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Research Note

Genetic diversity assessment of cucumber (*Cucumis sativus* L.) genotypes using molecular markers

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Abstract
In the present investigation, 13 genotypes of cucumber were screened for genetic diversity using eight ISSR primers. The six ISSR primers generated 52 alleles. A total of 52 loci were amplified that exhibited 92.30 per cent polymorphism. A maximum of 11 loci were detected by the primer UBC-855 and UBC-890. Unique bands were also observed by the primers namely UBC-808, UBC-840, and UBC-855. The similarity value ranged from 22 per cent to 80 per cent with the average value of 46 per cent. The genotype Pgyn-1 was found most diverse than others with 33 per cent similarity. The 13 genotypes were grouped into two major groups (A and B) based on ISSR markers. Group A consisted of the most diverse genotype, Pgyn-1. Group B contained the maximum number of genotypes and further divided in two major sub clusters IB and IIB. Sub cluster IB includes small sub cluster of seven genotypes in which Pgyn-4 was found separately whereas, sub cluster IIB contained PCUC-35 and Punjab Naveen as well as shared the cluster genetically while they differ a lot in respect to visual identification.

Key words
Cucumber, fingerprinting, ISSR, diversity, markers

Cucumber (*Cucumis sativus* L.) is one of the important cucurbitaceous vegetable grown throughout the world. It is an unique crop and is important features are not associated with discrete Mendelian traits, but are of a continuous or quantitative nature. Yielding ability is a prime example of such a trait and is of obvious importance. The information usually needed for developing high yielding cucumber in a particular species pertains to the extent of genetic variability for desirable traits in the available germplasm. Large variability ensures better chances to produce new forms. The phenomenon of hybrid vigour or heterosis resulting from the crosses between genotypically dissimilar genotypes forms an important measure of crop improvement. Along with this traditional methods of genetic studies in present scenario, DNA markers are becoming more popular and effective to study the genetic diversity and choosing right parents. There are limitations associated with DNA markers namely low reproducibility (RAPD), high cost (AFLP) and need to know the flanking sequences to develop species specific primers for SSR polymorphism. ISSR-PCR (Inter Simple Sequence-Polymerase Chain Reaction) however is a technique that overcomes most of these limitations.

An understanding of the extent of genetic diversity is critical for the success of a breeding program. Hence, selection based on genetic information using neutral molecular markers is essential, as it is more reliable and consistent. The genetic diversity of African cucumber germplasm was described by Knerr *et al.* (1989) using 18 isozyme loci. Molecular markers such as RAPD, RFLP, and SSRs have been employed for determining the extent of genetic diversity in African cucumber (*Cucumis sativus* L.) (Mliki *et al.*2003; Dijkhuizen *et al.* 1996) and Indian Snap Melon (*Cucumis melo* L.) (Dhillon *et al.* 2005; Staub *et al.* 2004). SSR Markers were used to compare the Indian Snap Melons with reference to accessions of melons from a diverse origin like South Asia, West Asia, and Europe (Gonzalo *et al*. 2005).

DNA marker-based fingerprinting can distinguish species rapidly using a smaller amount of DNA and therefore can assist to deduce reliable information on their phylogenetic relationships.DNA markers are not influenced by environmental conditions and therefore can be used to describe patterns of genetic variability among genotypes and identify duplicated accessions within germplasm collections. With this view, the present study was formulated to understand the molecular diversity among the cucumber genotypes.

Thirteen accessions of cucumber representing various growth habits were collected and grown under greenhouse conditions in G. B. Pant University of Agriculture and Technology, Pantnagar (Table 1).

From each genotype fresh young leaves were collected from 30 days old seedlings and frozen in liquid nitrogen. Then 100 mg of leaf tissue was ground into fine powder and genomic DNA was extracted by following CTAB procedure (Doyle and Doyle 1987). Eight ISSR primers were used for PCR analysis to detect polymorphism among the *Cucumis* spp. (Table 2). The PCR analysis was performed in 15 µL volume contained 40 ng DNA, 2.5 mM Primer, 10 mM dNTPs, 1.50 µL 10X assay buffer, 0.20 µL *Taq* polymerase, and 8.10 mL sterile distilled water. Amplification was carried out in thermal cycler with initial denaturation for 5 min at 94°C, 35 cycles of denaturing 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 72ºC.

Amplified products were separated by electrophoresis on 1.2% (w/v) agarose gel and visualized by staining with ethidium bromide and documented. Statistical analysis was carried out using NTSYS-pc 2.02i version (Rohlf 1998). The binary data scored was used to construct a dendrogram. The genetic associations between accessions were evaluated by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the phenotypic data. Similarity matrix was generated using the SIMINT program of NTSYS-pc software, version 2.02 (Rohlf 1998). The similarity coefficients were used for cluster analysis and a dendrogram was constructed by the Unweighted Pair-Group Method (UPGMA) (Sneath and Sokal 1973).

Identification and utilization of diverse germplasm is the principal issue in plant breeding. More accurate and complete descriptions of elite breeding materials and understanding the patterns of genetic diversity could help in determining future breeding strategies and facilitate introgression of diverse germplasm into the current genetic base.

Among the eight ISSR markers, only six were recorded polymorphic and remaining two were monomorphic. The six ISSR primer yielded a total of 52 alleles, of which 48 were polymorphic. The number of alleles produced by different primers ranged from 3 to 11 with an average of eight alleles per primer and level of polymorphism was found to be 92.30 per cent. The details of markers amplified by the six ISSR primers among the 13 genotypes are given in Table 3. The maximum number of amplicons were generated by the primer UBC 855 and UBC 890 (11 amplicons) whereas minimum number of amplicons were generated by the primer Prime 809 (3 amplicons). In addition, the maximum gene diversity was generated by the primer UBC 845 and minimum by Prime 809.

Variation observed in the ISSR profile for the markers and specific bands in the genotypes Pgyn-1, Pgyn-4, Pgyn-5, PCUC-35 and Pant khira-1 towards specific primers UBC-808, UBC-840 and UBC-855. Similarly, specific bands were produced by UBC- 808 in Pgyn-4 and PCUC-35 at 700 and 200 bp as shown in Fig. 1 whereas UBC-855 produced bands specific to Pgyn-1 and Pant khira-1at 100 bp and 650 bp (Fig.2) and finally UBC-840 produced bands specific to Pgyn -5 at 500 bp level (Fig. 3).

The binary data from the polymorphic primers were used for computing Jaccard's similarity indices. The similarity coefficients based on 52 ISSR alleles ranged from 0.22 to 0.80. Among the 13 *Cucumis* genotypes, PCUC-15 and PCUC-25 were identified to be most similar (80%) on the similarity coefficient, and the lowest similarity index (0.22) was observed between Pgyn-1 and Punjab Naveen. The Jaccard's similarity coefficient values clearly depicted rich genetic diversity within gynoecious and monoecious cucumber genotypes under investigation.

In the present study, the Polymorphic Information Content (PIC) of ISSRs primer was ranged from 0.31 to 0.90, with the average value of 0.60 (Table 3). Parvathaneni *et al*. (2011) reported that ISSR primer UBC 825 was highly informative with a PIC value of 0.8934 whereas, Innark *et al.* (2014) recorded polymorphism information content (PIC) ranged from 0.12 to 0.45 with the mean of 0.25.

The similarity values obtained for each pairwise comparison of ISSR markers among the 13 *Cucumis* genotypes were used to construct a dendrogram based on Jaccard's coefficient and the results are presented in Fig. 4. The 13 *Cucumis* genotypes formed into two clusters groups. Group A consisted of the most diverse genotype, Pgyn-1. Group B was again bifurcated into two sub clusters namely clusters IB and IIB. Cluster IB consisted of 7 genotypes namely Pgyn-4, Pgyn-5, PCUC-208, PCUC-8, PCUC-126, Pant Khira-1 and PCUC-83 and further divided into two sub clusters (IBI and IBII). The Pgyn-4 was the only genotype present in IBI whereas, the sub cluster IBII contained the genotype PCUC-83 was found dissimilar then rest of the five genotypes namely Pgyn-5, PCUC-208, PCUC-8, PCUC-126, Pant Khira-1. The genotypes which showed similar morphological and genetic trends were grouped more or less together. The genotypes Pgyn-1 and Pgyn-4 showed deviation from the existing cluster and also diverse with respect to their genetic makeup whereas PCUC-35 and Punjab Naveen shared the cluster genetically, while they differ a lot in respect to visual identification. The present findings are in consistent with the earlier reports of Dijkhuizen *et al.* (1996) and Bisht *et al.* (2004) reported high diversity in 29 accessions of cucumber collected

from different parts of India with RAPD marker. Therefore molecular markers are more authentic and useful, as the differentiation of plant species on the basis of visual observation/morphological characterization lacks the efficiency needed to identify individual genotypes since most of the traits are influenced by environmental and depend on the developmental stage of the plants.

On the basis of above results, it can be concluded that the molecular (ISSR) markers are more reliable and accurate method to identify genetic diversity in cucumber genotypes. 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.

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Table 1. List of Cucumber genotypes used diversity analysis in this study

S.No.	Germplasm Line	Nature	Source
1.	Pgyn-1	Gynoecious	Pantnagar
2.	Pgyn-4	Gynoecious	Pantnagar
3.	Pgyn-5	Gynoecious	Pantnagar
4.	PCUC-8	Monoecious	Pantnagar
5.	Pant Khira-1	Monoecious	Pantnagar
6.	PCUC-83	Monoecious	Pantnagar
7.	PCUC-126	Monoecious	Pantnagar
8.	PCUC-208	Monoecious	Pantnagar
9.	PCUC-15	Monoecious	Pantnagar
10.	PCUC-25	Monoecious	Pantnagar
11.	PCUC-35	Monoecious	Pantnagar
12.	US-832	Monoecious	UAS, Bangalore
13.	Punjab Naveen	Monoecious	PAU, Ludhiana

Table 2. List of primers used for ISSR analysis

ISSR Primer	Sequence (5'-3')
Prime 809	AGAGAGAGAGAGAGAGYG
UBC-840	GAGAGAGAGAGAGAGACTT
UBC-808	AGAGAGAGAGAGAGAGC
UBC-855	ACACACACACACACACCTT
UBC-846	CACACACACACACACACART
UBC-890	ACGACTACGGTGTGTGTTTGT
UBC-856	ACACACACACACACACCTA
UBC-866	CTCCTCCTCCTCCTCCTC

Table 3. Alleles produced by six ISSR primers produced among the genotypes

S.No.	Primer	Number of alleles	Number of polymorphic alleles	Polymorphism (%)	Gene diversity	PIC Values
1.	UBC-808	7	7	100.00	0.21	0.63
2.	Prime-809	6	3	50.00	0.16	0.31
3.	UBC-840	10	10	100.00	0.39	0.77
4.	UBC-845	7	6	85.70	0.62	0.78
5.	UBC-855	11	11	100.00	0.29	0.83
6.	UBC-890	11	11	100.00	0.31	0.90
	Total	**52**	**48**	**92.30**	**0.33**	**-**

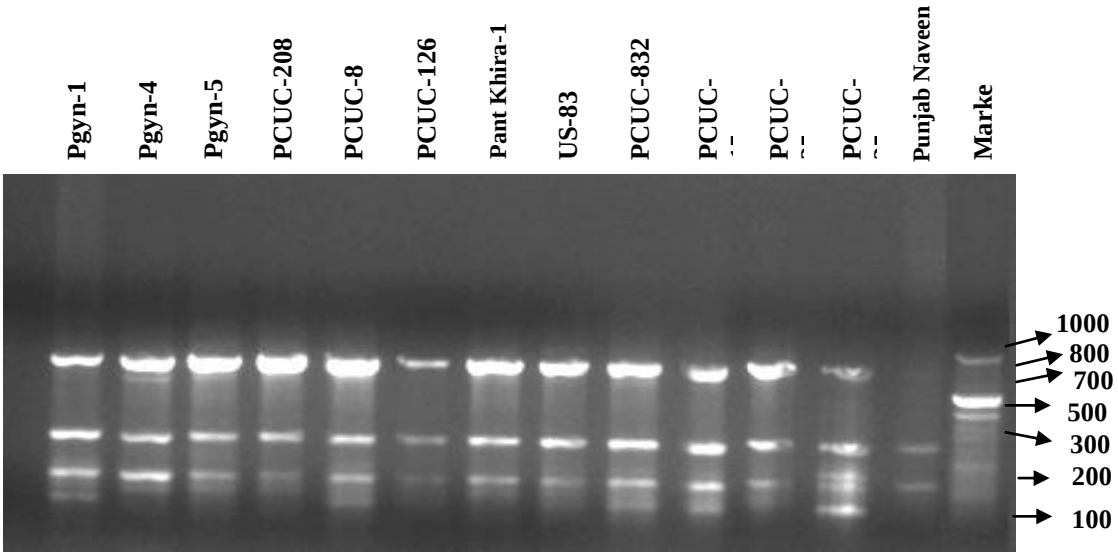

Fig 1. ISSR profile of gynoecious and monoecious cucumber genotypes with primer UBC- 808

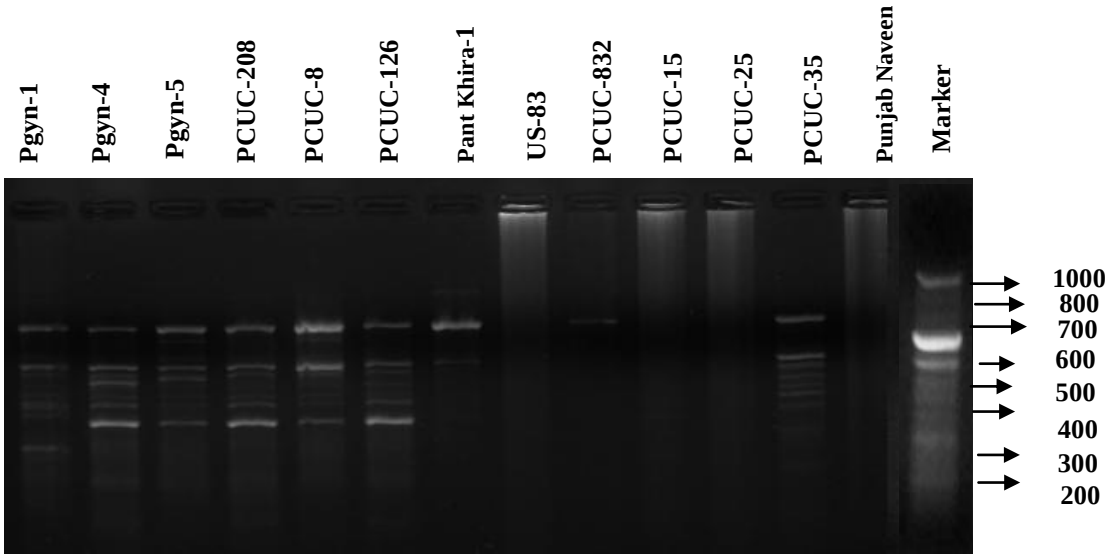

Fig.2. ISSR profile of gynoecious and monoecious cucumber genotypes with primer UBC-855

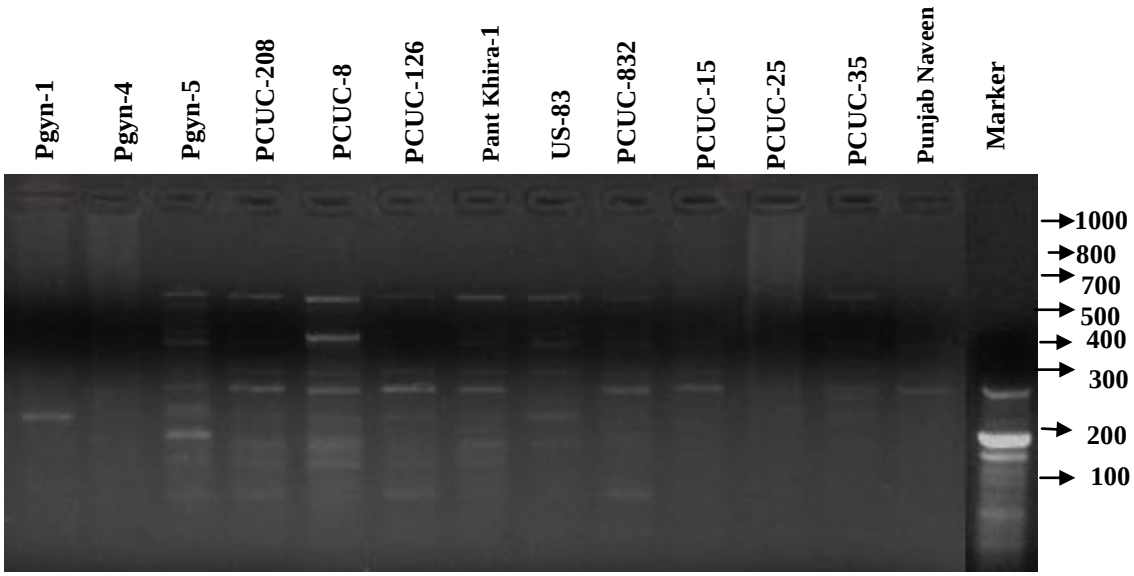

Fig 3. ISSR profile of gynoecious and monoecious cucumber genotypes with primer UBC- 840

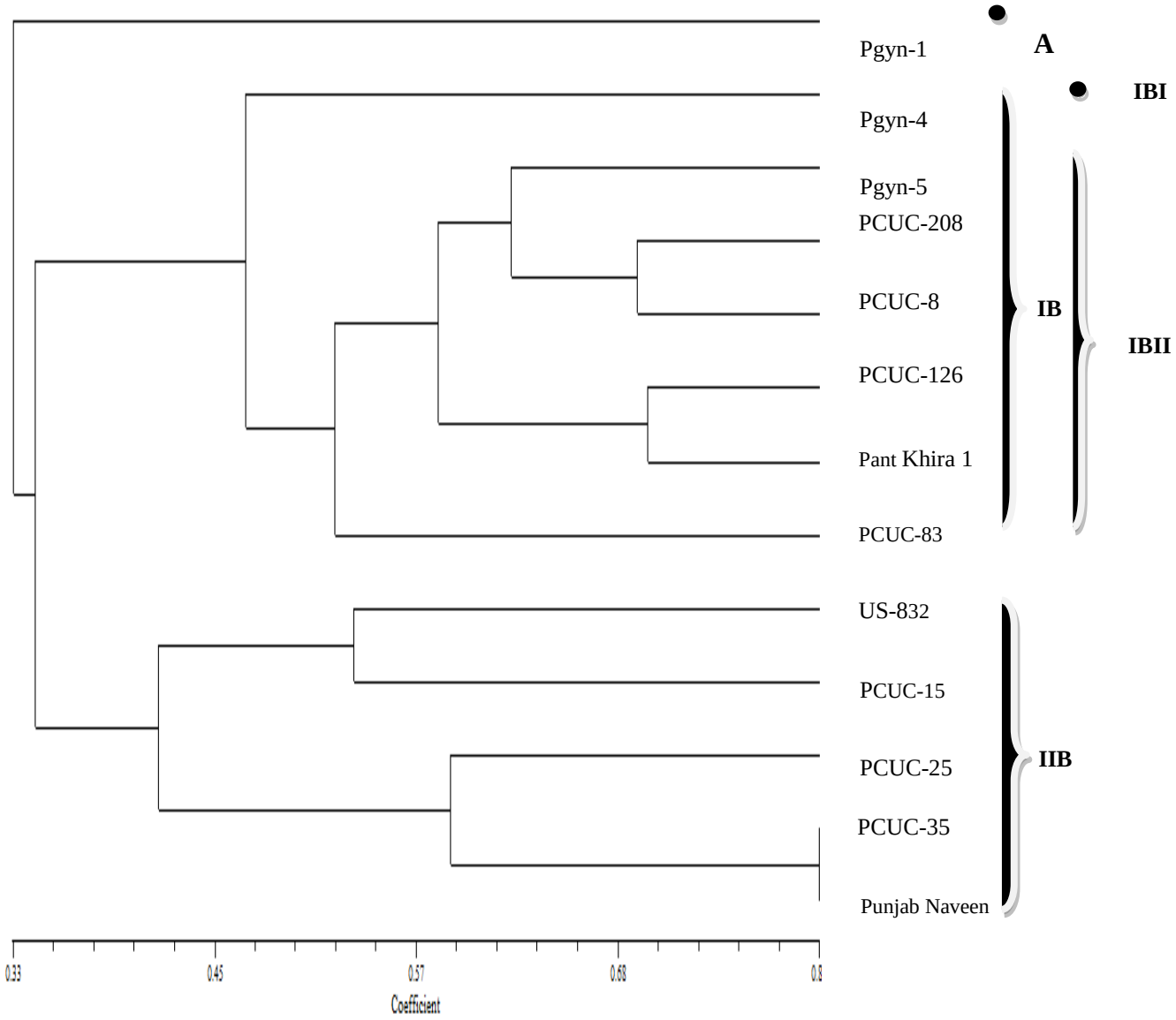

Fig. 4. Dendrogram illustrating the phylogenetic relationship among 13 cucumber genotypes based on UPGMA cluster analysis (ISSR)