Assessment of Genetic Relationship among Landraces of Bangladeshi Ridge Gourd (*Luffa acutangula* Roxb.) Using RAPD Markers

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Abstract

Information on genetic relatedness among ridge gourd (*Luffa acutangula*) genotypes from Bangladesh is currently not reported. Twenty eight accessions collected from different parts of Bangladesh were studied using random amplified polymorphic DNA (RAPD) technique. Four selected decamer primers, out of sixteen screened, could generate a total of 27 RAPD fragments of which 22 were polymorphic (81.5%). The bands ranged from 50 to 1500 bp in size. Genetic variation statistics for all loci estimated the average gene diversity (h) value as 0.278 and the Shannon's Information Index (I) as 0.415. Dendrogram based on unweighted pair-group method with arithmetic averages (UPGMA) segregated the accessions into five clusters. Cluster III was the largest with 13 members followed by cluster II, V, I and IV with 6, 4, 3 and 2 members, respectively. Accession LA27 and LA29 were found very close to each other with the highest inter-variety similarity index (96.05%) and the lowest genetic distance (0.077); whereas accession LA40 and LA72 were more distant to each other with the lowest inter-variety similarity index (44.43%) and the highest genetic distance (0.73). A DNA extraction method has been standardized. The marker was found to be useful tool for assessing genetic variations in *Luffa acutangula*.

Keywords: Genetic relationship; Germplasm; Ridge gourd; *Luffa acutangula;* RAPD.

1. Introduction

Ridge gourd (*Luffa acutangula* Roxb.) is an important member of the family Cucurbitaceae grown in Bangladesh as a year-round vegetable. It is nutritionally rich in vitamin A, C and Fe [1]; and has a considerable medicinal importance. Its abortifacient, antitumor, ribosome inactivating and immunomodulatory activities were reported earlier [2-4]. Recently it has gained attention from the nutritionists due to the presence of antioxidant (free radical scavenging-FRS) activity [5]. Effectiveness of its extract as larvicide [6] and its seed oils as grain protectant against certain insects [7] are not very far discoveries. Many wild relatives of this crop are grown in Bangladesh. So far, 106 local

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landraces were reported [8]. The average yield of the crop in Bangladesh, which was about 1.83 mt per acre in the year 2007-2008 [9], indicates low yield potentiality of the cultivars. The lack of high yielding variety, among many reasons, is an important one for such a low yield of this crop in Bangladesh. In a crop improvement programme, genetic diversity is one of the important tools to quantify genetic variability in both cross and self pollinated crops [10]. Polymerase chain reaction (PCR) technology had led to the development of several novel genetic assays based on selective DNA amplification. RAPD assay detects nucleotide sequences of polymorphic DNA using only a single primer of arbitrary nucleotide sequence. This technique always allows the examination of genomic variations without prior knowledge of DNA sequences, and is especially useful for revealing variations in a species with low genetic variability. In spite of some weaknesses, the relative case and speed, the high degree of polymorphism and the virtually inexhaustible pool of possible genetic markers make the technique advantageous over other molecular approaches. Growing interest to this vegetable crop (ridge gourd) was achieved through the studies on its constituents, but no systematic research on molecular basis has so far been done to evaluate the genetic potentialities of the available germplasm with a view to releasing modern varieties to meet up increasing demands to come. Under such circumstances, this study was conducted to identify variations within each of the ridge gourd germplasm under study, so that genetic relationship can be established at gene level using RAPD markers.

2. Materials and Methods

2.1. *Plant materials*

A total of 28 ridge gourd germplasm were used in this study. The materials were collected from different parts of Bangladesh by the Dept of Horticulture, Bangladesh Agricultural University, Mymensingh under a USDA-funded (BG-ARS-108) project entitled CVFB (Collection, Evaluation, Conservation and Utilization of Landraces and Wild Relatives of Some Important Vegetables and Fruits of Bangladesh).

2.2. *Genomic DNA isolation*

The total genomic DNA was isolated from young leaf tissue following the procedure described by Murray and Thompson [11]. The purity of DNA was confirmed by 1% agarose gel electrophoresis and the quantity was estimated by spectrophotometer at a wavelength of 260 nm using the Spectronic® Genesis™ (Spectronic Instruments Inc., USA). The final DNA concentration of each sample was adjusted to 25 ng/μl.

2.3. *DNA amplification*

After screening of 16 random primers from Operon Technologies Inc. (Alameda, California, USA), 4 decamers of oligonucleotides (OPA-04, OPA-07, OPC-02 and OPC-

06) produced clear and unambiguous bands were used for DNA amplification adopting the procedure of William *et al.* [12] with some modifications. Amplification reactions were carried out in a volume of 10 µl containing 10 mM of dNTPs (deoxynucleotide triphosphate) (2.5 mM each), 1 µl of (10×) *Taq* DNA polymerase buffer, 1 unit (U) of *Taq* DNA polymerase (Genei Pvt. Ltd., Bangalore, India), 0.5 µM of 10 mer primer, 100 ng (4 µl) of genomic DNA and a suitable amount of sterile deionized water. Amplifications were performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf) programmed for an initial denaturation at 94ºC for 5 min, 40 cycles of 30 sec denaturation at 94ºC, 30 sec annealing at 40ºC and 1 min extension at 72ºC, followed by final extension for 10 min at 72ºC. Reactions were held at 4^0C after completion of cycling. The amplified products plus loading dye were subjected to horizontal gel electrophoresis using 1.4% agarose gel in 1×TBE (89 mM Tris-Borate, 3 mM EDTA) buffer at 100 V for 1 hr using Genei Submarine Electrophoresis System. We used pUC18 marker and 100 bp DNA ladder (Genei Pvt. Ltd., Bangalore, India) as molecular size marker. The ethidium bromide stained gels were documented using UV Transilluminator (UVP Ltd., Trinity hall estate, Cambridge, UK).

2.4. *Data analysis*

All distinct RAPD bands were given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0) separately for each individual and each primer. The scores were then pooled to construct a single data matrix. This was used for estimating polymorphic loci, Nei's [13] gene diversity, Shannon's Information index [14] and the UPGMA dendrogram of the populations based on Nei's [15] genetic distances using the POPGENE (Version 1.31) [16] software package.

The similarity index values (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers according to the following formula:

Similarity index (SI) = $2 N_{xy}/(N_x + N_y)$

where N_x = the total number of fragments detected in individual '*x*'; N_y = the total number of fragments shown by individual '*y*' and N_{xy} = the number of fragments shared by individuals '*x*' and '*y*' [17]. Between population similarity (Sij) was calculated as the average similarity between randomly paired individuals from populations *i* and *j* [18].

3. Results and Discussion

3.1. *Polymorphism*

A considerable level of variability was observed among different landraces. Fig. 1 shows the RAPD profiles generated by the primer OPC-02 across 28 ridge gourd germplasm. A total of 27 bands were generated by selected four decamer primers of which 22 were

polymorphic (81.5%). Primer OPC-02 showed the highest polymorphism (100%) with the maximum number of fragments (8) whereas Primer OPA-07 showed the lowest (71.5%). Other two primers (OPA-04 and OPC-06) produced equal (75%) polymorphism, though the later gave the minimum number of bands (4). The average number of DNA bands amplified by each primer was 6.75 ranging from 50-1500 bp in size (Table 1).
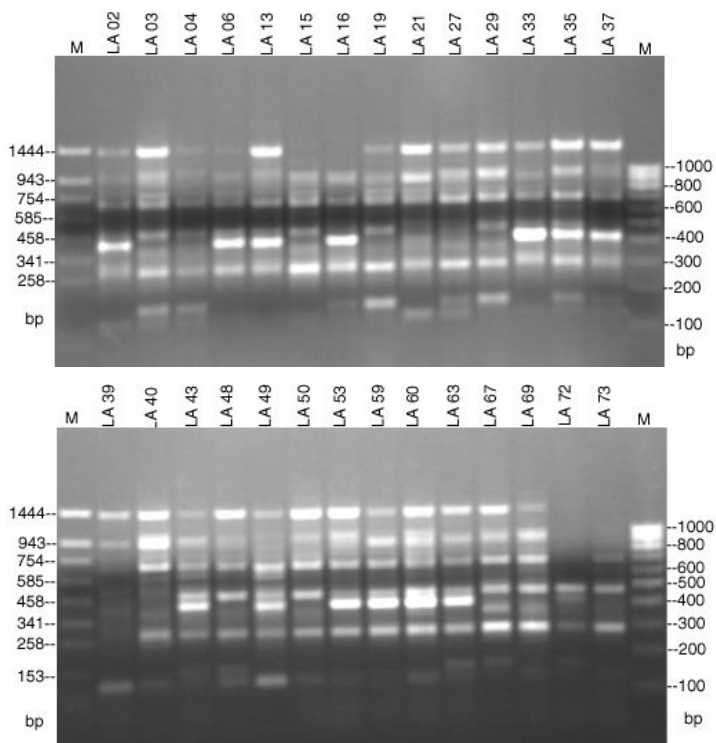

Fig. 1. RAPD marker profiles of 28 ridge gourd germplasm generated by primer OPC-02 in 1.4% agarose gel. (M: molecular weight marker-100 bp DNA ladder on right side and P^{UC} on the left)

Table 1. RAPD primers used to detect polymorphism, number of bands for polymorphism and size of the amplified fragments observed in ridge gourd germplasm from Bangladesh.

Primer code	Primer sequence (5′- 3′)	Number of scorable bands	Number of polymorphic markers	Percent poly-morphism	Size ranges (bp)
OPA-04	AATCGGGCTG	8	6	75	150-1500
OPA-07	GAAACGGGTG	7	5	71.5	50-1500
OPC-02	GTGAGGCGTC	8	8	100	100-1450
OPC-06	GAACGGACTC	4	3	75	200-1500
Total		27	22		

Average	6.75	81.5

3.2. *Genetic variation and similarity matrix*

Genetic variation statistics for all loci estimated the mean for Nei's gene diversity (h) value of 0.278 and that for Shannon's information Index (I) of 0.415. The loci OPA04-5, OPA04-6, OPA07-2, OPA07-6 and OPC06-3 were monomorphic showing nil h and I values. The maximum variation was exhibited by the locus OPA07-3 with h and I values of 0.5 and 0.693, respectively (Table 2). A similarity matrix based on the proportion of shared RAPD fragments was used to establish the level of relatedness between the germplasm. Pair-wise estimates of Similarity Indices (S_{ij}) ranged from 44.43% in Accession LA40-LA72 pair to 96.05% in Accession LA27-LA29 pair (Table 3).

Table 2. Summary of genetic variation statistics for all loci.

Locus	Nei's gene diversity (h)	Shannon's Information index (I)	Locus	Nei's gene diversity (h)	Shannon's Information index (I)
OPA04-1	0.459	0.652	OPC02-1	0.245	0.410
OPA04-2	0.490	0.683	OPC02-2	0.133	0.257
OPA04-3	0.490	0.683	OPC02-3	0.133	0.257
OPA04-4	0.191	0.341	OPC02-4	0.133	0.257
OPA04-5	0.000	0.000	OPC02-5	0.337	0.520
OPA04-6	0.000	0.000	OPC02-6	0.069	0.154
OPA04-7	0.408	0.598	OPC02-7	0.459	0.652
OPA04-8	0.408	0.598	OPC02-8	0.408	0.598
OPA07-1	0.477	0.670	OPC06-1	0.069	0.154
OPA07-2	0.000	0.000	OPC06-2	0.191	0.341
OPA07-3	0.500	0.693	OPC06-3	0.000	0.000
OPA07-4	0.497	0.691	OPC06-4	0.459	0.652
OPA07-5	0.477	0.670			
OPA07-6	0.000	0.000	Mean	0.278	0.415
OPA07-7	0.477	0.670	St. Dev.	0.197	0.267

Table 3. Summary of band-sharing based on similarity indices, S_{ij} (%) among individuals of 28 ridge gourd germplasm from Bangladesh.

Pop	LA02	LA03	LA04	LA06	LA13	LA15	LA16	LA19	LA21	LA27
LA02	***									
LA03	84.50	***								
LA04	77.40	93.08	***							
LA06	81.00	85.65	88.10	***						
LA13	86.15	74.00	69.05	80.95	***					
LA15	80.00	67.63	69.25	81.08	92.73	***				
LA16	73.30	89.50	90.18	81.25	75.60	72.75	***			
LA19	92.58	80.05	71.68	79.75	84.18	83.33	76.43	***		
LA21	73.35	85.83	85.33	80.53	82.93	75.68	88.38	72.95	***	
LA27	79.75	95.83	92.73	84.60	72.95	66.58	89.15	79.00	90.00	***
LA29	75.60	91.88	93.28	88.48	70.43	69.95	87.48	78.03	83.93	96.05
LA33	73.93	78.15	79.78	91.68	86.68	89.40	79.18	77.25	86.25	77.10
LA35	79.35	82.55	79.28	87.35	81.43	79.18	79.03	86.68	81.28	81.50
LA37	74.53	86.43	88.98	92.58	79.50	80.58	81.58	75.85	83.93	85.83

LA39	80.68	81.43	73.48	80.90	69.40	61.60	66.80	76.10	76.35	82.93

Table 3. Continued

Pop	LA02	LA03	LA04	LA06	LA13	LA15	LA16	LA19	LA21	LA27
LA40	60.95	69.45	75.60	78.93	65.85	65.85	66.78	58.45	74.13	72.63
LA43	80.00	67.38	61.70	65.28	81.25	74.50	66.68	83.30	72.78	70.55
LA48	73.33	62.40	62.58	66.08	68.58	67.63	55.95	72.35	62.08	66.15
LA49	92.30	83.90	80.00	71.43	81.43	75.00	75.90	83.93	81.08	83.55
LA50	78.75	87.30	83.80	87.30	74.50	74.18	82.75	78.33	78.28	82.40
LA53	87.83	88.68	80.65	88.08	81.43	74.98	80.05	83.65	81.43	83.93
LA59	75.58	84.15	79.78	83.35	77.10	70.35	79.18	74.60	85.58	87.90
LA60	74.85	83.33	83.75	83.50	77.85	70.85	85.90	74.78	90.18	87.08
LA63	78.10	77.50	72.45	72.20	91.25	84.00	79.35	84.85	85.83	79.40
LA67	78.15	89.58	85.83	85.58	73.08	72.60	85.58	85.23	81.65	89.23
LA69	87.58	79.75	72.00	71.73	80.90	74.45	76.40	87.30	73.08	75.58
LA72	73.40	66.40	58.33	60.83	70.00	67.60	65.83	76.93	63.00	66.05
LA73	59.70	68.83	64.18	66.68	60.43	57.73	66.68	58.45	69.13	72.00

Table 3. Continued

Pop	LA29	LA33	LA35	LA37	LA39	LA40	LA43	LA48	LA49
LA29	***								
LA33	80.53	***							
LA35	84.85	89.75	***						
LA37	89.58	91.15	87.35	***					
LA39	77.63	73.83	79.53	74.45	***				
LA40	76.20	77.50	69.95	79.28	73.03	***			
LA43	69.03	68.45	74.48	63.35	66.78	74.18	***		
LA48	69.58	63.68	69.73	64.58	66.25	70.98	79.40	***	
LA49	79.00	68.93	74.78	74.00	71.55	64.03	83.30	76.90	***
LA50	86.23	85.23	85.40	79.33	77.90	74.00	68.57	68.35	73.33
LA53	82.93	85.58	91.15	86.18	83.90	72.78	75.50	70.45	83.98
LA59	86.83	80.95	81.43	81.83	79.58	83.75	81.68	77.73	79.15
LA60	81.85	81.43	75.60	75.70	80.10	77.45	76.43	66.93	73.35
LA63	76.90	77.93	74.13	79.18	65.30	65.80	81.10	68.75	81.28
LA67	92.73	83.18	93.05	85.58	77.63	68.18	72.25	67.50	77.10
LA69	74.58	69.23	74.80	68.10	67.25	54.70	78.83	70.18	86.98
LA72	64.50	58.33	64.43	57.78	53.75	44.43	67.73	63.40	72.75
LA73	70.38	64.28	65.28	65.95	60.10	77.08	74.18	64.28	63.00

Table 3. Continued

Pop	LA50	LA53	LA59	LA60	LA63	LA67	LA69	LA72	LA73
LA50	***								
LA53	85.80	***							
LA59	81.08	87.73	***						
LA60	81.25	78.08	89.75	***					
LA63	69.95	73.10	77.25	78.28	***				
LA67	88.88	85.48	79.75	79.78	76.90	***			
LA69	74.45	79.75	70.68	70.58	80.58	81.38	***		
LA72	67.60	65.05	59.85	60.28	70.50	71.33	85.58	***	
LA73	68.45	71.33	82.50	72.78	60.80	63.60	54.28	68.18	***

3.3. *Cluster analysis*

Pair-wise comparisons of Nei's genetic distance (GD) between ridge gourd germplasm were calculated from combined data for the four primers. The highest GD (0.73) was observed between Accession LA40 and LA72, whereas the lowest (0.077) was between Accession LA27 and LA29. The UPGMA dendrogram based on Nei's genetic distance [15] segregated the populations into 5 clusters (Fig. 2). Cluster III was the largest comprising 13 populations followed by cluster II, V, I and IV with 6, 4, 3 and 2 members respectively.

A propitious natural environment for ridge gourd cultivation prevails in Bangladesh. It is a very common homestead vegetable consumed by wide range of people. Despite the nutritional as well as medicinal importance of the vegetable, information on the genetic background of this species is very scarce. We are the first in Bangladesh to report genetic variability among available landraces of *Luffa acutangula* with the help of randomly amplified polymorphic DNA (RAPD) markers. In the present study, RAPD technique has been found to be suitable in revealing genetic variability and relatedness among collected landraces of ridge gourd. It is predicted that the DNA fragments shared by two closely related individuals of a species are allelic while the fragments of equal sizes may have originated from a non-allelic genomic region [19]. The use of many RAPD primers may provide large number of species-specific RAPD markers for the analysis [20].
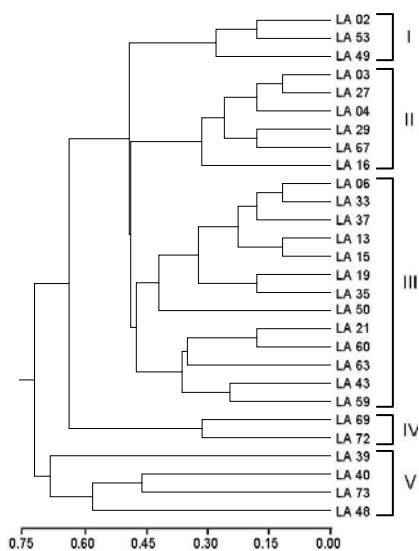

Fig. 2. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's [15] genetic distance between 28 ridge gourd germplasm according to RAPD analysis.

The proportion of polymorphic loci obtained in the present study was lower (81.5%) than that reported in teasle gourd-*Momordica dioica* Roxb. (95%) [21] but higher than that in bitter gourd-*Momordica charantia* L. (36.5%) [22], *Cucurbita maxima* (57%) [23] and melon-*Cucumis melo* (73%) [24]. Like the proportion of polymorphic loci, Nei's gene diversity and Shannon's information index were also higher, and that was expected because the samples were collected from different parts of the country covering the

maximum agro-ecological zones (AEZs) of Bangladesh. Relatively lower Similarity indices (44.43-96.05%) as compared to water melon (92-99.6%) [25] also indicated high genetic variation among collected germolasm of ridge gourd. The UPGMA dendrogram based on genetic distance (GD) grouped the populations into 5 clusters. Relatively high GD value (0.73) suggested that RAPD-based diversity assessments in this germplasm were generally consistent. The *Luffa acutangula* accessions examined were genetically distinct, and these differences provided for the development of strategies for genetic analyses and crop improvement in this species [26]. It also indicated that the genetic relationships identified using RAPD markers were highly concordant [27].

RAPD markers have been proved as effective tools to monitor the genetic variation in different organisms. Using only four primers and 28 samples, the present study revealed a remarkable level of genetic variability in *Luffa acutangula*. A more definite conclusion, however, may be reached with larger samples including all AEZ (if possible to collect) of Bangladesh with faster evolving molecular markers such as microsatellite loci. The level of genetic variation provides the raw material for the selective improvement of a stock for sustainable agriculture production. Through this study, we have revealed, for the first time, the genetic relationship among available landraces of cultivated ridge gourd (*L. acutangula*) in Bangladesh. The result of present study can be used as a baseline for further study with a view to improving the crop.

Acknowledgement

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