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Genetic Diversity of *Pseudomonas Syringae* pv. *Syringae* Causing Leaf Blight of Mango in Bangladesh

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Abstract **– Mango (*Mangifera indica* L.) belonging to the family Anacardiaceae is the king of fruits. The genetic variations of 25 isolates of *Pseudomonas syringae* pv. *syringae* obtained from five regions of Bangladesh were analyzed using RAPD markers by Polymerase Chain Reaction (PCR). The percentage of polymorphic loci was found different from one region to another. Mean diversity across all population for all the loci studied was 0.31. The co-efficient of gene differentiation (G_{st}) was 1.00 reflecting the existence of high level of genetic variations among the genotypes. Comparatively the highest genetic distance (0.6931) was observed in Isolates of Rangpur vs. Isolates of Dinajpur; the lowest genetic distance (0.2877) was estimated in Isolates of Rangpur vs. Isolates of Bogra. Considering the genetic distance values; the results indicated that the isolates of five regions were genetically different from each other. The dendrogram (UPGMA) constructed from Nei's (1972) genetic distance produced 2 main clusters of 25 isolates from five regions. UPGMA dendrogram revealed that isolates of Rangpur, Bogra and Mymensingh form same cluster with least genetic distance. Dinajpur and Rajshahi produce another cluster with least genetic distance. Genetic distance among the isolates of Rangpur, Bogra and Mymensingh was found very near and genetic distance among the isolates of Dinajpur and Rajshahi was found very near which means these isolates may be virulent and their genetic variation is minimum. The genetic variation among these isolates of different regions was low indicating geographical variation among the isolates collected from different regions.**

Keywords **- Genetic Diversity, *Pseudomonas Syringae* pv. *Syringae*, Leaf Blight of Mango, RAPD Marker, Bangladesh.**

I. Introduction

Mango (*Mangifera indica* L.) is a fleshy stone fruit belonging to the genus *Mangifera*, under the family Anacardiaceae. Mango is native to South Asia from where it has been distributed worldwide to become one of the most cultivated fruits. *Mangifera indica* – the 'common mango' or 'Indian mango' – is the only mango tree commonly cultivated in many tropical and subtropical regions. It is said to have originated in the region of eastern Indo-Bangladesh, Burma, Malaysia or Thailand [3]. It is widely grown all over Bangladesh with the quality mangoes solely concentrated in regions like Rajshahi, Dinajpur, Bogra, Mymensingh and Rangpur. The demand for mango fruit is increasing day by day with growing population but declining in production which results in scarcity every year. Disease is a major cause for lower production of mango in Bangladesh [12]. Bacterial leaf blight has been observed in mango in southern Spain in 1991. That resulted in significant economic loss and is one of the primary factors limiting mango fruit production in southern Spain and Portugal [6]. Bacterial leaf blight disease has been reported in nurseries of mango for the first time in Bangladesh [9]. Bacterial leaf blight caused by *Pseudomonas syringae* pv. *syringae* seems one of the great threat for mango saplings in nursery and orchard in Bangladesh. Disease symptoms include necrosis of vegetative and flower buds and bud failure before bud break. Generally, a white creamy gum exudes from necrotic lesions on buds, stems, and less frequently on petioles [7], [8].

DNA-based techniques form the basis of modern microbial characterization and identification. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and repetitive sequence PCR (rep-PCR) have demonstrated considerable genetic diversity among *P. syringae* pathovars [5], [2].

Though the disease is serious one that causes enormous loss of seedlings / saplings and profound financial loss of farmers, but it has not been studied thoroughly. In addition, the genetic biodiversity of the pathogen has not been studied in Bangladesh. Hence the present experiment was made to study the incidence and severity of leaf blight in major mango growing areas of Bangladesh, molecular characterization of *Pseudomonas syringae* pv. *syringae*, the causal organism of leaf blight of mango, and to determine the genetic variation among the isolates of *Pseudomonas syringae* pv. *syringae* collected from different regions of the country.

II. Materials and Methods

Survey of incidence and severity of leaf blight

A survey was carried out to know the status of bacterial leaf blight of mango in nurseries of major mango growing regions viz. Rajshahi, Dinajpur, Mymensingh, Rangpur and Bogra in Bangladesh. The surveyed nurseries were selected randomly and the incidence and severity of leaf blight recorded. The percent bacterial leaf blight incidence was calculated by the following formula of Rai and Mamtha (2005):

$$\text{Disease incidence (\%)} = \frac{\text{Total number of leaves on each plant}}{\text{Number of diseased leaves on each plant}} \times 100$$

Disease severity was calculated using the formula of Johnston (2000):

$$\text{Disease severity (\%)} = \frac{\text{Area of leaf tissue infected by disease}}{\text{Total leaf area}} \times 100$$

The infected leaf samples from different nurseries were brought to the laboratory for the isolation of the pathogen.

Isolation, Identification, Multiplication and Purification of bacteria

The infected portion of the leaf was surface disinfected by immersion in sterile distilled water, and plated on nutrient agar (NA). Plates were incubated at 28°C for 24 hrs. Cream or off white colored colony of bacteria was appeared after incubation on NA medium. The bacterium, *P. syringae* pv. *syringae* was characterized by a series of biochemical tests viz. KOH solubility test, Gram staining test, Kovac's oxidase test, Temperature sensitivity test, Levan test, Sugar utilization test, Arginine dihydrolase activity, Catalase test and Pectolytic test. Then the bacterial isolates were multiplied by using NA medium. The petridishes containing NA media were inoculated by the colony of bacteria with the help of sterilized platinum wire dipping in rectified spirit and flaming over a spirit lamp. All the inoculated plates were incubated at room temperature for 24 hours. In case of contamination, the bacterial cultures were purified by streaking a single colony of each isolate by sub culturing on NA medium as described by [1].

Molecular characterization of Pseudomonas syringae pv. *syringae*

DNA samples of each isolates were extracted following chloroform-isoamyle alcohol extraction and ethanol precipitation method from the pure culture of bacteria.

Bacteria were grown in 5 ml liquid broth medium at 27 ^{0}C for 18-24 hours. Cells were collected in 1.5 ml eppendorf tubes, after centrifuging the pellets were left and resuspended in 1 ml of 2M NaCl. The supernatant were removed after centrifuging and the pellet was resuspended in 525 µl of TE buffer. Then 60 µl of 20% SDS and 15 µl of proteinase K (200 µg/ml stock) were added in the mixture, after mixing by inverting, incubated at 37°C for 1 hour. Then 100 µl of 5 M NaCl was added in the mixture of each tube and mixed well. Then 80 µl of CTAB was added and mixed well followed by incubation at 65ºC and mixed gently every 5 minutes. DNA was mixed with an equal volume (750 µl) of chloroform-isoamyl alcohol, inverted to mix completely and centrifuged for 10 minutes at 4ºC. The aqueous layer was then transferred to a clean tube. Equal volume of Phenol-Chloroform was added in every tube and mixed well by inverting the tube. The mixture was centrifuged at 4ºC for 10 minutes. All the supernatant (exist in upper portion) were transferred from the tubes into the new tubes. Then equal volume i.e. 525 µl volume of Isopropanol were added into the supernatant and mixed well by inverting the tubes. The tubes were centrifuged at 4ºC. Then 1 ml of 70-75% ethanol was added and centrifuged at 4ºC. Ethanol was removed with the help of aspirator and the pellet was allowed to dry in the air for 5 minutes. The pellet (usually white in color) was resuspended in 20 µl of TE buffer and incubated at 65ºC for 10 minutes or at room temperature for 10 minutes. Lastly the isolated genomic DNA was preserved at -20ºC in refrigerator for further use.

The primer code used in this experiment was 62538038 and the sequence was TGCAGCACCG with 70% GC content. The Ladder used in the experiment was 100 bp.

The amplification conditions were based on Williams *et al.* (2000) with some modification. PCR reactions were performed on each DNA sample containing NH_4 buffer= 1.0 µl, dNTPs= 1.0 µl, Primer= 2.0 µl, $MgCl_2$=0.6 µl, Template DNA = 2.0 µl, AmpliTaq DNA polymerase=0.2 µl, ddH_2O=3.2 µl (Total= 10 µl).

During the experiment, PCR buffer, dNTPs, primer and DNA samples solutions were thawed from frozen stocks, mixed by vortexing and kept on ice. DNA template was pipetted first into PCR tubes compatible with the thermocycler used. A pre-mix was then prepared in course of following order: reaction buffer, dNTPs, DNA template and sterile distilled water. Taq polymerase enzyme was then added to the pre-mix. The pre-mix was then mixed up well and aliquoited into the tubes that already contain primer. The tubes were then sealed and placed in a thermocycle and the cycling was started immediately.

DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient Eppendorf). The reaction mix was preheated at 92 ºC for 4 minutes followed by 40 cycles of 1 min denaturation at 94ºC, 1 min annealing at 37 ºC and elongation or extension at 72 ºC for 3 minutes. After the last cycle, a final step of 5 minutes at 72 ºC was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4 ºC. The amplified product from each sample was separated electrophoretically on 1.4% agarose gel contain ethidium bromide in 1×TBE buffer at 120 V for 1½ hrs. When the bromophenol blue dye had reached three-fourths of the gel length, the electrophoresis was stopped and the power supply was disconnected. After completion of electrophoresis the gel was stained with Ethidium bromide (0.1µg/ml) solution for 15 min at room temperature. Thereafter the gel was removed from the Ethidium bromide tray and placed on the UV transilluminator in the dark chamber of image documentation system and DNA bands were observed and photographed by a Gel Documentation System.

Data Analysis

Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single allelic locus [11]. One molecular weight marker, 100 bp DNA ladder, was used to estimate the size of the amplification products by comparing the distance traveled by each fragment with that of the known sized fragments of molecular weight markers. All distinct bands or fragments (RAPD markers) were thereby given identical numbers according to their position on gel and scored visually on the basis of their presence (I) or absence (0), separately for each isolates of mango and primer.

The scores obtained using all primers in the RAPD analysis were then pooled to create -a single data matrix. This was used to estimate polymorphic loci. Gene flow, (N_m) was estimated according to the following formula:

Gene flow, N_m =0. 5 (1- G_{st})/ G_{st}

Where, G_{st} is the proportion of total genetic diversity attributable to subpopulation. It is also known as

coefficient of gene differentiation. The G_{st} values were calculated by using the following formula:

G_{st} =1-H_s/ H_t Where, H is the mean average heterozygosity of the total population and H 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 between the studied 12 cultivars using the Unweighted Pair-Group Method of Arithmetic means (UPGMA) [14]. The dendrogram was constructed using the POPGENE (Version 1.31) computer program 30[3].

III. Results and Discussion

The results of the biochemical tests of the causal bacteria are summarized in Table 1. All tests showed positive result towards identification of *Pseudomonas syringae* pv. *syringae*.

Incidence and Severity

The survey results indicated a regional variation in bacterial leaf blight incidence and severity. The results showed that the highest average leaf blight incidence was recorded in Rangpur (6.00%) and the lowest average incidence was in Bogra (4.23%) as shown in Fig. 1. At the same time the highest average severity was recorded in Rangpur (5.94%) and the lowest average severity was recorded in Bogra (3.80%).

RAPD patterns of the selected primer

In five isolates of Rangpur region, the total numbers of bands scored were 15 among them 10 were found polymorphic. That resulting polymorphic loci by 66.67% using the primer 62538038 (Fig. 2 A). In five isolates of Bogra region, the total numbers of bands scored were 12 among them 12 were found polymorphic that resulting polymorphic loci by 100.00% (Fig. 2 B). In five isolates of Mymensingh region, the total numbers of bands scored were 13 among them 7 were found polymorphic. That resulting polymorphic loci was 53.85% (Fig. 2 C). In five isolates of Rajshahi region, the total numbers of bands scored were 9 among them 9 were found polymorphic that resulting polymorphic loci was 100.00%(Fig. 2 D). In five isolates of Dinajpur region, the total numbers of bands scored were 7 among them 7 were found polymorphic that resulting polymorphic loci by 100.00% (Fig. 2 E).

Genetic diversity

All the isolates collected from five regions showed genetic variation in Observed number of alleles (* na), Effective number of alleles (* ne), Nei's (1973) gene diversity(* h), Shannon's Information index (* I) and the results are presented in Table 2.

Gene flow and total genetic diversity

The isolates collected from five regions of Bangladesh showed variation in Hardy-Weinberg average heterozygosity expectated in isolates (Ht), Hardy-Weinberg average heterozygosity obtained in isolates (Hs), Co-efficient of gene differentiation (Gst), estimation of gene flow from Gst or Gcs. Nm = 0.5(1 - Gst)/Gst (* Nm) are shown in Table 3. Out of the 25 collected isolates of *P. syringae* pv. *syringae* from five different regions of Bangladesh, the highest and lowest Ht values were recorded in Rajshahi and Mymensingh respectively. Variation of isolates in respect of Hs, Gst, and Nm were same in case of all isolates of all locations.

Analysis of genetic identity and genetic distance

The values of pair-wise comparisons of Nei's (1972) genetic distance and genetic identity of collected different isolates of *Pseudomonas syringae* pv. *syringae* from Rangpur, Bogra, Mymensingh, Rajshahi and Dinajpur were computed from combined data sets for one primer and the results are presented in Table 4. In Rangpur, comparatively the highest genetic distance (0.76) was observed in Isolate 2 vs. Isolate 5; the lowest genetic distance (0.14) was estimated in Isolate 1 vs. Isolate 2, Isolate 3 and Isolate 4. Genetic identity between isolates was found for one primer ranging from 0.60 to 0.87. Comparatively higher genetic identity was found in Isolate 2 vs. Isolate 1, and the lowest genetic identity was observed between Isolate 4 vs. Isolate 1, Isolate 2 and Isolate 5 vs. Isolate 1 and Isolate 4. In Bogra, comparatively the highest genetic distance (1.0986) was observed in Isolate 1 vs. Isolate 3, Isolate 4 and Isolate 5; the lowest genetic distance (0.1823) was estimated in Isolate 3 vs. Isolate 5. Comparatively higher genetic identity was found in Isolate 3 vs. Isolate 2 and Isolate 5 vs. Isolate 2, and the lowest genetic identity was observed between Isolate 3 vs. Isolate 1, Isolate 4 vs. 1 and Isolate 5 vs. Isolate 1 and Isolate 4.In Mymensingh, comparatively the highest genetic distance (0.6190) was observed in Isolate 2 vs. Isolate 5 and Isolate 3 vs. Isolate 5; the lowest genetic distance (0.0800) was estimated in Isolate 1 vs. Isolate 2 and in Isolate 1 vs. Isolate 4.Genetic identity between isolates was found for the two primers ranging from 0.54 to 0.92. Comparatively higher genetic identity was found in Isolate 4 vs. Isolate 1 and the lowest genetic identity was observed between Isolate 5 vs. Isolate 2 and Isolate 5 vs. Isolate 2. In Rajshahi, comparatively the highest genetic distance (0.81) was observed in Isolate 1 vs. Isolate 3 and Isolate 2 vs. Isolate 4 and Isolate 5; the lowest genetic distance (0.00) was estimated in Isolate 1 vs. Isolate 4 and Isolate 4 vs. Isolate 5. Genetic identity between isolates was found for the two primers ranging from 0.00 to 1.00. Comparatively higher genetic identity was found in Isolate 5 vs. Isolate 4 and the lowest genetic identity was observed between Isolate 4 vs. Isolate 1 and Isolate 5 and Isolate 5 vs. Isolate 1. In Dinajpur, comparatively the highest genetic distance (1.9459) was observed in Isolate 1 vs. Isolate 3 and Isolate 3 vs. Isolate 5; the lowest genetic distance (0.0000) was estimated in Isolate 1 vs. Isolate 5, Isolate 2 vs. Isolate 3 and Isolate 4 and Isolate 3 vs. Isolate 4.Genetic identity between isolates was found for the two primers ranging from 0.0000 to 1.0000. Comparatively higher genetic identity was found in Isolate 4 vs. Isolate 2 and Isolate 5 vs. Isolate 1, and the lowest genetic identity was observed between Isolate 3 vs. Isolate 2, Isolate 4 vs. Isolate 3.

Dendrogram of five isolates of Pseudomonas syringae pv. *syringae of Rangpur, Bogra, Mymensingh, Rajshahi and Dinajpur*

Dendrogram indicated segregation of the five isolates of bacteria collected from every five locations viz. Rangpur, Bogra, Mymensingh, Rajshahi and Dinajpur (Fig 3 A-E). The result of UPGMA dendrogram revealed that in Rangpur, Isolate 1 and Isolate 2 formed same cluster along with 3 and Isolate 4 with least genetic distance and Isolate 5 with comparatively higher genetic distance. In Bogra, Isolate 3 was closed to the Isolate 5, Isolate 2 and Isolate 4 with the least genetic distance. Again, Isolate 3 was related to the isolate 1, with high genetic distance 1.0986. In Mymensingh, Isolate 5 was closely related to the Isolate 2, Isolate 4 and Isolate 3. Again, Isolate 1 was distantly related to the isolate 5. In Rajshahi, Isolate 1 was close to the Isolate 4 and Isolate 5 with the least genetic distance. Again, Isolate 2 was close to the isolate 3. In Dinajpur, Isolate 1 was close to Isolate 5. Isolate 2 was closed to Isolate 3 and Isolate 4.

Mean of Nei's (1972) genetic distance and genetic identity of different isolates collected from five regions of Bangladesh

The values of pair-wise comparisons of Nei's (1972) genetic distance between 5 different locations from where isolates of *Pseudomonas syringae* pv. *syringae* were collected were computed for one primer ranging from 0.2877 to 0.6931 (Table 5). Comparatively the highest genetic distance (0.6931) was observed in Isolates of Rangpur vs. Isolates of Dinajpur and the lowest genetic distance (0.2877) was estimated in Isolates of Rangpur vs. Isolates of Bogra. Genetic identity between isolates was found for the two primers ranging from 0.5000 to 0.7500. Comparatively higher genetic identity was found in Isolates of Bogra vs. Isolates of Rangpur and the lowest genetic identity was observed between Isolates of Dinajpur vs. Isolates of Rangpur.

Dendrogram of isolates of five regions

Genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) based on Nei's (1972) indicated segregation of the isolates of bacteria collected from different locations of Bangladesh (Fig. 3 F). From dendrogram it was observed that isolates of Rangpur, Bogra and Mymensingh form same cluster with least genetic distance. Dinajpur and Rajshahi produce another cluster with least genetic distance. Genetic distance among the isolates of Rangpur, Bogra and Mymensingh is found very near and genetic distance among the isolates of Dinajpur and Rajshahi is found very near which means these isolates may be virulent and their genetic variation is minimum.

IV. Conclusion and Recommendation

Under the study, it was demonstrated that PCR clearly differentiated the isolates of *P. syringae* pv. *syringae* collected from the leaf of mango from different regions of Bangladesh with leaf blight disease symptoms. Clustering of bacterial isolates under different genetic groups was also agreed with the findings of [10]. They reported genetic diversity of *Psudomonas syringae* pv. *syringae*. Mean diversity (0.3057) across all population for all the loci indicating some degrees of genetic variation in isolates of *P. syringae* pv. *syringae* from different regions of Bangladesh. The genetic variation among these isolates of different regions is low and indicating some degrees of geographical variation among the isolates collected from different regions. These isolates are emerging as a great threat of mango cultivation and production. The findings of the present molecular based analysis of the pathogen *P. syringae* pv. *syringae* would definitely be useful to adopt a proper management strategy suitable for integrated disease management programs for the eco-friendly management of the leaf blight disease in Bangladesh. However, future research with additional isolates collected from other regions is needed to make conclusive remarks about the genetic variation and relatedness which will help for the better management of the disease.

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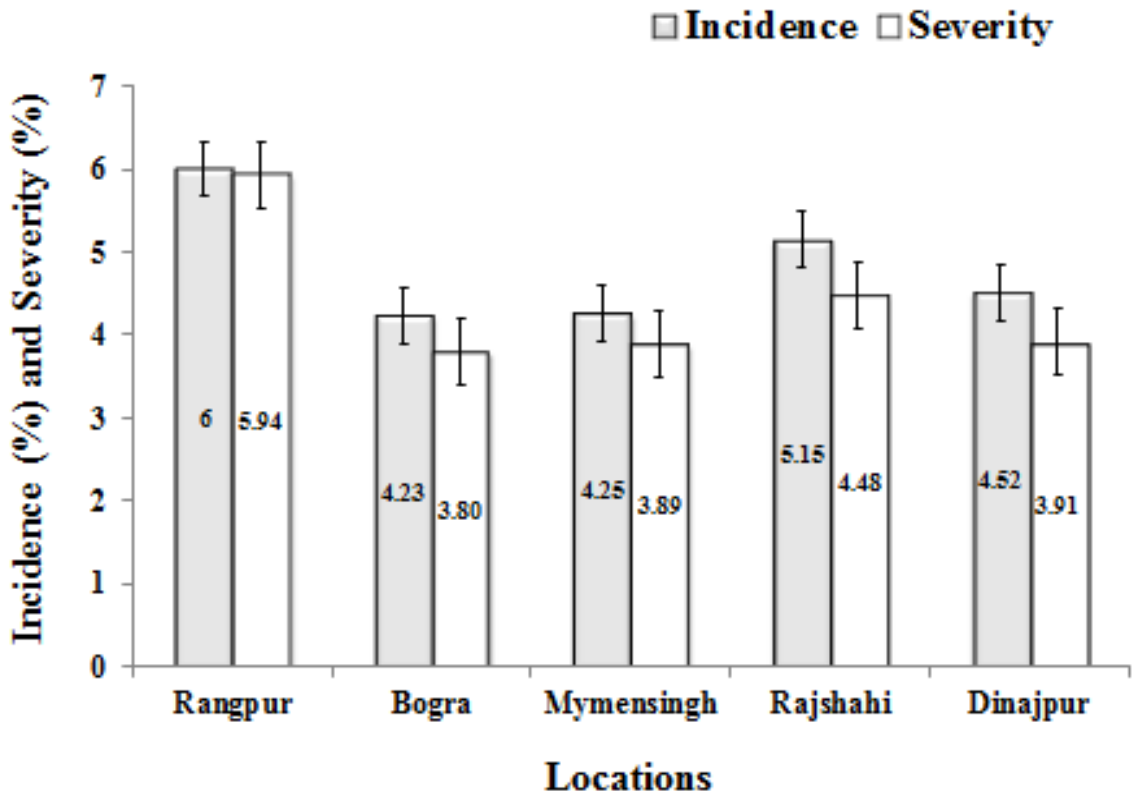
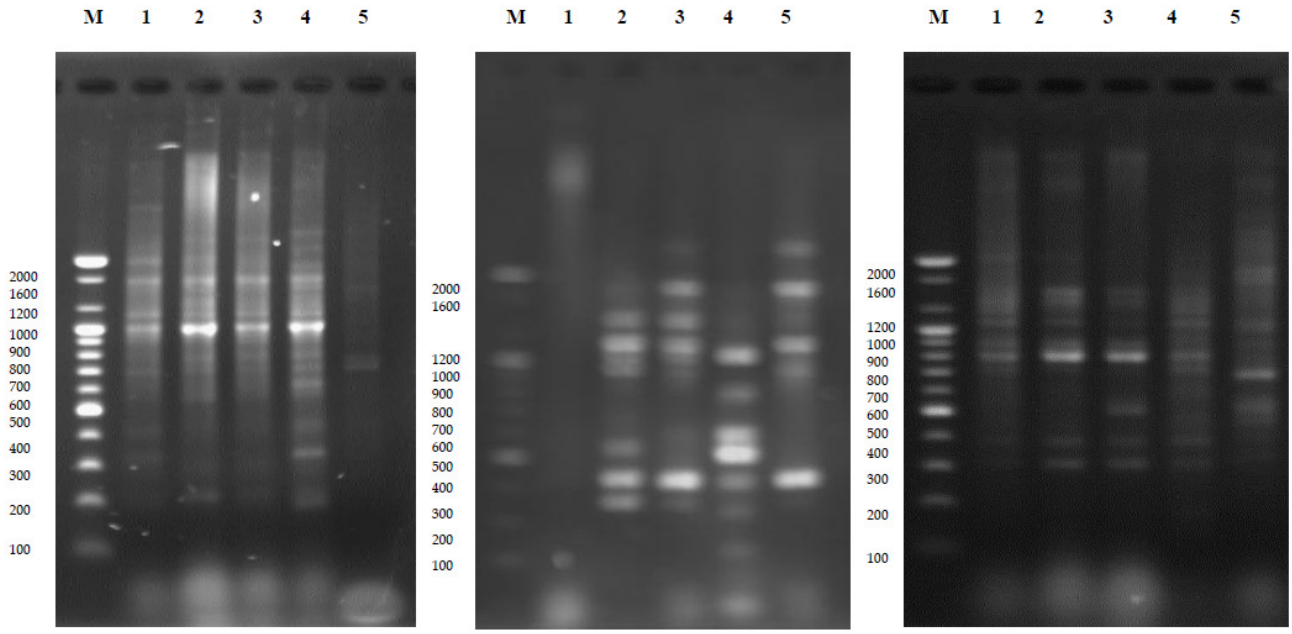


Fig.1. Incidence (%) and Severity (%) of leaf blight of mango caused by *P. syringae* pv. *syringae* in different regions of Bangladesh



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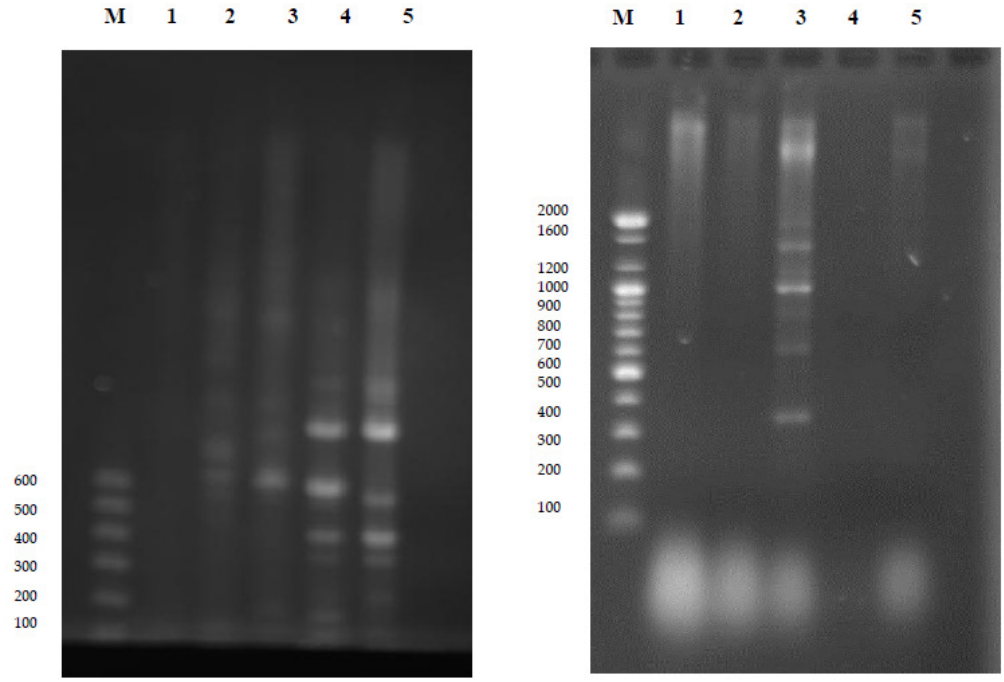

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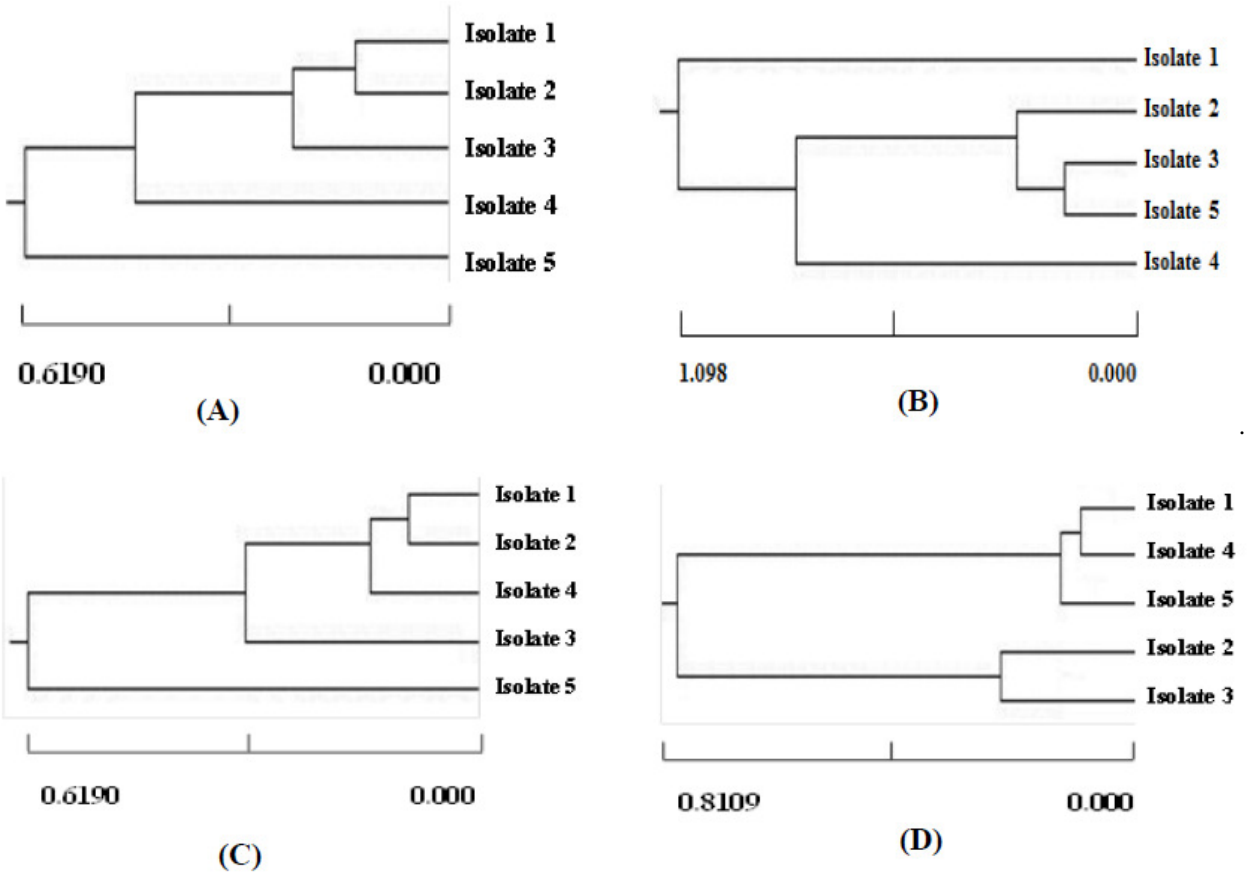
Fig.2. RAPD profile of 5 isolates of *Pseudomonas syringae* pv. *syringae* using primer 62538038. Molecular weight marker, M: 100 bp DNA Ladder. Lane 1 (M) DNA ladder in left side, Lane 1-5: Isolates 1, 2, 3, 4 and 5 on agarose gel collected from leaves of mango of A. Rangpur, B. Bogra, C. Mymensingh, D. Rajshahi and E. Dinajpur region



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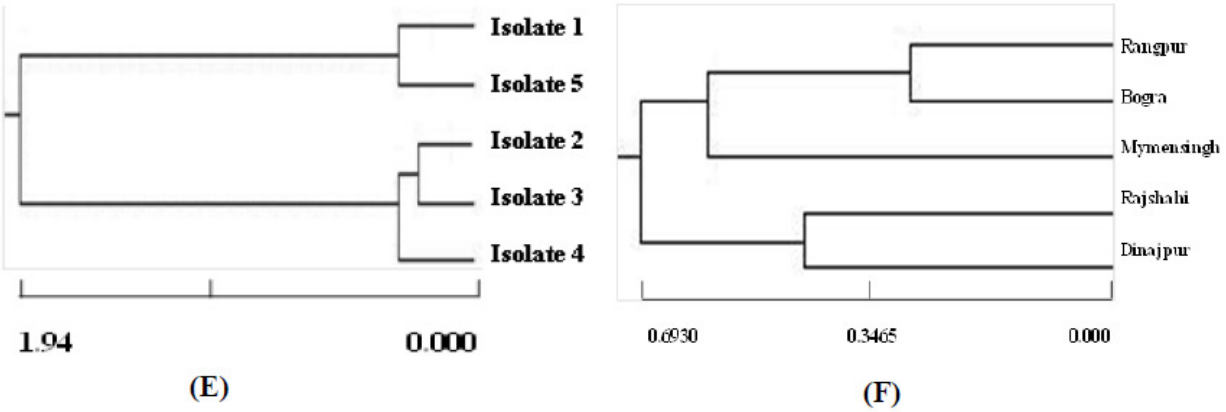

Fig.3. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Neis's (1972) genetic distance, summarizing data on differentiation in 5 isolates of *Pseudomonas syringae* pv. *syringae* according to RAPD analysis of isolates of (A) Rangpur, (B) Bogra, (C) Mymensingh, (D) Rajshahi, (E) Dinajpur regions and (F) five regions of Bangladesh

Table I: Biochemical tests of *Pseudomonas syringae* pv. *syringae*

Tests	Reaction	Inference
Pathogenecity tests	+ ve	*Pseudomonas syringae* pv.*syringae*
KOH tests	+ ve	*Pseudomonas syringae* pv.*syringae*
Kovac's tests	- ve	*Pseudomonas syringae* pv.*syringae*
Levan tests	+ ve	*Pseudomonas syringae* pv.*syringae*
Arginine tests	- ve	*Pseudomonas syringae* pv.*syringae*
Catalase tests	+ ve	*Pseudomonas syringae* pv.*syringae*
Sugarutilization tests	+ ve	*Pseudomonas syringae* pv.*syringae*
Potato soft rotting tests	+ ve	*Pseudomonas syringe* pv.*syringae*

Table II: Genetic diversity and frequency of polymorphic loci for one primer in five isolates of *Pseudomonas syringae* pv. *syringae* of five regions

Locations	No. of Loci	Sample size	na*		ne*		h*		I*	
			Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Rangpur	15	5	2.00	1.00	1.92	1.00	0.48	0.32	0.67	0.00
Bogra	12	5	2.00	2.00	1.92	1.47	0.48	0.32	0.67	0.50
Mymensingh	13	5	2.00	1.00	1.92	1.00	0.48	0.00	0.67	0.00
Rajshahi	9	5	2.00	2.00	1.92	1.47	0.48	0.32	0.67	0.50
Dinajpur	7	5	2.00	2.00	1.92	1.47	0.48	0.32	0.67	0.50

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

Table III: Average Gene flow (N_m) and the proportion of total genetic diversity (G_{st}) across RAPD marker of isolates of five regions

Locations	No. of Loci	Sample Size	Ht	Hs	Gst	* Nm
Rangpur	15	5	0.26	0.00	1.00	0.00
Bogra	12	5	0.39	0.00	1.00	0.00
Mymensingh	13	5	0.19	0.00	1.00	0.00
Rajshahi	9	5	0.41	0.00	1.00	0.00
Dinajpur	7	5	0.34	0.00	1.00	0.00

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

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Table IV. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) in different isolates pair of *Pseudomonas syringae* pv. *syringae* of Rangpur, Bogra, Mymensingh, Rajshahi and Dinajpur

Locations	Isolates	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Rangpur	Isolate 1	****	0.8667	0.8000	0.6000	0.6000
	Isolate 2	0.1431	****	0.8000	0.6000	0.4667
	Isolate 3	0.1431	0.2231	****	0.8000	0.6667
	Isolate 4	0.1431	0.5108	0.2231	****	0.6000
	Isolate 5	0.5108	0.7621	0.4055	0.5108	****
Bogra	Isolate 1	****	0.4167	0.3333	0.3333	0.3333
	Isolate 2	0.8755	****	0.7500	0.5833	0.7500
	Isolate 3	1.0986	0.2877	****	0.5000	0.8333
	Isolate 4	1.0986	0.5390	0.6931	****	0.3333
	Isolate 5	1.0986	0.2877	0.1823	1.0986	****
Mymensingh	Isolate 1	****	0.9231	0.7692	0.9231	0.6154
	Isolate 2	0.0800	****	0.8462	0.8462	0.5385
	Isolate 3	0.2624	0.1671	****	0.6923	0.5385
	Isolate 4	0.0800	0.1671	0.3677	****	0.6923
	Isolate 5	0.4855	0.6190	0.6190	0.3677	****
Rajshahi	Isolate 1	****	0.5556	0.4444	0.0000	0.0000
	Isolate 2	0.5878	****	0.8889	0.4444	0.4444
	Isolate 3	0.8109	0.1178	****	0.5556	0.5556
	Isolate 4	0.0000	0.8109	0.5878	****	1.0000
	Isolate 5	0.0000	0.8109	0.5878	0.0000	****
Dinajpur	Isolate 1	****	0.8571	0.1429	0.8571	1.0000
	Isolate 2	0.1542	****	0.0000	1.0000	0.8571
	Isolate 3	1.9459	0.0000	****	0.0000	0.1429
	Isolate 4	0.1542	0.0000	0.0000	****	0.8571
	Isolate 5	0.0000	0.1542	1.9459	0.1542	****

Table V. Mean of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) in different isolates pair of *Pseudomonas syringae* pv. *syringae* collected from five regions

Locations of Isolates collection	Rangpur	Bogra	Mymensingh	Rajshahi	Dinajpur
Rangpur	****	0.7500	0.5357	0.5714	0.5000
Bogra	0.2877	****	0.7143	0.6786	0.6071
Mymensingh	0.6242	0.3365	****	0.5714	0.5714
Rajshahi	0.5596	0.3878	0.5596	****	0.6786
Dinajpur	0.6931	0.4990	0.5596	0.3878	****