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ASSESSMENT OF GENETIC DIVERSITY AND RELATIONSHIP AMONG SOME COMMERCIAL CUCUMBER VARIETIES AND GENOTYPES USING RAPD MARKERS

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ABSTRACT

Genetic variability among the genotypes of any species could be utilized for its improvement. PCR-based Random Amplified Polymorphic DNA (RAPD) technique was used to determine the genetic diversity and relationship among 10 cucumber varieties and genotypes. Five decamer primers were used to amplify genomic DNA and the primers yielded a total of 54 bands of which 36 bands were polymorphic and 18 bands were monomorphic. The UPGMA dendrogram based on Nei's (1972) genetic distance indicated segregation of 10 cucumber varieties and genotypes into two main clusters. Variety Joti alone grouped in cluster 1 while variety Green Master, Shahi-50, Shikha, Shila, Shital, Naogaon-5, Shohag-50, Giant Long and genotype CS-043 grouped in cluster 2. Variety Shila was very close to variety Shital with the least genetic distance (0.1712). The highest genetic distance (0.5352) was found between Joti and Naogaon-5.

Key words: Genetic distance, polymorphism, PCR, dendrogram, cucumber.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) (2n = 14), a member of the family Cucurbitaceae, is one of the oldest vegetable crop supposed to be originated in India, between the Bay of Bengal and the Himalayas (Peirce 1987). It has been known in the history for over 3000 years. *Cucumis sativus* L. is one cucumber species which has commercial importance (Nonnecki 1989).

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Plant genetic diversity is a key component of any agricultural production system. This genetic diversity or similarity can be measured through genetic markers. These have been used to determine evolutionary relationship within and between species, genera or higher taxonomic categories (Paterson *et al*. 1991). The analysis of genetic diversity and relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising *et al*. 1995). In Bangladesh, there are lot of variability among cucumber germplasms. Some of these germplasms might have resistance to biotic and abiotic stresses that would be utilized in breeding program by traditional as well as modern techniques like *Agrobacterium* – mediated gene transfer. So, there are promising scopes to utilize these variability for improvement of this crop. The basis of the RAPD method is to use PCR with short oligonucleotide primers of arbitrary (random) sequence to generate genetic markers. Because RAPD–PCR primers are not designed to amplify a specific target sequence, the amplified loci are anonymous and presumably scattered throughout the genome (Williams *et al*. 1990). RAPD analysis is advantageous over isozyme electrophoresis because it generates much greater number of loci required for genetic analysis (Kimberling *et al.*, 1996). RAPD markers can be used as supposedly unbiased and neutral markers for genetic mapping applications (Michelmore *et al*. 1991), taxonomy (Chapco et al. 1992) as well as for genetic diagnostics. To attain this aim, the present study was carried out with the objective to asses the genetic diversity at the level of nuclear DNA and to establish a relationship among ten cucumber varieties and genotypes.

MATERIALS AND METHODS

Plant materials

The investigation was carried out at the Biotechnology Laboratory under the division of Plant Breeding, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during June, 2005 to July, 2009. Ten cucumber genotypes were used in the present investigation which had diverse genetic background. There were nine varieties collected from local seed market and one advanced line collected from the Department of Horticulture, Bangladesh Agricultural University (BAU), Mymensingh (Table 1).

TABLE 1: LIST OF CUCUMBER VARIETIES AND GENOTYPES USED IN THE STUDY

Sl.	Genotypes ID	Type	Source of collection

1	Shahi-50	HYV	Metal Agro Limited
2	Shila	HYV	East-West Seed Co.
3	Shital	HYV	Namdhari Malik Seed Co.
4	Naogaon-5	HYV	Metal Agro Limited
5	Shoag-50	HYV	Metal Agro Limited
6	Jiant Long	HYV	Metal Agro Limited
7	Shikha	HYV	United Seed Store
8	Green Master	HYV	United Seed Store
9	CS-043	Advance line	Dept. of Horticulture,
10	Joti	HYV	Energypac Agro Ltd.

Genotyping of cucumber genotypes

DNA extraction was done by using the mini preparation modified CTAB method (IRRI, 1997). DNA samples were evaluated both quantitatively and qualitatively using spectrophotometer and λ (lambda) DNA (concentration marker), respectively. Each RAPD reaction was done in a volume of 10 µl containing 10X PCR buffer 1 µl, 250 µM dNTP (mix) 1 µl, 10 µM primer 2.5 µl, 25 ng/ µl DNA template 2 µl, Taq DNA polymerase 1 unit or 0.2 µl and sterile deionized water 3.3 µl. DNA amplification was performed in an oil-free thermal cycler with the following program : initial denaturation at 94^{o}C for 3 min followed by 40 cycles of 30 sec denaturation at 94^{o}C, 30 sec annealing at 37^{o}C and primer elongation or extension at 72^{o}C for 2 min. After the last cycle, a final step for 7 minutes at 72^{o}C was allow to complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4°C. The amplified products were separated by electrophoresis in 1.5 % agarose gel. After electrophoresis, the gel was stained for 20 minutes in ethidium bromide solution. DNA bands were observed under UV light on a transilluminator and photographed. Seven primers 63AB10A3, 68AB10A8, 62AB10C2, 69AB10C9, 70AB10C10, 61AB10G1 and 64AB10G4 of random sequence were screened on two different genotypes to evaluate their suitability.

RAPD data analysis

The amplified bands were visually scored on the basis of their presence (1) or absence (0), separately for each individual and each primer. The scores obtained using all primers in the RAPD analysis were then pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's (1973) gene diversity, genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method With Arithmetic Means) dendrogram among populations using

computer program, POPGENE (Version 1.31) (Yeh, *et al.*, 1999). The same program was also used to perform the test of homogeneity in different locus between population pairs.

RESULTS AND DISCUSSION

Primer selection and RAPD pattern

Out of seven decamer primers five primers i.e. 63ABIOA3, 68AB10A8, 62AB10C2, 69AB10C9 and 61AB10GI which gave reproducible and distinct polymorphic amplified products were selected. Selected five primers exposed 54 bands. Out of 54 bands, 36 bands (66.67 %) were polymorphic and 18 bands (33.33 %) were monomorphic (Table 2). In an investigation of cucumber, Joyanti (2007) obtained 95.64 % polymorphic bands from big cucumber group (sosha) and 88.64 % polymorphic bands from small cucumber group (khira) using the same DNA primers. Five different primers generated various banding patterns, ranging from 10 (68AB10A8 and 61AB10G1) to 12 (69AB10C9). The primer 69AB10C9 generated the highest number of polymorphic bands (11). Thus it showed a higher level of polymorphism. On the other hand, the primer 68AB10A8 and 62AB10C2 produced the lowest number (4) of polymorphic bands (Table 2).

TABLE 2: RAPD PRIMERS WITH THEIR CORRESPONDING POLYMORPHIC BANDS OBSERVED IN CUCUMBER GERMPLASMS

Primer codes	Sequences (5′-3′)	Number of bands	Number of polymorphic bands	Polymorphic loci (%)
63AB10A3	GTCGCCGTCA	11	9	66.67
68AB10A8	GTGTGCCCCA	10	4	
62AB10C2	GGACCCAACC	11	4	
69AB10C9	CTCTGGAGAC	12	11	
61AB10G1	ACCGCGAAGG	10	8	
Total	-	54	36	
Average	-	10.8	7.2	

The banding patterns of different cucumber varieties and genotypes using primers 63AB10A3 and 68AB10A8 are shown in Figures 1 and 2**,** respectively. On an average each primer generated 10.8 scorable bands and 7.2 polymorphic bands per primer. Dark and light bands were produced from the RAPD reactions. Light bands produced from low homology between the primer and the pairing site

on the DNA strand (Thormann et al., 1994). In present investigation, the percentage of polymorphic loci was 66.67 %. A diverse level of polymorphism in different crops has been reported by Ashrafuzzaman (2007) (95.64 % in rice), Moonmoon (2006) (90.19 % in tomato) and Joyanti (2007) (95.64 % and 88.64 %, respectively in large and small group cucumber).

Polymorphism and Nei's gene diversity

The highest percentage polymorphic loci (18.52 %) was found in Naogaon-5 which gave 10 polymorphic loci whereas the lowest percentage (11.11%) polymorphic loci were observed both in Shahi-50 and Shital which gave 6 polymorphic loci (Table 3). The variety Naogaon-5 showed the higher level of gene diversity (0.0904) than that of other tested varieties and genotypes. Gene diversity (h) across all varieties and genotypes for all loci was 0.6834. Shahi-50 showed the lowest (0.0507) gene diversity. Shannon's information index (I) of all cucumber varieties and genotypes was 0.9548. The highest and the lowest I value were observed in variety Naogaon-5 (0.1261) and Shahi-50 (0.0719), respectively (Table 3). Since the variety Naogaon-5 exhibited the highest percentage polymorphic loci, gene diversity and shannon's information index, these suggested higher polymorphism. The above results indicated that plant breeder could utilize this variety (Naogaon-5) for breeding program. Estimation of Nei's (1973) genetic diversity and Shannon's information index across all loci supports the existence of high level of genetic variations among 10 cucumber varieties and genotypes.

Intra and inter genotype similarity indices

Intra genotype similarity indices (S_i) were higher ranging from 88.00 to 93.80 % with an average of 91.54% than inter variety similarity indices (Sij) ranging from 57.20 to 86.80 % with an average of 71.48 %. The highest intra genotype similarity indices (S_i) were observed both in Shahi-50 and CS-043 (93.80 %), and the lowest intra genotype similarity indices was found in Shikha (88.00 %). The highest inter genotype similarity indices (Sij) was found between Shila and Shital (86.80 %) and the lowest inter genotype similarity indices or band sharing value was observed between Naogaon-5 and Joti (57.20 %).

TABLE 3: ESTIMATION OF GENETIC VARIATION, PERCENTAGE OF POLYMORPHIC LOCI, NEI'S (1973) GENE DIVERSITY (H), AND SHANNON'S INFORMATION INDEX (I) OBTAINED FROM 10 CUCUMBER VARIETIES AND GENOTYPES

Genotype	Number of polymorphic loci	Percentage of polymorphic loci	Gene diversity (h)	Shannon's Information index (I)
Shahi-50	6	11.11	0.0507	0.0719
Shila	7	12.96	0.0633	0.0883
Shital	6	11.11	0.0542	0.0757
Naogaon-5	10	18.52	0.0904	0.1261
Shohag-50	8	14.81	0.0723	0.1009
Giant long	7	12.96	0.0633	0.0883
Shikha	8	14.81	0.0723	0.1009
Green Master	8	14.81	0.0723	0.1009
CS-043	7	12.96	0.0633	0.0883
Joti	9	16.67	0.0813	0.1135
Total	-	-	0.6834	0.9548

Gene flow and population differentiation

The average estimated gene flow (Nm) was 0.1386 and co-efficient of gene differentiation (Gst) was 0.7830 across all primers. Random amplified polymorphic DNA marker 68AB10A8 showed low level of differentiation (Gst) (0.3533) in the studied varieties and genotypes.

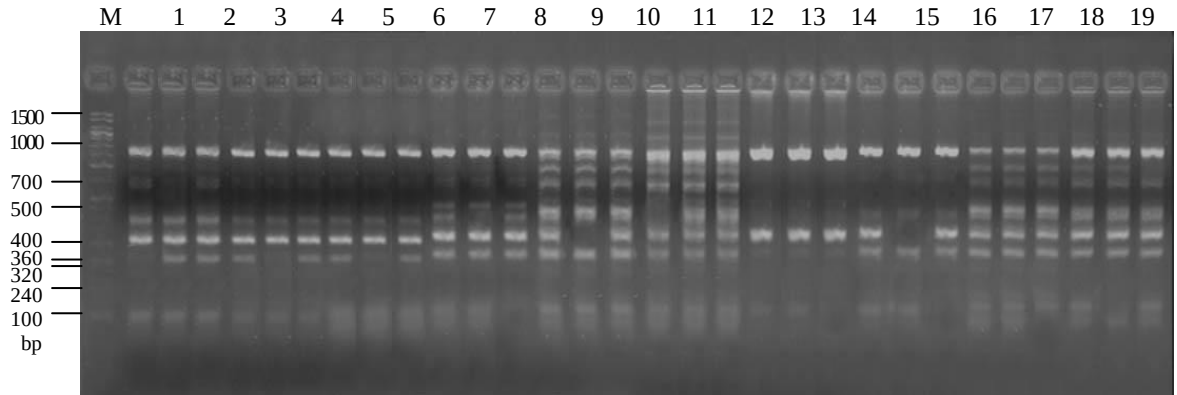

FIG. 1. RAPD PROFILES OF 10 CUCUMBER GERMPLASMS USING PRIMER 63AB10A3. LANE 1-3: SHAHI-50, 4-6: SHILA, 7-9: SHITAL, 10-12: NAOGAON-5, 13-15: SHOHAGH-50, 16-18: GIANT LONG, 19-21: SHIKHA, 22-24: GREEN MASTER, 25-27: CS-043, 28-30: JOTI. M: MOLECULAR WEIGHT MARKER (20 BP DNA LADDER)

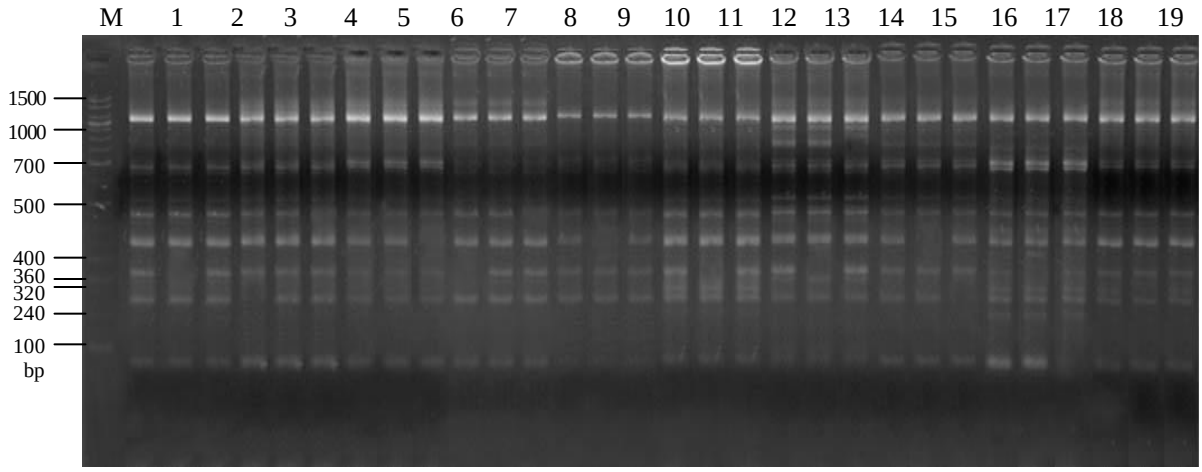

FIG. 2. RAPD PROFILES OF 10 CUCUMBER GERMPLASMS USING PRIMER 68AB10A8. LANE 1-3: SHAHI-50, 4-6: SHILA, 7-9: SHITAL, 10-12: NAOGAON-5, 13-15: SHOHAGH-50, 16-18: GIANT LONG, 19-21: SHIKHA, 22-24: GREEN MASTER, 25-27: CS-043, 28-30: JOTI.M: MOLECULAR WEIGHT MARKER (20 BP DNA LADDER)

Genetic distance

The values of pair-wise comparisons of Nei's (1972) genetic distance (GD) among 10 cucumber varieties and genotypes computed from combined data from the five primers ranged from 0.1712 to 0.5352 (Table 4). The

highest genetic distance (0.5352) was found between variety Joti and Naogaon-5 and the lowest (0.1712) GD was observed between variety Shital and Shila. From the difference between the highest and the lowest GD value it was revealed that there were wide variabilites among 10 cucumber varieties and genotypes. Mliki *et al.* (2003) reported different GD of 3 groups of African cucumber accessions. They mentioned that GD of group 1 ranged between 0.52 to 0.90, distances among group 2 accessions varied between 0.93 to 0.97 and the GD in group 3 was 0.65. Joyanti (2007) found GD ranged between 0.04 to 0.788 in sosha (large cucumber group) and GD ranged from 0.023 to 0.83 in Khira (small cucumber group). High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that variety Joti and Naogaon-5 showed the highest genetic variation, while the lowest genetic variation was observed between variety Shital and Shila, which can be used as parental source for breeding line to improve cucumber varieties. Moonmoon (2006) reported that for the assessment of genetic diversity, molecular markers were superior to morphological, biochemical and other method like pedigree and heterosis.

UPGMA dendrogram

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method with Arithmetic Means (UPGMA) indicated segregation of ten cucumber varieties and genotypes into two main clusters. Variety Joti alone formed cluster 1 and variety Green Master, Shahi-50, Shikha, Shila, Shital, Naogaon-5, Shohag-50, Giant Long and the genotype CS-043 grouped in cluster 2 (Fig. 3). In cluster 2 variety Green Master, Shahi-50, Shika, Shila, Shital, Naogaon-5 and Shohag-50 formed sub cluster-1 and variety Giant Long and genotype CS-043 formed sub cluster-2. Again in sub cluster-1 variety Green Master, Shahi-50 and Shikha formed sub sub cluster-1, and variety Shila, Shital, Naogaon-5 and Shohag-50 formed sub sub cluster-2. Again in sub sub cluster-1, variety Green Master alone formed group 1 and variety shahi-50 and shikha formed group 2, respectively and in sub sub cluster-2, variety Shila and Shital formed group 3 and variety

TABLE 4: SUMMARY OF NEI'S (1972) GENETIC DISTANCE (BELOW DIAGONAL) VALUES AMONG 10 CUCUMBER VARIETIES AND GENOTYPES

Genotype	Shahi-	Shila	Shital	Naogaon-5	Shohag-50	Giant	Shikha	Green	CS-	Joti

	50					Long		Master	043	
Shahi-50	****									
Shila	0.2148	****								
Shital	0.2633	**0.1712**	****							
Naogaon-5	0.3156	0.2334	0.2231	****						
Shohag-50	0.3239	0.4128	0.2126	0.2045	****					
Giant Long	0.4285	0.4103	0.5278	0.3930	0.3877	****				
Shikha	0.2052	0.2609	0.3816	0.4993	0.3878	0.3042	****			
Green Master	0.2723	0.2636	0.2970	0.3855	0.3559	0.3401	0.2525	****		
CS-043	0.3727	0.4774	0.5117	0.4184	0.3684	0.2170	0.2735	0.3501	****	
Joti	0.3549	0.3974	0.4446	**0.5352**	0.3985	0.4784	0.4657	0.4138	0.4884	****

Naogaon-5 and Shohag-50 formed group-4, respectively. Variety Shila was closer to the variety Shital with the least GD (0.1712), so they fall under group 3 and the highest GD (0.5352) was found among Joti and rest of the varieties and genotypes. That's why Joti alone formed one cluster and rest of varieties and genotypes formed another cluster. Except variety Joti other varieties and genotypes fall under cluster 2. These varieties and genotypes were probably identical based on some morphological characters. Variety Green Master, Shahi-50, Shikha, Shila, Shital, Naogaon-5 and Shohag-50 formed sub cluster-1, so that they were maintaining the closest genetic relationship. Similarly variety Giant Long and genotype CS-043 formed sub cluster-2, so that they had also close genetic relationship. In an investigation of molecular characterization of cucumber through RAPD maker Joyanti (2007) reported that two types of cucumber namely sosha (large cucumber group) and khira (small cucumber group) formed two different dendrogram. Regarding sosha germplasms, they were distinctly divided

into two major clusters A and B. Cluster A comprised the largest number (16) of germplasms and the rest formed cluster B. On the other hand, khira germplasms also divided into two major clusters. Mliki *et al.* (2003) arranged 26 African cucumber germplasms into 3 major groups. Group 1 consisted of 21 accessions (Egypt, Ethiopia and Libya), group 2 consisted of two accessions (Kenya and Algeria) and group 3 possessed three accessions (Egypt). These groups were different from each other (P>0.001). Accessions in group 1 differed genetically from all other accessions examined (P>0.01) and accessions of group 2 and 3 were uniquely associated with several RA accessions. In an investigation, Zhang *et al.* (1998) classified 34 cucumber germplasms into 3 groups viz. China group, South China group and European greenhouse group. From this investigation, it was revealed that the highest genetic diversity remains between variety Joti and Naogaon-5. On the other hand, the lowest genetic diversity was observed between the variety Shila and Shital. These findings indicated that variety Joti vs variety Naogaon-5 and variety Shila vs variety Shital could be used in plant breeding program for development of cucumber variety. RAPD markers provide a fast and efficient tool for genetic variability assessment and currently using in plant genetic resources management.

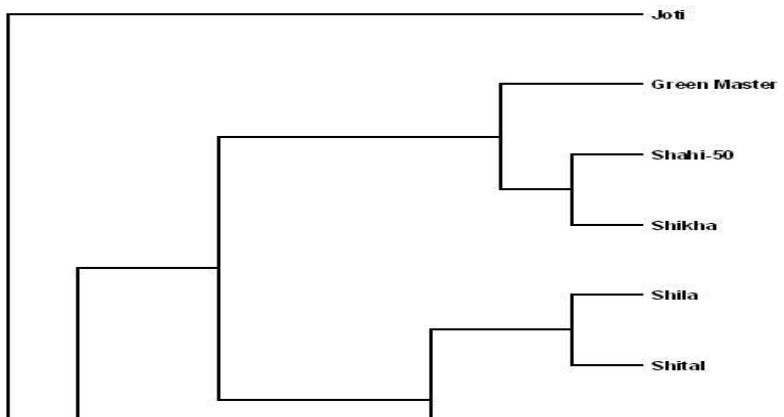

FIG. 3. UPGMA DENDROGRAM BASED ON NEI'S (1972) GENETIC DISTANCE SUMMARIZING THE DATA ON DIFFERENTIATION AMONG TEN CUCUMBER VARIETIES AND GENOTYPES, ACCORDING TO RAPD ANALYSIS

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