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Genetic Diversity of Some Taxa of Cucurbitaceae Family Based on " RAPD" Markers

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Abstract
To find out the taxonomic relationships , genetic diversity was used RAPD technique among seven taxa of Cucurbitaceae family, including Citrullus lanatus,Cucumis melo var. flexuous,Cucumis melo, Cucumis sativus,Cucurbita maxima, Cucurbita pepo, and Lagenaria siceraria, DNA of fresh leaves was extracted using modified protocol of CTAB due the taxa undr study have polyphenols and other secondary metabolites, four primers out of twenty decamer gave reproducible and appeared polymorphism in the RAPD profile, a total two hundred and five bands were produced out of which one hundred and five bands were monomorphic and other were polymorphic , our results arise two main clusters Cucurbita maxima has unique amplified and discriminated from other studied taxa with , thus they can be a good molecular tool to identification taxa, separated at the similarity value of 0.28, and the second cluster contained two gropes. Citrullus lanatus, Cucurbita pepo showed strongest correlation with highest value similarity among taxa under study was 0.12. furthermore, it could be recommended as a reservoir of alleles useful for breeding programs in parental crosses.
Keywords: Cucurbitaceae molecular, RAPD , genetic diversity , cluster analysis

1. Introduction

Cucurbitaceae today as known one of economically roles groups, including the most species providing human with edible products and beneficial fibers (Bisognin, 2002). Commonly grown in temperate climate, it have a reputation for overwhelming production (Paris, 2010).

Different squash types of this family taxa were introduced in to western Europe as early as the 16 th century (Whitaker, 1947) , when they were depicted and described in diverse botanical works, (Paris, 2001). Although the morphological features(qualitative and quantitative) have been provided useful data for genetic studies but this method has been decreased because it has been dealing with a limited phenotypic characters effected by environmental and climate influences(Klich,2002).

The molecular systematic studies give us deeper insight of genetic structures, it's have been an impotence role for many purposes in molecular biology, such as analyzing of granitic diversity by classification of the cultivars and germplasm collections, and clearing the phylogenetic relationships among groups, or closely related species. the determine sequence of nucleotides in the DNA of plants significantly increased our understanding of group plant evolution, (Carlson and Holsinger, 2010).

Majority of molecular analyses performed to data on cucurbitaceae have been focused on the establishment of phylogenetic of relationships among family species (Goldberg et al, 1972; Decker- Walters et al ,1990; Weeden and Robinson, 1990; Wilson, 1992; King et al, 1995; Katizir et al, 1996; Jobst et al, 1998; Sanjur et al, 2002; APG, 2009).

The taxonomic and genetic limits of some Cucurbitaceae taxa have been redefined and their closest-related wild species have been classified into intraspecific categories within these limits.

The random amplified polymorphic DNA (RAPD) have been widely used in DNA fingerprinting gene mapping isolation of phylogenetic relationships of many organisms and taxonomy within many families (Chandra, 2004), and can be used as a molecular technique for cultivar identification(Radwan et al,2014).

Ferriol et al (2003) used RAPD technique as a reliable, fast, and simplest technique molecular markers for estimating genetic as previous work with melon and cucumber closely related species. Other molecular markers (amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) , inter simple sequence repeat (ISSR) also have been used to analyzing the genetic diversity into Cucurbita species such as C.pepo (Katizir et al, 2000; Paris, 2003; Chiba et al .2003; Ferriol et al, 2004). , However a little is known about the genetic diversity within Cucurbitaceae family species (Radwan et al,2014), with very few Published paper regarding molecular genetic markers (Ferriol, et al 2003),as it considered the ranks Cucurbitaceae family are among the highest of plant families for number and percentage of taxa used as human food.

The objective of this study is investigation and identify reliable molecular markers for use in estimating genetic polymorphism within the molecular marker were used to test for genetic diversity within some taxa of Cucurbitaceae family, and systematic analysis of these taxa used on DNA profiling using RAPD technique.

2. Materials and Methods

2.1 Plants materials:

Seven taxa of Cucurbitaceae family were collected during April 2015 from different area of Dyiala city, the

collected taxa were identified and deposited at Baghdad university, college of education for pure sciences (BUE).

2.2 DNA extraction:

Approximately 50 to 100 mg of fresh leaf tissue and put in 1.5 ml tube, homogenized the tissue using liquid nitrogen with a conical hand tissue grinder.

Total genomic DNA was isolated from fresh leaves using modified of cetylrimethyl ammonium bromide (CTAB)method of Doyle and Doyle (1990),the powder suspend in 2.5 ml of CTAB extraction buffer (1.4 M NaCl, 2% CTAB) and 5ml of B- mecaptoethanol. The suspension was mixed well, ad put incubated at 60ºC for 20 min to homogenate, followed by chloroform: isoamyl alcohol extraction (24:1), and precipitation with two thirds of the volume of isopropanol at -20ºC, then bring down the sample formed after centrifugation for 5min, was washed with 1ml of 70% ethanol and 10m M of ammonium acetate, the CTAB removed from DNA by TE buffer (20m M EDTA, 0.1 M Tris- HCL p H= 8), for detection of the DNA samples that were electrophoresed in 1% agarose gel stained with ethidium bromide (100 v for 45min). (Liber et al , 2006)

2.3 Screening of PCR

Twenty different 10mers RAPD primers were tested in this study (table 1) supplied by Bioneer company were screened, four of them were gave it indicated results, Multi master mix were used, The thermoprofile for the PCR reaction was: 95°C for 5 minutes, then 35 cycles of 95°C for 30 sec, 37°C for 1 minute, and 72°C for 5 minute. Genotypes were visualized on 1% agarose gel, 1x TBE, 100 volts, for 55 minutes and scored as 1 or 0 based on presence or absence of a band.

Table 1. The sequences of twenty RAPD primers

Primer name	Sequence (5´- 3´)
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-07	AAGTCCGCTC
OPA-09	GGGTAACGCC
OPA-11	CAATCGCCGT
OPA-13	CAGCACCCAC
OPB-01	GTTTCGCTCC
OPB-02	TGATCCCTGG
OPB-03	CATCCCCCTG
OPB-04	GGACTGGAGT
OPC-12	TGTCATCCCC
OPG-01	CTACGGAGGA
OPG-02	GGCACTGAGG
OPZ-01	GAGCCCTCCA
OPZ-03	CAGCACCGCA
OPZ-04	AGGCTGTGCT

Table 2. The sequences of RAPD primers resulted amplified.

Primer name	Sequence (5´- 3´)	AN	Size range of bands(bp)	PM	%	MM	%
OPA-01	CAGGCCCTTC	21	300- 600	0	0	21	100
OPB-04	GGACTGGAGT	59	260- 1750	52	88.1	7	12.5
OPG-02	GGCACTGAGG	63	170- 2100	21	33.3	42	66.6
OPZ-03	CAGCACCGCA	62	190- 1300	27	43.5	35	56.4
	Total	205		100		105	

AN = alleles number; PM = Polymorphic bands; MM = Monomorphic bands.
%PM= PM/ANx100, % MM= MM/AN x100

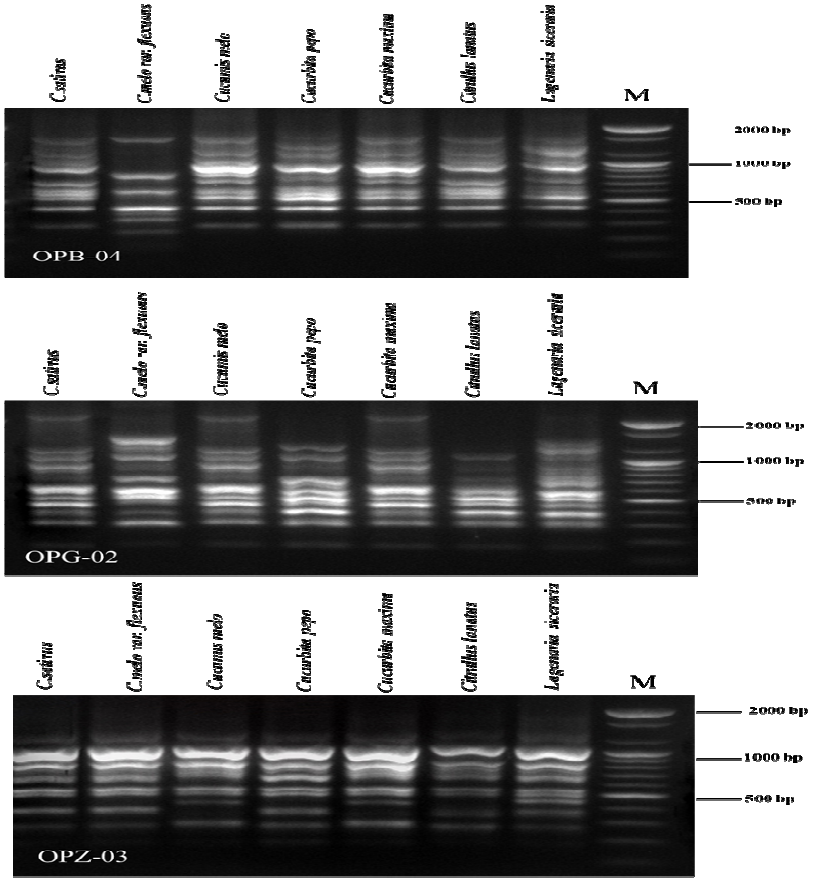

Figure 1. RAPD profiles amplified from genomic DNA of 7 Cucurbitaceae taxa

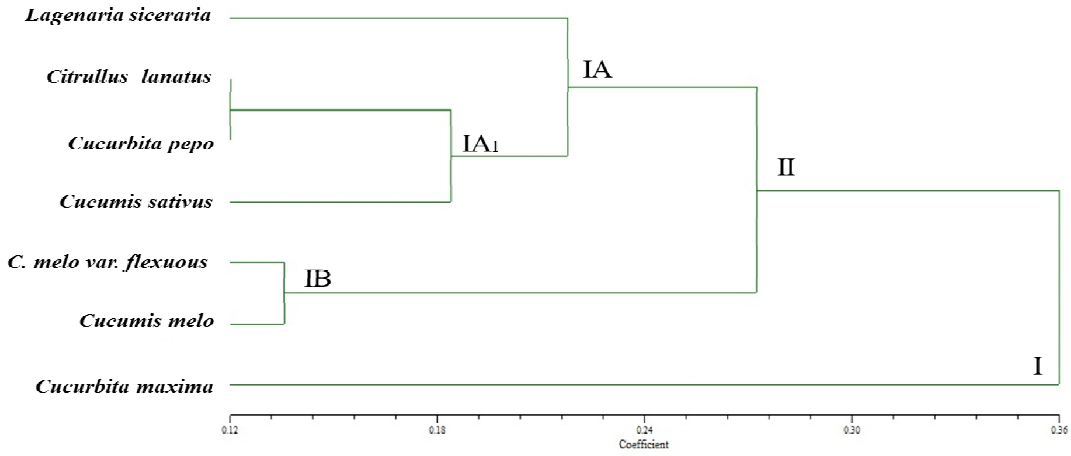

Figure 2. Dendrogram showing genetic relationships among 7 taxa of Cucurbitaceae.

2.4 Data analysis :

RAPD matrix was analyzed using the NTSYS-pc statistical package version 2.1. The data matrix was used to calculate the genetic similarity within and among species based on Jaccard's similarity coefficients, and a dendrogram displaying relationships among the 7 genotypes was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

3. Results

From this study notes the total of 205 amplified RAPD bands ranging from 170 bp to 2.1 kb in size were observed from the 7 Cucurbitaceae genotypes. The number of RAPD bands varied from 21 (primer OPA-01) to 63 (primers OPG-02) (table 2). One hundred polymorphic bands were obtained, Some representative

polymorphisms revealed by RAPD primers are presented in table 2, The dendrogram showing the genetic relationships among the 7 Cucurbitaceae genotypes (Figure 1) showed that Cucurbitaceae species were basically divided into 2 main clusters, the first (cluster I) consisted of *Cucurbita maxima,* The two main clusters separated at the similarity value of 0.28, and the second cluster (cluster II) contained two gropes with similarity value 0.21 which separated into 2 clades, (IA) containing *Lagenaria siceraria,* that separated from *Citrullus lanatus*, *Cucurbita pepo*, and *Cucumis sativus* by value similarity of 0.21, but *Citrullus lanatus*, *Cucurbita pepo* showed highest value similarity among taxa under study was 0.12, these 2 taxa isolated from *Cucumis sativus* with similarity range 0.18, according to the dendrogram linkage joining rule *Cucumis melo var. flexuous, Cucumis melo* were more distantly related and separated from the (IA) group at the similarity value of 0.13.

4. Discussion

Due high reproducibility and high levels of polymorphism, we can predict that a strong relationship or genetic diversity among taxa under study. The difference between reproducible bands based by each primer depends on sequence of primer and extent of variation in specific genotype(Chan and Sun,1997; Shukla et al, 2006; Shiran, 2007).

In RAPD technique , some fragments were incomparably amplified in single taxon such as the bands in *C. maxima* 170 bp- 1.7 kb, therefore these fragments have important interest in optimal management of germplasm collections, in addition they provide the identification of taxa and duplicates and verify possible pollen or seeds contamination during conservation activities (Ferriol et al, 2004).

The outcome of RAPD- PCR analysis and index matrix based on all DNA fragments that isolated by four primes observed the strongest homogeneity between *Citrullus lanatus*, and *Cucurbita pepo*, so it can be considered as a reservoir of alleles useful for breeding , due divergent genotypes may has a reliable breeding value (Gwanama et al, 2000), or has substitution rates and high levels of gene rearrangements.

However, When the new data are combined with existing botanical and molecular data it will be a clearer picture, Current study suggests fruit color and pattern vary considerably, and fruits with non-bitter flesh and sizes indicating a taxa, this result agreed with Sanjur et al (2002).

Moreover, Our results deviate in part from those of another study (Nagahavi and Jahansouz, 2005; Radwan, 2014) they regarding the identity of taxon but also by environmental differences such us geographic location, but agrred with Sanjur et al (2002) results that showed

the mitochondrial DNA of *Cucurbita*, evidence effected of environmental factors between the domesticated species within the genus, or between wild and domesticated taxa, the results were concordant analyses and further support an interpretation of two separate domestications in the species. *C. pepo.*

The *Cucurbita maxima* indicated its unique banding pattern over the rest taxa, the rustles ensured it's characterized by specific genotype , this lower genetic variability in this commercial hybrid compering with another taxa which is has narrower origin of hybrid and genetic erosion because intensive breeding (Formisano et al, 2010).

Actually hybridization, introgression, and migration with in Cucurbitaceae species, notably out crossing and open- pollinated these character genetically a great diversity of phenotypes, most of them being intermediate forms (Ferroil et al , 2003).

5. Conclusion

The reliable method of analysis that used in the present study provided an effective procedure to understand evaluating germplasm material, or gene flow in order to identify the species that could be further evaluated. This rustle indicates a high degree of correlation among studied taxa.

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