Archives of Agriculture Sciences Journal
Print ISSN: 2535-1680
Online ISSN: 2535-1699

ARCHIVES OF AGRICULTURE SCIENCES JOURNAL
Volume 4, Issue 1, 2021, Pages 102–114
Available online at www.agricuta.edu.eg
DOI: https://dx.doi.org/10.21608/aasj.2021.82396.1070

Genetic diversity analysis for nine F_1 hybrids of cucumber (*Cucumis sativus* L.) under Assuit conditions, Egypt

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Abstract

This experiment was performed on cucumber (*Cucumis sativus* L.) at Research Farm of the Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt (Longitude 031° 11’ 09” E and latitude 27° 10’ 39” N) in sandy clay loam soil. To evaluate nine F_1 hybrids of cucumber by using molecular analysis. Cucumber hybrids were planted in March 2019 and 2020 for the summer seasons 2019 and 2020. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Randomly amplified polymorphic DNA was used to assess the genetic diversity and relationship among 9 cucumber hybrids. Five RAPD primers generated a total number of 34 bands, out of them 3 bands were polymorphic with 8.82% polymorphism. The total number of bands generated by each primer ranged from 5 to 9 bands with an average of 6.8 band per primer. Additionally, only two primers gave polymorphism among cucumber samples i.e., OPA-03 and OPA-08. Some genetic diversity measures were estimated based on RAPD molecular data. In this regard, the polymorphism information content (PIC) ranged for 0.05 (OPA-03) to 0.16 (OPA-08) with an average of 0.04 for all primers. The primer resolving power was 0.67 for OPA-03 and 1.11 for OPA-08, with an overall value of 1.78 for RAPD primers. In addition, diversity index was almost the same as PIC, with 0.04 (OPA-03) and 0.16 (OPA-08) and an average of 0.04 for all RAPD primers.
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Keywords: cucumber, *Cucumis sativus*, RAPD, hybrid, genetic diversity.

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1. Introduction

Cucumber (*Cucumis sativus* L.) is grown widely in open field or glasshouse conditions. It has modest levels of vitamin A and C, magnesium, phosphorus, copper and manganese, fiber and, some phenolic compounds known to have antioxidant properties (Kumar *et al*., 2010). It helps in healing diseases of urinary bladder and kidney; digestive problems like heartburn, acidity, gastritis and ulcer (Garcia-Closes *et al*., 2004). The ascorbic acid and caffeic acid contained in cucumber help to reduce skin irritation and swollen (Okonmah, 2011). Cucumber originated in India and became popular throughout the Egyptian and the Greek-Roman Empire (Renner *et al*., 2007). It is an important vegetable crop grown in the temperate and tropical zones of the world. Soft and succulent, the vegetable crop is cherished by man and eaten in salads or sliced into stew in tropical regions. Its juice is often recommended as source of silicon to improve the health and complexion of the skin (Duke, 1997). Cucumber cultivars have distinctive characteristics/ traits which make them suitable for a particular environment or condition in terms of tolerances to drought, disease resistance, early maturing and yield. The variation in performance of cucumber cultivars has been widely documented by many scholars (Hamid *et al*. 2002; Manyvong, 1997; Maqsood *et al*., 2004; Mingbao, 1991; Ojeifo *et al*., 2008; Sharma *et al*., 2000; Odeleye and Odeleye, 2001) which could be as a result of environmental factors and genetic composition. Varietal differences affect or determine the growth and yield of crops. Majanbu *et al*. (1996) and Sajjan *et al*. (2002) reported that, growth characters of crops such as plant height, vine length, leaf area, member of leaves or branches, and fruit production were influenced by genetic factors of the different varieties. Odeleye and Odeleye (2001) indicated that, growth characters, yield and its component differed among crop varieties. Molecular markers are not affected by the environmental changes and they serve as a tool to overcome the deficiencies of morphological markers in the characterization studies (Agarwal *et al*., 2008; Gostimsky *et al*., 2005; Gulsen and Mutlu, 2005). For this reason, using morphological and molecular marker systems together is preferred for the better clarification of the relationships between the genotypes. Biochemical markers such as isozyme (Meglic *et al*., 1996) have been used for evaluation of genetic diversity in cucumber. Molecular markers have extensively been used for assessment of genetic diversity in cucumber including restriction fragment length polymorphisms (RFLPs) (Dijkhuizen *et al*., 1996) and PCR-based markers such as RAPD (Mliki, 2003). inter-simple sequence repeat (ISSR) (Wang *et al*., 2007), simple sequence repeat (SSR) (Danin *et al*., 2001) and EST-SSR (Hu *et al*., 2010) have been employed for the characterization of genetic diversity in cucumber and mushroom (Rajaratnam *et al*., 2012), however it seems that evaluation of morphological traits is not used, abundantly, for estimation of genetic variation in it. Molecular markers such as RAPD and SSRs have been employed for determination of genetic diversity in African cucumber (*Cucumis sativus* L.) (Mliki *et al.,* 2003). Al-Rawahi *et al.*

(2011) studied the genetic diversity among twenty-four accessions of cucumber for morphological traits. Al-Rawahi *et al.* (2011) clustered the genotypes to two main clusters. It seems that evaluation of genetic variation for morphological traits will help to provide valuable information about identification of new sources for high yield in cucumber germplasm in breeding programs. Also, observed results could suggest comparative advantages for further breeding options in cucumber. The objective of this research was to perform a compare germplasm evaluation of some cucumber hybrids in Egypt.

2. Materials and methods

2.1 Experimental site

This experiment was conducted at Research Farm of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt (Longitude 031° 11' 09" E and latitude 27° 10' 39" N) in sandy clay loam soil (Table 1). Cucumber hybrids were planted in March 2019 and 2020 for the summer seasons 2019 and 2020.

2.2 Source of seeds

Nine F_1 hybrid lines of cucumber were used in this investigation namely, Masira, Safeer, Basha, Aden, Krestal, Wafeer, Saso, Zena and Rocket. Seeds of the hybrid used were sourced from Horticulture Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt.

2.3 Soil sampling

Prior to land preparation, soil samples were randomly collected from the experimental field from 0-25 cm depth and thoroughly mixed to make a composite soil sample. The composite soil sample was analyzed for soil physico-chemical properties.

Table (1): The physical and chemical properties of the samples taken from experimental soil during the two cultivated seasons.

Characteristic	Value	Characteristic	Value
O.M. (%)	0.9	Mg^{+2} (%)	1.8
$CaCO_3$ (%)	1.62	Na^{+} (%)	6.5
Sand (%)	25.4	K^{+} (%)	0.22
Silt (%)	39.9	Available (ppm)	---
Clay (%)	34.8	NH_4 (%)	48.0
Texture class	Clay loam	N (%)	63.2
pH	7.8	P (%)	9.5
EC (dS/m)	1.3	Zn (%)	2.5
Soluble ions (me/L)	---	Fe (%)	9.6
CO_3 (%)	---	Mn (%)	4.2
HCO_3 (%)	2.35	$SO4^{-2}$ (%)	6.5
Cl (%)	2.3	Ca^{+2} (%)	3.3

2.4 Experimental design and cultural practices

The experiment was laid out in randomized complete block design (RCBD) with three replications. Nine hybrids of cucumber (*Cucumis sativus*) were sown. Seeds of cucumber hybrids was planted 50 cm apart on the northern side of 3.5 m long and 1.5 m wide ridges. Three seeds were planted per hill then they were thinned into one plant. Cucumber plants were fertilized with 300 kg /feddan (feddan = 4200 m^2 = 0.420 hectares = 1.037 acres) ammonium nitrate (33.5% N), 200 kg/feddan calcium superphosphate (15.5% P_2O_5) and 200 kg/feddan potassium sulfate (48% K_2O) (Lorenz and Maynard, 1980). Superphosphate was added during soil preparation. One third of N fertilizer was side banded 2 weeks after planting while the other two thirds were added at the flower bud initiation and the fruit setting stages, respectively. Soil preparation and other different cultural practices were followed as recommended for production of cucumber (Hassan, 1991).

2.5 DNA extraction

Total DNA was extracted from cucumber fresh leaves of all tested hybrids, following the protocol of Youssef *et al.* (2015). Cucumber new leaves were brought to the laboratory and washed by dipping in 70% ethanol and distilled water with 1 min for each, then the excess of water was removed by paper tissues. Samples were prepared by weighting 75-100 mg and kept in aluminum foil at -20°C till use. Frozen samples were grinded using liquid nitrogen and the powder was transferred into a 2ml Eppendorf tube. One ml pre-heated extraction buffer (65°C) plus 100 µl SDS (20%) were added to each sample and shaken vigorously and stand on ice. Tubes were incubated in a water bath at 65°C for 30min (with inversion each 5min). Samples were then left to take the room temperature (RT), and a half volume of 5 M Potassium Acetate (pH 8) was added with mixing and incubated on ice for 30 min. After samples taken RT, they were centrifuged at 12200 × g (13000 rpm) for 30 min at 10°C. The supernatant was transferred to a new 2ml tube and 5µl RNAase (10 mg/ml) was added and incubated at 37°C for 30 min. Equal volume cold Isopropanol was added and mixed gently and incubate -20°C for 1 h. Samples were centrifuged at 12200 × g (13000 rpm) for 20 min at 4°C. The supernatant then was discarded, and the pellet was washed 2 times with ethanol 70%, (500 µl, 9500 × g (10000 rpm), 5 min). The pellet was left to completely dry and dissolved in 600 µl TE buffer (incubation at 45°C can be used for acceleration). Samples were centrifuged at 12200 × g (13000 rpm) for 10 min at 10°C. The supernatant was transferred into a new 1.5ml tube, then equal volume of cold isopropanol with 1/10 volume sodium acetate (3 M, pH= 5.2) were added with gentle inversion of the tubes and then samples were incubated at -20°C for 1 hr.

The pellet was let to completely dry and dissolved in 50 µl ddH_2O and kept at -20°C till use.

2.6 DNA quantification

DNA concentration and purity were determined using a spectrophotometer. DNA concentration was estimated using the conversion factor formula of 1.0 OD at 260nm is equivalent to 50 µg/ml of dsDNA. To assess DNA quality, the optical densities at four additional wavelengths, 230_{nm}, 270_{nm}, 280_{nm} and 320_{nm}, (Stulnig and Amberger, 1994), were recorded. Pure preparations of DNA have an OD_{260}/OD_{280} value near to 1.8. Nucleic acid preparations free of phenol should have $OD_{260}:OD_{270}$ ratios of ~1.2, significant absorption at 230 nm indicates contamination by phenolate ion, thiocyanates, and other organic compounds, whereas absorption at higher wavelengths (320_{nm} or higher) is usually caused by light scattering, indicates the presence of particulate matter, and used for background correction. To confirm the concentration and quality of DNA samples, 100ng from each sample was run in 1% agarose gel stained in ethidium bromide solution and compared visually with 1kb ladder standard under UV illumination.

2.7 Randomly amplified polymorphic DNA (RAPD) assay

RAPD was performed as described by (Williams *et al*., 1990). Five RAPD primers were selected and used for the analysis (Table 2) according to band clearness. Each 20µl RAPD amplification reaction consisted of 2µl of 10× PCR buffer, 1.6µl of 50mM $MgCl_2$, 1.6µl of 10µM of primer, 2.5µl of 2mM dNTPs, 25 ng template DNA, and 0.25µl of 5U Taq-DNA polymerase. The PCR was carried out with the initial cycle at 94°C for 2 min, 94°C for 30 s, 36°C for 30 s and 72°C for 1 min, and the final extension at 72°C for 5 min. PCR products of RAPD were separated on 1.5% agarose gel and visualized by staining with ethidium bromide on UV-transilluminator.

Table (2): A survey of genetic diversity measures generated by five RAPD primers in nine cucumber hybrids.

Primer	TNB	NPB	%P	PIC	Rp	DI
OPA-03	9	1	11.11	0.05	0.67	0.04
OPA-08	5	2	40.00	0.16	1.11	0.16
OPA-15	5	0	0.00	0.00	0.00	0.00
OPE-05	6	0	0.00	0.00	0.00	0.00
OPY-05	9	0	0.00	0.00	0.00	0.00
All	34	3	8.82	0.04	1.78	0.04

TNB: total number of bands, NPB: number of polymorphic bands, %P: percentage of polymorphism, PIC: polymorphism information content, Rp: primer resolving power and DI: diversity index.

2.8 Analysis of molecular marker data

A binary matrix was made of presence "1" and absence "0" of bands for RAPD. Only strong and clear bands were considered and were applied in the analysis. Some genetic diversity measures were estimated, including percentage of polymorphism, polymorphism information content (PIC) (Roldan-Ruiz *et al*., 2000), primer resolving power (Rp) (Prevost and Wilkinson, 1999) and diversity index (DI) (Magurran, 1988). In addition, cluster analysis was performed to assess the relationship among cucumber samples based on RAPD data and Jaccard's coefficient (Jaccard, 1908). Dendrogram based on unweighted pair group method with arithmetic mean (UPGMA) using NTsys software.

3. Results

Randomly amplified polymorphic DNA was used to assess the genetic diversity and relationship among 9 cucumber hybrids. Five RAPD primers generated a total number of 34 bands, out of them 3 bands were polymorphic with 8.82% polymorphism. The total number of bands generated by each primer ranged from 5 to 9 bands with an average of 6.8 band per primer. Additionally, only two primers gave polymorphism among cucumber samples i.e., OPA-03 and OPA-08. Some genetic diversity measures were estimated based on RAPD molecular data (Tables 3:7). In this regard, the polymorphism information content (PIC) ranged for 0.05 (OPA-03) to 0.16 (OPA-08) with an average of 0.04 for all primers. The primer resolving power was 0.67 for OPA-03 and 1.11 for OPA-08, with an overall value of 1.78 for RAPD primers. In addition, diversity index was almost the same as PIC, with 0.04 (OPA-03) and 0.16 (OPA-08) and an average of 0.04 for all RAPD primers.

Table (3): A binary matrix showed the presence '1' and absence '0' of RAPD bands in 9 cucumber hybrids, primer OPA-03.

Primer	Band	Masira	Safeer	Basha	Aden	Krestal	Wafeer	Saso	Zena	Rocket
OPA-03	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1
	7	1	1	1	0	0	1	1	1	0
	8	1	1	1	1	1	1	1	1	1
	9	1	1	1	1	1	1	1	1	1
	All	9	9	9	8	8	9	9	9	8

Table (4): A binary matrix showed the presence ‘1’ and absence ‘0’ of RAPD bands in 9 cucumber hybrids, primer OPA-08.

Primer	Band	Masira	Safeer	Basha	Aden	Krestal	Wafeer	Saso	Zena	Rocket
OPA-03	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1
	4	0	0	0	1	1	0	0	1	0
	5	1	1	1	1	1	1	1	0	0
	All	4	4	4	5	5	4	4	4	3

Table (5): A binary matrix showed the presence ‘1’ and absence ‘0’ of RAPD bands in 9 cucumber hybrids, primer OPA-15.

Primer	Band	Masira	Safeer	Basha	Aden	Krestal	Wafeer	Saso	Zena	Rocket
OPA-03	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1
	All	5	5	5	5	5	5	5	5	5

Table (6): A binary matrix showed the presence ‘1’ and absence ‘0’ of RAPD bands in 9 cucumber hybrids, primer OPE-05.

Primer	Band	Masira	Safeer	Basha	Aden	Krestal	Wafeer	Saso	Zena	Rocket
OPA-03	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1
	All	6	6	6	6	6	6	6	6	6

Table (7): A binary matrix showed the presence ‘1’ and absence ‘0’ of RAPD bands in 9 cucumber hybrids, primer OPY-05.

Primer	Band	Masira	Safeer	Basha	Aden	Krestal	Wafeer	Saso	Zena	Rocket
OPA-03	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	1	1	1	1
	8	1	1	1	1	1	1	1	1	1
	9	1	1	1	1	1	1	1	1	1
	All	9	9	9	9	9	9	9	9	9

The binary matrix obtained for RAPD profiles was used to perform cluster analysis based on Jaccard’s coefficient. The UPGMA-dendrogram illustrate the relationship among cucumber samples and placed them according to their genetic

similarities (Figure 1:5). The nine cucumber hybrids were clustered into four groups, the first group contained ‘Masira’, ‘Safeer’, ‘Basha’, ‘Wafer’ and ‘Saso’. While the second group gathered both ‘Aden’ and ‘Krestal’. In addition, ‘Zena’ was placed in a single branch near to the first and second groups with similarity value of 0.87. However, ‘Rocket’ was the most different and farthest sample to the other, which placed out of group in a single branch (Figure 6).

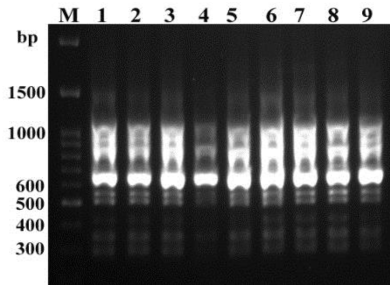

Figure (1): RAPD profile generated by OPA-03 among 9 cucumber hybrids.

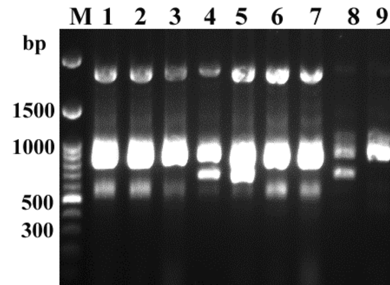

Figure (2): RAPD profile generated by OPA-08 among 9 cucumber hybrids.

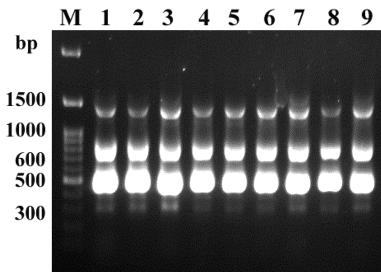

Figure (3): RAPD profile generated by OPA-15 among 9 cucumber hybrids.

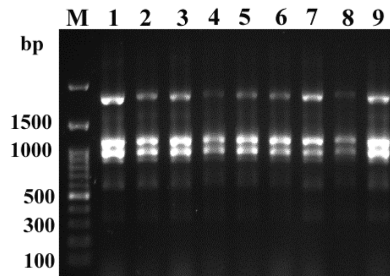

Figure (4): RAPD profile generated by OPE-05 among 9 cucumber hybrids.

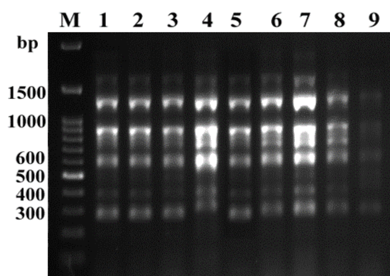

Figure (5): RAPD profile generated by OPY-05 among 9 cucumber hybrids.

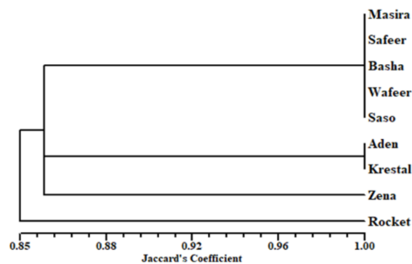

Figure (6): UPGMA-dendrogram based on RAPD molecular data and Jaccard’s coefficient, showing the genetic relationship among cucumber hybrids.

4. Discussion

Randomly amplified polymorphic DNA (RAPD) marker was used in the present study to assess the genetic diversity and relationship among nine cucumber hybrids. All primers were able to generate band profile successfully. Although only two primers were able to generate polymorphism among cucumber samples under study, a good relationship among samples was obtained molecularly. The relationship and diversity analysis performed in the present study could serve as a preliminary result, however number of molecular markers should be increased. There are several types of molecular markers being used in the assessment of genetic diversity. Among which, RAPD has abundant use with various plant and animal species. Although its low reliability, RAPD has a good advantage than other markers, that it is a universal nonspecific marker which its primers could bind and amplify any complementary region of the genome (Williams *et al*., 1990), which is recommended in evolutionary and diversity studies. RAPD has been used in genetic diversity assessment studies with many plant species (Singh *et al*., 2009; Youssef and Elazab 2015; Youssef *et al*., 2019), and proved to be an excellent tool to draw out variability and relationship among genotypes. Furthermore, RAPD has been used with cucumber to assess genetic diversity (Horejsi and Staub 1999; Someh *et al*., 2016; Yun *et al*., 2006), genetic stability of in vitro regenerated plants (Elmeer *et al*., 2009). In this regard, 118 cucumber accessions were analyzed using 71 RAPD loci (Horejsi and Staub, 1999). Their data indicate that RAPD markers have utility for analysis of genetic diversity and germplasm management in cucumber. Additionally, Genetic difference between cucumber inbred line and its hybrid progenies F1 using 30 RAPD primers (Yun *et al*., 2006). RAPD profiles showed a total of 71.37% polymorphism with a range of genetic distance among parents inbred lines from 0.123 to 0.164. They reported that, RAPD is appropriate tool to use for studying genetic diversities of cucumber, which can help in parental selection and molecular marker-assisted selection in breeding procedures. Furthermore, Someh *et al*. (2016) used 15 RAPD primer to assess the genetic diversity and relationship among 20 cucumber varieties. They found 73% polymorphism with 0.24 PIC value, in a context that this system could be used in selecting suitable parents in hybridization breeding programs in cucumber. On the other hand, RAPD was utilized to detect genomic instability in cucumber plants derived from somatic embryogenesis (Elmeer *et al*., 2009). The amplification products were monomorphic for all the plantlets of cucumber regenerated by embryogenesis. Thus, RAPD markers were useful in detection genetic instability – if any– in cucumber primary regenerant plants derived from somatic embryogenesis, and as a certification tool for monitoring genetic stability during the generation

process. The genetic diversity measures' values obtained in the present study were lower than those of the previous study. This might be due to the difference of cucumber genotypes analyzed and the type and number of RAPD primers. Therefore, it is recommended to increase the number of primers along with other molecular markers to figure out clear relationship among cucumber genotypes.

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