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Genetic diversity among cultivated and wild germplasm of cucumber based on RAPD analysis

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ABSTRACT

Seventeen germplasm of cucumber including gynoecious parthenocarpic lines, standard monoecious varieties and wild progenitor *C. satiuvs* var. *hardwickii* accessions were classified into five different groups on the basis of RAPD marker showing enough genetic diversity among them. The lowest similarity value based on Jaccard's coefficient was estimated between gynoeciopus partehenocarpic line PCUCP 3 and standard monoecious genotype PCUC 8 (0.39) which indicated that these two were the most divergent. All four gynoeciopus parthenocarpic fruited genotypes were grouped together and formed a separate cluster implicating higher similarity among them. Two genotypes, namely Kashmir Local and PCUC 28 from diverse geographical regions were grouped in a common cluster II. Four accessions belonging to *C. sativus* var. *hardwickii* have been grouped together in cluster IV. However, genotypes from both *Cucumis sativus* and *C. sativus* var. *hardwickii* were included in a common cluster III. A single genotype IC 277054 of *C. sativus* var. *hardwickii* was alone in cluster V, which was from Sholapur, Maharashtra while all other accessions of *C. satiuvs* var. *hardwickii* were from north western Himalayan region.

Key words: *C. sativus* var. *hardwickii,* parthenocarpic, gynoecious, DNA marker.

INTRODUCTION

Wild relatives of crop species are a rich source of valuable traits, from which we have realized only a small fraction of the potential gains to crop improvement. The diversity of the wild relatives has enabled them to survive longer than the oldest cultivated variety without any human assistance. Thus, wild germplasm act as sources of resistance to stresses and they offer a treasure of genes for crop improvement programmes. Cucumber is one of the important cucurbitaceous vegetable grown throughout the world. Fruit yield in cucumber is suppressed due to its unique fruiting habit as fruit developing from the first pollinated flower inhibit the development of subsequent fruits. Incorporation of quantitatively inherited characters into commercially adapted cultivars from exotic/wild germplasm can be an effective way to obtain greater variation and response to selection. Wild relative of cucumber, *Cucumis sativus* var. *hardwickii* is found in the natural habitats of Himalayan foothills of India, which characterizes with higher number of lateral branches and sequential fruiting habit and is considered useful for improving fruit yield.

Genetic diversity between individuals or populations can be determined using morphological or molecular markers. Phenotypic traits have certain limitations as they are influenced by environmental factors and the plant developmental stage. Molecular markers based on DNA sequence polymorphism are more informative and independent of environmental conditions. The advent of molecular markers such as RAPD have proved their potential to measure such genetic variations with good coverage of entire genome and thereby have been used for diversity analysis in a vast array of vegetable crops including cucumber Staub *et al*. (4). These PCR-based markers reveal variations at DNA level, those variations that are obtained from the huge extent of genetic polymorphism generated by these markers, leading to evaluation of phenotypic variability. RAPD analysis plays a key role in studying the DNA profile and thereby detecting the extent of polymorphism within species (Staub *et al*., 4). This marker system is being extensively used for many practical applications such as DNA fingerprinting, cultivar identification, genotype validation and estimation of genetic variability. The present study was aimed for characterization of 17 genotypes of *Cucumis* spp. including gynoecious parthenocarpic genotypes, standard monoecious varieties and wild germplasm of *C. sativus* var. *hardwickii* at molecular level using RAPD marker.

MATERIALS AND METHODS

Seventeen genotypes of *Cucumis sativus* L. including eight accessions of *Cucumis sativus* var. *hardwickii* were used in this study and described in Table 1. The genomic DNA was isolated by CTAB method from a bulk of 4-week-old seedling leaf tissues taken from five plants from each genotype.

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Table 1. Description of genotypes included in genetic diversity analysis.

S. No.	Genotype	Botanical variety	Description
1.	PCUCP 2	*Cucumis sativus* var. *sativus*	Gynoecious & parthenocarpic
2.	PCUCP 3	*Cucumis sativus* var. *sativus*	Gynoecious and parthenocarpic
3.	PCUCP 1	*Cucumis sativus* var. *sativus*	Gynoecious and parthenocarpic
4.	PCUCP 4	*Cucumis sativus* var. *sativus*	Gynoecious and parthenocarpic
5.	Kashmir Local	*Cucumis sativus* var. *sativus*	Monoecious
6.	PCUC 28	*Cucumis sativus* var. *sativus*	Monoecious
7.	PCUC 15	*Cucumis sativus* var. *sativus*	Monoecious
8.	PCPGR 2317	*C. sativus* var. *hardwickii*	Monoecious
9.	PCPGR 2314	*C. sativus* var. *hardwickii*	Monoecious
10.	PCPGR 2315	*C. sativus* var. *hardwickii*	Monoecious
11.	Poinsette	*Cucumis sativus* var. *sativus*	Monoecious
12.	PCUC 8	*Cucumis sativus* var. *sativus*	Monoecious
13.	IC 277054	*C. sativus* var. *hardwickii*	Monoecious
14.	IC 202060	*C. sativus* var. *hardwickii*	Monoecious
15.	PCPGR 2316	*C. sativus* var. *hardwickii*	Monoecious
16.	IC 331620	*C. sativus* var. *hardwickii*	Monoecious
17.	IC 331626	*C. sativus* var. *hardwickii*	Monoecious

The polymerase chain reaction (PCR) amplification procedure was optimized by determining the most appropriate concentration of template DNA (50 ng), *Taq*DNA polymerase (1U) and Mg^{++} ion (2.5 mM) required to generate repeatable PCR amplification profiles. The random decamer primers suitable for generation of polymorphic amplification profiles among the genotypes of two botanical varieties of *C. sativus* vars. *sativus* and *hardwickii* were identified by screening of 90 primers. However, only 18 primers were selected as these primers generated polymorphic, consistent and reproducible patterns. The polymerase chain reaction (PCR) was carried out in 0.2 ml tubes using Bio Rad Thermal cycler with 10-mer screened random primers. The amplification consisted of a 5 min. initial denaturation step at 94°C followed by denaturation at 94°C for 1 min. Annealing of the primer was done at 38°C for 1.30 min. followed by an extension period for 2 min. at 72°C. The reactions were subsequently subjected to 40 additional cycles after reaching the final annealing temperature. This was followed by a final extension at 72°C for 7 min.

The amplification products were run in 1.2 per cent agrose gel electrophoresis. Each amplification product was considered as a DNA maker. These were scored across all 17 genotypes manually. Bands were recorded as present (1) or absent (0) across the lanes. Very thin or faint bands were not considered for final scoring as these were inconsistent. The frequency of RAPD polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands. The binary data were 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 NTSYS-PC.

RESULTS AND DISCUSSION

The total number of RAPD bands generated by 18 random decamer primers for 17 genotypes were 126 with an average of 7 bands per primer. The amplification products with primer LC 118 (300-2000 bp), and LC 91(250-1600 bp) are depicted in Figs. 1 & 2 respectively. The total number of RAPD loci (Table 2) ranged from 4 (LC 171) to 10 (LC 111). The highest number of polymorphic bands were observed in LC 111 (9) followed by LC 91 (6), LC 118 (6), LC 90 (6) and LC 126 (6). The polymorphism (per cent) varied from 40 (LC 138) to 90 (LC 111) with an average of 71.42 per cent polymorphism. Out of total 18 random primers, ten primers were able to generate more than 70 per cent polymorphism. The similarity value based on Jaccard's coefficient ranged from 0.39 (PCUCP 3 and PCUC 8) to 0.94 (PCUCP 2 and PCUCP 3), which clearly demonstrated that gynoecious parthenocarpic genotype PCUCP3 was most divergent from standard monoecious genotype PCUC8 and two gynoecious

Table 2. Polymorphism shown by RAPD analysis in cucumber germplasm.

S. No.	Primer	Total No. of RAPD loci	Polymorphic loci	
			Number	Per cent
1.	LC 81	8	5	62.50
2.	LC 88	7	5	71.40
3.	LC 90	8	6	75.00
4.	LC 91	7	6	85.71
5.	LC 102	9	5	55.55
6.	LC 111	10	9	90.00
7.	LC 112	9	6	66.67
8.	LC 118	7	6	85.71
9.	LC 119	7	5	71.42
10.	LC 122	5	3	60.00
11.	LC 126	8	6	75.00
12.	LC 127	6	4	66.67
13.	LC 128	8	5	62.50
14.	LC 134	6	5	83.33
15.	LC 137	7	5	71.42
16.	LC 138	5	2	40.00
17.	LC 142	5	4	80.00
18.	LC 171	4	3	75.00
	Total	126	90	71.42

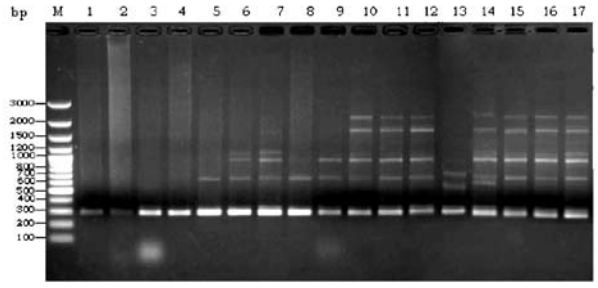


Fig. 1. RAPD profile of *Cucumis sativus* L. genotype obtained with primer LC 118.

parthenocarpic genotypes PCUCP 2 and PCUCP 3 were highly similar to each other as these two have been developed from same population. The lowest similarity value between four female lines of *C. sativus* used in Line x Tester design of another experiment (data not presented) was observed between PCUC 28 and PCUC 8 (0.53) followed by PCUC 28 and Poinsette (0.55), and PCUC 15 and PCUC 8 (0.63) and the highest similarity value was observed between Poinsette and PCUC 8 (0.79). It indicated that genotypes PCUC 28 and PCUC 8 were the most divergent parents out of

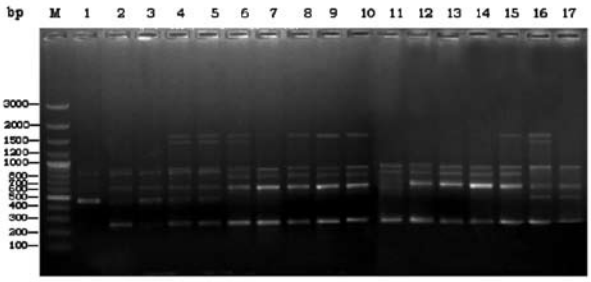


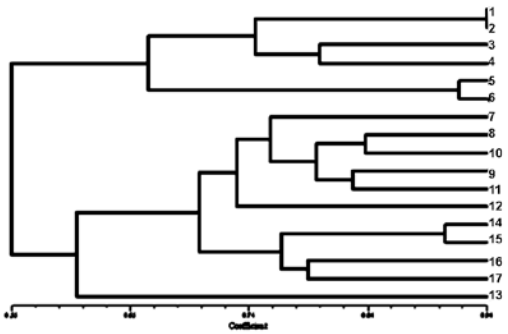
Fig. 2. RAPD profile of 17 genotype of *Cucumis sativus* L. obtained with primer LC 91.

four cucumber genotypes utilized as testers for line × tester analysis. It will be important to mention that these two parents have already been utilized in hybrid breeding programme and their cross combination (PCUC 28 × PCUC 8) have been released as a F_1 hybrid of cucumber named as Pant Sankar Kheera 1 from Central Varietal Release Committee. High amount of genetic diversity is reflected among female cucumber genotypes used as testers in line × tester analysis.

Among 8 accessions of *C. sativus* var. *hardwickii*, the similarity value ranged from 0.55 (P_6 and P_7) to 0.90 (P_3 and P_5), which exhibited large amount of genetic diversity among them. Moreover, it reflected higher genetic diversity among accessions of *C. sativus* var. *hardwickii* than *C. sativus* var. *sativus.* Higher polymorphism level in *C. sativus* var. *hardwickii* (17-25%) than *C. sativus* var. *sativus* has been reported by Horejsi *et al*. (3). High variation in *hardwickii* lines based on RAPD marker was also reported by Bisht *et al.* (1).

Most of the commercial cultivars grown in India are monoecious and they are not suitable for polyhouse production. Recently, four gynoecious and parthenocarpic genotypes of cucumber (PCUCP 1, PCUCP 2, PCUCP 3 and PCUCP 4) have been developed at G.B. Pant University of Agriculture and Technology, Pantnagar for the first time in India and these four genotypes have also been included for molecular characterization and diversity analysis. The similarity value among parthenocarpic gynoecious lines have been observed between 0.72 (PCUCP 3 and PCUCP 4) and 0.94 (PCUCP 2 and PCUCP 3), which indicated more similarity among them which is evident as all were developed from same population.

The UPGMA (un-weighted pair group method with arithmetic mean) analysis was done and dendrogram was constructed using Jaccard's similarity matrix of RAPD markers involving data generated out of 18 polymorphic primers on 17 genotypes of *C. sativus* vars. *sativus* and *hardwickii* and was depicted in Figure 3. The seventeen genotypes were grouped into five



1. PCUCP 2, 2. PCUCP 3, 3. PCUCP 1, 4. PCUCP 4, 5. Kashmir Local, 6. PCUC 28, 7. PCUC 15, 8. PCPGR 2317 , 9. PCPGR 2314 , 10. PCPGR 2315 , 11. Poinsette, 12. PCUC 8, 13. IC 277054 , 14. IC 202060 , 15. PCPGR 2316 , 16. IC 331620 , 17. IC 331626

Fig. 3. UPGMA dendrogram for 17 *Cucumis sativus* L. genotypes.

different clusters (Table 3) showing enough genetic diversity among them. The first cluster consisted of 4 genotypes and all of them were gynoecious and parthenocarpic in nature and were developed from the same population. Cluster II included two genotypes Kashmir Local and PCUC 28, which were from different geographical regions. Cluster III included three cucumber genotypes and three lines of *C. sativus* var. *hardwickii*. Four different accessions of *C. sativus* var. *hardwickii* have been grouped together in cluster IV, while a single genotype IC 277054 of *C. sativus* var. *hardwickii* was alone in cluster V, which was from Solapur, Maharashtra, while all other accession of *C. sativus* var. *hardwickii* were from north western Himalayan region (Uttarakhand and Himachal Pradesh).

Table 3. Classification of 17 cucumber genotypes based on RAPD analysis.

Cluster	No. of genotype(s)	Genotype(s)
I	4	PCUCP 1, PCUCP 2, PCUCP 3 and PCUCP 4
II	2	Kashmir Local and PCUC 28
III		PCUC 8, PCUC 15, Poinsette, PCPGR 2314 (P_1), PCPGR 2315 (P_2) and PCPGR 2317 (P_4)
IV	4	PCPGR 2316 (P_3), IC 202060 (P_5), IC 331620 (P_7) and IC 331626 (P_8)
V	1	IC 277054 (P_6)

Cultivated cucumber has a narrow genetic base and the *C. sativus* var. *hardwickii* revealed higher genetic variation both in morphological and RAPD analysis.

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