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Genetic diversity studies in cucumber (*Cucumis sativus* L.) using RAPD markers

Susheel Sharma*, Suhasini Pradhan, Prakriti Khajuria and Aejaz Ahmad Dar

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Abstract

The present study was undertaken to assess genetic diversity in 19 genotypes of cucumber using 14 randomly amplified polymorphic DNA (RAPD) markers. About 125 amplicons in the range of 5 to 13 with the average of 8.93 bands per primer were produced with 100 per cent polymorphism. Polymorphic information content had maximum value of 0.44 with primer OPC02 and minimum of 0.17 with primer OPA02. The highest marker index value of 4.13 was obtained for primer OPC08 and lowest of 1.21 for primer OPA09. The highest value of resolving power of 6.53 was reported with primer OPC08 and lowest value of 1.47 with primer OPA09. Primers OPC02 and OPC08 were reported as best informative markers for overall estimation of genetic diversity of cucumber crop. Jaccard's similarity coefficient ranged from 0.09–0.80 with the maximum value of 0.80 between Sikkim Local and Pouni Local genotypes and minimum of 0.09 between AAUC-1 and Gyn-C, AAUC-1 and DWD, Akhnoor Local and Gyn-C, WI-2757 and Gyn-C, K75 and DWD. The study can be helpful for variety identification, development of heterotic combinations by exploiting the genotypes with low similarity values and molecular breeding for improvement of cucumber.

Keywords: RAPD, Diversity, cucumber, similarity index

Introduction

The *Cucumis* genus includes 30 wild and cultivated species that are spread throughout the world and has two major species: cucumber and melon. Cucumber has a small chromosome complement with n = x = 7 and a small haploid genome of 367 Mbp/C. Cucumber is an important vegetable crop having unique properties within its genome. Nevertheless cucumber has a narrow genetic base, with a genetic variability of only 3–8% (Dijkhuizen et al. 1996, Meglic et al. 1996, and Horejsi and Staub 1999). The study of genetic diversity is thus necessary for efficient utilization, conservation and management of germplasm collections in narrow genetic base crops like cucumber. Molecular marker systems are an important tool for the characterization and utilization of germplasm banks (Tanksley and McCouch, 1997). Molecular markers based on DNA sequence polymorphism such as RAPD have proved their potential to measure genetic diversity with good coverage of entire genome and thereby have been used in number of vegetable crops including cucumber for diversity analysis studies (Staub et al. 1997). These RAPD markers generate huge extent of genetic polymorphism, leading to evaluation of phenotypic variability are suitable for genetic analysis as, only small amount of DNA is required and are fast and simple. They have also been used extensively for variety identification, determination of genetic variability, relationship among the crop genotypes and construction of linkage maps (Jaroslava et al. 2002). The present study was aimed to find out genetic diversity among 19 cucumber genotypes (local and gynoecious lines and varieties of cucumber) using 14 RAPD markers.

Materials and Methods

Isolation and Quantification of Genomic DNA: Nineteen genotypes of cucumber were used in the present study for diversity analysis (Table 1). Seeds of these genotypes were grown in cups for 3-4 weeks and leaf tissue at 3-4 leaf stage of seedlings was used for genomic DNA isolation using CTAB method (Doyle and Doyle, 1990). The isolated genomic DNA samples were quantified using microvolume spectrophotometer (MySpec; www.sigma-svi.com) and were also visualized under UV light after electrophoresis on 0.8% agarose gel. DNA samples were further diluted using sterilized Milli Q water, to a working concentration of 50ng/µl.

PCR Amplification: A set of 14 arbitrary random 10-mer primers were selected for use in amplification of genomic DNA based on earlier studies. Primers were diluted to a concentration of 5 pmol using sterilized Milli

School of Biotechnology, SKUAST-J, Jammu- 180 009, J&K
*Corresponding author, Email: drsusheelsharma@rediffmail.com

Table 1: Similarity index of nineteen *Cucumis* genotypes as obtained by Jaccard's coefficient

	AAU C-1	HP Local	Gyn-A	Gyn-B	Gyn-C	DWD	K 75	K 90	Long Green	Sel 75-2-10	Sikkim Local	Rajouri Local	Pouni Local	Akhnoor Local	Reasi Local	Pusa Uday	Pusa Barkha	Gy-14
HP Local	0.32																	
Gyn-A	0.14	0.26																
Gyn-B	0.36	0.76	0.24															
Gyn-C	0.09	0.18	0.50	0.20														
DWD	0.09	0.10	0.07	0.14	0.07													
K 75	0.25	0.56	0.17	0.52	0.20	0.09												
K 90	0.26	0.50	0.20	0.53	0.18	0.11	0.59											
Long Green	0.16	0.34	0.28	0.39	0.24	0.15	0.46	0.39										
Sel-75-2-10	0.24	0.48	0.17	0.51	0.19	0.13	0.52	0.53	0.58									
Sikim Local	0.29	0.54	0.17	0.57	0.17	0.15	0.49	0.57	0.50	0.70								
Rajouri Local	0.27	0.46	0.16	0.49	0.16	0.13	0.47	0.48	0.46	0.78	0.68							
Pouni Local	0.30	0.48	0.18	0.48	0.16	0.17	0.43	0.50	0.45	0.67	0.80	0.69						
Akhnoor Local	0.30	0.47	0.21	0.44	0.09	0.12	0.41	0.45	0.39	0.57	0.63	0.58	0.71					
Reasi Local	0.25	0.40	0.20	0.43	0.16	0.14	0.30	0.30	0.35	0.45	0.51	0.49	0.60	0.57				
Pusa Uday	0.28	0.37	0.18	0.40	0.16	0.17	0.27	0.38	0.33	0.48	0.54	0.49	0.57	0.47	0.57			
Pusa Barkha	0.27	0.42	0.21	0.43	0.13	0.18	0.29	0.36	0.39	0.53	0.55	0.51	0.58	0.55	0.61	0.73		
Gy-14	0.30	0.42	0.16	0.45	0.11	0.11	0.35	0.33	0.37	0.48	0.46	0.41	0.51	0.50	0.51	0.54	0.58	
WI-2757	0.33	0.38	0.17	0.38	0.09	0.11	0.27	0.27	0.31	0.33	0.34	0.28	0.33	0.36	0.36	0.33	0.44	0.60

Q water. DNA amplification was carried out in 0.2 ml PCR tubes. For each primer, 10 µl amplification reactions contained 1X Taq DNA polymerase buffer (with MgCl2), 3µl of template DNA (50ng/µl), 0.2 mM dNTPs, 0.6 pM primer and 2.5 units of Taq DNA polymerase. Amplifications were carried out in Peq lab thermocycler. The amplification thermal profile consisted of 5 min initial denaturation step at 94p C followed by denaturation at 94p C for 1 minute. Annealing of primers was done at 37p C for 1 min followed by an extension period of 1 min at 72p C. The reactions were subjected to 42 cycles after reaching the final annealing temperature. This was followed by final extension at 72p C for 7 min. The amplified PCR products were resolved finally by electrophoresis on 1.5% agarose gel.

Statistical analysis: The banding patterns of all genotypes against each primer were compared. Amplified bands were manually scored for band presence (1) or absence (0) for each genotype and binomial data matrix was generated which was further used for calculating total number of bands, number of polymorphic bands, and monomorphic bands for each primer. In order to check the informativeness and discriminatory power of RAPD primers, different parameters like polymorphism percentage, polymorphic information content (PIC), marker index (MI) and resolving power (Rp) were calculated. The PIC was calculated as $1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2\ p_j^2\right]$ where *pi* is the frequency of the i^{th} allele (Botstein et al. 1980). The MI was obtained as the product of PIC and EMR (Varshney et al. 2007). EMR was obtained by multiplying the proportion of polymorphic markers (â) and the total number of polymorphic bands (Powell et al. 1996). Band informativeness (Ib) was calculated by 1 " (2 x |0.5 " *p*|) where *p* is the proportion of the total genotypes containing a particular band. Resolving power (Rp) estimates the ability of a primer to distinguish various genotypes and calculated as "Ib (Prevost and Wilkinson 1999). The binary data was used to compute pair wise similarity coefficient (Jaccard 1908). The similarity matrix thus obtained was subjected to cluster analysis using the UPGMA (Unweighted Pair Group Method with Arithmetic average) algorithm using NTSYSpc version 2.2.

Results and Discussion

The DNA profile of cucumber genotypes in present study showing prominent polymorphism is presented in Fig. 1. The total number of amplified products obtained were 125 in the range of 5 to 13 at the mean value of 8.93 bands per primer with primers OPA11, OPC08, OPE01 giving the maximum (13) and primers OPA09, OPC02, OPD18 giving the minimum (5) number of amplicons. The molecular size of amplicons ranged from 150 (OPA09) to 2500 bp (OPC08) with 100% polymorphism (Table 2). The mean value of bands per primer was found higher than previous reported study in 41 genotypes of Spanish cucumber and 17 genotypes of Indian cucumber (Bernet et al. 2003 and Choudhary et al. 2011). The polymorphism information content value is often used to measure the informativeness of a genetic marker for linkage studies. The highest PIC value of 0.44 was found with primer

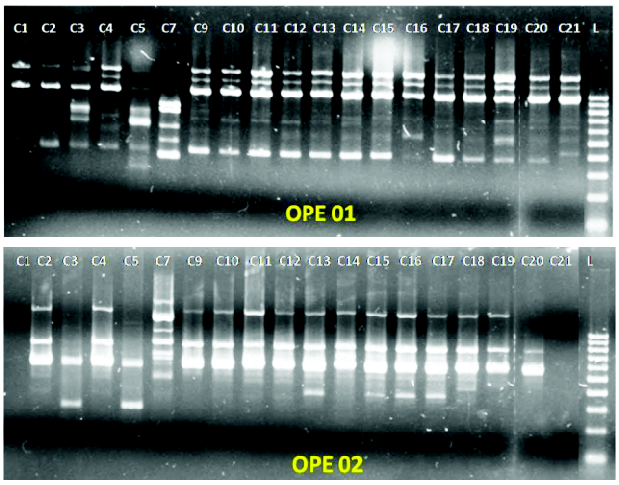

Fig. 1: DNA profile of cucumber genotypes revealed by RAPD primers OPE 01 and OPE 02 (C1–C21 are cucumber genotypes while as L is 100 bp ladder).

OPC02 and the lowest value of 0.17 with primer OPA02, with an average value of 0.28 per primer. The PIC obtained by using RAPD markers with cucumber germplasm was lower than reported earlier by using SSR markers (Hu et al. 2010). Marker index (MI) for each RAPD primer was calculated to determine the general usefulness of the system of markers used. The highest MI was reported for primer OPC08 (4.13) and lowest for primer OPA09 (1.21), with mean value of 2.42 per primer. Resolving power (Rp) was calculated for determining discriminatory potential of primers. The highest Rp value was observed with primer OPC08

Table 2: Marker attributes calculated for each RAPD primer used

Primer	Sequence (5'—3')	NB	NPB	PIC	MI	Rp
OPA02	TGCCGAGCTG	8	8	0.17	1.37	1.58
OPA03	AGTCAGCCAC	10	10	0.3	3	4.32
OPA05	AGGGGTCTTG	6	6	0.24	1.42	1.89
OPA07	GAAACGGGTG	7	7	0.31	2.19	3.05
OPA09	GGGTAACGCC	5	5	0.24	1.21	1.47
OPA10	GTGATCGCAG	12	12	0.22	2.61	3.37
OPA11	CAATCGCCGT	13	13	0.29	3.73	5.37
OPC02	GTGAGGCGTC	5	5	0.44	2.18	3.47
OPC08	TGGACCGGTG	13	13	0.32	4.13	6.53
OPE01	CCCAAGGTCC	13	13	0.24	3.08	3.89
OPE02	GGTGCGGGAA	12	12	0.21	2.54	3.05
OPE03	CCAGATGCAC	9	9	0.2	1.81	2.53
OPD07	TTGGCACGGG	7	7	0.36	2.54	3.58
OPD18	GAGAGCCAAC	5	5	0.43	2.13	3.47
Total		125	125	-	-	-
Mean		8.93	8.93	0.28	2.42	3.40
Minimum		5.00	5.00	0.17	1.21	1.47
Maximum		13.00	13.00	0.44	4.13	6.53

NB: total no. of bands, NPB: number of polymorphic bands, PIC: polymorphism information content, MI: marker index, Rp: Resolving power.

(6.53) and the lowest with the primer OPA09 (1.47), with an average of 3.39 per primer (Table 2). Thus among 14 primers used in the present study, OPC02 and OPC08 were reported as best markers in terms of polymorphic information content, marker index and discriminatory potential.

Genetic distance and similarity estimates obtained in the present study led to a more and clear understanding of genetic relationships. The binary data was used to calculate Jaccard's similarity coefficient (SJ) and in turn for plotting dendrogram. SJ ranged from 0.09–0.80 with an average value of 0.45 and had the maximum similarity coefficient of 0.80 between Sikkim local and Pouni local genotypes and minimum of 0.09 between AAUC-1 and Gyn-C, AAUC-1 and DWD, Akhnoor local and Gyn-C, WI-2757 and Gyn-C, K75 and DWD. However, Choudhary et al., 2011 reported higher Jaccard's similarity coefficient that ranged from 0.39 to 0.94 with a mean of 0.67 in Indian cucumber by using 18 RAPD markers. The dendrogram revealed cluster analysis into two major groups A and B. Genotype DWD was found solitary in group B, while as group A was splitted further into A1 and A2. The A1 cluster contained majority of genotypes (16) and A2 contained only two genotypes namely, Gyn-A and Gyn-C (Fig. 2 and Table 1). The grouping of maximum genotypes in one cluster indicates a narrow genetic base in cucumber. The genetic erosion

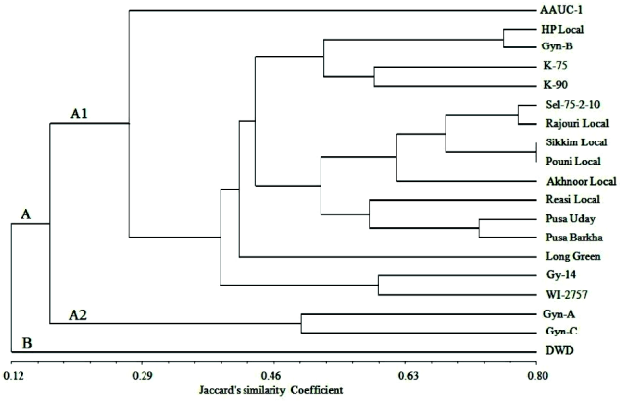

Fig. 2: UPGMA based dendrogram of cucumber genotypes based on RAPD data

of cucumber germplasm in India was also reported earlier (Staub et al. 1997; Pandey et al. 2013). Therefore efforts are required mainly to conserve highly variable genotypes in order to minimize the genetic erosion. Thus there is a need of using varied collection of cucumber accessions that will broaden the genetic base and can improve the breeding program.

सारांश

खीरा के 49 प्रभेदों की अनुवांशिक विविधता का मूल्यांकन 14 रैण्डोमली एम्पीलीफाइड पालीमार्फिक डी.एन.ए. (आर.ए.पी.डी.) के

माध्यम से किया गया। लगभग 125 एम्पलीकान का विस्तार 5–13 तथा प्रति प्राइमर 8.93 बैन्ड्स उत्पन्न हुए जिनमें शत–प्रतिशत बहुतरूपता स्पष्ट हुई। बहुरूपता सूचना में सबसे अधिक मूल्य 0.44 प्राइमर ओ. पी.सी.ओ.–2 तथा सबसे कम मूल्य 0.17 प्राइमर ओ.पी. ए.–2 से प्राप्त हुआ। खीरा की फसल में कुल अनुवांशिक विविधता ज्ञात करने हेतु प्राइमर ओ.पी.सी.ओ.–2 एवं ओ.पी.सी.ओ.–8 उत्तम सूचक मार्कर पाया गया। जैकार्ड समान गुणांक विस्तार 0.097 से 0.80 था तथा अधिकतम मूल्य सिक्किम लोकल व पौनी लोकल एवं निम्नतम प्रभेद ए.ए.यू.सी.–1 व जिन–सी, ए.ए.यू.सी.–1 व डी.डब्ल्यू. डी., अखनूर लोकल तथा जिन–सी, डब्ल्यू.आई.–2757 व जिन–सी, के.–75 व डी.डब्ल्यू.डी. में पायी गयी। खीरा में किस्म, पहचान, कम समान मूल्य वाली प्रभेदों का उपयोग कर ओज संयोजन संकरण तथा अण्विक प्रजनन हेतु उपयोग किया जा सकता है।

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