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RAPD-PCR Finger Printing of Brinjal Germplasm Against Fruit Rot of Brinjal

Sanjeev P. Jakatimath[1*], R. K. Mesta[1], P. S. Ajjappalavar[2], Sadanand K. Mushrif[3] and I. B. Biradhar[4]

[1]Department of Plant Pathology (University of Horticultural Sciences) Bagalkot-587104, Karnataka, India

[2]Horticultural Research Station (University of Horticultural Sciences) Devihosur-581110, Haveri, Karnataka, India

[3]Department of Plant Pathology (University of Horticultural Sciences) Kolar-563101, Karnataka, India

[4]Department of Agronomy (University of Horticultural Sciences), Arabavi-591307, Karnataka, India. India

*Corresponding Author E-mail: jakatimathsanjeev7@gmail.com

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ABSTRACT

A total of 60 genotypes screened under filed condition against fruit rot of brinjal. Among 22 genotypes which showed different categories of resistant reaction were selected for the molecular characterization that helped in the reliable varietal selection programme for resistance breeding. All genotypes were analyzed by molecular parameters. For molecular characterization 34 RAPD markers were used for screening. Only 25 RAPD decamer primers were selected for the genetic analysis of all representative genotypes. The extent of genetic diversity and construction of phylogenetic tree was done by NTYSES software. Genetic similarity varied from 0.01 to 0.93 from each of the primer analyzed. Among the twenty two RAPD screened genotypes few showed the polymorphism. The whole phylogeny tree falls in two clusters namely C_1 & C_2 using Jaccard similarity coefficient of 0.01. The cluster C_2 again divided into different sub clusters namely SCA and SCB. Among all the genotypes represented in the phylollogenic tree, line-3 ($K_{12}d_{10}$12-6) & line-11 ($K_{12}D_{10}$52-1) were farthest to each other; however line-17 ($K_{12}D_{10}$36-1) & line-21($K_{12}D_{10}$11-5) were very closely related. Even though line 3 and line 19 belongs to different clusters with less co-efficient they look alike with respect to resistance against the fruit rot.

Key words: *Eggplant, Resistance, Fruit rot, Characterization, RAPD.*

INTRODUCTION

Brinjal or egg plant (*Solanum melongena* L.) is an important vegetable crop belongs to the family solanaceae. Brinjal is one of the most common, popular and principal vegetable crops grown in the tropical and sub tropical areas. It's a highly productive and usually finds its place as the poor man's crop. This crop is extensively grown in India, Pakistan, Bangladesh, China and Phillipines. South Asia accounts for almost 50 per cent of world brinjal area under cultivation[6]. This sturdy crop is cultivated throughout the year, even in the hot wet monsoon season when other vegetables are in short of supply.

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In India, brinjal is mainly grown in the states like West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Andhra Pradesh, Karnataka etc. with an area of 7.22 lakh hectare with a production of 135.58 metric tonnes and productivity of 19.10 tonnes per ha[1]. It contributes about 12.47 per cent of the total production of vegetables in India. In Karnataka, brinjal is cultivated over an area of 15,800 ha with a production of 4002.50 tonnes[2]. It is mainly grown in the Bagalkot district. The productivity is quite low because of some biotic and abiotic stresses which are limiting factors for the successful production of brinjal. A principal limiting factor in profitable cultivation of this crop is attack of several diseases mainly caused by group of fungi, bacteria and phytoplasma. This crop is prone to many diseases right from seedling stage to harvesting stage. Damping off, *Phomopsis* blight, fruit rot, leaf spot, wilt and phyllody are some of such diseases which, when become severe can cause heavy losses to the farmer. The fruit rot caused by a group of fungi, is becoming severe disease in northern dry zone. The disease was first reported from the Gujarat state in 1914 and since then from many parts of India. In general, the crop loss due to this disease ranges from 15-20%[7]. The fruit rot caused by *Colletotrichum melongenae* is a serious, important and destructive fungal disease causing 5-15% average yield losses[4]. When the environmental conditions are favourable the fruit rot incidence can be up to 50% and this can result serious economic losses[18],. It has been reported that *Phomopsis vexans* reduces yield and marketable value of the crop nearly 20-30 %[12]. The fruit infection takes place during fruit formation just some days prior to the harvest of the crop. This infection becomes severe at the time of harvesting to marketing. Though, it is suspected that many fungi are involved, the exact role of these fungi is not documented. And some farmers are using known fungicides indiscriminately and unscientifically which may result in residual toxicity problems in brinjal fruit. On the other hand no resistant variety / line/ germplasm are available for this disease. Hence there is alternative look for the assessment of genetic variation, is a major concern of plant pathologists, breeders and population geneticists. Availability of sufficient variation is required for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and a biotic factor. Hence the 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[3,5]. RAPD have been used to construct genetic maps and for the molecular tagging of various agronomic traits in various crop species[21]. 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*[14]., Sharmin *et al*[17]., Kabir[13] and Islam[9] characterized resistant and susceptible cultivars of eggplant along with their interspecific offspring in Bangladesh. The literature in this line of work reveals that since then not much work has been done on these aspects. Therefore, the following investigation were carried out in the Department of Plant Pathology, COH, Bagalkot, UHS, Bagalkot to thrash the threaten posed by the fruit rot of brinjal caused by the *Alternaria alternata, Colletotrichum melongenae* and *Phomopsis vexans* .

MATERIALS AND METHOD

Screening of Brinjal Lines Against Fruit Rot

The experiment on screening of 60 brinjal lines against fruit rot was conducted at Haveli farm of College of Horticulture Bagalkot, under the natural infection. Seedlings were raised in plastic trays in the net house with proper care and management. A piece of medium high land with good drainage system was selected. The field was prepared by ploughing and harrowing. During field preparation, fertilizers and manures were applied at recommended doses[1]. Seedlings of

30 days old were transplanted in the field and watered properly. The lines were planted in the 6m single line in two replications along with the available susceptible line (line no-26) in between every 5 lines. Five seedlings of each line were planted at 60×60 cm spacing and each line was replicated twice. Observations were recorded by screening the lines under natural disease pressure conditions. The lines were graded according to the 0 to 5 scales as suggested by Islam *et al*[10]., and finally PDI was calculated. Sixty genotypes/lines were evaluated under field condition to know their disease reaction against fruit rot of brinjal. Per cent disease index was calculated as formulae below. Further the varieties were placed in different categories of resistance and susceptibility on the basis of method given by Pathak *et al*[16]. Among sixty genotypes screened, 22 lines were selected as representative samples based on the different disease reaction. The leaf samples were collected from the 22 representative lines were bagged separately in poly bags and brought to the laboratory cleaned under running tap water and air dried then stored in the refrigerator for further RAPD analysis.

$$\text{Per cent disease index} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of samples} \times \text{Maximum disease grade}} \times 100$$

Scale for scoring the fruit rot of brinjal[10]

Sl. No	Grade	Description
1	0	0% infection on fruit
2	1	1-10% infection on fruit
3	2	10-15% infection on fruit
4	3	15-30% infection on fruit
5	4	30-40% infection on fruit
6	5	➢ 50% infection on fruit

Rapd-Pcr Finger Printing of Brinjal Germplasm Against Fruit Rot

The germplasm lines were characterized with respect to their resistance against fruit rot using decamer RAPD primers for finger printing of genotypes. The 22 representative germplasm belonging to different categories of resistant reaction *viz.*, resistant, moderately resistant and moderately susceptible line were used. The polymorphic banding pattern was obtained to analyze and characterize the tested germplasm lines based on their genetic relatedness.

DNA Extraction, Purification and Quantification

Genomic DNA was extracted from leaves of the different brinjal lines by the CTAB method, according to Murray and Thompson with some modifications to eliminate phenolics. From each cultivar, 1 g of leaves were ground in liquid nitrogen to a fine powder in a pre-chilled mortar and transferred into a 30 ml centrifuge tube containing 10 ml of DNA extraction buffer containing 0.1 M Tris chloride (pH 8.0), 0.02 M EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB (w/v), 2% poly vinyl pyrolidone (w/v) and 0.2% b-mercaptoethanol (v/v) and incubated in a water bath at 65^{o}C for 60 min. The tubes were cooled at room temperature and an equal amount of chloroform: isoamyl alcohol (24:1) was added, mixed thoroughly by gentle inversion and finally centrifuged at 10,000 rpm for 15 min at 20^{o}C. The upper aqueous layer was transferred to a fresh sterile centrifuge tube and 5µl vol chilled isopropanol was added and incubated overnight at -20^{o}C. The tubes were centrifuged at 10,000 rpm for 15 min at 4^{o}C and the pellet was washed with 70% ethanol, air dried and dissolved in 200 µl

TAE buffer and stored at 4°C. For DNA purification, 5ll RNase (10 mg/ml) was added to total isolated DNA (200ll) and incubated at 37°C for 60 min. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed gently. The tubes were centrifuged at 10,000 rpm for 5 min and the aqueous layer was transferred to fresh eppendorf tube and 1/10 volume of sodium acetate (3 M, pH 5.2) and a double volume of chilled absolute ethanol was added. After 30 min, the mixture was centrifuged at 10,000 rpm for 5 min and finally, the pellet was washed with 70% ethanol, dried and dissolved in 50X TE buffer. For quantification of genomic DNA, the absorbance of the DNA samples was measured at 260 nm in a Nano Drop 1000 (Thermo Scientific). After quantification, the quality of the purified DNA was analysed in a 0.7% (w/v) agarose gel.

Polymerase Chain Reaction

A total of 34 decamer oligonucleotides of arbitrary sequence tested for PCR amplification. Among them 13 RAPD primers were used for the polymorphism survey. The RAPD assay was carried out in 0.2 ml PCR vials containing 25 reaction buffer, 200 µM dNTPs mix, 0.4 µl primer, 50 ng DNA template, 1 U Taq DNA polymerase and sterile distilled water to a final volume of 25 micro litres. The content was gently mixed by spinning for a few seconds. The PCR amplification was performed with a thermo cycler (Eppendorf, Germany). The standardised amplification was performed at an initial denaturation at 94 °C for 4 min, followed by 40 cycles of denaturation at 94 °C for 30 seconds; primer annealing based on Tm for 1 min; primer extension at 72 °C for 2 min and final primer extension at 72 °C for 10 min. Polymerase chain reaction amplified products were analysed by agarose gel electrophoresis using 1.5% agarose in 19X TAE buffer. The amplicon sizes were measured with 5 kb DNA ladder. Respective gels were stained with 10 ppm ethidium bromide followed by image capturing using a gel documentation system (Herolab, Germany). The procedure was repeated twice for each DNA set and reproducible primers were subjected for genetic relatedness analysis.

Primers used for RAPD analyses

Sl. No	Primers
1	RAPD-1
2	OPB-04
3	OPJ-10
4	OPM-16
5	OPJ-06
6	RAPD-11
7	OPB-03
8	RAPD-03
9	OPD-16
10	OPJ-05
11	OPJ-04
12	OPA-06
13	OPA-AC-07
14	OPA-11
15	OPA-14
16	OPB-07
17	OPC-06
18	OPC-03
19	C-20
20	RAPD-07
21	OPB-03
22	OPB-17
23	OPC-20
24	OPE-AC-07

Descriptions of Reagents, Chemicals and Primers Used for Extracting Genomic DNA and Rapd Analyses

The details of reagents, chemicals and primers used for extracting genomic DNA and RAPD analyses are given below.

1. Reagent

For a Volume of 500 ml,

NaEDTA 3.7224 g

Tris HCl 6.0550 g

NaCl 40.9080 g

CTAB 10.0000 g

And CTAB was dissolved by heating to 60° C & stored at 37° C (*Autoclave*)**.** 0.5% -mercaptoethanol added just before use

2. Chloroform: Iso Amyl Alcohol: 24:1 v/v

3. 5 M NaCl (Autoclave)

4. TE Buffer: 10 mM Tris HCl and 1 mM EDTA prepared and mixed. pH was adjusted to 8.0 for a volume of 250 ml.

Tris HCl 0.3025 g

NaEDTA 0.0931 g (Autoclave)

5. 7.5 M Ammonium Acetate: pH 7.7 (Autoclave)

6. Wash Solution: 70% v/v ethanol; chilled

7. Alcohol: 95% stored at -20 ℃

8. Absolute Alcohol: Stored at -20 °C

9. RNAase: (10 mg/ml) Dissolved RNAase in 10 mM Tris HCl + 15 mM NaCl pH 7.5. Boiled for 5 min & cooled to room temperature.

10. PVPP (Poly Venyl Pyrrolidone Powder)

11. TBE Buffer/ TAE Buffer (Stock Solution*)*

a. 50X TAE in 500 ml water 10X TBE in 500 ml of Water.
b. 242.0 g Tris base 54.0 g Tris base 57.1 ml of Glacial acetic acid 27.5 g Boric acid.
c. 100 ml of 0.5 M EDTA (pH 7.0) 7.44 g EDTA (pH 8.0) or 20 ml of 50 mM EDTA (*Autoclave*) Working solution: *1X* dilute stock 10 times.

12. Bromophenol blue: *Stock solution:* 0.25% in 50% glycerol

13. Ethedium bromide: 10 mg/ml

Data Analysis

The various sizes of amplified products were scored for presence (1) or absence (0) in the twenty two genotypes to generate a binary matrix. Binary matrix data were analysed by the software NTSYS-pc, version 2.11w, to calculate the Jaccard's similarity coefficient. Genetic similarity between cultivars was calculated according to Jaccard's similarity coefficient[11]. The computer programme WINBOOT was used to determine the robustness of the dendrogram, with 2,000 replications along with Jaccard's coefficient. Per cent polymorphism was calculated for each primer combination according to the formula given below.

% Polymorphism = p / (m+p)

Where, p is total number of polymorphic bands and m is the total number of monomorphic bands of the primer combination.

RESULT AND DISCUSSION

Randomly amplified polymorphic DNA assays were performed with 34 random primers. Of these, 24 produced polymorphic and reproducible bands and were selected for further screening. Out of 34 primers 13 primers were selected and assessed to know the difference between the 22 selected cultivars. Genetic similarity varied from 0.01 to 0.93 (Table 3) from each of the primer analyzed. Detailed pictorial representation is shown in the **Fig. (1)** with 9 primers (OPERON-AC07, OPA-06, OPB-03, OPB-03, OPB-07, OPB-17, OPC-03, OPJ-05, and RAPD-1) which have shown 100% polymorphism and remaining 4 primers (C-20, OPB-04, OPJ-04 and RAPD-3) which showed 58-78% polymorphism. Tabulation is presented in Table (2). The whole phylogeny tree falls in two clusters namely C_1 & C_2 using Jaccard similarity coefficient of 0.01. The cluster C_2 again divided into different sub clusters namely SCA ($K_{12}D_{10}$ 35-1, $K_{12}d_{10}$12-6, $K_{12}d_{10}$77-3, $K_{12}D_{10}$75, Melavanki local, $K_{12}D_{10}$25-1, Bijapur local, $K_{12}D_{10}$39-1, $K_{12}D_{10}$87-2, $K_{12}D_{10}$52-1, $K_{12}D_{10}$32-5, $K_{12}D_{10}$97-3, $K_{12}D_{10}$104-1, $K_{12}D_{10}$ 36-3, $K_{12}D_{10}$75-3, R-2590, $K_{12}D_{10}$38-5, $K_{12}D_{10}$11-5 and $K_{12}D_{10}$129-4) and SCB ($K_{12}D_{10}$2-3). Remaining clusters fall under the SBA2 cluster. Among all the genotypes represented in the phylollogenic tree, line-3 ($K_{12}d_{10}$12-6) & line-11 ($K_{12}D_{10}$52-1) were farthest to each other; however line-17 ($K_{12}D_{10}$36-1) & line-21($K_{12}D_{10}$11-5) were very closely related (Fig.1). Even though line 3 and line 19 belongs to different clusters with less co-efficient they look alike with respect to resistance against the fruit rot. The detail representation of RAPD

profile of 13 primers showed in the Plate 8 (a&b). Breeding for the disease resistance has been an effective, economical and practical method of disease control. Cultivation of resistant variety seems to be the best alternative and most economical to keep the activity of fruit rot pathogen under control. In all crop improvement programmes, growing of resistant varieties has been found to be appropriate choice to combat the disease. The use of resistant cultivars is perhaps the most desirable method of controlling diseases in crops[19]. This approach, according to Voorrips *et al*[20]., has been less exploited in fruit and vegetable crops mainly due to the longer time required for breeding and selecting for resistance and the short term advantage of chemical control. Efforts have been made to locate the source of resistance for this disease in India. In the present investigation, the reaction of different genotypes against fruit rot was carried out in field conditions. Sixty brinjal genotypes were screened against brinjal fruit rot under natural condition as described in material and methods. The data revealed that, among the 60 genotypes evaluated, none was found immune. Two genotypes *viz.*, CBB-3 and CBB-26 were found resistant, 31 genotypes were moderately resistant and 27 genotypes showed susceptible reaction. None of the genotypes showed highly susceptible reaction. The results are in contrary with findings of Pandey *et al*[15]., who conducted the experiment to evaluate 41 entries of brinjal under natural epiphytotic condition against *Phomopsis* blight disease. Among 41 lines evaluated, none of the entries were found resistant to fruit rot. Two varieties *viz.*, Ramanagar giant and KS-233 showed moderate resistance and others showed susceptibility. However both DBR-91 and baramasi recorded high susceptibility with fruit rot intensity of 4.72 / plant and per cent fruit infection of 47.5% and 85% respectively. In the present investigation, according to phenotypic analysis CBB-1 & CBB-26 were found resistant so, by above result it revealed that same can be used in the breeding strategies for the crop improvement programme to develop resistant varieties. Randomly amplified polymorphic DNA assays were performed with 34 random primers. Of these, 28 produced polymorphic and reproducible bands. In an assay of the 13 RAPD primers on the 22 cultivars, genetic similarity varied from 0.01 to 0.93 from each of the primer analysed. The whole phylogeny tree fell in two clusters namely C_1 & C_2 using Jaccard similarity coefficient of 0.01. The cluster C_2 again divided into different sub clusters namely SCA and SCB while remaining fell under the SBA2 cluster. Among all the genotypes represented in the phylollogenic tree line-3 ($K_{12}d_{10}12$-6) & line-11 ($K_{12}D_{10}52$-1) were farthest to each other while line-17 ($K_{12}D_{10}36$-1) & line-21($K_{12}D_{10}11$-5) were very closely related. However Sharmin *et al*[17]., reported that molecular variability and relatedness of three parents and two F_5 offspring's by RAPD technique to see the continuity of the resistance character. The results revealed that *Phomopsis* resistant cultivar BAU Begun-1 when crossed with two cultivars-Dohazari G and Laffa, all the F_1, F_2, F_3 and F_4 plants showed resistance[9]. Ibrahim *et al*[8]., reported the molecular characterization of F_4 lines of egg plants and revealed that random amplified polymorphic DNA technique was used for assessing genetic variation and relationship among parent cultivars and their F4 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). Resistant cultivars such as Kalenda, Aranquez, Zebrina, Aomura and Porcelaine screened through RAPD analysis can be used, if available, to the farmer. However, these cultivars must be used judiciously to prevent breakdown by the pathogen.

Table 1: Genotypes screened in the field used for RAPD analysis and their reaction to disease in the field

Sl. No.	Lines	Genotypes	Disease intensity
1	Line-1	Malapur local	Moderately resistant
2	Line-2	$K_{12}D_{10}$ 35-1	Moderately susceptible
3	Line-3	$K_{12}d_{10}$12-6	Resistant
4	Line-4	$K_{12}d_{10}$77-3	Moderately susceptible
5	Line -5	$K_{12}D_{10}$75-2	Moderately resistant
6	Line-6	MELAVANKI LOCAL	Moderately resistant
7	Line-7	$K_{12}D_{10}$25-1	Moderately resistant
8	Line-8	BIJAPUR LOCAL	Moderately resistant
9	Line-9	$K_{12}D_{10}$39-1	Moderately susceptible
10	Line-10	$K_{12}D_{10}$87-2	Moderately susceptible
11	Line-11	$K_{12}D_{10}$52-1	Moderately susceptible
12	Line-12	$K_{12}D_{10}$32-5	Moderately resistant
13	Line-13	$K_{12}D_{10}$97-3	Moderately susceptible
14	Line-14	$K_{12}D_{10}$104-1	Moderately susceptible
15	Line-15	$K_{12}D_{10}$ 36-3	Moderately susceptible
16	Line-20	$K_{12}D_{10}$75-3	Moderately resistant
17	Line-32	$K_{12}D_{10}$36-1	Moderately resistant
18	Line-34	R-2590	Moderately resistant
19	Line-26	$K_{12}D_{10}$2-3	Resistant
20	Line-21	$K_{12}D_{10}$38-5	Moderately resistant
21	Line-46	$K_{12}D_{10}$11-5	Moderately resistant
22	Line-22	$K_{12}D_{10}$129-4	Moderately susceptible

Table 2: Summary of genetic analyses obtained by using 13 RAPD primers for 22 brinjal (*Salanum melongenae* L.)

Sl. No.	Primer name	Sequence	Total no. of bands	Total no. of polymorphic bands	No. of Monomorphic bands	Percent polymorphism
1	OPERON-AC07	GTGCCCGATC	80	00	00	100
2	C-20	ACTTCGCCAC	105	61	44	58.09
3	OPA-06	GGTCCCTGAC	54	00	00	100
4	OPM-16	GTAACCAGCC	111	00	00	100
5	OPB-03	CATCCCCCTG	62	00	00	100
6	OPB-04	GGACTGGAGT	100	78	22	78
7	OPB-07	GGTGACGCAG	68	00	00	100
8	OPB-17	GACCGCTTGT	50	00	00	100
9	OPC-03	GGGGGTCTTT	33	00	00	100
10	OPJ-04	CCGAACACGG	74	52	22	70.27
11	OPJ-05	CTCCATGGGG	58	00	00	100
12	RAPD-1	CCACACTACC	80	36	44	100
13	RAPD-3	CGGCCCCGGC	113	69	44	61.06

Table 3: Jaccord similarities

Rows/ cols	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22
G1	1.00																					
G2	0.01	1.00																				
G3	0.16	0.07	1.00																			
G4	0.59	0.02	0.15	1.00																		
G5	0.64	0.01	0.13	0.56	1.00																	
G6	0.64	0.01	0.14	0.62	0.84	1.00																
G7	0.66	0.01	0.15	0.58	0.80	0.76	1.00															
G8	0.14	0.01	0.01	0.19	0.13	0.14	0.10	1.00														
G9	0.13	0.01	0.01	0.26	0.17	0.19	0.15	0.62	1.00													
G10	0.34	0.01	0.12	0.46	0.40	0.45	0.35	0.32	0.42	1.00												
G11	0.37	0.01	0.17	0.34	0.25	0.27	0.22	0.32	0.29	0.58	1.00											
G12	0.03	0.01	0.01	0.04	0.02	0.02	0.02	0.16	0.14	0.06	0.09	1.00										
G13	0.04	0.01	0.01	0.10	0.14	0.11	0.05	0.04	0.03	0.15	0.03	0.07	1.00									
G14	0.49	0.01	0.07	0.48	0.74	0.67	0.62	0.12	0.15	0.35	0.23	0.02	0.10	1.00								
G15	0.12	0.01	0.00	0.22	0.13	0.15	0.15	0.53	0.47	0.25	0.18	0.18	0.04	0.12	1.00							
G16	0.16	0.01	0.26	0.10	0.13	0.14	0.17	0.20	0.18	0.09	0.10	0.07	0.08	0.04	0.21	1.00						
G17	0.50	0.01	0.07	0.52	0.78	0.67	0.64	0.13	0.15	0.36	0.23	0.02	0.14	0.93	0.12	0.05	1.00					
G18	0.48	0.01	0.18	0.47	0.54	0.56	0.56	0.18	0.21	0.43	0.32	0.03	0.04	0.50	0.18	0.20	0.50	1.00				
G19	0.56	0.01	0.12	0.52	0.87	0.77	0.74	0.12	0.14	0.35	0.22	0.02	0.13	0.83	0.11	0.12	0.86	0.52	1.00			
G20	0.55	0.01	0.12	0.52	0.82	0.73	0.73	0.12	0.14	0.34	0.22	0.02	0.11	0.85	0.11	0.12	0.89	0.59	0.90	1.00		
G21	0.50	0.01	0.07	0.50	0.75	0.68	0.63	0.13	0.14	0.36	0.23	0.02	0.13	0.92	0.12	0.05	0.96	0.50	0.85	0.88	1.00	
G22	0.67	0.01	0.00	0.18	0.10	0.13	0.13	0.57	0.15	0.26	0.14	0.20	0.04	0.10	0.61	0.15	0.10	0.14	0.10	0.10	0.11	1.00

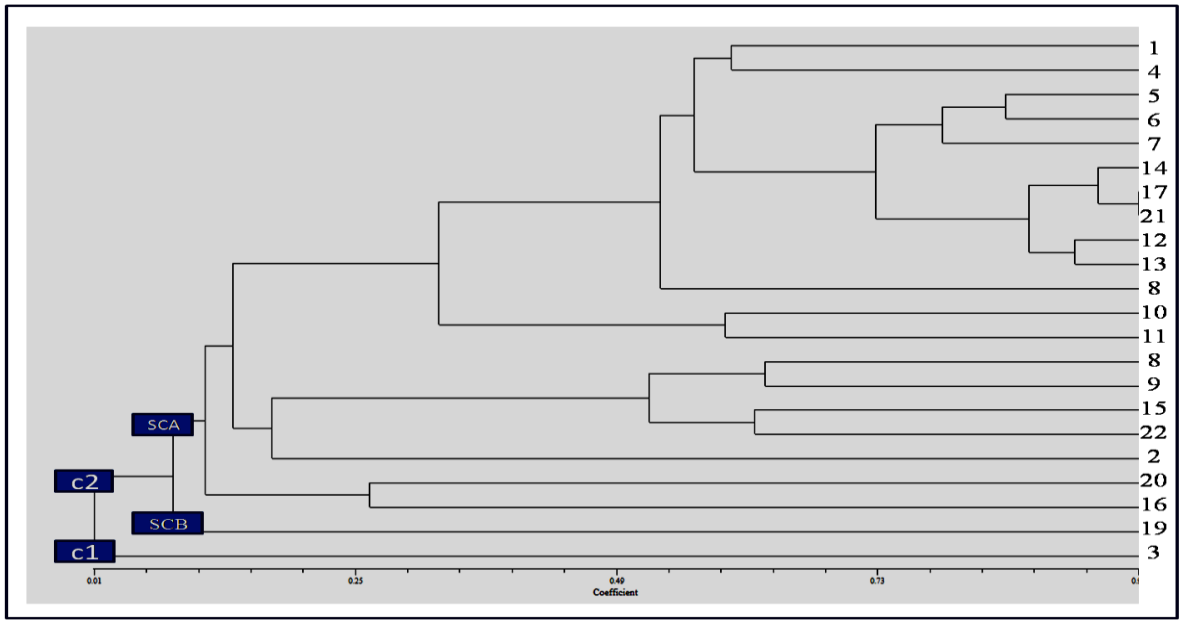

Fig. 1: NTYES cluster analysis showing the relationship of disease intensity and diversity among 22 genotypes of brinjal (*Salanum melongena)* produced by RAPD

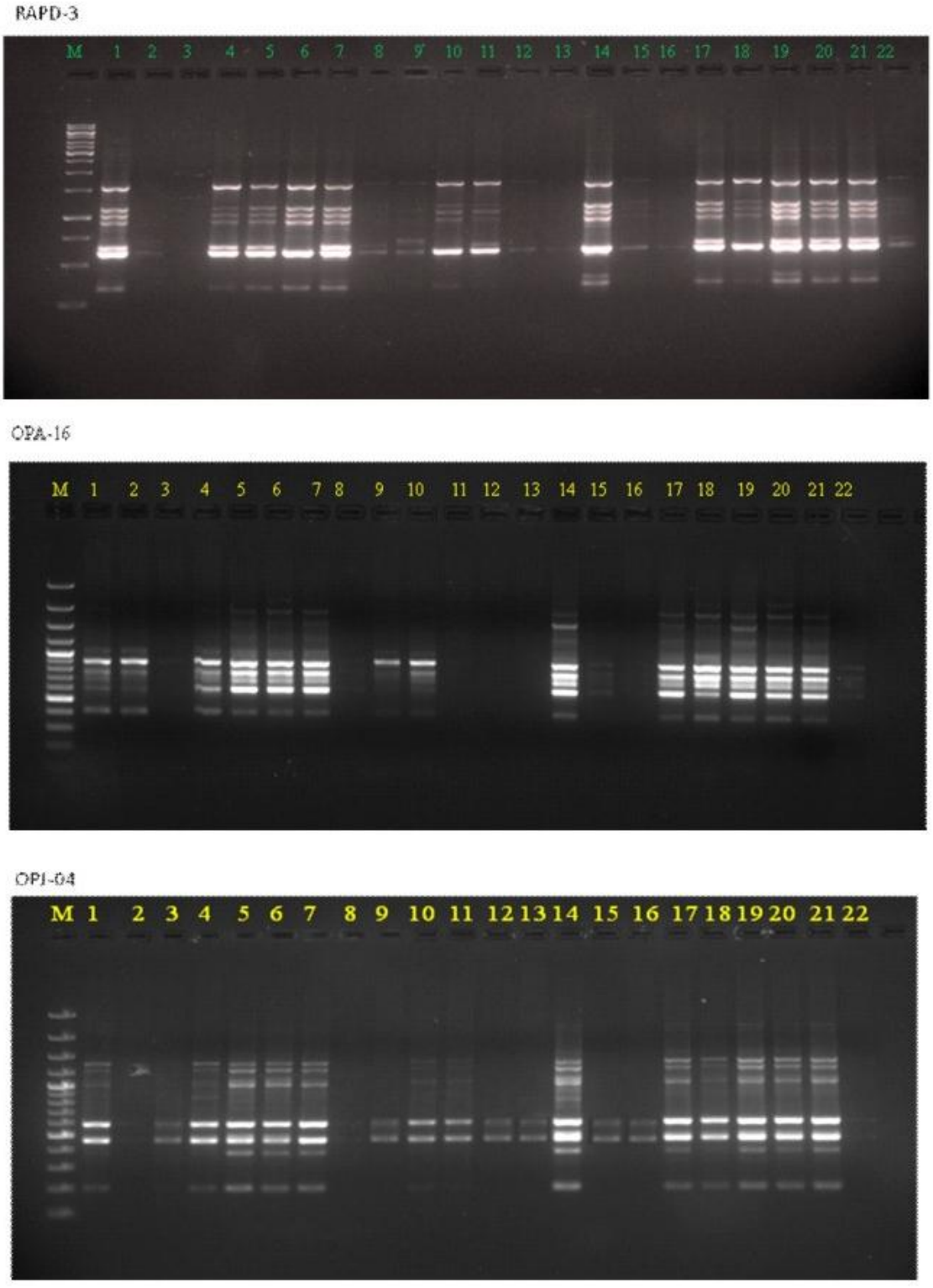

Plate 8(b): RAPD profile of 22 brinjal genotypes generated by random primer (RAPD-3, OPA-16, and OPJ-04. The lanes represent, lane M100-bp ladder; lanes 1-22 (Brinjal genotypes)

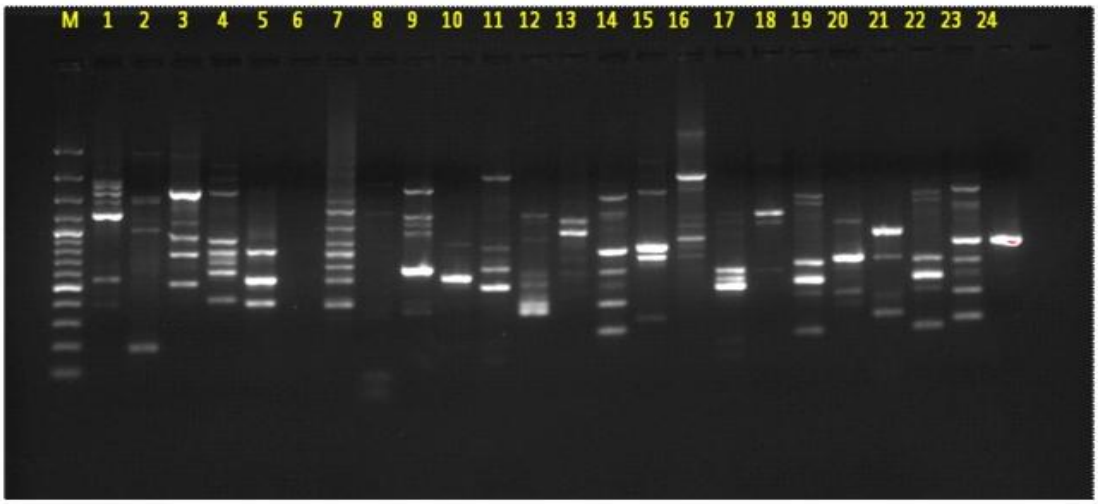

Plate 7: RAPD amplification products of primer (RAPD-1, OPB-04, OPJ-10, OPA-16, OPJ-06, RAPD-11, OPB-03, RAPD-3, OPD-16, OPJ-05, OPJ-04, OPA-06, OPERON AC-07, OPA-11, OPA-14, OPB-07, OPC-06, OPC-03, C-20, RAPD-7, OPB-03, OPB-17, OPC-20 and OPE-AC-07)

Opb-4

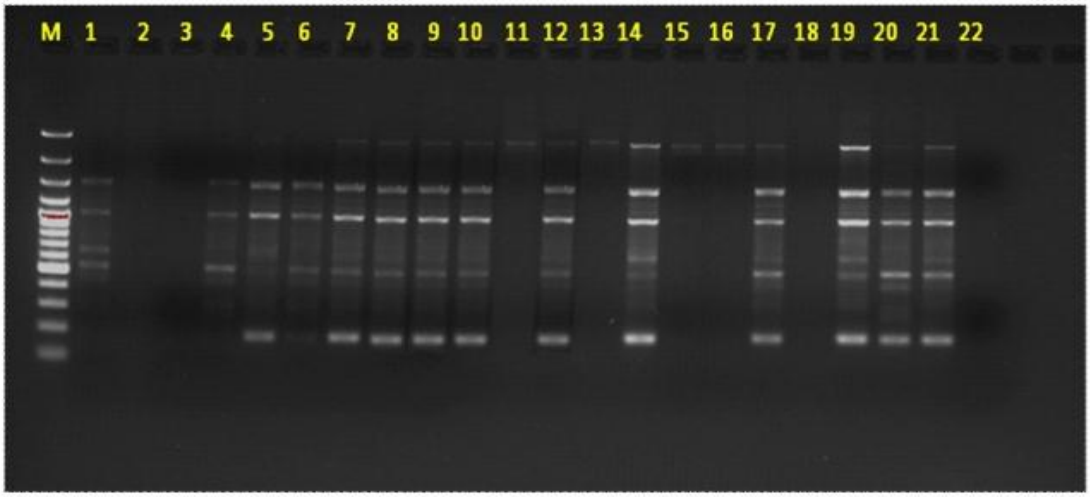

Plate 8(a): RAPD profile of 22 brinjal genotypes generated by random primer (OPB-4) (The lanes represent, lane M,100-bp ladder; lanes 1-22 (Brinjal genotypes))

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