Bangladesh J. Bot. **51**(3): 519-526, 2022 (September) DOI: https://doi.org/10.3329/bjb.v51i3.61998

VARIATION OF GENETIC DIVERSITY OF *RALSTONIA SOLANACEARUM* IN TOMATO AND EGG PLANT OF BANGLADESH

PURNIMA DEY, ISMAIL HOSSAIN[1], HYAT MAHMUD*[2], SAJAL KUMER DEY[3] AND MOHAMMAD DELWAR HOSSAIN[4]

Professor Golam Ali Fakir Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh

Keywords: Genetic diversity, *Ralstonia solanacearum*, Solanaceous, Tomato, Egg plant

Abstract

Genetic diversity of *Ralstonia solanacearum,* on seeds of tomato and brinjal from different seed sources in Bangladesh were studied with Random Amplified Polymorphic DNA (RAPD) marker. The primers *viz.*, OPA02, OPA08, OPA09, OPA10, OPA18 and OPE19 were used in this study and were also evaluated on the basis of intensity and resolution of the band. The genetic variations in 24 isolates of *R. solanacearum* were analyzed with (OPA18) RAPD marker in Polymerase Chain Reaction (PCR). The percentage of polymorphic loci was 100.00 for both tomato and brinjal. Combined Dendrogram based on Nei's (1972) genetic distance in Unweighted Pair Group Method of Arithmetic Means (UPGMA) segregated the 24 isolates of *R. solanacearum* into two main clusters: RsT-6, RsB-2 and RsB-3 in cluster 1, and rest of the 24 isolates were grouped in cluster 2**.** The isolate of brinjal (RsB-8) was close to the isolate RsB-12 and the isolate RsB-11 was near to RsT-5 (tomato) with the least genetic distance (0.000), while the brinjal isolate RsB-4 was also close to RsB-5 with the low genetic distance (0.0606). The coefficient of gene differentiation (G_{st}) was obtained at 1.0000 which reflects the existence of higher genetic variations among 24 isolates of *R. solanacearum*. Comparatively higher genetic distance (1.7346) and the lower genetic identity (0.1765) were observed in RsB-1 vs. RsT-12, whilst the higher genetic identity (1.000) and the lower genetic distance (0.000) were estimated in RsB-8 vs. RsB-12 and RsB-11 vs. RsT-5, respectively. Consequently, interactive significant genetic variations in 24 isolates of *R. solanacearum* of tomato and brinjal seed sources were found to be evident in both the crop species' of diversified different varieties as the genetic variability of specific crop species.

Introduction

Tomato and brinjal are the important vegetable in terms of production, commercial use and consumption in Bangladesh. Solanaceous crops are highly prone to a number of bacterial diseases. Bacterial wilt caused by *Ralstonia solanacearum* is the destructive and harmful disease of the solanaceous family, resulting in complete loss of the crop (Kelman 1954, Hayward 1991). *R. solanacearum* has its exceptional diversity among the strains related to host range, and higher virulence with geographical distribution (Hayward 1994). *R. solanacearum* bacteria become motile during infection process, and travel throughout the vascular system in the plant (Grey and Steck 2001). The pathogen mostly survives through soil and crop residue (Granada and Sequeira 1983). The pathogen is also carried in both the seeds of tomato and eggplant (Shakya 1992, Roopali 1994). Yield losses (60-100%) are endemic due to tomato bacterial wilt *R, solanacearum*

*Author for correspondence: <mhyat81@gmail.com>. [1]College of Agricultural Sciences, International University of Business Agriculture and Technology (IUBAT), 4 Embankment Drive Road, Sector 10, Uttara Model Town, Dhaka-1230, Bangladesh. [2]Department of Agricultural Extension, Khamarbari, Kushtia, Bangladesh. [3]World Vision Bangladesh. [4]Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

(Popoola *et al*. 2015). Bacterial wilt caused by *R. solanacearum* in brinjal is also a very devastating disease that reduces eggplant production from 4.24 to 86.14% (Sabita *et al*. 2000). However, there is a principal need to develop reliable, fast and specific identification methods to prevent spread of diseases caused by several phytopathogenic bacteria.

Assessment of molecular polymorphism is more meaningful than the phenotypic level data on morphological traits that are closely related to the environment. So the study of polymorphism is ascertained at nucleotide bases in DNA which attributes all biological information. In this level, identical accessions show wider distinct variations. It is urged to evaluate the distributed genetic variation in order to use the genetic resources, while this distribution is influenced by environmental and species characteristics (Vilela-Morales *et al*. 1997). Research on genetic variability of *R. solanacearum* in the specific species of tomato or brinjal has been studied with significant variation, but combined effect of genetic diversity on *R. solanacearum* of tomato and brinjal have not been yet conducted in different seed sources in Bangladesh.

Polymerase chain reaction (PCR) amplifies random fragments from genomic DNA. Among the available DNA molecular techniques, RAPD has many advantages over others such as easy and rapidity in analysis, low cost, availability of large numbers of primers and the small amount of DNA for analysis (Weising *et al.* 2005). In the present study, the genetic variation among the isolates of *R. solanacearum* of different sources of tomato and brinjal seeds available in Bangladesh was led with RAPD marker.

Materials and Methods

Twelve seed samples of tomato (Novelty hybrid, Digonta, Utsab, Udayan F1, Ratan, RomaVF –Lal teer, Roma VF- Metal, Marglove, Roma VF- Khrishan Agro, Roma VF- Pashapashi, Bina Tomato–5 and BARI Tomato-3 (Harvested seed sample for own experiment), and 12 seed samples of brinjal (Chalanger eggplant F1, ACI beguni, Uttara, Kranti, Shingnath, Khatkhatia, Kata begun, Laffa, Zhumki, Kaikka nandina, Islampuri and BARI Begun-1) were collected from different seed companies, Research Institute, seed agency and seed stores in Bangladesh. Consecutively, 12 brinjal seed samples of 88 and 12 tomato seed sample among 77 were tested to determine the variation of *R. solanaceurum* in the present study.

All seed samples of tomato and brinjal were studied to find out the molecular character of *R. solanacearum* at molecular plant pathology laboratory, Department of plant pathology, BAU, Mymensingh during 2013-2016. The primers such as OPA02, OPA08, OPA09, OPA10, OPA18 and OPE19 were evaluated in this study. Of these, OPA18 was selected for its good amplification product and showed a good quality banding pattern.

DNA samples of each isolate were extracted following the method of Ausubel *et al.* (1987). The bacteria were cultured for 18 hrs at 27°C with shaking and 1.5 ml of bacterial culture was transferred in a micro centrifuge tube with spin at 8000 rpm for 5 min. One ml of 2M NaCl was used for pellet purification and was resuspended in the 525 µl of TE buffer by vortexing. Then 60 µl of 20% SDS and 15 µl of proteinase K (200 µg/ml) were mixed gently in the mixture of each tube. The tube was incubated at 37°C for 1 hr and 100 µl of 5 M NaCl was added in the mixture of each tube. Then 80 µl of CTAB was mixed well gently at every 5 min followed by incubation in a heated water bath at 65°C. DNA was taken with an equal volume (750 µl) of chloroform-isoamyl alcohol and mixed by inverting the tube completely. The tubes were centrifuged at 12000 rpm for 10 min at 4°C and the supernatant was placed in a new tube very carefully to avoid the chloroform in the supernatant. The aqueous layer was then transferred to a clean tube. Then equal volume of phenol-chloroform was added in every tube and centrifuged with 12000 rpm at 4°C for 10 min. All the supernatants of tubes were transferred to the new tubes. Then equal volume (500 µl) of

isopropanol was mixed well in the supernatant by inverting the tube. The tubes were centrifuged at 4°C for 20 min of 13000 rpm and the DNA pellet was washed with 1 ml of 75% ethanol. Ethanol was removed and the pellet was allowed to dry in the air for 5 min. The pellet in white color was resuspended in 20 µl of TE buffer and incubated at room temperature for 10 min. The extracted genomic DNA was preserved in the refrigerator at - 20°C until use.

Polymerase chain reaction (PCR) reactions were determined on each amplified DNA with the primer AGGTGACCGT (5'–3') (Khayamie *et al.* 2009). PCR reaction mixture consists of Template DNA 2.5 µl, Taq buffer 1.5 µl, 250 µM each of dNTPs0.5 µl, MgCl2 1.0 µl (3 mM), Ampli Taq DNA polymerase 0.2 µl, ddH2O 3.8 µl and Primer 2.5 µl in total volume of 12.0 µl. The thermal cycling profile was started at 92°C for 5 min in initial denaturation followed by 45 cycles at 94°C for 1 min, 1 min annealing at 37°C, elongation at 72°C for 2 and 10 min for 72°C in final extension for all amplified fragments. Amplified DNA of all isolates and 1 kb ladder were used in the gel electrophoresis at 100 mV for 7 hrs. The gel was stained with ethidium bromide for 15 min at room temperature and it was transferred from the ethidium bromide tray and kept on the UV transilluminator for image documentation of DNA bands and photographed with the Gel Documentation System.

All isolates of *R. solanacearum* were applied for cluster analysis based on RAPD results. The amplified bands were visually scored as present (I) and absent (0) for each individual and each primer. The obtained scores were pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's genetic distance (Nei 1973), genetic diversity, Population differentiation (Gst), gene flow (Nm), genetic distance and the unweighted pair group method (UPGMA) with arithmetic means dendrogram using the computer program, POPGENE (Version I.31) (Yeh *et al.* 1999). The same program was also used to perform test for homogeneity in different loci between population pairs.

Gene flow, (Nm) was estimated according to the following formula

Gene flow, Nm = 0. 5 (1- Gst)/Gst

Where, Gst is the proportion of total genetic diversity attributable to subpopulation. It is also known as the coefficient of gene differentiation.

The Gst values were calculated by using the following formula:

Gst = 1-Hs/ Ht Where, Hs is the mean average heterozygosity of the total population and Ht is the mean of Hardy-Weinberg expectation of heterozygosity obtained with population average allele frequencies.

Nei's genetic distance and identity values were computed from frequencies of polymorphic markers to estimate genetic relationship among the isolates using the Unweighted Pair-Group Method of Arithmetic means (UPGMA) (Nei 1973). The dendrogram was constructed using the POPGENE (Version 1.31) computer program 30 (Yeh *et al.* 1999).

Results and Discussion

The mean of Nei's (1972) gene diversity and Shannon's Information indices in 24 isolates of *R. solanacearum* on seeds of different seed samples of tomato and brinjal were found to be 0.4500 and 0.6415, respectively in combined analysis (Table 1). Gene diversity (h) in the isolates estimated the gene flow (N_m) value in Nei's analysis as presented in Table 2. Average heterozygosity (H_t) of both crops showed 0.4500. Heterozygosity (H_S) was also obtained 0.000 among them. The highest level co-efficient of gene differentiation (1.00) was noted and higher level differentiation in variety supported the presence of sufficient polymorphisms in the isolates (Table 2). These findings were in agreement with the results as reported by Xu *et al.* (2009) and

Mahbou *et al.* (2009). Higher levels of genetic diversity in the strains of pathogen *R. solanacearum* were observed by them. Genetic variability in *R. solanacearum* with higher genetic divergence in host range was reported by Prakasha *et al.* (2016). Nishat *et al.* (2015) also reported that higher levels of genetic variation were found in the gene differentiation of 0.5487.

Table 1. Mean Genetic diversity and Shannon's Information index of 24 isolates of *Ralstonia solanacearum* collected from seeds of different seed samples of tomato and brinjal.

Crop	Loci	Sample size	na*	ne*	h*	I*
Tomato + Brinjal	17	24	2.000	1.8285	0.4500	0.6415

* na = Observed number of alleles, * ne = Effective number of alleles, * h = Nei's (1973) gene diversity,
* I = Shannon's Information index

Table 2. Mean Gene flow (N_m) and the proportion of total genetic diversity (G_{st}) across different RAPD markers of isolates of tomato and brinjal.

Crop	Loci	Sample size	Ht	Hs	Gst	Nm*
Tomato + Brinjal	17	24	0.4500	0.0000	1.0000	0.0000

Ht = Hardy-Weinberg average heterozygosity expectated in isolates, Hs = Hardy-Weinberg average heterozygosity obtained in isolates, Gst = Co-efficient of gene differentiation, * Nm = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst.

Pair-wise comparisons of Nei's (1972) genetic distance in 24 different isolates of *R. solanacearum* of tomato and brinjal seeds of different seed sources with varieties were computed from combined data sets with one primer ranging from 0.000 to 1.7346 (Table 3). The highest (1.7346) genetic distance was observed in the isolate of Metal seed company Ltd. vs Harvested seed sample of own experiment (BARI Tomato-3), while the lowest genetic distance (0.000) was estimated in Krishan Agro service vs. Harvested seed sample of own experiment (BARI Begun-1) and Yasin Beez Vander vs. Lal Teer seed company. Genetic identity among the isolates was found in one primer ranging from 0.1765 to 1.000 as presented in Table 3. Comparatively, the isolate of Krishan Agro service vs. Harvested seed sample of own experiment (BARI Begun-1) and Yasin Beez Vander vs. Lal Teer seed company exhibited higher genetic identity (1.000) and the lowest (0.1765) genetic identity was observed between the isolate of Metal seed company limited vs. Harvested seed sample of own experiment (BARI Tomato-3) (Table 3). These were the complementing works of Papke and Ward (2004), and Goss *et al.* (2005). The genetic distance of *R. solanacearum* was obtained very near between two same clusters in the isolates of the same host with their lower genetic variation in different locations, but wider variation was absolutely found in different clusters with host range as reported by Nishat *et al.* (2015). Dey *et al.* (2021) also observed the highest genetic identity (0.8500) and the lowest (0.3000) genetic identity in the isolates of *R. solanacearum*. These findings were also supported with the observation of Afrose *et al.* (2014), Jannat *et al.* (2014) and Khayamie *et al.* (2009).

Dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) resulted in segregating the 24 isolates of *Ralstonia solanacearum* of tomato and brinjal seed samples of different varieties in two main clusters: RsB-1, RsT-6, RsB-2 and RsB-3 were formed in cluster 1 and rest of the 24 isolates resulted in cluster 2 (Fig. 1). In cluster 1, RsB-1, RsT-6 and RsB-2 belonged to sub cluster 1 which generated into sub sub cluster 1with RsB-1 and RsT-6, and sub sub cluster 2 was with RsB-2. RsB-3 was also observed in the

Table 3. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) in different isolates pair of *Ralstonia solanacearum* of tomato (Isolate 13-24: RsT1-RsT12) and brinjal (Isolate 1-12: RsB1-RsB12)

Isolate	1	2	3	4	5	6	7	8	9	10	11	12
1	****	0.6471	0.5882	0.4118	0.3529	0.5294	0.4118	0.2941	0.4118	0.4706	0.4118	0.2941
2	0.4353	****	0.5882	0.6471	0.5882	0.4118	0.6471	0.5294	0.5294	0.5882	0.5294	0.5294
3	0.5306	0.5306	****	0.3529	0.2941	0.5882	0.3529	0.4706	0.3529	0.5294	0.3529	0.4706
4	0.8873	0.4353	1.0415	****	0.9412	0.2941	0.7647	0.5294	0.4118	0.4706	0.5294	0.5294
5	1.0415	0.5306	1.2238	0.0606	****	0.2353	0.7059	0.4706	0.3529	0.5294	0.4706	0.4706
6	0.6360	0.8873	0.5306	1.2238	1.4469	****	0.5294	0.6471	0.6471	0.7059	0.6471	0.6471
7	0.8873	0.4353	1.0415	0.2683	0.3483	0.6360	****	0.7647	0.6471	0.7059	0.7647	0.7647
8	1.2238	0.6360	0.7538	0.6360	0.7538	0.4353	0.2683	****	0.8824	0.5882	0.8824	1.0000
9	0.8873	0.6360	1.0415	0.8873	1.0415	0.4353	0.4353	0.1252	****	0.4706	0.8824	0.8824
10	0.7538	0.5306	0.6360	0.7538	0.6360	0.3483	0.3483	0.5306	0.7538	****	0.5882	0.5882
11	0.8873	0.6360	1.0415	0.6360	0.7538	0.4353	0.2683	0.1252	0.1252	0.5306	****	0.8824
12	1.2238	0.6360	0.7538	0.6360	0.7538	0.4353	0.2683	0.0000	0.1252	0.5306	0.1252	****
13	0.8873	0.6360	0.7538	0.6360	0.7538	0.4353	0.4353	0.1252	0.1252	0.7538	0.1252	0.1252
14	1.4469	1.0415	0.8873	0.7538	0.6360	0.7538	0.7538	0.3483	0.3483	0.8873	0.3483	0.3483
15	1.0415	0.7538	0.8873	0.1942	0.1252	1.4469	0.5306	0.7538	1.0415	0.6360	0.7538	0.7538
16	1.2238	0.8873	0.7538	0.4353	0.3483	1.2238	0.8873	0.6360	0.6360	1.0415	0.6360	0.6360
17	0.8873	0.6360	1.0415	0.6360	0.7538	0.4353	0.2683	0.1252	0.1252	0.5306	0.0000	0.1252
18	0.3483	0.5306	0.6360	1.0415	1.2238	0.7538	0.7538	0.7538	0.5306	0.8873	0.7538	0.7538
19	0.7538	1.0415	1.2238	0.7538	0.6360	0.7538	0.7538	1.0415	1.0415	0.6360	0.7538	1.0415
20	0.4353	0.8873	0.7538	1.2238	1.0415	0.6360	0.8873	0.8873	0.6360	0.5306	0.6360	0.8873
21	0.6360	0.8873	0.7538	0.8873	0.7538	0.6360	0.8873	0.6360	0.6360	1.0415	0.6360	0.6360
22	1.0415	1.0415	0.8873	0.7538	0.8873	0.5306	0.5306	0.5306	0.3483	0.8873	0.5306	0.5306
23	1.0415	0.7538	0.8873	1.0415	0.8873	0.5306	0.5306	0.3483	0.3483	0.4353	0.5306	0.3483
24	1.7346	1.2238	0.7538	0.6360	0.5306	0.6360	0.6360	0.4353	0.6360	0.7538	0.6360	0.4353

Table 3 right side contd.

13	14	15	16	17	18	19	20	21	22	23	24
0.4118	0.2353	0.3529	0.2941	0.4118	0.7059	0.4706	0.6471	0.5294	0.3529	0.3529	0.1765
0.5294	0.3529	0.4706	0.4118	0.5294	0.5882	0.3529	0.4118	0.4118	0.3529	0.4706	0.2941
0.4706	0.4118	0.4118	0.4706	0.3529	0.5294	0.2941	0.4706	0.4706	0.4118	0.4118	0.4706
0.5294	0.4706	0.8235	0.6471	0.5294	0.3529	0.4706	0.2941	0.4118	0.4706	0.3529	0.5294
0.4706	0.5294	0.8824	0.7059	0.4706	0.2941	0.5294	0.3529	0.4706	0.4118	0.4118	0.5882
0.6471	0.4706	0.2353	0.2941	0.6471	0.4706	0.4706	0.5294	0.5294	0.5882	0.5882	0.5294
0.6471	0.4706	0.5882	0.4118	0.7647	0.4706	0.4706	0.4118	0.4118	0.5882	0.5882	0.5294
0.8824	0.7059	0.4706	0.5294	0.8824	0.4706	0.3529	0.4118	0.5294	0.5882	0.7059	0.6471
0.8824	0.7059	0.3529	0.5294	0.8824	0.5882	0.3529	0.5294	0.5294	0.7059	0.7059	0.5294
0.4706	0.4118	0.5294	0.3529	0.5882	0.4118	0.5294	0.5882	0.3529	0.4118	0.6471	0.4706
0.8824	0.7059	0.4706	0.5294	1.0000	0.4706	0.4706	0.5294	0.5294	0.5882	0.5882	0.5294
0.8824	0.7059	0.4706	0.5294	0.8824	0.4706	0.3529	0.4118	0.5294	0.5882	0.7059	0.6471
****	0.8235	0.4706	0.6471	0.8824	0.4706	0.3529	0.4118	0.6471	0.5882	0.5882	0.5294
0.1942	****	0.5294	0.8235	0.7059	0.4118	0.5294	0.4706	0.7059	0.6471	0.5294	0.5882
0.7538	0.6360	****	0.7059	0.4706	0.2941	0.5294	0.4706	0.3529	0.4118	0.5294	0.5882
0.4353	0.1942	0.3483	****	0.5294	0.4706	0.4706	0.4118	0.6471	0.5882	0.3529	0.6471
0.1252	0.3483	0.7538	0.6360	****	0.4706	0.4706	0.5294	0.5294	0.5882	0.5882	0.5294
0.7538	0.8873	1.2238	0.7538	0.7538	****	0.6471	0.4706	0.5882	0.5294	0.5294	0.3529
1.0415	0.6360	0.6360	0.7538	0.7538	0.4353	****	0.4706	0.5882	0.4118	0.5294	0.4706
0.8873	0.7538	0.7538	0.8873	0.6360	0.7538	0.7538	****	0.2941	0.5882	0.5882	0.2941
0.4353	0.3483	1.0415	0.4353	0.6360	0.5306	0.5306	1.2238	****	0.4706	0.3529	0.6471
0.5306	0.4353	0.8873	0.5306	0.5306	0.6360	0.8873	0.5306	0.7538	****	0.5294	0.5882
0.5306	0.6360	0.6360	1.0415	0.5306	0.6360	0.6360	0.5306	1.0415	0.6360	****	0.5882
0.6360	0.5306	0.5306	0.4353	0.6360	1.0415	0.7538	1.2238	0.4353	0.5306	0.5306	****

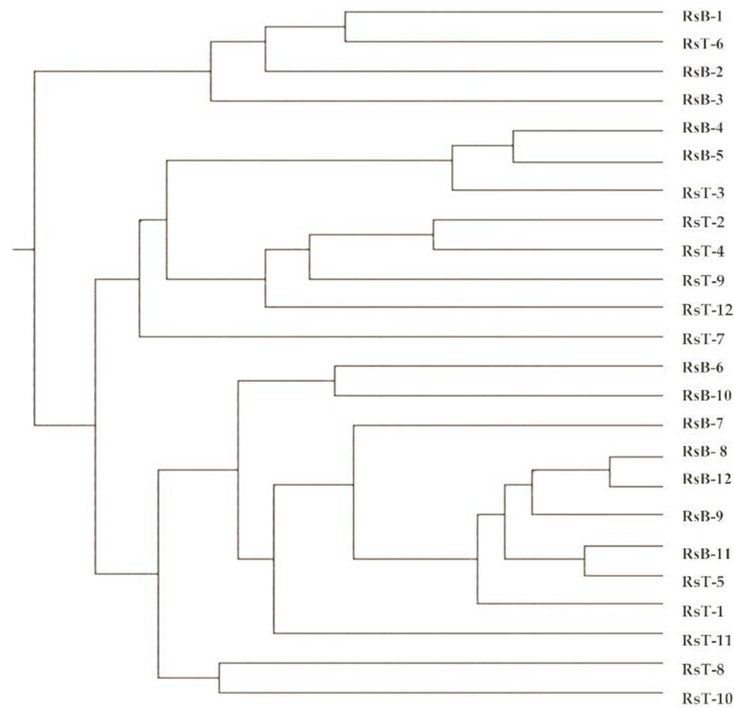

Fig. 1. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation in 24 brinjal and tomato accession according to RAPD analysis.

sub cluster 2. In cluster 2, RsB-4, RsB-5, RsT-3, RsT-2, RsT-4, RsT-9, RsT-12 and RsT-7 were formed in the sub cluster1 and RsB-6, RsB-10, RsB-7, RsB-8, RsB-12, RsB-9, RsB-11, RsT-5, RsT-1, RsT-11, RsT-8 and RsT-10 were grouped in the sub cluster 2. Sub cluster 1 was again divided into two sub sub clusters. RsB-4, RsB-5, RsT-3, RsT-2, RsT-4, RsT-9 and RsT-12 were included in sub sub cluster 1 and RsT-7 was in sub sub cluster 2. Sub sub cluster 1 generated two groups. RsB-4, RsB-5 and RsT-3 were in group 1 and RsT-2, RsT-4, RsT-9, and RsT-12 produced group 2. Moreover, group 1 and 2 generated two sub groups. In group 1, RsB-4 andRsB-5 were in sub group 1 and RsT-3 belonged to subgroup 2. In group 2, RsT-2, RsT-4 and RsT-9 were pronounced in sub group 1, and RsT-12 was generated in sub group 2. Sub group 1 was again divided into two sub sub groups. RsT-2 and RsT-4 belonged to sub sub group 1 and RsT-9 was alone in sub sub group 2. In sub cluster 2, two distinct sub sub cluster were produced. RsB-6, RsB-10, RsB-7, RsB-8, RsB-12, RsB-9, RsB-11, RsT-5, RsT-1 and RsT-11 were generated in the sub sub cluster 1, and RsT-8 and RsT-10 were in the sub sub cluster 2. Sub sub cluster1 formed two groups. RsB-6 and RsB-10 were in group 1, and RsB-7, RsB-8, RsB-12, RsB-9, RsB-11, RsT-5, RsT-1 and RsT-11 belonged to group 2. However, group 2 generated two sub groups. RsB-7, RsB-8, RsB-12, RsB-9, RsB-11, RsT-5 and RsT-1 belonged to sub group 1and RsT-11 was also observed in sub group 2. Sub group 1 was further divided into two sub sub groups**.** RsB-7was alone in the sub sub group 1, and RsB-8, RsB-12, RsB-9, RsB-11, RsT-11, RsT-5 and RsT-1 revealed sub sub group 2 where RsT-1was in one group, and RsB-8, RsB-12 andRsB-9 were found in separate group and RsB-11 and RsT-5 were also distinctly marked in another group**.**

Ramsubhag *et al.* (2012) reported that the significant difference was found in clustering population in the isolates of *R. solanacearum* of bacterial wilt of tomato. Similar findings were in agreement with the observation of Nishat *et al.* (2015) and Dey *et al.* (2020). Dey *et al.* (2020) observed the genetic variation in the isolates of brinjal seeds with different seed sources of several varieties. They reported that clustering and sub clustering in 12 isolates of *R. solanacerum* was found distinctly, and the highest genetic distance (1.447) and the lowest genetic distance (0.000) were noted in 12 isolates of *R. solanacearum.* The isolates of seed borne *R. solanacearum* were amplified in dendrogram with RAPD analysis and exerted the genetic variation in different seed sources of eggplant and tomato with different locations in Bangladesh.

The results revealed the lower or higher level of genetic distance exists among the isolates of *R. solanacearum* collected from different seed samples of tomato and brinjal with their different locations. The isolates were not host specific, and were distributed to seed samples in different varieties through seed contamination, and infected with seed and soil borne pathogens from their crop species'. Genetic variation may be the limiting factors in the heredity constitutions of the individuals within or intra crop species. The bacteria are well known to survive under diverse ecological conditions, interacting with biotic and abiotic stresses, and higher variability with their diversified locations. Further studies of *R. solanacerum* populations of brinjal and tomato seeds of isolates through molecular sequence analysis assess the wider genetic variations that will result in the genetic pattern and composition of infections in making decisions to use seeds of brinjal and tomato production.

Acknowledgements

The authors acknowledge the financial support and scholarship granted by the Bangabandhu fellowship on Science, Information and Communication Technology, Ministry of Science and Technology, Dhaka and also acknowledge the Professor Golam Ali Fakir Seed Pathology Centre and Molecular Plant Pathology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh to conduct this research with laboratory facilities.

References

Afrose S, Hossain I, Hossain MD and Khan MAH 2014. Genetic diversity of *Pseudomonas syringae* pv. *Syringae* causing leaf blight of litchi in Bangladesh. SAARC J. Agric. **12 (1):** 150-161.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman G, Smith JA and Struhl K 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., NewYork.

Dey P, Hossain I, Hossain MD, Mahmud H and Dey SK 2020. Assessment of genetic diversity of seed borne *Ralstonia solanacearum* of eggplant in Bangladesh. J. Plant Physiol. Pathol. **8(1):** 1-7.

Dey P, Hossain I, Hossain MD, Mahmud H and Dey SK 2021. Tomato Seed Borne *Ralstonia solanacearum* in Bangladesh. Russ. Agric. Sci. **47(4):** 386-393.

Goss EM, Kreitman M and Bergelson J (2005) Genetic diversity, recombination and cryptic clades in Pseudomonas viridiflava infecting natural populations of Arabidopsis thaliana. Genetics **169:** 21-35.

Granada GA and Sequeira L 1983. A new selective medium for *Pseudomonas solanacearum*. Plant Dis. **67:** 184-1088.

Grey BE and Steck TR 2001. The viable but nonculturable state of *Ralstonia solanacearum* may be involved in long-term survival of plant infection. Appl. Environ. Microbiol. **67(9):** 3866 - 3872.

Hayward AC 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum.* Ann. Rev. Phytopath. **29:** 65-87.

Hayward AC 1994. Characteristics of *Pseudomonas solanacearum.* J. Appl. Bacteriol. **27:** 265-277.

Jannat N, Hossain I, Dey P and Khan MAH 2014. Genetic diversity of *Pseudomonas syringae*pv. *syringae* causing leaf blight of mango in Bangladesh. Int. J. Agric. Innov. Res. **4 (2):** 628-635.

Kelman A 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopath. **44:** 693-695.

Khayamie S, Niknejad KN, Rabie B, Ebadie AA (2009) Genetic characterization of P. syringae pv. syringae strains from stone fruits based on RAPD analysis in Iran. Agri Tropica et Subtropica **42:** 162-166.

Mahbou STG, Cellier G, Wicker E, Guilbaud C, Kahane R, Allen C and Prior P 2009. Broad diversity of *Ralstonia solanacearum* strains in Cameroon. Plant Dis. **93:** 1123-1130.

Nei M 1973. Analysis of gene diversity in subdivided populations. Proc. Natl Acad.Sci, USA **70:** 3321-3323.

Nishat S, Hamim I, Khalil MI, Ali MA, Hossain MA, Meah MB and Islam MR 2015. Genetic diversity of the bacterial wilt pathogen *Ralstonia solanacearum* using a RAPD marker. C. R. Biol. **338:** 757-767.

Papke TR and Ward DM 2004. The importance of physical isolation to microbial diversification. FEMS Microbiol. Ecol. **48:** 293-303.

Popoola AR, Ganiyu SA, Enikuomehin OA, Bodunde JG, Adedibu OB, Durosomo HA and Karunwi OA 2015. Isolation and characterization of *Ralstonia solanacearum* causing bacterial wilt of tomato in Nigeria. Niger. J. Biotechnol. **29:** 1-10.

Prakasha A, Umesha S, Raghava S and Marahel S 2016. Discrimination of *Ralstonia solanacearum* isolates by genetic signatures produced by single-strand conformation polymorphism and low-stringency single specific primer PCR analysis. Afr. J. Microbiol. Res. **10(30):** 1128-1139.

Ramsubhag A, Lawrence D, Cassie D, Fraser R, Umaharan P, Prior P and Wicker E 2012 Wide genetic diversity of *Ralstonia solanacearum* strains affecting tomato in Trinidad, West Indies. Plant Pathol. **61(5):** 844-857.

Roopali S 1994. Seed transmission of *Pseudomonas solanacearum* in tomato and eggplant. ACIAR Bacterial Wilt Newsletter **11:** 12-13.

Sabita JN, Boruah BM and Rachid HA 2000. Yield potentiality of some brinjal cultivars in severely bacterial wilt infected condition. Vegetable Sci. **27:** 76-77.

Shakya DD 1992. Occurrence of *Pseudomonas solanacearum* in tomato seeds imported into Nepal. Bacterial wilt. ACIAR No. **45:** 371-372.

Vilela-Morales EA, Valois ACC and Nass LL 1997. Recursosgeneticos Vegetables, Embrapacenargen. Brasilia 78.

Weising K, Atkinson G and Gardner C 2005. Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation. PCR Methods Applica. **4:** 249-255.

Xu J, Pan ZC, Prior P, Xu JS, Zhang Z, Zhang H, Zhang LQ, He LY and Feng J 2009. Genetic diversity of *Ralstonia solanacearum* strains from China. Eur. J. Plant Path. **125:** 641-653.

Yeh FC, Yang RC and Boyle T 1999. POPGENE 32-version 1.31.Population Genetics Software.

(Manuscript received on 08 January, 2022; revised on 05 September, 2022)