

Genetic Diversity among Some Cucurbits Species Determined by Random Amplified Polymorphic DNA RAPD Marker

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Abstract RAPD markers were used to determine the genetic relationships and evaluating similarity among some cucurbits species. Thirteen RAPD primers were used to amplify DNA extracted from the leaves of 10 cucurbit species using CTAB method. A total of 227 bands were amplified of which 225 showed polymorphism among the 10 species. PCR-RAPD analysis showed a number of differences in the size and number of bands among the species, which means that there are genetical differences among the studied cucurbit species. Based on these markers, genetic similarity coefficients were calculated and a dendrogram was constructed. The dendrogram analysis delineated three major clusters. The first cluster consisted of one group which comprised *Cucurbita moschata* and *C. pepo* at a level of 38.6 % genetic similarity. The second cluster consisted of four groups: Group I comprised *Luffa aegyptiaca* at a level of 20.6 % genetic similarity. Group II comprised the closely related species *Cucumis melo* var. *reticulatus* and *C. melo* var. *flexuosus* at a level of 62 % genetic similarity. Group III consisted of *Cucumis sativus* with about 37.8 % genetic similarity to group II. Group IV consisted of *Ctenolepis cerasiformis* at a level of 26.6 % genetic similarity. The third cluster consisted of two groups. Group I comprised *Citrullus lanatus* and *Colocynthis vulgaris* at a level of 36.2 % genetic similarity. Group II consisted of *Coccinia grandis* at the level of 19.4 % genetic similarity. The three clusters were similar to each other at a level of 15% genetic similarity. Genetic similarity ranged between 15% and 62 %. This study demonstrates that RAPD markers are useful in assessing genetic diversity among cucurbits.

Keywords Cucurbits, Genetic Diversity, RAPD Marker

1. Introduction

The family Cucurbitaceae belongs to the order Cucurbitales, class Magnoliopsida (subclass Rosidae). Although most of its species originated in the Old World[1], many originated in the New World, and at least seven genera in both hemispheres[2]. There is tremendous genetic diversity within the family in tropical, subtropical, arid deserts and temperate regions. Archaeological evidence indicated that cucurbits were present in ancient and prehistoric cultures.

Molecular markers can be an effective mean to determine genetic relatedness among cultivars and strains used in cucurbits breeding programmes. A number of studies have been designed to examine genetic diversity and phylogenetic

relationships among cucurbit cultivars. Some of these studies used biochemical methods such as isoenzyme[3], while others used molecular approaches based on DNA[4] to evaluate genetic diversity in crop species. Most of the isoenzymes tested produced monomorphic patterns ([5,6].

Several methods are now available concerning molecular markers studies, and scientists can choose the method of particular interest, depending on available material and objectives. Among the existing molecular approaches, random amplified polymorphic DNA (RAPD) is one of the most widely used molecular methods in genetic studies and has been applied for the less-known species. However, this method is less reproducible and shows a lower degree of variability as compared with to other methods. RAPD procedure provides a sufficient number of informative markers that could distinguish watermelon cultivars[7, 8]. In a study[9], genetic diversity and relatedness were examined among *Citrullus lanatus* var. *lanatus*, *C. lanatus* var. *citroides*, and *C. colocynthis* using RAPD analysis. RAPD markers were also used in the construction of an initial

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genetic linkage map for watermelon (*Citrullus lanatus*)[10], and to determine genetic relatedness among Asian watermelon cultivars and breeding lines[11]. Reference[12] used RAPD to study polymorphism and to construct a linkage map for watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai by using 148 primers. Amnon *et al.*[13] used RAPD markers to study genetic diversity among accessions of some American cultivars of watermelon (*Citrullus lanatus* var. *lanatus*). The objectives of this study was studied the genetic diversity among some Cucurbits and the utility of the RAPD markers system for evaluating similarity.

2. Materials and Methods

Plant material: Accessions of ten cucurbit species were obtained from various sources (Table 1). For DNA extraction the plants were grown in pots.

RAPD analysis: For RAPD analysis DNA was extracted from cucurbits leaves according to[14]. Purity and concentration of DNA was tested spectrophotometrically at wave lengths of 260 and 280 nm (Eppendorf – Germany). RAPD reactions were performed using 13 random primers (Nine of these primers were 10-mers (Operon Technologies; OP, and University of British Columbia, UBC), while the other 4 were 12-mers (Bexnet, Co., Japan.) according to[15] using PCR thermocycler. Amplification was performed using touchdown annealing temperature along two or three steps, starting with the melting temperature of the primer and then reducing the temperature by 3 degrees for each step. After all cycles had been completed, 7 µl of the products were mixed with 1 µl of DNA loading buffer and loaded into 2% agarose gel. The agarose gel was then run in 1x TAE buffer at 100 V for one and a half hour. The gels were soaked in 3% ethidium bromide for 30- 60 minutes, and then placed on the UV- transilluminator for band visualization.

Cluster analysis: The RAPD fragments 200 to 1500 base pairs (bp) were visually scored as present (1) or absent (0). A dendrogram was constructed based on similarity matrix data by applying the UPGMA[16] and cluster analysis using the software package NTSYS – PC version 2[17].

3. Results and Discussion

The DNA concentration of the 10 accessions ranged between 80.8 and 352.1 µg/ml, and purity ranged from 1.7 to 2.3 (Table 2). The analysis of the 10 accessions with 13 RAPD primers produced a total of 227 reproducible bands (Table 3). Among these, 225 bands were polymorphic (99.1%) and ranged in size from 200 to 1500 bp. Heikal, *et al.*[18] reported that the total number of reproducible fragments among the fourteen genotypes of *Cucurbita* species by six primers reached 463 bands out of them 405 (87.5%) were polymorphic fragments. They found the levels of polymorphism among the fourteen genotypes of *Cucurbita* species are relatively high (69.8% - 97.0%),

indicating the wide gene pool existence in the fourteen genotypes. The total number of bands per primer ranged between 9 and 24, with an average of 17.5 bands per primer (Table 3). The number of polymorphic bands per primer varied from 9 to 24, with an average of 17.3 bands (Table 3). The number of polymorphic bands produced per primer was relatively high among the species (Figs 1 and 2). This high level of RAPD markers polymorphism is in accordance with the results of[19] and[20] who reported that *Cucurbita pepo* and *Cucurbita maxima* are highly polymorphic species. However, DNA polymorphism has been reported as moderate to high in other allogamous genera such as *Brassica*, *Allium*, *Asparagus* and *Cucumis*.

Reference[21] found high level of the polymorphism among the different *Cucurbita* species (97.44%). However, in case of *Citrullus lanatus*,[22] reported an average of polymorphic bands produced per primer was 21.2.

To estimate genetic diversity among the accessions, genetic similarity coefficients were calculated, and a dendrogram was constructed (Fig 3). The range of DNA similarity in the dendrogram was between 0.15 and 0.62. Reference[23] reported a range 0.13 - 0.41 of dissimilarity in 31 landraces of *Cucurbita moschata* using 31 random primers. The wide range of dissimilarity values suggests that germplasm collection represent a genetically diverse population. The dendrogram consisted of three major clusters. The first cluster consisted of one group (Group I) which comprises the species: *Cucurbita moschata* and *C. pepo* which both have a DNA of a genetic similarity of 38.6 %. The second cluster consisted of four groups as follows: Group I comprises the species *Luffa aegyptiaca* which has a genetic similarity of 20.6 %. Group II comprises the closely related species *Cucumis melo* var. *reticulatus* and *C. melo* var. *flexuosus* with a genetic similarity of 62 %. Group III consists of *Cucumis sativus*. This group is similar to group II in their genetic similarity (37.8 %). Group IV consists of *Ctenolepis cerasiformis* with a genetic similarity of 26.6 %.

The third cluster consisted of two groups. The first group comprises the species *Citrullus lanatus* and *Colocynthis vulgaris* which had a genetic similarity of 36.2 %. The second group consists of *Coccinia grandis* which has a genetic similarity of 19.4 %. Reference[24] analysed accessions of *Cucurbita moschata* with 11 SRAP primers and reported a total of 148 reproducible bands. Among these, 98(66.2%) were polymorphic and ranged in size from 140 to 950 bp. The total number of bands per primer ranged between 9 and 21, with an average of 13.5 bands per primer. They also analysed accessions of the same plant with 6 AFLP primers and identified a total of 156 reproducible bands which ranged in size from 60 to 380 bp. Of these, 134 (85.9 %) were polymorphic. They found a range of 14 to 41 amplified bands per primer, with an average of 26 bands.

Reference[25] analysed accessions of three African edible-seeded cucurbits (*Citrullus lanatus* L. Matsumra & Nakai, *Cucumeropsis mannii* L. Naudin and *Cucumis melo* var. *agrestis* L. Naudin.), with 11 ISSR primers on DNA extracted from an accession of the three species and

observed 66 bands with 4 to 11 bands per primer.

Reference[13] analysed accessions of *Citrullus lanatus* var. *lanatus* using 138 RAPD primers that were initially screened among some cultivars of watermelon. Of the 138 RAPD primers, only 35 primers produced polymorphic RAPD patterns. Of the later, 25 primers produced 288 reproducible RAPD bands that ranged in size from 100 to 3000 bp.

Reference[13] also analysed accessions of *Citrullus lanatus* and *C. colocynthis* with 30 RAPD primers that were used for genetic diversity among the two species. The primers produced 662 bands that could be rated with high confidence and ranged in size from 100 to 2650 bp. The number of bands produced by each primer in the above study was relatively high (an average of 22 bands per primer).

Reference[26] analysed molecular variation in accessions

of *Cucumis melo* L. and *C. metuliferus* by 18 RAPD primers and found a total of 176 bands. The number of polymorphic bands was 75 in *Cucumis metuliferus* and 96 in *Cucumis melo*. In general, the cluster analysis grouped the accessions according to many morphological traits and showed a clear separation between the species. The RAPD marker revealed considerable genetic diversity among the species, a finding which strongly agrees with the great morphological variability observed among the cucurbits in this study. Similar results were observed for the oriental *Cucurbita moschata* using RAPD marker[27]. RAPD marker systems proved to be useful for analyzing the genetic diversity of some genera of the family Cucurbitaceae. The knowledge of the diversity of this germplasm will facilitate its use in breeding programmes and the improvement of management of large collections of the species of this family.

Table 1. Plant accessions of cucurbits used in the study

No.	Species	Distribution and Description
1	<i>Coccinia grandis</i>	Wild. Nile banks, Khartoum State
2	<i>Colocynthis vulgaris</i>	Wild. Nile banks, wadis and valleys in Khartoum State. Fruit edible by camels; the pulp is extracted and used in medicine.
3	<i>Ctenolepis cerasiformis</i>	Wild. Nile banks, Khartoum State
4	<i>Cucumis melo</i> var. <i>flexuous</i>	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. Fruits edible as salad or pickled
5	<i>Cucumis sativus</i>	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. Fruits edible as salad or pickled
6	<i>Cucumis melo</i> var. <i>reticulatus</i>	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. The fresh flesh of the fruit is eaten as dessert.
7	<i>Luffa aegyptiaca</i>	Wild. Widespread in Khartoum state. Used as bath sponges and for cleaning purposes.
8	<i>Cucurbita pepo</i>	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. The immature fruits are eaten as a fresh vegetable; mature fruits used for baking, making jam and pies or forage for livestock.
9	<i>Citrullus lanatus</i>	Cultivated. Widespread in Khartoum State. Edible. Dried parched seeds are chewed.
10	<i>Cucurbita moschata</i>	Widespread. Cultivated, Faculty of Agric, Dept. of Horticulture. The immature fruits are eaten as a fresh vegetable; mature fruits used for baking, making jam and pies or forage for livestock.

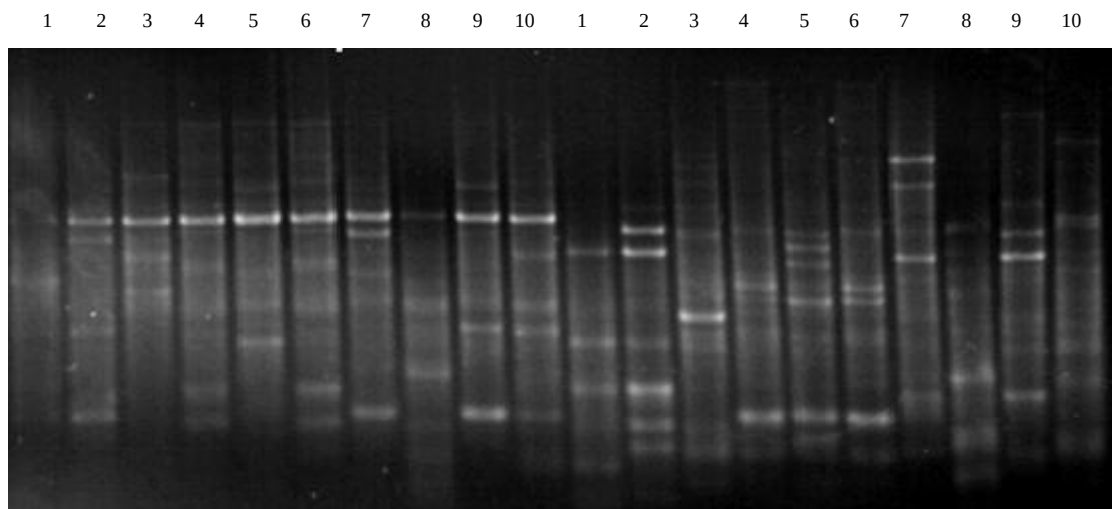
Table 2. DNA concentration and purity of cucurbits accessions

No.	Species	Concentration (µg/ml)	Purity
1	<i>Coccinia grandis</i>	80.8	1.9
2	<i>Colocynthis vulgaris</i>	154.6	2.3
3	<i>Ctenolepis cerasiformis</i>	193.0	1.7
4	<i>Cucumis melo</i> var. <i>flexuous</i>	186.0	1.8
5	<i>Cucumis sativus</i>	352.1	1.8
6	<i>Cucumis melo</i> var. <i>reticulatus</i>	228.0	1.8
7	<i>Luffa aegyptiaca</i>	201.2	1.8
8	<i>Cucurbita pepo</i>	294.3	2.1
9	<i>Citrullus lanatus</i>	274.8	1.8
10	<i>Cucurbita moschata</i>	152.1	1.7

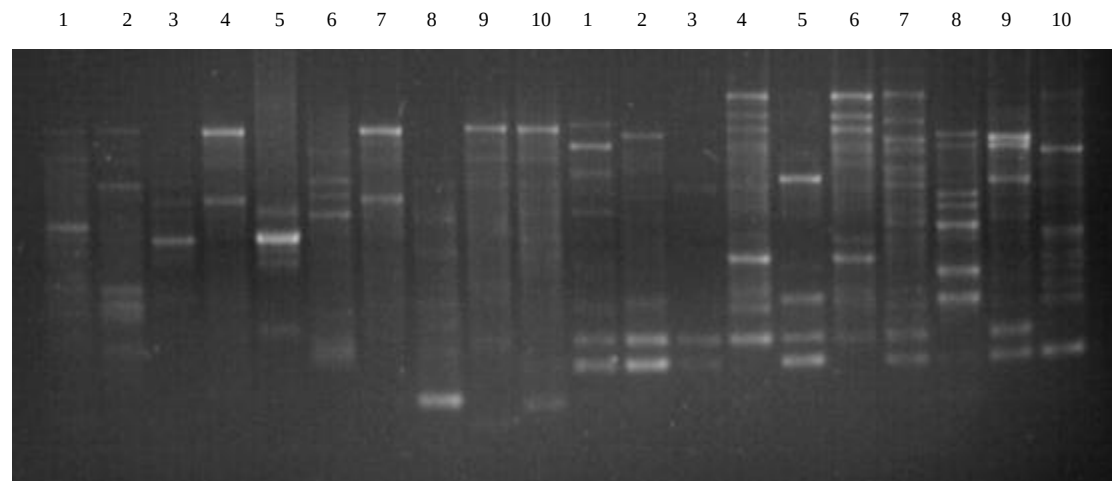
Table 3. Number of total and polymorphic fragments using RAPD marker. Primer names are according to manufacturer's identification system (Operon technology; OP, and University of British Columbia, UBC)

No.	Primer	Sequence (5 – 3)	Temperature (°C)	Total number of bands	Number of polymorphic bands	Percentage of polymorphism
1	A0 1	AGC AGC GCC TCA	42.4	16	15	93.8
2	A02	GCC AGC TGT ACG	42.4	18	18	100
3	A0 3	TGC CTC GCA CCA	42.4	17	17	100
4	A04	GCC CCG TTA GCA	42.4	23	23	100
5	OPB 05	TGCGCCCTTC	33.0	22	22	100
6	UBC106	CGTCTGCCCG	37.1	17	17	100
7	UBC115	TTCCGCGGGC	37.1	14	14	100
8	UBC152	CGCACCAC	37.1	24	24	100
9	UBC155	CTGGCGGCTG	37.1	24	24	100
10	UBC157	CGTGGGCAGG	37.1	17	16	94.1
11	UBC199	GCTCCCCAC	37.1	16	16	100
12	UBC222	AAGCCTCCCC	33.0	10	10	100
13	UBC228	GCTGGGCCGA	37.1	9	9	100
	Total			227	225	99.1
	Average			17.5	17.3	98.9

(a)



(b)

**Figure 1.** Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbits (a) using UBC157 primer (left) and UBC155 primer (right) and (b) using UBC 222 primer (left) and UBC OPB 05 primer (right)

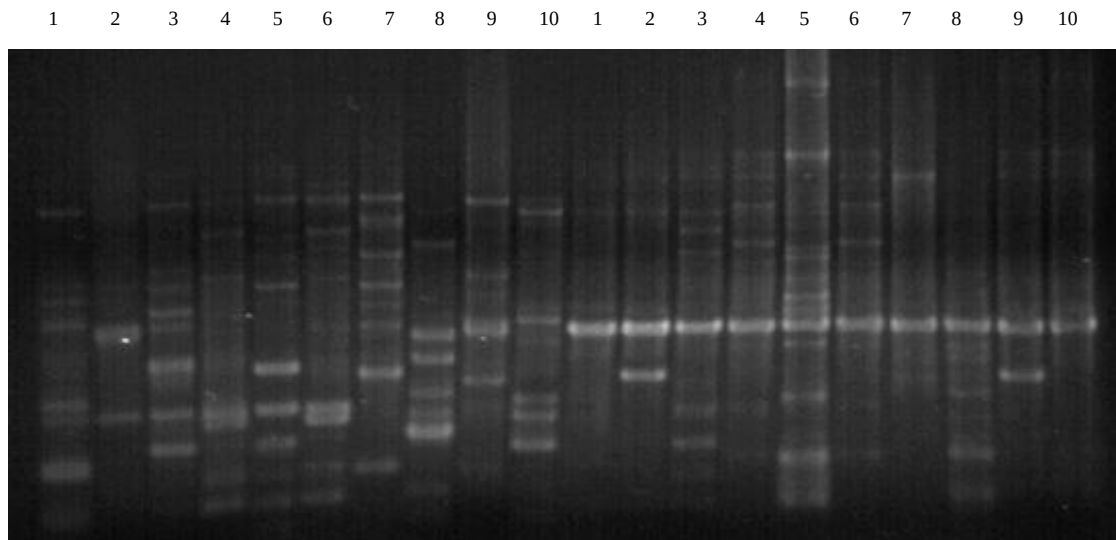
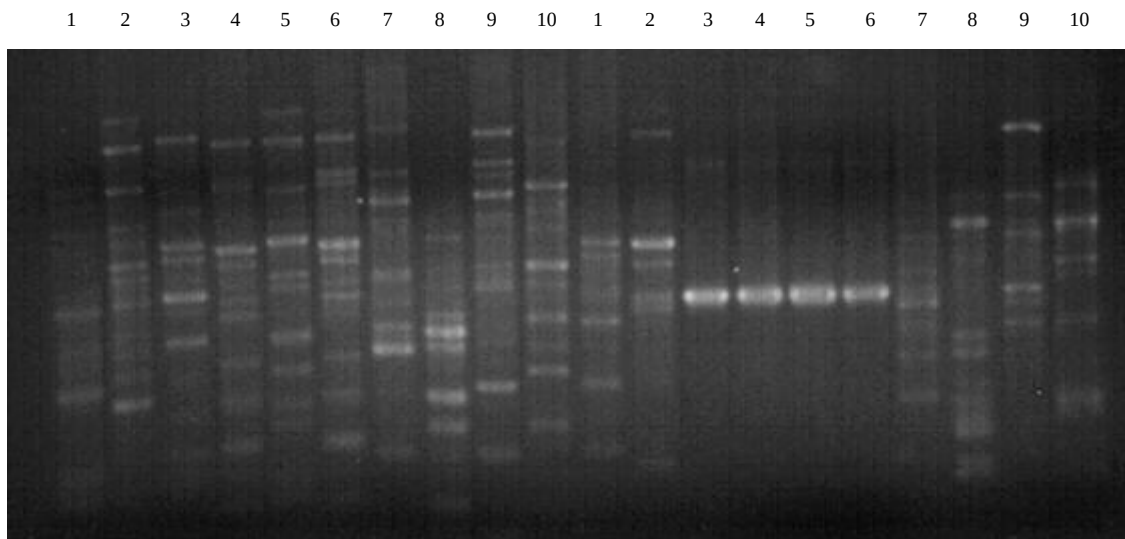
(a)**(b)**

Figure 2. Agarose gel showing polymerase chain reaction (PCR) products of 10 accessions of Cucurbits (a) using A02 primer (left) and A01 primer (right) and (b) using A04 primer (left) and A03 primer (right)

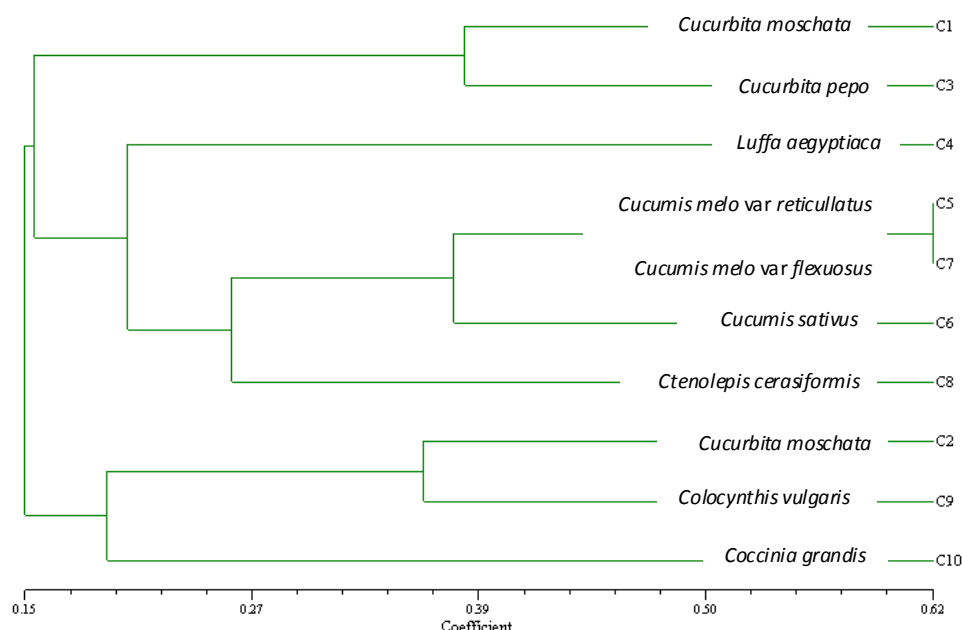


Figure 3. Dendrogram analysis of 10 species of the family Cucurbitaceae

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