

# Study of genetic variation of eggplant cultivars by using RAPD-PCR molecular markers and the relationship with *Phomopsis* blight disease reaction

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**ABSTRACT.** Disease susceptibility and genetic variability in 10 eggplant genotypes were studied after inoculating *Phomopsis vexans* under confined field conditions. Random amplified polymorphic DNA (RAPD) markers were used to assess genetic variation and relationships among eggplant genotypes. The disease index of leaves ranged 0.208-13.79%, while fruit infection ranged 2.15-42.76%. Two varieties, Dohazari G and Laffa S, were found to be susceptible, 6 were moderately resistant, 1 was moderately susceptible, and BAU Begun-1 was resistant to *P. vexans*. Amplification of genomic DNA by using 3 RAPD primers produced 20 bands: 14 (70%) were polymorphic and 6 (30%) were monomorphic. The highest intra-

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variety similarity indices values were found in ISD 006, Ishurdi L, Jessore L, and BAU Begun-1 (100%), while the lowest was in Dohazari G (90%). The lowest genetic distance (0.0513) and the highest genetic identity (0.9500) were observed between the ISD 006 and Ishurdi L combinations. A comparatively higher genetic distance (0.3724) and the lowest genetic identity (0.6891) were observed between the ISD 006 and Dohazari G combinations. A dendogram was constructed based on Nei's genetic distance, which produced 2 main clusters of the genotypes - Cluster I: ISD 006, Ishurdi L, Marich begun L, BAU Begun-1, Marich begun S, and Chega and Cluster 2: Laffa S, Dohazari G, Jessore L, and Singhnath. Genetic variation and its relationship with disease susceptibility were assessed using RAPD markers, to develop disease-resistant varieties and improve eggplant crops.

**Key words:** Eggplant; Genetic variation; Phomopsis blight; Resistance; Random amplified polymorphic DNA

## INTRODUCTION

Eggplant or brinjal (*Solanum melongena*) is an important vegetable and is extensively grown in many countries of the world, with a total production of approximately 32 million tons (Choudhary and Gaur, 2009). It is cultivated and available year-round in Bangladesh, with annual production reaching 341,000 t in the year 2009-2010 (BBS, 2011). Phomopsis blight caused by *Phomopsis vexans* is a major limitation to successful cultivation of eggplant in Bangladesh (Das, 1998). The disease has been reported to cause 15-20% yield loss (30-50% in severe cases) (Das, 1998). The loss is estimated to be US\$ 134 million per year resulting from *Phomopsis* blight and fruit rot of eggplant in Bangladesh (Meah et al., 2002). The pathogen is seed-borne and can survive in the soil for long periods of time (Kalda et al., 1977).

Eggplant shows high diversity because there are many varieties, cultivars, landraces, hybrids, advanced lines, and wild races in the Asiatic region. More than 60 eggplant cultivars are grown in Bangladesh (Meah et al., 2007); a wide range of genetic variability exists among cultivated varieties. Genetic information regarding disease susceptibility and characteristics that contribute to yield must be examined to improve eggplant crop production. For rapid and efficient plant breeding programs, knowledge of the interrelationships among disease susceptibility and factors affecting yield are necessary.

The qualitative and quantitative improvement of crops depends on the available gene pool and its manipulation. Genetic diversity studies based on morphological traits and molecular marker studies enable the evaluation of genetic variation prior to the initiation of a new breeding program. The random amplified polymorphic DNA (RAPD) technique is a widely used, easy, and inexpensive. In this method, DNA fragments are amplified by polymerase chain reaction (PCR) using primers with arbitrary sequences for rapid screening of polymorphisms. An advantage of this method is that no prior knowledge of the sequence is required (Sobral and Honeycutt, 1993). Because primers can be chosen arbitrarily, any organism can be mapped with the same set of primers, but some loss of information occurs because RAPD markers are dominant. Though RAPD has some limitations, it has been used extensively for genetic studies, such as for analyzing genetic variation in plants

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and microbes (Demir et al., 2010; Ali et al., 2011; Aruga et al., 2012; Joshi et al., 2013; Izadiyan and Taghavi, 2013) and constructing linkage maps for certain plant species (Yang and Quiros, 1995). RAPD methods have been successfully used to examine genetic variability in eggplant in different countries (Karihaloo and Gottlieb, 1995; Singh et al., 2006; Yasmin et al., 2006; Demir et al., 2010; Ali et al., 2011; Sharmin et al., 2011; Laila et al., 2012; Khalil et al., 2013). However, the relationship between genetic variation and susceptibility to *Phomopsis* blight has not been examined. Therefore, the present study was conducted to evaluate the molecular variability and relatedness of 10 eggplant varieties and their susceptibility to *Phomopsis* blight disease using RAPD-PCR.

## MATERIAL AND METHODS

## Evaluation of eggplant genotypes for resistance to phomopsis blight

#### Plant materials and experimentation

Seeds of 10 eggplant cultivars, ISD-006, Laffa S, Dohazari G, Jessore L, Singhnath, Marich begun S, Marich begun L, BAU Begun-1, Ishurdi L, and Chega, were obtained from the IPM Lab, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

The experiment was conducted in the field laboratory of the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The experiment was laid out in a randomized complete block design with 3 replications. The unit plot size was 4.0 x 2.5 m, maintaining a row-torow distance 60 cm and plant-to-pant distance of 50 cm. Fertilizers were applied at recommended doses (Anonymous, 2005). Irrigation and intercultural operations were conducted when necessary.

## Inoculation of eggplant with Phomopsis vexans

A virulent isolate of *Phomopsis vexans* was used for inoculation. The cultures were collected from the IPM lab and multiplied in potato dextrose agar medium. In each plot, inoculation of 5 plants was conducted during the pre-flowering stage and 5 plants during the fruiting stage. A 70-mL spore suspension (5 x 10<sup>6</sup> spores/mL) was sprayed onto each plant (Islam, 2006). Spraying was done at afternoon, and inoculated plants were covered with a moist transparent polythene bag for 48 h to ensure better infection (Islam, 2006).

## Assessment of Phomopsis blight and fruit rot

After inoculation, symptoms on leaves, flowers, and fruits were observed at 7-day intervals for up to 21 days. Data regarding leaf infection (%), percent leaf area diseased (LAD), flower infection (%), fruit infection (%), and percent fruit area diseased (FAD) were recorded. Disease severity was recorded according to the standard rating scale (1-5) (Islam et al., 1990). The percent disease index (PDI) was calculated according to the formula described by Singh (1984).

## Harvesting and data recording

Characteristics affecting eggplant yield were examined and data were recorded for all plants. Plant height, number of primary branches per plant, number of secondary branches per

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plant, number of fruits per plant, fruit length, fruit breadth, and individual fruit weight were recorded in the field. Mature fruits were harvested at the edible stage at an interval of 7 days. Five fruits per variety were allowed to ripen and the seeds were collected to grow plants in the following year.

#### Data analysis

Data were analyzed to determine statistical significance. Analysis of variance followed by Duncan's multiple range test (DMRT) to test the differences between the genotypes using the computer based software MSTATC.

## Molecular characterization of eggplant cultivars using RAPD markers

## Genomic DNA extraction and quantification

The seeds of 10 selected eggplant cultivars were used for the RAPD analysis. To obtain genomic DNA, the youngest healthy leaf samples collected from 15-day-old seedlings were used. A modified cetrimonium bromide mini-prep method was followed to extract DNA from leaf samples (Kabir, 2007). The concentrations of DNA in the samples were determined using a UV spectrophotometer at 260 nm. DNA quality was verified by electrophoreses on a 0.8% agarose gel in Tris-boric acid-EDTA buffer.

#### PCR amplification and electrophoresis

RAPD amplification reactions were conducted following the procedure described by William et al. (1990) with some modifications. Screening was conducted with 14 arbitrary decamer primers (Bangalore Genei, India). Three primers (OPA17, OPB07, OPE8) showing scorable and good reproducible bands were selected for subsequent RAPD analysis of eggplant germplasms.

PCR reactions were performed on each DNA sample in a  $10-\mu$ L reaction mixture containing 1X PCR buffer (10 mM Tris HCl pH 8.5, 50 mM KCl, and 15 mM MgCl<sub>2</sub>), 10 mM of each dNTP, 5 pmol primer, 2 U *Taq* DNA polymerase (Bengalore Genei, India), 100 ng genomic DNA, and the remaining volume in sterile deionized water. DNA amplification was carried out in a DNA thermocycler (Biometra, Röttingen, Germany) with 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 conducted for complete extension of all amplified fragments. The PCR products were stored at 4°C until electrophoresis. Reaction mixtures were mixed with 2.0  $\mu$ L 6X loading dye. Amplified fragments were separated on a 1.5% agarose gel (Bangalore Genei, India) in 0.5X Tris-boric acid-EDTA buffer along with 20 bp DNA weight marker (Bengalore Genei, Bangalore, India) for 2 h at 100 V. The gel was stained with 0.1  $\mu$ g/mL ethidium bromide solution for 15 min. Fragments were visualized under a UV-transilluminator and photographed using the Gel Documentation System (Biometra).

#### Scoring and data analysis

Each band was considered to represent a phenotype at a single locus because all RAPD markers were dominant (Williams et al., 1990). The amplified bands were visually scored as present

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(1) and absent (0) 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 genetic distance (1973), genetic diversity, genetic distance (D), and the unweighted pair group method with arithmetic mean dendrogram using the computer program, POPGENE (Version 1.31) (Yeh et al., 1999). The same program was used to test for homogeneity in different loci between population pairs.

# **RESULTS AND DISCUSSION**

#### Evaluation of eggplant genotypes against P. vexans

The inoculated eggplant genotypes showed different percentages of leaf infection and severity (leaf area diseased) (Table 1). Among the eggplant genotypes, the highest leaf infection (30.64%) was observed in cultivar Dohazari G followed by Laffa S (15.21%). BAU Begun-1 showed the lowest (1.36%) leaf infection and was statistically identical to Chega. The highest PDI in leaves was recorded in the cultivar Dohazari G (13.79%), followed by the cultivar Laffa S (6.14%). The lowest PDI was calculated for BAU Begun-1. Based on the PDI values, the cultivar BAU Begun-1 was graded as resistant, while cultivar ISD-006, Jessore L, Marich begun S, Marich begun L, Ishurdi L, and Chega were graded as moderately resistant. Singhnath was moderately susceptible and Dohazari G and Laffa S were susceptible (Table 1).

Table 1. Disease reaction of eggplant cultivars against Phomopsis vexans in induced field condition at flowering stage.							
Genotypes	Leaf infection (%)	PDI (leaf)	Reaction				
ISD-006	9.98 <sup>cd</sup>	3.97°	MR				
Laffa S	15.21 <sup>b</sup>	6.14 <sup>b</sup>	MR				
Dohazari G	30.64ª	13.79ª	S				
Jessore	7.18 <sup>d</sup>	2.97 <sup>cd</sup>	MR				
Singhnath	12.61 <sup>bc</sup>	5.86 <sup>b</sup>	MS				
Marich Begun S	10.10 <sup>cd</sup>	3.84°	MR				
Marich Begun L	13.28 <sup>bc</sup>	4.05°	MR				
BAU Begun-1	1.36 <sup>e</sup>	0.21 <sup>e</sup>	R				
Ishurdi L	7.93 <sup>d</sup>	3.20 <sup>cd</sup>	MR				
Chega	2.50 <sup>e</sup>	1.34 <sup>de</sup>	MR				

Column having common letter(s) do not differ significantly at 5% level of probability. R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible.

Upon inoculation, fruits of different eggplant genotypes showed different lesion sizes and percentage of fruit infection (Table 2). Among the eggplants genotypes, the highest lesion size was observed in cultivar Dohazari G (3.367 cm<sup>2</sup>) followed by cultivar Laffa S (2.223 cm<sup>2</sup>); other cultivar lesions were less than 1 cm<sup>2</sup>. Among the genotypes, BAU Begun-1 showed the smallest lesion, which was statistically similar to the genotypes Jessore L, Ishurdi L, and Chega.

Fruit infection ranged from 2.15 to 42.76%. The highest percentage of fruit infection was recorded in genotype/cultivar Dohazari G followed by Laffa S (Table 2). The lowest percentage of fruit infection was recorded in genotype BAU Begun-1. The highest PDI on fruit was recorded in the genotype of Dohazari G followed by Laffa S. The lowest PDI on fruit was found in genotype BAU Begun-1. Based on the PDI values on fruit, the genotype BAU Begun-1 was graded as resistant whereas cultivar ISD-006, Jessore L, Ishurdi L, Marich begun S, Marich begun L, and Chega graded were moderately resistant, Singhnath was moderately susceptible, and Dohazari G and Laffa S were susceptible (Table 2).

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Genotypes	Lesion size (cm2)	Fruit infection (%)	PDI (fruit)	Reaction
ISD-006	0.895 <sup>cd</sup>	11.88 <sup>ef</sup>	7.18°	MR
Laffa S	2.223 <sup>b</sup>	36.40 <sup>b</sup>	28.46ª	S
Dohazari G	3.367ª	42.76ª	28.53ª	S
Jessore L	0.287 <sup>d</sup>	24.03 <sup>cd</sup>	8.20°	MR
Singhnath	1.667 <sup>bc</sup>	28.43°	14.61 <sup>b</sup>	MS
Marich Begun S	0.839 <sup>cd</sup>	22.53 <sup>cd</sup>	7.73℃	MR
Marich Begun L	0.773 <sup>cd</sup>	31.17 <sup>cd</sup>	7.84°	MR
BAU Begun-1	0.039 <sup>d</sup>	2.15 <sup>f</sup>	0.49 <sup>d</sup>	R
Ishurdi L	0.336 <sup>d</sup>	18.89°	6.14°	MR
Chega	0.446 <sup>d</sup>	5.44 <sup>f</sup>	2.75 <sup>d</sup>	MR

Column having common letter(s) do not differ significantly at the 5% level of probability. R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

Evaluation of the 10 eggplant genotypes revealed that BAU Begun-1 was resistant and Dohazari G was susceptible against *P. vexans*. The genotype BAU Begun-1 is only the variety that was resistant to *P. vexans* in Bangladesh according to Islam (2006), Meah and Islam (2005), Meah (2003), and Meah et al. (1998). Some genotypes were moderately resistant, while some were moderately susceptible to *P. vexans*. Similar results were observed by Islam (2006) in Bangladesh. Meah (2003) found that BAU Begun-1 and Katabegun (wild) were resistant to *P. vexans*. Similar studies in India revealed the existence of differential response among eggplant varieties to infection by *P. vexans* in an inoculation test (Kalda et al., 1977; Quaiser and Ahmad, 1987; Khodke, 1990).

# Molecular characterization of eggplant cultivars using RAPD markers

The number of fragments amplified per primer varied. Three primers generated 20 bands ranging in size from 100 to 1500 base pairs (Figures 1, 2, and 3). Of the 20 distinct bands, 14 bands (70.00%) were considered to be polymorphic and 6 bands (30.00%) were monomorphic among the 10 eggplant varieties/genotypes.



M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 1920 21 22 23 24 25 26 27 28 29 30 M

**Figure 1.** RAPD profiles of 10 eggplant varieties using primer OPA17. *Lanes 1-3* = ISD 006; *lanes 4-6* = Laffa S; *lanes 7-9* = Dohazari G; *lanes 10-12* = Jessore L; *lanes 13-15* = Singhnath; *lanes 16-18* = Marich begun S; *lanes 19-21* = Marich begun L; *lanes 22-24* = BAU Begun-1; *lanes 25-27* = Ishurdi L; *lanes 28-30* = Chega. *Lane M* = molecular weight marker (20-base pair DNA ladder).

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**Figure 2.** RAPD profiles of 10 eggplant varieties using primer OPB07. *Lanes* 1-3 = ISD 006; *lanes* 4-6 = Laffa S; *lanes* 7-9 = Dohazari G; *lanes* 10-12 = Jessore L; *lanes* 13-15 = Singhnath; *lanes* 16-18 = Marich begun S; *lanes* 19-21 = Marich begun L; *lanes* 22-24 = BAU Begun-1; *lanes* 25-27 = Ishurdi L; *lanes* 28-30 = Chega. *Lane* M = molecular weight marker (20-base pair DNA ladder).



M 1 2 3 4 5 6 7 8 9 10 11 1213 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 M

**Figure 3.** RAPD profiles of 10 eggplant varieties using primer OPE8. *Lanes 1-3* = ISD 006; *lanes 4-6* = Laffa S; *lanes 7-9* = Dohazari G; *lanes 10-12* = Jessore L; *lanes 13-15* = Singhnath; *lanes 16-18* = Marich begun S; *lanes 19-21* = Marich begun L; *lanes 22-24* = BAU Begun-1; *lanes 25-27* = Ishurdi L; *lanes 28-30* = Chega. *Lane M* = molecular weight marker (20-base pair DNA ladder).

Among the 3 primers, OPB07 produced the largest number of polymorphic bands (6), indicating a high level of polymorphism, whereas the primers OPA17 and OPE8 produced 5 and 3 polymorphic bands, respectively (Table 3).

Table 3. RAPD primers with	corresponding ba	nds scored	and their	size range	together v	with polymorphic	bands
observed in 10 eggplant culti	vars.						

Primer codes	Sequences (5'-3')	Number of bands	Number of polymorphic bands	Polymorphic loci (%)
OPA17	GACCGCTTGT	7	5	70.00
OPB07	GGTGACGCAG	6	6	
OPE8	TCACCACGGT	7	3	
Total		20	14	
Average		6.67	4.66	

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The primer OPA17 and OPE8 produced the largest number of bands (7), whereas OPB07 generated 6 bands. In contrast, the primer OPB07 amplified the largest number of polymorphic bands (6). The average number of bands and polymorphic bands produced by the 3 primers were 6.67 and 4.66, respectively (Table 3). The percentage of polymorphic loci was 70.00%.

The number of bands per primer varied from 6-7, with a mean value of 6.66. The average number of polymorphic bands was 4.66. A similar number of polymorphic RAPD bands per primer with a mean (5.9) was reported by Karihaloo and Gottlieb (1995) in eggplant varieties and in related weedy forms known as insanum (5.7). A similar polymorphism level expressed by arbitrary primers was obtained in other RAPD studies, such as in maize (Valdmar et al., 2004) and eggplant (Chakraborty, 2006; Kabir, 2007; Demir et al., 2010; Sharmin et al., 2011; Khalil et al., 2013).

#### Intra- and inter-variety similarity indices

The highest intra-variety similarity index  $(S_i)$  values were found in the ISD 006, Jessore L, BAU Begun-1, and Ishurdi L varieties (100%), followed by Marich begun S, Chega, Singhnath, Laffa S, Marich begun L, and Dohazari G. The lowest intra-variety similarity index  $(S_i)$  value of 90.47% was found in Dohazari G (Table 4). Eggplants genotypes showing higher intra-variety similarity and a lower frequency of polymorphic loci are likely to show less heterozygosity compared to those showing lower intra-variety similarity. Varieties with lower similarity are less homogenous.

Varieties		Similarity (%)		Mean (S <sub>i</sub> )
	OPA17	OPB07	OPE8	
ISD 006	100.0	100.0	100.0	100.0
Laffa S	83.33	100.0	100.0	94.44
Dohazari G	100.0	71.41	100.0	90.47
Jessore L	100.0	100.0	100.0	100.0
Singhnath	92.58	100.0	94.86	95.81
Marich begun S	100.0	93.93	100.0	97.97
Marich begum L	100.0	77.45	100.0	92.48
BAU Begun-1	100.0	100.0	100.0	100.0
Ishurdi BS	100.0	100.0	100.0	100.0
Chega	100.0	92.58	100.0	97.52
Average				96.86

The average value for intra-variety similarity indices  $(S_i)$  was higher (average 96.86) than intervariety similarity indices  $(S_{ij})$  (88.13). The highest level of both intra- and inter-variety similarity indices were generated using the primer OPE8 (Table 5). The lowest intra-variety similarity indices  $(S_i)$  and inter-variety similarity indices  $(S_{ij})$  were produced using primer OPB07.

Table 5. Band sharing based percentage similarity intra- and inter-variety indices generated using the primer.						
Primers	Intra-variety similarity indices (S <sub>j</sub> )	Inter-variety similarity indices $(S_{ij})$				
OPA17	97.59	86.98				
OPB07	93.53	85.29				
OPE 8	99.48	92.04				
Average	96.86	88.13				

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# Genetic distance and genetic identity

The values of pairwise comparisons for Nei's (1972) genetic distance between varieties/ genotypes were determined using the combined data for the 3 primers and ranged from 0.0513-0.3724 (Table 6). The genetic distance values between the ISD 006 and Dohazari G genotypes was found to be the highest (0.3724), and the lowest genetic distance (0.0513) was observed for the ISD 006 and Ishurdi L genotype pair. A comparatively higher genetic distance was observed between the Laffa S *vs* Marich begun L (0.3605), Jessore L Vs Chega, and Marich begun L Vs Chega variety combinations. The genetic distance values indicated that the varieties were genetically different from each other. Stedje and Bukenya (2003) examined variation in *Solanum* and found no correlation between genetic distance can be used as parents in crossing programs. Similar results have been reported by Latif et al. (2013), Rafii et al. (2012), and Shabanimofrad et al. (2013).

**Table 6.** Summary of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) values for different variety pairs of eggplant.

Varieties	ISD 006	Laffa S	Dohazari G	Jessore L	Singhnath	Marich begun S	Marich begun L	BAU Begun-1	Ishurdi L	Chega
ISD 006	-	0.8284	0.6891	0.7500	0.8203	0.8313	0.8444	0.8500	0.9500	0.8153
Laffa S	0.1882	-	0.8217	0.7774	0.8494	0.8169	0.6835	0.7774	0.7774	0.8441
Dohazari G	0.3724	0.1963	-	0.8449	0.8131	0.7806	0.8012	0.8449	0.7410	0.8579
Jessore L	0.2877	0.2518	0.1685	-	0.9149	0.7807	0.8119	0.9000	0.8000	0.7649
Singhnath	0.1981	0.1632	0.2069	0.0890	-	0.9123	0.7797	0.8715	0.8715	0.8360
Marich begun S	0.1847	0.2022	0.2477	0.2475	0.0918	-	0.7919	0.8820	0.8820	0.9059
Marich begun L	0.1691	0.3605	0.2217	0.2084	0.2488	0.2333	-	0.9147	0.8958	0.7757
BAU Begun-1	0.1625	0.2518	0.1685	0.1054	0.1375	0.1256	0.0892	-	0.9000	0.8657
Ishurdi L	0.0513	0.2518	0.2997	0.2231	0.1375	0.1256	0.1100	0.1054	-	0.8657
Chega	0.2042	0.1694	0.1532	0.2680	0.1792	0.0988	0.2540	0.1442	0.1442	-

Genetic identity between varieties was observed for the 3 primers, ranging from 0.6891 to 0.9500. A comparatively high genetic identity (0.9500) was found for the ISD 006 and Ishurdi L variety pair and the lowest was observed between ISD 006 and Dohazari G (0.6891). The average genetic identity was 0.82. Similar results were reported by Karihaloo and Gottlieb (1995) and (Chakraborty, 2006).

## Unweighted pair group method with arithmetic mean dendrogram

An unweighted pair group method with arithmetic mean dendrogram based on Nei's (1972) genetic distance was constructed (Figure 4). The 10 eggplant varieties were grouped into 2 main clusters: ISD 006, Ishurdi L, Marich begun L, BAU Begun-1, Marich begun S, and Chega grouped into cluster 1, while Laffa S, Dohazari G, Jessore L, and Singhnath were in cluster 2 (Figure 4).

In cluster 1, ISD-006, Ishurdi L, Marich begun L, and BAU Begun-1 formed sub-cluster 1, while Marich begun S and Chega formed sub-cluster 2. Among the varieties of sub-cluster 1, ISD 006 and Ishurdi L produced sub-sub cluster 1, while Marich begun L and BAU Begun-1 belonged to sub-sub cluster 2. Among the varieties of sub-sub cluster 1, ISD 006 was in 1 group, while Ishurdi L was in another group. In sub-sub cluster 2, Marich begun L was in 1 group while BAU Begun-1 was in another group.

In cluster 2, Laffa S was in sub-cluster 1 while Dohazari G, Jessore L, and Singhnath formed sub-cluster 2. Again among the varieties of sub-cluster 2, Dohazari G was in sub-sub

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cluster 1 and Jessore L and Singhnath belonged to sub-sub cluster 2. Two varieties were sub-sub cluster 2; Jessore L was in 1 group and Singhnath was in another group.



Figure 4. Unweighted pair group method with arithmetic mean dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between 10 eggplant varieties, according to RAPD analysis.

Overall, BAU Begun-1, which is resistant to *Phomopsis* blight and Ishurdi L, Marich begun S, Marich begun L, and Chega were tolerant to phomopsis blight found in cluster 1. In contrast, Dohazari G, Laffa S, Singhnath, and Jessore L were found to be in cluster 2, where all varieties were susceptible and moderately susceptible to *Phomopsis* blight, except Jessore L, which was moderately resistant/tolerant. This relationship of distinct groupings based on disease susceptibility was not reflected on the dendogram. This may have been because the RAPD primers were arbitrary and non-specific (not specific primers to *Phomopsis* blight) traits and adequate number of primers was not used. Additionally, their parental types may have been derived from the same origin. Similar results were found by Debasis et al. (2002) for potato late blight resistant and susceptible cultivars, for which there were no clear groupings based on the dendogram.

The results of the present study reveal the diversity among the eggplant varieties, which will be helpful for programs aimed at developing *Phomopsis* blight disease-resistant eggplant using biotechnological approaches and traditional breeding methods.

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