

# Pathogenic Variability of *Ralstonia solanacearum* Causing Bacterial Wilt of Brinjal in Red and Lateritic Agro-climatic Zone of West Bengal

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## ABSTRACT

Brinjal (*Solanum melongena* L.) is one of the most economically important solanaceous vegetables in India. The crop is suffering severely from bacterial wilt disease caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* that considerably limits its cultivation and production. Symptoms of the disease in brinjal appeared usually at flowering and fruiting stage of the crop growth. Drooping of the top most leaves followed by total or partial wilting was common. Infected plants showed vascular browning. Variability of the pathogen was recorded in respect to their aggressiveness and pathogenicity. Presence of more aggressive isolates in Bahadurpur (BAHRS<sub>3</sub>) and Halsidanga (HALRS<sub>6</sub>) and moderately aggressive isolates in Mukundapur (MUKRS<sub>2</sub>) and Gorabari (GORRS<sub>3</sub>) may be due to higher cropping intensity of solanaceous vegetables. Stem incision appeared superior to the stem injection method for pathogenicity test through cross inoculation studies. No host specificity was observed among the isolates of the pathogen from brinjal, tomato and *Amaranthus spinosus* revealed race 1 while host specificity was recorded in case of the pathogen isolated from *Costus speciosus* revealed race 4.

## Highlights

- ① Bacterial wilt of brinjal is a threatening disease causing havoc loss of the crop.
- ① Symptoms usually appeared at flowering and fruiting stages of the crop growth.
- ① Isolates of the pathogen showed variability in respect to aggressiveness and pathogenicity.
- ① The pathogen isolated from diverse sources and climatic situations did not show any variation in respect to physico-biochemical traits.
- ① Isolate of the pathogen from *Costus speciosus* showed host specificity.

**Keywords:** Aggressiveness study, bacterial wilt, brinjal, cross inoculation, host specificity, pathogenicity, *Ralstonia solanacearum*, variability

Brinjal (*Solanum melongena* L.) is one of the important solanaceous vegetables in Red and Lateritic Agro-climatic Zone of West Bengal. Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* (= *Pseudomonas solanacearum* E.F. Smith) is the main constrain for its cultivation (Mondal *et al.* 2011a). It is a destructive bacterial disease distributed throughout the world, infects more than 450 plant species of different botanical families (Kelman 1953; Hayward 1991; Daughtrey

2003), limits production of many economically important crops such as brinjal, tomato, tobacco, potato, jute, banana etc. (Kelman 1953; Kelman *et al.* 1954; Khatua and Maiti 1982). The disease was reported from different parts of India as well as West Bengal (Chappel, 1892; Butler, 1903; Das and Chattopadhyay 1955; Mukherjee and Chattopadhyay 1955; Chattopadhyay and Mukherjee 1968; Sharma and Mukherjee 1970; Chaudhuri and Khatua 1982; Chatterjee 1996; Samaddar *et al.*, 1998; Mondal *et*



al. 2004; Mondal *et al.* 2011b, Mondal *et al.* 2012a; Mondal *et al.* 2016). Management of the disease is very much complicated because of extreme variability, extensive host range and soil borne in nature of the pathogen (Mondal *et al.* 2014). The present investigation was conducted to study the variability among different isolates of *R. solanacearum* infecting brinjal in four different districts namely Purulia, Paschim Medinipur, Bankura and Birbhum of West Bengal under Red and Lateritic Agro-climatic zone.

## MATERIALS AND METHODS

### Collection of infected plant samples

Bacterial wilt infected brinjal plants were collected from diverse sources of Red and Lateritic Agro-climatic zone of West Bengal (Fulberia and Mukundapur of Purulia, Banshpahari of Paschim Medinipur, Gorabari and Saldiha of Bankura, Bahadurpur and Halsidanga of Birbhum). The disease was confirmed in field condition through symptomatological studies and ooze test, and brought to the laboratory for isolation of the pathogen in TZC medium. Morphological and biochemical studies of seven different isolates of the pathogen from brinjal (FULRS<sub>1</sub> - Fulberia, MUKRS<sub>2</sub> - Mukundapur, GORRS<sub>3</sub> - Gorabari, SALRS<sub>4</sub> - Saldiha, BAHRS<sub>5</sub> - Bahadurpur, HALRS<sub>6</sub> - Halsidanga, BANRS<sub>7</sub> - Banshpahari) were done in laboratory (Kelman, 1953, Kelman, 1954, Hayward, 1964).

### Aggressiveness study

The aggressiveness study was conducted under controlled condition in the laboratory. Brinjal seeds (var. Muktakeshi, a popular variety susceptible to bacterial wilt) were sown in rectangular plastic trays in rows containing sterilized soil (Mandal 2015). The experiment was replicated thrice. Two sets of experiment were conducted. Forty-eight hours old cultures of *R. solanacearum* grown in TZC medium was used as inoculum at a concentration of  $1.5 \times 10^8$  cells/ml collected from seven different places under Red and Lateritic Agro-climatic Zone of West Bengal. Fifteen days old brinjal seedlings were inoculated by making 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  $26 \pm 1^\circ\text{C}$  with 98 % RH. Numbers of wilted seedlings were recorded at an interval of 24 hours.

### Pathogenicity through cross inoculation study

Pathogenicity test was conducted by stem injection and stem incision methods (Kelman 1953). Inoculum was prepared by putting a few pieces of stem tissue from basal part of wilted plant in distilled water. Bacteria oozed out and formed opaque suspension. The suspension containing  $1.5 \times 10^8$  bacterial cell/ml was used for the experiment.

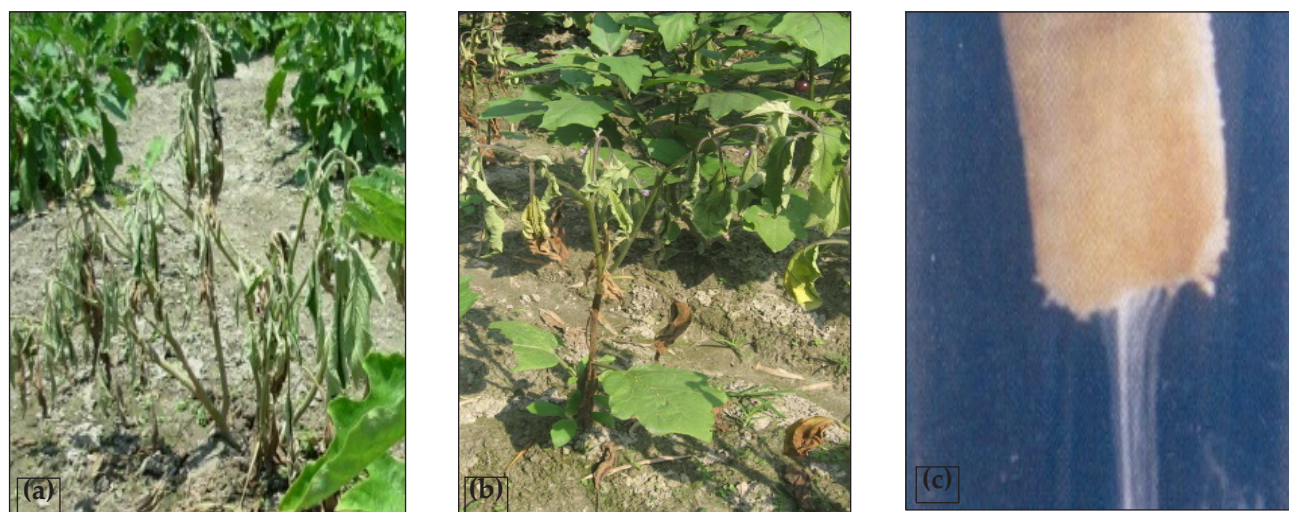
## RESULTS AND DISCUSSION

### Symptoms of bacterial wilt of brinjal

Symptoms of bacterial wilt of brinjal appeared in the fields at flowering and fruiting stage (Plate 1a & 1b) of the crops. Drooping of the top most leaves was recorded in early stage of the disease development that was prominent in day hours and recovered at night hours as normal plant. After 4 to 5 days the infected plant failed to recover leading to death of the infected plants without much changing of its green colour (Plate 1a & 1b). Wilting of one or two side branches were common leaving the other branches normal (Plate 1b). Infected plants showed vascular browning. The present investigation was corroborated with previous workers (Kelman 1953, Nolla 1931). Wilting of seedlings at 25-30 days after transplanting was observed may be due to infected transplants. All the wilted plants were ooze test positive (Plate 1c). The finding was corroborated with the earlier observation made by Mondal *et al.* (2011a) from West Bengal.

### Physico-biochemical characterization of the isolates

All the isolates (Table 1) were gram negative, straight rod, motile having flagella, showed positive oxidase reaction, could not produce levan from sucrose, could not liquefy gelatin, could not hydrolyse starch, produced lipase, could not grow at  $4^\circ\text{C}$ , showed negative arginine hydrolase activity, methyl red (MR) test was negative, Voges-Praskauer (vp) test was positive (produced acetyl methyl-carbinol or acetoin by utilizing glucose), produced hydrogen sulphide by dissimilation of



**Plate 1:** Symptoms of bacterial wilt of brinjal. (a) Wilting at flowering and fruiting stage of plant, (b) Plant showing partial wilting, (c) Bacteria oozing out from the cut end of infected plant

**Table 1:** Characteristics of different isolates of *R. solanacearum*

Characteristics	FULRS <sub>1</sub>	MUKRS <sub>2</sub>	GORRS <sub>3</sub>	SALRS <sub>4</sub>	BAHRS <sub>5</sub>	HALRS <sub>6</sub>	BANRS <sub>7</sub>
<b>Shape</b>	Rod and bent rod	Rod and bent rod	Rod and bent rod	Rod and bent rod	Rod and bent rod	Rod and bent rod	Rod and bent rod
<b>Gram reaction</b>	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Oxidase reaction	+	+	+	+	+	+	+
Levan formation from sucrose	-	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-
Lipase (tween 80 hydrolysis)	+	+	+	+	+	+	+
Growth at 4°C	-	-	-	-	-	-	-
Arginine hydrolase reaction	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-
Production of H <sub>2</sub> S	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-
Catalase reaction	+	+	+	+	+	+	+

FULRS<sub>1</sub>- Fulberia, MUKRS<sub>2</sub>- Mukundapur, GORRS<sub>3</sub>- Gorabari, SALRS<sub>4</sub>- Saldiha, BAHRS<sub>5</sub>- Bahadurpur, HALRS<sub>6</sub>- Halsidanga, BANRS<sub>7</sub>-Banshpahari

cystine and methionine, could not produce indole from tryptophan, catalase reaction was positive (could convert hydrogen peroxide into water and oxygen). Above mentioned results confirmed that wilt causing bacterium was *Ralstonia solanacearum* previously known as *Pseudomonas solanacearum* (Hayward 1964; Palleroni 1984; Bargey's Manual of Determinative Bacteriology, 9<sup>th</sup> Edition 2000). Isolates collected from different geographical and environmental situation did not show any type of variation in respect of physico-biochemical traits.

### Aggressiveness study of the isolates

The results presented in Table 2 depicted that isolates FULRS<sub>1</sub>, SALRS<sub>4</sub>, MUKRS<sub>2</sub>, GORRS<sub>3</sub> and BANRS<sub>7</sub> did not show any wilting up to 8 Days of inoculation. In case of the isolate HALRS<sub>6</sub> wilting started at 6 DAT (8.78%), whereas in BAHRS<sub>5</sub> wilting started at 7 DAT (15.45%). Very fast wilting was observed in BAHRS<sub>5</sub> up to 11 DAI. More than 70% seedlings were wilted at 12 DAI in HALRS<sub>6</sub> (80.83%) and BAHRS<sub>5</sub> (73.89%) that indicated the rapid development of the disease. In case of



**Table 2:** Aggressiveness of different isolates of *R. solanacearum* on brinjal

Isolates	Percentage of wilted plant as on days after treatment (DAI)						
	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI	11 DAI	12 DAI
FULRS <sub>1</sub>	0.00 (0.40)*	0.00 (0.40)	0.00 (0.40)	12.78 (17.27)	22.20 (28.11)	33.30 (35.24)	45.46 (42.42)
BAHRS <sub>5</sub>	0.00 (0.40)	15.45 (23.19)	20.56 (26.89)	38.52 (38.22)	50.18 (45.09)	73.89 (59.39)	73.89 (59.39)
HALRS <sub>6</sub>	8.78 (17.26)	9.23 (17.66)	12.12 (20.37)	17.36 (20.44)	37.50 (37.74)	60.97 (51.36)	80.83 (61.55)
SALRS <sub>4</sub>	0.00 (0.40)	0.00 (0.40)	0.00 (0.40)	9.52 (11.40)	14.17 (22.07)	21.56 (27.69)	42.12 (32.71)
MUKRS <sub>2</sub>	0.00 (0.40)	0.00 (0.40)	0.00 (0.40)	9.52 (11.40)	25.53 (28.45)	46.03 (42.73)	65.47 (54.07)
GORRS <sub>3</sub>	0.00 (0.40)	0.00 (0.40)	0.00 (0.40)	27.78 (31.68)	52.22 (46.32)	58.89 (50.16)	62.22 (52.07)
BANRS <sub>7</sub>	0.00 (0.40)	0.00 (0.40)	0.00 (0.40)	5.74 (11.51)	25.14 (30.01)	29.38 (32.83)	48.83 (44.52)
SEm (±)	4.65	0.70	0.96	4.36	3.09	3.45	3.15
CD (p=0.05)	15.16	2.35	3.14	14.21	10.07	11.25	10.28

\*Figures in parentheses indicate the corresponding angular transformed values, FULRS<sub>1</sub> – Fulberia, MUKRS<sub>2</sub> – Mukundapur, GORRS<sub>3</sub> – Gorabari, SALRS<sub>4</sub> – Saldiha, BAHRS<sub>5</sub> – Bahadurpur, HALRS<sub>6</sub> – Halsidanga, BANRS<sub>7</sub> – Banshpahari, DAI=Days after inoculation.

**Table 3:** Pathogenicity of different isolates of *R. solanacearum*

Isolates of brinjal	Stem injection method				Stem incision method			
	Response of pathogenicity test				Response of pathogenicity test			
	Brinjal	Tomato	<i>Amaranthus spinosus</i>	<i>Costus speciosus</i>	Brinjal	Tomato	<i>Amaranthus spinosus</i>	<i>Costus speciosus</i>
FULRS <sub>1</sub>	++	++	++	-	+++	+++	+++	-
BAHRS <sub>5</sub>	++	++	++	-	+++	+++	+++	-
HALRS <sub>6</sub>	++	++	++	-	+++	+++	+++	-
SALRS <sub>4</sub>	++	++	++	-	+++	+++	+++	-
MUKRS <sub>2</sub>	++	++	++	-	+++	+++	+++	-
GORRS <sub>3</sub>	++	++	++	-	+++	+++	+++	-
BANRS <sub>7</sub>	++	++	++	-	+++	+++	+++	-

+++ = Very rapid wilting, ++ = Rapid wilting, + = Moderate wilting, - = No wilting, FULRS<sub>1</sub> – Fulberia, MUKRS<sub>2</sub> – Mukundapur, GORRS<sub>3</sub> – Gorabari, SALRS<sub>4</sub> – Saldiha, BAHRS<sub>5</sub> – Bahadurpur, HALRS<sub>6</sub> – Halsidanga, BANRS<sub>7</sub> – Banshpahari.

MUKRS<sub>2</sub> and GORRS<sub>3</sub> more than 60% seedling mortality was recorded. Though, in all other cases wilting of 60% seedlings were not observed till 12 DAI but more than 40% wilting were recorded. Isolates BAHRS<sub>5</sub> and HALRS<sub>6</sub> were more aggressive followed by MUKRS<sub>2</sub> and GORRS<sub>3</sub> than others. The seven isolates were categorized in three different groups in respect to aggressiveness. Most aggressive group (i.e. Group A) includes BAHRS<sub>5</sub> and HALRS<sub>6</sub>, moderate aggressive group (i.e. Group B) consisting of MUKRS<sub>2</sub> and GORRS<sub>3</sub> and less aggressive group (i.e. Group C) consisting of FULRS<sub>1</sub>, SALRS<sub>4</sub> and BANRS<sub>7</sub>.

Bacterial wilt of brinjal and other solanaceous vegetables is prevalent in West Bengal and it is the main constraint of brinjal cultivation. Presence of more aggressive isolates in Bahadurpur (BAHRS<sub>5</sub>) and Halsidanga (HALRS<sub>6</sub>) and moderately

aggressive isolates in Mukundapur (MUKRS<sub>2</sub>) and Gorabari (GORRS<sub>3</sub>) may be due to higher cropping intensity of solanaceous vegetables. Because of agro-climatic variations and disparity in cropping pattern, it is expected that *R. solanacearum* isolated from wilted brinjal plants may have variation in aggressiveness (Mondal 2007; Mondal *et al.* 2011a). Different eco-friendly strategies need to be developed to minimize the disease incidence in these areas. Though lesser aggressiveness is being observed in some cases, still much more attention is required and similar strategies needs to be followed for sustainable production and productivity of brinjal and other solanaceous vegetables.

### Pathogenicity and cross inoculation studies

It is apparent from the result that all the isolates (FULRS<sub>1</sub> – Fulberia, MUKRS<sub>2</sub> – Mukundapur, GORRS<sub>3</sub>

**Table 4:** Pathogenicity through cross inoculation studies

Isolates	Stem injection method				Stem incision method			
	Response of pathogenicity test				Response of pathogenicity test			
	Brinjal	Tomato	<i>Amaranthus spinosus</i>	<i>Costus speciosus</i>	Brinjal	Tomato	<i>Amaranthus spinosus</i>	<i>Costus speciosus</i>
Brinjal	++	++	++	-	+++	+++	+++	-
Tomato	++	++	++	-	+++	+++	+++	-
<i>Amaranthus spinosus</i>	++	++	++	-	+++	+++	+++	-
<i>Costus speciosus</i>	-	-	-	++	-	-	-	++

+++ = Very rapid wilting, ++ = Rapid wilting, + = Moderate wilting, - = No wilting.

-Gorabari, SALRS<sub>4</sub>-Saldiha, BAHRS<sub>5</sub>-Bahadurpur, HALRS<sub>6</sub>-Halsidanga, BANRS<sub>7</sub>-Banshpahari) of *R. solanacearum* from brinjal were pathogenic on brinjal, tomato and *Amaranthus spinosus* (Table 3). Cross inoculation studies also revealed that the isolates of *R. solanacearum* from brinjal, tomato and *Amaranthus spinosus* were non-host specific, whereas isolates from *Costus speciosus* showed host specificity, produced disease only on the same host. This indicated that the isolates were dissimilar and host specific. Extent of wilting was varied with the host species (Table 4).

The result showed in Table 3-4 indicates that the isolate infecting tomato, brinjal and *Amaranthus* are race 1 of the pathogen, which is ubiquitous throughout the lateritic region. Isolate infecting *Costus speciosus* appeared to be different. It was evident from the earlier experiment that *Ralstonia solanacearum* infecting ginger (*Zingiber officinale*), elephant foot yam (*Amorphophalus campanulata*) and *Costus speciosus* is race 4 (Buddenhagen *et al.* 1962, Buddenhagen, 1986; Lazano and Sequeira 1970; Daughtrey 2003; Mondal *et al.* 2012a and Mondal *et al.* 2012c). The isolates of *Ralstonia solanacearum* from brinjal also showed variation in respect of virulence. Stem incision method appeared superior where very rapid wilting (+++) observed than the stem injection method for cross inoculation studies and pathogenicity test. Kelman (1953) and Klement (1963) suggested stem inoculation method for pathogenicity test. The study also corroborated with Mondal *et al.* (2012b).

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