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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF EGGPLANT LINES FOR RESISTANT TO PHOMOPSIS BLIGHT AND FRUIT ROT

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Abstract

The F_4 lines of eggplant derived from the crosses of Dohazari G x BAU Begun-1 and Laffa S x BAU Begun-1 were evaluated for resistance to phomopsis blight and fruit rot under confined field conditions. The inoculated plants exhibited differential disease reactions. Among the parents, BAU Begun-1 was resistant whereas Dohazari G and Laffa S were susceptible to *Phomopsis vexans*. All the phenotypes of F_4 progenies showed resistant reaction to the disease. Significant differences were observed among the phenotypes in all the yield components. High genotypic and phenotypic coefficient of variation, heritability and per cent genetic advance were estimated for number of fruits per plant, number of secondary branch per plant, fruit length and fruit breadth. Significant positive correlation was observed between yield contributing characters. Random amplified polymorphic DNA technique was used for assessing genetic variation and relationship among parent cultivars and their F_4 progenies of eggplant. Amplification with five decamer primers generated 69.0% polymorphic bands. Comparatively higher genetic distance was observed between Laffa S vs. green globose (Dohazari G x BAU Begun-1). The dendogram constructed from Nei's genetic distance produced two main clusters, the parent cultivars and six F_4 lines formed cluster 1 and one line in cluster 2. F_4 lines of the tested phenotypes showed similar disease reaction and divided into same sub cluster. The parent cultivars were different in case of disease reaction and finally divided into two groups, susceptible cultivars Laffa S and Dohazari G belonged to group 1 and the resistant parent BAU Begun-1 formed another group.

Keywords: Eggplant, Resistance, Phomopsis, Characterization, RAPD

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Introduction

Eggplant also known as Brinjal (*Solanum melongena* L.) is a common fruit vegetable and widely grown in Asia, Africa, and the subtropics, including the southern USA and the Mediterranean region with world production of 42.9 million tons for the year 2009 (FAO, 2010). Asia has the largest eggplant production, which comprises more than 90% of the world production area and 87% of the world production (Choudhary and Gaur, 2009). It is the second most important vegetable crop next to potato in Bangladesh in respect of total acreage and production. It is cultivated on about 15% of total vegetable cultivated land and contributes about 8% to total vegetable production (BBS, 2011).

Among the diseases of eggplant Phomopsis blight and fruit rot caused by *Phomopsis vexans* (Sacc. and Syd.) Harter is very devastating and widespread (Chen *et al.*, 2002). Phomopsis blight ranks second only to bacterial wilt in destructiveness of eggplant and varies in severity depending on area, soil type and weather (Meah *et al.*, 2002). *Phomopsis vexans* is both externally and internally seed borne and remains viable for about 14 months in soil with plant debris and in the seed from infected fruits (Kalda *et al.*, 1977). The pathogen also causes damping off, seedling/stem blight, collar rot, stem canker and leaf spot. Studies showed that about 21% of fruit rot and 7% of seed rot in eggplant is caused by *P. vexans* and gives final yield losses of 15-50% (Das, 1998), equivalent to about US $ 20 million. Farmers have been using expensive pesticides to control the disease, but no satisfactory results could be obtained. Rather, repeated use of pesticides creates environmental pollution and a health hazard. Host plant resistance is the best control method for environmental and financial reasons. BAU Begun-1 is a round shaped variety and only

resistant variety in Bangladesh cultivated in north-western region (Meah *et al.*, 2007). This variety was crossed with two commercial and popular varieties, Dohazari G and Laffa S. Almost all the F_1, F_2 and F_3's were found to be the resistant to phomopsis blight and fruit rot (Kabir, 2007; Islam, 2006).

Different scientists (Roychowdhury *et al.*, 2011; Sharmin *et al.*, 2010; Islam and Uddin, 2009; Baswana *et al.*, 2002) studied the genetic diversity, heritability and genetic advance in eggplant genotypes. The extensive variability in growth habit, spiny or non-spiny nature, foliage shape/colour, floral structure, fruit shape/size/colour and yields of eggplant were observed to have influence on phomopsis disease reaction (Singh *et al.*, 1999).

The use of molecular markers to track loci and genome regions in crop plants is now routinely applied in many breeding programs. For an effective breeding program, information concerning the extent and nature of genetic diversity or variation within a crop species is essential. Random amplified polymorphic DNA (RAPD**)** is a widely applied approach for characterization of DNA from plants and other organisms using PCR. The RAPD technique has provided a relatively simple and inexpensive method for analysis of genetic variation in plants, fungi and bacteria (Fukuan *et al.*, 2003; Bidochka *et al*., 1994). RAPD have been used to construct genetic maps and for the molecular tagging of various agronomic traits in various crop species (Williams *et al.*, 1990).

RAPD technique has been successfully used for the study of genetic variability analysis in different crops including different species of *Solanum* in Bangladesh and other countries. Laila *et al.* (2012); Sharmin *et al.* (2011); Kabir (2007) and Islam (2006) characterized resistant and susceptible cultivars of eggplant along with their interspecific offspring in Bangladesh.

BAU Begun-1 is yet to be widely cultivated and its popularity is limited to a particular area of the country. So the resistance trait in BAU Begun-1 is needed to be transferred to some widely cultivated varieties covering larger areas of Bangladesh. So BAU Begun-1 was crossed with two popular commercial varieties of eggplant e.g. Dohazari G and Laffa S. The present research work was undertaken to evaluate the advanced lines of the crosses at F_4 generation for the inheritance of the resistance trait against *Phomopsis vexans* and molecular characterization using RAPD markers.

Materials and Methods

Screening of F_4 population of eggplant for resistance to Phomopsis blight and fruit rot

The experiment was conducted in the Field Laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh during the winter season of 2006-07.

Source of seed, seedling raising and transplantation

The seeds of the varieties Dohazari G, Laffa S and BAU Begun-1 and advanced lines (F_4 generation) of the intervarietal crosses of eggplant, viz. (1) Dohazari G x BAU Begun-1 (2) Laffa S x BAU Begun-1 were collected from IPM Laboratory, Department of Plant Pathology, BAU. Seedlings were raised in the seedbed following proper management.

The seedlings at 28 days age were transplanted in the field. The experiment was laid out in a Randomized Complete Block Design with four replications. The unit plot size was 12.0 m x 2.0 m with 2 rows in each plot. Fertilizers and manures were applied at recommended doses (Anonymous, 2005). Application of fertilizers and transplantation were done according to Islam (2006). Weeding, irrigation and other intercultural operations were done whenever necessary.

Inoculation of eggplant by Phomopsis vexans

A mixer of cultures of five isolates of *P. vexans* was used for inoculation. The isolates of *P. vexans* were collected from IPM Lab., BAU. The multiplication of *P. vexans* and preparation of spore suspension were done according to the procedure of Islam (2006). All the plants of F_4 generations and parents were inoculated at the flowering and fruiting stage. Spore suspension (5 x 10^6 spores ml^{-1}) was sprayed @ 70 ml per plant (Islam, 2006). The spraying was done at afternoon and inoculated plants were covered with transparent polythene bag for 48 hours for making favourable condition of infection.

Assessment of Phomopsis blight and fruit rot

After inoculation, symptoms on leaves, flowers and fruits were observed at seven-day interval up to 21 days. Data on leaf infection (%), per cent leaf area diseased (LAD), flower infection (%), fruit infection (%) and per cent fruit area diseased (FAD) were recorded. The 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 of Singh (1984).

Harvesting and data recording

The characters responsible for yield of eggplant were studied and data were recorded from the parents and F_4 populations from all the plants. Plant height, number of primary branches per plant, number of secondary branches per plant, number of fruits per plant, fruit length, fruit breadth and individual fruit weight were recorded in the field. The mature fruits were harvested at the edible stage at an interval of seven days. Five fruits per variety/genotype were allowed to ripe and seeds were collected from them for growing plants in the next year.

Data analysis

Data were analyzed to find out the statistical significance. Analysis of variances followed by Duncan's Multiple Range Test (DMRT) was performed to test the differences between the genotypes with the computer based software MSTATC.

Besides, different components of genotypic and phenotypic variance, heritability, genetic advance and correlation were also estimated. Genotypic and phenotypic variances and heritability in broad sense were estimated according to formula given by Johnson *et al.* (1955), genotypic and phenotypic coefficients of variation by the formula of Burton (1952). The expected genetic advance (GA) for different characters under investigation was estimated according to the formula used by Johnson *et al.* (1955) and Allard (1960). Genetic advance in percentage of mean was calculated by the formula used by Comstock and Robinson (1952).

Using the formula of Singh and Chaudhary (1985), genetic and phenotypic covariances were calculated. Genotypic and phenotypic correlation coefficients between different characters in all possible combinations were calculated with the formula given by Miller *et al.* (1958).

Molecular characterization of F_4 population of eggplants through RAPD markers

Plant materials

Three parents (BAU Begun-1, Laffa S and Dohazari G) and seven F_4 lines obtained from their crosses (Dohazari G x BAU Begun-1 and Laffa S x BAU Begun-1) were used for the molecular characterization through random amplified polymorphic DNA (RAPD) technique. The experiment was carried out in the Biotechnology Laboratory of the Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.

Genomic DNA Extraction

Fresh leaf samples collected from 14-day old seedling were used for the study. Modified CTAB mini-prep method was followed to extract DNA from leaf samples (Kabir, 2007).

Approximately 25 mg of leaf tissues were cut into small pieces and ground with a pre cooled mortar and pestle. The ground powder was mixed with 670 µl extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 5 M NaCl) and 40 µl SDS (20%). The mixture was then transferred in a 2.0 ml micro centrifuge tube (Eppendorf, Germany) and mixed thoroughly. The samples were vortexes for 20 seconds and incubated at 65°C for 10 minutes in water bath (DSB-1000). The digested mixture was removed from water bath and 100 µl 5 M NaCl was added and mixed by inversion. One hundred micro liters CTAB (BioBasic, Canada) was added and mixed by vortexing. The tubes were again incubated in water bath at same temperature for 10 minutes. Nine hundred micro liter chloroform (chloroform: isoamyl alcohol = 24: 1, v/v) was added to the mixture after cooling in room temperature and mixed gently to separate the DNA from protein. The extracts were centrifuged for 10 minutes at 14000 rpm with a micro centrifuge (SIGMA 1-14) to allow precipitation of the cell debris. The supernatant was carefully transferred to a new 1.5 ml micro centrifuge tubes without disturbing the lower portion. Equal volume of ice cooled Isopropanol was added to the tubes, mixed gently by trapping with finger, and centrifuged for 10 minutes at 14000 rpm. The supernatant was decanted and pellets were air dried for few minutes, washed with 70% ethanol (200 µl), and then centrifuged for 5 minutes at 14000 rpm. Then the liquid was completely removed without disturbing the DNA pellet when air dried for 2 hours at room temperature. Finally, the pellets were suspended in 30.0 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at -20°C.

The quality of the DNA was verified by electrophoreses on a 0.8% agarose gel in TBE (Tris-boric acid-EDTA) buffer. The concentration of DNA samples was determined using a UV Spectrophotometer at 260 nm.

PCR amplification and Electrophoresis

RAPD reactions were maintained following the process of Williams *et al*. (1990) with some modifications. Thirty arbitrary decamer primers (Bengalore Genei, India) against DNA from parental cultivars were screened and five primers producing good scoreable and reproducible bands were selected for subsequent RAPD analysis of eggplant cultivars and phenotypes (Table 5).

PCR reactions were performed on each DNA sample in a 10.0 µl reaction mixture containing 1X PCR buffer (10 mM Tris HCl, pH 8.5; 50 mM KCl and 15 mM $MgCl_2$), 10 mM each dNTPs (Bengalore Genei, India), 5 pmols primer, 2 U of Taq DNA polymerase (Bengalore Genei), 100 ng of genomic DNA and rest amount of sterile deionized water. DNA amplification was carried out in a DNA thermocycler (Biometra, Germany) at the 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 10 min was allowed for complete extension of all amplified fragments (Kabir, 2007). Amplified fragments were separated on a 1.5% agarose (Invitrogen, Canada) gel in 1X 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 20 min. Finally fragments were visualized under UV-transilluminator and photographed by Gel Documentation System (Biometra, Germany).

Scoring and Data analysis

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 (1972) genetic diversity, genetic distance and a UPGMA (Unweighted Pair Group Method with Arithmetic Means) dendrogram using a computer program, POPGENE (Version 1.31) (Yeh *et al.*, 1999).

Results and Discussion

Screening of F_4 lines of eggplant for resistance to Phomopsis blight and fruit rot

F_4 advanced lines of eggplants and respective parents showed different percentage of leaf infection and severity (leaf area diseased) after inoculation (Table 1). Among the seven F_4 lines and their respective parents, the highest (32.47%) leaf infection was found in cultivar Dohazari G followed by Laffa S (30.57%). The eggplant cultivar BAU Begun-1 did not produce any infection on leaf. In contrast, the F_4 lines (Dohazari G x BAU Begun-1) and (Laffa S x BAU Begun-1) showed leaf infection of 2.87 to 3.12% and 2.83 to 3.16%, respectively. Similarly, the per cent disease index (PDI) was recorded the highest in the cultivars Dohazari G and Laffa S. Less than 1% PDI was recorded for all the F_4 lines of eggplant.

Table 1. Reaction of F_4 lines and parents of eggplant against *P. vexans* in induced condition during 2006-2007 at Mymensingh, Bangldesh

Phenotypes/ varieties	Infected leaves (%)	Per cent disease index (leaf)	Infected fruits (%)	Per cent disease index (fruit)	Disease reaction
(Dohazari G x BAU Begun-1)					
Green round	2.97 c	0.42 c	1.42 bc	0.42 b	Resistant
Green globose	3.12 c	0.43 c	1.83 b	0.42 b	Resistant
Green long	2.87 c	0.47 c	1.79 b	0.39 b	Resistant
(Laffa S x BAU Begun-1)					
Green globose	2.86 c	0.38 c	1.37 bc	0.38 b	Resistant
Green white long	2.83 c	0.45 c	1.38 bc	0.29 b	Resistant
Purple globose	3.12 c	0.41 c	1.61 b	0.30 b	Resistant
Purple long	3.16 c	0.39 c	1.42 bc	0.44 b	Resistant
BAU Begun-1	0.00 d	0.00 d	0.00 c	0.00 b	Resistant
Dohazari G	32.47 a	33.86 a	27.22 a	30.68 a	Susceptible
Laffa S	30.56 b	30.57 b	28.04 a	31.19 a	Susceptible

Means followed by the same letter in a column did not differ significantly at the 1% level by DMRT

The fruit infection of the tested phenotypes ranged from 0.00 to 28.04%. The highest percentage of fruit infection was recorded in parent cultivar Laffa S followed by Dohazari G (Table 1). There was no fruit infection observed in the cultivar BAU Begun-1. All the F_4 lines of eggplants (Laffa S × BAU Begun-1) and (Dohazari G × BAU Begun-1) produced fruit infection less than 2% that were statistically similar. The per cent disease index was also the highest in Laffa S (31.19) followed by Dohazari G (30.68). However, all the F_4 lines had less than 1% PDI, whereas BAU Begun-1 was found free from disease. F_1 and F_2 offspring derived from two crosses, Laffa S x BAU Begun-1 and Dohazari G x BAU Begun-1 were reported free from diseases and graded as resistant to *P. vexans* (Meah, 2007). In F_3 populations, Kabir (2007) also reported similar results.

The tested phenotype were categorized according to Islam *et al*. (1990) and found parent cultivars Dohazari G and Laffa as susceptible to *Phomopsis vexans* and cultivar BAU Begun-1 was resistant. All the F_4 plants were grouped based on colour and shape and graded as resistant though a few plants showed infection (PDI less than 1%). Fruit shape and colour had no effect on the intensity of infection (Table 1). Meah (2007) also reported that Laffa S and Dohazari G were susceptible and BAU Begun-1 (previously known as IPM-31) as resistant.

Performance of the phenotypes

All the yield contributing characters had significant differences amongst the phenotypes (Table 2). This means differences exist between the tested phenotypes even within the same environment where they were grown. Plant height ranged from 58.16 to 70.43 cm. Laffa S was the tallest cultivars among the tested phenotypes which was statistically identical with Dohazari G (Table 2). In contrast, green round line of Dohazari G x BAU Begun-1 was the shortest.

Number of primary branches per plant ranged from 11.68 to 14.58. Green globose of Laffa S x BAU Begun-1 cross produced maximum number of primary branches. Green globose of Dohazari G x BAU Begun-1 cross produced the lowest number of primary branches and it was statistically similar with most of the phenotypes. The highest number (14.76) of secondary branches was recorded in BAU Begun-1 and it was statistically different from all other phenotypes. The lowest number of secondary branches (11.30) was found in green round line (Dohazari G x BAU Begun-1). Number of fruits per plant ranged from 10.52 to 21.13. The parent Laffa S produced the highest number of fruits. All the F_4 phenotypes produced statistically similar number of fruits per plant.

Table 2. Yield attributing characters of F_4 lines and their parents of eggplant affected due to Phomopsis blight and fruit rot in induced condition in 2006-2007 at Mymensingh

Phenotypes/ varieties	Plant height (cm)	Primary branch plant^{-1}	Secondary branch plant^{-1}	Fruits plant^{-1}	Fruit length (cm)	Fruit width (cm)	Individual fruit weight (g)
Dohazari G x BAU Begun-1							
Green round	58.16 e	12.27 de	11.30 e	10.52 d	11.42 d	8.20 b	246.0 e
Green globose	62.24 cd	11.68 e	12.10 de	12.80 d	12.62 c	6.91 ef	259.9 d
Green long	59.97 de	13.18 bcd	13.08 d	13.51 d	15.59 ab	7.45 cde	280.1 bc
Laffa S x BAU Begun-1							
Green globose	60.85 de	14.58 a	13.18 d	12.49 d	12.13 cd	8.09 b	275.0 bc
Green white long	59.04 de	12.58 cde	15.12 c	13.32 d	16.11 a	7.11 def	284.8 ab
Purple globose	61.83 de	11.93 e	11.70 e	12.85 d	11.78 cd	7.79 bc	277.8 bc
Purple long	60.93 de	11.77 e	12.48 de	12.58 d	15.80 ab	6.64 f	266.4 cd
BAU Begun-1	65.45 bc	13.80 ab	17.76 a	17.81 b	11.88 cd	9.43 a	297.8 a
Dohazari G	67.82 ab	11.81 e	16.38 b	20.77 a	14.97 b	9.12 a	272.4 bcd
Laffa S	70.43 a	13.49 bc	16.39 b	21.13 a	15.23 ab	7.59 bcd	283.4 b

Means followed by the same letter in a column did not differ significantly at the 1% level by DMRT

Fruit length varied from 11.42 to 16.11 cm. The highest fruit length was recorded in green white long of F_4 lines (Laffa S x BAU Begun-1). In contrast, the lowest fruit length was observed in the green round of F_4 lines (Dohazari G x BAU Begun-1). Fruit width was recorded as 6.64 to 9.43 cm. The highest fruit width was observed in BAU Begun-1 followed by Dohazari G and both were statistically similar. The lowest fruit diameter was found in purple long (Laffa S x BAU Begun-1). Individual fruit weight ranged from 246.03 to 297.83 gram. The highest fruit weight was recorded in BAU Begun-1 (Figure 5) and the lowest in green round of F_4 lines (Dohazari G x BAU Begun-1).

The significant differences were observed in all the characters studied amongst the phenotypes. Significant difference in plant height, number of branches per plant, number of fruits per plant, fruit length, fruit width and individual fruit weight have also been reported in different eggplant varieties (Islam and Uddin, 2009; Sharmin *et al.*, 2010; Roychowdhury *et al.*, 2011).

Variability, heritability and genetic advance for yield contributing characters

The highest genotypic variance (δ^2g) and phenotypic variance (δ^2p) was found in individual fruit weight (195.06 and 243.00) followed by plant height (15.11 and 18.05), number of fruits per plant (13.75 and 14.35). Low magnitude of δ^2g and δ^2p were observed in number of secondary branches per plant, fruit length, number of primary branches per plant and fruit width (Table 3).

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) ranged from 5.09 to 25.09 and 5.68 to 25.63, respectively. The highest genotypic coefficient of variation and phenotypic coefficient of variation was found in number of fruits per plant followed by number of secondary branch per plant. In contrast, the lowest GCV and PCV were calculated for individual fruit weight. Islam and Uddin (2009); Sharmin *et al.* (2010); Roychowdhury *et al.* (2011) also reported higher phenotypic coefficients of variation than genotypic ones for the yield contributing characters. High GCV indicated high genetic variability and thus results the scope for improvements of the characters.

Table 3. Estimation of genetic parameters for yield contributing characters in seven F_4 lines of eggplant and their parents

Characters	Mean range	Genotypic variance (σ^2g)	Phenotypic variance (σ^2p)	Genotypic coefficient of variation (%)	Phenotypic coefficient of variation (%)	Heritability (%)	Genetic advance	Genetic advance (%)
Plant height (cm)	58.16-70.43	15.11	18.05	6.20	6.78	83.71	7.33	11.69
Number of primary branches plant^{-1}	11.68-14.58	0.94	1.22	7.63	8.69	77.05	1.75	13.79
Number of secondary branches plant^{-1}	11.30-17.76	5.11	5.44	16.20	16.72	93.93	4.51	32.35
Number of fruits plant^{-1}	10.52-21.13	13.75	14.35	25.09	25.63	95.82	7.48	50.59
Fruit length (cm)	11.42-16.11	3.67	3.91	13.93	14.38	93.86	3.82	27.81
Fruit width (cm)	6.64-9.43	0.80	0.89	11.42	12.05	89.89	1.75	22.31
Individual fruit weight (g)	246.03-297.83	195.06	243.00	5.09	5.68	80.27	25.78	9.40

Heritability of the phenotypes ranged from 77.05 to 95.82%. Almost all the characters showed more than 80% heritability. The highest heritability was calculated for number of fruits per plant followed by number of secondary branches per plant (93.93) and fruit length (93.86). High heritability for most of the characters of different eggplants was also reported (Islam and Uddin, 2009, Sharmin *et al.*, 2010; Roychowdhury *et al.*, 2011). The genetic advance and per cent genetic advance varied from 1.75 to 25.78 and 9.40 to 50.59, respectively. The highest genetic advance was recorded for individual fruit weight; whereas genetic advance in percentage was found in number of fruits per plant, which means there is possibility for improving the characters. Similar results were also reported by Islam and Uddin, (2009); Sharmin *et al.* (2010) and Roychowdhury *et al.* (2011). Sharmin *et al.* (2010) and Roychowdhury *et al.* (2011) showed high heritability for number of fruits per plant with high genetic advance as percentage of mean, which is in agreement with the present study.

Relationship between different yield contributing characters

Different yield contributing characters and their relationships were studied through analysis of correlation coefficients between characters (Table 4).

Plant height was positively correlated with number of primary branches per plant, fruit length, fruit width and individual fruit weight. But the relationship of plant height was significant with number of secondary branch per plant and number of fruits per plant. Positive correlation between plant height and eggplant yield also reported by Muniappan *et al.* (2010).

The relationship of number of primary branches per plant with number of secondary branches per plant, number of fruits per plant, fruit width and individual fruit weight was positive. The negative significant correlation was observed for primary branches per plant with fruit length. The number of secondary branches per plant was positively related with other characters. Significant relationships were calculated with number of fruits per plant and individual fruit weight. Insignificant relationship was observed with fruit length and fruit width.

The number of fruits per plant had positive correlation with fruit length, fruit width and individual fruit weight. Fruit length had negative correlation with fruit width. There was positive insignificant correlation between fruit length with individual fruit weight. Number of fruits has positive correlation with fruit yield also reported by Muniappan *et al.* (2010). Individual fruit weight was positively correlated with plant height, number of primary branches per plant, number of secondary branches per plant, number of fruits per plant, but insignificant with fruit length and fruit width.

Table 4. Correlation coefficients between different yield contributing characters in eggplant cultivars and their F_4 lines

Characters	Plant height (cm)	No. of primary branches plant^{-1}	No. of secondary branches plant^{-1}	No. of fruits plant^{-1}	Fruit length (cm)	Fruit width (cm)	Individual fruit weight (g)
Plant height (cm)		0.140	0.720**	0.951***	0.159	0.407	0.418
No. of primary branches plant^{-1}			0.392	0.170	- 0.154	0.336	0.531
No. of secondary branches plant^{-1}				0.854***	0.291	0.564	0.742**
Number of fruits plant^{-1}					0.315	0.491	0.524
Fruit length (cm)						- 0.430	0.240
Fruit width (cm)							0.304
Individual fruit weight (g)							

*, ** and *** indicate significant at 5%, 1% and 0.1% level of probability, respectively.

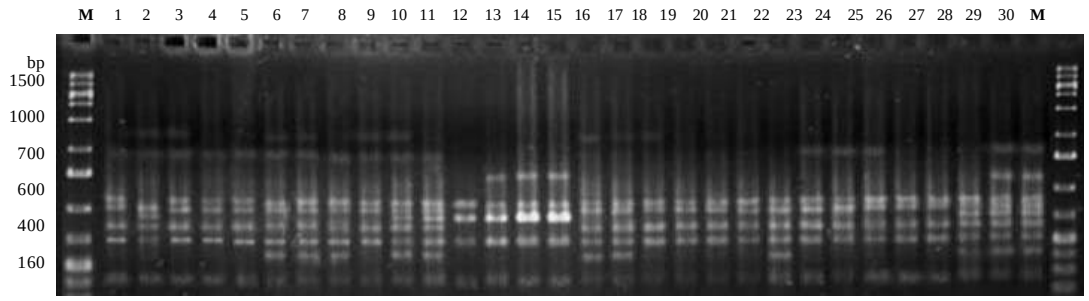
Molecular characterization of F_4 lines of eggplants through RAPD markers

RAPD profiles and analysis

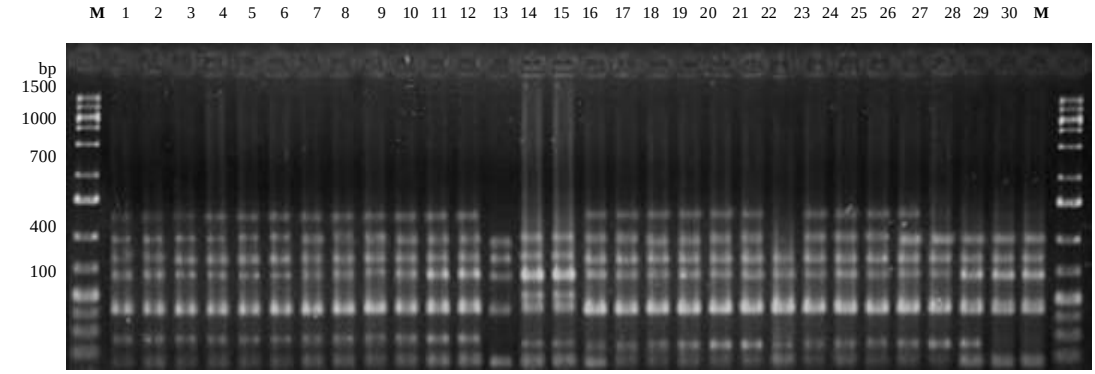
The genome DNA from three eggplant cultivars and their F_4 lines when amplified with five different RAPD primers, various banding patterns were observed. Among the RAPD profiles, only two are presented in Photograph 1 and 2. The number of band ranged from 4 to 10. The primer 67AB10G7, OPB10 and OPC05 produced the highest numbers of bands as well as polymorphic bands whereas the other two primers (OPA03 and OPB09) produced the lowest number of bands. So first three primers showed a higher level of polymorphism (Table 5). The band size ranged from 100 to 800bp. RAPD analysis of BAU Begun-1, Laffa S, Dohazari G and their F_4 lines revealed that the selected five primers generated 37 bands of which 26 (69.0%) were polymorphic and 11 (31.0%) were monomorphic.

Table 5. RAPD primers with corresponding bands scored and their size range together with polymorphic bands observed in three parents (Dohazari G, Laffa S and BAU Begun-1) and their seven F_4 lines of eggplant

Primer code	Primer sequences (5´-3´)	Band size (bp)	Total bands scored	Polymorphic bands	Polymorphic loci (%)
67AB10G7	TTG GCA CGG G	100-600	10	8	80.00
OPA03	AGT CAG CCA C	100-300	4	3	75.00
OPB09	TGG GGG ACT C	140-300	5	3	60.00
OPB10	CTG CTG GGA C	160-800	10	8	80.00
OPC05	GAT GAC CGC C	110-500	8	4	50.00
Total			37	26	345.00
Average			7.4	5.2	69.00



Photograph 1. RAPD profiles of 3 parents and their 7 F_4 progenies using primer OPB10 (M: 20bp ladder, Lane 1-3: BAU Begun-1, Lane 4-6: Dohazari G, Lane 7-9: Laffa S, Lane 10-12: Green round (Dohazari G x BAU Begun-1), Lane 13-15: Green globose (Dohazari G x BAU Begun-1), Lane 16-18: Green long (Dohazari G x BAU Begun-1), Lane 19-21: Green globose (Laffa S x BAU Begun-1), Lane 22-24: Green white long (Laffa S x BAU Begun-1), Lane 25-27: Purple globose (Laffa S x BAU Begun-1) and Lane 28-30: Purple long (Laffa S x BAU Begun-1).



Photograph 2. RAPD profiles of 3 parents and their 7 F_4 progenies using primer OPC05 (M: 20bp ladder, Lane 1-3: BAU Begun-1, Lane 4-6: Dohazari G, Lane 7-9: Laffa S, Lane 10-12: Green round (Dohazari G x BAU Begun-1), Lane 13-15: Green globose (Dohazari G x BAU Begun-1), Lane 16-18: Green long (Dohazari G x BAU Begun-1), Lane 19-21: Green globose (Laffa S x BAU Begun-1), Lane 22-24: Green white long (Laffa S x BAU Begun-1), Lane 25-27: Purple globose (Laffa S x BAU Begun-1) and Lane 28-30: Purple long (Laffa S x BAU Begun-1).

The number of band and polymorphic band per primer obtained 7.4 and 5.2, respectively (Table 5). The number of polymorphic band per primer have been reported 4.0, 5.3 and 5.9 in eggplant by Laila *et al.* (2012), Sharmin *et al.* (2011) and Karihaloo *et al.* (1995), respectively. The difference might be due to the use of different primers and eggplant varieties. The percentage of polymorphic loci was 69.00 for Laffa S, Dohazari G, BAU Begun-1 and their F_4 lines. Similar level of polymorphism expressed by arbitrary primers in eggplant also available (Laila *et al.*, 2012; Sharmin *et al.*, 2011).

Frequency of polymorphic loci

The number and proportion of polymorphic loci was found to be the highest in green white long line of Laffa S x BAU Begun-1 (27.03%) and the lowest on green long (F_4 line of Dohazari G × BAU Begun-1) and green globose (F_4 line of Laffa S x BAU Begun-1) (Table 6). The highest proportion of Nei's gene diversity value and Shannon's Information index were found in green white long (F_4 line of Laffa S x BAU Begun-1) which was 0.1166 and 0.1675, respectively. On the other hand, the lowest proportion of polymorphic loci and Nei's gene diversity value were found in purple globose (F_4 line of Laffa S × IPM-31) (Table 6).

Table 6. Estimates of genetic variation, number and proportion of Polymorphic loci, gene diversity and Shannon's Information index obtained in 3 cultivars and 7 F_4 lines of eggplant

Phenotypes/ varieties	No. of polymorphic loci	Proportion of polymorphic loci (%)	Gene diversity (h)	Shannon's Information index (I)
BAU Begun-1	7	18.92	0.0720	0.1068
Laffa S	6	16.22	0.0639	0.0939
Dohazari G	5	13.51	0.0558	0.0810
Green round (D)*	8	21.62	0.1004	0.1417
Green globose (D)	4	10.81	0.0477	0.0681
Green long (D)	1	2.70	0.0132	0.0184
Green globose (L)**	1	2.70	0.0132	0.0184
Green white long (L)	10	27.03	0.1166	0.1675
Purple globose (L)	3	8.11	0.0294	0.0442
Purple long (L)	4	10.81	0.0426	0.0626

*Green round (D) = Green round progeny of Dohazari G x BAU Begun-1
**Green globose (L) = Green globose progeny of Laffa S x BAU Begun-1

The DNA polymorphisms are detected by band presence versus 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).

Genetic identity and genetic distance

Genetic identity between varieties was found for the 5 primers, ranged from 0.5661 to 0.9883 (Table 7). Comparatively the higher genetic identity was found in green globose (Laffa S x BAU Begun-1) vs. purple globose (Laffa S x BAU Begun-1) followed by purple globose (Laffa S x BAU Begun-1) vs. green white long (Laffa S x BAU Begun-1). The lowest genetic identity was observed between green globose (Laffa S x BAU Begun-1) vs. Laffa S.

The values of pair-wise comparisons of Nei's (1972) genetic distance between varieties were computed from combined data for the 5 primers, ranged from 0.0177 to 0.5689 (Table 7). Comparatively, higher genetic distance was observed between Laffa S vs. green globose (Dohazari G x BAU Begun-1), BAU Begun-1 vs. green globose (Dohazari G x BAU Begun-1) and Dohazari G vs. green globose (Dohazari G x BAU Begun-1) phenotype pairs than other phenotype combinations. The lowest genetic distance was found in green globose (Laffa S x BAU Begun-1) vs. purple globose (Laffa S x BAU Begun-1) phenotype pair. Considering the genetic distance values, the varieties were genetically different from each other.

Table 7. Summary of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) values for ten phenotype pairs of eggplant

Phenotypes/ varieties	BAU Begun-1	Laffa S	Dohazari G	Green round (D)	Green globose (D)	Green long (D)	Green globose (L)	Green white long (L)	Purple globose (L)	Purple long (L)
BAU Begun-1	****	0.9148	0.9326	0.8616	0.6029	0.8608	0.8934	0.8943	0.8964	0.7665
Laffa S	0.0891	****	0.9322	0.8404	0.5661	0.8147	0.8562	0.8626	0.8590	0.7025
Dohazari G	0.0698	0.0703	****	0.8663	0.6262	0.8500	0.8723	0.8593	0.8752	0.7435
Green round (D)	0.1490	0.1738	0.1435	****	0.7770	0.8563	0.8929	0.8674	0.8897	0.8525
Green globose (D)	0.5060	0.5689	0.4681	0.2523	****	0.6507	0.7005	0.6760	0.7217	0.7379
Green long (D)	0.1499	0.2050	0.1626	0.1552	0.4298	****	0.9586	0.8904	0.9389	0.8326
Green globose (L)	0.1127	0.1552	0.1366	0.1133	0.3559	0.0423	****	0.9438	0.9883	0.8326
Green white long (L	0.1117	0.1478	0.1516	0.1422	0.3915	0.1161	0.0578	****	0.9604	0.8445
Purple globose (L)	0.1094	0.1520	0.1333	0.1169	0.3262	0.0630	0.0117	0.0404	****	0.8482
Purple long (L)	0.2659	0.3532	0.2964	0.1596	0.3039	0.1832	0.1832	0.1690	0.1646	****

*Green round (D) = Green round progeny of Dohazari G x BAU Begun-1
**Green globose (L) = Green globose progeny of Laffa S x BAU Begun-1

Dendrogram of F_4 lines and their parents

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the phenotypes of eggplant into two main clusters: nine phenotypes grouped into cluster I and only green globose (F_4 line of Dohazari G x BAU Begun-1) grouped in cluster 2 (Fig. 1).

In cluster 1, BAU Begun-1, Dohazari G, Laffa S, green round and green long (F_4 line of Dohazari G x BAU Begun-1), green globose, purple globose and green white long (F_4 lines of Laffa S x BAU Begun-1) formed sub cluster 1 while purple long (F_4 line of Laffa S x BAU Begun-1) was in sub cluster 2. Among the phenotypes of sub cluster 1, the three parent cultivars BAU Begun-1, Dohazari G and Laffa S formed sub sub cluster 1 and F_4 lines of eggplant belonged to sub sub cluster 2.

F_4 lines of the tested phenotypes showed similar disease reaction and also produced similar number of fruits and formed same sub cluster. The parent cultivars are produce similar number of fruits, but they are different in case of disease reaction and finally divided into two groups, susceptible cultivars Laffa S and Dohazari G belonged to group 1 and the resistant parent BAU Begun-1 formed another group. On the other hand sub sub cluster 2 were divided into two groups, green round of Dohazari G x BAU Begun-1 formed sub group 1 and other four phenotypes were in sub group 2.

Eggplant germplasms of the Indian subcontinent are very diverse. Wide variation in the desirable phenotypes in different regions substantiates the high level of genetic variability (Karihaloo *et al.*, 1995). Thus, the present study showed different types of reaction and grouping.

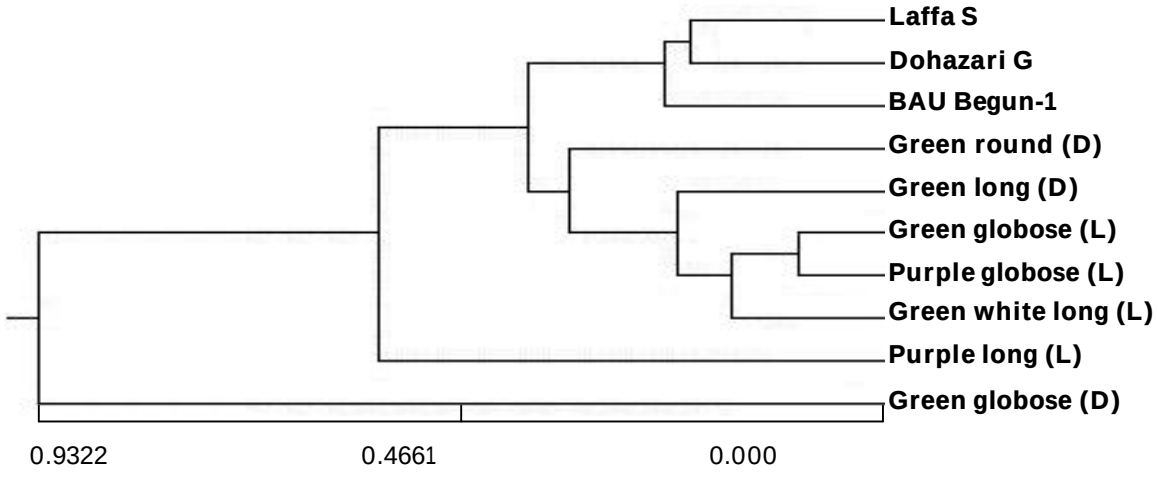


Fig. 1. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between 3 cultivars and 7 F_4 lines of eggplant, according to RAPD analysis [Green round (D) = Green round progeny of Dohazari G x BAU Begun-1, Green globose (L) = Green globose progeny of Laffa S x BAU Begun-1].

Among the three parent and their 7 F_4 lines studied, the cultivar BAU Begun-1 and F_4 lines showed resistant reaction and rest parents showed susceptible reaction to phomopsis blight and fruit rot. According the RAPD analysis, three cultivars and 6 F_4 lines grouped in same cluster and finally resistant parent formed separate sub sub cluster. Therefore, the result of the present study indicates that the variability and diversity present among the phenotypes, which will be useful for development of new eggplant cultivars.

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