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FINGERPRINTING AND DETECTING OF CLOSELY RELATED CUCUMBER (*CUCUMIS SATIVUS* L.) GENOTYPES USING APPLICATION OF ISSR MARKERS

Nidhal Abdul Hussein Al–Bdairi[1] and Balqees Hadi Al-Musawi[2]

[1]Department of Biology, College of Education for girls, Kufa University, Iraq.
[2]Department of Biology, College of Science, Kerbala University, Iraq.

Abstract

This research was conducted in molecular laboratory of college of education for girls-Kufa university. ISSR (Inter Simple Sequence Repeat) markers were used to evaluate the genetic relationships among six genotypes of cucumber (*Cucumis sativus* L.) cultivated in Iraq. This study was conducted for fingerprinting ten Iraqi cucumber genotypes using ten ISSR markers; products of ISSR markers amplification produced 67 polymorphic bands out of 106. The ISSR primers UBC-807, UBC-835 and UBC-872 success in giving a unique fingerprint for most studied genotypes. The results revealed that ISSR markers could be efficiently applied to quickly access for genetic variation available in the cucumber germplasm. The information on genetic similarity among genotypes will be useful for selecting the accessions to establish a germplasm bank of cucumber landraces and to develop breeding programs.

Key words: Cucumber, ISSR, Fingerprint, Genetic diversity.

Introduction

Cucumber (*Cucumis sativus* L.) considered is an significant vegetable crop grown and a widely cultivated plant in worldwide, which belongs to the gourd Cucurbitaceae family that consist of several crops of economic value, many medicinal and nutritional benefits (Gill and Bali, 2011). Cucumber is one of important and desirable economic crops in Iraq as its fruits are used as aperitif, consumed freshly or with salad or pickles, but is the fourth extreme useful vegetable crop existence sold in the world (FAO, 2016).

Cucumis genus consist of further than 55 species universally included 25 Asian and Australian and sacrifially 30 African species as detected by DNA sequences of plastid and nuclear markers, then have two species of cucumber (*Cucumis sativus*) and melon, muskmelon (*Cucumis melon*) that are economical significant vegetable crops in numerous regions particularly wherever the cucumber is most used fresh (Dhaliwal, 2017).

China, India, Russia and USA respectively regarded are the maximum producers of cucumber (Food and Drug Administration, 2016). Cucumber (*Cucumis sativus* L.) have a small chromosome complements with n = x = 7, 2n = 2x = 14 and a little haploid genome of 367 Mbp/C. Also it is a perfect model organism for experiences which cultivated for genetic studies, because of characteristic and it is various regulate of unisexual or bisexual flowering sex phenotypes (Nam, *et al.,* 2005).

Molecular markers considered a good tools applied to recognize genetic variation in several organisms (Irshad and Idrees, 2014). DNA fingerprinting and assessment of genetic relationships have become an significant tool to varietal identity in plant breeding programs (McGregor, 2000; Wünsch and Hormaza, 2002). ISSR is one of types of DNA markers that depend on the PCR technique and due to its efficiency to recognize individuals with major accuracy and regulator (Nadeem *et al.,* 2018).

ISSR have been used to successfully to assess variation in a vast range of plants, such as evaluate genetic variety in most plant species, included a Potato (Rocha *et al.,* 2010), Barley (Guasmi *et al.,* 2012), Walnut (Ahmed *et al.,* 2012), in Ocimum species (Chen *et al.,*

***Author for correspondence :** E-mail : nidhal.albdairi@uokufa.edu.iq, balqees.hadi@uokerbala.edu.iq

2013) Rice (Girma *et al.,* 2013) and *Cucurbita pepo* (Nontuthuko *et al.,* 2015).

ISSR was described as a powerful technique to assess genetic diversity and similarities between and within species. The aims of this study was evaluation of ISSR marker ability to assess genetic diversity between Iraqi cucumber genotypes in order to fingerprint and estimate genetic relationships between them. Also, results can generate invaluable basic reference or more efficient germplasm management and for origination utilized for cucumber a breeding program, such as genotypes identification, parental choice for developing heterotic hybrids or germplasm preservation.

Materials and Methods

Preparation of plant material and genomic DNA isolation

Plant material consisted of six genotypes of Cucumber (*Cucumis sativus* L.) genotype seeds were selected from different origin and certified sources in the country, such as: 1 (Rami - local), 2 (Tender green- USA), 3 (Straight eight- USA), 4 (Bush champion- USA), 5 (Spacer master - USA) and 6 (Stimora- USA).

Genome DNA Isolation

Table 1: Primers and their sequences used as ISSRs markers.

Primer Name	Sequence 5'-3'	Ann-ealing	Primer name	Sequence 5'-3'	Ann-ealing
UBC-807	$(AG)_8T$	$50C^0$	UBC-828	$(TG)_8A$	$50C^0$
UBC-808	$(AG)_8C$	$52C^0$	UBC-835	$(AG)_8YC$	$48C^0$
UBC-810	$(GA)_8T$	$53C^0$	UBC-848	$(CA)_8RG$	$52C^0$
UBC-816	$(CA)_8T$	$52C^0$	UBC-872	$(GATA)_4$	$48C^0$
UBC-817	$(CA)_8A$	$52C^0$	UBC-881	GGG (TG GGG)2TG	$50C^0$

DNA was extracted using Genomic DNA Mini Kit from the fresh studied cucumber genotypes leaves and DNA quantity, about 132 µg/ml with a purity ranged between 1.8–1.9 which measured by Nanodrop device.

ISSR Application

In this study, using ten ISSR markers with temperature detailed in table 1. PCR amplification conducted using Thermo cycler (Agilent technology sure cycler 8800) in PCR pre mix master mix containing (250 µM of each dNTP, 1Unit of *Taq* DNA polymerase and 1X reaction buffer, with 1.5mM $MgCl_2$). Then, it was placed in the Thermo-cycler on a specific program for each primers according to annealing temperatures of primer illustrated in details from table 1, According (Golabadi *et al.,* 2012). Electrophoresis done using 1.5% agarose gel at 70 Volts for 3 hours.

Statistical Analysis

The results of the amplification process gathered in a table depending on the Scoring data as 1, 0 for presence or absence of DNA bands in the studied samples, the number 1 indicates to the presence of clear DNA band, while the number 0 indicates absence of the band. Individually the tables were organized for each pimer and the dendrogram was drawn by applying UPGMA (Unweighted Pair Group Method with Arithmetic Averaging) utilize the Statistical PAST program, Version 62.1. (Hammer *et al.,* 2001).

Results and Discussion

Fingerprinting analysis

DNA fingerprinting with the objective of genotypes or varietal identification, has become an significant tool for genetic identification, germplasm management and recording systems (Wu *et al.,* 2010). Ten primers were tested showing varied multiplication results between the studied categories.

Table 2: Summary of primers amplification and discrimination value of each ISSR primer in this study.

No.	Primer name	No. of ampl-ified bands	No. of main bands	No. of Mono-morphic Bands	No. of Poly-morphic bands	Poly -morp hism (%)	Pri-mer effic-iency	Discr--imin--atory value(%)
1	UBC-807	57	14	4	10	71.43	0.175	14.925
2	UBC-808	44	10	3	7	70	0.159	10.448
3	UBC-810	50	8	4	4*	50	0.080	5.970*
4	UBC-816	32*	6*	2*	4*	66.67	0.125	5.970*
5	UBC-817	43	9	3	6	66.67	0.140	8.955
6	UBC-828	55	11	5	6	54.55	0.110	8.955
7	UBC-835	39	9	2*	7	77.78*	0.179	10.448
8	UBC-848	53	13	9*	4*	30.77*	0.075*	5.970*
9	UBC-872	63*	17*	4	13*	76.47	0.206*	19.403*
10	UBC-881	41	9	3	6	66.67	0.146	8.955
	Total	477	106	39	67			

These primers showed 477 amplified bands from the original 106 main bands and the high number of amplified bands was 63 obtained through all genomes by the UBC-872 primer while the lowest number of the amplified bands was 32 by the UBC-816 primer. Also the highest number of main bands 17 was obtained by the UBC-872 primer whereas, the lowest number of main bands 6 by the UBC-816 primers.

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, 2014). The results suggest that the UBC-872 primer should be used in the future and other races for the two reasons table 2.

In this study, the ISSR primers (UBC-807, UBC-835 and UBC-872) that showed specified fingerprints can be applied in among to screen lengthy spectrum of cucumber access. That alleles number are significant for they likely impress for specific areas with a genome specified to a special species of cucumber by ISSR markers, Fig. 1. Also, accession on particular DNA fingerprints will be of rise value into cucumber breeders cooperative in the improvement of the yield.

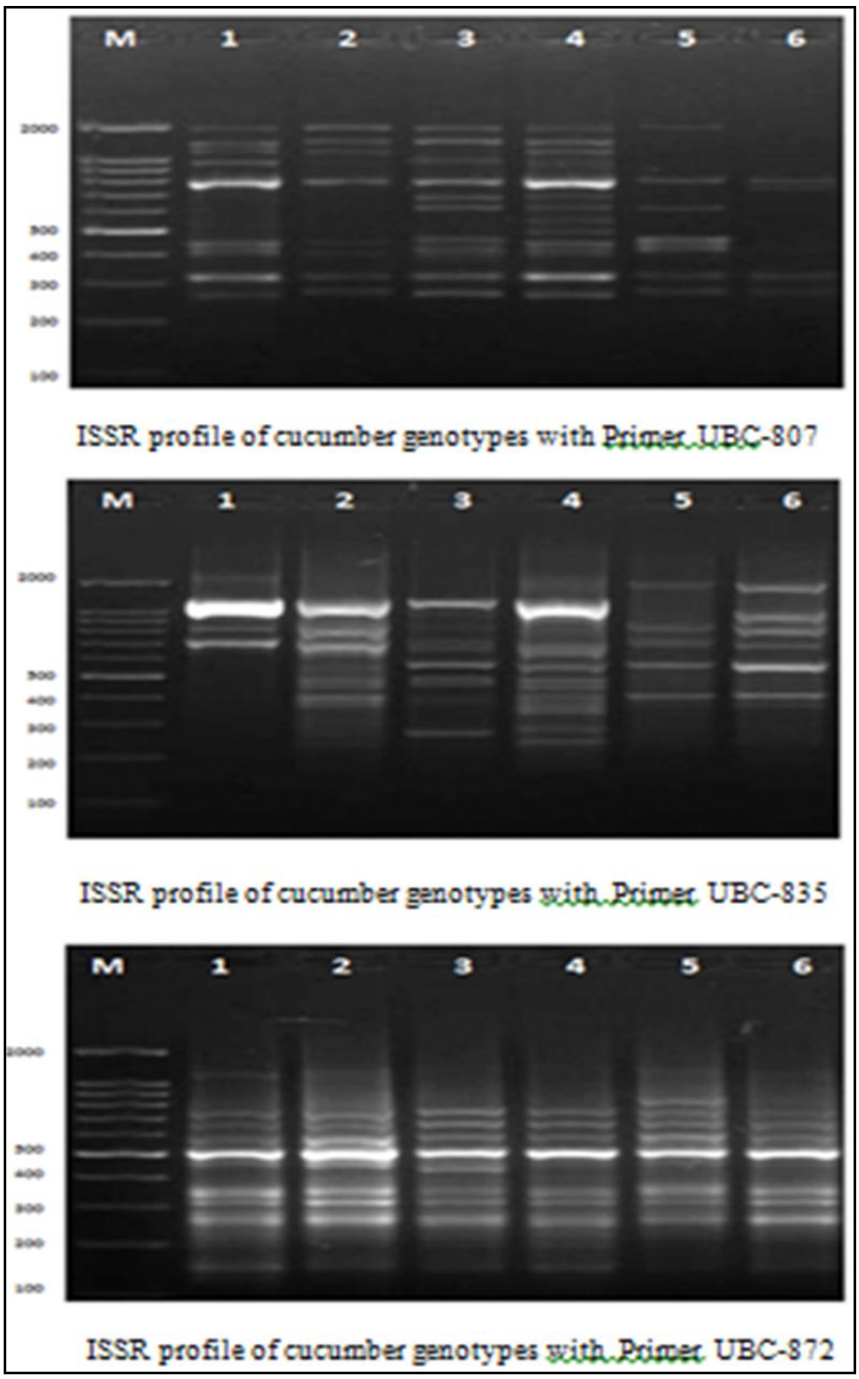

Fig. 1: ISSR amplification pattern generated on 6 cucumber genotypes lanes: 1 (Rami - local), 2 (Tender green- USA), 3 (Straight eight- USA), 4 (Bush champion- USA), 5 (Spacer master USA) and 6 (Stimora- USA). M = 100-2000 bp DNA ladder marker.

The primer UBC-835 gave higher value for polymorphism 77.78%, while the lowest value 30.77% for polymorphism in primer UBC-848 table 2, that showed a more polymorphism than the ISSR markers may provide better than genetic patterns (Demir *et al.,* 2010). The most significant fact to be possessed into account is that difference in the level of polymorphism can be the score of discrete areas of the cucumber genome that have been evaluated by select indicators or variance among the materials utilized (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 genotypes. Discrimination power of a primer will increased by increasing the number of specific genotypes using the specific primers (Arif *et al.,* 2010) It is useful to test several primers to identify the genotypes obtained. Higher discriminatory in primer UBC-872 of 19.403 while, primers UBC-810, UBC-816 and UBC-848 gave the lowest discriminating capacity of 5.970.

Also 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 UBC-872 primer gave the highest calculated efficiency of 0.206 while, the UBC-848 primer gave the least efficiency Calculated as 0.075 table 2.

The primer efficiency range may show the primer's ability to give a large

Table 3: The genetic distance values among 6 cucumber genotypes: 1) Rami - local(, 2) Tender green- USA), 3 (Straight eight- USA), 4 (Bush champion- USA), 5 (Spacer master - USA) and 6 (Stimora- USA).

Geno-types	1	2	3	4	5	6
1	0.00000					
2	0.48525	0.00000				
3	0.44778	0.34324	0.00000			
4	0.39086	0.28677	0.24255	0.00000		
5	0.56393	0.49794	0.40650	0.26674	0.00000	
6	0.58950	0.33564	0.39914	0.33472	0.33426	0.00000

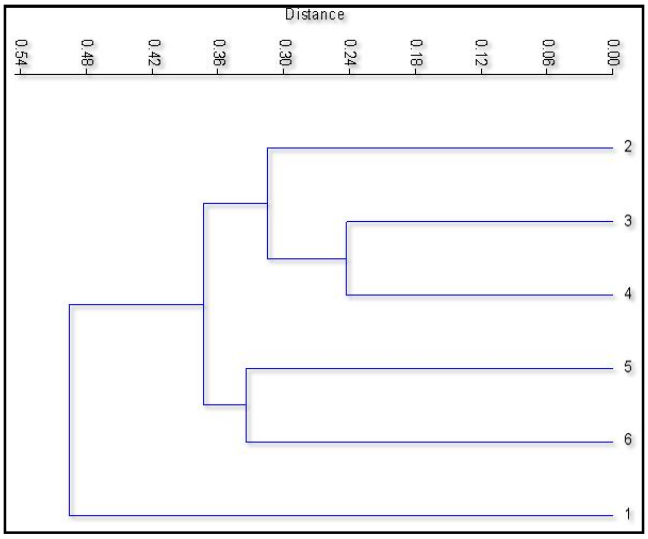

Fig. 2: UPGMA dendrogram of genetic relationship tree between cucumber genotypes: 1 (Rami - local), 2 (Tender green- USA), 3 (Straight eight- USA), 4 (Bush champion- USA), 5 (Spacer master - USA) and 6 (Stimora- USA). based on ISSR data.

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). Power discrimination was also used to reveal the fingerprinting of other plants genomes such as cucumber (Al-Jaf and AL-Jubouri, 2017) and Rice (Al-Musawi *et al.,* 2019). Discrimination power reflects genetics and is very important for plant breeders to see large variation for selection in future studies.

Relationship among cucumber genotypes using ISSR markers

Based on the estimated genetic similarity matrix table 3, Value of the highest genetic distance was 0.58950 observed between 1 (Rami - local) and 6 (Stimora- USA) genotypes (both originated from different locations. While the lowest genetic distance was 0.24255 observed between 3 (Straight eight- USA), 4 (Bush champion- USA) genotypes (originated from USA), these relations between genotypes which dependent to geographical origin and related to that they share a common parent or ancestor, genetic similarity, the presence of common ancestors or not might have influence the similarity or dissimilarity among the genotypes (Morales *et al.,* 2011).

This variation between genotypes in agronomic characteristic are perhaps due to the variance of climate types, ground sample and the position to another (Ezzat *et al.,* 2010). Also, this possibly due to the effect of varies ecological conditions on the phenotypic characteristic, essential to clear variation even between similar genotypes (Shehzad *et al.,* 2009). Also the domestic names cannot be looked perfect leader to the appearance of diversity (Chakauya *et al.,* 2006).

Results in Fig. 2 show that cucumber genotypes divided between two major cluster, the first small one included only 1 (Rami) (local from Najaf), the other large major cluster contained the rest five genotypes which further divided in to two sub clusters, the first small contained two genotype while the other large sub cluster contained three genotypes.

The introductions include cucumber from USA, Therefore, the high levels of genetic variability detected in the studied collection are justified by the multiple origin of the genotypes associated with cultivation in different localities for several years and they can be applied for pedigree analysis and to define phylogenetic relationships among genotypes (Senior *et al.,* 1998). Molecular markers are therefore, important tools for preservation and for management of the existing genetic variability.

In this study, ISSR primers as announced here so, collect helpful information for evaluating the genetic diversity among cucumber germplasm in Al-Najaf areas and for the use of cucumber landraces in Iraq in future breeding of new genotypes of cucumber that may be most tolerant to abiotic stresses, more productive and have better nutritive value contrast to the present landraces.

Conclusion

Molecular markers (ISSR) powerful and useful tool for clarifying relationships within species and also for providing useful genetic indicators to identify genotypes in the cucumber and polymorphisms found among species that can be used in breeding programs to maximize the use of genetic resources. Also that are a further reliable and careful method for diversity estimation.

The information acquired from this study possibly

helpful for then identification of favorable cucumber genotypes for realization the reach of genetic diversity current in cucumber genotypes.

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