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PRODUCTION OF NEW STRAWBERRY STRAINS VIA ANTHER CULTURE:
3- THE CYTOLOGYCAL STUDY AND DNA FINGERPRINTING

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ABSTRACT

This study was carried out during 1998 to 2002 to establish an efficient protocol for generating somaclonal variations in strawberry via anther culture and selecting the promising ones for the strawberry breeding programs in Egypt. Ten strains from Camarosa, and a strain from each of Capitola and Chandler were subjected to nursery and fruit production trials in a randomized complete blocks design. Cytological studies and DNA fingerprintings were carried out for the selected strains.

All examined root tips of all selected strains showed that the chromosome number was 56 (octaploid) as in the original cultivars. The pairwise differences among strawberry genotypes were calculated by (SPSS). The analysis was based on the number of markers that were different between any given pair of genotypes. The results of analysis showed two groups. The highest value of similarity was observed between the two Camarosa strains C8 and C9; which clustered together and closely grouped with Chandler strain B1, and the three genotypes could also be grouped with Camarosa Cultivar. Also, C5 and C6 Camarosa strains showed a second group together, with a high similarity value, and they were clustered with Camarosa strain C10, and the three strains could be grouped with Camarosa strain C3. Moreover, Camarosa strain C4 was closely grouped with the last mentioned four strains.

The study conclude that, consensus fingerprint profiling using the randomly amplified polymorphic DNA (RAPD) markers is a useful and reliable method for establishing genetic identities of the strawberry cultivars and advanced selections, regenerated from anther culture.

Keywords: *Strawberry, somaclonal variation, chromosomes number and* DNA fingerprint.

INTRODUCTION

Because of the large number of strawberry cultivars grown today, there is a pressing need for the development of reliable methods for identifying and distinguishing strawberry cultivars and for assessing genetic diversity in *strawberry germplasm for future breeding purposes.*

Generally, the anther culture method not only produce haploid plants, but also frequently others with a differentiated ploidy. Rosati *et al.* (1975), Oosawa and Takayanagi (1982) and Quarta *et al.* (1991) started that the chromosome number, of strawberry regenerated clones by anther culture , to be of four different octaploid cultivars. They found that all the regenerated plants had the octaploid chromosome number of 2n = 8x = 56.

In another study, Niemirowicz-Szczytt and Zakrzewska (1981) examined 42 somaclones of Redgautlet strawberry cultivarwhich were

obtained by anther culture. They found that the chromosomes numbers for 25 plants were 15 tetraploids (2n = 4x = 28), 1 hexaploids (2n = 6x = 42) and 9 octaploids (2n = 8x = 56).

Svensson and Johansson (1994) stated that it could be possible to obtain different ploidy levels from cultured strawberry anthers cultured. They recorded also that 92 clones were found to be octaploid (2n = 8x = 56), 2 heptaploid (2n = 7x = 49), 7 hexadecaploid (2n = 16x = 122) and 4 probably mixoploid (4x – 8x).

Soon, the polymerase chain reaction (PCR) gave rise to the development of other PCR related methods that made it possible to amplify the fragments of DNA unknown sequences. One of the most commonly used methods (developed by Williams *et al.*, 1990) is the random amplifed polymorphic DNA (RAPD). The analysis of polymorphism by means of this method does not require preliminary information concerning the DNA sequence under analysis. During the reaction, the sequences of many loci of the genome are amplified.

Hancock and Callow (1994), Degani *et al.* (1998) and Poreebski and Catling (1998) screened some strawberry cultivars using the polymerase chain reaction (PCR). They stated that this technology shows particular promise as a tool in strawberry genetic studies, because high levels of diversity were observed in a relatively narrow breeding population. Further, the power of this technique to differentiate among close relatives suggests that its use showed overcome the limitations cited using isozymes polymorphisms.

As respect to similarity values, Degani *et al.* (1998) found that it ranged from 0 to 95% with values closer to 100% indicating greater similarity. However, some cultivars clustered with one of their immediate parents. They added that there was no particularly strong correlation between estimates of genetic similarity from RAPD data and known pedigree data for some of studied strawberry cultivars.

Degani *et al.* (2001) evaluated nineteen strawberry cultivars using RAPD system. They found that California cultivars viz. Seascape and Chandler which share Douglas as an immediate parent clustered together; but separately from the other California cultivar.

El-Tarras *et al.* (2001) stated that using 11 differentiating RAPD primers to identify six strawberry cultivars produced 204 polymorphic DNA fragments with a high potential to differentiate strawberry genotypes. A dendrogram displaying the relative genetic similarities among the genotypes showed the existence of genetic diversity among the tested cultivars. Red Lands Hop and Red Lands Joy cultivars produced 40 and 36 DNA bands, respectively, showing high genetic similarities in dendrogram.

Therefore, the aims of this study were to identify and distinguish chromosome number among all obtained strains via RAPD analysis.

MATERIALS AND METHODS

1. Cytological study:

Okasha *et al.* 2003 evaluated of 12 new strains in 1999/2000 and 2000/2001 for yield performances and fruits physical and chemical

characteristics. They also present studies conducted on cytological investigation and DNA fingerprinting.

Mitotic studies were made on three root tips of 100 plantlets from each selected genotype. Somatic chromosome number was demonstrated using acetocarmen reagent for 30 min. as mentioned by Raghuvanshi (1962). Only one mm of the root tips was executed and split with a sharp blade then squashed on a glass slide. Chromosome numbers were counted using a light microscope at 1000 X magnification.

2. RAPD analysis:

Genomic DNA was extracted from fifteen strawberry genotypes, following the method of (Doyle and Doyle, 1987). PCR-RAPD reactions were conducted using eleven arbitary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA), from kits A, C and O. Names and sequences of these eleven primers are listed as the following:

Primer Code No.	Sequence (5' to 3')
OPA -04	AATCGGGCTG
OPA -09	GGGTAACGCC
OPA -10	GTGATCGCAG
OPA -14	TCTGTGCTGC
OPC -10	TGTCTGGGTG
OPC -12	TGTCATCCCC
OPO -04	AAGTCCGCTC
OPO -05	CCCAGTCACT
OPO -06	CCACGGGAAG
OPO -10	TCAGAGCGCC
OPO -11	GACAGGAGGT

The reaction conditions were optimized and mixtures (25 µl total volume) were composed of: 2.5 µl 2.5 mM dNTPs, 2.5 µl 25 mM $MgCl_2$, 2.5 µl 10x buffer, 2.0 µl 10 µM primer, 1.0 µl 100 ng template DNA, 1.25 unit Taq polymerase and H_2O (d.w) up to 25 µl. The mixture was covered with 20 µl of mineral oil.

Amplification was carried out in a Perkin Elmer thermocycler 2400 for 40 cycles according to the manufactured manual of Operon technology, Inc., company as follows: one cycle 94°C for 2 min, 40 cycles (94°C for 1 min, 37°C for 1 min, 72°C for 2 min), one cycle 2°C for 10 min and hold at 4°C.

3. Data analysis:

RAPD patterns were scored by the Computer Gel Documentation System supplied by UVP, USA. Variations among genotypes were evaluated from pairwise comparison for the proportion of shared bands i.e., two times the number of common bands (shared fragments) divided by the total number of bands for each pair of of genotypes against all markers. Therefore, DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among genotypes (Yang and Quiros, 1993).

The gel was photographed under UV light with UVP Gel Documentation System and the data of molecular markers was analyzed by Gelworks 1D advanced software UVP version 4.

RESULTS AND DISCUSSION

1. Cytological study:

All examined root tips of all selected strains illustrated that the chromosome number of the plantlets developed from calli was 56 (octaploid) as in the original cultivars Table (1) and (Fig., 1). It was also noticed that non of the regenerated plants seemed to be tetraploid, mixoploid, hexaploid or heptaploid.

Fig. 1: A photograph of strawberry chromosomes (2n = 56). X 1000.

Table (1): Chromosome counts of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars.

Genotype designation	No. of plantlets	No. of roots examined	No. of cells examined	No. & % of polyploidy cells	Ploidy group and %
Capitola (A)	100	300	185	125 (67.6%)	100% Octaploid
A1	100	300	182	112 (61.5%)	100% Octaploid
Chandler (B)	100	300	191	130 (68.1%)	100% Octaploid
B1	100	300	194	112 (57.7%)	100% Octaploid
Camarosa (C)	100	300	185	99 (53.5)	100% Octaploid
C1	100	300	190	103 (54.2)	100% Octaploid
C2	100	300	188	112 (59.6)	100% Octaploid
C3	100	300	210	126 (60 %)	100% Octaploid
C4	100	300	198	124 (62.6%)	100% Octaploid
C5	100	300	220	138 (62.7%)	100% Octaploid
C6	100	300	260	175 (67.3%)	100% Octaploid
C7	100	300	205	130 (63.4%)	100% Octaploid
C8	100	300	280	184 (65.7%)	100% Octaploid
C9	100	300	197	112 (56.9%)	100% Octaploid
C10	100	300	188	122 (65.9%)	100% Octaploid

A =Capitola **A 1 = Capitola strain**
B = Chandler **B1 = Chandler strain**
C = Camarosa ***C1-C10 = Camarosa straine***

The obtained results are in agreement with those obtained by Rosati *et al.* (1975), Oosawa and Takayanagi (1982), and Quarta *et al.* (1991); who found that all the regenerated plants had the octaploid chromosome number

of 2n = 8x = 56. On the other hand, the obtained results did not seem to agree with those of Niemirowicz-Szczytt and Zakrzewska (1981); who found tetraploid, hexaploid, octaploid, Niemirowicz-Szczytt *et al.* (1983); who found diploid, tetraploid, hexaploid and mixoploid; and Simon *et al.* (1987); who found octaploid plants and hexadecaploid 2n = 16x = 112; as well as Svensson and Jahansson (1994); who found octaploid, heptaploid, hexaploid, hexadecaploid and mixoploid in their studies on strawberry plants.

The origin of those obtained octaploid plants could be explained to be due to either their development from the somatic tissue of the anthers or to the cytological instability of the haplotetraploid callus, derived from microspores. Since, it is known that, in most of the high ploidy plants, the cells of a callus, cultured *in vitro*, involve high cytological instability.

2. DNA fingerprinting:

The genetic variabilities among the newly developed twelve strains of strawberry and their original cultivars, based on randomly amplified polymorphic DNA (RAPD) relationships analysis were studied, using eleven random 10-mer primers. Six primers failed to produce (PCR) products for all the genotypes; while, the other five primers had amplified sixty PCR bands, where all of them were polymorphic (100%). The distribution of the polymorphic bands are shown in Tables (2-6) which reflect the variability among the studied genotypes.

Primer OPO-04:

Table 2 and Fig. 2 indicated that the molecular weights of PCR products, generated by primer OPO-04, ranged from 225 to 1535 bp. Number of total bands varied among the genotypes; where, the lowest number was three bands, as found in C1, C4 and C7 Camarosa strains, while the highest number was seven, as recorded in C2 Camarosa strain. It is also noted from Table 1 and Fig. 2 that no common bands among the studied genotypes were identified. In this connection, Capitola, C6, C8, C9 and C10 Camarosa strains had 5 fragments each, but only the fragments Nos.4 and 7 were present in all of them; while, the others were present in some of them and absent from the others.

The bands were polymorfic, as they were present in some genotypes and absent in the others. Some genotypes had some specific bands and could be used to distinguish them; as found in Capitola cultivar, which could be distinguished from the others by the existence of one unique fragment at the molecular weight of 225 bp, as well as C2 Camarosa strain, at the molecular weight of 380 bp.

On the other hand, a band with the molecular weight 935 bp was found to be absent in C2 and C4 Camarosa strains; as well as a band with the molecular weight 510 bp in Camarosa and its strain no.7; which indicated that these bands might be considered as specific negative markers for these genotypes, compared with the others. Both of C3 and C5 Camarosa strains were in the same class pattern; while, the other tested genotypes were in separate classes, as shown in Table 2 and Fig. 2.

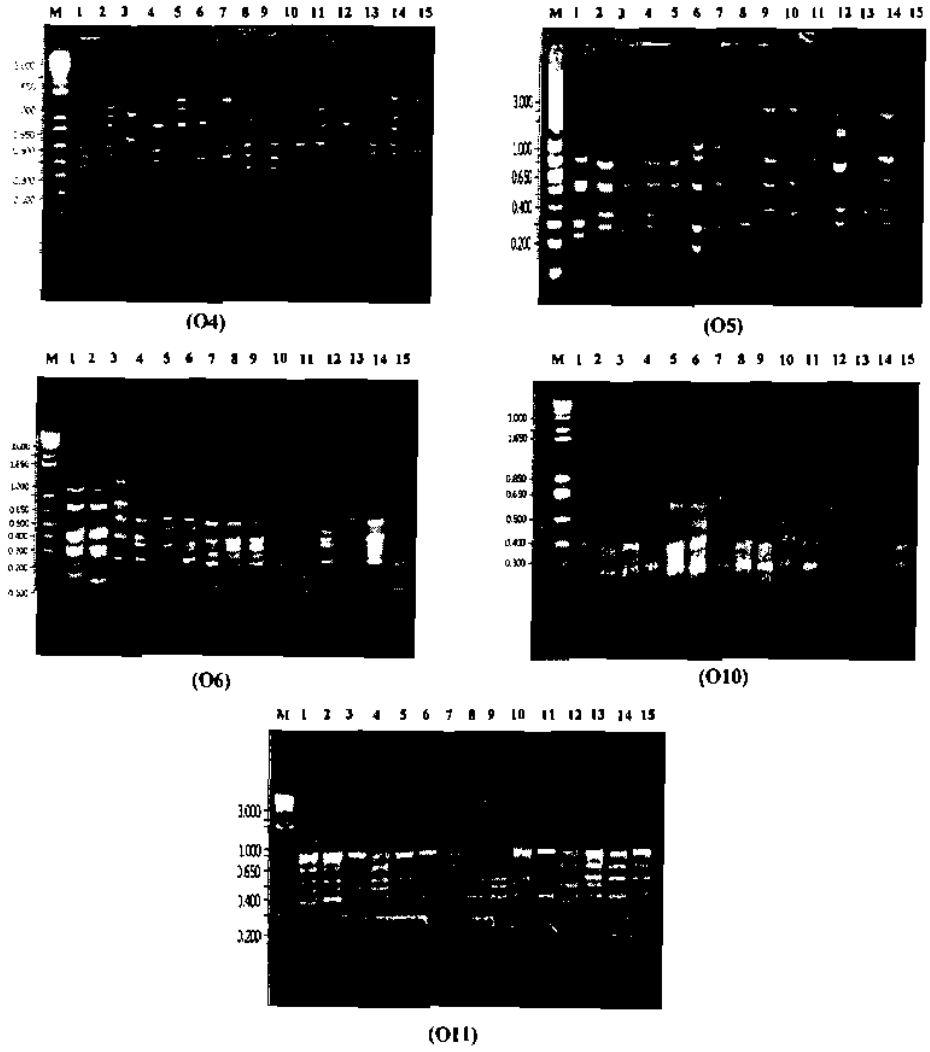

Fig. (2): RAPD patterns of genomic DNA of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars using the random primers OPO-4, 5, 6, 10 and 11.

1=A = Capitola
2= A 1 = Capitola strain
3 = B = Chandler
4= B1 = Chandler strain
5 = C = Camarosa
6-10= C1-C10 = Camarosa strains

Table 2: Genomic fingerprints of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars using the random primer OPO-04.

Band No.	Molecular size (Kb)	A	A1	B	B1	C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
1	1.535	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1
2	1.265	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0
3	1.110	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1
4	0.935	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1
5	0.680	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
6	0.610	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0
7	0.510	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
8	0.420	1	1	1	0	0	0	0	1	1	1	1	1	0	0	0
9	0.380	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
10	0.315	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0
11	0.225	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Primer OPO-05:

The results of RAPD analysis by using primer OPO-05 are illustrated in Table (3) and Fig. (2). This primer produced fourteen bands with molecular weights ranging from 185 to 2165 bp. The lowest number of bands was two which was detected in Camarosa cultivar, while, the highest number was six in Chandler and C7 Camarosa strain.

Moreover, the detected bands were polymorfic, as there were no common bands in all tested genotypes.

Table 3: Genomic fingerprints of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars using the random primer OPO-05.

Band No.	Molecular size (Kb)	A	A1	B	B1	C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
1	2.165	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
2	1.940	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
3	1.380	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
4	1.095	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0
5	0.950	1	0	0	0	0	1	0	0	1	1	1	0	1	1	1
6	0.815	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0
7	0.580	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1
8	0.400	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
9	0.370	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
10	0.330	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0
11	0.290	1	0	0	0	0	0	0	1	0	0	1	1	0	0	1
12	0.265	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0
13	0.220	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0.185	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

A =Capitola A1 = Capitola strain B = Chandler
B1 = Chandler strain C = Camarosa C1-C10 = Camarosa strains

Bands with the molecular weights of 1940, 1380, 220 and 185 bp were present in (C7&C9 Camarosa strains), (Chandler & C7 Camarosa strain), Capitola and C1 Camarosa strains, respectively; and they could be used to distinguish them among the other tested genotypes. While, the band at the

molecular weight 580 bp could be considered s a specific negative marker for C3 and C7 Camarosa strains; as it absent from these two particular genotypes, compared with all other tested genotypes. The tested genotypes appeared in separate class patterns; except C4 and C5 Camarosa strains *which were in the same class, as shown in Table* 3.

Primer OPO-06:

Table 4 and Fig. 2 illustrate the RAPD analysis derived from primer OPO-06. The PCR products of this primer ranged from two to seven fragments with molecular weights ranging from 125 to 1205 bp. No common bands were observed among all studied genotypes. In this respect, each of A1 *Capitola strain, Camarosa,* C1, C2, C4, C7 *and* C9 *Camarosa* strains had five fragments; but, fragments no.2, 4 and 7 at the molecular weights of 1000, 720 and 405 bp were only present in A1. Also, fragment no.16 at the molecular weight 125 bp was only present in C2 Camarosa strain. On the other hand, the band with the molecular weight 535 bp was absent in A1 and C7 strains, indicating that this particular band might be considered as a specific negative marker for these two later genotypes as compared with all *others.*

Accordingly, fifteen class patterns were observed when the number and distribution of fragments were taken in consideration resulting in a distinct identification for all tested genotypes, as appeared in Table 4 and Fig.2.

Table 4: Genomic fingerprints of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars using the random primer OPO-06.

Band No.	Molecular size (Kb)	A	A1	B	B1	C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
1	1.205	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2	1.000	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.850	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	0.720	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
5	0.535	0	0	1	1	1	1	1	1	1	0	0	0	1	1	0
6	0.450	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0
7	0.405	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
8	0.365	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
9	0.325	0	0	1	1	1	1	0	1	0	1	0	1	1	1	0
10	0.295	1	1	0	0	0	1	0	1	1	0	0	0	0	0	0
11	0.260	1	0	0	0	0	0	1	1	1	1	0	0	0	1	0
12	0.225	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
13	0.185	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
14	0.165	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
15	0.145	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0
16	0.125	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1

Primer OPO-010:

The results presented in Table 5 and Fig. 2 illustrated that primer OPO-10 produced molecular weights of PCR that ranged from 195 to 860 bp.

Although the results indicated eleven markers produced by this primer, the contained number of bands ranged from two to four among the tested genotypes. In this connection, no common bands in all tested genotypes were detected; while, bands at the molecular weights 860 and 605 bp were only present in C7 Camarosa strain. Also bands at 715 and 195 bp molecular weights were only obtained in C2 Camarosa strain and Capitola strain, respectively.

On the other hand, using this primer were eleven classes produced, Capitola and Chandler cultivars appeared in the same class; while, Camarosa strains no. 3, 6, 8 and 9 were in another class. The other tested genotypes appeared in individual classes.

Table 5: Genomic fingerprints of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars using the random primer OPO-10.

Band No.	Molecular size (kb)	A	A1	B	B1	C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
1	0.860	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2	0.715	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
3	0.660	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
4	0.605	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
5	0.500	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0
6	0.365	1	0	1	0	0	1	0	1	1	1	1	0	1	1	0
7	0.335	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1
8	0.265	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1
9	0.245	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
10	0.215	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0
11	0.195	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Primer OPO-011:

The results of RAPD analysis by using OPO-11 are shown in Table 6 and Fig. 2. It reacted with all the fifteen tested genotypes.

Table 6: Genomic fingerprints of 12 the newly selected strawberry strains, regenerated by anther cultures, and their original cultivars using the random primer OPO-11.

Band No.	Molecular size (kb)	A	A1	B	B1	C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
1	1.000	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1
2	0.765	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1
3	0.585	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1
4	0.505	1	1	1	1	1	0	0	0	1	1	0	1	1	1	0
5	0.415	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1
6	0.380	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
7	0.325	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0
8	0.225	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0

A =Capitola
B = Chandler
C = Camarosa

A 1 = Capitola atrain
B1 = Chandler strain
C1-C10 = Camarosa straina

The molecular weights of the PCR products generated by this primer ranged from 225 to 1000 bp. The number of total detected bands varied among the genotypes, where the lowest number was two in C1 Camarosa strain and the highest number was six in Chandler strain and Camarosa cultivar. Ten class patterns were detected according to the distribution of PCR products of this primer. In this respect, Chandler strain and Camarosa cultivar appeared in the same class; while, Capitola strain, Chandler cultivar and C5, C8 & C9 Camarosa strains appeared together in another class. The other tested genotypes were in distributed individual classes.

Phylogenetic relationships:

The pairwise differences among strawberry genotypes, calculated by SPSS are shown in Table 7 and Fig. 3. The analysis was based on the number of markers that were different between any given pair of genotypes. The results of analysis showed two groups. The highest value of similarity was observed between the two Camarosa strains C8 and C9 with similarity of 0.829; which clustered together and closely grouped with Camarosa strain C5 and the three genotypes could also be grouped with Camarosa Cultivar. Also, C6 and C10 Camarosa strains showed a second group together, with a high similarity value (similarity 0.667), and the two strains could be grouped with Camarosa strain C4. Moreover, Camarosa strain C3 was closely grouped with the last mentioned four strains.

The results in Table 7 and Fig. 3 also showed that there were no particular strong correlations between the estimates of genetic similarity from RAPD data and the known pedigree data from the tested cultivars, probably because of the relatively small number of RAPD markers.

Table 7: RAPD similarity matrix of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars.

	A	A1	B	B1	C	C1	C2	C3	C4	C5	C6	C7	C8	C9
A														
A1	0.578													
B	0.52	0.812												
B1	0.485	0.524	0.633											
C	0.372	0.571	0.553	0.7										
C1	0.429	0.341	0.478	0.564	0.564									
C2	0.419	0.333	0.511	0.6	0.5	0.564								
C3	0.524	0.39	0.435	0.513	0.462	0.526	0.564							
C4	0.591	0.419	0.458	0.634	0.537	0.55	0.537	0.66						
C5	0.591	0.465	0.5	0.634	0.537	0.6	0.585	0.7	0.782					
C6	0.571	0.488	0.435	0.462	0.462	0.526	0.462	0.632	0.6	0.75				
C7	0.4	0.318	0.449	0.429	0.429	0.263	0.286	0.33	0.279	0.372	0.233			
C8	0.524	0.537	0.585	0.716	0.667	0.632	0.513	0.632	0.66	0.75	0.632	0.38		
C9	0.578	0.5	0.653	0.714	0.619	0.586	0.619	0.634	0.666	0.744	0.585	0.409	0.829	
C10	0.45	0.482	0.5	0.486	0.541	0.444	0.541	0.666	0.474	0.632	0.667	0.369	0.667	0.667

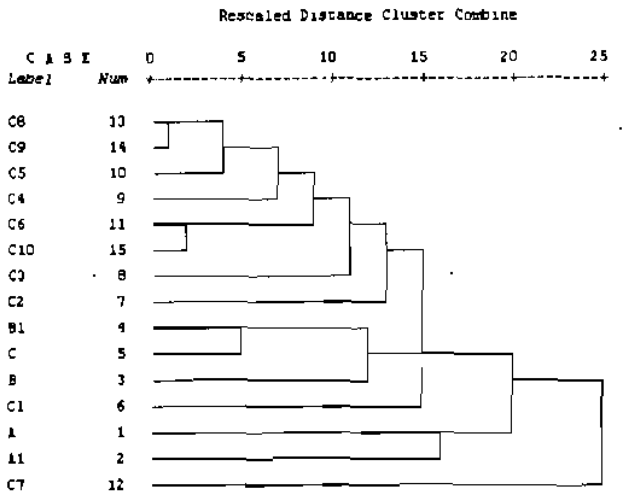

Fig. 3: Dendogram of the 12 newly selected strawberry strains, regenerated by anther cultures, and their original cultivars for the RAPD similarity matrix shown in Table (7).

A 1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains
A = Capitola B = Chandler C = Camarosa

The present results seemed to agree with those of Levi and Rowland (1997) in their study on blueberry, using nearly 200 RAPD markers and 18 cultivar or selections representing 3 species. They found that RAPD markers were usefull for separating out the different species; but, did not accurately assess the genetic relationships of cultivars within the *Vaccinium corymbosum L.* species.

Genetic distances obtained from molecular marker data may be used for germplasim characterization in order to establish a core germplasm collection and identity clusters of unrelated genotypes. In eukaryotic species like strawberry, the proportion of segregating loci is more important for the detection of trail association, (e.g. marker-assisted selection, MAS) than the phenotypic differences between two parents.

In conclusion, the consensus fingerprint profiling, using the RAPD markers, is a useful and reliable method for establishing genetic identities of the strawberry cultivars and advanced selections regenrated from anther culture. It provided also an improved discrimination way for evaluating genitic diversity and relatedness.

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إنتاج بعض سلالات الفراولة الجديدة من خلال زراعة المتوك:
٢- الدراسة السيتولوجية والبصمة الوراثية

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أجريت هذه الدراسة خلال الفترة من ٢٠٠١-٢٠٠٢ لتقييم بعض سلالات الفراولة المنتجة حديثا، من حيث العدد الكروموسومى والبصمة الوراثية للسلالات المنتخبة والآباء.

أوضحت النتائج أنه بفحص عدد الكروموسومات فى قمم الجذور وجد أنه ٥٦ كروموسوم (ثمانية التضاعف) لجميع السلالات الناتجة كما فى الآباء. وأظهرت نتائج البصمة الوراثية أن هناك تشابها إلى حد كبير بين سلالتى الصنف كماروزا أرقام ٨ و ٩، وكذا بين السلالات أرقام ٥ و ٦ للصنف ذاته وقد ارتبطت المجموعة الأولى مع الصنف شاندلر وسلالاته، بينما ارتبطت كل من السلالات أرقام ٥ و ٦ و ١٠ من الصنف كماروزا بالسلالة رقم ٣ لنفس الصنف. وقد ارتبطت السلالات الأربعة الأخيرة مع السلالة ٤ من الصنف كماروزا أيضا. كما أن طريقة البادئات العشوائية تعد طريقة ناجحة للتفرقة بين الطرز الجينية المختلفة من الفراولة.

وتجمل الدراسة أنه أمكن الحصول على طرز جينية جديدة بها اختلافات وراثية هامة لمربى الفراولة باستخدام طريقة زراعة المتوك فى المعمل.