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GENETIC DIVERSITY IN 6 LOCAL CUCUMBER VARIETIES (CUCUMIS *SATIVES*) IN KARNATAKA MARKET BY RAPD-PCR TECHNIQUE

Latha, K.
Department of Biotechnology, Government Science College, Hassan-573201, Karnataka, India

ABSTRACT
Local varieties are genetically diverse and well adapted to local agro-ecosystems. Local varieties are closely associated with the livelihoods of the farmers.DNA polymorphism among local varieties of *Cucumis satives* was assessed using RAPD Local varieties that represent diverse cucumis-types were surveyed using 12 RAPD primers. This study was aimed at finding genetic diversity among 6 local cucumber varieties available in Karnataka state, Hassan district India. The technique was employed with RAPD analysis. DNA extracted from leaves of seedlings was assessed using polymerase chain reaction (PCR) with single 10- base oligonucleotide random primers to analysis genetic difference among varieties of cucumber.The OPA-19 primer recorded the highest polymorphism (86.6%) and OPA -12 the least (10%). Electrophoresis of amplified product revealed polymorphism among genetically related varieties. Around 130 bands different bands were observed under UV light. 115 bands were polymorphic for a specific primer and can be used as differential markers. The local names, place of cultivation and phenotypic characters expressed by the varieties were not suitable for varietal differentiation. The results of this study can serve determining genetic diversity among local varieties of cucumber in Karnataka state, Hassan district. India.

KEY WORDS: RAPD, Cucumber, genetic diversity, dendrogram analysis

INTRODUCTION

The cucumber (*cucumis satives*) is a widely cultivated plant in the gourd family cucurbitaceae. The cucumber is originated in India but now is grown in most of the continents. It is used both as fruit and vegetable and one of the most important vegetable crops grown.The cucumber has been cultivated for at least 3000 years and the cucumber is listed among the foods of ancients. [15] (Nguyen Thi Lang *et al*., 2007;)studied, genetic diversity is analyzed among 14 cucumber cultivars by RAPD. [16] (Suat Sensoyand *et al*. studied 2007 ;) the genetic relationships among 56 of Turkey (Cucumis melo L.) genotypes by using RAPD markers. [**17](Zhang Haiying** and *et al.,*1998;)Studied genetic relationships of cucumber by 20 primers and generated 130 RAPD markers that were reproducible. [23] (Choudhary H and *et al.*2011) studied *C. satiuvs* var. *hardwickii* accessions were differentiated on basis of RAPD markers [**18]** (YANG Fuqiang and *et al.,* 2009**)** study indicated AFLP markers were successfully employed to study genetic diversity. The research on Isozyme and restriction fragment length polymorphisms RFLPs was done to understand genetic relationships and germplasm management in cucumber Cucumis sativus L.).[1] (Thomas Horejsi and Jack E. Staub, 1998 ;). The results and data the study indicated RAPD markers can be used for the analysis of genetic diversity and germplasm management in cucumber. There are some evidences on RFLP markers have been reported (Lopez-seze *et al*. 2002 ;) [2], (Staub *et al.,* 2004) [3]. The standard protocol of RAPD markers used for the detection of the genetic relationships of Greek cucumber varieties was also extended in the present analysis (Pavlikaki *et al.* 2004 ;) [4]. DNA polymorphism among *Cucumis melo* accessions was extensively studied with RFLPs and RAPD DNA bases analysis and it was indicated that there is a genetic diversity among melon-types. (Leah Silbersteina *et al*. 1999) [8]. A research on recombinant inbred lines (RIL) were developed from a narrow cross in cucumber (*Cucumis sativus* L) and genetic linkage and quantitative trait locus (QTL**)** was studied. (G. Fazio *et al*., 2003) [7]. The study revealed on the Variation at isozyme and random amplified polymorphic DNA (RAPD) loci in eight cucumber and seven melon cultivars, breeding lines, and plant introductions were used to determine the utility of these markers for assessing genetic variation among populations of each species. (Jack et *al*..1997) [6]. Genetic diversity among 26 cucumber (*Cucumis sativus* L. var. *sativus*) accessions from five African countries Algeria, Egypt, Ethiopia, Kenya , Libya, present in the U.S. National Plant Germplasm System were examined by assessing variation at 71 polymorphic random amplified polymorphic DNA (RAPD) loci. (Ahmed Mliki *et al*., 2003)[5].

Cucumber cultivar is extremely important both for cultivation and breeding of crop plants. Cultivar variety identification based on morphological character can be difficult and complicated. Polymerase chain reaction technologies such as Random Amplified Polymerase DNA (RAPD) readily and quickly identify cultivars

using seeds and young leaves. With these backgrounds the objective of the study was set to find out genetic variation and diversity of local cultivated varieties of cucumber in local area of Karnataka State in India.

MATERIALS AND METHODS

Market survey and sample collection.

Cucumbers are available in market in Hassan. Seed were collected from farmers nearby villages. Some cultivated varieties were purchased in clean zip-lock bags and taken to the laboratory for further studies. As part of the market survey, the traders were asked specific questions to found the locations, where they are transported from and local names for each cucumber variety. (S1) Local green variety, 2(S2)Dharwad green variety, 3(S3)Short green variety,4(S4)Local short green(mullu southe)variety, 5(S5) local white (yare southe) variety, 6(S6) Local large green (bhud southe) variety.

Table-1

Sl No	Plant Material
S1	Local green
S2	Dharwad Green
S3	Short Green Variety
S4	Local Short Green(mullu southe)
S5	Local White(yare southe)
S6	Local large green (Bhudu Southe)

Plant material and DNA extraction

Six different cultivated and commercial verities of cucumber were collected from local farmers Hassan District, Karnataka, India for DNA extraction. Genomic DNA from fresh young leaves of seven day old seedlings was extracted according to modified CTAB (mCTAB) method (Seon-Kap *et al*. 2000;)[9] Leaves were folded into 10-15 cm sections and were placed in aluminum foil along with the tag identifying the sample. It was then placed in container with ice to keep samples cool during the transit. The leaf samples were stored at -80ºC until they were ground.

DNA Quantification

UV Spectrophotometric method: Nucleic acids have an absorption maximum at 260nm. Most samples contain contaminates such as proteins and single stranded DNA/RNA that absorb maximally at 280nm. The equation for calculating DNA in the presence of contaminates is A [260] /A [280] = pure dsDNA , The higher the ratio, the more pure the DNA sample. It is acceptable to have a ratio between 1.8 and 2.0.

Agarose gel electrophoresis method:

0.8% agarose gel was prepared and DNA (2 µl) was loaded with standard check (λ DNA) and electrophoresed for 1-½ hours. The intensity of bands was compared to assess relative concentration of DNA in each of the samples.

Amplification of DNA using Polymerase Chain Reaction

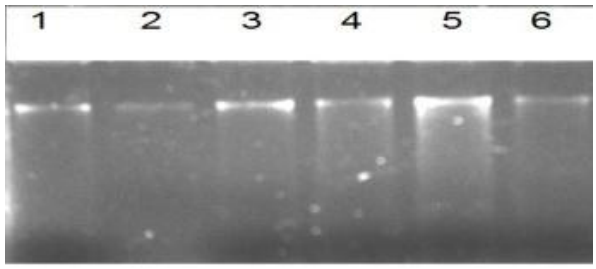
(PCR): Amplification of selected region from a complex DNA mixture was carried out in vitro by the PCR method (Darelle Thomson and Robert Henry, 1993;)[10] (Corbett Gradient model)

Diversity analysis of samples using agarose gel electrophoresis

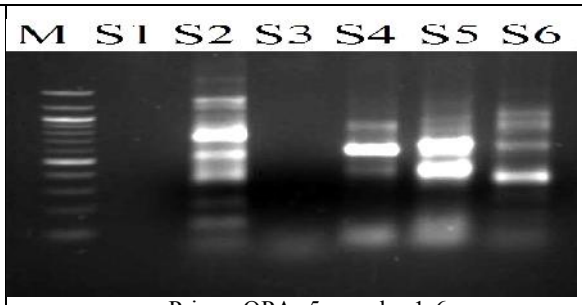
DNA band scoring and interpretation for genotypic data. There are different types of interpretation and scoring data following the banding pattern for different types of PCR amplification

RESULTS AND DISCUSSION

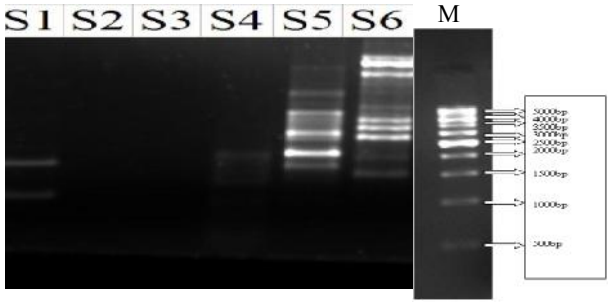
DNA Isolation from Cucumber leaves 1-6



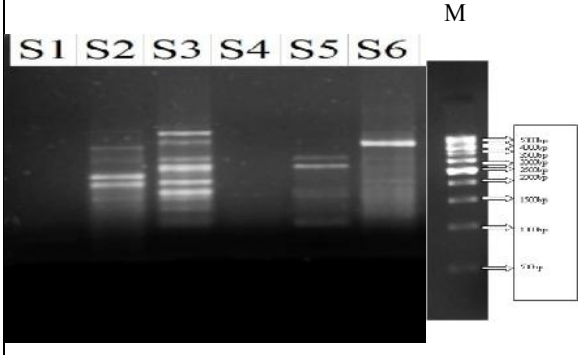
RAPD BAND PATTERN,Primer OPA4: Samples: 1-6



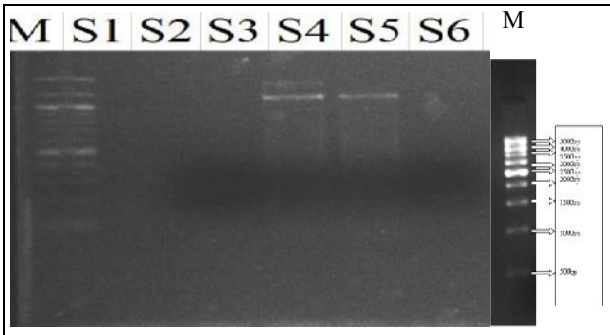
Primer OPA -5 samples 1-6



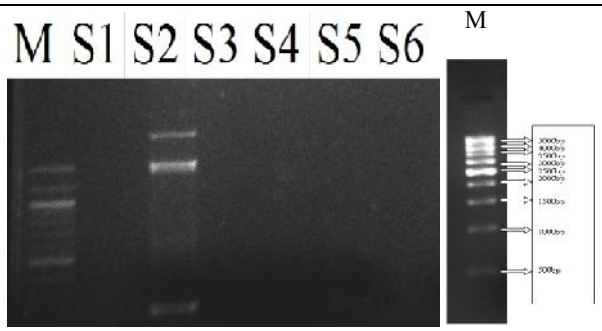
Primer OPA -5 samples 1-6



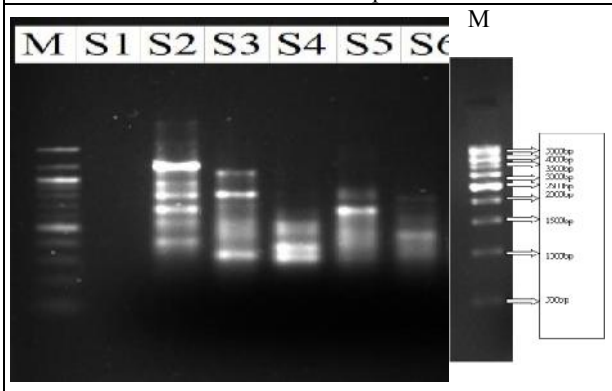
Primer OPA11: Samples: 1-6



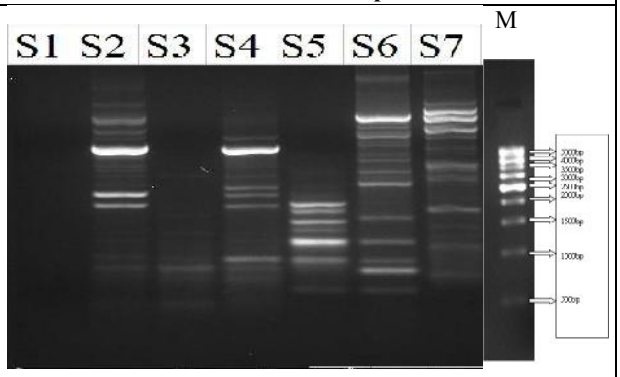
Primer OPA12: Samples: 1-6



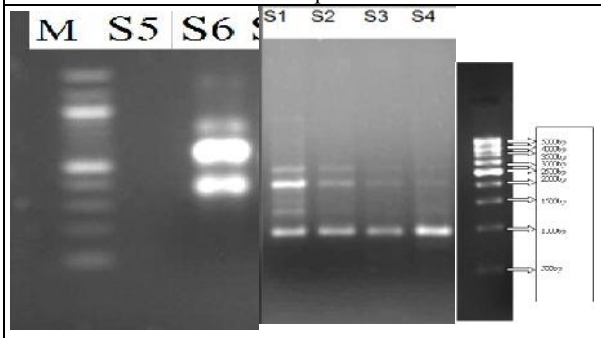
Primer OPA13: Samples: 1-6



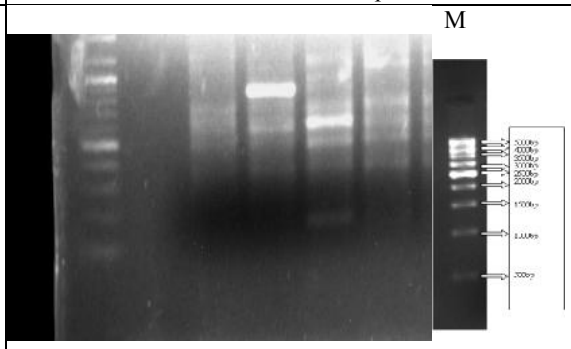
Primer OPA18: Samples: 1-**6**



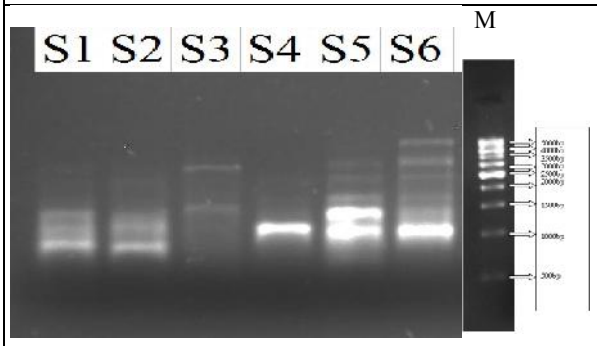
Primer OPA19: Samples: 1-6



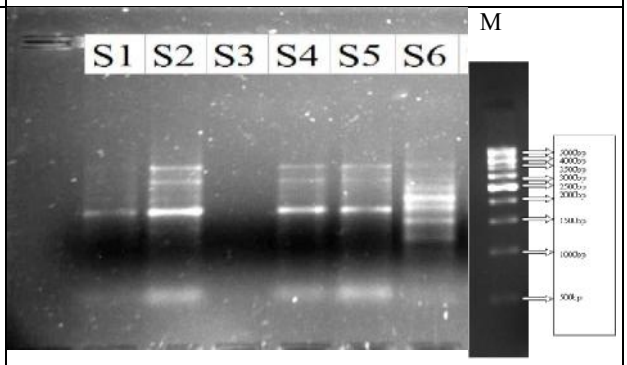
Primer OPU2: Samples: 1-6



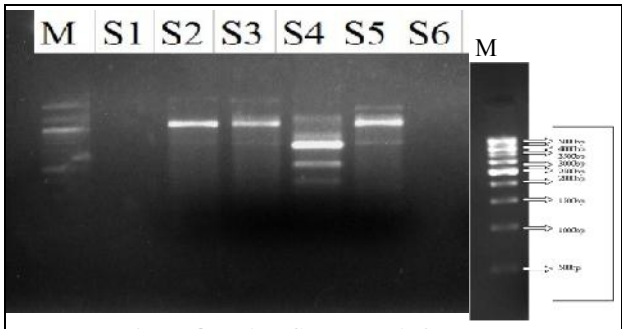
Primer OPU11: Samples: S1 S2 S3 S4 S5 S6



Primer OPU13: Samples: 1-6



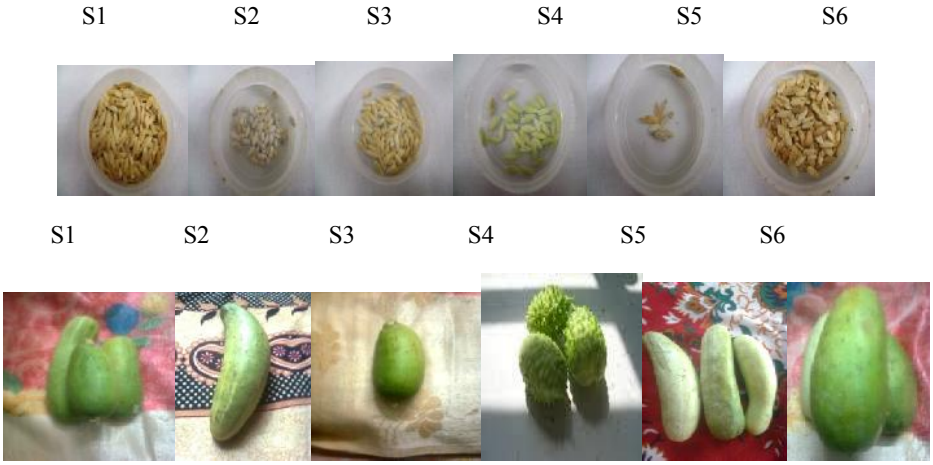
Primer OPU16: Samples 1-6



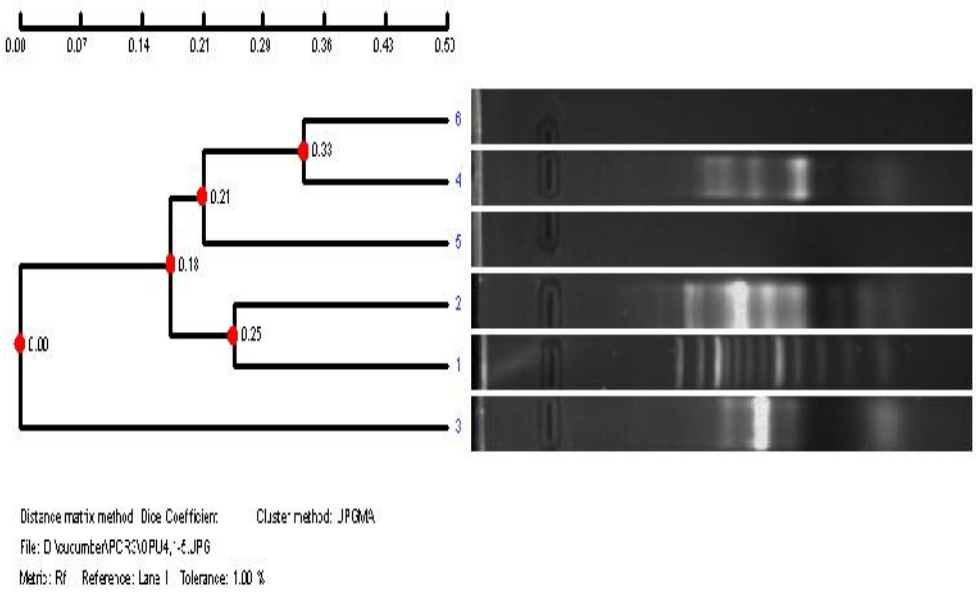
Primer OPU17: Samples: 1-6

TABLE 2: List of RAPD primers that showed polymorphism. (Operon technologies)

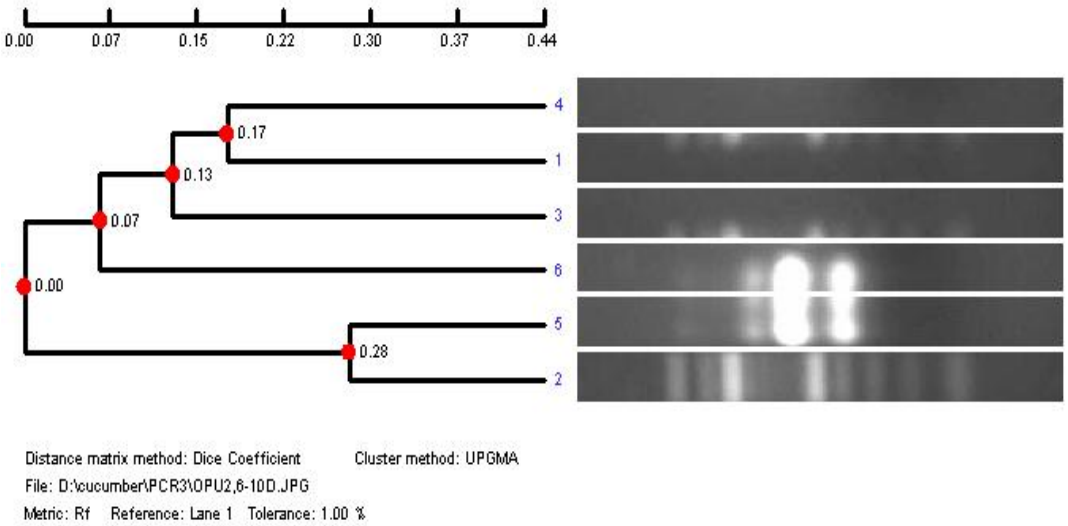
Primer code	Sequence
1 OPA- 4	5'-AATCGGGCTG -3'
2 OPA- 5	5'- AGGGGTCTTG -3'
3 OPA-11	5'- CAATCGCCGT -3'
4 OPA-12	5'-TCGGCGATAG -3'
5 OPA-13	5'-CAGCACCCAC -3'
6 OPA-18	5'- AGGTGACCGT-3'
7 OPA-19	5'- CAAACATCGG-3'
8 OPU-02	5'- CTGAGGTCTC -3'
9 OPU-11	5'- AGACCCAGAG-3'
10 OPU-13	5'- GGCTGGTTCC-3'
11 OPU-16	5'- CTGCGCTGGA-3'
12 OPU-17	5'- ACCTGGGGAG-3'



Dendrogram of 6 samples with primer OPA - 4



Dendrogram of 6 samples with primer OPU-2



RESULTS AND DISCUSSION

DNA polymorphism was assessed by RFLPs and RAPDs in thirteen melon-types varieties using 18 primers.Study indicated largest difference among melon-types occurred between *C. melo* var. *momordica* from India, (**Leah Silberstein** et a1.999;*)*[12].Reported that DNA (RAPD) was used to detect seed genetic purity of a new commercial hybrid cultivar variety 'Biyu' in cucumber. Tried 395 primers, and reported 44 were found to be useful for detecting genetic purity identification of cucumber hybrid seeds. **(Yongjian,Xu Yong,Ouyang Xinxing, *et al.*1998;) [14]. [19](Zhuang Feiyun and *et al.*2003;)studied** the relationship of cucumber by RAPD markers in Cultivated Cucumber and Wild Cucumis Species, [20] (Rajiv Krishna and *et al.*2011;)Studied genetic diversity of 13 *Cucumis* genotypes by ISSR Markers finger printing analysis grouped them into six clusters. **[21](Ma Yu-hua Gai Jun-yi 1979 ;) studied** quantitative characters of local soybean varieties.[24](Xu, D. H. and Gai, J. Y. 2003;), studied genetic interrelationship of wild and cultivated soybeans growing in China. This study indicates that geographical differentiation plays a important role in the genetic differentiation of both wild and cultivated soybeans. [22] (Diaga Diouf1 and *et al.* 2005 ;) Genetic diversity of local varieties of cowpea was studied by using RAPD microsatellite (SSR) techniques relationships among genetic lines was assessed, microsatellite markers are promising in determining the relationship among cowpea accessions.

The 12 primers were used to study the polymorphism in cucumber varieties out of which OPA-19 produced maximum 35 polymorphic bands. The least amplification that is single monomorphic band was observed with OPA-12. OPA-4 amplification was observed in all the varieties except in S1 and S3. Totally 1 monomorphic and 12 polymorphic bands were observed this primer can employ for varietal identification of S2, S4, S5, and S6. OPA-5 amplification was observed only two varieties S5, and S6. No amplification was observed in other varieties. Totally single monomorphic band 17 polymorphic and were observed. This primer can be employed for varietal identification of S5, and S6. With OPA-11 amplification was observed only in S2, S3, and S5 single band in S6 Totally 1 monomorphic and 14 polymorphic bands were observed. This primer can employed for varietal identification of S2, S3, and S5, The amplification with OPA-12 was not observed.Single band was observed in S4, S5,.Totally no polymorphic and 1 monomorphic band was observed in S4 and S5.It cannot be used as molecular marker, for identification of these selected local varieties. Mostly the first report for using RAPD analysis for varietal idenfication in cucumber reported by (Temiesak P**,** *et al 1993*) [11] indicated higher similarity between C4 cucumbers improved varieties than the two local varieties. In OPA-13 amplification was observed only in S2, No amplification was observed in other varieties. 2 polymorphic bands observed only in S2 and, So it can be used as molecular marker, for identification S2,.With OPA-18 amplification was observed in all the varieties except S1. Single monomorphic band was observed and 14 polymorphic bands were observed it can be used as differential marker for the identification of S2, S3, S4 and S5. With OPA-19 maximum amplification was observed in all the varieties except S1, where no amplification was observed. Totally 32 bands were observed in which 3 monomeric bands and 29 bands were polymorphic. Hence it can be efficiently used for varietal identification of cucumber.Among OPA series we have tried, only in OPA-19, maximum amplification was observed.OPU-2 amplification was observed in all the varieties except S5. Totally 4 monomeric bands in S1, S2, S3, S4, and 3 polymorphic bands were observed.Hence it can be efficiently used for varietal identification of cucumber of S6. OPU-11 amplification was observed in S2, S3, S4.and S5 Totally no monomorphic band was observed and 2 polymorphic bands were observed. Hence it can be successfully used as molecular marker, for the identifying of S2, and S3, OPU-13 amplification was observed in all the varieties.Totally 1 monomorphic and 9 polymorphic bands were observed. Hence it can be successfully used as molecular marker, for the identifying of all the

varieties. OPU-16 amplification was observed in all the varieties except in S3, Totally 1 monomorphic and 7 polymorphic bands were observed. This primer can be successfully employed for varietal identification of S6, OPU-17 amplification was observed in all the varieties except in S1, S6, Totally1 monomorphic and 4 polymorphic bands were observed. This primer can be successfully employed for varietal identification of S4. Comparision between a molecular and a morphological characterization of 41 seed samples belonging to 36 cucumber varieties was done and the results indicated that molecular characterization is not offering the same uniformity and relations between varieties as in morphological characterization.(Bernet, et *al.*(2003) [13]

Dendrogram Analysis

The dendrogram of 6 different Cucumber species based on UPGMA cluster analysis of RAPD data was generated using 12 arbitrary primers. dendrogram of 6 samples with Primer OPA-4. The results of the cluster analysis are shown by a dendrogram, which lists all of the samples and indicates at what level of similarity any two clusters were joined. The x-axis is some measure of the similarity or distance at which clusters join. In the dendrogram shown above, with OPA-4 samples S6-S4 are the most similar and join to form the first cluster, followed by samples S2-S1 .The last three clusters to form are S4-S5-S2-S3 these Clusters may join pair wise, such as the joining of S4-S6 and S2-S1. Alternatively, individual samples may be sequentially added to an existing cluster, such as the join of S5 with S6-S4 followed by the joining of S4-S5 and S2-S1. Lastly the join of S3 with all the above which shows no similarity with any of the above joins. Such sequential joining of individual samples is known as chaining. In the above second dendrogram of 6 samples, with OPU-2 the pair wise cluster is formed between the samples S4 and S1, S3-S6 which show the maximum similarity. Followed by the finally sub sequential cluster is formed which shows similarity between S5 and S2.

Conclusion

Local varieties have multiple benefits, including low input requirements, superior culinary and nutritional qualities, and specific adaptation to marginal areas with little or no access to chemical fertilizer inputs; these have all contributed to the continued cultivation of local varieties. In contrast traditional landraces or local varieties can perform more reliably under poor conditions, and incur few of the additional input costs, such as inorganic fertilizers, required by many improved modern varieties.6 varieties were used for RAPD marker polymorphism with 12 primers. The 12 primers produced a total of 130 bands were observed. 15 monomorphic and 115 polymorphic fragments were observed. A total and mean character difference matrix was calculated based on the RAPD data and a dendrogram was constructed using the unweighted pair-group method with arithmetic averages (UPGMA).Results demonstrated that RAPD markers could be effectively used for the identification of cultivars. The data can be utilized for local crop development, or exchange of varieties, their maintenance and utilization, their enhancement seed multiplication, processing and storage. Analysis of RAPD data was generated using 12 arbitrary primers suggests that varietal identification can be done with RAPD technique.

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