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Variation in *Ralstonia solanacearum* Isolated from Brinjal Plants in West Bengal

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Abstract

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al. (=*Pseudomonas solanacearum* E.F.Smith) is prevalent in West Bengal infecting brinjal, tomato, potato, tobacco and chilli every year and causing extensive damage to crops. Affected brinjal plants showed leaf drooping, followed by complete or partial wilting. Browning of inner tissues of the vascular bundle can be observed in infected plants. Isolates of *R. solanacearum* collected from different geographical and environmental milieu did not show any type of variation with respect to their physico-biochemical characteristics of the pathogen. However, regional level variations in aggressiveness of *R. solanacearum* were found among the isolates collected from infected brinjal plants.

1. Introduction

Brinjal (*Solanum melongena* L.) is a very important vegetable crop grown throughout the world and almost in all states of India with a cultivated area of 0.6 million hectares and production of about 10.38 million metric tonnes. Whereas the cultivated area of brinjal in West Bengal was 155.30 thousand hectares and production 2.76 million tonnes in the year 2008-09 (National Horticulture Board, 2009; Precision Farming Development Centre, 2010). In West Bengal, brinjal is cultivated throughout the year as cash crop and is attacked by a number of pests and diseases. The bacterial wilt of brinjal caused by race-1 of *Ralstonia solanacearum* (Smith) Yabuuchi et al. (*Pseudomonas solanacearum* E.F. Smith) is the devastating disease of brinjal where crop loss may reach upto 100% in highly infested field in West Bengal and the extent of losses depends on seasons (Chatterjee et al., 1999). The disease is complicated to control primarily because the pathogen is soil-borne, vascular in nature and infects several hundreds of plant species (Kelman, 1953; Hayward, 1991). Planting resistant varieties is the most important management strategy. But the success of bacterial wilt resistant brinjal varieties has, however, been limited by the location-specific nature of the resistance to bacterial wilt. This is due in part to large pathogen strain variation (AVRDC Annual Report, 2000). Keeping this view in mind the present investigation was under taken to extend the understanding of status of bacterial wilt of brinjal and regional variation of *R. solanacearum* isolated from brinjal plants from different agro-climatic region of West Bengal.

2. Materials and Methods

2.1. Survey and study of symptomatology

Surveys were conducted in four different agro-climatic regions (Table 1) of West Bengal for four consecutive years (2004-2007) to record the incidence of bacterial wilt in brinjal along with some other commonly cultivated and economically important solanaceous crops. The infected plants were observed minutely and the symptoms of bacterial wilt in the field were recorded at different stages of crop growth.

2.2. Confirmation of the bacterium

Pure culture of the bacterium was maintained in Tetrazolium chloride (TZC) medium (Kelman, 1954). Bacterial nature of the disease was confirmed by ooze test, through selective medium (Granada and Sequeira, 1983), through morphological and bio-chemical studies and pathogenicity test (Kelman,

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Table 1: Incidence of bacterial wilt of different solanaceous crops in West Bengal

Agro-climatic region	District	Range (% disease incidence)				
		Brinjal	Tomato	Potato	Tobacco	Chilli
I	Murshidabad	17.20-62.50	4.72-60.45	12.80-25.74	*NR	2.29-16.00
II	South 24 Parganas	14.85-56.25	10.50-80.76	34.54-73.82	NAC	4.67-24.07
I	Nadia	21.16-89.64	20.32-85.63	12.00-38.46	2.83-7.45	2.30-15.00
IV	Coochbehar	16.32-75.15	6.88-61.55	5.37-13.94	4.94-27.50	NR
II	North 24 Parganas	25.00-86.45	17.30-98.90	10.72-63.19	#NAC	7.16-18.29
III	Birbhum	22.25-68.32	10.25-20.70	2.63-32.17	NAC	15.3-42.85
IV	Jalpaiguri	24.85-72.61	26.17-45.52	2.26-10.83	4.80-10.17	NR

#Non-availability of crop during survey; *Data not recorded; I= Gangetic Alluvial region; II= Coastal Alluvial region; III=Lateritic Red and Gravelly undulating region; IV= Terai and Teesta Alluvial region

1953; Kelman, 1954; Hayward, 1964, Bernner and Statey, , 2005.).

2.3. *Aggressiveness study*

The evaluation of aggressiveness of different isolates of *R. solanacearum* from brinjal plants was conducted under controlled condition in the laboratory. Brinjal seeds (var. *Muktakeshi,* a commonly cultivated brinjal variety that is susceptible to bacterial wilt) were sown in rectangular plastic trays in rows containing sterilized soil and replicated thrice. Two sets of experiment were conducted. Forty-eight hours old cultures of *R. solanacearum* grown in TZC media was used as inoculum at a concentration of 1.5 x 10^8 cells ml^{-1} collected from seven different districts in four agro-climatic regions (Gangetic alluvial region, Terai and Teesta alluvial region, Lateritic Red and Gravelly undulating region and Coastal alluvial region) of West Bengal. Fifteen days old brinjal seedlings were inoculated by giving small incision on the roots of one side of each row (Kelman, 1953). Bacterial suspension was applied over the wounded roots and kept in controlled humid chamber at a temperature of 28±1^{0}C with 98 % RH. Numbers of wilted seedlings were recorded at 24 hours interval.

Cluster analysis was performed for grouping the isolates based on aggressiveness (Johnson and Wichern, 1996; SAS 9.2, 2009; SAS EG 4.2, 2009)

3. Results and discussion

3.1. Natural incidence

During surveys, it was found that the incidence of the disease in different solanaceous crops varied from 2.26 to 98.90% in different agro-climatic zones of West Bengal (Table 1). Bacterial wilt of brinjal, tomato and potato were quite common in almost all the areas of seven districts surveyed i.e. Murshidabad, South 24 Parganas, North 24 Parganas, Nadia, Coochbehar, Birbhum and Jalpaiguri. However, the bacterial wilt of tobacco was recorded from Nadia, Coochbehar and Jalpaiguri, and that of chilli from Murshidabad, South 24 Parganas, North 24 Parganas, Nadia and Birbhum. The overall range of average per cent disease incidence (PDI) of bacterial wilt of brinjal, tomato, potato, tobacco and chilli were 14.85-89.64, 4.72-98.90, 2.26-73.82, 2.83-27.50 and 2.29-42.85 respectively. The severity of the bacterial wilt disease of brinjal was maximum in Nadia (Gangetic alluvial region) followed by North 24 Parganas (Coastal alluvial region).

3.2. Symptom of bacterial wilt of brinjal

Usually the disease symptom appears in the field during flowering and early fruiting stage (Plate 3). In early stage of disease development drooping of the leaf at the top of the plant was found. Such drooping was found in day time but at night the plant becomes normal. The drooping symptom continues for 3 to 7 days. At the end of this phase the infected plant fails to recover at night hours. Ultimately the plant dies. In some cases drooping of leaf is found at one side of the plant involving one or two branches and the infected branch die leaving the other branches normal (Plate 2). Subsequently the apparently healthy branches also wilted and the plant died. The disease spreads very rapidly in field and within a week 40 - 50 % plants showed wilting symptom. Prominent vascular browning was observed in the inner tissue of the infected plants. In severely infected field wilting was found at early growth stage even in seed bed, poor growth of the plant at early stage (25 days after transplanting) was observed when infected seedlings from nursery bed were transplanted in main field (Plate 1). Such infected plants showed swelling of the basal portion of stem, drying / blighting of the leaf tissue near the margin of the leaf lamina with slightly curling of the leaf. This type of symptom was noticed first time in many fields in Nadia and North 24 Parganas districts of West Bengal. In all type of symptoms, disease was confirmed by ooze test (Plate 4) in field condition as well as biochemical test in laboratory.

3.3. Characterization of isolates of R. solanacearum from

Plate 1: Wilting of brinjal plant at early growth stage

Plate 2: Partial wilting of brinjal plant just before flowering stage

Plate 3: Wilting of brinjal plant at reproductive stage

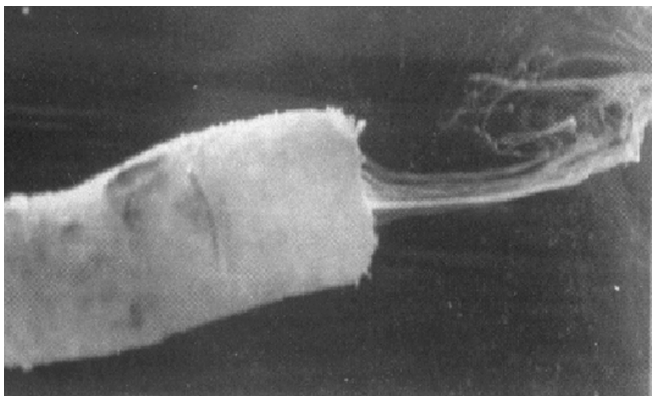

Plate 4: Oozing of *R. solanacearum* from infected cut end of brinjal

brinjal and pathogenicity test

Sixteen most pivotal physico-biochemical characterizations (Table 2) were made in seven different isolates of *R. solanacearum* isolated from brinjal which were collected from Murshidabad (RS_1), South 24 Parganas (RS_2), Nadia (RS_3), Coochbehar (RS_4), North 24 Parganas (RS_5), Birbhum (RS_6) and Jalpaiguri (RS_7). Results confirmed that wilt causing bacterium was *Ralstonia solanacearum* previously known as *Pseudomonas solanacearum* (Kelman, 1953; Hayward, 1964; Bergey's Manual of Systematic Bacteriology, 2nd Edition, 2005). Isolates collected from different geographical and environmental milieu and from brinjal did not show any type of variation in respect of physico-biochemical characters of the very microorganism. On pathogenicity test (Table 3) by stem injection and root inoculation method (Kelman, 1953), isolates of *R. Solanacearum* from brinjal, tomato, potato, chilli and tobacco were found to be pathogenic on tomato and brinjal.

3.4. Aggressiveness study

It appears from the results presented in Table 4 that wilting started at 4 DAI (Days after inoculation). At 5 DAI wilting was not found in isolates RS_3 and RS_4 (Figure 1) and 100% seedlings were wilted at 10 DAI in RS_3 and RS_4 indicating

Table 2: Characteristics of different isolates of *R. solanacearum* from brinjal

Characteristics	RS_1	RS_2	RS_3	RS_4	RS_5	RS_6	RS_7
A. Morphological characteristics							
Shape	SR	SR	SR	SR	SR	SR	SR
Gram reaction	-	-	-	-	-	-	-
Motility	M	M	M	M	M	M	M
Presence of flagella	+	+	+	+	+	+	+
B. Bio-chemical Characteristics							
Oxidase reaction	+	+	+	+	+	+	+
Levan formation from sucrose	_	_	_	_	_	_	_
Gelatin lequifaction	_	_	_	_	_	_	_
Starch hydrolysis	_	_	_	_	_	_	_
Lipase (tween 80 hydrolysis)	+	+	+	+	+	+	+
Growth at 40^0C	_	_	_	_	_	_	_
Arginine hydrolase reaction	_	_	_	_	_	_	_
Methyl red test	_	_	_	_	_	_	_
Voges-Proskauer test	+	+	+	+	+	+	+
Production of H_2S	+	+	+	+	+	+	+
Indole production	_	_	_	_	_	_	_
Catalase reaction	+	+	+	+	+	+	+

SR= Straight rod, M= Motile, - = Negative, + = Positive

Table 3: Pathogenicity of isolates of *R. solanacearum* from crop plants on brinjal and tomato

Sources of isolates From	Stem injection method		Root inoculation method	
	Disease reaction on		Disease reaction on	
	Brinjal	Tomato	Brinjal	Tomato
Brinjal	+ +	+ +	+ +	+ +
Tomato	+ +	+ +	+ +	+ +
Potato	++	++	++	++
Chilli	+	+	+	+
Tobacco	+ +	++	++	+ +

+ + = Rapid wilting (i.e. wilting occurs within 9-11 DAI), + = Moderate wilting (i.e. wilting occurs within 12-15 DAI)

rapid development of disease. In all other isolates 100% seedlings were wilted at 12 DAI. Isolates RS_3 and RS_4 were more aggressive than other isolates. At 11 DAI RS_5 isolates showed 100% wilting symptom. Isolates RS_5 can be considered as more aggressive than RS_1, RS_6 and RS_7. The seven isolates can be categorized in three groups in respect to aggressiveness. Most aggressive group (i.e. Group I) includes RS_3 and RS_4 followed by second moderate aggressive group (i.e. Group II) consisting of RS_5 and less aggressive group (i.e. Group III) consisting of RS_1, RS_2, RS_6 and RS_7. Further, the same data for isolates from different districts of West Bengal (Table 4) has been considered and cluster analysis has been performed using average linkage method. The same result is obtained

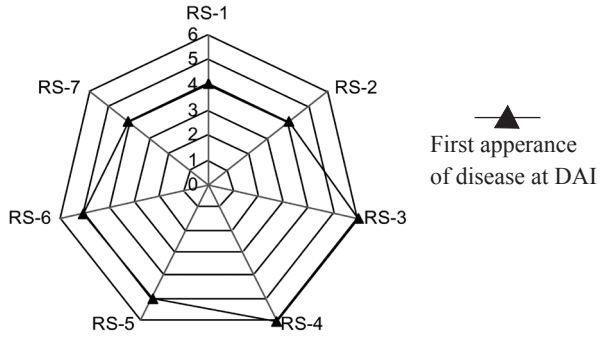

Figure 1: Aggressiveness study of different isolates of *R. solanacearum* isolated from brinjal

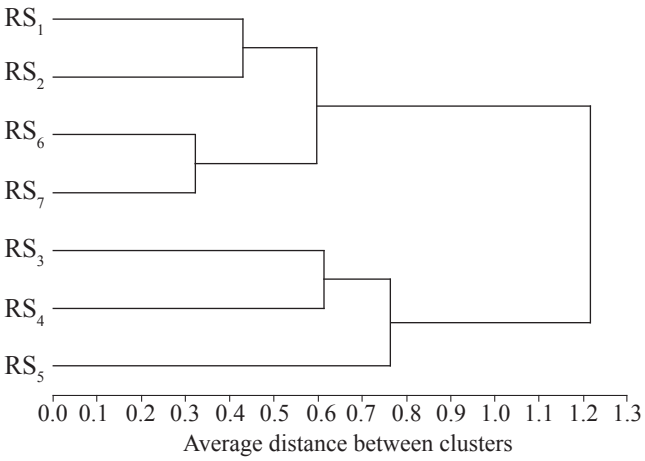

Figure 2: Clustering of isolates of *R. solanacearum* from different districts based on percentage of wilted plants as on DAI

Table 4: Aggressiveness of different isolates of *R. solanacearum* from brinjal

Isolates	Percentage of wilted plants as on DAI								
	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI	11 DAI	12 DAI
Murshidabad (RS_1)	11.11 (19.16)*	14.44 (21.87)	20.00 (25.59)	36.11 (36.84)	50.00 (45.00)	55.56 (48.24)	73.33 (59.14)	94.44 (50.79)	100.00 (89.59)
South 24 Parganas (RS_2)	7.77 (16.17)	20.55 (26.88)	28.33 (32.11)	43.89 (41.48)	56.67 (48.88)	73.89 (59.71)	81.11 (66.01)	94.44 (58.20)	100.00 (89.59)
Nadia (RS_3)	0.00 (0.40)	0.00 (0.40)	6.67 (9.12)	41.11 (39.83)	64.58 (53.54)	91.45 (73.25)	100.00 (89.59)	100.00 (89.59)	100.00 (89.59)
Cochbehar (RS_4)	0.00 (0.40)	0.00 (0.40)	28.41 (31.88)	49.60 (44.77)	57.14 (49.22)	76.90 (61.25)	100.00 (89.59)	100.00 (89.59)	100.00 (89.59)
North 24 Parganas (RS_5)	0.00 (0.40)	12.90 (20.71)	26.81 (31.08)	47.82 (43.75)	63.62 (52.94)	77.97 (62.05)	88.98 (70.65)	100.00 (89.59)	100.00 (89.59)
Birbhum (RS_6)	0.00 (0.40)	2.56 (5.63)	12.35 (20.42)	28.37 (31.67)	48.84 (44.18)	59.19 (50.52)	71.19 (58.03)	87.65 (50.91)	100.00 (89.59)
Jalpaiguri (RS_7)	2.78 (5.86)	8.22 (13.67)	17.96 (24.58)	35.55 (36.58)	44.45 (41.80)	59.44 (50.48)	82.22 (65.07)	94.44 (52.45)	100.00 (89.59)
SEm±	2.30	3.29	4.66	2.91	3.77	4.41	4.07	4.58	-
CD (p= 0.05)	7.08	10.15	14.36	8.96	11.60	13.58	12.53	14.12	-

DAI= Days after inoculation; *Figure in parenthesis indicates the corresponding angular transformed value

using cluster analysis of the isolates and it was observed that 3 clusters were formed at the average distance of 0.75 (Figure 2). The first cluster includes isolates RS_3 and RS_4, the second cluster consists of only isolate RS_5 and the remaining 4 isolates (RS_1, RS_2, RS_6 & RS_7) form the 3rd cluster at the distance of 0.75 which is exactly same as it was observed.

4. Conclusion

Bacterial wilt of brinjal and other solanaceous crops is prevalent in West Bengal and it is the main constraint of brinjal cultivation. Presence of more aggressive isolates in Nadia (RS_3), Coochbehar (RS_4) and North 24 Parganas (RS_5) districts of West Bengal may be due to high cropping intensity of solanaceous vegetables and diverse weed population which acts as reservoir of inoculum. Due to agro-climatic disparity and cropping pattern, it is expected that *R. solanacearum* isolated from wilted brinjal plants may have variation in aggressiveness. Though lesser aggressiveness is being observed in other districts such as Murshidabad, South 24 Parganas, Birbhum and Jalpaiguri, still much more attention is required and similar strategies needs to be followed to cope up with the future production and productivity of brinjal and other solanaceous crops. Similar study for all districts growing solanaceous crops in West Bengal is needed for sustainable production and productivity.

5. References

AVRDC, 2000. Variation of *Ralstonia solanacearum* strains isolated from tomato plants in Indonesia, Philippines, and Taiwan. In: AVRDC Annual Report (Wang, J.F. ed.), Taiwan 9-12.

Bernner, D.J., Statey, J.T., 2005. Bergey's Manual of Systematic Bacteriology (2nd Edn vol. II). The proteobacteria. Part C, The α-, β-, Δ-, and epsilonproteobacteria, Springer, New York, USA. www.worldcat.org/.../bergeys-manual-of-systematic-bacteriology-vol-2... part-c.../262680421.

Chatterjee, S., Mukherjee, N., Khatua, D.C., Mondal, R.K., 1999. Source and survival of *Pseudomonas solanacearum* infecting jute. In: Palit, P., Pathak, S., Sing, D.P. (Eds.), Jute and allied: fibre Agriculture and processing, CRIJAF, Barracpore 743101, India, 221-235.

Granada, G.A., Sequeira, L., 1983. Survival of *Pseudomonas solanacearum* in soil, rhizosphere and plant roots. Canadian Journal of Microbiology 29, 433-440.

Hayward, A.C., 1964. Characteristics of *Pseudomonas solanacearum*. Journal of Applied Bacteriology 27, 265-277.

Hayward, A.C., 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 29, 65-87.

Johnson, R.A., Wichern, D.W., 1996. Applied Multivariate Statistical Analysis. Prentice-Hall of India Private Ltd, New Delhi, India.

Kelman, A., 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. A literature review and bibliography. Technical Bulletin of North Carolina Agricultural Experiment Station No. 99, 194.

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

National Horticulture Board, 2009. Ministry of Agriculture, Govt. of India

Precision Farming Development Centre, 2010. Agricultural & Food Engineering Department, IIT Kharagpur, West Bengal, India.

SAS 9.2 , 2009. SAS Institute Inc. Cary, NC, USA.

SAS Enterprise Guide 4.2, 2009. SAS Institute Inc. Cary, NC, USA.