GENETIC ANALYSIS IN SOME *Cucurbitaceae* PLANTS

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The *Cucurbitaceae* or cucurbit family (also commonly referred to as the cucumber) found in the warmer regions of the world, mostly in South Africa. It is a major family for economically important species, particularly those with edible fruits. The *Cucurbitaceae* are mostly climbing herbaceous annuals and are sometimes perennials comprising about 118 genera and 825 species (Jeffrey, 1990). The *Cucurbitaceae* family comprises important crop species, including melon or cantaloupe (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* L.), and squash (*Cucurbita pepo* L., *Cucurbita maxima*). Members of *Cucurbitaceae* have several unique traits. The genus *Cucumis* contains 33 species, the most economically, edible important species are *Cucumis sativus* (cucumber) and *Cucumis melo* (cantaloupe), *Cucumis prophetarum* , native to Africa ,with medium yellow toxic fruit (Ghebretinsae *et al*., 2007). *Cucurbita pepo* L. beside its edible, it is used in treating bladder disorders, stomach upsets, intestinal worms, bed-wetting, rheumatism, benign prostatic hyperplasia, burns, wounds, *Citrullus colocynthis* L. (*Cucurbitaceae*) is a widely grown desert plant with multi-use potential, is one of the native plants of the Middle East countries which is used in traditional medicine, contains active substances such as saponins, alkaloids and glycosides (Hassan *et al*., 2000). It is used in traditional medicine inhibit the implantation of embryos; treat (constipation, rheumatism, cancer, oedema, bacterial infections and diabetes). The plant showed the presence of large amounts of phenolics and flavonoids indicating its antioxidant activity (Sunil and Mamal, 2008). It is used as antidiabetic and immunostimulant and antioxidant (Bendjeddou *et al*., 2003). The main active compound in *Cucurbitaceae* is cucurbiticine glycosides (B and E) with insecticidal and antioxidant properties (Torkey *et al*., 2009).

Morphological markers were routinely used for diversity analysis and for establishing relationships among different species and genotypes affected by environmental changes in many cases, it is better to use different biochemical and molecular markers which have been developed as more powerful tools for such studies. Biochemical markers such as proteins and isozymes have been extensively studied in plants genotypes polymorphism. They have been successfully used to identify wild, cultivated species and crop species, including *Cucurbitaceae* species (Pasha and Sen, 2003). On the other hand, many investigators employed

RAPD-PCR to assay genetic fingerprint and diversity in wide range of plants such as *Cucurbita* species (Heikal *et al*., 2008). Molecular markers based on DNA sequence polymorphisms are independent of environmental conditions and show a higher level of polymorphism. Morphological and biochemical markers reflect variation of expressed regions of genome while molecular markers indicate variation of all genome including expressed and non-expressed regions. The advent of molecular techniques has provided a new dimension to detect genetic polymorphism based on DNA facilitating in biological research branches such as phylogenetic relationships, taxonomy and genetic diversity (Williams *et al*., 1990). Molecular markers provide a quick and reliable method for estimating genetic relationships among plant genotypes (Thormann *et al*., 1994).They also reported that among the different types of molecular markers, randomly amplified polymorphic DNAs (RAPDs) are useful for the assessment of genetic diversity owing to their simplicity, speed, and relatively low-cost when compared to other types of molecular markers. ISSR markers have also proven useful for detecting genetic polymorphisms among species by generating a large number of markers that target multiple microsatellite loci distributed across the genome. Also, the utility costs of ISSRs are lower that than RAPD (more reproducible polymorphisms with specific primers (Dey *et al*., 2006; Singh *et al*., 2007). AFLPs have proven to be extremely proficient in revealing diversity at the species level and within species population that provide an effective means of covering a wide area of the genome in a single assay. It was preferred over other DNA-based markers mainly because of its high multiplex ratio and non-requirement of prior sequence information (Gaikwad *et al*., 2008).

In this study, biochemical markers based on (protein and isozymes) and molecular markers based on (RAPD, ISSR and AFLP) were carried out on seven *Cucurbitaceae* species to assess the genetic variation and relationship among them.

MATERIALS AND METHODS

A. Materials

Seven species of *Cucurbitaceae* were collected from Sinai, by Egyptian Desert Genebank, Desert Research Center such as: *Cucumis melo* L., *Citrullus lanatus* L., *Citrullus sativtus* L., *Citrullus colocynthis* L., *Cucumis prophetarum, Cucurbita pepo* L. and *Corallocarpus schempri*

B. Methods

1- Extraction of total protein

Bulked leaf sample (0.5 g) of each sample was ground with liquid nitrogen and mixed with extraction buffer pH 7.5 (50 mM tris, 5% glycerol and 14 mMB-mercaptoethanol) in a morter with pestle, left overnight then vortexed for 15 sec and centrifuged at 12.000 rpm at 4°C for 10 min. The supernatants were transferred to new eppendorf tubes and kept at -20°C

until use for electrophoresis analysis according to Laemmli (1970).

1-1- Isozyme analysis.

Seven isozymes were used such as: Acid phosphatase (Acph), alcohol dehydrogenase (Adh), α- esteras and β-esteras (Est), aldehyde oxidase (Ao), malate dehydrogenase (Mdh), and peroxidase (Px). Isozymes were separated according to Stegemann *et al*. (1985). In gels staining, protocols of Wendel and Weeden (1989) was used for Acph and Adh and Ao. Scandalios (1964) was used for α and β-Est, Jonathan and Wendell, (1990) was used for Mdh, Heldt (1997) was used for Px. 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.

2- DNA extraction

Genomic DNA was extracted from fresh young leaves of ten plants which were chosen randomly for each species by CTAB method of Doyle and Doyle (1987). Leaves of ten plants from a single species were bulked prior to extraction. DNA was measured by spectrophotometer before gel electrophoresis.

2-1- RAPD analysis

PCR reactions were performed according to Williams *et al*. (1990) using six 10-mer primers (Operon Technology, USA) such as: OP-A4, OP-A15, OP-A20, OP-B7, OP-Z4 and OP-Z10 with the sequences as shown in Table (3). The reaction conditions were optimized and mixtures were prepared (25 µl total volumes) consisted of the following: 1.0 µl dNTPs (8 mM), 1.0 µl Taq DNA polymerase (1 U/1µl), 2.5 µl 10 X buffer, 3 µl $MgCl_2$ (15 mM), 1.0 µl Primer (10mM), 1.0 µl Template DNA (10-50 ng/ µl) and 15.5 µl H_2O up to 25 µl. Amplification were carried out in a Strategene Robocycler Greadient 96 Robocycler device programmed for 45 cycles as follows: Denaturation, (one cycle) 94°C for 4 minutes, followed by 35 cycle as follow: 94°C for 1 minute, 36˚C for 1 minute and 30 seconds and 72°C for 2 minutes and 30 second, extension, (one cycle) 72°C for 7 minutes. Agarose Gel electrophoresis (1.2%) was used for resolving the PCR products according to Sambrook *et al*. (1989). The run was performed for one hour at 100 volt in Biometra submarine (40x20 cm). Fragments were detected o an UV-transilluminator and photographed by using Biometra Bio Doc Analyze 2005.

2-2- ISSR analysis

ISSR-PCR reactions were conducted according to Sharma *et al*. (1995) using specific primers which were synthesized by metabion GmbH Germany with the sequences shown in Table (5). The reaction conditions were optimized and mixtures were prepared (25 µl total volumes) consisted of the following: 1.0 µl dNTPs, 1 µl Taq DNA polymerase 2.5 µl 10 X buffer, 3 µl $MgCl_2$, 1.0 µl Primer, 1.0 µl Template DNA and 15.5 µl H_2O up to 25 µl. Amplification were carried out in Stratgene Robocycler Gradient 96 which

was programmed for 30 cycles as follows: Denaturation (one cycle) 94°C for 2 minutes, followed by 30 cycles: as follows 94°C for40 second, 44°C for 45 secs, 72°C for 2 minute and 30 secs, and finally one cycle extension at 72°C for 20 minutes, and 4°C (infinitive). Agarose Gel electrophoresis (1.2%) was used for resolving the PCR amplification products. The run was performed for one hour at 120 volt in Biometra submarine (40x20 cm). Fragments were detected on UV-transilluminator and photographed by using Biometra Bio Doc Analyze 2005.

2-3- AFLP analysis

AFLP method was carried out following the standard procedure described according to Vos *et al*. (1995). High - quality genomic DNA (0.5 g) was digested with a pair of restriction enzymes *(Pst*1/*Mse*1) then ligated to double stranded *Pst*1and *Mse*1 adaptores. The ligate was preamplified with nonselective primers and selective amplification was carried out using pairs of 2 bp and 3 bp selective primers (Table 8). The products were separated on polyacrylamide gels using an M13 sequencing ladder as a standard size. Gel images were resolved and analyzed by Egy Gene Gel Analyzer Version One software to determine relative mobility (RF), molecular size (MS) of fragments in base pairs.

Statistical analysis

Similarity matrix was developed by the statistical package for social science program SPSS based on combined analysis of overall molecular and biochemical markers.

RESULTS AND DISCUSSION

1. SDS-PAGE analysis

Leaf protein analysis was carried out on the seven studied species of *Cucurbitaceae* (Fig. 1) and scored in (Tables 1 a and b). The results indicated that a total number of fifty five protein bands were observed among the seven studied species. Moreover, a band at the molecular weight of 89 KDa was considered as a monomorphic band, which indicated the relations among these species of *Cucurbitaceae*. In addition, thirty three bands were considered as a polymorphic bands with a high polymorphism 98%, indicated the genetic variations among the seven studied species of *Cucurbitaceae*. Twenty one bands were unique bands for a particular species of the seven one (species specific markers).The obtained results were in accordance with the results obtained by Megha and Ram (2006) in bottle gourd, Pasha and Sen (2003) in several taxa of *Cucurbitaceae*, Jagesh *et al*. (2006) in pumpkin and in discordance to Pichl (1978) who found little variation in the overall seed and leaf protein pattern in *Cucurbitaceae.*

2. Isozyme analysis

Seven isozyme systems including *Acp* (acid phosphatase), *Adh* (alcohol dehydrogenase), α-Est (esterase), β-Est (esterase), Ao (aldehyde oxidase), *Mdh*

(Malate dehydrogenase) and *Prx* (Peroxidase) were conducted to study the genetic variability and similarity among the seven *Cucurbitaceae* species (Fig. 2 and Table 2). A total of 37 bands were overall the seven isozyme systems, with a 70% of total polymorphism.

Data presented in Table (2) and Fig. (2) indicated that Acph (acid phosphatase) that has antioxidant activity, its electrophoretic pattern included a total of three bands, two out of these bands were polymorphic (No. 2 and 3) and one band was monomorphic (No. 1). The results agreed with Amaral-Junior *et al*. (1996) in pumpkin to reveal polymorphic bands.

Adh (Alcohol dehydrogenase) results presented in Table (2) and Fig. (2) indicated that Adh has antioxidant activity revealed three bands, bands No. 1 and No. 2 were monomorphic while band No. 3 was polymorphic. The results agreed with the finding of (Mukhlesur *et al.*, 2005) who concluded that Adh gene family has two or three loci in angiosperm species and multiple loci in monocots.

Esterases (Est) is a gene family hydrolyze ester bond in lipid to produce plant energy for biochemical reactions (Durate *et al*., 2007). Analysis of α-esterase (Est) in Table (2) and Fig. (2) revealed the presence of six bands, four were polymorphic (No. 3, 4, 5 and 6) and two were monomorphic bands (No.1 and No. 2). Finally, β-est revealed four bands, three were polymorphic (No. 3, 4 and 5) and one was monomorphic (No. 1) among the seven studied species. The obtained results were in accordance with the results obtained by Amaral-junior *et al*. (1994) in pumpkin who revealed that α-esterase (EST) which gave three polymorphic bands and Carvalho *et al*. (2003) in *Lagenaria siceraria* who reveal α- and β-esterases showed highly polymorphism percentages.

Data presented in Table (2) and illustrated in Fig. (2) indicated that Ao revealed thirteen bands which recorded 100% polymorphism. The results agreed with the finding of (Gunter, 2011) who separated aldehyde oxidase from potato tubers (*Solanum tuberosum*) revealed high polymorphism percentages.

Mdh (Malate dehydrogenase) plays a major role in central metabolism and stress tolerance. From the data detected in Table (2) and Fig. (2), Mdh banding pattern gave four total bands, two were monomorphic (No. 1 and 2) and two were polymorphic (No. 3 and 4). These results agreed with the finding of Duart *et al.* (2007) who indicated that Mdh in *Cactaceae* and *Broccoli* is a dimeric enzyme of three bands with two or three monomorphic loci.

Px forms a defense system in living organisms against oxygen radicals, mediated peroxidation of unsaturated lipids in lignification. Records in Table (2) and Fig. (2) showed that peroxidase banding patterns comprise three bands with different intensities, one was monomorphic (No. 1) and two polymorphic bands (No. 2 and 3). The results agree with (Amaral-Junior *et al*., 1996) in pumpkin who indi-

cated the efficient participation of antioxidant mechanisms, including the synergistic activities of prx, might play an important role during stress and microbial infection.

Based on isozyme markers, polymorphism percentages were estimated for the seven isozyme systems among the seven *Cucurbitaceae* species as shown in Table (2) which revealed 37 total bands, 26 were polymorphic (70% polymorphism) and nine were monomorphic bands. The highest polymorphism percentage was 100% for Ao and the lowest polymorphism percentage was 33% for Adh. The obtained results were in accordance with the results obtained by Khalil *et al*. (2010) who successfully detected the genetic variability in ten *Brassicaceae* species collected from North coast in of Egypt and Sinai, revealed by α- β-est, ACP, Prx, Adh and Mdh which produced 26 bands (73% of polymorphism) and Azazy *et al*. (2011) who studied genetic variation in five bottle ground (*Lagenaria siceraria*) cultivar with five isozyme system and produced eight bands (42% of polymorphism).

1. RAPD analysis

Six RAPD primers were used in the present investigation to study the genetic relationships among the seven *Cucurbitaceae* species as shown in Fig (3). One monomorphic and 129 polymorphic distinct fragments (almost 99% of polymorphism) were revealed in the seven tested species (Table 4). The results showed that OP-Z10, OP-B7, OP-A20, OP-A4 and OP-A15 primers were highly polymorphic (100% of polymorphism) and produced wide fragment length ranged from 400-2300 bp, 215-1083 bp, 206-1032 bp, 158-968 bp and 194-981 bp, respectively. Moreover, OP-Z4 primer showed the lowest polymorphism percentages (90%) which produced molecular fragment length ranged from 114-392 bp. These result agreed with the results obtained by Amnon *et al*. (2005) who determined genetic relatedness among *Citrullus* and *Cucumis* species in India, They examined phylogenetic relationships and found *Citrullus* species and subspecies are closer as compared with those among most *Cucumis* species and proofed that, although wide genetic differences and reproductive barriers exist among *cucurbit* species in this study. They are still considered as potential germplasm source for enhancing watermelon and melon crops using traditional breeding, genetic and biotechnology procedures. Shiragambi *et al*. (2011) used a combination of morphological and RAPD analysis for three *Cucumis* species and revealed that a polymorphism with OPB and OPA primer series was able to distinguish among three *Cucumis* species sufficiently.

2. ISSR analysis

Five ISSR primers were used in the present investigation to study the genetic relationships among the seven *Cucurbitaceae* species as shown in Fig. (4). Two monomorphic and 119 polymorphic distinct bands (98% of polymor-

phism) were revealed in the seven investigated species (Table 6). The results showed that HB11, HB12 and HB14 primers were highly polymorphic (100% of polymorphism) which produced wide bp length ranged from 594 to 3870 bp, 366 to 3307 bp and 343 to 3379 bp, respectively. On the other hand, HB9 and HB10 primers were less polymorphic. These results agreed with the results obtained by Djè *et al*. (2006) who applied a molecular approach using inter-simple sequence repeat (ISSR) markers on three African edible-seeded cucurbits (*Citrullus lanatus* L. Matsumura and Nakai, *Cucumeropsis mannii* L. Naudin and *Cucumis melo* var. *agrestis* L. Naudin) and successfully produced polymorphism among them and wth the statement of Dalamu *et al*. (2012) who analysed fifty indigenous *Momordica charantia* L. relative to *Cucurbitaceae* using RAPD and ISSR markers and demonstrated a large genetic variability among the Asian bitter gourd genotypes, which indicates that they should be considered as a valuable gene pool for bitter gourd breeding programs.

Species-Specific-Markers:

Some specific markers for the seven *Cucurbitaceae* species by ISSR analysis are listed in Table (7). All ISSR markers were found to be species specific. These markers were scored for the presence of unique bands for a given species. *Cucumis melo* L. had eleven total unique bands; one could be scored by HB10, three unique bands by HB12 and six unique bands by HB11. Then, *Citrullus lanatus* L. had three unique bands could be scored by HB9, HB11, and HB14. *Cucumis prophetarum* had eight total unique bands six could be scored by HB10 and HB11 and two unique bands by HB14. *Cucurbita pepo* L. had five total unique bands three could be scored by HB10, HB11, HB12 and two bands by HB14. *Corallocarpus schempri* had ten total unique bands, eight could be scored by HB9, HB11 and HB14 and two unique bands by HB14. These results agreed with the finding of Hossein *et al*. (2013) who investigated the genetic variation among 42 *cucumus* which were collected from different regions of Iran plus. They reported that, banding patterns of 22 ISSR primers and 13 RAPD primers revealed 202 (67.32%) and 85 (54.90%) polymorphic bands, respectively and concluded that among three different molecular data sets, the RAPD and ISSR data were good discriminators with a significant and closer relationship to morpho-agronomic data.

3. AFLP analysis

A total number of 82 amplified fragments were obtained by using this primer pair within a fragment size ranged from 100 to 1500 bp (Fig. 5 and Table 8). Forty five polymorphic bands were obtained represented (of polymorphism 54.8%). Resulted common bands were 37 amplified fragments. These results agreed with Paris (2003) who studied genetic relationships across a broad spectrum of the *Cucurbita pepo* squash gene pool, with emphasis on domesticates, using AFLP, ISSR and SSR markers. Forty-five acces-

sions were compared for presence or absence of 448 AFLP, 147 ISSR and 20 SSR bands. Similarity values were estimated and UPGMA cluster analysis was conducted. The results obtained from these three marker systems were highly correlated ($P<0.001$). Clustering was in accordance with the division of *C. pepo* into three subspecies

4. Combined analysis based on (protein, isozymes, RAPD, ISSR and AFLP)

Based on total analyses (protein, isozymes, RAPD, ISSR and AFLP), similarity matrix was developed by SPSS computer package system as shown in Table (9). The closest relationship was scored between *Citrullus lanatus* L. and *Cucumis melo* L. with similarity value of 59%, *Citrullus sativtus* L. and *Cucumis melo* L. with similarity value of 59% and *Citrullus sativtus* L. and *Citrullus lanatus* L. with similarity value of 56%.While *Corallocarpus schempri* and *Citrullus lanatus* L. gave the lowest similarity of 39% which were considered distantly related and not closely related species. The dendrogram based on overall analysis (protein, isozymes, RAPD, ISSR and AFLP), separated the seven Cucurbitaceae species into two main clusters as shown in Fig. (6). Moreover, *Cucumis melo* L., *Citrullus lanatus* L. and *Citrullus sativtus* L. were separated together meaning that they were closely related in the first main cluster, *Citrullus colocynthis* L. separated in the second cluster and *Cucumis prophetarum* in subcluster. While, *Cucumis prophetarum, Cucurbita pepo* L. and *Corallocarpus schempri* were cluster in another sub cluster as distainly related to the main first cluster. These results agreed with the results of Amar (2008) who used RAPD, ISSR and AFLP markers to detect variation among *Balanites aegyptiaca* plants collected from twenty different sites in Egypt. They exhibited different polymorphism percentages and classified the twenty sites into two main cluster and two sub clusters which showed the relationships and genetic diversity among them.

SUMMARY

The present study was carried out to analysis the genetic differentiation between seven species of *Cucurbitaceae* by using biochemical and molecular markers. Analysis of SDS-PAGE of soluble proteins for the seven *Cucurbitaceae* species such as: *Cucumis melo* L., *Citrullus lanatus* L., *Citrullus sativtus* L., *Citrullus colocynthis* L., *Cucumis prophetarum, Cucurbita pepo* L. and *Corallocarpus schempri*, revealed polymorphism of 98%. Seven isozyme systems including Acp (acid phosphatase), Adh (alchol dehydrogenase), α- and β-Est (esterases), Ao (Aldehyde oxidase), Mdh (malate dehydrogenase) and Prx (peroxidase) successfully indicated genetic variability estimated as 70% polymorphism discriminating between seven *Cucurbitaceae* species. Six preselected RAPD primers were used in the present study to discriminating between the seven *Cucurbitaceae* species. RAPD markers revealed (99%) polymorphism that is considerable the highest pol-

ymorphism among seven *Cucurbitaceae* species and the lowest similarity (1%), ISSR markers revealed (98%) polymorphism and finally, AFLP gave (54.8%) of polymorphism with the lowest polymorphism indicating the highest similarity (46%). Based on the results and polymorphism percentages, RAPD, ISSR, SDS-PAGE of soluble proteins, isozymes and AFLP were useful molecular and biochemical tools to discriminate between the seven *Cucurbitaceae* species.

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Table (1a): SDS-PAGE patterns of leaf soluble proteins extracted from the studied seven *Cucurbitaceae* species*.

MW	1	2	3	4	5	6	7	Polymorphism
143.483	-	+	+	+	-	-	-	Polymorphic
141.965	-	+	+	+	-	-	-	Polymorphic
131.493	+	-	-	-	-	+	-	Polymorphic
126.820	-	-	+	-	-	-	-	Unique
124.945	+	+	+	+	-	+	-	Polymorphic
115.976	-	+	-	-	-	-	-	Unique
100.349	-	+	-	-	-	-	-	Unique
99.286	-	-	-	-	-	+	-	Unique
92.552	-	-	+	-	-	+	+	Polymorphic
89.644	+	+	+	+	+	+	+	monomorphic
86.274	-	-	+	-	-	-	-	Unique
84.999	-	-	+	+	-	-	+	Polymorphic
78.228	-	-	+	+	-	-	+	Polymorphic
70.631	-	-	-	+	-	-	-	Unique
67.976	-	-	-	+	-	-	+	Polymorphic
63.771	-	-	-	+	-	-	+	Polymorphic
60.725	-	-	+	+	-	-	+	Polymorphic
59.319	+	-	+	-	+	-	+	Polymorphic
58.442	-	-	-	+	-	-	-	Unique
56.365	+	+	+	-	-	+	+	Polymorphic
54.711	+	-	-	+	+	+	+	Polymorphic
52.992	-	+	+	-	+	-	+	Polymorphic
51.436	-	-	-	-	-	+	-	Unique
50.891	-	-	-	-	-	+	-	Unique
50.139	-	+	+	-	-	-	+	Polymorphic
48.667	+	-	-	-	+	-	-	Polymorphic
47.440	+	+	+	-	-	+	-	Polymorphic
47.037	-	-	-	-	-	+	-	Unique
45.949	-	-	+	-	-	-	+	Polymorphic
44.269	-	-	+	-	-	-	+	Polymorphic
43.291	-	-	-	-	-	-	+	Unique
41.841	-	-	-	-	+	+	-	Polymorphic
40.440	-	-	-	+	-	-	-	Unique
37.858	-	-	-	+	-	-	-	Unique
37.537	+	-	-	-	+	-	-	Polymorphic
35.973	-	-	-	+	-	-	-	Unique
35.140	+	+	+	-	+	+	+	Polymorphic
34.036	-	-	+	+	-	-	-	Polymorphic
33.462	+	+	-	-	-	-	+	Polymorphic
32.135	+	-	-	-	+	-	+	Polymorphic
31.126	-	+	-	-	-	-	-	Unique
29.450	-	-	-	+	+	-	-	Polymorphic
28.707	+	+	+	-	-	+	+	Polymorphic
27.865	-	-	-	-	-	+	-	Unique

Table (1a): Cont'

26.647	-	-	-	-	-	+	-	Unique
26.030	-	-	-	-	+	-	-	Unique
25.266	+	+	+	+	+	-	-	Polymorphic
24.420	+	+	+	+	-	-	-	Polymorphic
24.316	-	-	-	-	-	+	+	Polymorphic
23.056	+	-	-	-	-	-	-	Unique
21.815	-	-	-	-	-	+	-	Unique
21.084	-	-	-	-	-	+	-	Unique
19.865	+	-	-	-	+	+	-	Polymorphic
18.795	-	+	+	+	-	+	+	Polymorphic
18.205	+	+	+	-	-	-	-	Polymorphic

*(M) = Maker, 1 = (*Cucumis melo*), 2 = (*Citrillus lanatus*), 3 = (*Citrillus sativus*), 4 = (*Citrillus colocynthis*), 5 = (*Cucumis prolitarium*), 6 = (*Cucurbita pepo*) and 7 = (*Corallocarpus schempri).*

Table (1b): Number, types and polymorphism percentage of leaf soluble protein bands extracted from the seven studied *Cucurbitaceae* species.

Mono-morphic bands	Polymorphic bands		Total bands	Polymorphism %
	Non-unique bands	Unique bands		
1	33	21	55	98%

Table (2): Polymorphism percentages generated by seven isozyme systems among the seven *Cucurbitaceae* species.

Types of isozyme	No. of mono-morphic bands	No. of poly-morphic bands	Total bands	% polymorphism
Acph	1	2	3	66%
Adh	2	1	3	33%
α-est	2	4	6	66%
β-est	1	3	4	75%
AO	0	13	13	100%
Mdh	2	2	4	50%
Px	3	1	4	66%
Total	11	26	37	70%

Table (3): The six used RAPD Primer codes and their sequences.

No	Primer codes	Primer Sequences
1	OP-A4	5'- AATCGGGCTG-3'
2	OP-A15	5'- TTCCGAACCC-3'
3	OP-A20	5'- GTTGCGATCC-3'
4	OP-B7	5'- GGTGACGCAG-3'
5	OP-Z4	5'- AGGCTGTGCT-3'
6	OP-Z10	5'- CCGACAAACC-3'

Table (4): Primer codes, length range (bp), total amplified fragments, number of monomorphic fragments, and number of polymorphic fragments and polymorphism percentages of six RAPD primers among the seven *Cucurbitaceae* species.

Primer code	Length range (bp)	Total Amplified fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism percentages
OP-A4	158-968	28	0	28	100
OP-A15	194-981	27	0	27	100
OP-A20	206-1032	28	0	28	100
OP-B7	215-1083	24	0	24	100
OP-Z4	114-392	10	1	9	90
OP-Z10	400-2300	13	0	13	100
Total-		130	1	129	99

Table (5): The five used ISSR primer codes and their sequences.

No.	Primer codes	Sequence
1	HB9	$(GT)_6$ GG
2	HB10	$(GA)_6$ CC
3	HB11	$(GT)_6$ CC
4	HB12	$(CAC)_3$ GC
5	HB14	$(CTC)_3$ GC

Table (6): Total bands, number of monomorphic fragments, number of polymorphic fragments and polymorphism percentages of ISSR primers among the seven studied *Cucurbitaceae* species.

Primer code	Length range (bp)	Total amplified fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism percentages
HB9	290-2382	15	1	14	93
HB10	182-4803	20	1	19	95
HB11	594-3870	34	0	34	100
HB12	366 -3307	23	0	23	100
HB14	343- 3379	29	0	29	90
Total		121	2	119	98

Table (7): ISSR primers for the seven *Cucurbitaceae* species specific marker.

No. of specific primers	ISSR Primer	Genotypes	Unique bands
1	HB9	*Citrullus lanatus* L. *Corallocarpus schempri*	1 3
2	HB10	*Cucumis melo* L. *Citrullus colocynthis* L. *Cucumis prophetarum* *Cucurbita pepo* L.	1 1 2 1
3	HB11	*Cucumis melo L.* *Citrullus lanatus L.* *Citrullus colocynthis* L. *Cucumis prophetarum* *Cucurbita pepo* L. *Corallocarpus schempri*	6 1 2 4 1 3
4	HB12	*Cucumis melo* L. *Citrullus colocynthis* L. *Cucurbita pepo* L. *Corallocarpus schempri*	3 1 1 2
5	HB14	*Cucumis melo* L. *Citrullus lanatus* L. *Citrullus colocynthis* L. *Cucumis prophetarum* *Cucurbita pepo* L. *Corallocarpus schempri*	1 1 2 2 2 2
Total			44

Table (8): Selective nucleotides of AFLP primer combinations, number of total bands, polymorphic bands and polymorphism percentages.

Primer comb.	Selective nucleotides		Number of bands		% of polymorphism
	EcoRI	*MseI*	Total	Polymorphic	
EcoRI/*MseI*	AAC	CAG	82	45	54.8%

Table (9): Similarity matrix of total analysis (protein, isozymes, RAPD, ISSR and AFLP) markers among the seven *Cucurbitaceae* species.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Cucumis melo L. (1)	100						
Citrullus lanatus L. (2)	59	100					
Citrullus sativtus L. (3)	59	56	100				
Citrullus colocynthis L. (4)	45	48	52	100			
Cucumis prophetarum (5)	46	42	48	51	100		
Cucurbita pepo L. (6)	43	40	42	46	54	100	
Corallocarpus schempri (7)	43	39	41	54	49	62	100

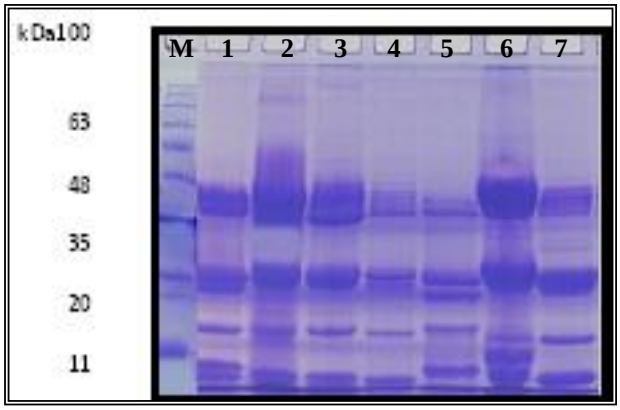

Fig. (1): SDS-PAGE profiles of soluble proteins extracted from the seven studied *Cucurbitaceae* species*. *(M) = Maker, 1 = (*Cucumis melo*), 2 = (*Citrillus lanatus*), 3 = (*Citrillus sativus*), 4 = (*Citrillus colocynthis*), 5 = (*Cucumis prolitarium*), 6 = (*Cucurbita pepo*) and 7 = (*Corallocarpus schempri).*

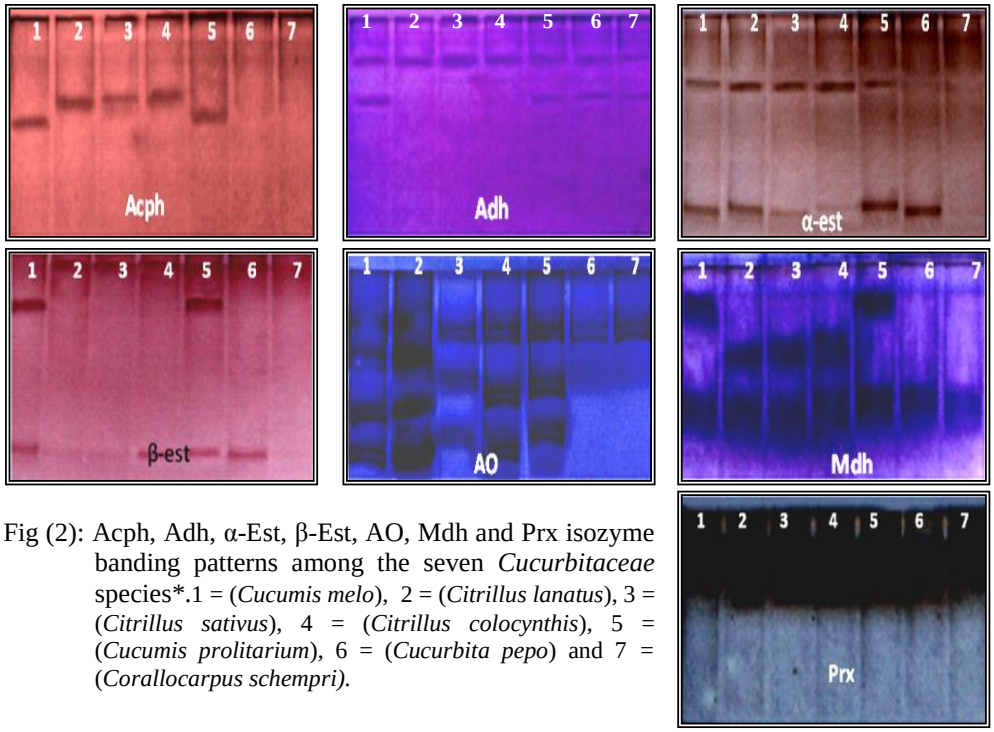

Fig (2): Acph, Adh, α-Est, β-Est, AO, Mdh and Prx isozyme banding patterns among the seven *Cucurbitaceae* species*.1 = (*Cucumis melo*), 2 = (*Citrillus lanatus*), 3 = (*Citrillus sativus*), 4 = (*Citrillus colocynthis*), 5 = (*Cucumis prolitarium*), 6 = (*Cucurbita pepo*) and 7 = (*Corallocarpus schempri).*

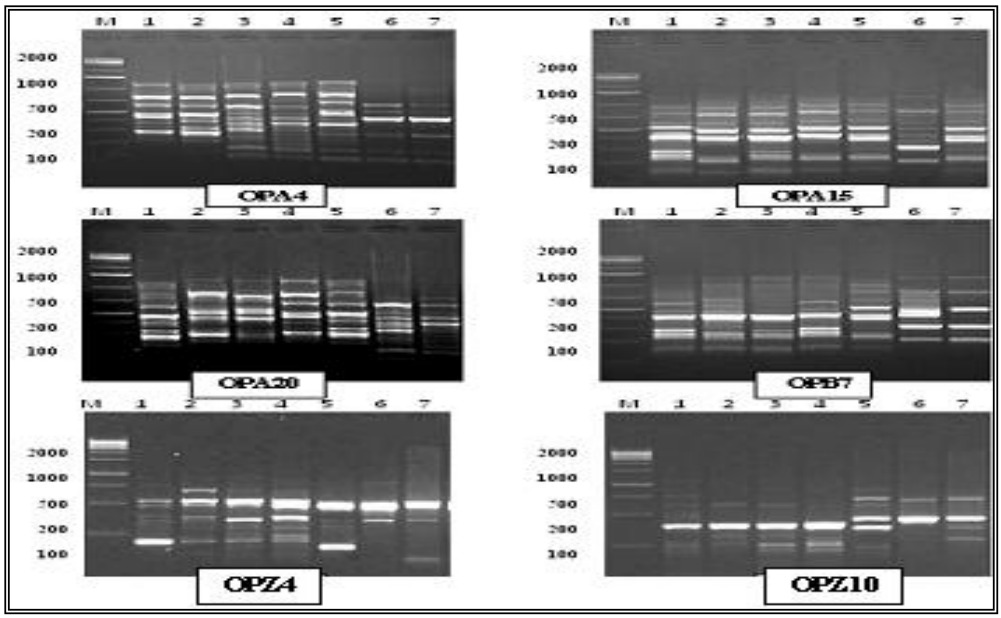

Fig. (3): OP-A4, OP-A15, OP-A20, OP-B7, OP-Z4 and OP-Z10 RAPD primers among the seven *Cucurbitaceae* species*.*(M) = Maker, 1 = (*Cucumis melo*), 2 = (*Citrillus lanatus*), 3 = (*Citrillus sativus*), 4 = (*Citrillus colocynthis*), 5 = (*Cucumis prolitarium*), 6 = (*Cucurbita pepo*) and 7 = (*Corallocarpus schempri).*

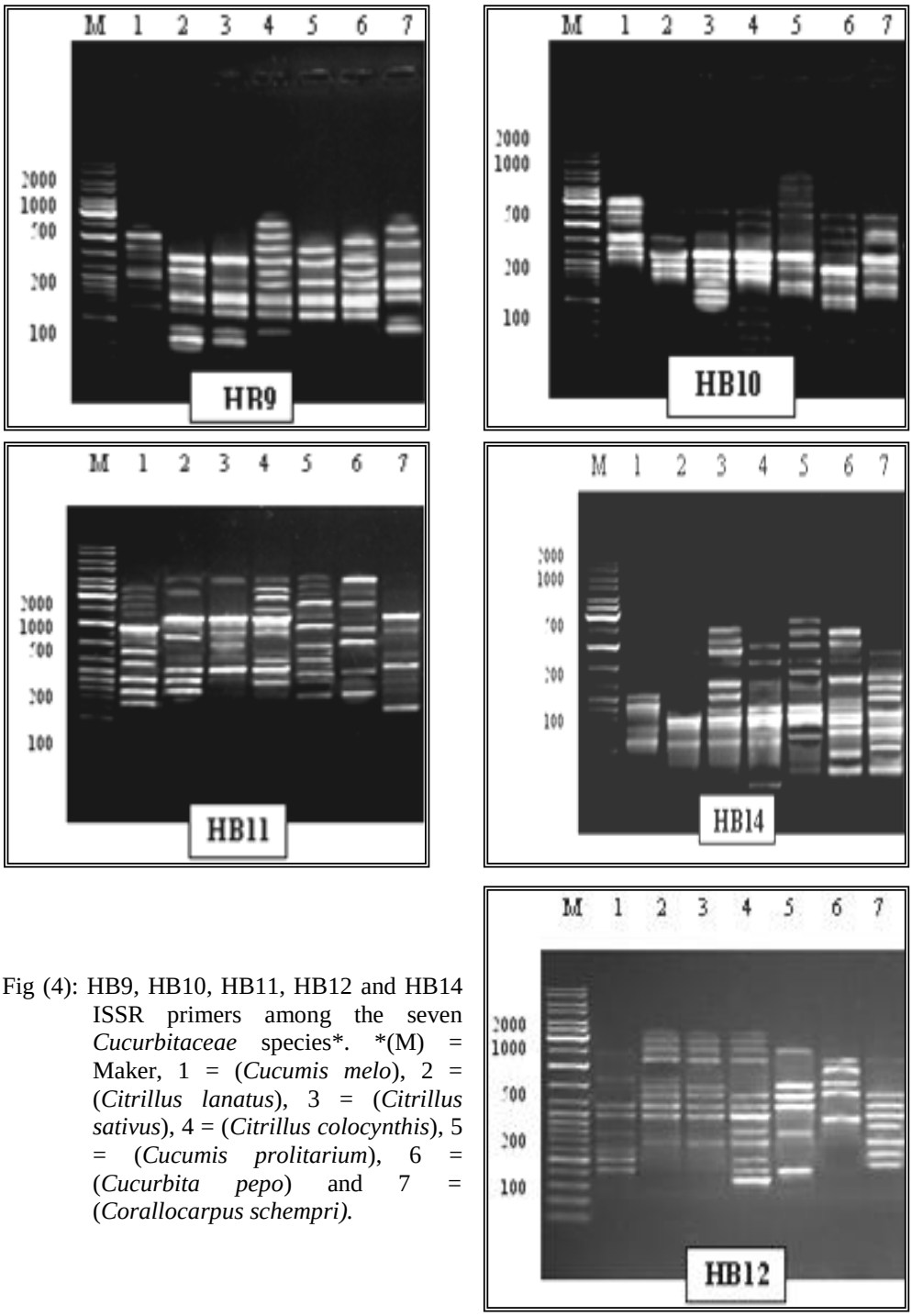

Fig (4): HB9, HB10, HB11, HB12 and HB14 ISSR primers among the seven *Cucurbitaceae* species*. *(M) = Maker, 1 = (*Cucumis melo*), 2 = (*Citrillus lanatus*), 3 = (*Citrillus sativus*), 4 = (*Citrillus colocynthis*), 5 = (*Cucumis prolitarium*), 6 = (*Cucurbita pepo*) and 7 = (*Corallocarpus schempri).*

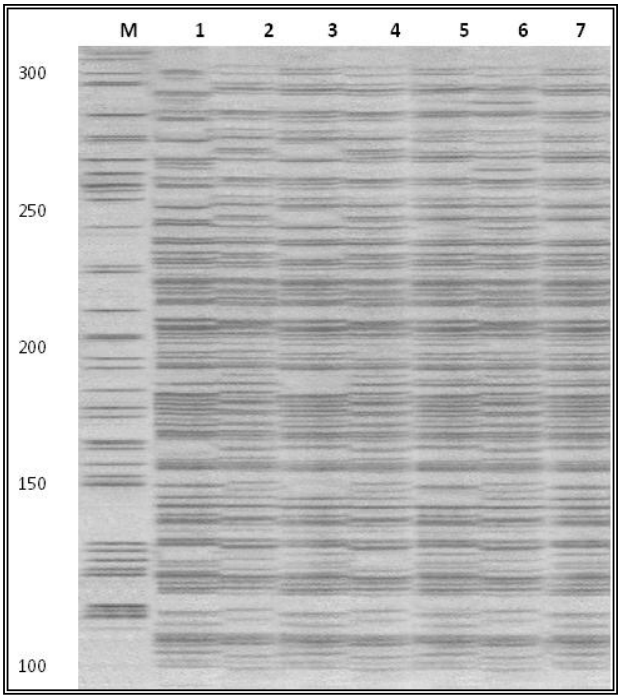

Fig. (5): AFLP fingerprints among the seven *Cucurbitaceae* species using one primer combinations.

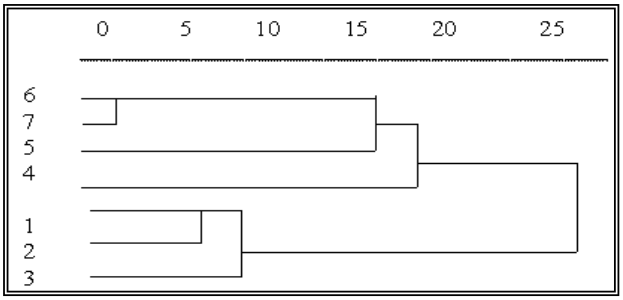

Fig. (6): Dendrogram based on total analysis (protein, isozymes, RAPD, ISSR and AFLP) among seven *Cucurbitaceae* genotypes.