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Genetic distinctness revealed by SSR characterization in *Cucumis sativus* L. and its wild relatives

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Abstract
The present work was undertaken to assess the genetic diversity by characterization using SSR markers in diverse collections of *Cucumis* genotypes. The results revealed that the accessions varied distinctly at the genomic level. The size of the amplicons ranged from 121.13 bp (SSR12810) to 362.84 bp (SSR11742) with a mean number of 4.10 alleles per locus. Number of amplicons ranged from 2.00 (SSR19493) to 6.00 (SSR11742 and AF202378) respectively. The PIC ranged from 0.20 in SSR06660 to 0.81 in AF20237. The Jaccard's similarity coefficient values were maximum (0.83) between IC618084A and IC613480; IC613484 and IC595512 and IC613470 and 469517, which was in consonance with the clustering pattern followed in the dendrogram. Marker index ranged from 0.35 in SSR19493 to 4.05 in AF202378. Highest PIC and marker index values in AF202378 (0.81 and 4.05 respectively) followed by SSR11742 (0.75 and 3.77 respectively) are in agreement with their maximum number of amplicons produced, indicating that these markers are very informative and can be used in future genetic diversity analysis studies in cucumber.

Keywords: *Cucumis sativus*, Polymorphism information content, Marker Index, Similarity coefficient, Dendrogram, SSR markers

Introduction
Cucumber (*Cucumis sativus* L.) is an important vegetable crop of family Cucurbitaceae with chromosome number 2n=2x=14. It is mainly grown for its tender fruits consumed as salads and pickles. It is an important component of cosmetic industry owing to its soothing, cleansing and softening properties (Wang *et al*., 2007) [12]. Recently, in view of the changing food habits and increasing health concern of the people, cucumber is placed as a main component of salad preparations. Despite its economic, medicinal and nutritional values, studyon molecular genetic diversity of this cropis very limited.

Availability of genetic diversity is a pre-requisite for any crop improvement programme. But assessment of genetic diversity based on phenotype has limitations since most of the morphological characters are greatly influenced by environmental factors and developmental stage of the plant. Hence the substantial variation in the morphology of crop needs to be supported by molecular markers as well. Further, it is also known that progenies developed from geographically and genetically diverse parents in hybridization programme will be promising in terms of their agronomic performance due to varied combination of genes.

In the present scenario, DNA markers have become more popular and effective to study the genetic diversity among genotypes. Use of SSR markers is one of the common and best choices for molecular characterization, to reveal the genetic distinctness of even morphologically similar genotypes. They are PCR based markers, highly polymorphic, multi-allelic, co-dominant, easily reproducible and widely distributed along the genome (Powell *et al*., 1996) [7]. SSR markers have wide applications in the areas such as gene mapping, marker assisted selection, genetic diversity analysis, cultivar identification and gene pyramiding. SSR markers are increasingly being used in number of crop species for the purpose of gene mapping, marker assisted selection, germplasm analysis and varietal identification.

Materials and methods
Twenty seven *Cucumis* genotypes comprising 21 accessions of *Cucumis sativus* and five accessions of wild species were selected for study (Table 1).

Total genomic DNA from the young leaves of the accessions was extracted following CTAB method (Dellaporta *et al*., 1983) [2] with minor modifications. Quantification and quality checking of the isolated genomic DNA was done using Nanodrop-Genway (Genova Nano) spectrophotometrically by estimating the optical density value at 260 nm and 280 nm. The DNA samples were diluted to working concentration of 50 ng/µl using the obtained concentration of original sample from Nanodrop. The amplified products were separated by agarose gel electrophoresis at a concentration of 1.50 per cent agarose. A total of 20 primers reported as polymorphic from earlier studies (Hu *et al*., 2010; Innark *et al*., 2013 and Pandey *et al*., 2013) [3, 4, 6] were used for SSR profiling.

Statistical analysis

The banding pattern generated by the DNA fragments were scored based on the molecular weight of the fragments generated. The molecular weight of each band was estimated by using the software Uvitech by selecting molecular weight analysis option. Clear and unambiguous bands of various molecular weights were scored for the presence (1) and absence of the bands (0) respectively. Manually scored bands were prepared in the form of a binary matrix and the data matrix was further subjected to analysis using NTSYS (Numerical Taxonomy and Multivariate Analysis System) version 2.1 (Rohlf, 2000) [8]. SIMQUAL programme was used to calculate pair wise Jaccard's similarity coefficient (Jaccard, 1908) [5] and generated a similarity matrix.

This similarity matrix was used in cluster analysis following Unweighted Pair Group Method (UPGMA) (Sneath and Sokal, 1973) [10] using SAHN clustering algorithm (Sequential agglomerative Hierarchical and Nested) based on the similarity indices and genetic relatedness among the 27 genotypes to construct dendrogram.

Polymorphic information content (Anderson *et al.,* 1993) [1] and Marker index (MI) (Powell *et al*., 1996) [7] of each SSR marker were the parameters used for measuring the performance of markers. Both PIC and MI confirm the suitability of the primer, PIC represents the ability of a marker to detect the polymorphism within a population and MI helps to understand the capacity of primer to detect polymorphic loci among varieties. Markers were classified as informative when PIC was ≥ 0.5. Markers were classified as informative when PIC was ≥ 0.5

$$PIC = 1 - \sum_{j=1}^{n} (P_{ij})^2$$

where, n is the number of marker alleles for marker '*i*' and P_{ij} is the frequency of the jth allele for marker '*i*',

MI = PIC × No. of polymorphic bands

Results and discussion

Twenty polymorphic primers which produced polymorphic patterns in at least two accessions were used to study the molecular divergence among the genotypes. SSR profiling analysis revealed high level of genetic distinctness among the genotypes studied. The varied sizes of amplicons ranging from 121.13 bp to 362.84 bp were observed (Table 2). Maximum number of alleles (6) was obtained with primer SSR11742 and AF202378, indicating the informative nature of these markers. All primers considered for study revealed polymorphism in wild species also. However, a total of 83 amplicons with an average of 4.1 alleles per locus was detected in the study. Similar reports of divergence at genome level was earlier reported by Pandey *et al*. (2013) [6], on studies on molecular characterization of 44 cucumber genotypes using 53 polymorphic SSR primers. They have identified 163 amplification products with an average of 3.05 alleles per locus. In contrary, Singh *et al*. (2016) [9] obtained 9.3 alleles per locus by characterization using eight ISSR (Inter Simple Sequence Repeats) markers.

Table 1: List of accessions used in the study

S. No.	Accession/ Collection No.		State	S. No.	Accession/ Collection No.		State
1	IC613481	*Cucumis sativus*	West Bengal	15	IC613488	*Cucumis sativus*	Mizoram
2	IC613480	*Cucumis sativus*	West Bengal	16	IC595510	*Cucumis sativus*	Tripura
3	IC618084A	*Cucumis sativus*	Arunachal Pradesh	17	IC613474	*Cucumis sativus*	Nagaland
4	IC595512	*Cucumis sativus*	Tripura	18	IC595518	*Cucumis sativus*	Kerala
5	IC613484	*Cucumis sativus*	West Bengal	19	IC613485	*Cucumis sativus*	West Bengal
6	IC595504	*Cucumis sativus*	Mizoram	20	IC618083	*Cucumis sativus*	Tripura
7	IC613471	*Cucumis sativus*	Arunachal Pradesh	21	IC613462	*Cucumis sativus*	Mizoram
8	IC541367	*Cucumis sativus*	A&N Islands	22	IC595508A	*Cucumis sativus*	Tripura
9	IC331445	*Cucumis sativus*	Odisha	23	JB-11/156	*C.hystrix*	Mizoram
10	IC613477	*Cucumis sativus*	West Bengal	24	N-09/110	*C.setosus*	Maharashtra
11	IC613476	*Cucumis sativus*	West Bengal	25	JP-13/47	*C.leosperma*	Kerala
12	IC613470	*Cucumis sativus*	Tripura	26	JB-11/36	*C.muriculatus*	Mizoram
13	IC469517	*Cucumis sativus*	Karnataka	27	JBT-51/29	*C.agrestis*	Jharkhand
14	IC613479	*Cucumis sativus*	West Bengal				

Two markers namely UW053690 and SSR11742 showed heterozygous condition in the banding pattern. UW053690 exhibited heterozygous condition in the genotype IC613476 (West Bengal) whereas SSR11742 in IC613480 (West Bengal), IC618084A (Arunachal Pradesh), IC541367 (A&N islands) and IC613479 (West Bengal). It was revealed through nucleotide BLAST (blastn) that the sequence of SSR11742 had sequence homology with *Cucumis melo* ras-related proteinRab7-like (LOC103489588) mRNA. The distinctness of this region in the genome of 27 *Cucumis* genotypes was revealed by this marker.

PIC value greater than 0.50 was exhibited by 14 markers. Highest PIC and marker index values in AF202378 (0.81 and 4.05 respectively) followed by SSR11742 (0.75 and 3.77 respectively) are in agreement with their maximum number of amplicons produced, indicating that these markers are very informative and can be used in future genetic diversity analysis studies in cucumber. Unique bands produced by SSR11742 in the genotype IC613485 and JBT-51/29 (*C. agrestis*) may be characterised in detail in future studies.

Pair wise similarity matrix based on Jaccard's coefficient for 27 genotypes is presented in Table 3. The similarity

coefficient was maximum (0.83) between IC618084A and IC613480, IC613484 and IC595512 and IC613470 and 469517, which was in consonance with the clustering pattern followed in the dendrogram. Both IC618084A and IC613480 were accessions from West Bengal. However, IC595512, a collection from Tripura, showed similarity with IC613484, from West Bengal, a state lying near the North Eastern belt of India. In contrary, IC613470 from Tripura exhibited high similarity with IC469517 from Karnataka. Similar studies were earlier conducted by Staub *et al*. (1997) [11]; Pandey *et al*. (2013) [6] and Mliki *et al*. (2003).

Cluster analysis resulted in grouping of genotypes into 4 distinct clusters. Cluster I had 5 accessions whereas cluster II and III with four accessions each (Table 4). A random grouping of genotypes was observed in the dendrogram as genotypes from same state grouped in different clusters and from different states falling in same cluster (Fig. 1). The clustering pattern did not exhibited any parallelism between genetic distinctness and geographical diversity. The reason may be due to the long term differential selection and local seed flow between farmers, whereby similarity between genotypes from different regions occurred. Further, India being the centre for maximum diversity in cucumber and its related species, genetic recombinations might have led to the randomness in grouping of the genotypes.

Table 2: Characteristics of 20 SSR markers used for molecular characterization of 27 *Cucumis* genotypes

Primer ID	PIC	No. of amplicons	Marker Index	Amplicon size	
				Min	Max
UW053690	0.65	5	2.61	190.66	300.00
UW029476	0.73	5	2.93	161.55	251.72
SSR12810	0.63	3	1.26	121.13	170.18
SSR12227	0.46	3	0.92	147.74	213.74
SSR05830	0.52	3	1.05	145.97	200.00
SSR22071	0.43	4	1.28	187.83	300.00
SSR05737	0.44	3	0.87	135.79	188.61
SSR19493	0.35	2	0.35	169.01	234.05
SSR06660	0.20	3	0.41	174.18	221.68
SSR33278	0.32	3	0.64	159.34	257.09
SSR11742	0.75	6	3.77	128.36	362.84
DN910157	0.64	4	1.93	142.89	217.41
DN910437	0.68	4	2.03	193.69	325.57
DN909941	0.66	5	2.66	200.00	292.66
AF202378	0.81	6	4.05	182.99	300.00
BI740103	0.62	5	2.49	174.16	292.78
AY942801	0.52	3	1.05	174.15	250.85
CK758649	0.62	4	1.87	200.00	314.48
yfSSR108	0.73	5	2.92	121.34	276.17
SSR17292	0.70	4	2.09	185.99	259.81
Max	0.81	6	4.05	200.00	362.84
Min	0.20	2	0.35	121.13	170.18

PIC-Polymorphism information content

Table 3: Distribution of 27 *Cucumis* genotypes in different clusters

Cluster No.	Number of genotypes	Genotypes
I	5	IC613485 (WB), IC595518 (Ke), IC613474 (N), IC595510 (T) and IC613488 (Mi)
II	4	JP-13-47(Ke), JBT-51/29 (Jh), N-09-110 (Ma), JB-11/156 (Mi)
III	4	JB-11/36 (Mi), IC595508A (T), IC613462 (Mi) and IC618083 (T)
IV	14	IC613479 (WB), IC469517 (Ka), IC613470 (T), IC613476 (WB), IC613477 (WB), IC331445 (O), IC613471 (ArP), IC541367 (A&N), IC595504 (Mi), IC613484 (WB), IC595512 (T), IC618084A (ArP), IC613480 (WB) and IC613481 (WB)

A&N- Andaman &Nicobar Islands; ArP- Arunachal Pradesh; Ka-Karnataka; Ke- Kerala; Ma-Maharashtra; Mi-Mizoram; N-Nagaland; O-Odisha; T-Tripura; WB-West Bengal

Table 4: Jaccards similarity coefficient betweeen 27 genotypes

IC613481	1.00																										
IC613480	0.62	1.00																									
IC618084A	0.75	0.83	1.00																								
IC595512	0.68	0.56	0.62	1.00																							
IC613484	0.68	0.50	0.62	0.83	1.00																						
IC595504	0.50	0.50	0.56	0.50	0.50	1.00																					
IC613471	0.43	0.39	0.43	0.54	0.54	0.59	1.00																				
IC541367	0.62	0.62	0.68	0.56	0.56	0.68	0.48	1.00																			
IC331445	0.50	0.31	0.40	0.40	0.45	0.40	0.39	0.45	1.00																		
IC613477	0.56	0.45	0.50	0.50	0.50	0.45	0.48	0.68	0.62	1.00																	
IC613476	0.56	0.40	0.50	0.45	0.50	0.40	0.43	0.56	0.68	0.75	1.00																
IC613470	0.45	0.40	0.40	0.35	0.40	0.31	0.34	0.45	0.68	0.56	0.68	1.00															
IC469517	0.40	0.35	0.35	0.31	0.35	0.35	0.34	0.40	0.56	0.45	0.56	0.83	1.00														
IC613479	0.31	0.40	0.35	0.31	0.27	0.56	0.34	0.45	0.40	0.45	0.45	0.40	0.45	1.00													
IC613488	0.20	0.20	0.20	0.17	0.24	0.31	0.23	0.20	0.35	0.27	0.31	0.45	0.50	0.45	1.00												
IC595510	0.14	0.14	0.14	0.17	0.17	0.27	0.19	0.17	0.24	0.20	0.20	0.27	0.27	0.31	0.62	1.00											
IC613474	0.13	0.13	0.13	0.16	0.16	0.26	0.22	0.16	0.23	0.19	0.23	0.19	0.23	0.30	0.34	0.43	1.00										
IC595518	0.11	0.11	0.11	0.14	0.14	0.24	0.19	0.14	0.20	0.17	0.17	0.17	0.17	0.24	0.24	0.27	0.48	1.00									
IC613485	0.05	0.08	0.05	0.05	0.08	0.11	0.16	0.08	0.11	0.11	0.11	0.11	0.11	0.11	0.14	0.14	0.26	0.45	1.00								
IC618083	0.35	0.31	0.40	0.31	0.31	0.27	0.23	0.31	0.31	0.35	0.35	0.24	0.20	0.20	0.24	0.17	0.19	0.14	0.08	1.00							
IC613462	0.24	0.27	0.27	0.27	0.27	0.24	0.19	0.20	0.20	0.24	0.27	0.20	0.17	0.17	0.24	0.24	0.23	0.17	0.08	0.62	1.00						
IC595508A	0.24	0.20	0.20	0.27	0.27	0.17	0.19	0.14	0.24	0.20	0.24	0.24	0.20	0.14	0.24	0.24	0.23	0.20	0.11	0.45	0.75	1.00					
JB-11/156	0.20	0.17	0.20	0.27	0.27	0.24	0.19	0.35	0.24	0.27	0.27	0.24	0.20	0.14	0.08	0.11	0.05	0.08	0.05	0.24	0.20	0.24	1.00				
N-09/110	0.11	0.14	0.14	0.17	0.17	0.11	0.16	0.17	0.14	0.17	0.17	0.14	0.14	0.08	0.02	0.05	0.00	0.02	0.02	0.17	0.24	0.27	0.56	1.00			
JP-13/47	0.24	0.17	0.21	0.21	0.21	0.21	0.20	0.32	0.28	0.32	0.24	0.24	0.21	0.21	0.14	0.17	0.11	0.17	0.14	0.24	0.14	0.21	0.37	0.24	1.00		
JB-11/36	0.17	0.11	0.11	0.08	0.11	0.17	0.10	0.17	0.20	0.17	0.20	0.24	0.27	0.20	0.24	0.11	0.13	0.11	0.05	0.35	0.27	0.24	0.27	0.20	0.28	1.00	
JBT-51/29	0.17	0.17	0.20	0.20	0.20	0.24	0.23	0.27	0.24	0.27	0.31	0.24	0.27	0.27	0.17	0.17	0.16	0.17	0.08	0.17	0.14	0.20	0.56	0.40	0.37	0.24	1.00

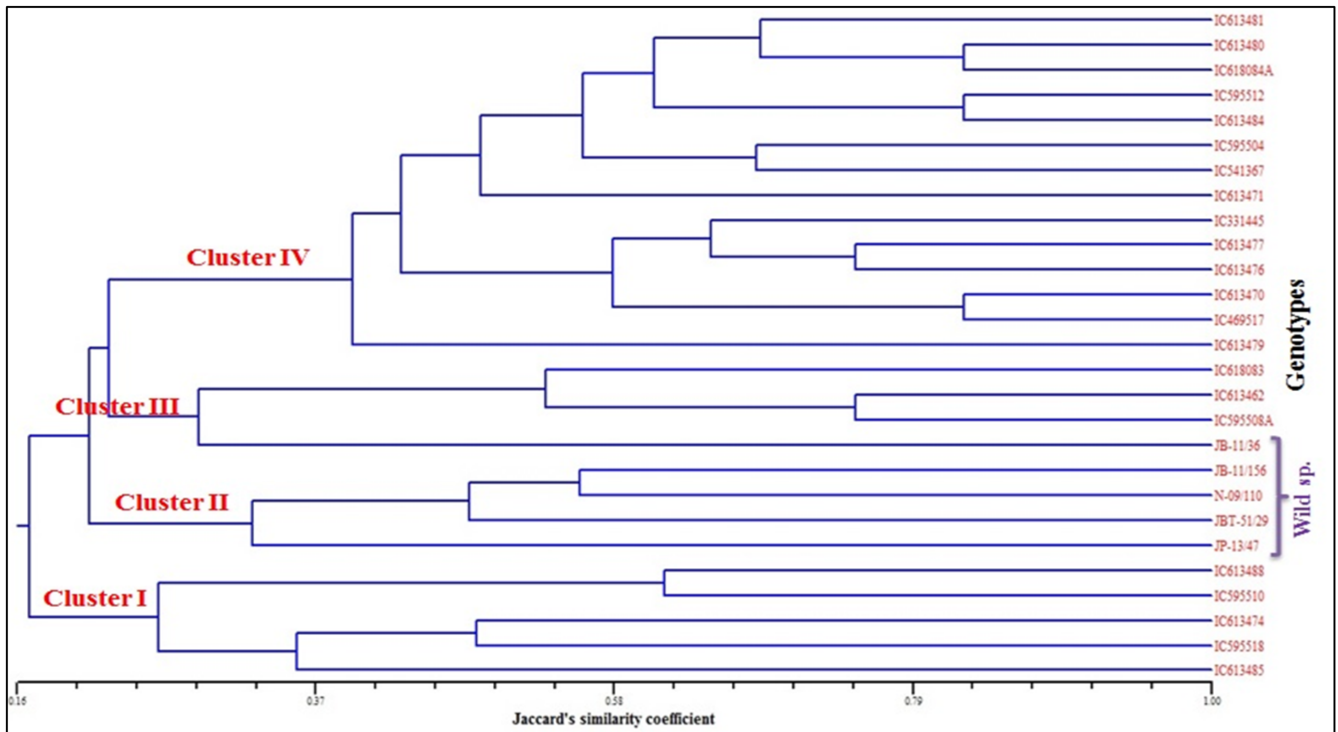

Fig 1: Dendrogram generated based on molecular characterization of *Cucumis* genotypes

Inclusion of considerable number of genotypes of *Cucumis sativus* into cluster IV indicated the narrow genetic base of cucumber germplasm used by farmers. Hence a diverse collection is still needed to be augmented from rich diversity areas. All the accessions of wild species grouped in cluster II, whereas, JB-11/36, an accession of *C. muriculatus* fallen in cluster III.

Conclusion

The results of the study indicated that, the genotypes used in the experiment were highly variable. SSR11742 and AF202378 were found to be highly polymorphic and informative markers which can be used for genetic diversity analysis of cucumber. Future collection missions should be targeted for broadening the genetic base of cucumber, from the diversity rich areas. An in-depth molecular analysis using more polymorphic EST-SSRs (Expressed sequence tags) may reveal unique bands in the accessions which may be linked to exonic regions, as wild species harbour many valuable genes.

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References

1. Anderson JA, Churchil GA, Autrique JE, Tanksley SO, Sorrels ME. Optimizing parent selection for genetic linkage maps. Genome. 1993; 36:181-186.
2. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation Version II. Plant Molecular biology Reporter.1983; 1:19-21.
3. Hu J, Li J, Liang F, Liu L, Si S. Genetic relationship of a cucumber germplasm collection revealed by newly developed EST-SSR markers. Journal of Genetics. 2010; 89:28-32.
4. Innark, Khanobdee C, Samipak S, Jantasuriyarat C. Evaluation of genetic diversity in cucumber (*Cucumis sativus* L.) germplasm using agro-economic traits and microsatellite markers. Scientia Horticulturae. 2013; 162: 278-284.
5. Jaccard P. Nouvellsrecherchess sur la distribution florate. Bulletin de la Société Vaudoise des Sciences Naturelles.1908; 44:223-270.
6. Pandey S, Ansari WA, Mishra VK, Singh AK, Singh M. Genetic diversity in Indian cucumber based on microsatellite and morphological markers. Biochemical Systematics and Ecology. 2013; 51:19-27.
7. Powell W, Morgante, Andre C, Hanafey M, Vogel J, Tingey S, Rafalasky A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding. 1996; 3:225-238.
8. Rohlf FJ. NTSYS-pc: Numerical Taxonomy System Ver. 2.1. Exeter Software, Setauket, New York. 2000, 44.
9. Singh DK, Tewari R, Singh NK, Singh SS. Genetic diversity in cucumber using Inter Simple Sequence Repeats (ISSR). Transcriptomics doi:10.4172/2329-8936.1000129. 2016; 4:129.
10. Sneath PHA, Sokal RR. Numerical Taxonomy: The Principles and Practice of Numerical Classification. San Francisco, Freeman. 1973, 573.
11. Staub JE, Serquen FC, McCreight JD. Genetic diversity in cucumber (Cucumis sativus L.): III. An evaluation of Indian germplasm. Genetic Resources and Crop Evolution. 1997; 44:315-326.
12. Wang YH, Joobeur T, Dean RA, Staub JE Cucurbits. In: Kole, C. (ed.), Genome Mapping and Molecular Breeding in Plants. Springer Verlag Berlin-Heidelberg. 2007, 315-329.