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Assessment of genetic diversity in cucumber varieties using RAPD markers

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Abstract

Cucumber (*Cucumis sativus* L.) is one of the most economically important plants in many countries of the world. The identification of cultivars is extremely important both for cultivation and breeding of crop plants. There is little known about the genetic relationships between cucumber genotypes in Iran. The genetic diversity and the relationships among 20 cucumber varieties were evaluated by RAPD markers. A total of 155 bands were generated with 15 RAPD primers, out of which 114 bands were polymorphic (73 %). The mean polymorphism index content (PIC) was 0.24. Considerable levels of polymorphism were observed within cucumber varieties. Cluster analysis based on Jaccard similarity coefficients grouped varieties into three main clusters. The data obtained from this study can be used to select suitable parents in hybridization breeding programs in cucumber.

Key words: Cucumber, genetic diversity, cluster analysis, RAPD markers.

Introduction

Cucumber (*Cucumis sativus* L.) is an edible fruit belonging to the family *Cucurbitaceae*. The genus C*ucumis* contains more than 30 species, such as cucumber, which is an economically important crop and grown widely around the world (Huang *et al.,* 2009). The identification of cultivars is extremely important in both cultivation and breeding of crops. Cultivar identification based on morphological characteristics can be difficult and complicated (Latha and Makari, 2011). Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between cultivars (Burstin *et al.,* 2001; Rosetto *et al.,* 2002; Bakht *et al.,* 2011). Polymerase chain reaction (PCR) technologies, such as random amplified polymorphic DNA (RAPD) analysis, can readily and quickly identify cultivars using seeds and young leaves (Latha and Makari, 2011). Various types of molecular markers have been used to characterize cucumber (Innak *et al.,* 2013). Genetic diversity among 26 cucumber accessions from five African countries Algeria, Egypt, Ethiopia, Kenya and Libya were examined by assessing variation at 71 polymorphic RAPD loci (Mliki *et al.,* 2003). Lang *et al.* (2007) studied the genetic diversity among 14 cucumber cultivars by RAPD. Sensoy *et al.* (2007) studied the genetic relationships among 56 genotypes of melons (*Cucumis melo* L.) from Turkey using RAPD markers. Latha (2012) studied DNA polymorphism among local varieties of cucumber using RAPD in India and reported 10 to 86% polymorphism among them. Pandey *et al.* (2013) assessed the genetic variation among 44 cucumber accessions by using morphological and SSR markers in India. They reported high genetic variability at morphological level but low molecular diversity observed among cucumber accessions. In another study, RAPD markers have been used for the cultivar identification and assessing genetic diversity in Pakistani watermelon (Mujaju *et al.,* 2010) and *Cucumis* spp. (Lang *et al.,* 2007). There is little known about the genetic relationships between cucumber genotypes in Iran. Therefore, this study aimed to investigate the molecular diversity and genetic relationships between cucumber varieties using RAPD markers. The results obtained from this study could provide the information for cucumber breeders to select the appropriate parents in cucumber breeding program for the generation of new cucumber cultivars.

Materials and Methods

Plant materials and DNA extraction: Twenty cucumber varieties consisted of 13 monoecious (designed as ASM, PLA, MAX, BAR, ASG, HUM, HUP, SUPS, BOS, SUPD, SIM, VIC and SUPA), 4 gynoecious (SOL, ROY, NEG and CLO) and 3 landrace varieties (LOCS, LOCR and LOCG) were used for the study of genetic diversity. Genomic DNA from the fresh young leaves of 14 day old seedlings was extracted according to modified CTAB method (Hwang and Kim, 2000). About 0.5 g of fresh young leaves was powdered by using mortar and pestle containing liquid nitrogen. The frozen leaf powder was transferred to tube containing 0.6 mL of extraction buffer [2% CTAB, 100 mM Tris-HCL, 20 mM EDTA, 1.4 M NaCl, 1% PVP-40, pH 8.0] containing 1% of β-mercaptoethanol added just before use. The extract was incubated for 40 min at 60 °C with occasional swirling, mixed with an equal volume of chloroform: isoamyl alcohol (24:1, V/V) and centrifuged at 12,000 rpm for 10 min at 4 °C. The aqueous phase was transferred to a new tube and mixed with 2/3 volume of ice-cold isopropanol. The mixture was left at -20 °C for 30 min and again centrifuged at 12,000 rpm for 10 min at 4 °C. The pellet was washed with 70% ethanol, air dried at room temperature for 20 min and finally, the dried pellet dissolved in 80 µL TE buffer and stored at -20 °C.

DNA Amplification and Gel Electrophoresis: Fifteen RAPD primers (Operon technologies, USA) were used in this study (Table 1). Each reaction mixture (25 µL) for PCR amplification

Table 1. RAPD primers used in diversity assessment of twenty cucumber varieties

Primer	Total number of bands	Number of polymorphic bands	Polymorphism percent	Fragment length (bp)	PIC
OPT-01	14	11	78	400-2000	0.24
OPT-05	14	13	92	600-2000	0.27
OPD-20	15	9	60	480-3000	0.21
OPG-14	12	9	75	400-2500	0.24
OPA-06	4	4	100	750-1100	0.24
OPA-11	6	6	100	350-1800	0.44
OPA-12	8	4	50	700-1700	0.22
OPA-17	8	8	100	1100-3000	0.29
OPH-03	15	6	40	350-2500	0.13
OPH-06	11	11	100	1200-3000	0.30
OPH-09	7	5	71	650-2200	0.27
OPH-16	8	4	50	400-2000	0.12
OPJ-06	10	4	40	300-1200	0.22
OPK-14	12	9	75	600-1500	0.22
OPE-04	11	11	100	450-2000	0.33
Total	155	114			

consisted of 1X reaction buffer, 1.5 mM $MgCl_2$, 1 mM dNTPs, 1.0 mM of primer, 0.3 unit of *Taq* DNA polymerase, and 4 ng genomic DNA template. The PCR amplification conditions were as follows: denaturtion at 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 32 °C for 2 min and 72 °C for 7 min and the final extension of 72 °C for 4 min. Amplified products were resolved in 1.5% agarose gel with 1X TBE buffer and stained with ethidium bromide. A 100bp DNA ladder was used as a molecular standard. The gels were stained with ethidium bromide and photographed under UV light.

Data analysis: Data were scored for the presence or absence. Bands were scored '1' for its presence and `0' for its absence. These data were then utilized to generate genetic similarity matrix among varieties. Cucumber cultivars were grouped according to Jaccard's similarity coefficients based on cluster analysis via the un-weighted pair group method with arithmetic mean (UPGMA) method with NTSYS version 2.1 (Rohlf, 1998).

Results and discussion

The genetic diversity and the relationships among cucumber varieties were evaluated by means of RAPD markers using 15 primers. The number of bands scored per primer ranged from 4 (OPA-06) to 15 (OPD-20 and OPH-03), with a mean of 10.33. Overall, the polymorphic fragment number varied between 4 (OPA-06, OPH-16, OPA-12 and OPJ-06) to 13 (OPT-05) with a mean of 7.6. The PIC values for the 15 primers ranged from 0.12 to 0.44, with an average of 0.21 (Table 1). The maximum amount of PIC was belonged to primer OPA-11 (0.44) and the minimum amount of PIC was belonged to primer OPH-16 (0.12) (Table 1). Primers OPE-4, OPA-6, OPA-17, OPA-11 and OPH-06 recorded the highest polymorphism (100%) and OPJ-06 was the least (40%). From total of 15 screened primers 155 bands with high intensity were scored and 114 (73%) of them were polymorphic suggesting high genetic diversity among these tested varieties. RAPD patterns using primers OPG-14 and OPD-20 are presented in (Fig. 1). The molecular weight of the generated bands in the present study ranged from 300-3000bp.

The genetic similarity matrix based on Jaccard's similarity coefficients is presented in Table 2. The results of similarity matrix showed that studied varieties had similarity ranging from 0.56 to 0.88. The highest genetic similarity (0.88) existed between the varieties PLA and ASG and the lowest genetic similarity (0.56) was observed between the varieties of MAX with CLO and LOCG with HUP. Also genetic similarity of 0.59 was observed between varieties of LOCR with BOS, LOCG with VIC and CLO with SIM. Onto *et al*. (2008) using 12 cucumber genotypes through RAPD markers reported the similar values for similarity (0.67 to 0.93).

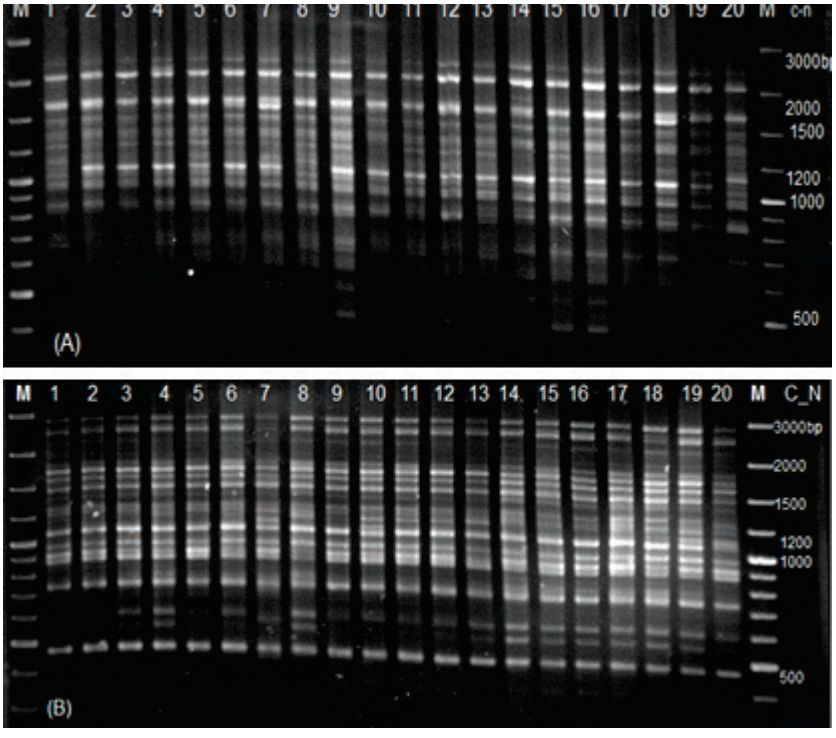

Fig. 1. RAPD patterns obtained using primers OPG-14(A) and OPD-20 (B) for cucumber varieties.1: ASM, 2: PLA, 3: MAX, 4: BAR, 5: ASG, 6: HUM, 7: HUP, 8: SUPS, 9: BOS, 10: SUPD, 11: SIM, 12: VIC, 13: SUPA, 14: SOL, 15: ROY, 16: NEG, 17: CLO, 18: LOCS, 19: LOCR and 20: LOCG.

Cluster analysis based on similarity matrix coefficients using UPGMA grouped 20 varieties into 3 main clusters (Fig. 2). Cophenetic correlation between ultrametric similarities of tree and similarity matrix was high ($r = 0.71$, $P < 0.01$), suggesting that the cluster analysis strongly represents the similarity matrix. The largest cluster (cluster A) consisted of two sub clusters, A_1 including ASM, PLA, ASG, MAX, BAR and SUPA. In this cluster the varieties PLA and ASG are similar to each other, indicating a high genetic similarity (0.88) among these genotypes. This could be due to a common parent. The sub cluster A_2 consisted of HUM, VIC, BOS, SUPD and SIM. Group B consisted of HUP, SOL, ROY, NEG and SUPS and the maximum similarity was 0.84 which was seen between ROY and NEG. Local varieties LOCS, LOCR and LOCG were clustered with CLO in group C. Within this cluster, two varieties LOCS and LOCR showed genetic similarity of 0.76. Similar study was done by Innark *et al.* (2013) who examined diversity of 38 cucumber genotypes. They grouped them into three main clusters using eight

Table 2. Genetic similarity matrix of 20 cucumber varieties using RAPD markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1																			
2	0.83	1																		
3	0.80	0.74	1																	
4	0.74	0.74	0.84	1																
5	0.84	0.88	0.76	0.80	1															
6	0.79	0.78	0.79	0.81	0.82	1														
7	0.72	0.75	0.71	0.76	0.77	0.81	1													
8	0.69	0.69	0.68	0.68	0.71	0.72	0.69	1												
9	0.74	0.78	0.73	0.75	0.8	0.81	0.76	0.67	1											
10	0.71	0.71	0.72	0.74	0.76	0.79	0.72	0.69	0.82	1										
11	0.74	0.72	0.74	0.72	0.78	0.79	0.68	0.64	0.76	0.78	1									
12	0.74	0.76	0.71	0.73	0.79	0.81	0.76	0.73	0.81	0.78	0.78	1								
13	0.71	0.73	0.72	0.73	0.78	0.75	0.7	0.65	0.74	0.69	0.71	0.78	1							
14	0.73	0.73	0.69	0.76	0.79	0.77	0.78	0.66	0.78	0.74	0.72	0.77	0.78	1						
15	0.70	0.70	0.70	0.70	0.72	0.72	0.72	0.70	0.68	0.72	0.69	0.69	0.65	0.74	1					
16	0.76	0.73	0.73	0.72	0.77	0.79	0.73	0.69	0.75	0.76	0.76	0.76	0.73	0.83	0.84	1				
17	0.62	0.68	0.56	0.64	0.69	0.67	0.69	0.66	0.68	0.65	0.59	0.72	0.73	0.78	0.65	0.73	1			
18	0.68	0.71	0.63	0.68	0.73	0.73	0.75	0.62	0.73	0.7	0.65	0.72	0.68	0.72	0.73	0.76	0.76	1		
19	0.7	0.71	0.71	0.64	0.68	0.74	0.69	0.69	0.59	0.65	0.64	0.68	0.67	0.76	0.67	0.74	0.73	0.76	1	
20	0.63	0.65	0.64	0.63	0.63	0.62	0.56	0.60	0.60	0.60	0.60	0.59	0.65	0.6	0.62	0.63	0.64	0.62	0.66	1

1: ASM, 2: PLA, 3: MAX, 4: BAR, 5: ASG, 6: HUM, 7: HUP, 8: SUPS, 9: BOS, 10: SUPD, 11: SIM, 12: VIC, 13: SUPA, 14: SOL, 15: ROY, 16: NEG, 17: CLO, 18: LOCS, 19: LOCR and 20: LOCG.

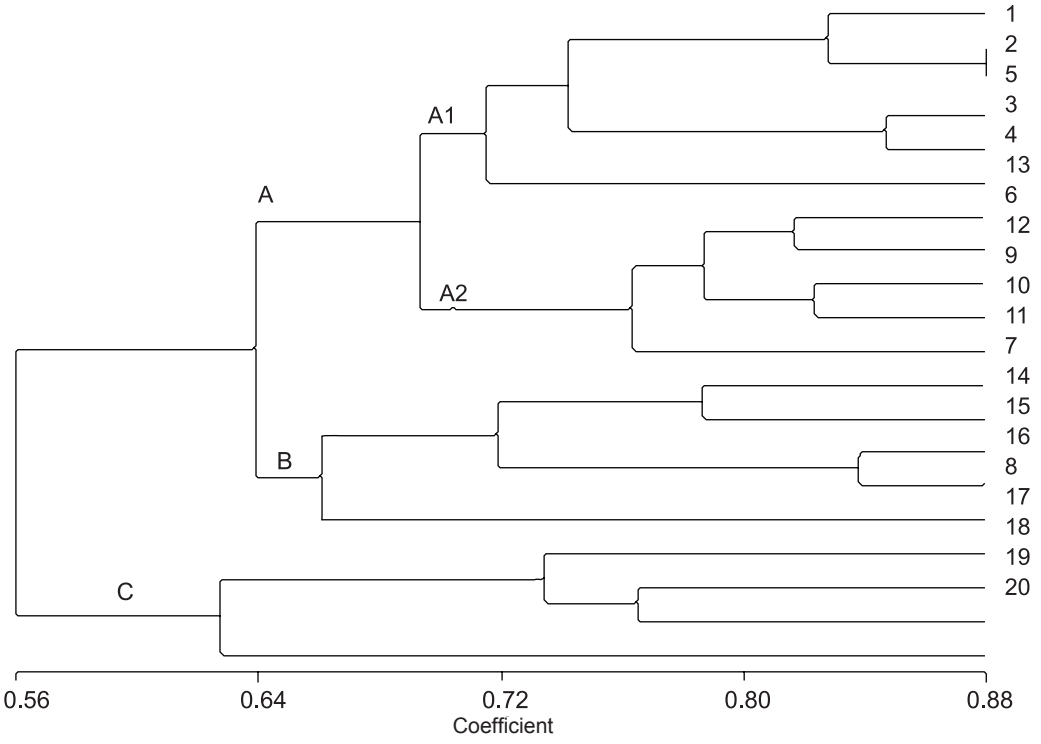

Fig. 2. Cluster analysis of 20 cucumber varieties using RAPD data. 1: ASM, 2: PLA, 3: MAX, 4: BAR, 5: ASG, 6: HUM, 7: HUP, 8: SUPS, 9: BOS, 10: SUPD, 11: SIM, 12: VIC, 13: SUPA, 14: SOL, 15: ROY, 16: NEG, 17:

agro-economic traits and twenty SSR markers. Parvathaneni *et al.* (2011) using 13 *Cucumis* genotypes grouped them into six clusters through ISSR markers. While, low molecular diversity among the Indian cucumber accessions was reported by Pandey *et al.* (2013) based on SSR markers.

According to the results, cucumber varieties were separated into 3 clusters. The results obtained from this study could be helpful in management of germplasm collections as well as providing the information for breeders to select the suitable parents in hybridization breeding programs. However, it must

be remembered that RAPD or other molecular techniques alone are not sufficient for determining genetic diversity. Thus morphological diversity of the cucumber genotypes also needs to investigated along with molecular studies.

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