MOLECULAR AND MORPHOLOGICAL INDICATORS (QUTHA) *Cucumis melo* PLANTED IN IRAQ

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ABSTRACT
A field experiment was carried out at the Department of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad .during 2016. to investigate the genetic diversity for six genotype of snake melon *Cucumis melo* var. *flexuosus* (Qutha), cultivated in Iraq. These selected genotypes were collected from Iraq's Governorates (Baghdad, Sulaymaniyah, Mosul, Salahadeein, and Diyala)in addition to commercial genotype and designated as A,B,C,D,E, and F, respectively. The treatments were arranged in randomized complete block design (RCBD) with three replications Genetic diversity among the six selected genotype was studied utilizing the random amplification of polymorphic DNA (RAPD) markers and linked with fruits morphological traits. The results showed that the highest genetic diversity (0.285) observed between the genotype (A and B) and between (A and D) was (0.235) ,while the genotype (C and D) had the lowest genetic distances which was (0.031), as well as between(E and F) was (0.059). Also the results revealed that Genetic distances depend on the variance phenotypic of fruits recorded about (1) as highest number among the genotype (A and D) , (B and D), (C and D), (D and E), and (D and F), while the lowest was about (0.091) for (A and C.)Genotype

Key world: genetic and phenotype distances *Cucumis melo* ،Randomly Amplified Polymorphic DNA

المؤشرات الجزيئية والمظهرية للقثاء *Cucumis melo* المزروع في العراق

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المستخلص
نفذت تجربة حقلية في قسم البستنة وهندسة الحدائق –كلية الزراعة – جامعة بغداد في الموسم 2016 بهدف دراسة البعد الوراثي لست تراكيب وراثية من القثاء المزروعة في العراق والتي جمعت من محافظات السليمانية وبغداد وموصل وصلاح الدين وديالى فضلا عن صنف تجاري رمز للتراكيب الوراثية A و B و C وD و E و F بالتتابع. رتبت التراكيب الوراثية تصميم القطاعات الكاملة المعشاة وبثلاث مكررات . حسب البعد الوراثي بأستعمال مؤشرات التضاعف العشوائي(RAPD) بالأعتماد على الصفات المظهرية للثمار اظهرت النتائج ان هناك بعد وراثي بين التراكيب الوراثية A و B بلغ 0.285 و البعد بين A و D بلغ 0.235 وان اقل بعد وراثي بلغ 0.031 بين التراكيب D و C وبين E و F بلغ 0.059 اما البعد بين التراكيب الوراثية على اساس الاختلافات المظهرية لثمار لوحظ ان اعلى بعد بلغ 1 بين التراكيب Aو D وبين B و D و بين C و D وبين E و D وبين D و F وان اقل بعد بلغ 0.091 بين التركيبين الوراثيين A و C .

كلمات مفتاحية : البعد الوراثي والمظهري ، القثاء ،مؤشرات التضاعف العشوائي للدنا

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INTRODUCTION

snake melon *Cucumis melo* var. *flexuosus* related to the Cucurbitaceae family. It is a morphologically diverse crop with very polymorphic fruit types (6,8) It is commonly known by the name qutha in Iraq, where many genotype can be found. Plant biodiversity is essential for classical and modern plant breeding programs including genetic engineering **(**4). In a broad sense, marker - assisted breeding can be traced long time back to the use of classical or morphological markers as an assisting tool for selection of plants with desired traits in breeding (11). During the early history of plant breeding, the markers were mainly used visible morphological traits such as leaf shape, flower color, pubescence color, pod color, seed color, seed shape, helium color, awn type and length, fruit shape, rind (exocarp) color and stripes, flesh color, stem length, etc. These morphological markers generally represent genetic polymorphisms which are easily identified and manipulated.Randomly Amplified Polymorphic .DNA (RAPD) predominantly provides dominant markers. This method of investigation the relationship has been highest levels of polymorphism and considered simple, relatively cheap, and easy to conduct **(**20). The Randomly Amplified Polymorphic DNA (RAPD) technique markers is available among different types of molecular marker techniques **(**19**)** and has been the most applicator be the researchers due to its simplicity, cost effective, and fast and easy to perform (5, 19). The diversity among *Cucumis melo* was analyzed using several morphological traits and molecular markers (**7,** 14, 15, and 18). Molecular markers could be provide complete morphological data because they are plentiful and free from the effect of environmental factors and allow for cultivar identification in the early stages of development (12). Ali-Shtayeh et al (2) showed that 50 snake melon accessions collected from Palestine (West Bank), 17 of the phenotypic characters were polymorphic. These accessions related to four important genotype of *Cucumis melo* var. *flexuosus*. Bhakarl et al (3)investigated the genetic diversity at molecular level using RAPD primers across 23 landrace of watermelon. Abdel-Ghani and A.Mahadeen (1) studied the diversity among eight Jordanian snake melon (*Cucumis melo* var. *flexuosus*) genotype by analyzing morphological traits with random amplified polymorphic DNA (RAPD) markers. Pair-wise distances based on morphological data ranged from 4.6 to 8.10 among studied populations. The objective of this study was to investigate the genetic diversity for six genotypes of snake melon *Cucumis melo* var. *flexuosus* (Qutha), cultivated in Iraq.

MATERIALS AND METHODS

This research was carried out at the Department of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad using six genotypes of snake melon *Cucumis melo* var. *flexuosus* (Qutha). These selected genotypes were collected from Iraq Governorates (Baghdad, Sulaymaniyah, Mosul, Salahadeein, and Diyala) in addition to commercial genotype and designated as A,B,C,D,E, and F, respectively. The genotype seeds were sown at the field using Randomized Complete Block Design with three replications Morphological characters were recorded using randomly ten plants within each plot, ten immature fruits were harvested from each plot, and the following traits were studied: fruit shape skin texture end deeply wrinkled Description of morphological characters depended on the classification of International Plant Genetic Resources Institute (IPGRI) (2, 17). Fruit hairs presence or absence. Qualitative character, transformation to a numerical (1, 0) presence or absence respectively.

Molecular diversity

A second experiment was included molecular fingerprinting of snake melon accessions which based on the Randomly Amplified Polymorphic of DNA (RAPD), investigate in Biotechnology Center, University of AL-Nahrain,

Leaves samples

The experiment was carried out in pots under greenhouse condition, where 5 seeds were sown in each pot. Seeds and plants were irrigated with tap water (100 ml/ pot) according to the filed capacity. Leaves sample were taken after 4 weeks from the sowing

date, which will be used for the molecular assessment.

Genomic DNA Isolation

DNA was extracted from the leaves of snake melon cultivars using the genomic DNA kit (CATB, Iraq). The purity of extracted DNA was measured using the nanodrop and its quality was also taken by agarose gel electrophoresis with red safe nucleic acid stain (initron) and visualized under UV light

RAPD assay

Ten RAPD primers were used in this study which were synthesized in Bioneer (Korea) and dissolved to obtain the final concentration of (10pmol/ml). The primers and their sequences are shows in Table 1

Table 1. The names and sequences of the primers used in this study

No.	Primers name	Sequences(´5 -´3)
1.	**Pr-1**	**GAGTCAGCAG**
2.	**Pr-2**	**GTGACGTAGG**
3.	**Pr-3**	**GGTCCCTGAC**
4.	**Pr-4**	**CCTGGGCTTC**
5.	**Pr-5**	**GGGTAACGCC**
6.	**Pr-6**	**GGACCCAACC**
7.	**Pr-7**	**ACCTGAACGG**
8.	**Pr-8**	**GTGTGCCCCA**
9.	**Pr-9**	**GGTCTACACC**
10.	**Pr-10**	**GTGATCGCAG**

PCR technique was performed in 20µl volume containing (PCR Premix – Bioneer) 5 µl master mix, 2 µl primer (10 pmol), 2 µl DNA template (2 µl) 100 ng and 11 µl deionised water. The amplification was carried out in a thermo cycler programmed as follows: one cycle of 94°C/5min, 40cycle of (94°C/1min, 41°C /1min, 72°C/2min), one cycles of 72°C/10 min. The primer annealing degrees were varied according to the melting point of each primer. Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. The agarose gel was prepared by dissolving 1.2 g agarose in 100ml TBE solution (1X). The gel was stained with red safe nucleic acid stain, photograph under UV light, and scanned using a Gel-Documentation system.

Data of RAPD products

The presence of a band was marked as (1) and the absence was marked as (0). Accordingly, data were scored for all genotypes depends on amplification product and primers. Then entered into SPSS Program V20. Dissimilarity measure between the accessions to carry out the clustering By UPGMA in accordance to the algorithm proposed by Lance and Williams (13).

RESULTS AND DISCUSSION

Genetic analysis showed that the highest genetic distance (0.285) was observed between the landrace A and B and between A and D(0.235) whereas the lowest genetic distance 0.031 was observed between the landrace D and C as well as between E and F 0.059 (Table 2). This genetic distance shows the importance of Iraqi genotype of snake melon *Cucumis melo var. flexuosus* (Qutha cucumber) for plant in breeding programs. These results are consistent with the results of other researchers (1 ,9 ,16). Genetic distance is a measure of the genetic diversity between Iraqi genotype of snake melon *Cucumis melo* var. *flexuosus* which usually depend on the phenotype differences of its fruit. Genetic analysis showed that the highest genetic distance 1 was observed between the landrace A and D , B and D , C and D , D and E , and D and F whereas the lowest genetic distance 0.091 was observed between A and C. The phenotypic and molecular marker data can be used for the development of association mapping and could be play a crucial role in the development of snake melon in the short and long terms (16).

Table 2. Genetic distance coefficient based on RAPD markers among the six snake melon *Cucumis melo var. flexuosus* (Qutha) genotypes

	A	B	C	D	E	F
A	**.000**	**.176**	**.200**	**.235**	**.258**	**.206**
B		**.000**	**.125**	**.161**	**.143**	**.193**
C			**.000**	**.031**	**.138**	**.153**
D				**.000**	**.143**	**.158**
E					**.000**	**.059**
F						**.000**

Table 3. Genetic distance coefficient based on morphological traits among the six snake melon *Cucumis melo var. flexuosus* (Qutha) genotype

	A	B	C	D	E	F
A	**.000**	**1.000**	**.091**	**1.000**	**.714**	**.111**
B		**.000**	**.714**	**1.000**	**.333**	**1.000**
C			**.000**	**1.000**	**.500**	**.200**
D				**.000**	**1.000**	**1.000**
E					**.000**	**1.000**
F						**.000**

Cluster analysis is one of the statistical indicators used in the distribution of plant groups by their characteristics and then determine the degree of genetic variability. Genetic distances dendrogram which is built on RAPD marker exhibit the distribution of selected genotype in two major groups .The first group included (A) genotype. The second group included the genotype (B, C, D, E and F.). Furthermore, the second group could be subdivided into two sub-groups where first subgroup included (F and E) and second subgroup included (B, C, and D) (Fig 1). The results indicate at that RAPD markers are good tools to identify snake melon genotypes genetic diversity

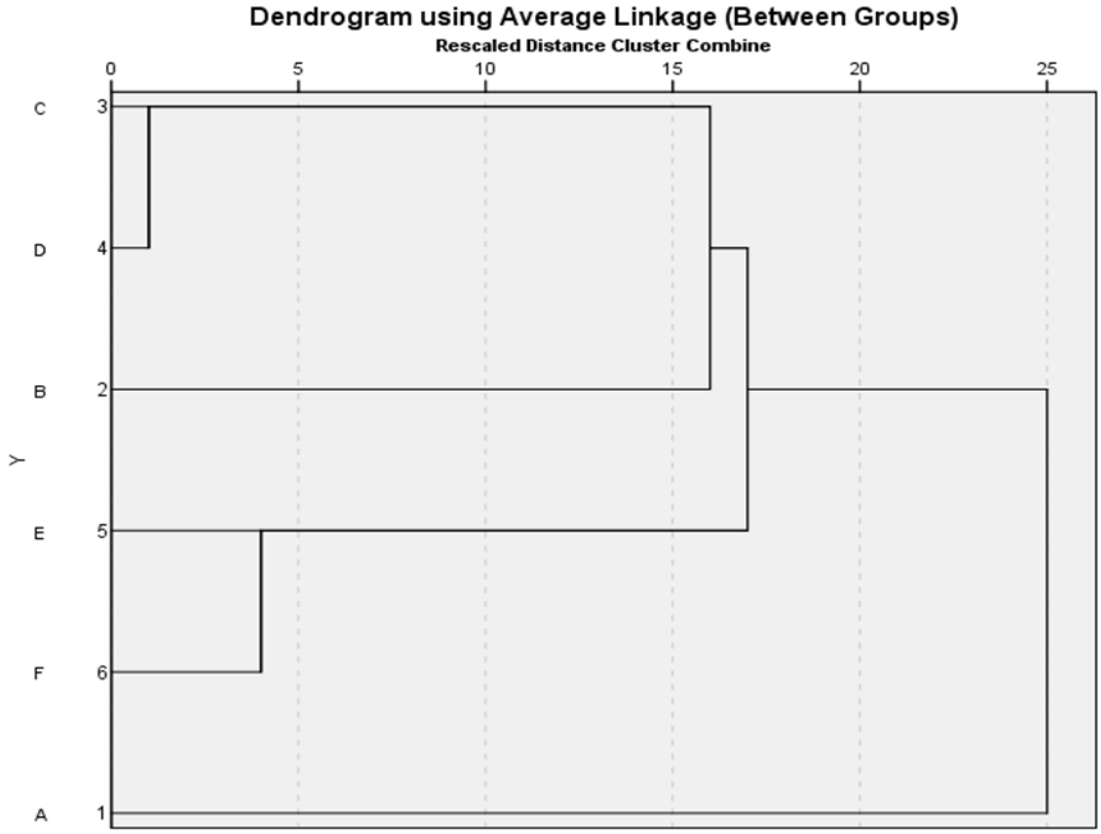

Fig 1. Dendrogram resulting from an UPGMA cluster analysis of landrace of *Cucumis melo var. flexuosus* (Qutha) of RAPD marker data

Cluster analysis refers to the specific phenotypic parameters of fruits, which could be utilized for the distribution of genotype according to their phenotypic characteristics. The results indicated a significant correlation between the dimension that depends on molecular analysis and phenotypic characteristics. It had been noted that the distribution of the genotype was fall into two main groups in which the first group included genotype (A), while the second group included genotype (B, C, D, E and F). The second group could be subdivided into two subgroups included (B and C) and (D and E and F) landrace (Fig 2)

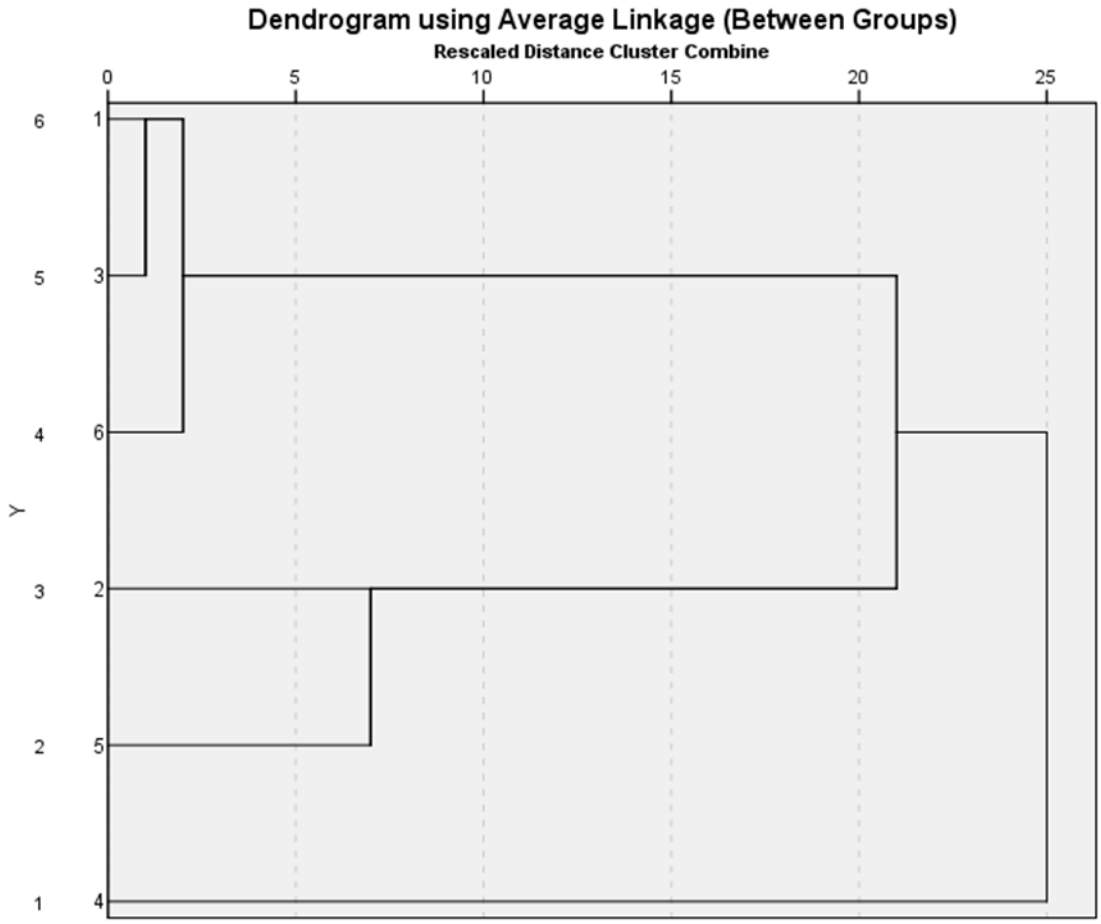

Fig 2 Fruit Morphological traits dendrogram of *Cucumis melo* var. *flexuosus* (Qutha cucumber)

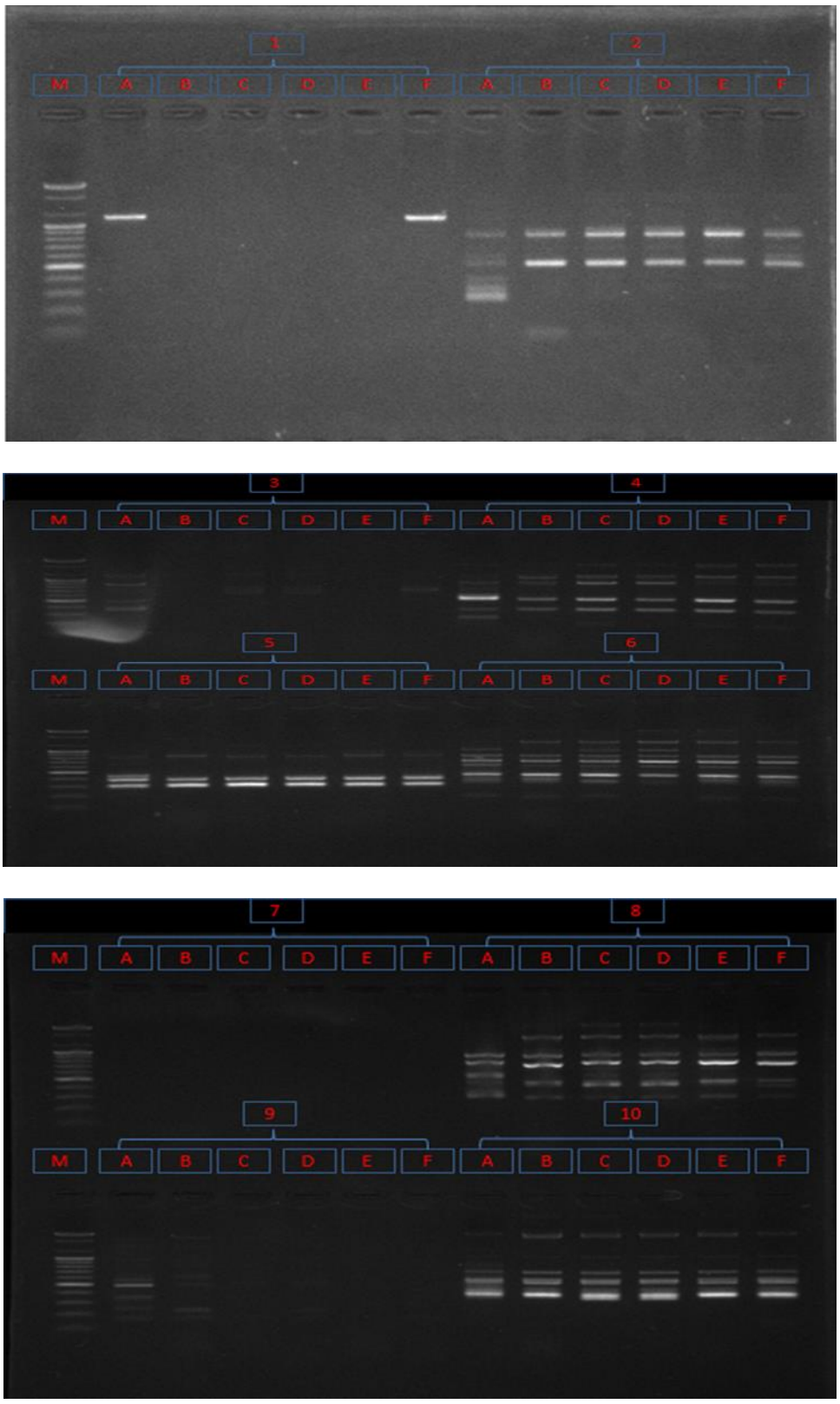

Fig 3.Amplification products generated from 6 genomic DNA of *Cucumis melo var. flexuosus* (Qutha c)accessions using RAPD primer

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