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RESEARCH ARTICLE

Bacterial wilt of banana in West Bengal, India

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ABSTRACT

Bacterial wilt of banana caused by Race 2 of *Ralstonia solanacearum* (Smith) Yabuuchi *et al*. is prevalent in West Bengal affecting commonly cultivated cultivars - Champa (AAB), Martaman (AAB), Kanthali (ABB), Giant Governor (AAA) and Kanch Kala - a cooking variety (ABB). In variety Champa, symptoms of the disease appear as reddening of the basal part of the pseudostem followed by yellowing of leaves leading to wilting of plant. Causal bacterium oozes out at the cushion of peduncle. Vascular blackening is found on any part of the rhizome but concentrated at the middle in wilted plant. Planting of diseased suckers show slow and stunted growth with a few leaves, usually two. The leaves show necrotic symptoms. Gradual yellowing of leaves followed by wilting along with typical vascular browning are the common symptoms in cutivar Kanthali, Martaman and Kanch Kala. Occasionally reddening and splitting of peudostem longitudinally at the base of plant are found in cultivar, Martaman. The affected plants of Giant Governor do not show any wilt symptom. Leaves do not turned yellow. Affected plants grow slowly and drying of leaves along margin is the common symptom in plants. A selective medium (Casein hydrolysate 1g, Glucose 5g, Peptone 10 g, Agar agar 20 g, 2,3,5 - Triphenyltetrazolium chloride (TZC) 50 mg, Crystal violet 50 mg, Polymyxin B sulphate 10 mg, Tyrothricin 20 mg, Chloramphenicol 5mg, Cycloheximide 50mg, Distilled water up to 1000 ml) was standardized for isolation of the causal bacterium.

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INTRODUCTION

Banana (*Musa* spp.) is an important fruit crop in tropical and subtropical countries. In some countries like Uganda and Tangenia it holds the status of food crop. Banana is the second largest produced fruit after citrus, contributing about 16 per cent of the world's total fruit production (FAO, 2009). India is largest producer of banana, contributing to 27 per cent of the world's banana production. Tamil Nadu is the leading producer of banana followed by Maharashtra and Gujarat in India (Mahapatra *et al.,* 2010). In West Bengal, the area under banana cultivation has been surprisingly increased during the last ten years. Three districts of Gangetic Alluvial Zone *viz.,* Hoogly, Nadia, North 24 Parganas are the major contributors in West Bengal. In this state, cultivar Champa (AAB), Martaman (AAB) and Kanthali (ABB) are the popular cultivars along with Giant Governor (AAA). With the increase in commercial cultivation of banana, disease pest gradually becomes limiting factor in achieving higher and quality production. Among the diseases, Sigatoka and Panama wilt are considered so far, to damage this crop (Misra, 2002) in West Bengal. In addition to these diseases, bacterial wilt is appearing every year. After the first report of this disease as early as in 1968 (Chattopadhyay and and Mukhopadhyay, 1968), existence of Moko disease (bacterial wilt) in West Bengal was questionable. Present study includes the record of symptoms of the disease and its simple diagnostics, varieties affected, mode of isolation of the pathogen and race determination to confirm the incidence of the disease as serious threat to banana cultivation in West Bengal.

MATERIALS AND METHODS

Selective medium was prepared through modification of medium proposed by Granada and Sequeira (1983). The composition of the medium was Casein hydrolysate 1g, Glucose 5 g, Peptone 10 g, Agar agar 20 g, 2, 3, 5-Triphenyltetrazolium chloride (TZC) 50 mg, Crystal violet 50 mg, Polymyxin B sulphate 10 mg, Tyrothricin 20 mg, Chloramphenicol 5 mg, Cycloheximide 50 mg and Distilled water up to 1000 ml. For preparation of this selective medium, 50mg of TZC and 50mg of Crystal violet were dissolved in 10ml distilled water and then sterilized in autoclave at 15 lb/sq. inch for 15 minutes. All the antibiotics were sterilized by dissolving them in 1ml of 70 per cent ethanol and kept for 30 minutes, and then 100 ml sterile water was poured over the dissolved antibiotics. Sterilized TZC and crystal violet solution and antibiotic solutions were kept in 4°C for future use. Sterilized agar medium was prepared excluding TZC, crystal violet and antibiotic and in 100 ml of melted medium, 1ml of TZC and crystal violet solution and 10 ml of antibiotic solution were mixed before experimental use.

After selection of virulent isolate, *Ralstonia solanacearum* was multiplied in PDA medium. Forty eight hours old culture was used for artificial inoculation. Bacterial suspension prepared from this culture was injected at base of the pseudostem of banana plant.

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Symptomatology :

In variety Champa (AAB) :

The banana (cv. Champa) orchards did not give a healthy look. Many plants died before flowering causing gaps in every line of plantation. In severely infected orchards gaps were more and prominent. Middle leaf became abnormally straight accompanied with one or two leaves at the late stage of the growth. Drooping of leaves was very much prominent before shooting of fruits. Drying of these leaves was not observed. The straightened leaves turned yellow from the tip or from the margins and gradually dried. The dried area gradually proceeded towards the midrib. The drying was recorded from leaf margin of one or both side(s) of the midrib. Once the entire leaf dried up it looked papery. These dried leaves remained straight for several days and then hung down. Light red streaks appeared on midribs of the leaves of diseased plant, in early stage of disease development, in contrast to green colour of a healthy leaf.

The symptoms appeared in the plants in the field during rainy season and were very much prominent and typical in the winter season. Yellowing and drooping of leaves were also found in suckers. Severity of the disease increased in ratoon crop.

The colour of the pseudostem towards base of plants turned dark red compared to green colour of a healthy plant. When these pseudostems (at the base) were cut transversely, a typical vascular blackening was noticed concentrated near the centre of the pseudostem (*i.e.* on the peduncle base).

In the rhizome, the infected strands usually constituted a conspicuously coloured mass where the vascular strands were closely aggregated near the periphery of the stele, *i.e.* about one inch from the outer surface. But individual yellow and brown strands were also observed to be abundant towards the centre in some cases. The vascular discolouration could be seen extending into the suckers. Dark black patches might develop at any part of the rhizome. Bacterial ooze from cushions of peduncles of diseased plants and base of the bracts were seen. Flowers of infected plants when put into water also showed oozing.

In diseased plants fingers were small, thin and poorly grown. The fruits were cracked and showed red streaks on the skin similar to that of the midribs of the infected leaves. Later on, these fruits turned yellow and became internally brown. Internally dry rot was also found.

Planting of diseased suckers showed slow and stunted growth with a few leaves, usually two. The leaves showed necrotic symptoms. The pseudostem was reddish in colour but later showed rotting symptom leading to the death of the whole plant. Severely infected suckers might not produce the leaves at all.

In variety Kanthali, Martaman and Kanchkala :

In the early stage of the of disease the leaves turned pale green to bright yellow and remained as such or after few days they collapsed near the junction of the lamina with the petioles. Within two months most of the leaves dried up resulting death of the plant. Interestingly reddening of the pseudostem was not observed in Kanthali (ABB) and Kanch Kala (ABB, a cooking variety). Occasinally, reddening and splitting of peudostem longitudinally at the base of plant were found in Martaman (AAB). Browning of vascular tissue was similar to Champa. Oozing of bacterial mass from scar of the flower was not found.

In variety Giant Governor :

The plants in this variety (AAA) did not show any wilt symptom. Leaves did not turn yellow. The leaves were pale green in colour, slow in growth and unhealthy looking. Drying of leaves along margin was the common symptom. Occasional ripening of a few immature fruits in the affected plant was found. At fruiting stage, pseudostem affected plant might break at middle. Vascular tissue of the rhizome did not turn dark brown or black but general colour of the affected rhizome

changed to light brown compared to cream colour of healthy rhizome

Stover (1972) considered wilt of banana reported by Chattopadbyay and Mukhopadhyay (1968) in West Bengal as yellow mat disease caused by solanaceous strain of *Pseudomonas*. According to Buddenhagen (1961), one of the confirmatory symptoms of Moko disease was fruit rot and fruit stalk discolouration. Similar symptom was found in Champa (AAB) variety in Hooghly district and flowers of infected bunch showed positive oozing when put in water. Like insect transmitted SFR strain of *Pseudomonas solanacearum* (Buddenhagen and Elsasser, 1962), bacterial ooze was also noticed from the cushion of peduncle. Reddening of the pseudostem and reddish streak on the midribs of the wilt affected banana plant was not reported earlier.

Symptoms recorded under field condition were similar to Moko disease as stated by Buddenhagen (1961). The varieties affected by bacterial wilt in West Bengal are triploid banana (Chatterjee *et al*., 1997, Mondal *et al*., 2011). Sivamani and Gnanamanickam (1987) reported Moko disease from Tamil Nadu in Poovan variety. The banana variety Champa, widely cultivated in West Bengal is included under the group Poovan, a triploid banana (AAB group).

Diagnosis of the disease :

In most cases, it is difficult to distinguish Moko disease from Panama wilt under field condition based on the characters mentioned by Strover (1972). Moko disease can be confirmed based on observing brown discolouration in the rhizome followed by positive ooze test. Negative response in ooze test indicates the possibility of Panama wilt. Now, to confirm Panama wilt, 15–20 thick transverse disc of rhizome was taken from infected plant after removing surface tissues followed by washing. This disc was then put in a polythene bag and its mouth was closed after pumping air into the bag. The total set was kept at room temperature or BOD at 28±1°C. After 36-72 hour, prominent growth of *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *cubense* (E.F. Sm.) W.C. Snyder and H.N. Hans. comes up over the brown coloured tissues of the rhizome. No such growth will develop in Bacterial wilt infected rhizome.

Isolation of the pathogen

In Nutrient agar medium :

Small pieces (1-2 cm^2) of blackened infected rhizome from the infected plant were surface sterilized with 70 per cent ethyl alcohol and then immediately dipped in mercuric chloride solution (1:1000) for 15 seconds and passed through three changes of sterile water. These sterilized bits were then kept in sterile distilled water in a culture tube to allow the bacterium to ooze out in water for exudation. The turbid bacterial suspension was diluted (10^{-5}) with sterile water following serial dilution method. Loopful of this bacterial suspension was streaked over the solidified nutrient agar slants and subsequently on two other slants. The culture tubes were then kept in B.O.D. at 28±1°C. Single colonies came up in some slants. Single isolated colony was picked up and streaked on new slants. A small amount of bacterial mass was suspended in sterile distilled water and then streaked on agar slants for purification of the culture. Artificial inoculation with the bacterium obtained through isolation in Nutrient agar medium was unsuccessful in most cases.

In selective medium :

A number of media have been evolved and reported by different authors (Karganilla and Budlenhagen, 1972; Nesmith and Jenkins, 1979; Granada and Sequeira, 1983), from time to time for selective isolation of this bacterium. The selective medium suggested by Granada and Sequeira (1983) was taken for isolation of *Pseudomonas solanacearum*. Isolation of *P. solanacaarum* in this medium from an infected tomato plant initially failed. No bacterial growth came up on this medium. Later some of the ingredients of the medium were screened separately against tomato isolates (*P. solanacearum*) and thimerosal was found to be inhibitory even at as low as concentration of 1 ppm (Table 1). While Crystal violet, Polymyxin B sulphate, Tyrothricin and Cycloheximide were non-inhibitors of *R. solanacearum* isolates even at 500-1000

Table 1: Sensitivity of *Ralstonia solanacearum* (tomato isolate) towards some ingredients of selective medium proposed by Granada and Sequeira (1983)

Ingredients	Diameter of inhibition zone in mm* Concentration of chemicals in ppm									
	1000	500	200	100	50	20	10	5	2	1
Chloramphenicol	16.5	14.2	12.9	11.3	9.9	8.5	0.0	-	-	-
Thimerosal	-	-	-	-	-	-	25.3	19.9	12.2	11.1
Crystal violet	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
Polymysin B sulphate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
Tryrothricin	-	-	-	-	0.0	0.0	0.0	0.0	-	-
Cycloheximide	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-

* Average of 9 replications, - not tested

ppm concentration. Chloramphenicol was inhibitory at 20 ppm and above.

By eliminating thimerosal, the medium suggested by Granada and Sequeira (1983), was used for isolation of *Pseudomonas solanacearum* (*Ralstonia solanacearum*) from banana and tomato.

After surface sterilization and preparation of diluted bacterial suspension (10^{-5}) as stated earlier, one ml of this diluted suspension was put in sterilized Petriplates with the help of a sterilized pipette. Melted selective medium was then poured in these Petriplates up to 0.5 cm height, and the bacterial suspension was thoroughly mixed with the medium. After solidification of the medium, the Petriplates were kept in B.O.D. at 28 ±1°C.

Bacterial colonies were observed within 36-48 hrs. Three different types of colonies grew on this medium - round, butyrous, deep red colony; round, fluidal white colony with a pink center and translucent with smooth surface.

Similarly, isolation was done with infected tomato stem tissue. In this case also three types of colonies were obtained.

Relative population of different types of colonies obtained in isolation from infected tomato and banana in selective medium is presented in Table 2. It appears that in most of the cases number of red type colony was more, followed by pink dot and then by white colony.

Table 2 : Population of *Ralstonia solanacearum* in bacterial ooze collected from tomato and banana plants

Sample No.	Population of *Ralstonia solanacearum*, cfu/ml x 10^5 / ml of ooze					
	Tomato			Banana		
	White	Red	Pink dot	White	Red	Pink dot
1.	0.2	2.56	1.2	3.0	2.0	3.0
2.	0.07	0.54	0.5	7.0	8.0	8.0
3.	0.3	2.9	1.73	0.0	20.0	4.0
4.	0.4	2.6	2.26	4.0	17.0	10.0
5.	0.2	2.5	1.5	1.0	4.0	9.0

To know the degree of virulence of different type of colonies, three types of bacteria obtained from tomato were separately grown in PDA slants. After 48 hours growth, suspension was prepared with individual colony and injected to 30 days old tomato seedlings. Tomato seedlings inoculated with pink dot colony showed wilting symptoms at 15 days after inoculation in July. Seedlings inoculated with other types of bacterial isolates did not show any symptom. The bacterial colony showing pink centre with white boundary was considered to be virulent one and the other two types were avirulent. Artificial inoculation in banana with pink dot colony obtained from infected banana was successful. Failure of inoculation by cultured isolated in Nutrient agar medium was due to dominancy of avirulent cells due to sub-culturing. Kelman (1954) demonstrated relationship of virulence with colony morphology. He also showed that colony having pink centre with white boundary was virulent. Granada and Sequeira (1983) have also expressed similar views. The modified medium was considered to be selective for *Ralstonia solanacearum* prevalent in West Bengal.

Race determination of bacterial wilt pathogen :

In glass house experiment, potato and tomato plants were grown in earthen pots. Thirty days old plants were inoculated by stem injection method (Kelman, 1953) with bacterial suspension obtained from an infected banana sucker (from cv. Champa). Another set of plants was inoculated with bacterial suspension obtained from wilted tomato plants. Suitable control was maintained where only water was injected. Tomato and potato plants inoculated with a solanaceous strain showed the typical wilt symptoms within 2 to 3 weeks. But potato and tomato plants when inoculated with banana isolate did not show any wilt symptoms. These plants appeared healthy even 45 days after inoculation. Small adventitous roots developed from the stems of tomato plants near the point of inoculation.

Ralstonia solanacearum (*Pseudomonas solanacearum*) infects about 200 plant species and causes wilt disease (Kelman, 1953). Buddenhagen *et al.* (1962) identified 3 races, e.g. Race 1 affects tomato, tobacco, many solanaceous and other weeds and certain diploid bananas (Musa groups BB or AA) ; Race 2 is pathogenic on triploid bananas (Musa groups AAA, AAB and ABB) and Heliconia and Race 3 on potato. They did not accept the report of Chattopadhyay and Mukhopadhyay (1968) on incidence of Moko disease from India (Stover, 1972). According to their opinion, it was possibly caused by a solanaceous strain of *Pseudomonas solanacearum* (*Ralstonia solanacearum*). Result of the present study clearly indicates bacterial wilt of banana in West Bengal is caused by *Ralstonia solanacearum* Race 2.

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