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MOLECULAR CHARACTERIZATION OF EGGPLANT CROSSES BY USING RAPD ANALYSIS

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ABSTRACT

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Random amplified polymorphic DNA (RAPD) technique was used to determine the genetic variation and relationships among three parents (BAU Begun-1, Dohazari G and Laffa S) and five F_5 offsprings (BAU Begun-1 x Laffa S and BAU Begun-1 x Dohazari G) of eggplant. Amplification with 3 decamer primers generated 28 and 31 bands in Laffa S x BAU Begun-1 and Dohazari G x BAU Begun-1 respectively from which 16 (57.14%) and 18 (58.06%) were polymorphic, respectively. The co-efficient of gene differentiation (G_{st}) was 0.4534 reflecting the existence of high level of genetic variations among the genotypes. The dendrogram (UPGMA) constructed from Nei's genetic distance produced 2 main clusters of the parents and F_5 offsprings. Higher genetic distance (0.5256) and lower genetic identity (0.5799) was observed between the genotype combinations. Genetic variation and relatedness to disease reaction as assessed using RAPD markers could be potential sources for the development of *Phomopsis vexans* resistant variety which is one of the major biotic stresses in eggplant in Bangladesh.

***Key words**: molecular characterization, eggplant, RAPD, genetic distance*

INTRODUCTION

Eggplant or brinjal (*Solanum melongena* L.), an important popular vegetable crops cultivated in the tropics and subtropics. Its position is second in vegetable crops in terms of production next to potato. Asia has the largest eggplant production which comprises more than 90% of the world production area and 87% of the world production (Food and Agriculture Organization (FAO) Agriculture Database, 2007). A large number of eggplant cultivars are grown in Bangladesh, which show a wide range of variations in yield performance and disease reaction. Fruit rot caused by *Phomopsis vexans* (Sac & Syd) is one of the major constraints in successful cultivation of eggplant in Bangladesh (Das 1998). The pathogen causes disease on foliage and also in fruit. Almost all eggplant cutivars of Bangladesh are reported susceptible to this disease. The disease is reported to cause 15-20% (30-50% in severe case) yield loss (Das1998; Khan 1999). The pathogen is seed-borne, can survive in the soil for long time (Kalda *et al.* 1977). The phomopsis resistant cultivar BAU Begun-1 was crossed with two cultivars-Dohazari G and Laffa S. All F_1, F_2, F_3 and F_4 plants of both the crosses showed resistance (Islam 2006; Hasan 1990). F_3 plants segregated for color and fruit shape into five categories. F_5 plants will be evaluated for molecular characterization to see the segregating nature for resistance.

Eggplants present a high morphogenetic potential that is useful for developmental studies as well as for establishing biotechnological approaches to produce improved varieties. Variability in eggplant was previously studied by Karihaloo and Gottlieb (1995), Stedje and Bukenya-Ziraba (2003) and Singh *et al*. (2006) using RAPD technique. This method was also successfully used for genetic variability analysis in rice (Rahman 2004), wheat (Mitra 2005), maize (Mandal 2005) and potato (Yasmin *et al.* 2006) etc.

The aim of the present research work is to evaluate the molecular variability and relatedness of three parents and two F_5 offspring's by RAPD technique to see the continuity of the resistance character and the degree of homozygosity attained thereof.

MATERIALS AND METHODS

Genomic DNA Extraction and Quantification

Three parents (Laffa S, Dohazari G and BAU Begun-1) and five F_5 offsprings (BAU Begun-1 x Laffa S and BAU Begun-1 x Dohazari G) were used for the RAPD analysis. As a source of genomic DNA youngest healthy leaf samples-collected from the 15-day old seedling were used. The experiment was carried out in the Biotechnology Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Modified CTAB mini-prep method was followed to extract DNA from leaf samples (Kabir 2007). The concentration of DNA in the samples was determined using a UV Spectrophotometer at 260nm. The quality of the DNA was verified by electrophoreses on a 0.8% agarose gel in TBE (Tris-boric acid-EDTA) buffer.

PCR amplification and Electrophoresis

RAPD amplification reactions were maintained essentially following Williams *et al*. (1990) with some modifications. The screening was done with fifteen arbitrary decamer primers (Bengalore Genei, India) using DNA from parental cultivars. Three primers resulting scorable and reproducible bands were selected for subsequent RAPD analysis of eggplant germplasms (Table 1).

PCR reactions were performed on each DNA sample in a 10 µl reaction mixture containing 1x PCR buffer (10 mM Tris HCl pH 8.5, 50mM KCl and 15 mM $MgCl_2$), 10 mM each dNTPs, 5 pmols primer, 2 U of Taq DNA polymerase (Bengalore Genei, India), 100 ng of genomic DNA and rest amount of sterile deionized water. DNA amplification was carried out in a DNA thermocycler (Biometra, Germany) as the following thermal profile: initial denaturation for 3 min at 94°C followed by 41 cycles of 1 min denaturation at 94°C, 1 min annealing at 35°C and extension at 72°C for 2 min. A final extension step at 72°C for 7 min was allowed for complete extension of all amplified fragments. The PCR products were kept at 4°C until electrophoresis. Reaction mixtures were mixed with 2.0 µl 6X loading dye. Amplified fragments were separated on a 1.5% agarose (Bengalore Genei, India) gel in 0.5 X TBE buffer along with 20 bp DNA weight marker (Bengalore Genei, India) for 2 hours at 100V. Gel was stained with Ethidium bromide solution (0.1 µg ml^{-1}) for 15 min. Finally fragments were visualized under UV-transilluminator and photographed by Gel Documentation System (Biometra, Germany).

Scoring and Data analysis

It is assumed that each band represented the phenotype at a single locus because all RAPD markers are nearly dominant (Williams *et al.* 1990). The amplified bands were visually scored as present (1) and absent (0) separately for each individual and each primer. The scores obtained were pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's (1973), genetic diversity, genetic distance (D) and a UPGMA (Unweighted Pair Group Method with Arithmetic Means) dendrogram using a computer program, POPGENE (Version 1.31) (Yeh *et al.* 1999). The same program was also used to perform the test of homogeneity in different loci between population pairs.

RESULTS AND DISCUSSION

Among the 13 primers initially tested 67AB10G7, OPC-05, OPF-20 primers yielded comparatively maximum number of amplification products with high intensity and minimal smearing, good resolution and also clear bands. The number of fragments amplified per primer varied. In case of Laffa S, BAU Begun-1 and their F_5 offsprings, the selected three primers generated 28 bands. Out of the 28 bands, 16 (57.14%) were polymorphic and 12 (42.86%) were monomorphic. In case of Dohazari G, BAU Begun-1 and their F_5 offsprings, three primers generated 31 bands of which 18 (58.06%) were polymorphic and 13 (41.94%) were monomorphic. The primers generated 9.33 scorable bands per primer and 5.33 polymorphic bands per primer were produced for Laffa S, BAU Begun-1 and their offsprings. On the other hand, 10.33 scorable bands per primer and 6 polymorphic bands per primer for Dohazari G, BAU Begun-1 and their offsprings. Strong and weak bands were produced in the RAPD reactions. Weak bands results from low homology between the primer and the pairing site on the DNA strand (Thormann *et al.* 1994). The percentage of polymorphic loci was 57.14 for Laffa S, BAU Begun-1 and their offsprings and 58.06 for Dohazari G, BAU Begun-1 and their offsprings (Table 1 and 2).

Table 1. RAPD primers with corresponding bands scored and their polymorphic bands observed in 2 parents (Laffa S and BAU Begun-1) and their 5 F_5 offspring of eggplant

Primer code	Nucleotide sequences (5/-3/)	Number of bands	Number of polymorphic bands	Polymorphic loci (%)
67AB10G7	TTGGCACGGG	9	4	
OPC-05	GATGACCGCC	10	7	57.14
OPF-20	GGTCTAGAGG	9	5	
Total		28	16	
Average		9.33	5.33	

Table 2. RAPD primers with corresponding bands scored and their polymorphic bands observed in 2 parents (Dohazari G and BAU Begun-1) and their 5 F_5 offspring of eggplant

Primer code	Nucleotide sequences (5/-3/)	Number of bands	Number of polymorphic bands	Polymorphic loci (%)
67AB10G7	TTGGCACGGG	10	5	
OPC-05	GATGACCGCC	12	9	58.06
OPF-20	GGTCTAGAGG	9	4	
Total		31	18	
Average		10.33	6	

The primer 67AB10G7 showed variable number of bands in Green globose and in Green white long. Laffa S, BAU Begun-1 and their crosses exhibited 10 bands, all of which were present in Green globose and Green white long. Green globose displayed additional 2 bands not found in the rests. On the other hand, Green white long

displayed a single additional band also found in Green globose but not found in the rests. This type of banding pattern is the indication of similarity between the two genotypes and the dissimilarity from the rests (Figure 1, 2, 3).

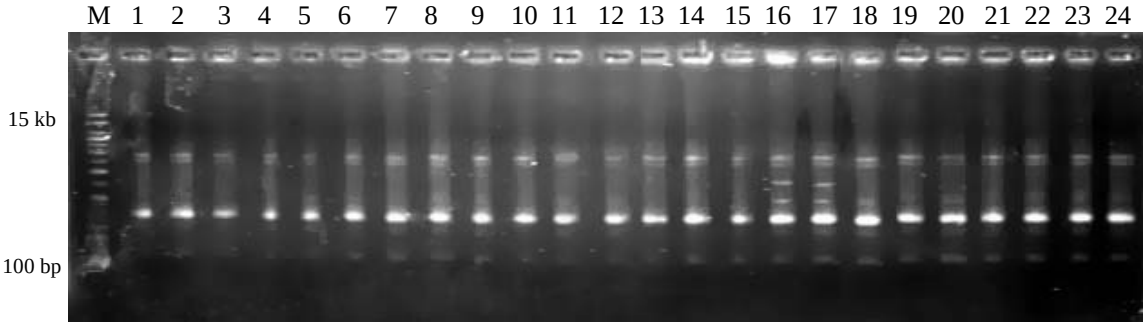

Figure 1. RAPD profiles of parents and their F_5 progenies using primer 67AB10G7 (Lane M: 20bp ladder, Lane 1-3: BAU Begun-1, Lane 4-6: Laffa S, Lane 7-9: Dohazari G, Lane 10-12: Green globose (Dohazari x BAU Begun-1), Lane 13-15: Green long (Dohazari x BAU Begun-1), Lane 16-18: Green globose (Laffa S x BAU Begun-1), Lane 19-21: Green white long (Laffa S x BAU Begun-1), Lane 22-24: Purple globose (Laffa S x BAU Begun-1) on 1.5% agarose gel.

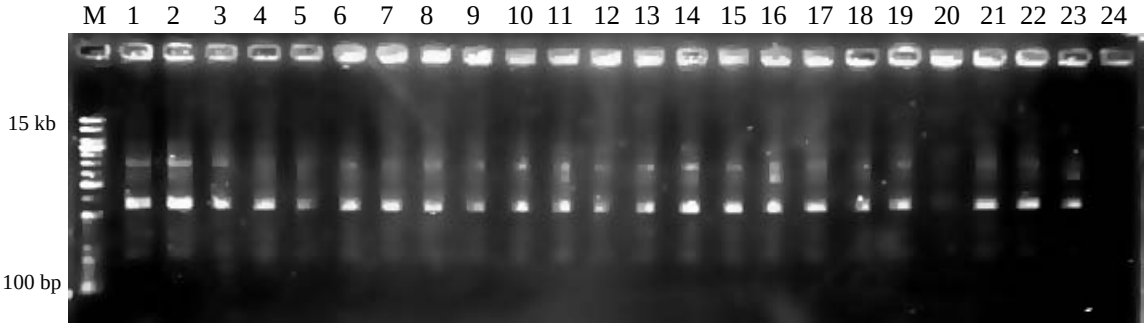

Figure 2. RAPD profiles of parents and their F_5 progenies using primer OPC5 (Lane M: 20bp ladder, Lane 1-3: BAU Begun-1, Lane 4-6: Laffa S, Lane 7-9: Dohazari G, Lane 10-12: Green globose (Dohazari x BAU Begun-1), Lane 13-15: Green long (Dohazari x BAU Begun-1), Lane 16-18: Green globose (Laffa S x BAU Begun-1), Lane 19-21: Green white long (Laffa S x BAU Begun-1), Lane 22-24: Purple globose (Laffa S x BAU Begun-1) on 1.5% agarose gel.

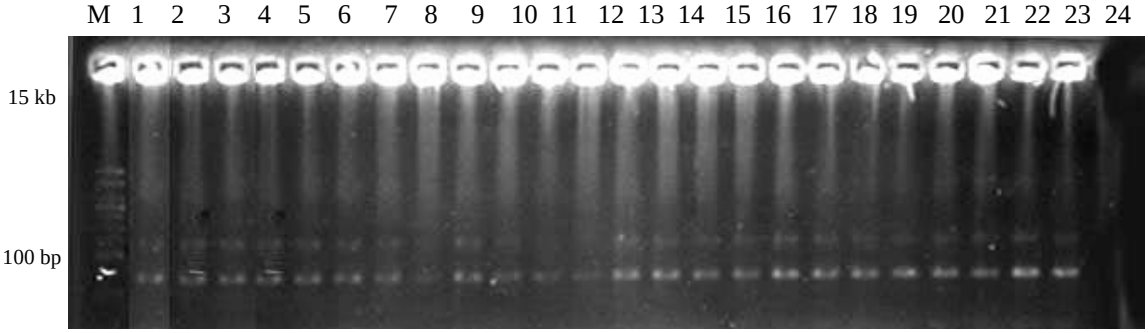

Figure 3. RAPD profiles of parents and their F_5 progenies using primer OPF20 (Lane M: 20bp ladder, Lane 1-3: BAU Begun-1, Lane 4-6: Laffa S, Lane 7-9: Dohazari G, Lane 10-12: Green globose (Dohazari x BAU Begun-1), Lane 13-15: Green long (Dohazari x BAU Begun-1), Lane 16-18: Green globose (Laffa S x BAU Begun-1), Lane 19-21: Green white long (Laffa S x BAU Begun-1), Lane 22-24: Purple globose (Laffa S x BAU Begun-1) on 1.5% agarose gel.

Similar level of polymorphism expressed by arbitrary primers compared to reports of the RAPD studies are available, e.g. in rice (67%) (Ko *et al.* 1994), in wheat (78%) (Sivolap *et al*. 1997), in maize (72.2%) (Valdmar *et al*. 2004). Varieties having lower similarity are less homogenous group. The highest intra-variety similarity indices (S_i) value were found Laffa S (82.82%) followed by Dohazari G and crosses of BAU Begun-1 and Dohazari G (Table 3). From this study, it is likely that individuals of Laffa S and Dohazari G

genotypes were more homogenous while that of BAU Begun-1 was found to be less. Because BAU Begun-1 showed low intra-variety similarity (S_i) value (65.58%) (Table 3).

Table 3. Summary of the band sharing based on percentage similarity indices within and between individuals of 3 parents and 5 crosses of eggplant. Similarity (S_i) between individuals of the same variety

Genotypes	Similarity (%)			Average S_i (%)
	OPG 7	OPC 5	OPF 20	
BAU Begun-1	66.67	70.09	59.98	65.58
Laffa S	73.02	84.98	90.47	82.82
Dohazari G	89.32	73.50	84.61	82.48
BAU Begun-1 x Dohazari G				
Green globose	84.61	70.09	73.02	75.91
Green long	84.98	84.19	56.95	75.37
BAU Begun-1 x Laffa S				
Green globose	70.71	72.73	68.49	70.64
Green white long	90.47	73.02	60	74.50
Purple globose	70.09	70.71	60.61	67.14

Gene diversity and frequency of polymorphic loci

Overall average gene diversity across all varieties for all loci studies was 0.4312. High level of gene diversity value and Shannon's Information index was found in locus OPC05-7 and OPF20-5 (0.4972 and 0.6904, respectively). The lowest level of gene diversity value and Shannon's Information index was found in locus OPC05-8 (0.2877 and 0.4625 respectively). The number and proportion of polymorphic loci was found to be the highest in BAU Begun-1 and Dohazari G (20.83%). Green globose obtained 2 polymorphic loci (8.33%). The highest proportion of Nei's gene diversity (h) value and Shannon's Information index (I) were found in Green long which was 0.4795 and 0.6724, respectively. Singh *et al.* (2006) found high level of genetic diversity in eggplant. On the other hand, the lowest proportion of polymorphic loci and Nei's gene diversity value were found in Purple globose (Table 4). The DNA polymorphisms are detected by band presence versus band absence and may be caused by failure to prime a site in some individuals due to nucleotide sequence difference or by insertions or deletions between priming sites (Clark and Langigan, 1993).

Table 4. Estimates of genetic variation: number and proportion of Polymorphic loci, gene diversity and Shannon's Information index obtained in different eggplant genotypes

Accession ID	No. of polymorphic loci	Proportion of polymorphic loci (%)	Gene diversity (h)	Shannon's Information index (I)
BAU Begun-1	5	20.83	0.3799	0.5663
Laffa S	3	12.50	0.4234	0.6132
Dohazari G	5	20.83	0.4599	0.1434
F_5 (Dohazari G x BAU Begun-1)				
Green globose	3	12.50	0.4354	0.6269
Green long	4	16.67	0.4795	0.6724
F_5 (Laffa S x BAU Begun-1)				
Green globose	2	8.33	0.4392	0.6301
Green white long	4	16.67	0.4613	0.6535
Purple globose	3	12.50	0.3710	0.5557

Genetic distance and genetic identity

The values of pair-wise comparisons of Nei's (1972) genetic distance between varieties were computed from combined data for the 3 primers, ranged from 0.1510 to 0.5448 (Table 5). Comparatively higher genetic distance was observed between Laffa S vs. Dohazari G, Green globose vs. Green long, Dohazari G vs. Green long and Green globose vs. Green white long genotype pairs than other genotype combinations. The lowest genetic distance (0.1510) was found in BAU Begun-1 vs. Green globose genotype pair. Considering the genetic distance values, the varieties were genetically different from each other. Genetic identity between varieties was found for the 3 primers, ranged from 0.5799 to 0.8599. Comparatively the higher genetic identity was found in BAU Begun-1 vs. Green globose, (0.8151) in Green long vs. Green white long and in Laffa S vs. Green long and the lowest genetic identity was observed between Dohazari G vs. Laffa S.

Table 5. Summary of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) values for eight (8) genotype pairs of eggplant

Genotypes	BAU Begun-1	Laffa-S	Dhohazari G	Dohazari G x BAU Begun-1		Laffa S x BAU Begun-1		
				Green globose	Green long	Green globose	Green white long	Purple globose
BAU Begun-1	****	0.6986	0.7605	0.8599	0.7866	0.7474	0.7489	0.7281
Laffa-S	0.3587	****	0.5799	0.6989	0.8002	0.6108	0.7515	0.7091
Dhohazari G	0.2738	0.5448	****	0.7423	0.6102	0.6974	0.6727	0.7479
Dohazari G x BAU Begun-1								
Green globose	0.1510	0.3583	0.2980	****	0.7253	0.7231	0.7673	0.6403
Green long	0.2401	0.2229	0.4939	0.3212	****	0.5912	0.8151	0.6546
Laffa S × BAU Begun-1								
Green globose	0.2912	0.4930	0.3604	0.3242	0.5256	****	0.6147	0.6820
Green white long	0.2892	0.2856	0.3965	0.2649	0.2044	0.4866	****	0.7089
Purple globose	0.3174	0.3437	0.2904	0.4459	0.4237	0.3828	0.3441	****

Dendrogram

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 8 varieties of eggplant into two main clusters: BAU Begun-1, Dohazari G, Green globose (Laffa S x BAU Begun-1), Green globose (Dohazari G x BAU Begun-1) and Purple globose grouped into cluster 1 and Laffa S, Green long and Green white long grouped in cluster 2. In cluster 1, BAU Begun-1, Dohazari G, Green globose (Laffa S x BAU Begun-1) and Green globose (Dohazari G x BAU Begun-1) formed sub cluster 1 while Purple globose was in sub cluster 2. Again, among the genotypes of sub cluster 1, BAU Begun-1, Dohazari G, and Green globose (Dohazari G x BAU Begun-1) formed sub sub cluster 1 and Green globose (Laffa S x BAU Begun-1) belonged to sub sub cluster 2. Further, the varieties of sub sub cluster 1 were divided into two groups, BAU Begun-1 and Green globose (Dohazari G x BAU Begun-1) belonged to group 1. Group 1 further divided into two sub groups. BAU Begun-1 belonged to sub group 1 while Green globose (Dohazari G x BAU Begun-1) formed sub group 2. On the other hand Dohazari G belonged to group 2. BAU Begun-1, Dohazari G and three crosses under study formed one cluster which means they are similar in some characters like plant height, fruit color, fruit shape etc. On the other hand, Laffa S and rest two crosses under study formed second cluster showing similarities in some characters also.

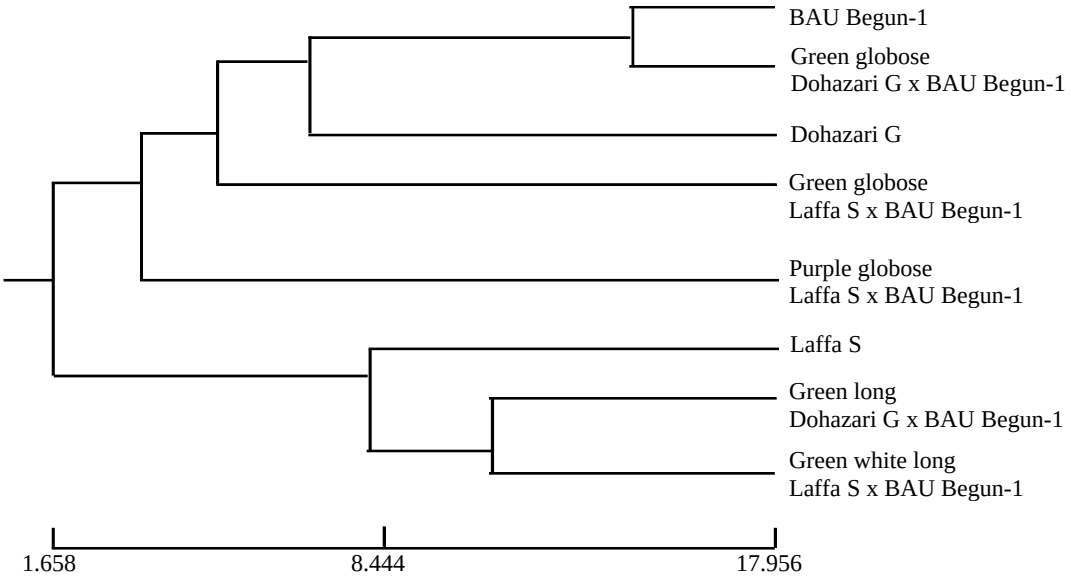

Figure 4. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between 8 eggplant genotypes, according to RAPD analysis

Eggplant germplasms of the Indian subcontinent are very diverse. Wide variation in the desirable genotypes/agronomy types in different regions substantiates the high level of genetic variability observed. High degree of diversity of species belonging to *Solanum* may be attributable to the fact that it is an ancient plant (Whalen 1979). RAPD and other discontinuous markers can serve as a means of genetic distances to establish

phylogenetic relationships among taxa (Karihaloo and Gottieb, 1995; Rodriguez *et al.* 1999; Rabey *et al.* 2002). Estimation of genetic differences and discrimination of genetic relationship between *Solanum* species are for utilization of plant genetic resources.

CONCLUSION

Among the 3 parents and 5 F_5 progenies of eggplant studied, the cultivar BAU Begun 1 contains highest number and proportion of polymorphic loci and gene diversity with the lowest intra-variety similarity. This shows more heterozygosity while the rests show less. High genetic variability and significant genetic differentiation between varieties indicate rich genetic resources of eggplant germplasm.

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