

HORTICULTURAL STUDIES AND GENETIC FINGERPRINTING OF APRICOT CULTIVARS USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER

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ABSTRACT

The three apricot cultivars (Amal, Amar and Canino), which budded on apricot seedlings (Baladi) were compared for their vegetative growth, yield as well as fruit quality.

Results showed that Amar cultivar was the most vigorous tree, while it gave the smallest leaf area. Amal and Canino cultivars, were the least for vigorous but gave the largest leaf area. Yield for Canino cultivar was very high comparing with the yield of Amar cultivar. Consequently, the best fruit quality for Canino was more than the other two cultivars.

Random amplified polymorphic DNA (RAPD) analysis was carried out to evaluate polymorphism and genetic similarity between three apricot cultivars (Amal, Amar and Canino) and rootstock (Baladi). Seventy-five amplification products were identified using four random primers, with an average of 18.8 bands per primer. The genetic variations arranged between them as Baladi > Canino > Amar > Amal. Hierarchical cluster analysis revealed the low level of genetic variation between Amal and Amar, whereas their rootstock Baladi with Canino showed genetic dissimilarity. This polymorphism make this marker useful for genetic studies in apricot cultivars.

INTRODUCTION

Apricot is one of the most important deciduous fruits planted in Egypt a long time ago. Amar, one of well adapted apricot cultivars, is widely grown in Egypt. It gives good vegetative growth and high yield with best fruit quality.

Various apricot cultivars have been recently introduced to Egyptian agriculture, as Amal and Canino, which showed great success specially in newly reclaimed desert areas. Amal was introduced in early eighty's by some growers, its trees showed good vegetative growth, high yield and good fruit quality. Also, Canino cultivar, which was also introduced nearly in the same period, is considered a beneficial high cash crop and is highly appreciable by both the grower and consumer. The most apricot cultivars in Egypt are diploid ($2n=16$), and almostly propagated by grafting on apricot seedlings (Baladi).

Although there is a considerable variations among fruit trees, all have several common characteristics. Apricot, which named (cv), represents a unique gene combination which ordinarily must be perpetuated by some means of asexual propagation such as budding or grafting. Since the majority of cvs were selected over very long periods of time, new gene combinations result in fruit and other plant characteristics, which may be superior or in some cases inferior (Tanksey and Orton, 1983).

Apricot cultivars are very difficult to differentiate before fruiting without using morphological characteristics. The past decades have

witnessed the development of new approaches to study the genetic and taxonomic relationship among cultivars and rootstocks at the molecular level. These new approaches involved: DNA base sequence homology by hybridization, amino acid sequences of protein's having the same function and of electrophoretic profiles of proteins. Khalil and El-Sheik (2000) compared between two apricot cultivars Amal and Canino for their tree growth, fruiting, yield as well as fruit quality and protein profile. They postulated a close genetic relationship between the two cultivars.

For many years the principle of genetics have been applied to crop variety improvement with great success. Several crop species like wheat and tomato have been used as model genetic systems because of their central importance to food production. Until recently, virtually all progress in both breeding and model genetic systems has relied on a phenotypic assay of genotype. Since the efficiency of a selection scheme or genetic analysis based on phenotype is a function of the heritability of the trait, factors such as the environment, multigenic and quantitative inheritance, or partial and complete dominance often confound the expression of a genetic trait (Tanksley *et al.* 1989). Many of the complications of a phenotype-based assay can be mitigated through direct identification of genotype with a DNA-based diagnostic assay. For this reason DNA-based genetic markers are being integrated into several genetic systems and are expected to play an important role in the future of plant breeding (Tingey and Tufo 1993).

The utility of DNA-based diagnostic markers is determined to a large extent by the technology that is used to reveal DNA-based polymorphisms. Nowadays the technology of choice for many species is random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990). RAPDs provide an efficient method of estimating genetic relationships among genotypes from any organism. This marker can be detected readily throughout plant genomes, it provide discrete data that are less ambiguous than many other types of data.

RAPD markers (Williams *et al.*, 1990) are based on amplification of DNA by the polymerase chain reaction (PCR) using primers homologous to random target sites in the genome, a method also known as arbitrarily-primed PCR. RAPD markers are simpler to detect than RFLPs, requiring only PCR amplification and electrophoresis to generate data (Thormann *et al.*, 1994). Information on apricot molecular biology is limited.

Apricot species (*Prunus armeniaca*) are diploids with relatively few chromosomes ($2n=2x=16$) and small genomes. Thus the use of molecular methods could lead to valuable genetic insights and increased breeding efficiency (Liou *et al.*, 1996).

The present study was aimed to evaluate the adapted apricot cultivar (Amar) and the two new introduced apricot cultivars (Amal and Canino) and their rootstock (Baladi), with regards to vegetative growth, fruiting, yield and fruit quality. At the molecular level, RAPD markers were used for estimating genetic variability between the three apricot cultivars and their rootstock.

MATERIALS AND METHODS

The present investigation was carried out for successive seasons 1999 and 2000, on apricot trees budded on apricot seedlings, at Khatatba, Menofia Governorate, in a private farm. Trees were planted in 1994, grown on sandy soil at 5.0x5.0m. apart, irrigated by drip irrigation and treated with normal agricultural practices. Three apricot cultivars, which named Amal, Amar and Canino were chosen with uniform size as possible. A complete randomized block design was used with three replications and three sub replications i.e., nine trees were chosen for each cultivar (three trees in each replicate). This means that 27 trees for data collection.

Data collected

I. Apricot tree growth:

- a. **Shoot length and diameter.** Ten shoots per tree were tagged as soon as emerged. Length and diameter were periodically measured during the season.
- b. **Leaf area.** At end of July, leaf samples from the middle of tagged shoots were taken for the determination of leaf area using leaf area meter (CD 2001 USA).
- c. **Tree dimension.** Canopy dimension were measured at the end of the season i.e., starting from December 1999. Tree size was calculated according to the formula mentioned by Westwood, 1978.

$$\frac{4}{3} \pi a^2 b$$

where $\pi = 3.14$, $a = \frac{1}{2}$ major axis and $b = \frac{1}{2}$ minor axis.

- d. **Trunk circumference.** The circumference of each trunk for apricot trees was measured by a tape at fixed point i.e. 25 cm, above soil surface at the end of each season under study (Dec. 1999 and 2000). Data will be presented as cross section area.
- II. **Yield.** Apricot yield at harvest time, number and weight of apricot fruits of the three cultivars under study were determined in each selected tree for yield data.
 - III. **Fruit quality.** Samples of fruits were collected from each tree for the determination of fruit characteristics which include the following:
 - a. **Physical properties.** Fruit weight, size and fruit dimensions i.e. diameter and length in cm were determined. Also skin colour was estimated by matching with colour-chart according to Robert, 1938. Percentage of flesh was calculated.
 - b. **Chemical properties.** Total soluble solids, and juice acidity according to A.O.A.C. (1965) were determined and recorded.

Data were analyzed statistically according to Snedecor and Cochran (1990), in each season and L.S.D was used for comparison between means of each treatment.

Random amplified polymorphic DNA (RAPD) markers:

DNA isolation. DNA was extracted from leaf tissue of trees from three cultivars Amal, Amar and Canino and their rootstock (Baladi) using a hexadecyl-trimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1987). Five grams of frozen apricot leaves from each cultivars were ground in 15ml of buffer (1.4M NaCl, 0.2% (V/V) 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-Cl PH 8.0) in blender. Five millilitres of CTAB buffer (the above buffer plus 2% (w/v) CTAB (Sigma) were then added to the ground tissue, and this mixture was incubated at 60°C for 30 min. DNA was extracted with one volume of chloroform- isoamyl alcohol (24:1) then centrifuged at 3500 gs for 5 min. DNA was precipitated by the addition of 2/3 volume of cold isopropanol. The DNA pellet was dried under vacuum and resuspended in 50 ul of TE buffer.

Amplification reactions. Polymerase chain reaction were performed in 25 ul containing 80 mM Tris-Cl PH 9.0, 20 mM (NH₄)₂ SO₄, 100 mM each of dATP, dCTP, dGTP, and dTTP (promega), 3.5 mM MgCl₂, 400nM of a single primer, 5U of Taq DNA polymerase (promega) and 50 ng of genomic DNA. The reactions were performed in a thermal cycler (COY) with the following temperature conditions: 94°C for 4 min, followed by 45 cycles of 92°C for 1 min, 35°C for 1 min, 72°C for 2 min and ending with 72°C for 6 min. PCR products were visualized with ethidium bromide after electrophoresis in a 1.4% agarose gel. The RAPD fragments were scored visually as the presence or absence of a band. RAPD fragment size was estimated by comparison with a 500 bp ladder DNA size standard or a 1-Kb ladder (promega, Madison, Wis.). Gels were photographed using an ultraviolet transilluminator with plus-X pan, 35 mm, 125 ASA film (Kodak).

Primer Screening :

The following sequences were used :

Primer Code	Primer Sequences
PO 3	CTGATATGCC
PO 8	AGGGCGTAAG
RO 9	TGAGCACGAG
18	CTGATACGCC

RESULTS

Orchard studies.

i) Vegetative growth:

It appears from Tables 1 and 2 that when the different examined cultivars were budded on the same rootstock (apricot seedlings), Amar cultivar, in general, was the most vigorous tree (tree size) during the years of study, followed by Amal, whereas Canino cultivar was the smallest.

The one year old shoot lengths were the longest in Amal and Amar cultivars. In both seasons, Amar cultivar had a thinner one-year-old shoot diameter, while the thicker shoot diameters were detected for the other two

cultivars. Amal gave highly significant increases of leaf area while the lowest for Amar cultivars, Canino gave intermediate value. The value of trunk circumference was high in the case of Canino and low of Amal in the two seasons, whereas of Amar was intermediate.

Table 1: Vegetative growth of apricot cultivars in 1999.

Vegetative characters	Amal	Amar	Canino	L.S.Dat5%
Length of one year old shoot (cm).	111.57	109.17	64.33	2.82
Diameter of one year old shoot (cm).	0.40	0.37	0.47	0.10
Leaf area (cm ²)	58.47	37.87	42.17	5.26
Trunk circumference (cm)	22.47	25.33	27.53	1.31
Tree size (m ³)	8.47	9.07	6.90	0.46

Table 2: Vegetative growth of apricot cultivars in 2000.

Vegetative characters	Amal	Amar	Canino	L.S.Dat5%
Length of one year old shoot (cm).	112.43	110.10	65.77	3.98
Diameter of one year old shoot (cm).	0.40	0.37	0.50	0.05
Leaf area (cm ²)	60.37	40.10	48.10	2.58
Trunk circumference (cm)	26.20	28.13	31.10	1.44
Tree size (m ³)	8.77	9.20	7.17	0.34

II) Yield.

Data for yield per tree of the three apricot cultivars budded on apricot seedlings shown in Table 3 for the two seasons. It ranged between 1158.7 to 1416.7 and 1240.0 to 1570.0 respectively for the No. of fruits per tree in the two seasons, whereas Kg. per tree had almost similar ranging between 29.17 to 44.00 and 30.43 to 46.10 Kg.

Table 3: Yield per tree for apricot cultivars (No/tree and Kg/tree) in 1999 and 2000 seasons.

Yield	Season	Amal	Amar	Canin	L.S.D at 5%
Fruit No./tree	1999	1158.7	1323.3	1416.7	114.07
	2000	1240.0	1400.0	1570.0	41.43
Kg./tree	1999	29.17	39.27	44.00	1.23
	2000	30.43	41.67	46.10	1.10

Major mature fruit characters.

a) **Physical properties.** Table 4 shows the characteristics of mature fruits produced through self pollination by the apricot cultivars budded on apricot seedlings in 1999 and 2000. Statistical analysis revealed that significant differences occurred in eight and size of fruits. It can be arranged in a descending order as Canino > Amar > Amal. On the other hand, there were slight significant variations for fruit diameter and height, in the two seasons.

Table 4 presents the colour of the fruit for different cultivars. Canino and Amar cultivars gave the best orange colour than Amal cultivar. In the two seasons, the percentage of flesh was the lowest for Amar cultivar (89.43 and

86.40% respectively in the two seasons), while it was the highest for other two cultivars (ranged between 94.27 to 95.73%, in the two seasons).

Table 4: Fruit characters of apricot cultivars in 1999 and 2000 seasons.

Fruit characters	Season	Amal	Amar	Canin	L.S.D at 5%
Fruit weight (gm)	1999	25.17	29.68	31.06	1.04
	2000	24.54	29.76	29.36	0.87
Fruit size (cm ³)	1999	23.77	28.50	30.67	1.07
	2000	22.93	26.27	28.93	1.05
Fruit diameter (cm)	1999	3.4	3.0	3.6	0.15
	2000	3.3	3.5	3.8	0.10
Fruit height (cm)	1999	3.2	2.9	3.6	0.22
	2000	3.2	3.1	3.5	0.14
T.S.S %	1999	17.0	14.9	17.7	0.60
	2000	16.23	14.07	17.0	0.12
Acidity %	1999	0.644	0.718	0.671	0.04
	2000	0.643	0.733	0.743	0.04
Fruit characters	Season	Amal	Amar	Canin	L.S.D at 5%
Flesh percent %	1999	94.27	89.43	95.37	1.21
	2000	95.47	86.40	95.73	1.42
Skin Colour	1999	Marigold	Cadmium	Cadmium	
	2000	Orange Part I	8/1 part I	8/1 Part I	
Flesh Colour	1999	Persimon	Cadmium	Cadmium	
	2000	Orange 710/3 Part I	Orange 8 Part I	Orange 8 Part I	

Chemical properties. Total soluble solids in the juice from different apricot cultivars was significantly low for Amar in the two seasons. Amal and Canino fruits had a T.S.S very close in the two seasons, being around 17.0%. Acidity was determined as g. Maleic acid/100 ml of the juice, it was the highest in Amar cultivar comparing the other two cultivars.

Table 4 present the colour of the skin and flesh fruit for different cultivars. Canino gave the best colour than the other two cultivars in both seasons.

RAPD markers:

Polymerase chain reaction (PCR) based genetic markers and randomly amplified polymorphic DNA (RAPD) markers have been shortly introduced and gained widespread application in genetic and plant breeding. RAPD relies on amplification using arbitrary primers. Thus, a large number of fragments can be quickly generated since no specific target DNA sequence is needed. Four of the tenth primers that were screened for RAPD markers produced clear and consistent amplification products. A total of 75 bands (putative loci) were generated by these primers in the assay of DNA samples for four cultivars. Each primer gave 11 to 26 amplification products with an average of 18.8 bands per primer. Variation of amplification products between cultivars was observed (Fig.1).

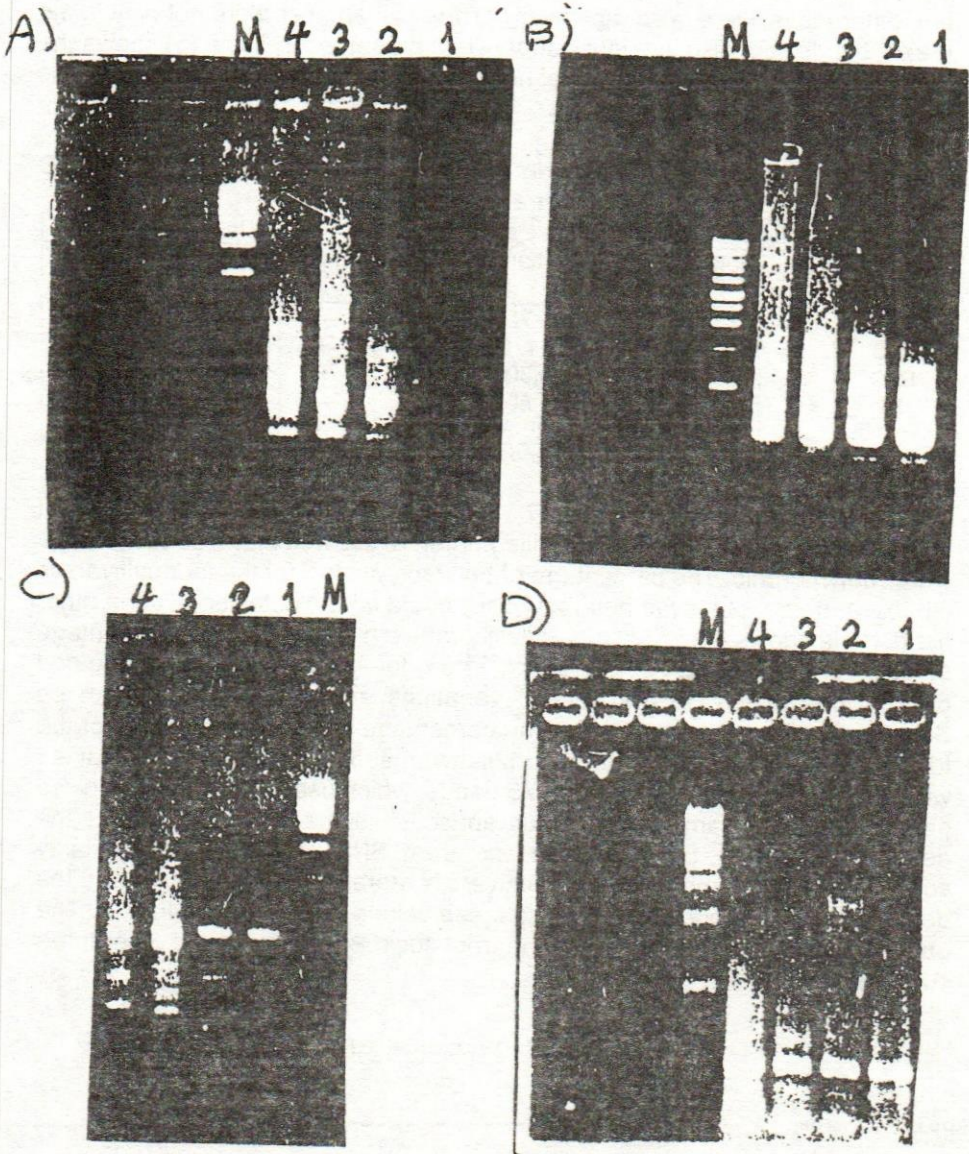


Fig (1): RAPD profiles apricot cultivar: M-1 kbp marker; 1, Amal; 2, Amar, 3; Baladi and 4, canino A) Primer PO3; b) primer PO8; C) primer RO9 and d) primer 18

Amplification of genomic DNA from four cultivars (Amal, Amar, Canino and Baladi) indicated that some bands were common to all cultivars but differences were also apparent. These differences were not only in the major bands but also in minor bands. Data presented in Table (5) shows the total number of bands, number of polymorphic bands and size of polymorphic band.

Table 5: Random DNA primers selected to distinguish RAPD Fingerprints of four apricot cultivars.

Primer	DNA bands		Size of polym. band (bp)	Cultivars			
	Total	Polymorphic		Amal	Amar	Canino	Baladi
P03	8	4	1100,750,600,450	----	---+	--++	++++
P08	9	4	2000,1750,570,500	--+-	---+	+--+	----
R09	5	4	1250,300,200,150	--+-	+--+	----	----
18	9	7	1100,500,450,350 320,300,250	----	----	+--+	----
				---	---	+--	---

+ present of band - absent of polym. band.

From this Table primers, P03; P08 and R09 showed that 4 bands out of 8,9 and 5 were polymorphic, while primer 18 showed that 7 bands out of 9 were polymorphic. The percentage of polymorphic loci for the four cultivars is 61.3% and the observed heterozygosity could not be obtained. On the other hand, data shows the levels of variability for each cultivar and the percentage of polymorphic loci is 10.5% for Amal; 31.6% for Amar; 36.8% for Canino and 57.9% for Baladi. So the genetic variations arranged between them as Baladi > Canino > Amar > Amal. The percentage of monomorphic loci for the four cultivars is 38.7%. The accumulative matrix of all the profiles obtained with the four primers resulted in 75 bands, which used to compute pairwise genetic distance among the four apricot cultivars and to construct the dendrogram using the computer program SPSS release 6.1. Similarity coefficient matrix among the four cultivars is represented in Table (6) and the dendrogram developed according to these values is illustrated in Fig (2). The obtained dendrogram separates the rootstock Baladi from the other three cultivars.

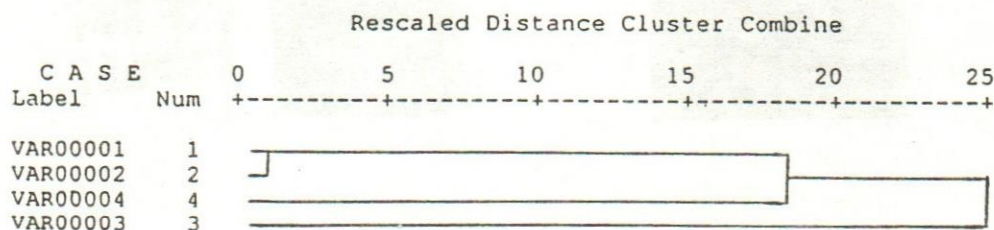


Fig (2): Dendrogram using average linkage (between groups) 1,2,3 and 4 Amal, Amar, Baladi, and Canino

Table 6: Rescaled reversed absolute squared euclidean similarity coefficient matrix

Variable	Amal	Amar	Baladi
Amar	1.0000		
Baladi	.1111	.1111	
Canino	.4444	.2222	.0000

Table (6) and Fig (2) indicate that as the genetic distance between Amal and (Canino and Baladi) increases, the Horticultural characters were improved.

DISCUSSION

Various apricot cultivars have been recently introduced to Egyptian agriculture, so the present study is an attempt to differentiate between these cultivars and the adapted apricot cultivar (Amar), for the horticultural and genetic features.

The data shows that Amar cultivar gave the greatest tree size and longest shoots than other two cultivars, while it had the thinnest one year old shoot diameter. Canino had the smallest tree size and shortest shoots, whereas its leaf area was large. Amal gave the largest leaf area and smallest circumference, Khalil and El-Shiek (2000) pointed out that Amal apricot cultivar gave the largest tree and leaf area.

Variation in yield per tree for apricot cultivars was very high when Amal cultivar gave around 29.5 Kg per tree, and Canino cultivar gave around 45.0 Kg per tree. Amar apricot cultivar gave intermediate value (around 40 Kg per tree). These variation may be due to the apricot seedings rootstock, which had a different genotype Schechter *et al.*, 1991 reported that both shoot and spur foliage characteristics differed significantly among trees on different rootstocks.

Fruit weight and fruit size varied according to the cultivars. Canino cultivar showed largest fruit in 1999, while in the second year for the two cultivars (Amar and Canino). This behaviour may be explained by those reported by Nesterov (1982) who stated that fruit size varied according to variety and geographical conditions. Patzold (1986) pointed out no consistent influence of rootstock or rootstock/interstock combination on fruit size. The differences between the diameter, high and flesh percentage were slight significant for the three apricot cultivars. Embree and Craig (1989) pointed out in apple that fruit diameter was influenced by the cultivar and not by the rootstock.

The percentage of total soluble solids (T.S.S) content was highest for Amal and Canino cultivars, and was lowest for Amar cultivar. As regards to acidity, the results obtained in both seasons 1999 and 2000, indicate that differed with slight significantly. These findings were in agreement with those of Euden (1991) in apple and Khalil *et al.*, (1999) in apricot. Canino cultivar gave the best Orange fruit colour (skin or flesh) compared with other two apricot cultivars. Khalil and El-Sheik (2000) found similar observation.

The genetic variability is related to phenotypic characteristics such as fruit size and shape, tree vigour, susceptibility to diseases, level of acidity in fruits, etc. Isoenzyme analysis has found similar genetic variability at the biochemical level even in morphologically uniform seedling populations (Moore and Castle, 1988). Since the efficiency of a selection scheme or genetic analysis based on phenotype is a function of the heritability of the trait, factors such as the environment, multigenic and quantitative inheritance, or partial and complete dominance often confound the expression of a genetic trait. For this reason, DNA-based genetic markers are being integrated into several genetic systems and are expected to play an important role in the future of plant breeding.

Williams *et al.*, 1999 demonstrated the utility of single short oligonucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly throughout the genome. He showed that the differences (polymorphisms) in the pattern of bands amplified from genetically distinct individuals behaved as mendelian genetic markers (named RAPDs, for Random Amplified Polymorphic DNA). The many advantages of RAPD markers over RFLP or isozyme markers accelerated the adoption of RAPD technology for the construction of genetic maps, fingerprinting, and population genetic studies (Hedrick, 1992). Apricot species (*Prunus armeniaca*) is a diploid ($2n=16$), self-pollinated, so few studies have been done with self-pollinated fruits like apricot species.

The four cultivars of apricot under this investigation were common and commercially important in Egypt (i.e. Amal, Amar, Canino and Baladi). Our studies showed that a total of 75 bands (putative loci) were generated by four primers in the assay of DNA samples, with an average of 18.8 bands per primers. The amplified fragments ranged in size from 1750 to 100 bp. Arrangement of primers depending on the number of the generated DNA fragments is $8 > P03 > 18 > R09$.

Variation of amplification products between cultivars was observed and the percentage of polymorphic in Baladi rootstock was more than Canino and Amar while Amal was less polymorphic. The polymorphic loci for the three cultivars Amal, Amar and Canino were 10.5%; 31.6% and 36.8% respectively, so there were slight inter-cultivar variation between them. The polymorphic loci for the three cultivars and the rootstock together was 61.3% and the monomorphic loci was 38.7%. The genetic variation arranged as Baladi > Canino > Amar > Amal, so Amal was more stable than Amar and Canino while Baladi has a high genetic variation. The genetic variations were also found in horticultural characters between them. Observed heterozygosity could not be obtained for RAPD data because these markers are dominant. The constructed dendrogram showed that the cultivar Amal and the cultivar Amar could be grouped together (Fig. 2), furthermore, Amal was closely related to Amar than to Canino. The dendrogram revealed also that the cultivar Canino and the rootstock Baladi were distantly separated from each other as well as from the two other cultivars Amal and Amar. Not surprisingly, Canino and Baladi displayed different genetic distances when were compared to Amal or Amar, though the cultivar Canino and the

rootstock Baladi have different origins. In this regard, the data based on RAPD analysis were found to be partially in accordance with the horticulture of the studied cultivars. These variations among cultivars using molecular markers was observed in other plant species such as Mandarins (Machado *et al.*, 1996), Walnut (Woeste *et al.*, 1996), Apple (Yang *et al.*, 1997), Grape (Siles *et al.*, 2000) and Apricot (Hurtado *et al.*, 2001). Polymorphic RAPD markers are usually inherited in a Mendelian fashion in many crop species, indicating that they are of nuclear origin. Polymorphisms detected by RAPDs reflect either sequence variation at the sites of priming, small insertions or deletions in the region of the genome which amplified or both (Lu *et al.*, 1996). The results presented in this report suggest that DNA fingerprints in apricot are likely to be useful for identification and breeding purposes.

REFERENCES

- A.D.A.C (1965). Association of Official Analytical Chemists, Official Methods of Analysis. Published by A.D.A.C. Washington. D.C. USA.
- Doyle, J.J. and J.L. Doyle (1987). Isolation of plant DNA from fresh tissue. *Phytochem. Bull.* 19: 11.
- Embree, C.G. and W.E. Craig (1989). Field performance of "Summerland Red McIntosh", MacSpur McIntosh", "Empire and Idared on six rootstocks. *Fruit varieties. Journal* 43 (4): 159-164 (cf. *Hort. Abst.* 60 (5): 358. 1990).
- Euden, T.V. (1991). M27 is especially suitable for triploid cultivars., *Fruiteelt* 81:16-18 (cf. *Hort. Abst.* 62 (8): 745. 1992).
- Hedrick, P. (1992). Shooting the RAPDs. *Nature*, (355): 679- 680.
- Hurtado, M.A.; M.L. Badenes; G. Llacer; A. Westman; E. Beck and G.A. Abbott (2001). Contribution to apricot genetic analysis with RFLP, RAPD and AFLP markers. *ISHS Acta Horticulturae* 546.
- Khalil B.M.; A.M. El-Seginy; M.M. Makarem and E. Abdel Aziz (1999). Effect of hydrogen Cyanamide on flowering, yield and fruit quality of Canino apricot trees. *J.Agric. Sci. Mansoura, Univ.*, 24(11): 6859- 6866.
- Khalil, B.M. and A.A. El-Sheik (2000). Comparative study between two apricot cultivars in relation with protein profile. *J. Agric. Sci. Mansoura Univ.*, 25(6): 3559- 3568.
- Liou, P.G.; F.G. Gmitter and G.A. Morre. (1996). Characterization of the citrus genome through analysis of restriction fragment length polymorphisms. *Theor. Appl. Genet.*, 92: 425 – 435.
- Lu, J.; M.R. Knox; M.J. Ambrose; M.J. Brown and T.H. Ellis (1996). Comparative analysis of genetic diversity in pea assessed by RFLP and RAPD- based methods. *Theor. Appl. Genet.*, 93: 1103-1111.
- Machado, M.A.; C.H. Filho; M.L. Targon and J.Jr. Pompeu (1996). Genetic relationship of Mediterranean mandarins (*Citrus deliciosa* Tenore) using RAPD markers. *Euphytica*, 92: 321-326.
- Moore, G.A and W.S. Castle (1988). Morphological and isozymic analysis of open-pollinated Citrus rootstock populations. *J. Hered.*, 79:59-63.

- Nesterov, Y.S. (1982). Geographical variation in fruit quality and yield in apple varieties. *Vestnik Sel'sko- Khozyaistvennoi Nauki* (10) 47-49 (cf. Hort. Abst. 55 (10): 888. 1985).
- Patzold, G. (1986). Vista Bella., a new early apple cultivar. *Garenbau* 33(12) 369-370 (cf. Hort. Abst. 58 (9) 5433, 1988).
- Robert, F.W. (1938). Colour. Chart of Royal Horticultural Society, Issued by the British Colour Council in Collaboration with the Royal Horticultural society. London. Part III.
- Schechter, L.; D.C. Elfving and J.T.A. Proctor (1991). Rootstock affects vegetative growth characteristics and productivity of Delicious. *Hortscience*. 26 (9): 1145-1146.
- Siles, B.A.; K.A.O'Neil; M.A. Fox; D.E. Anderson; A.F. Kuntz and A.C. Morris (2000). Genetic fingerprinting of grape plant using RAPD analysis an dynamic size-sieving capillary electrophoresis. *J. Agric. Food Chem.* 48 (12): 5903 - 5912.
- Snedecor, G.W. and W.G. Cochran (1990). *Statistical Methods*. 7th. The Iowa State, Univ. Press, Ames, Iowa, USA, P. 593.
- Tanksley, S.D. and T.J. Orton (1983). *Isozymes in Plant Genetics and Breeding* Elsevier Science Publishers B.V. PP. 401-402.
- Tanksley, S.D.; N.D. Young; A.H. Paterson and M.W. Bonierbale (1989). RFLP mapping in plant breeding : New tools for an old science. *Bio Technology* 7: 257- 264.
- Thormann, C.E.; M.E. Ferreira; L.E. Camargo; T.G. Tivang and T.C. Osborn (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. *Theor. Appl. Genet.*, 88: 973-980.
- Tingey, S.V. and J.P. Tufo (1993). Genetic analysis with random amplified polymorphic DNA markers. *Plant Physiol.*, 101:349-352.
- Westwood, M.N. (1978). *Temperate Zone Pomology*. W.H. Freeman Company San Fransisco. USA. PP. 428.
- Williams, J.G.; A.R. Kubelik; K.J. Livak; J.A. Rafalski and S.V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
- Woeste, K.; G.H. Mcgranahan and R. Bernatzky (1996). RAPD Loci from a walnut backcross [(*Juglans hindsii* X *J. regia*) X *J. regia*]. *J. Amer. Soc. Hort. Sci.* 121(3): 358 -361.
- Yang, H.Y.; S.S. Korban; J. Krüger and H. Schmidt (1997). A RAPD marker tightly linked to the Scab-resistance gene *V₇* in apple. *J. Amer. Soc. Hort. Sci.* 122 (1): 47-52.

دراسات بستانية والبصمات الوراثية لأصناف المشمس باستخدام تكنيك ال د ن ا المتعاضم العشوائى متعدد المظاهر

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أجريت هذه الدراسة على ثلاثة أصناف من المشمش (أمل - عمار - كاتينو) والمطعمومة على اصل المشمس البذرى والمنزوعة سنة ١٩٩٤ فى مزرعة خاصة بمنطقة الخطاطبة محافظة المنوفية. لمقارنة الثلاثة أصناف من المشمش والمطعمومة على الأصل البذرى من حيث النمو الخضرى والمحصول وجوده الثمار.

ولقد أظهرت النتائج الآتى :

- ١ - ان صنف المشمش العمار يتميز بكون حجم الشجرة بينما اعطى متوسط مساحة أوراق اقل والعكس بالنسبة لصنفى أمل وكاتينو حيث تميزا بحجم أقل للأشجار بينما كانت مساحة الأوراق كبيرة .
- ٢ - أعطى صنف المشمش الكاتينو أعلى كمية محصول فى العدد والوزن بالمقارنة بالصنفين الآخرين .
- ٣ - أعطى صنف المشمس الكاتينو أكبر حجم ووزن للثمار بينما كانت المواد الصلبة الكلية فى كلا الصنفين أمل والكاتينو أعلى من صنف العمار .
- ٤ - استخدم تكنيك الدنا المتعاضم العشوائى متعدد المظاهر لتحديد البصمة الوراثية لأصناف الأربعة من المشمش (أمل - عمار - كاتينو - والبلدى كأصل) ومدى التشابه بينهم وقد استخدمت أربعة بادئيات عشوائية اعطت (٧٥) خمسة وسبعون حزمة بمعدل ثمانية عشر وثمانية من عشر (١٨٨) حزمة لكل بادىء وكان ترتيب الاختلافات الوراثية بين الأربعة أصناف تحت الدراسة هى بلدى < كاتينو < عمار < أمل - وتحليل هذه الحزم باستخدام الكمبيوتر اظهرت قليل من الاختلافات الوراثية بين الصنفين أمل والعمار بينما اظهرا هذان الصنفين اختلافات وراثية كبيرة مع كل من بلدى والكاتينو - وهذه التباينات جعلت من هذا التكنيك فائدة فى الدراسة الوراثية لأصناف المشمش المختلفة.