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Genetic Diversity Cucumber Using Inter Simple Sequence Repeats (ISSR)

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Abstract

Cucumber is an important vegetable crop grown worldwide. Genetic diversity and similarity among 11 cucumber genotypes viz. PCUCP-2, PCUCP-3, Nun-3139, Nun-3121, Nun-3141, Infinity, Isatis and Kian and PCUC-8, PCUC-15 and PCUC-28 were grown under greenhouse conditions in G. B. Pant University of Agriculture and Technology, Pantnagar. Fresh and young leaves were collected from each genotype. The leaves were cut into small bits and ground to powder using liquid nitrogen. Genomic DNA was then isolated using modified CTAB method. The genetic diversity and similarity was estimated by using 8 ISSR markers. The 6 ISSR primers generated a total of 49 polymorphic alleles. On the basis of ISSR data, dendrogram clustered the genotypes into two major clusters and five sub-clusters. Among all the genotypes a total of 40-100% polymorphism was observed. The number of alleles produced by different primers ranged from 5 to 14 with an average of 9.5 alleles per primer and the level of polymorphism was observed 88.88%. The similarity value ranged from 35% to 96%. Three monocious genotypes viz. PCUC-8, PCUC-15 and PCUC-28 (Pant Khira-1) were grouped in one cluster with 96% similarity, whereas parthenocarpic genotypes in another cluster. Therefore it is concluded that characterization and determination of diversity among parthenocarpic and monocious cucumber is done, this information can be utilized for formulation of efficient breeding programme of cucumber.

Keywords: Cucumber; *Cucumis* spp; Genetic diversity; ISSR markers; Morphological

Introduction

Cucumber (*Cucumis sativus* L., 2n=2x=14) is one of the most important member of the family *cucurbitaceae* including several crops of economic importance. It is thought to be one of the oldest vegetable crops, being grown for at least five thousand years. It is the fourth most important vegetable crop after tomato, cabbage and onion in Asia. About 30 species of cucumber are distributed across the southeast Himalayas with basic chromosome number 7 and across Africa with basic chromosome number 12. Cucumber is a unique crop and many important features of cultivated crops are not associated with discrete mendelian traits, but are of a continuous or quantitative nature. Genetic diversity between individuals can be determined using 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 Choudhary et al., [1]. Along with the traditional method of quantifying genetic diversity DNA markers are becoming more popular and effective to study since it rely on genetic materials which are not influenced by environment. In cucumber, molecular genetics technology will be highly desirable tool to support breeding, since cucumber cultivation generally requires more time, labour, space and equipment. Genetic markers have been successfully used to select morphological characteristics Fan et al., [2]. Efforts have led to the development of genomic SSR markers. The Inter Single Sequence Repeat i.e. ISSR Danin Poleg et al., [3] is a PCR based DNA diagnostic assay involving PCR amplification of genomic DNA segments followed by Agarose Gel Electrophoresis. The advent of ISSR marker has proved their potential to measure genetic variations with good coverage of entire genome. This marker reveals a much larger number of polymorphic fragments per primer and also do not require prior knowledge of DNA sequence for primer design. Success of hybrid as well OPV development programme is based on the availability of potential diverse germplasm. The degree of heterotic effect of F_1 population is correlated with genetic divergence of parental lines, as parents are more divergent, the heterosis is higher and vice-versa Prasad and Singh [4]. Parthenocarpy has long been known to occur within the species of *Cucumis sativus* L. Sturtevan [5]. Parthenocarpy be regarded as the ability to develop fruits without pollination. Fruits with developing seeds inhibit the growth of later fruits: however, to a lesser extent if fruits are grown parthenocarpically Strong [6], Denna [7]. In general, the average yield of field bred cucumber is 400 q/ha under polyhouse condition as compared to 80 q/ha under open field condition. However, in comparison parthenocarpic cucumber scales a yield of 1800 q/ha under the protected cultivation. Singh and Padiyar [8]. The development of genetic markers associated with cucumber has a narrow genetic base Staub et al., [9] which limits development of new cucumber cultivars. To date, the degree of genetic diversity in cucumber has been assessed with a number of DNA markers Zhuang et al., [10] which provide useful information for cucumber cross breeding. Molecular markers such as RAPD and SSRs have been employed for determination of genetic diversity in African cucumber (*Cucumis sativus* L.) Ahmad Mliki et al., [11]. There are many approaches used to quantify the diversity at intra as well as interspecies level, however, molecular markers are considered to have enormous potential to explore genetic diversity by detecting polymorphisms at DNA level. They are useful tools for breeding, genotype identification, and the determination of genome organization and evolution in plants. Among the marker systems, ISSR are polymerase chain reaction (PCR) based and considered to be the simplest marker system. Analysis of ISSR involves the PCR amplification of regions between adjacent, inversely oriented microsatellites, using a single simple sequence repeat (SSR) motifs (di-, tri-, tetra-, or pentanucleotides) containing primers anchored at the 3′or 5′ end by two to four arbitrary, often degenerate nucleotides Zietkiewicz et al., [12]. The major areas of the application of ISSR are in genomic fingerprinting, genetic diversity and phylogenetic analysis, genome mapping, gene tagging and marker assisted selection, determining SSR motif frequency and studies of natural population/ speciation. With this view, the present study was formulated to understand the molecular diversity among the *Cucumis* genotypes.

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Materials and Methods

Eleven genotypes of cucumber viz. PCUCP-2, PCUCP-3, Nun-3139, Nun-3121, Nun-3141, Infinity, Isatis and Kian (parthenocarpic) and PCUC-8, PCUC-15 and PCUC-28 (monoecious) were grown under greenhouse conditions in G. B. Pant University of Agriculture and Technology, Pantnagar (Table 1). Fresh and young leaves were collected from each genotype. The leaves were cut into small bits and ground to powder using liquid nitrogen. Genomic DNA was then isolated using modified CTAB method Doyle and Doyle [13]. Eight ISSR primers were used for PCR analysis to detect polymorphism among the *Cucumis* spp. (Table 2). The PCR amplification was performed in a volume of 25 µl reaction setup of various PCR reagents based on the stock and final concentration of different components was prepared. The PCR was programmed as follows: Initial denaturation for 5 min at 94°C, followed by 35 cycles of amplification consisting of a denaturation step for 1 min at 94°C annealing for 2 min at 50°C, extension for 2 min at 72°C, and final extension for 5 min at 72°C. Amplified products were separated by electrophoresis on 1.2% agarose gel and visualized by staining with ethidium bromide and documented. Statistical analysis was carried out using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) 2.02 i version Rohlf [14]. The binary data score was used to construct a dendrogram. The pairwise genetic relationship between accessions were determined by calculating Jaccard's similarity coefficient. The similarity coefficients were used for cluster analysis and a dendrogram was constructed by the Unweighted Pair-Group Method (UPGMA) Sneath and Sokal [15].

S. N.	Genotypes	Nature	Source
1.	PCUCP-2 (Pant Parthenocarpic Cucumber-2)	Parthenocarpic	Pantnagar
2.	PCUCP-3 (Pant Parthenocarpic Cucumber-3)	Parthenocarpic	Pantnagar
3.	Nun-3139	Parthenocarpic	Nunhems
4.	Nun-3121	Parthenocarpic	Nunhems
5.	Nun-3141	Parthenocarpic	Nunhems
6.	Infinity	Parthenocarpic	Procured from local market
7.	Isatis	Parthenocarpic	Nunhems
8.	Kian	Parthenocarpic	Nunhems
9.	PCUC-8	Monoecious	Pantnagar
10.	PCUC-15	Monoecious	Pantnagar
11.	Pant Khira-1 (PCUC-28)	Monoecious	Pantnagar

Table 1: List of cucumber genotypes used for diversity analysis.

S.N.	Oligo reference code	Sequence 5'- 3'
1.	UBC - 808	AGAGAGAGAGAGAGAGC
2.	UBC - 811	GAGAGAGAGAGAGAGAC
3.	UBC - 812	GAGAGAGAGAGAGAGAA
4.	UBC - 825	ACACACACACACACACT
5.	UBC - 834	AGAGAGAGAGAGAGAGYT
6.	UBC - 840	GAGAGAGAGAGAGAGAYT
7.	UBC - 842	GAGAGAGAGAGAGAGAYG
8.	UBC - 880	GGAGAGGAGGAGAGGAGA

Table 2: List of primers used for ISSR analysis.

S. N.	Primer	No. of amplified alleles	No. of polymorphic alleles	Percent (%) polymorphism	PIC value
1.	UBC-842	12	12	100.00	0.34
2.	UBC-834	9	8	88.88	0.27
3.	UBC-880	8	6	75.00	0.28
4.	UBC-840	9	7	77.77	0.28
5.	UBC-808	5	2	40.00	0.19
6.	UBC-811	14	14	100.00	0.19
	Total	**57**	**49**	**85.95**	0.25

Table 3: Alleles produced by 6 ISSR primers produced among the genotypes.

Results and Discussion

The information on genetic diversity helps in choosing parents for generations of new varieties, needs continuous evaluation of germplasm for useful characters, which in earlier days was solely based on the available morphological data. But descriptions based on morphological data are fundamentally flawed in their ability to provide reliable information for the calculation of genetic distance. Advances in molecular biology have provided descriptors based on DNA markers. These markers are highly heritable, available in high numbers, and often exhibit enough polymorphism to discriminate even closely related genotypes. The DNA-based markers have largely overcome the problems encountered with morphological and biochemical markers. The major advantages are speed with which results are generated, low amounts of genomic DNA required, and the ability to share the information on primer sequences without the need to exchange DNA Godwin et al., [16].

Molecular Diversity Analysis Using ISSR Markers

Out of the eight ISSR markers primer UBC 812 and UBC 825 were found to be monomorphic, whereas remaining 6 primers yielded a total of 57 alleles of which 49 were polymorphic. The number of alleles produced by different primers ranged from 5 to 14 with an average of 9.5 alleles per primer. The level of polymorphism was found to be in between 40% to 88.88%. The details of markers amplified by the 6 ISSR primers among the 11 genotypes are given in (Table 3). The maximum number of 14 alleles was generated by the primer UBC 811 whereas minimum number of 2 amplicons was generated by the primer UBC 808. The binary data from the polymorphic primers were used for computing Jaccard's similarity indices. The similarity coefficients based on alleles ranged from 0.0 to 0.91 (Table 4). Among the 11 *Cucumis* genotypes, the highest similarity index (0.96) was observed between Pant Parthenocarpic Cucumber-2 and Pant Parthenocarpic Cucumber-3 and the lowest similarity index (0.35) was observed between Pant Parthenocarpic Cucumber-2 and Kian. The similarity values obtained for each pairwise comparison of ISSR markers among the 11 *Cucumis* genotypes were used to construct a dendrogram based on Jaccard's coefficient (Figure 1). Markers data based dendrogram analysis classified eleven cucumber genotypes into two main groups at 52% similarity Group A comprised of cluster I and cluster II at 76% similarity. Whereas group B was bifurcated into 2 Subgroups: Subgroup B1 and Subgroup B2. Subgroup B1 consisted of Cluster III and Cluster IV at similarity of 71%. Cluster III was again forked into Cluster III A and Cluster III B with 78% similarity having the genotype Nun-3141 and Infinity. Cluster IV was divided into cluster IV A and Cluster IV B with 82% similarity consisting of genotype Isatis and Kian. Subgroup B2 was further bifurcated into Cluster V and Cluster VI with 81% similarity having 3 monoecious cucumber genotypes (PCUC-8, PCUC-15 and PCUC-28). The marker analysis discriminated between 8 parthenocarpic and 3 monoecious genotypes of cucumber. It is clear from the dendrogram (Figure 2) that 8 parthenocarpic cucumber genotypes (PCUCP-2, PCUCP-3, Nun-3139, Nun-3121, Nun-3141, Infinity, Isatis and Kian) had wide molecular diversity with monoecious

Genotypes	PCUCP -2	PCUCP-3	NUN -3139	NUN -3121	NUN -3141	Infinity	Isatis	Kian	PCUC -8	PCUC -15	PCUC -28
PCUCP-2	1.00										
PCUCP-3	0.96	1.00									
NUN-3139	0.77	0.77	1.00								
NUN-3121	0.77	0.80	0.82	1.00							
NUN-3141	0.61	0.61	0.80	0.77	1.00						
Infinity	0.43	0.47	0.59	0.63	0.78	1.00					
Isatis	0.49	0.49	0.61	0.57	0.70	0.80	1.00				
Kian	0.35	0.38	0.47	0.50	0.59	0.77	0.82	1.00			
PCUC-8	0.45	0.45	0.57	0.50	0.56	0.59	0.68	0.78	1.00		
PCUC-15	0.40	0.43	0.45	0.45	0.50	0.54	0.63	0.77	0.84	1.00	
PCUC-28	0.45	0.49	0.50	0.50	0.52	0.52	0.57	0.68	0.78	0.87	1.00

Table 4: Pairwise Jaccard's similarity coefficient among 11 genotypes of parthenocarpic and monoecious cucumber.

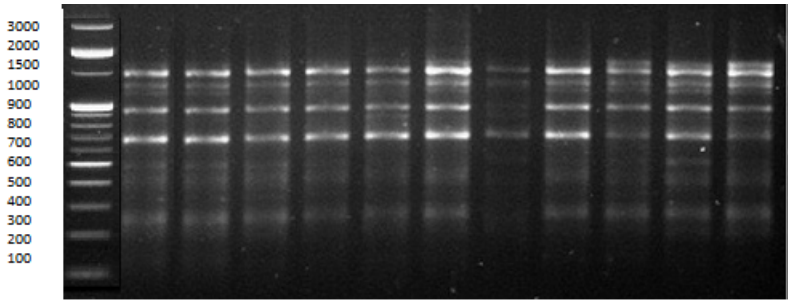
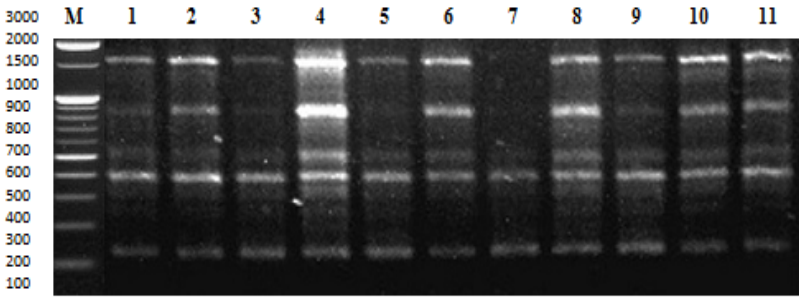


Figure 1: ISSR profile of parthenocarpic and monoecious cucumber genotypes with primer UBC-842.



M = Marker, 1 = PCUCP-2, 2 = PCUCP-3, 3 = Nun-3139, 4 = Nun-3121, 5 = Nun-3141, 6 = Infinity, 7 = Isatis, 8 = Kian, 9 = PCUC-8, 10 = PCUC-15, 11 = PCUC-28.

Figure 2: ISSR profile of parthenocarpic and monoecious cucumber genotypes with primer UBC-834.

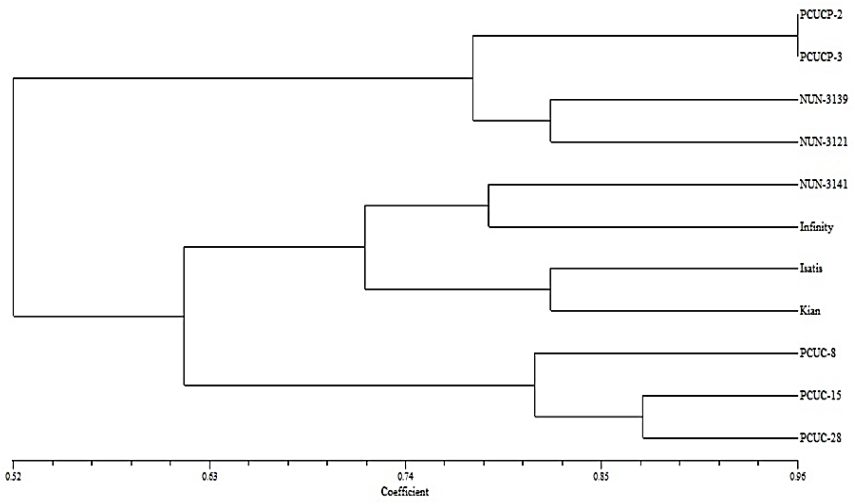


Figure 3: Dendrogram of 11 cucumber genotypes based on morphological data.

cucumber genotypes (PCUC-8, PCUC-15 and PCUC-28) (Figure 3). Molecular markers (ISSR) are a more reliable and accurate method for diversity assessment. The information obtained from this study may be useful for further identification of promising cucumber genotypes for understanding the extent of genetic diversity present in cucumber genotypes. Pair wise similarity matrix for 11 parthenocarpic and monoecious genotypes of cucumber showed the minimum similarity for Pant Parthenocarpic Cucumber-2 with Kian (0.35) followed by Pant Parthenocarpic Cucumber-3 with Kian (0.38) and Pant Parthenocarpic Cucumber-2 with PCUC-15 (0.40) (Table 4). In the present investigation we therefore successfully characterise and determine the diversity between parthenocarpic and monoecious genotypes of cucumber that may be further utilized in breeding programme of cucumber.

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