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MOLECULAR FINGERPRINT OF SOME NEW HOT PEPPER "*Capsicum annuum* L." CULTIVARS IN EGYPT

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ABSTRACT

The present investigation was conducted to develop biochemical and molecular genetic fingerprints for the four *Capsicum annuum* cultivars; Bohemian, Cherry, Long-Red Cayenne and Anaheim. Polyacrylamide gel electrophoresis was employed to identify the biochemical fingerprint based on SDS-proteins (sodium dodecyl sulfate) and two isozyme (peroxidase and esterase) profiles. The gained data of SDS-protein patterns revealed low level of polymorphism whereas the two isozyme systems were effective in identifying the four *Capsicum* cultivars. Each cultivar was distinguished by a unique class pattern, which indicates that isozyme fingerprinting is a reliable technique to discriminate the four *Capsicum* cultivars.

Extracted DNA from fresh leaf samples was used to identify the molecular fingerprint of the four cultivars of *Capsicum* under study. Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis with five random primers (UBC-20, -35, -50 OPB-08 and -09) was performed. The results of RAPD analysis partialy discriminate among the four cultivars of *Capsicum* by one or more unique bands. Some of the random primers were more successful in identifying cultivars (UBC-20) than the others, where they generated high number of unique bands.

The similarity indices for all pairs of four groups of *Capsicum* cultivars scored from polymorphism across SDS-protein, two isozymes and RAPD analysis were used to identify the genetic relationships among these cultivars.

Keywords: Capsicum, pepper, electrophoresis, isozymes, RAPD-PCR, similarity.

INTRODUCTION

Genetic fingerprinting studies of *Capsicum* cultivars offer possibilities for use in classifying and improving *Capsicum* cultivars. SDS-polyacrylamide gel electrophoresis of proteins, isozymes and PCR-RAPD (polymerase chain reactions-randomly amplified polymorphic DNA) analyses offer reliably rapid means for establishing genetic profiles and elucidation of genetic relationships within and between taxa that would help in conservation of new varieties and save efforts, expanses and time in the breeding programs of *Capsicum*. Badr (1995) used SDS-protein profiles to study the relationships between some taxa of *Trifolium*. Saeed *et al.* (1998) used SDS protein profiles to discriminate between two jujube cultivars. Afiah *et al.* (1999) studied the protein profiles of some wheat cultivars and their F_1 hybrids. Also Aly *et al.* (2000) used grain storage protein to discriminate between nine rice cultivars.

Isozymes have been extensively utilized to deduce similarity indices and to construct dendrogram trees in many plant taxa. In this regard, Abdelsalam *et al.* (1998) used isozymes to discriminate between some barley cultivars. Also, Li and Li (1999) used 9 isoenzyme systems to investigate variation in twelve natural populations of *Eucalyptus microtheca* from Australia. Cluster analysis based on the UPGMA (un-weighted pair group method of arithmetic means) dendrogram revealed low levels of genetic

distance among these populations. Ohnishi and Asano (1999) investigated nineteen natural populations of *Fagopyrum homotropicum* from the Yunnan and China for their allozyme variation at 16 loci of 11 enzymes. The position of populations in the phylogenetic tree constructed from genetic distance was found to be nearly corresponding with the geographical origin of the populations.

The development of randomly amplified polymorphic DNA (RAPD) markers by Williams *et al.* (1990) based on the use of short primers (10 mer) of arbitrary nucleotide sequences in PCR have a number of advantages over the other DNA-based marker systems (Sane *et al.*, 1997). RAPD analysis is an effective, cost-efficient method for genotype identification and pedigree analysis (Welsh *et al.*, 1991).

Because of technical advantages, RAPD markers have been extensively used in population genetics, analysis of biodiversity and studies of relationships among species and varieties. De Bustos *et al.* (1999) used sixty-four arbitrary 10-mer primers to assess the variations within and between *Hordeum* species and subspecies. Esselaman *et al.* (2000) used RAPD markers to identify and differentiate among some *Dendroseris* species. Abdel-Tawab *et al.* (2001) used forty-nine 10-mer random primers to fingerprint eight *Sorghum bicolor* cultivars.

This study was planned to: 1) establish biochemical and molecular genetic fingerprint for four *Capsicum annuum* cultivars using SDS-protein, isozymes and RAPD analyses; 2) elucidate phylogenetic relationships among these cultivars.

MATERIALS AND METHODS

Four of *Capsicum annuum* L. Cultivars; Bohemian, Cherry, Long-Red Cayenne and, Anaheim representing different genomes were used in this study. Seeds of cv Bohemian "OSU 6-2" selection were kindly donated from Prof. Dr. James Motes, Oklahoma state University, USA. This cultivar is almost twenty times more pungent than any other common cultivars. Seeds of Cherry cultivar were imported from Moravo Seed Co., Mirulove, Czech Republic. On the other hand, seeds of cv. Long-Red Cayenne and Anaheim cultivars were delivered through Burpee Exclusive, Co., Warminster, USA. They were maintained by the author through propagation for successive years in an isolated area. The seeds were grown in the green house of the Medicinal and Aromatic Plant Research Department and the fingerprinting investigations were carried out in the Biotechnology Lab., both are belonging to Horticulture Research Institute, Agricultural Research Center.

SDS-electrophoretic protein procedure:

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to examine *Capsicum* seed storage proteins according to the method of Laemmli (1970). Ten dry seeds of each *Capsicum* cultivar were mixed together and milled to fine powder. A volume of 0.2 ml of sample buffer (0.2 M Tris-HCl pH 6.8, 2 % SDS, 10 % glycerol) was added separately to 0.02 g of seed meal of each cultivar and stored overnight at 4 ^{o}C. Centrifugation was performed at 9000 rpm for 6 minutes, then 30 µl supernatant were loaded in SDS-slab gels of 12.5% polyacrylamide containing 10 % SDS. Run

power was 15 mA for about half-hour then raised to 25 mA for 6-7 hours Molecular weights of the different bands were calibrated with a protein standard with the following molecular weights; 14.3 kDa (lysozyme), 21.5 kDa (trypsin inhibitor), 30.0 kDa (carbonic anhydrase), 46.0 kDa (ovalbumin), 66.2 kDa (bovine serum albumin), and 97.4 kDa (phosphorylase b). The SDS-protein gels were scanned and analyzed using Gel Doc 2000 BioRad system.

Isozyme electrophoreses:

For Peroxidase (*Px*) and esterase (*Est*) isozymes extraction, 0.2 g fresh leaf samples from each *Capsicum* sample was homogenized separately in 1 ml of 0.125 M Tris-borate buffer pH 8.9. Then each sample was centrifuged for 10 minutes at 10,000 rpm. These isozymes were separated in 10 % polyacrylamide gel electrophoresis according to Stegemann *et al.* (1985). A volume of 50 µl extract of each sample was mixed with 12.5 µl glycerol and 60 µl from this mixture was applied to each gel well. Staining of the gels for peroxidase (*Px*) was performed as described by Larsen and Benson (1970). The staining solution was composed of 50 ml of 1M Na-acetate pH 4.7, 50 ml of methanol, 50 ml of 3, 3`, 5, 5`-tetramethylbenzidine (TMBZ) and 2 ml of 30 % H_2O_2, while for esterase (*Est*) the staining solution was composed of 50 ml of 100 mM Na-phosphate pH 6.0, 25 mg of α-naphthyl acetate and 50 ml of fast blue RR salt according to Scandalios (1964).

Plant DNA extraction and amplification procedure:

Seeds of each cultivar were germinated separately in the green house. Ten fresh and young seedlings of each *Capsicum* cultivar were mixed together. Extraction of DNA was performed separately for each cultivar.

About 0.1 gm (fresh weight) of plant tissues was ground to fine powder in liquid N_2 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, then 1 ml of chloroform and isoamyl alcohol (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, and then 1 ml of isopropanol was added and then kept overnight in 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 µl TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) buffer. DNA was quantified by spectrophotometer and gel electrophoresis (Dellaporta *et al.* 1983)..

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors include PCR temperature cycle profile and concentration of each of the template DNA, primer, and MgCl2 and *Taq* polymerase A total of twenty-one 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. Table (A) lists the base sequence of these six DNA primers that produced informative polymorphic bands.

Table A: Code and sequence of the six UBC primers.

Primer	Base sequence
UBC 20	3`-TCC GGG TTTG-5`
UBC 35	3`-CCG GGG TTAA-5`
UBC 50	3`-TTC CCC GCGC-5`
OPB-08	3`-GTC CAC ACGG-5`
OPB-09	3`-TGG GGG ACTC-5`

The PCR amplification was performed in a 25 µl reaction volume containing the following: 200 µl of dNTPs (2.5 mM), 1.5 µl of Mg Cl_2 (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 ddH_2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Perkin Elmer Gene Amp PCR System 2400. The reaction was subjected to one cycle at 95 °C for 5 minutes, followed by 35 cycles at 96 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds, then a final cycle of 72 °C for 5 minutes. PCR products were run at 100 V for one hour on 1.4 % agarose gels to detect polymorphism between *Capsicum* samples 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.

A mix of a 1018 bp fragment and its multimers and pBR328 DNA fragments with lengths of 12216, 11198, 10180, 9162, 8144, 7126, 6108, 5090, 4072, 3054, 2036, 1636, 1018, 517/506, 396, 344, 298, 220/201, and 154/134 bp was used as marker.

Data analysis:

The protein, isozyme or DNA bands generated by each *Capsicum* sample, were counted and their molecular weights or sizes were estimated according to those of the protein or DNA markers, respectively. The presence or absence of each protein, isozyme, or DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) for computation using Dice (1945) equation as implemented in the computer program SPSS version 10. The bands scored from SDS-protein, isozyme and RAPD-PCR analyses were pooled together to construct dendrogram tree.

RESULTS AND DISCUSSION

SDS-Protein analysis:

Figure (1) demonstrates the SDS-protein profile of the four *Capsicum annuum* cultivars; Bohemian, Cherry, Long-Red Cayenne and, Anaheim. While Table (1) represents the occurrence of bands as (+) and absence as (–). A maximum of 25 bands were detected with molecular weights ranging from 100 to 10 kDa. Considering band's number, the resulted protein profile revealed slight polymorphism at the molecular weight range of about 85 to

66.2 kDa. While the molecular weight range of about 66.2 to 10 kDa did not show any polymorphism. A total of 21 monomorphic bands were detected in the resulted protein profile. With regard of band's intensity a silght intra-cultivar variations were recorded among the studied cultivars. Also, the low molecular weight region was characterized by the presence of high insensitive bands. The same finding was obtained by Hassan (2001) who reported that slight polymorphism was observed among seed storage protein of nine mung bean cultivars.

Many authors used seed storage protein variability for the identification and characterization of species and cultivars or germplasm collections. Felix *et al.* (1996) used grain storage protein to characterize a set of F_1 derived haploid lines of bread wheat. Radovic and Vapa (1996) used SDS-PAGE to study the composition of hordein fractions of 33 Yugoslav barley cultivars. On the basis of hordein profiles, the cultivars were divided into 18 groups. Twelve cultivars showed unique hordein subunit composition while remainder fell into six groups of 2 to 9 cultivars.

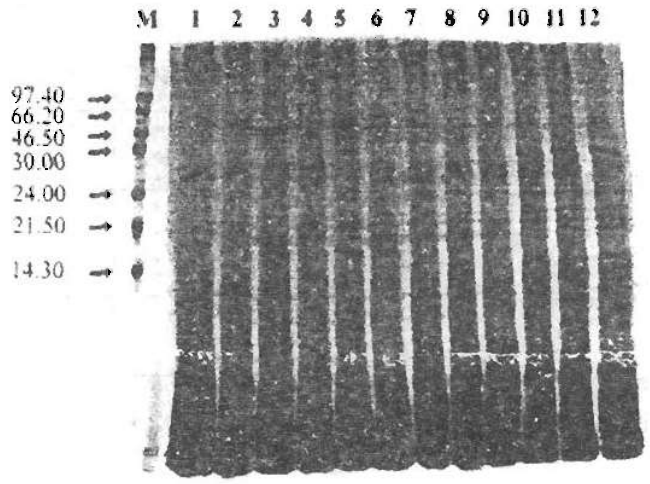

Fig. 1: SDS-Protein banding patterns of the four *Capsicum annuum* cultivars. Lanes (1-3): Bohemian, lanes (4-6): Cherry, lanes (7-9): Long-Red Cayenne lanes (10-12): Anaheim while lane (M): referrers to the standard protein marker.

Table 1: The presence (+) and absence (-) of bands in SDS-protein and isozymes banding patterns of fresh leaf samples of the four *Capsicum annuum* cultivars. R_1, R_2 and R_3 are the three replicates of each cultivar.

Biochemical Marker	approximate molecular Weight (KDa.)	Band No.	Bohemian			Cherry			Long-Red Cayenne			Anaheim		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Protein	93	1	+	+	+	+	+	+	+	+	+	-	-	-
	85	2	-	-	-	-	-	-	+	+	+	+	+	+
	80	3	+	+	+	-	-	-	+	+	+	+	+	+
	60	4	+	+	+	+	+	+	+	+	+	+	+	+
	55	5	+	+	+	+	+	+	+	+	+	+	+	+
	53	6	+	+	+	+	+	+	+	+	+	+	+	+
	50	7	+	+	+	+	+	+	+	+	+	+	+	+
	48	8	+	+	+	+	+	+	+	+	+	+	+	+
	46	9	+	+	+	+	+	+	+	+	+	+	+	+
	43	10	+	+	+	+	+	+	+	+	+	+	+	+
	39	11	+	+	+	+	+	+	+	+	+	+	+	+
	33	12	+	+	+	+	+	+	+	+	+	+	+	+
	27	13	+	+	+	+	+	+	+	+	+	+	+	+
	26	14	+	+	+	+	+	+	+	+	+	+	+	+
	33	15	+	+	+	+	+	+	+	+	+	+	+	+
	27	16	+	+	+	+	+	+	+	+	+	+	+	+
	26	17	+	+	+	+	+	+	+	+	+	+	+	+
	24	18	+	+	+	+	+	+	+	+	+	+	+	+
	23	19	+	+	+	+	+	+	+	+	+	+	+	+
	20	20	+	+	+	+	+	+	+	+	+	+	+	+
	18	21	+	+	+	+	+	+	+	+	+	+	+	+
	16	22	+	+	+	+	+	+	+	+	+	+	+	+
	15	23	+	+	+	+	+	+	+	+	+	+	+	+
	13	24	+	+	+	+	+	+	+	+	+	+	+	+
	12	25	+	+	+	+	+	+	+	+	+	+	+	+
Esterase of seeds		*Est* 1	+	+	+	+	+	+	+	+	+	+	+	+
		Est 2	+	+	+	+	+	+	-	-	-	-	-	-
Esterase of leaves		*Est* 1	+	+	+	+	+	+	+	+	+	+	+	+
		Est 2	+	+	+	+	+	+	-	-	-	+	+	+
Peroxidase of seeds		*Px* 1	+	+	+	+	+	+	+	+	+	+	+	+
		Px 2	+	+	+	-	-	-	+	+	+	+	+	+
		Px 3	-	-	-	+	+	+	+	+	+	-	-	-
Peroxidase of leaves		*Px* 1	+	+	+	+	+	+	+	+	+	+	+	+

Isozyme analyses:

Esterase (*Est*):

Table (1) and Figure (2-a) demonstrate esterase-banding patterns of seed samples from the studied *Capsicum* cultivars. With regard to band's number and intensity a clear inter-cultivar variation was observed. The obtained patterns exhibited a maximum number of two bands, which were not necessarily present in all samples. The band number 1 was scored as a monomorphic band while band number 2 was identified as a polymorphic one. According to the presence or absence of the band number 2, the *Capsicum* cultivars were grouped in two categories. The first group included

the two cultivars Bohemian and Cherry and characterized by the presence of such a band while the second group comprised the cultivars Long-Red Cayenne and Anaheim and characterized by the absence of this band (Table 2).

Table (1) and Figure (2-b) represent esterase electrophoretic banding patterns among the examined leaf samples of the four *Capsicum* cultivars. An obvious inter-cultivar variation was observed with regard to band's number and intensity. Slight intracultivar differences in band intensity were noticed among replicates of Anaheim. A total of two bands were identified for the studied cultivars, which were present in some cultivars and absent in others. The first was scored as a common band (monomorphic) for all cultivars. Band no. 2 was absent only in cultivar Long-Red Cayenne. The absence of this band could be used to discriminate between cultivars Long-Red Cayenne and the other three cultivars. Accordingly two peroxidase groups were recognized with regard to the presence or absence of band no. 2. (Table 2).

Table 2: Distribution of the four *Capsicum annuun* cultivars according to their SDS-protein and isozyme groups.

Biochemical system	No. of cultivars	Class pattern	Cultivars groups
Esterase	2	*Est* 1S	Bohemian and Cherry.
of seeds	2	*Est* 2S	Long-Red Cayenne and Anaheim.
Esterase	3	*Est* 1L	Bohemian, Cherry and Anaheim
of leaves	1	*Est* 2L	Long-Red Cayenne.
Peroxidase	2	*Px* 1S	Bohemian and Anaheim
of seeds	1	*Px* 2S	Cherry.
	1	*Px* 3S	Long-Red Cayenne.
Peroxidase of leaves	4	*Px* 1L	Bohemian, Cherry, Long-Red Cayenne and Anaheim

Peroxidase (*Px*):

Peroxidase banding patterns of the seed from the four *Capsicum* cultivars are illustrated in Table (1) and Figure (3-a). This enzyme revealed high polymorphism level. Considering band's number and intensity, intercultivar variation was recorded between the studied cultivars. Three bands were recognized which were present in some cultivars and absent in the others. The first band was identified in all the studied genotypes (monomorphic) while the second band was absent only in Cherry. Similarly, the third band was absent in both Bohemian and Anaheim. Accordingly, the studied cultivars were categorized in three categories (Table 2).

Peroxidase electrophoretic patterns of leaf samples from the four *Capsicum* cultivars are given in Table (1) and Figure (3-b). This pattern does not revealed polymorphism among the studied genotypes. Only, one common band was observed among the profiles of the all studied cultivars. Therefore, one peroxidase group was recognized among the studied *Capsicum* cultivars (Table 2).

Figure (2). Photograph and diagram of esterase isozyme banding patterns of the four Capsicum annuum cultivars. (a) From seeds and (b) from leaves. Lanes (1-3): Bohemian, lanes (4-6): Cherry, lanes (7-9): Long-Red cayenne lanes (10-12): Anaheim.

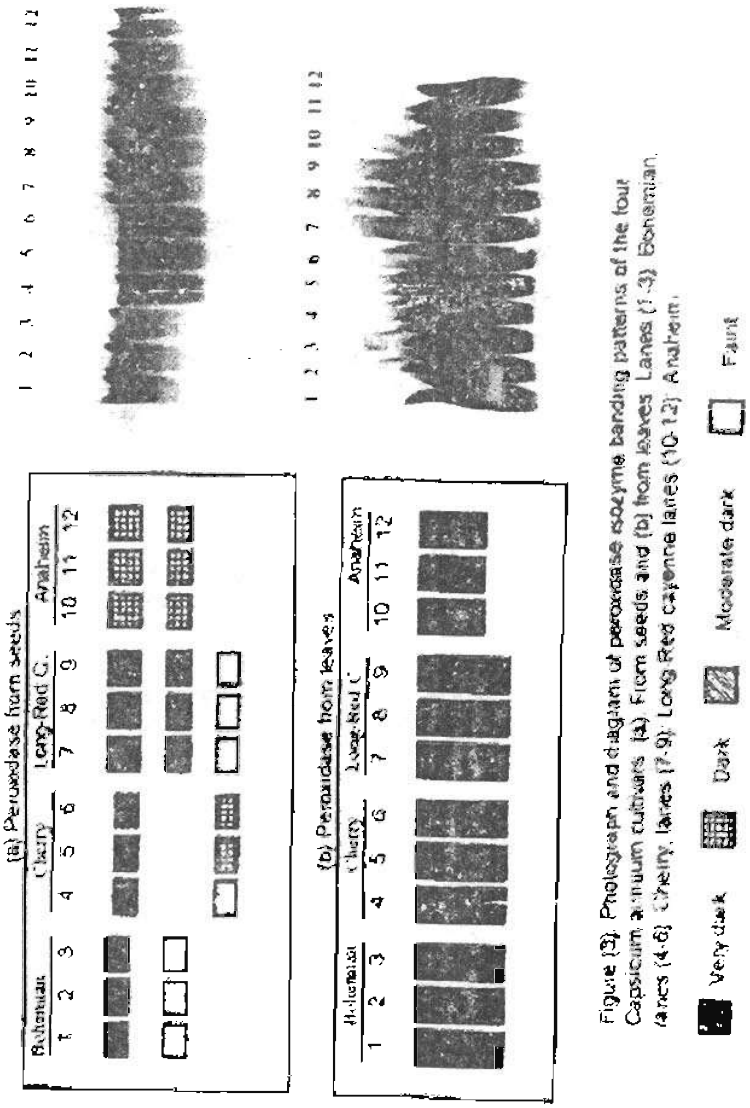

Figure (3): Photograph and diagram of peroxidase isozyme banding patterns of the four Capsicum annuum cultivars (a) From seeds and (b) from leaves. Lanes (1-3) Bohemian, lanes (4-6) Cherry, lanes (7-9) Long Red cayenne lanes (10-12) Anaheim.

It is obvious that neither esterase patterns nor peroxidase isozyme indices used above can stand alone to provide sufficient polymorphic expressions to distinguish between each *Capsicum* cultivars in a unique pattern. Therefore, combined class patterns seem to solve this problem as they offer higher resolution to characterize each cultivar and assign it to a unique class pattern (Table 3). Several investigators reached the same conclusion and recommended the use of isozyme analyses for the elucidation of cultivar identifications for crop plants (Abdel-Tawab *et al.*, 1993).

Table 3: **Combined class patterns for the four *Capsicum annuum* cultivars across esterase enzyme of seeds (*Est* S), of leaves (*Est* L), peroxidase of seeds (*Px* S) and of leaves (*Px* L) analyses.**

Class	Combined class pattern				Sample
	Est. of Seeds	*Est.* of Leaves	*Px.* of Seeds	*Px.* of Seeds	
1	*Est.* 1S	*Est.* 1L	*Px.* 1S	*Px.* 1L	Bohemian
2	*Est.* 1S	*Est.* 1L	*Px.* 2S	Px. 1L	Cherry
3	*Est.* 2S	*Est.* 2L	*Px.* 3S	*Px.* 1L	Long-Red Cayenne
4	*Est.* 2S	*Est.* 1L	*Px.* 1S	*Px.* 1L	Anaheim

The identification and discrimination of many plant taxa based on isozyme polymorphism were reported by many authors. In this regard, Elisiario *et al.* (1999), used isozymes to study genetic variability in Mandarin (*Citrus reticulata*) cv. Carvalhais. This cultivar is used in several *Citrus* breeding programs throughout the world. All of the analyzed trees of Carvalhais showed similar monomorphic patterns for each isoenzyme system, indicating that this cultivar originated as a single initial clone.

RAPD-PCR analysis

Fifteen random primers were initially tested, but five-revealed distinct inter- and intra- specific polymorphism among the four *Capsicum* cultivars under study. These primers are named UBC-20, -35, -50, OPB-08 and -09. Figure (4) illustrates the RAPD profiles generated by these primers. With the exception of the primer CPB-08, all the used primers revealed considerable polymorphism among the studied *Capsicum* cultivars. A total of 18 DNA bands were detected. These bands were sorted as; 6 monomorphic and 12 polymorphic bands. Out of the polymorphic ones, 5 unique bands were scored (Table 4). The range of polymorphism for these primers varied from 100 % (primers UBC-20, -50 and OPB-09) to 0.00 % (primer OPB-08). The size of DNA bands ranged from 1600 bp (UBC-50) to 370 bp (OPB-08). The profiles of DNA bands varied with the primer used.

The primer UBC-20 generated a total of 5 polymorphic DNA band in the studied *Capsicum* genotypes (Table 4 and Fig. 4). Three unique bands were identified out of the polymorphic ones. These three unique DNA bands were scored with a molecular size of about 750, 620 and 530 bp in Bohemian (lane 3). These bands clearly discriminate this cultivar from the remainder of the studied *Capsicum* cultivars. The primer UBC-35 generated one

monomorphic (detected in all the studied genotypes) and 2 polymorphic bands. (Table 4 and Fig. 4). One unique band was identified out of the polymorphic ones. This band was detected at about 620 bp in Cherry (lane 2). This bands clearly discriminate between Cherry and the other studied genotypes. The two primers UBC-50 generated 2 polymorphic bands in the studied genotypes. UBC-50 does not revealed any amplification reaction with Bohemian (lane 1). The primer OPB-08 generated a total of 3 monomorphic bands in the studied genotypes. (Fig. 4). Primer OPB-09 generated a total of three polymorphic bands. One of these bands was scored as unique one. This band was detected at about 1380 bp in Cherry (lane 2). Also, this primer does not generate any bands in the two cultivars Bohemian (lane 1) and Anaheim (lane 4) (Fig. 4).

Table 4: Molecular size in bp of the amplified polymorphic (unique) DNA bands generated by five DNA random primers used for identifying the four *Capsicum annuum* cultivars.

Sample	UBC-20	UBC-35	UBC-50	OPB-08	OPB-09
Bohemian	-	-	-	-	-
Cherry	-	-	-	-	1380
Long-Red Cayenne	750-620-530	-	-	-	-
Anaheim	-	-	-	-	-

Many studies reported that large numbers of random primers were tested to establish the genetic fingerprint of many plant genotypes, but limited number of the utilized primers succeeded to generate reproducible profiles with sufficient polymorphism. In this study, only five out of fifteen primers were succeeded to generate polymorphic and reproducible amplification products. Many authors reported the use of large number of primers to identify and characterized different genotypes, but a limited number of primers succeeded to generate distinct and repeatable polymorphic products. Sharma and Jana (2002) used RAPD markers to distinguish between 28 different accessions elonging o 4 pecies nd wo ub-species of *Fagopyrum*. Of the 75 random 10-mer primers tested, only 9 enerated clear and easily interpretable amplification products.

The use of RAPDs to determine genetic fingerprint and relationships has been demonstrated by many authors. In this regard, Sigurdsson *et al.* (1995) used ten random primers to evaluate the use of RAPD analysis for distinguishing nine trees from a single population of *Populus trichocarpa* in Alaska. Adams (2000) used RAPD analysis to identify 13 taxa of the smooth leaf margin *Juniperus* in section Sabina from the western hemisphere. The results revealed similar patterns among these species. Based on these data, a new species, very similar to *J. blancoi* and *J. scopulorum*, from northern Mexico was recognized i.e. *J. mucronata*.

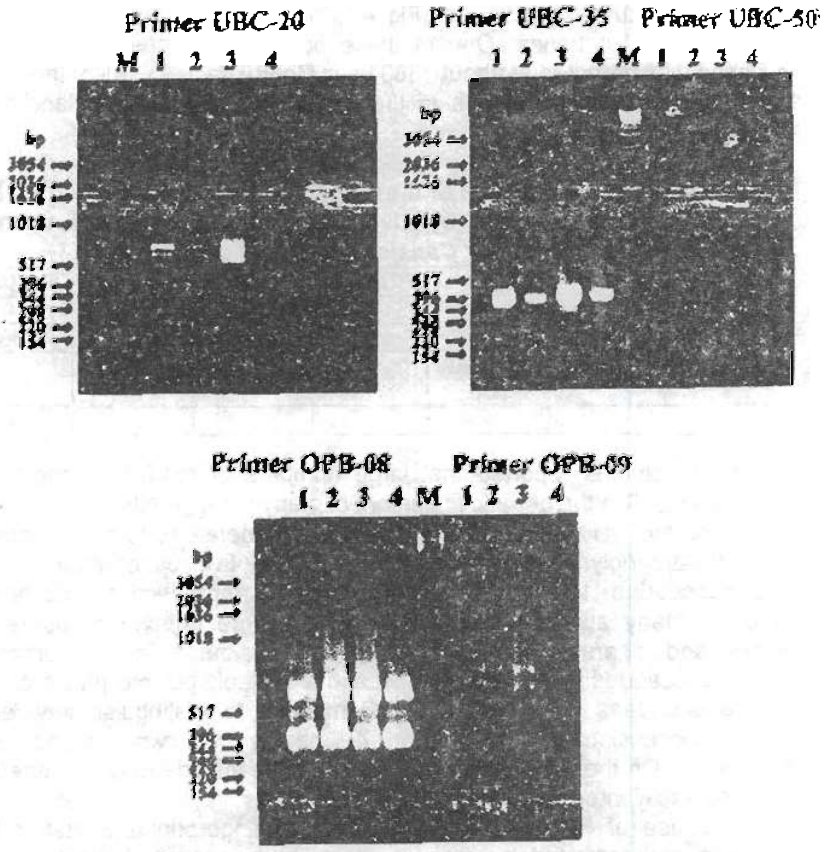

Figure (4): RAPD fingerprints of the four *Capsicum annuum* cultivars using five random primers. Lane (1): Bohemian, lane (2): Cherry, lane (3): Long-Red Cayenne lane (4): Anaheim. Lane M is the standard DNA marker

The similarity coefficients for all pairs of the *Capsicum* cultivars scored from polymorphism across SDS-protein, two isozyme (esterase and peroxidase from both seeds and leaces) and RAPD analyses are represented in Table (5) and the dendrogram developed according to these values is illustrated in Figure (5). The obtained dendrogram separated the five cultivars under study into two categories. The first category includes Bohemian, Cherry, and Anaheim while the second one includes only Long-Red Cayenne. The most similar cultivars appear to be Bohemian and Anaheim with similarity coefficient equal to 0.873 (Table 5).

Table 5: Dendrogram of the four *Capsicum annuum* cultivars as estimated across polymorphism of SDS-Protein, two isozymes (peroxidase and esterase) and RAPD analyses.

	Matrix file input		
	Bohemian	Cherry	Long-Red C.
Cherry	0.813		
Long-Red C.	0.721	0.776	
Anaheim	0.873	0.754	0.793

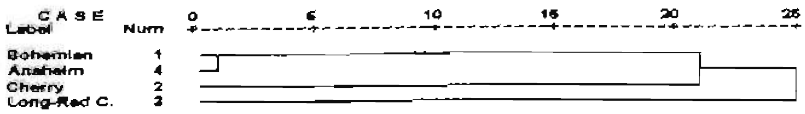

Figure 5: Dendrogram of the four *Capsicum annuum* cultivars as estimated across SDS-protein, two isozyme systems (peroxidase and esterase) and RAPD analyses

Several investigators reached the same conclusion and recommended the use of isozyme analyses for the elucidation of similarity coeffecients for crop plants. In this regard, Onus (1999) examines the utility of enzyme polymorphism for taxonomic identification of purple-flowered *Capsicum cardenasii, C. eximium, C. pubescens* and *C. tovarii* and for revealing genetic distance between these species. Results showed that while *C. cardenasii, C. eximium and C. pubescens* were closely related, *C. tovarii* was halfway from these species. Lefebvre *et al.* (2001) used RAPD markers to estimate genetic distances of 47 pepper (*Capsicum annuum*) inbred lines belonging to five varietal types. Based on pattern scores, dendrograms were produced by the UPGMA method. Phenetic trees based on molecular data were consistent with the classification of variety group. The molecular genetic distances were

correlated with distances based on a set of discriminating agronomic traits measured for identification. Hassan *et al.* (2002) used RAPD markers to fingerprint ten accessions representing five *Populus* species (*Populus nigra*, *Populus alba*, *Populus euramericana*, *Populus euphratica* and *Populus angulata*) from different locations. The similarity coefficient values were used to produce a dendrogram that illustrates the genetic distance among the studied poplar accessions. The dendrogram showed that poplar samples of each species collected from different locations are grouped together in one cluster.

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البصمة الوراثية الجزيئية لبعض أصناف جديدة من الفلفل الحريف فى مصر

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إستهدف هذا البحث التعرف على البصمة الوراثية البيوكميائية والجزيئية لأربعة من أصناف الفلفل الحريف (*Capsicum annuum*) وهى Bohemian, Cherry, Long-Red Cayenne, Anaheim. وقد إستخدم الجيل عديد الأكريلاميد للتعرف على البصمة الوراثية البيوكميائية القائمة على طرز حزم البروتين و إثنين من مشابهات الإنزيمات وهى البيروكسيديز والإستيريز.

أوضحت نتائج طرز البروتين وجود مستوى منخفض من التباين بينما كان لمشابهى الإنزيمين البيروكسيديز والإستيريز فاعلية كبيرة فى تعريف أصناف الفلفل الأربعة. فقد أمكن تمييز كل صنف بمجموعة فريدة من الطرز النوعية المجمعة، مما يدل على كفاءة تقنية البصمة الوراثية البيوكميائية فى التعرف على أصناف الفلفل.

تم إستخدام الدنا (DNA) المستخلص من الأوراق الطازجة للتعرف على البصمة الوراثية الجزيئية لأصناف الفلفل الأربعة قيد الدراسة. فقد عمل تضاعف عشوائى فى جهاز سلسلة تفاعلات إنزيم البلمرة (RAPD-PCR) لأجزاء من الدنا بإستخدام خمسة من البادئات العشوائية وهى (UBC-20, -35, -50, OPB-08 and -09) . أدت نتائج التضاعف العشوائى للدنا الى تمييز جزئى لكل أصناف الفلفل الأربعة بواسطة حزمة متفردة أو أكثر. وكانت بعض البادئات (UBC-20) أكثر قدرة من البادئات الأخرى فى تمييز أصناف الفلفل، فقد أعطت حزم متفردة أكثر بالتضاعف العشوائى للدنا. وبناء على تلك النتائج فإنه يمكن التمييز بين الأصناف الأربعة.