الواسعة فيما بينها ومن هنا فلابد من الاعتماد على طرق اضافية وخاصة بالنسبة لاصفات المتعلقة بالنواحى الاقتصادية .

و باستخدام واسيمات الاحماض النووية (RAPD) و البروتين الذائب الكلى وأنزيمى البيروكسيدز والاستريز أمكن تقسيم المجموعة قيد الدراسة الى خمس مجموعات تحتوى المجموعة الأولى على خوخ تروبيكسنو وديزرت رد وسويلنج بينما تحتوى المجموعة الثانية على نكتارين ١ جى سى /١٠٠ ونكتارين ٩٦٦ ونكتارين ٩٠/٢٤ بينما تحتوى المجموعة الثالثة على مشمش كاتينو وعمار بينما تحتوى المجموعة الرابعة برقوق إيرلى سويت وسنتاروزا وبيوتى وتضم المجموعة الخامسة خوخ فلوردا ٨٣٤ وفلوردا برنس وفلوردا ستار وفلورداجلو وإيرلى جراند والخوخ المشمشى.وبتحديد العلاقة الوراثية بين هذه الأصناف وجد أن بعض الحزم مرتبطة ببعض الصفات الاقتصادية والتى يمكن استخدامها فى انتخاب الاصناف التى تحتوى على هذه الحزمة وبالتالى الصفة الاقتصادية المرغوبة .