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Biochemical characterization and variability of Egyptian new hybrids of *Capsicum* L.

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ABSTRACT

Six Egyptian new hybrids belonging to two species of *Capsicum* L. were analyzed using biochemical markers. Low polymorphism percentage (38%) was recorded in the SDS-PAGE pattern. Two species-specific, one for each species, were scored and could be used as biochemical markers. Eight-isozyme systems produced 21 bands, among them only three patterns; alcohol dehydrogenase, malic enzyme and malate dehydrogenase, recorded polymorphism percentages ranged between 57 to 80%. Five characterized unique bands were detected; two in yoser 4 of *C. frutescens* and three in kotof 2 of *C. annuum.* The UPGMA dendrogram revealed low genetic variability of the six hybrids that separated into two main clusters with genetic distance of 0.25.

Keywords: *Capsicum*, biochemical variability, SDS-PAGE, isozyme, dendrogram.

INTRODUCTION

Capsicum is the genus of the family Solanaceae finding diverse uses from nutritional and culinary to pharmaceutical uses. In this genus, more than 30 species have been described, but only five of them, *Capsicum annuum* var. *annuum*, *C. Chinense*, *C. frutescens*, *C. baccatum* var. *pendulum*, and *C. pubescens* are considered to be domesticated (Moscone *et al.,* 2007). *Capsicum annuum* is the most widely cultivated and is used as vegetable and spice. The other four species are used to produce spice or used as genetic resources for disease resistance genes. *Capsicum frutescens* is widespread throughout central and lowland South America, and also in other tropical and subtropical regions, such as Asia, Africa, and the Pacific Islands.

Knowledge of cytological and molecular relationships between plant species is very useful in planning effective breeding strategies designed to transfer desirable genes or gene clusters from one species into another, thereby producing fruitful genomic reconstructions and disease free plants. Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme. It is a valuable technique to get knowledge closeness between investigated genera (i.e., through similarity index) (Knapp, 2002). So, genetic diversity in *Capsicum* has previously been studied using morphological, cytological and biochemical marker systems (Kaur and Kapoor, 2001; Gopinath *et al.,* 2006).

Electrophoresis of seed storage protein banding patterns was used to investigate the genetic and taxonomic relationships in the genus *Capsicum* (Panda *et al*., 1986; Vladova *et al.,* 2004; Zubaida *et al*., 2006). However, polymorphism of seed storage

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protein profiles in *Capsicum annuum* L. and *Capsicum frutescens* L. germplasm has been associated with geographical origin (Odeigah *et al*., 1999; Anu and Peter, 2003). On the other hand, isozyme analyses were also conducted to study the variability in *Capsicum* species (Fernando *et al*., 1989; Adilson *et al.,* 1999; Onus and Pickersgill, 2000). Sota and Nawata (2005) used eight isozyme analyses to record the geographic variation of *Capsicum frutescens* L. in Southeast and East Asia, and to investigate its dispersal routes into Japan.

Keeping in view the importance of protein profiling, the present study was conducted to characterize and estimate variability in six Egyptian new hybrids belonging to two species of *Capsicum* L., and this data may provide a scientific basis for future selection and crop management.

MATERIAL AND METHODS

Plant materials

The seeds of Egyptian hybrids; khairat, yoser 1, yoser 4 of *Capsicum frutescens* and kotof 1, kotof 2, kotof 3 of *Capsicum annuum*; were supplied by Agricultural Research Center (Horticulture Research Department), Dokki, Giza, Egypt.

SDS-PAGE

SDS-polyacrylamide gel electrophoresis was performed in 14 % acrylamide slab gels following the system of **(**Laemmli, 1970). Protein extraction was conducted by mixing ten seeds of each hybrid with an equal weight of pure, clean, sterile fine sand. The seeds were then ground to fine powder using a mortar and pestle and homogenized with 1.5 M Tris-HCl buffer, pH 8.8 in clean Eppendorf tube and left in refrigerator overnight (Badr, 1995)**.** Then 20 µl of each sample supernatant was loaded in the gel. After run finished, gel was stained, distained and photographed.

Isozyme analysis

The examined isozymes were: α-and β-esterases (Est.), acid phosphatase (Acph.), alcohol dehydrogenase (Adh.), aldehyde oxidase (Ao.), malic enzyme, malate dehydrogenase (mdh) and peroxidase (Px). For their extraction, three mature seeds of each hybrid were germinated under the same incubation conditions i.e. in pots holding 2500 g of air-dried soil in a greenhouse for 6-8 weeks with suitable irrigation; 0.25 g of fresh leaves of the seedlings was homogenized in 1 ml extraction buffer (1 M Tris-HCl, pH 8.8) using a mortar and pestle; centrifuged at 3000 rpm for five minutes; the supernatant was kept at –20°C until use. For isozymes separation, 10% (w/v) Native-polyacrylamide gel electrophoresis method was used (Stegemann *et al.,* 1985). For electrophoresis, 50 µl of extract was mixed with 20µl of treatment buffer and 50 µl of this mixture was applied to the well. In gels staining, protocols of Scandalios (1964) were used for α and β-Est.; Wendel and Weeden (1989) for both Ao and Acph; Weeden and Wendel (1990) for Adh; Jonathan and Wendell (1990) for Malic and Mdh and Heldt (1997) for Px. After run finished, gels were washed two or three times with tap water; fixed in ethanol: 20% glacial acetic acid (9:11 v/v) for 24 hours and photographed.

Data analysis

Differences in bands intensity among profiles of the different samples were not considered. The produced clear well defined bands are used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Then the presence or absence of each protein band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram among the six hybrids. Genetic distance was calculated

by the following formula: Genetic distance = 1- similarity coefficient according to Nei and Li (1979) as implemented in the computer program SPSS-11.

RESULTS AND DISCUSSION

The produced SDS-protein profile of the six new Egyptian hybrids belonging to *C.frutescens* and *C. annuum* is shown in Fig. (1). Table (1) revealed a total number of 18 detectable bands (subunits) with molecular mass (Mr) ranging from 76.950 to 13.824 kDa.

Table 1: Molecular mass (Mr.) in kilo-Daltons (kDa) of the produced SDS-PAGE of seed protein bands and their presence (+) or absence (-) in the two *Capsicum* species.

Rf	Ms	*C. frutescens*			*C. annuum*		
		khairat	yoser 1	yoser 4	kotof 1	kotof 2	kotof 3
0.221	76.950	-	-	+	+	+	+
0.233	74.897	-	-	+	+	+	+
0.256	71.115	-	+	+	+	+	+
0.302	64.114	-	+	+	+	+	+
0.399	51.529	+	+	+	+	+	+
0.409	50.381	+	+	+	+	+	+
0.558	36.015	+	+	+	+	+	+
0.587	33.737	+	+	+	+	+	+
0.594	33.209	+	+	+	+	+	+
0.613	31.817	+	+	+	+	+	+
0.779	21.890	+	+	+	+	+	+
0.793	21.210	+	+	+	-	-	-
0.811	20.368	-	-	-	+	+	+
0.822	19.869	+	+	+	+	+	+
0.869	17.873	+	+	+	+	+	+
0.916	16.077	+	+	-	-	-	-
0.971	14.203	+	+	+	+	+	+
0.983	13.824	+	+	+	+	+	+

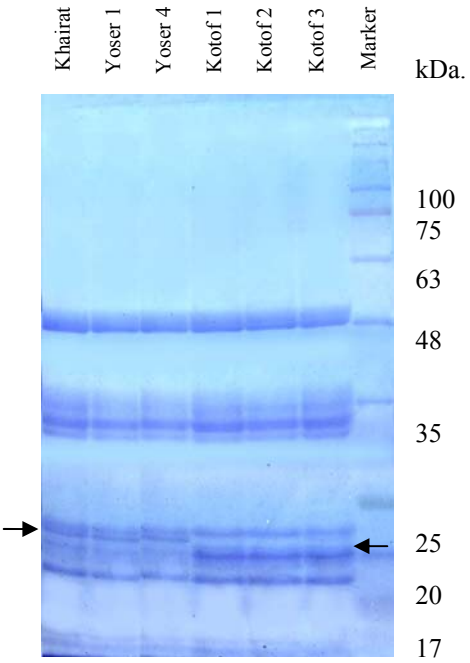

Fig. 1: Seed protein profile of six hybrids of two *Capsicum* species using SDS-PAGE technique. Short arrows indicate two species specific bands. kDa: kilo Dalton.

Low polymorphism percentage (38%) was recorded in the protein pattern as shown in Table (3). The results were in accordance to those of Panda *et al.,* 1986; Anu and Peter, 2003; Zubaida *et al.,* 2006. Aniel *et al.* (2010) detected a total of 15 protein polypeptide bands with molecular weights ranging from 22.4 to 80.8 kDa in seed material of 10 cultivars of *C. annuum* L. And so, limited intra and inter specific variations were observed in protein pattern. Hybrids of *C. frutescens* were characterized by the band at 21.210 kDa, while the band with molecular mass of 20.368 kDa distinguished those of *C. annuum*. The two distinguishable bands were considered as species-specific bands and could be used as biochemical marker for each species. According to findings of the SDS-PAGE, the overall blueprint of seed storage proteins show low degree of heterogeneity may be attributed to cultivar homogeneity or purity. Odeigah *et al.* (1999) and Fufa *et al*. (2005) reported a similar conclusion.

In the present study, eight isozymes were used to characterize biochemically the *Capsicum* hybrids. The results of utilized isozymes were pooled together in Table (3). Twenty one bands were recorded, the highest number was seven in malic enzyme pattern, while the lowest was one in α-and β-esterases, acid phosphatase, aldehyde oxidase, and peroxidase patterns, that did not detect any polymorphism percentage. Only five unique bands from 11 polymorphic ones; two in yoser 4 of *C. frutescens* and three in kotof 2 of *C. annuum*, could be considered as biochemical markers as illustrated in Table (2). These findings were similar to those of Fernando *et al,* 1989; Onus and Pickersgill, 2000; Sota and Nawata, 2005.

Table 2: The recorded bands of eight isozymes and their presence (+) or absence (-) in the two *Capsicum* species.

Isozyme system	Rf	*C. frutescens*			*C. annuum*		
		khairat	yoser 1	yoser 4	kotof 1	kotof 2	kotof 3
α-est	0.019	+	+	+	+	+	+
β-est	0.022	+	+	+	+	+	+
Acph	0.035	+	+	+	+	+	+
Adh	0.008	+	+	+	+	+	+
	0.137	+	-	+	-	-	+
	0.161	-	+	-	+	-	-
	0.191	-	-	-	-	+	-
Ao	0.030	+	+	+	+	+	+
Malic	0.018	+	+	+	+	+	+
	0.453	-	-	+	-	-	-
	0.545	-	-	-	+	-	+
	0.627	+	+	-	-	-	-
	0.718	-	-	-	-	+	-
Mal	0.013	+	+	+	+	+	+
	0.262	+	+	+	+	+	+
	0.346	+	+	+	+	+	+
	0.474	-	-	+	-	-	-
	0.564	-	-	-	+	-	+
	0.685	+	+	-	-	-	-
	0.751	-	-	-	-	+	-
Px	0.022	+	+	+	+	+	+

Table 3: Number and types of the SDS-PAGE and isozymes bands as well as the total polymorphism percentages generated in the two *Capsicum* species.

system	Monomorphic bands	Polymorphic bands		Total bands	Polymorphism %
		Unique	Shared		
SDS-PAGE	11	0	7	18	38
α-est	1	0	0	1	0
β-est	1	0	0	1	0
Acph	1	0	0	1	0
Adh	1	1	2	4	75
Ao	1	0	0	1	0
Malic	1	2	2	5	80
Mal	3	2	2	7	57
Px	1	0	0	1	0

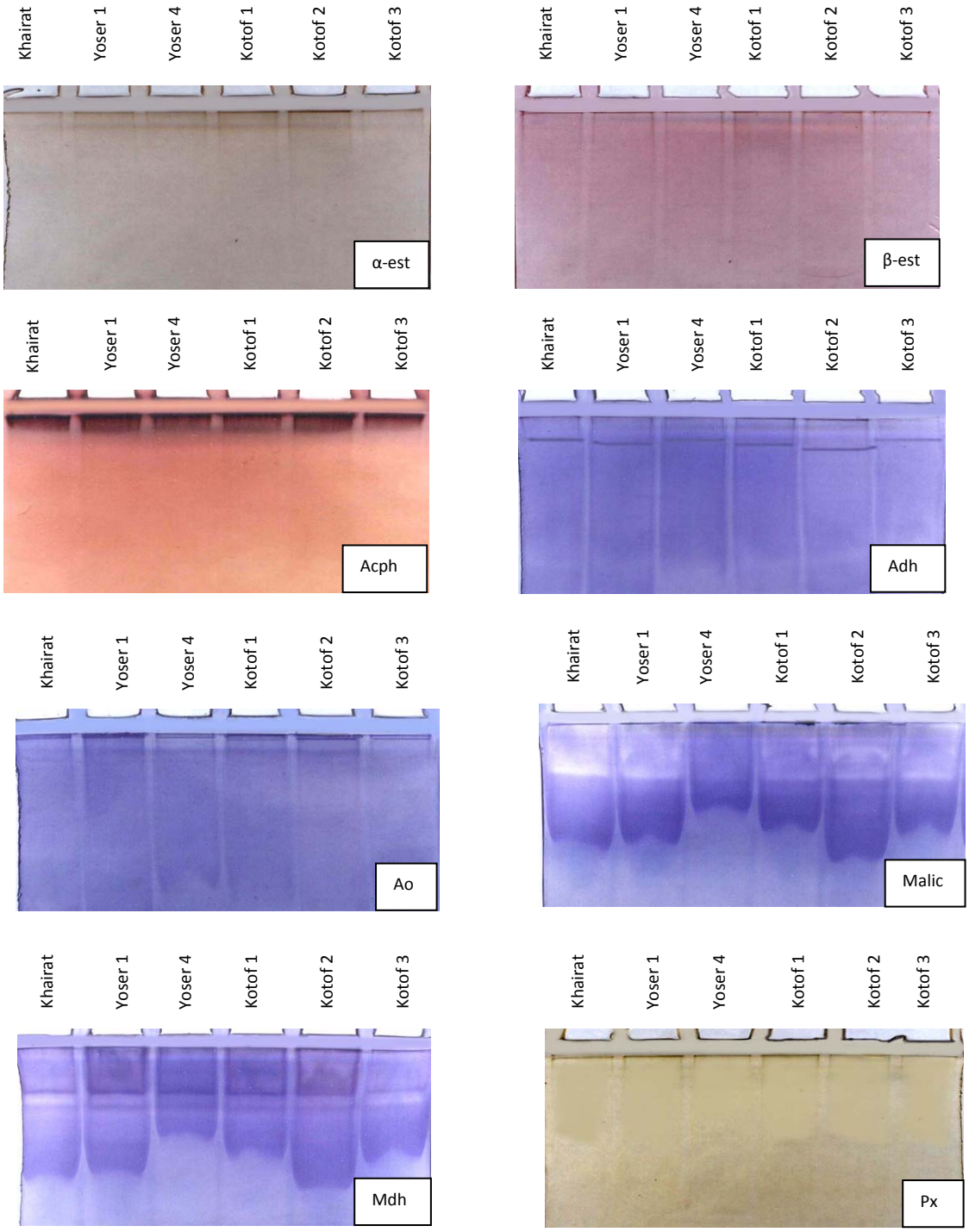

Fig. 2: Zymograms of six hybrids of two *Capsicum* species using eight isozymes techniques.

Three patterns: alcohol dehydrogenase, malic enzyme and malate dehydrogenase, recorded polymorphism percentages ranged between 57 to 80%. This agreed with Adilson *et al.* (1999) who observed high polymorphism in malate dehydrogenase pattern in wild species of *Capsicum*. Also, Sota and Nawata (2005) revealed variability in malic enzyme among accessions of *C. frutescens* in Southeast and East Asia. It was obvious that no one of the protein or isozyme indices used above can stand alone to provide sufficient polymorphic profile to distinguish between the two species of *Capsicum*, therefore, combined class patterns seem to solve this problem as they offer higher resolution to characterize the two species. For this reason, genetic similarity matrix based on protein and isozymes data among the six hybrids was prepared and presented in Table (4). The highest similarity value (0.966) was recorded between kotof 4 and 6 of *C. annuum*, meanwhile the lowest genetic similarity coefficient (0.764) was observed between khairat of *C. frutescens* and kotof 1 and 2 of *C. annuum*. The UPGMA dendrogram illustrated in Fig. (3) revealed a low genetic variability as it separated the six hybrids into two main clusters with genetic distance of 0.25. The first cluster included khairat and yoser 1 of *C. frutescens* with genetic distance of 0.057.

Table 4: Matrix of the genetic similarity of six hybrids of two *Capsicum* species based on combination of SDS-PAGE and isozymes data analyses.

Hybrid	Khairat	Yoser 1	Yoser 4	Kotof 1	Kotof 2	Kotof 3
Khairat	1.00					
Yoser 1	0.926	1.00				
Yoser 4	0.836	0.842	1.00			
Kotof 1	0.764	0.842	0.862	1.00		
kotof 2	0.764	0.807	0.862	0.897	1.00	
Kotof 3	0.800	0.807	0.897	0.966	0.897	1.00

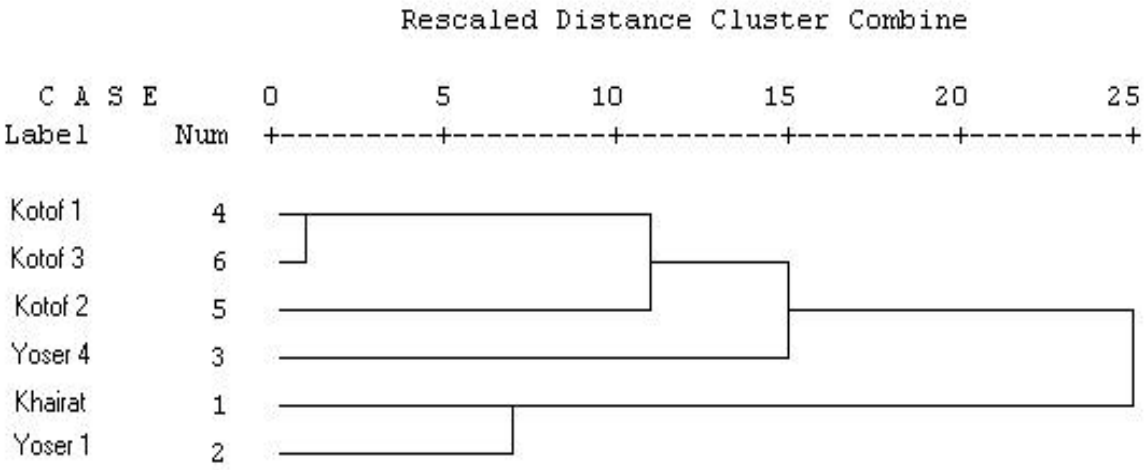

Fig. 3: Dendrogram demonstrated the relationships among the six hybrids of two *Capsicum* species based on combination of SDS-PAGE and isozymes characters.

The second cluster was divided into two subclusters. Yoser 4 of *C. frutescens* is categorized into the first subcluster, while the other subcluster included the three hybrids of *C. annuum*. This was in accordance with Aniel *et al.* (2010) who revealed that large intra-specific differences were not found in the cultivars of *C. annuum* L., and similarity index and UPGMA produced two distinct clusters each comprising four cultivars. Furthermore, Lorena *et al.* (2005) mentioned the close genetic relationship that exists between these two species, which are commonly known as the *Capsicum annuum-chinense-frutescens* complex.

In conclusion, although nine biochemical techniques were used, slight intra and inter specific variations were detected in the six Egyptian hybrids of *C. frutescens* and *C. annuum*, therefore application of molecular markers is recommended for more characterization and discrimination between the two *Capsicum* species.

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ARABIC SUMMARY

التوصيف والتباين البيوكيميائى لستة هجن مصرية جديدة تابعه لجنس الفلفل

شوكت محمود احمد
قسم العلوم البيولوجية والجيولوجية- كلية التربية- جامعة عين شمس روكسى- هليوبوليس
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تم اجراء تحليل باستخدام الكاشفات البيوكيميائية لستة هجن مصرية جديدة تابعه لنوعين من جنس الفلفل (الكابسيكم). وقد سجل التفريد الكهربى للبروتين نسبة تباين منخفضة (38%) بين الهجن موضع الدراسة، وسجل ايضا حزمتين مميزتين للنوعين- بمعدل حزمة لكل نوع- وقد اعتبرتا من الكاشفات البيوكيميائة لانواع جنس الفلفل. أعطت ثماني مشابهات انزيمية واحدا وعشرين حزمة، وسجلت ثلاث أنماط وهى: انزيم الكحول ديهيدروجينيز وانزيم حمض الماليك وانزيم المالات ديهيدروجينيز نسب تباين متفاوتة ما بين 57 الى 80 فى المائة، وتميزت بعض الهجن بخمس حزم متفردة، اثنتان منها فى هجين يسر4 التابع لنوع كابسيكم فروتيسينس (الفلفل الشجيرى)، وثلاث حزم لهجين قطوف2 التابع لنوع كابسيكم انييوم (الفلفل الحولى). وكشفت علاقات القرابة لهذة الهجن عن التباين الجينى المنخفض من خلال تقسيمها الى مجموعتين فقط بمسافة وراثية تقدر ب 0.25.