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Assessment of genetic variation in some *Cucumis sativus* L. genotypes in Iraq using RAPD markers

Nidhal Abdul Hussein Al–Badeiry
College of Education for Girls
Kufa University

Abstract

The current study was performed using the RARD markers in detect the Genetic relationship, Genetic diversity, and DNA fingerprint of ten categories of the cucumber genotypes *Cucumis sativus L*. Isolating Genomic DNA from cucumber's dry, young leaves, and an amount of DNA was extracted, between 172.6 - 448 Nano gram/microliter,with a purity ranging between 1.788-2.126. 10primers were chosen, which showed contrastive multiplying results among the studied categories. Those primers showed 105 different contrastive bands from the original 158 bands. Each selected primers produced between 52bands OPA-04 to 104 bands OPD-20 . The size of the amplified results was between 110 bp OPA-06 to 1911 bp OPA-06. The primer OPA-05 was given the highest value of the main bands, which were 24 band, and the lower value was given to the primers OPA-10,OPD-02 which were 12band. Higher value for polymorphism in primer OPA-04 While, lowest value for polymorphism in primer OPA-10. The primer OPA-10 was given the highest efficiency which was 0.56 while, Primer OPD-02 was given the lowest efficiency 0.093. Higher number for Monomorphic bands to 5 in primer OPD-20, while primers OPA-04 show no monomorphic bands . Primers gave OPA-01, OPA-03, OPA-05, OPA-10 highest number of unique bands, reached 4 bands while ,OPA-10 gave no unique bands.

Key words: Cucumber, RAPD markers, Genetic diversity

1. Introduction

Cucurbitaceae family contains about 90 genera and over 700 species of economic importance (Sikdar et al., 2010). As plants of this family produce unisexual flowers, cross pollination is a regular feature. Few studies have been reported on the genetic relationship of Cucurbitaceae (Helm and Hemleben, 1997). The evaluation of genetic relationships among the members would promote the efficient use of genetic variations in the breeding program (Saengprajak and Saensouk, 2012).

Biodiversity is a combination of genetic differences and environmental influences and is one of the components of a living society in order to maintain its stability and functioning. Whether it is at the individual or group level, biodiversity is a measure of the validity of biological systems and the basis for many biology studies (Weihua *et al*., 2011). The estimates of genetic diversity using new molecular tools, especially molecular markers, have proved useful for identifying existing heterogeneous groups, identifying new heterogeneous groups, and assigning embryos of unknown genetic origin to fixed heterogeneous groups (Pejic *et al*., 1998; Casa *et al*., 2005,2002).

DNA markers have many advantages compared to other genetic indicators. The most important of these advantages is that they show the heterogeneity that occurs on DNA directly. DNA is also known to be a stable genetic material that is not affected by the environment. (Staub *et al*., 2010; Pecchioni *et al*., 1996). DNA indicators are used in many different fields, such as genetic mapping, the identification of mutant genes associated with genetic diseases, and the tests of the father and epidemiology (Hartl and Jones, 2005).

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RAPD random amplified polymorphic DNA (RAPD) is a DNA-based polymerase chain reaction (PCR) technique, one of the most common molecular techniques for the development of DNA markers (Kumar and Gurusubramanian , 2011) known as the multiplication of specific DNA sites using random of ten nitrogen bases associated with their complement to the genome with the presence of DNA polymerase and the appropriate temperature to produce multiple multiplexes of different molecular weights (Williams *et al*.,1990). These markers were utilized in many other fields of sciences such as finding the DNA, creating the genetic map as well as the genetic relationship (Carlson *et al*.,1991) and in the studies and researches that tackles the evolution and genealogy (Iqbal *et al*., 1997). These indicators were applied to study the genetic diversity among the types of cucumber that is common in Iraq for the following reasons:

1. Estimating the genetic diversity of the cucumber which appears as a difference in number and size of the multiplying pieces.
2. Finding the DNA of the studied genetic patterns.

2. Materials and Methods

2.1 Collecting Plant Samples

Cucumber genotype seeds were selected from different origin and certified sources in the country, such as: (1) Rami, (2) Tender green, (3) Straight eight, (4) Bush champion, (5) National pickling, (6) Spacer master, (7) Poinsett 76, (8) Sumter, (9) Market more 76 and (10) Stimora. The seeds were planted in small pots, immersed in water, until the leaves grew in order to collect the DNA from them.

2.2 Isolating the DNA from the Plant Samples

The DNA from all study samples of cucumber's dry, young leaves were collected and isolated using the a specific protocol designed by Geneaid. This kit provides a rather easy and quick way to collect a pure DNA from the sample tissues. After that, Bio Drop was used in order to measure the concentration of DNA and estimating its clarity at 260 and 280 Nano Meter wavelength.

2.3 Primers

The primers of this study were prepared by Bioneer on the shape of Lyophilized, and were 10 primers to be precise. There were prepared using TE in order to get the final concentration (Original Solution) 100 picomole/ microliter. From this solution, the primer that is to be used in the polymerase chain reaction was prepared at a concentration 10 picomole/microliter. As illustrated in the below table.

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Table 1: Name of Primer and the Nucleotides sequence with the Source

Nucleotides Sequence (5'→ 3')	Name of primer	Nucleotides Sequence (5'→ 3')	Name of primer
GGTCCCTGAC	OPA-06	AATCGGGCTG	OPA-04
GGGTAACGCC	OPA-09	AGGGGTCTTG	OPA-05
Mahmoud and Abdel - Fatah, 2012			
AGTCAGCCAC	OPA-03	GTGATCGCAG	OPA-10
GGACCCAACC	OPD-02	GGTGCGGGAA	OPE-02
(AL-Badeiry, 2013 ,Wangsomnuk *et al*., 2011)			
ACCCGGTCAC	OPD-20	CAGGCCCTTC	OPA-01
(Srivastava *et al*., 2009)			

2.4 Polymerase Chain Reaction

IF the certified particle indicator was applied in this study RAPD using the PCR technique according to the following steps:

1. The work was done using gloves and in the sterilization chamber, while keeping all the solution on ice.
2. Adding 5 microliter from the DNA template, 4microliter from the primer to the prepared main reaction tube. The final size of the reaction is completed by adding distilled, de-ionized water in order for the solution to reach 20 microliter.
3. Then, it was placed in the Thermo-cycler on a specific program for each primers according to annealing temperatures as follows: (1) One cycle of 5 min at 94 C˚ , for 40 cycle of each 20 sec at 94 C˚ , 2 min at 40 C˚ , 1 min at 72C˚, with a final extension for one cycle of 10 min at 72 C˚.

After the completion of the reaction time, the tubes were removed from the Thermo-cycler, the agarose-made gel was put in a concentration of 1.2%, where the agarose slide is made by dissolving this material in a conducting solution and heating it until it becomes clear, then, the agarose is added in a separate bowl until it cools down, which creates a flexible gelatinous substance, usually used in the separation process for (2- 4) hours at 70 voltage. After that, the PCR products displayed the process on the gel for the ultra violet rays using the *Gel Documentation System* for photographs (Weigand *et al.*, 1993).

3. Results and Discussion

Isolating Genomic DNA from cucumber's.*Cucumis sativus L.* dry, young leaves, and an amount of DNA was extracted, between 172.6-448 Nano gram/microliter, with a purity ranging between 1.788- 2.126 Using the Bio Drop device, it was estimated that between 260

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http://www.kufabiojournal.org

and 280 wavelengths. The estimated molecular size of the DNA were 50-150 KB, and were determined using 0.9% of the agarose Gel and electric signals.

3.1 The Results of Multiplying DNA depending on RAPD

10 primers were tested showing varied multiplication results between the studied categories. These primers showed 105contrastive bands from the original 158 main bands. The highest number of amplified bands was 104 obtained through all genomes by the OPD-20 primer and the lowest number of the amplified bands 52by the OPA-04 primer (Figures 1 and 2). The highest number of main bands 24 was obtained by the OPA-05 primer and the lowest number of main bands 12 by the OPA-10,OPD-02 primers (Figures3 and 4). The difference in the number of main and amplified bands is mainly due to the primer structure and that some primers recognize a large number of link locations which are more useful than those recognizing a lower number of these locations, giving better chance of detecting DNA polymorphisms among individuals (Williams et al., 1990 and Tahir, 2010). The results suggest that the OPD-20 primer should be used in the future and other races for the two reasons (Table 2) above.

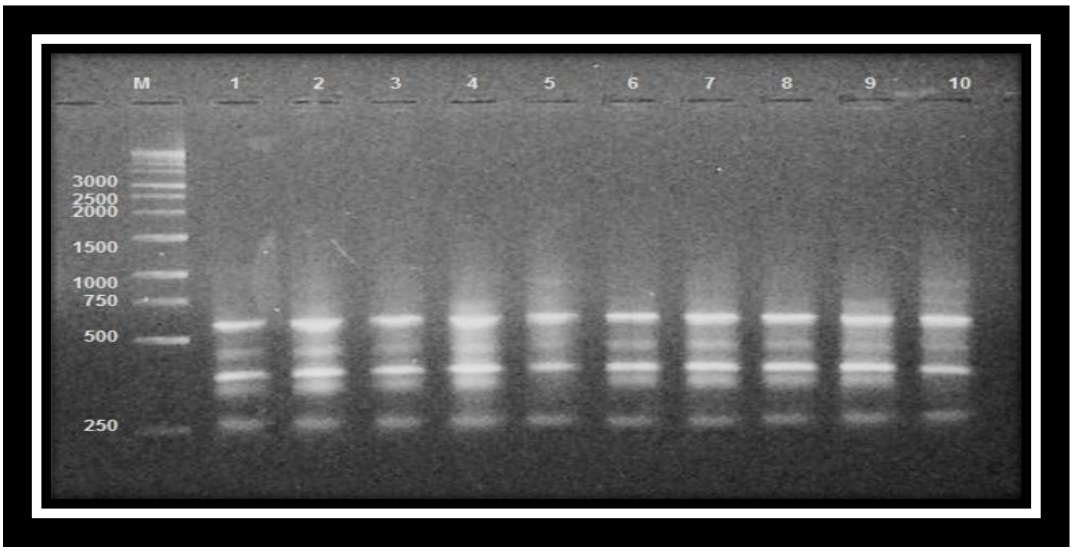

Figure 1: OPA-04 primer multiplication results on 1.5% agarose gel with standard of 30-ampere and 70-volt volumetric index for 10 varieties of cucumber.

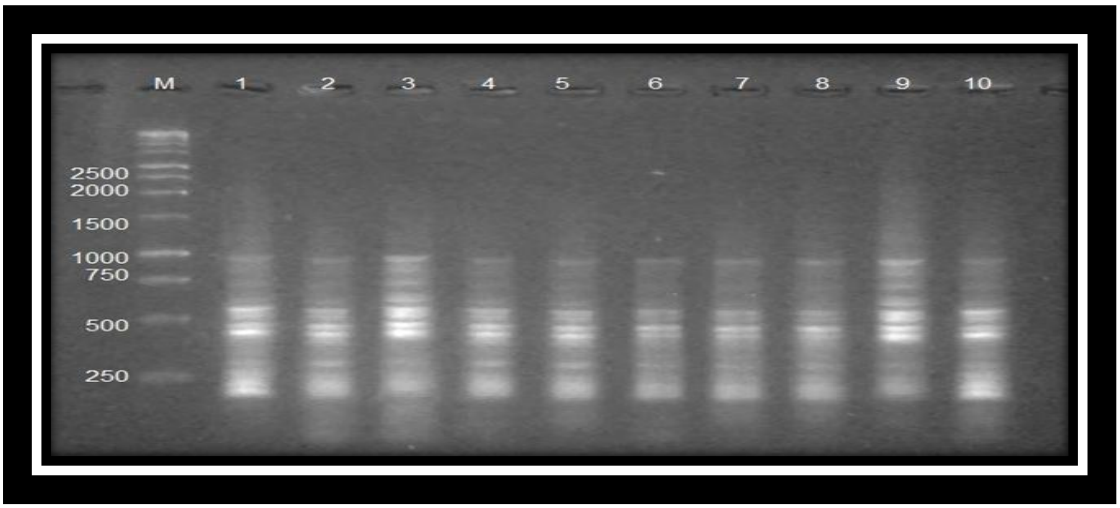

Figure 2: OPD-20 primer multiplication results on 1.5% agarose gel with standard of 30-ampere and 70-volt volumetric index for 10 varieties of cucumber.

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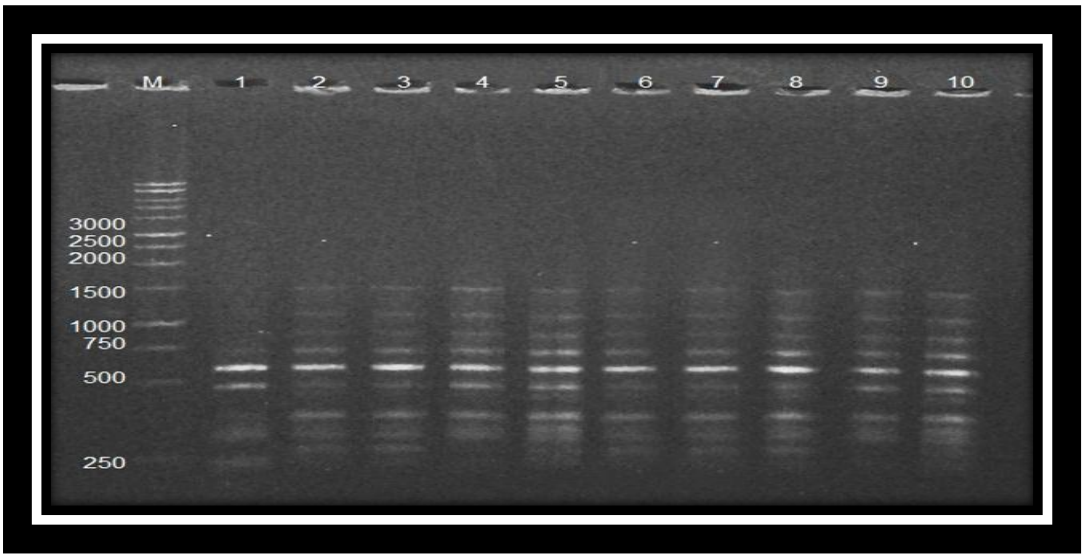

Figure 3: OPA-10 primer multiplication results on 1.5% agarose gel with standard of 30-ampere and 70-volt volumetric index for 10 varieties of cucumber.

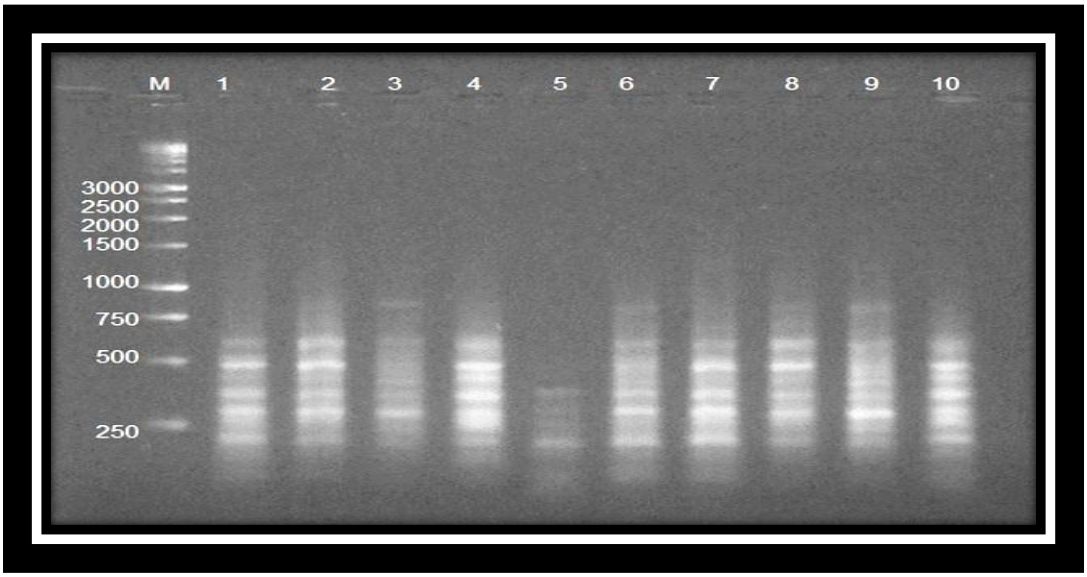

Figure 4: OPD-02 primer multiplication results on the 1.5% agarose gel with standard of 30-ampere and 70-volt volumetric guide for 10 varieties of cucumber.

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Table 2: The primer, the size of the pieces, the number of main bands, the number of amplified bands, the number of monomorphic bands, the number of polymorphic bands, uniqe bands, polymorphism, primer efficiency and discrimination value of each RAPD primer in this study.

No.	Primer	Fragment size range bp)(	No. of main bands	No. of amplified bands	No. of monomorphic bands	No. of polymorphic bands	No. of unique bands	Polymorphism%	Discri mi-natory value %	Primer efficiency
1	OPA-01	274-1736	18	79	3	11	4	61.1	3.971	0.139
2	OPA-03	221-1265	13	59	2	6	4	64.1	2.166	0.101
3	OPA-04	263-951	13	52	0	12	1	92.3	4.332	0.23
4	OPA--05	232-1258	24	95	2	18	4	75	6.498	0.189
5	OPA-06	110-1911	20	84	2	14	3	70	5.054	0.166
6	OPA-09	250-1178	15	56	1	12	2	80	4.332	0.214
7	OPA-10	223-1469	12	89	4	5	0	41.6	1.805	0.56
8	OPD-02	179-869	12	64	3	6	2	50	2.166	0.093
9	OPD-20	176-952	18	104	5	10	2	55.5	3.61	0.096
10	OPE-02	236-860	13	62	1	11	1	84.6	3.971	0.177
			158	744	23	105		67.42%		Total

The difference in the number of bands resulting from the use of each primer is different from the difference in the molecular weights of these bands, which depends on the number and locations of the sequence of primers on the DNA bar. The very light bands are ignored and this corresponds to (Barone et al., 1999; Swoboda and Bhalla, 1997) . In addition, the number of DNA binding sites depends on the type of genome and the extent to which the enzyme is defined on those sites (Caetano – Anolles, 1997). Either the variance based on the differences in the intensity of the bands. The bands shine as a result of the appearance of some bands amplified together in the same molecular weight and appear in the form of a single dense band is actually more than a band may be the result of homozygostity case where the same site is multiplied by the other allele and has the same Molecular weight So the amplified pieces are grouped together in those locations. The increase in the concentration of the template DNA leads to a repetition of the number of copies of the target DNA, which leads to the same multiplication of the site more than once. Since it is difficult to determine the precise concentration of DNA for several factors, the difference in the thickness of the resulting bands cannot be used as a measure of genetic variation. RAPD is one of the markers of complete sovereignty. Thus, it is not possible to estimate the number of alleles per site (AL-Hassani, 2002). This is consistent with what (Vogt *et al*., 1997). does not rely on the intensity of bands glazing as a measure of contrast due to the difficulty of controlling the precise concentration of DNA.

The existence of joint bands between some species only, without the rest of the varieties, can be invested by linking them to specific traits common to these varieties such as resistance to pathogens or inappropriate environment. In addition, the presence of common bands makes the RAPD markers more appropriate than the other DNAmarkers. (1996) Powell et al., when compared to RAPD with other markers such as SSR and RFLP. RAPD was found to be more accurate for studying the genetic relationship between the presence of co-beams between the studied species compared with SSR and RFLAP Which is usually characterized by the appearance of a band or tow band . Primer gave OPA-04 higher value for polymorphism

92.3%, while the lowest value for polymorphism in primer OPA-10 41.6% (Table 2) showed a more polymorphism than the RAPD markers may provide Better than genetic patterns (Demir et al., 2010). The most important fact to be taken into account is that variations in the level of polymorphism can be the result of distinct areas of the cucumber genome that have been evaluated by selected indicators or differences between the materials used (Sun *et al*., 2001). The number of primers required to identify selected varieties is dependent on discrimination power of each tested primers which means its ability to finding unique pattern for the studied varieties. Discrimination power of a primer will increased by increasing the number of specific varieties using the specific primers (Arif *et al*.,2010) It is useful to test several primers to identify the varieties obtained. Higher discriminatory in primer OPA-05 of6.498 while, primer OPA-10 gave the lowest discriminating capacity of 1.805.

Of the other important criteria to be analyzed, the efficiency of the primer and Discriminatory capacity determined by the materials and methods of all the primers tested in this study. It was found that the OPA-10 primer gave the highest calculated efficiency of 0.56 while, the OPD-02 primer gave the least efficiency Calculated as 0.093 (Table 2). The primer efficiency range may show the primer's ability to give a large proportion of the polymorphic bands according to a number of multiplication ranges. Thus, the primer efficiency is not the primer which gives the largest number of multiplied packs, but the ability to show differences between the studied species (Newton and Graham, 1997). In our current study and previous studies, the rate of variation was 66-67.4% (Srivastava *et al*., 2009). The same principle was true with other primers OPA-03, OPA-04, OPA-10 (Sarkar Roy *et al*., 2012) OPE-02 (AL-Badeiry, 2013) Others are certainly related to the germplasm used.

4. Conclusion

RAPD is a powerful and useful tool for clarifying relationships within species and also for providing useful genetic indicators to identify varieties in the cucumber and polymorphisms found among species that can be used in breeding programs to maximize the use of genetic resources.

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