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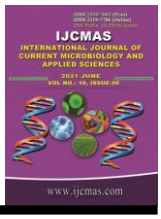

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Determination of Races and Biovars of *Ralstonia solanacearum* causing Bacterial Wilt of Brinjal in Chhattisgarh, India

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A B S T R A C T

Bacterial wilt of brinjal caused by *Ralstonia solanacearum* is endemic in most brinjal growing areas of Chhattisgarh state causing 40 to 80% loss in yield. Control measure requires definite information on race and biovar characteristics of the pathogen in those endemic areas. Five *Ralstonia solanacearum* isolates (*Rs* 3, *Rs* 7, *Rs* 8, *Rs* 11 and *Rs* 12,) of brinjal host were tested on different host plants which are host pathogenic. Under pathogenicity tests of all five *Rs* isolates on different host ranges, the brinjal, tomato, chili, potato, geranium, cucurbits and cucumber plants were infected and showed wilted symptoms except *Rs* 11 in geranium. While ginger, banana, rose, soybean and sweet potato plants were not infected with any *Rs* isolates and remained healthy. According to the described in EPPO, 2004, the pattern of the infection caused by different *Rs* isolates on different host range were studied and found that the dominant *Rs* isolates in Chhattisgarh state was grouped under Race 1 (caused wide variety: Ginger, olive, chili pepper, peanut, *Solanum* spp., tobacco, *Musa* spp. (banana and plantain), peanut, *Heliconia* tomato and distributed in Asia, Africa, Australia, north and south America) followed by Race 3 and Race 2 with 53.84%, 38.46% and 7.69%, respectively. Therefore, dominant groups of *Rs* isolates causing bacterial wilt of brinjal belonged to race 1 and biovar III.

Introduction

Eggplant, commonly known as brinjal (*Solanum melongena* L.) in India, is an often cross pollinated crop and belongs to the angiospermic family solanaceae. It is a popular and principal vegetable crop widely grown in tropics and subtropics especially in Asia, Europe, Africa and America. Indian sub-continent and China are its primary centers of diversity.

India was the second largest producer of brinjal in the world with 12510000 tonnes

production after china with 32908763 tonnes production while, India was first in area of brinjal growing countries with 733000 ha harvested area followed by China with 786266 ha area in 2017 (FAO, 2019). In India, the brinjal crop is cultivated throughout the year with annual production of 12801 '000 MT over an area of 730 '000 hectare in 2017-18 (Horticultural statistics at a glance, 2018). In Chhattisgarh, brinjal is an important cash crop grown over an area of 37.93 '000 hectare with an annual production of 705.40 '000 MT (2017-18). District Kondagaon was ranked first in area (5.30 '000 hectares) followed by district Durg (4.38 '000 hectares), while Durg district was first in production (110.32 '000 MT) followed by Kondagaon (77.75 '000 MT) in 2017-18 (Horticultural statistics at a glance 2018). There is much variation in the chemical constituents of the fruits of different types and cultivars of brinjal. Therefore, it is grown throughout the year in different parts of Bastar plateau by growing situation based cultivar.

Ralstonia solanacearum (Smith, 1896; Yabuuchi *et al.*, 1995) causes bacterial wilt disease is one of the most destructive diseases of solanaceous crops and highly challenging worldwide. Bacterial wilt is a major problem in India and directly affects economic production of brinjal. It is also considered one of the important diseases in Chhattisgarh state especially in Bastar plateau. The wilt problem is persistent throughout the year in different parts of Bastar plateau and affects every stage of the crop. It has a wide host range remaining a major biotic limiting factor of several important crops of family solanaceae, leguminacea, several trees and herbs.

Ralstonia solanacearum is a highly species complex bacteria and has a complex of variants, variously described by several workers *as* groups, races, biovars, biotypes, sub-races, phylotypes and strains. The different classifications of *Ralstonia solanacearum* have created a considerable amount of confusion in the literature. *Ralstonia solanacearum* distinguished by several workers in to five bivars on the basis of carbohydrate utilization (Buddenhagen and Kelman, 1964; Denny and Hayward, 2001), five races on the basis of pathogenicity of different host range (Buddenhagen *et al.*, 1962; Denny and Hayward, 2001; EPPO, 2004; Denny, 2006) and four phylotypes on the basis of geographical origin and genetic make-up (Fegan and Prior, 2005).

At present, there is a lot of confusion regarding the most prevalent strains of *Ralstonia solanacearum* in the different parts of the world. However, in India little knowledge is available about the most prevalent biovars, races and strains in various locations of India.

Races were assigned based on host range (Buddenhagen *et al.*, 1962; Schaad *et al.*, 2001). They used four species of the family Solanaceae (*Solanum lycopersicum*, *Capsicum annuum*, *Solanum melangena* and *S. tuberosum*) and one from Moracea family (*Morus alba* L.) for determining race of *Ralstonia solanacearum*. The classification of race and biovar has gained wide acceptance for subdividing *Ralstonia solanacearum*. On the basis of this classification system, *Ralstonia solanacearum* strains grouped into races according to their capability to infect different host plants. Race 1 is comprised of many strains having a wide host range and pathogenic on different solanaceous crops and weed hosts, race 2 is restricted to triploid banana and *Heliconia*, race 3 (potato race) affects potato, race 4 infects ginger, and race 5 is pathogenic on mulberry (He *et al.*, 1983).

Klement *et al.*, (1963) used pathogenicity tests for the identification of races through one month old plants were inoculated with *Ralstonia solanacearum* by trimming the roots

and dipping in bacterial suspension for 30 minutes. The inoculated plants were then kept in a net house until the symptoms developed. *Ralstonia solanacearum* from the wilted plant was reisolated on TZC agar and colonies were compared with the original culture.

The study of the differential host plant illustrated to used different hosts for the initial separation of *X. campestris* pv. *campestris* isolates into races. Isolates corresponding to Kamoun's races 0, 1, 2, and 4 were identified among those tested, but isolates showing the pattern of reaction of Kamoun's race 3 were not found (Kamoun *et al.*, 1992).

Singh *et al.*, (2010) collected isolates of *Ralstonia solanacearum* from wilted solanaceous crops (potato, tomato, brinjal, chilli and capsicum) caused by *R. solanacearum* from different parts of the Northern and Eastern states of India such as Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Jharkhand and West Bengal and on the basis of pathogenicity tests and Hayword's classification isolates were categorized into race 1 and biovar III.

Isolates of *Ralstonia solanacearum* causes bacterial rhizome rot of patumma (*Circus alismatifolia*) from Thailand were tested on different host range i.e. ginger, tomato, eggplant, paper and marigold for pathogenicity test and found typical wilt symptoms on different host. Patumma strains were also tested for sugar utilization test and found that three sugar alcohols oxidized but did not utilize three disaccharides in the test and they shared the same biochemical characters with strains of other hosts. Based on these characters, isolates of *Ralstonia solanacearum* of patumma were identified as Race 1 and biovar 4. *Ralstonia solanacearum* infects 29 natural hosts other than potato and tomato (Pradhanang *et al.*, 2000). *Ralstonia solanacearum* has a broad host range of 450 crop species across 54 families (Wicker *et al.*, 2009). The crop plant belonging to the solanaceous family is particularly threatened, including cultivated species such as potato, tomato, eggplant, chilii and tobacco (Hayward, 1994). Several weed host such as *S.dulcamara, S.nigrum, Portulaca oleracea* and *Rumex dentatus* (Elphinstone, 1998) and volunteer plants or (in colder climates: perennial) served as reservoir of *Ralstonia solanacearum.*

The major hosts of *Ralstonia solanacearum* worldwide were listed in Table 1 reported by different workers (Kumar *et al.*, 2004, Kelman, 1953; Bradbury, 1986; Elphinstone, 2005) that the host range of *Ralstonia solanacearum* is not restricted to solanaceous crops but affected to many other plant families among broad and narrow leaf plants.

Materials and Methods

Races were assigned based on host range (Buddenhagen *et al.*, 1962). Five isolates of *Ralstonia solanacearum* were multiplied in TZC medium to inoculate on differential hosts under artificial conditions. 13 plants species *viz*., brinjal (*Solanum melongena*), tomato (*Lycopersicon esculentum* Mill.), chilli (*Capsicum frutescens*), potato (*S. tuberosum*), ginger (*Z. officinale*), banana (*Musa ac-cuminata*), geranium (*Pelargonium*), rose (*Rosa*), soyabean (*Glycine max*), bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), sweet potato (*Ipomoea batatas*) and mulberry (*Morus alba*) were used as differential hosts as method described in EPPO, 2004 (Table 2).

The seedlings were grown in a greenhouse under artificial conditions. Twenty days old seedlings of brinjal, tomato and chili were pulled out gently washed free of soil and a few tertiary roots were clipped with sterilized scissors and dipped in the bacterial culture for

10 minutes. Tuber and rhizome of potato and ginger crops and seeds of geranium, soybean, bottle gourd and cucumber crops were dipped in the bacterial culture for 20 minutes. Stem cutting of sweet potato, rose and mulberry were dipped in the bacterial culture for 20 minutes. Thereafter, the inoculated seedlings, tubers, rhizomes, seeds and plant cuttings were transplanted to plastic bags containing slightly acidic soil. *In situ* inoculation of different isolates in all bags was carried out through different inoculation methods as explained in below.

Direct inoculation on plant material

Twenty to thirty days old seedlings of *viz.*, brinjal, chili and tomato were pulled out gently and washed for free of soil. A few tertiary roots were clipped with sterilized scissors and dipped in the bacterial culture for 10 minutes. The inoculated seedlings were transplanted to plastic bags containing slightly acidic soil. The method was explained by Klement *et al.,* (1963).

Root inoculation through infested soils

Plants were stopped watered one day before inoculation to reduce moisture in the pots. Roots of the plant were slightly injured by inserting a scalpel in the pots in order to facilitate bacterial infection. About 50 ml of bacterial suspension was poured onto the surface of each bag. Inoculated plants were regularly watered and kept at a temperature range from 28 to 30^{o}C and 80-90% relative humidity (Winstead and Kelman, 1952).

Detached leaf method

For this method terminal leaves containing 2 to 3 leaflets were selected and detached from the plants. These leaves were dipped in a conical flask or in test tubes containing bacterial suspensions for 3 to 5 minutes. This method was applied in all family of plants. Inoculated plants were observed for appearance of symptoms as explained by Winstead and Kelman (1952).

Cotyledons inoculation

Bacterial suspension was inoculated into the plant through the use of wounding done by needle puncture or carborundum to introduce the pathogen.

Stem inoculation

Bacterial suspension was introduced into the main stem of the plant with a pediatric syringe by inserting the needle gently into the vascular tissue of the main stem while carefully holding and supporting the whole plant with a hand to prevent damage.

A check was also established in the same manner but injected with sterile distilled water in place of Bacterial suspension. The same procedure was repeated after 20 days interval as described by earlier investigators (Thind and Payak, 1978; Kutama *et al.,* 2011).

Plants similarly inoculated with sterile water served as the control. The plants were watered regularly and observations for appearance of wilt symptoms were recorded. The bacterium was re-isolated from wilted plants and compared with the original culture of *Ralstonia solanacearum* so as to satisfy the coach postulates.

Results and Discussion

There is no biochemical test for race identification of *R. solanacearum* caused bacterial wilt disease for a broad host range. In the present investigation on pot culture experiment, the races of *R. solanacearum* were identifying by pathogenicity test in 13 different wide host ranges as brinjal (*Solanum*

melongena), tomato (*Lycopersicon esculentum* Mill.), chili (*Capsicum frutescens*), potato (*S. tuberosum*), ginger (*Z. officinale*), banana (*Musa accuminata*), geranium (*Pelargonium*), rose (*Rosa*), soybean (*Glycine max*), bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), sweet potato (*Ipomoea batatas*) and mulberry (*Morus alba*) in the present study. Similar methods for pathogenicity tests were made by Wang and Berk (1997), Vicente *et al.,* (2001), Kumar, 2006 and Nouria *et al.,* (2009).

The result of the pathogenicity test showed in table 3 and 4, that the wilt symptoms were produced in brinjal, tomato, chili, Geranium, cucurbit and cucumber seedlings in the inoculation of *Rs* 3, *Rs* 7, *Rs* 8, *Rs* 11and *Rs* 12 isolates of *R. solanacearum,* whereas potato infected with *Rs* 3, *Rs* 8 and *Rs* 12 and geranium infected with *Rs* 3, *Rs* 7, *Rs* 8 and *Rs* 12 at 75 DAT. While, none of the group of *R. solanacearum* isolates was not able to develop wilt symptoms in inoculated ginger, banana, rose, soybean and sweet potato seedlings with zero percent disease incidence. Range of percentage disease incidence in all host range were recorded in inoculation of isolates *Rs* 3 with minimum 25% to maximum 60%, *Rs* 7 with 0 to maximum 95%, *Rs* 8 with minimum 10 to maximum100%, *Rs* 11 with 0 to maximum 40% and *Rs* 12 with minimum15 to maximum 60% at 75 DAT. Among the all isolates of *R. solanacearum* inoculated from wilted brinjal plant, Rs 3 was more prominent to cause brinjal seedlings with 30 % followed by Rs 12, Rs 8, Rs 7 and Rs 11 with 25%, 20%, 15% and 10% wilt incidence respectively at 75 DAT (Table 3 and 4). Table 3and 4 showed that all five isolates of *R. solanacearum* i.e. *Rs*3, *Rs*7, *Rs*8, *Rs*11and *Rs*12 were categorized into races on the basis of pathogenicity test. The brinjal, tomato, chili and potato plants were infected by all isolates of *R. solanacearum* and developed the wilt symptoms that indicate predominant races of *R. solanacearum* as race 1 followed by race 3 whereas cucurbits and cucumber plants were infected by all isolates of brinjal indicating predominant race of *R. solanacearum* as race 1. While, positive infection of geranium plants with all isolates of *R. solanacearum* except *Rs*11 were indicating predominant race as race 3. While ginger, banana, rose, soybean and sweet potato plants were not infected with any *R. solanacearum* isolates and remained healthy. In the present investigation, the pattern of the infection caused by different *R. solanacearum* isolates based on different host range as described in EPPO, 2004 were studied and found that the predominant *R. solanacearum* isolates in Bastar plateau of Chhattisgarh state was grouped under race 1 followed by race 3 and race 2 with 53.84%, 38.46% and 7.69% disease incidence.

Similar studies made by Prasanna Kumar (2004) and reported that 57 isolates belonging to race 1 obtained from solanaceous and non solanaceous plants could cause infection in ginger and the 4 Ginger isolates infected solanaceous host plants but not banana and mulberry and tobacco plants. Stanford and Wolf (1917) also reported in similar studies that the strain variation of *Pseudomonas solanacearum* based cross inoculation method for the isolates of brinjal, tobacco, tomato and potato but found no differences among the isolates. The findings of the present study on determination of predominant races of *R. solanacearum* are also supported by Buddenhagen *et al.,* (1962) who classified *R. solanacearum* into three races who found only one race. Race 1 infects many solanaceous plants such as brinjal, tomato, tobacco, pepper and other plants including some weeds.

Table.1 List of major hosts of *Ralstonia solanacearum*

Hosts	References
***Lycopersicon esculentum* (tomato), *Solanum tuberosum* (potato)**	Sequeira, 1998, Lopes *et al.*, 2005
***Capsicum annum* (sweet pepper), *Solanum melongena* (eggplant)**	
***Nicotiana tabacum* (tobacco), *Arachis hypogaea* (groundnut)**	
***Pelargonium hortorum* (geranium)**	Swanson, 2007
Arabidopsis thaliana	Norman *et al.*, 2009
Bananas and *Heliconia spp*	EPPO, 1999
Sunflower	Elphinstone, 2005
Pepper* spp. and *Morus spp	Aragaki and Quinon, 1965
***Anacardium occidentale* (cashew)**	Shiomi *et al*., 1989
Annona spp. (custard apple)	Mayers and Hutton, 1987
***Archontophoenix alexandrae* (Alexandra palm)**	Akiew and Hams, 1990
Artichokes	Aly and El ghafar, 2000
Cerastium glomeratum, Drymaria cordata, Polygonum capitatum and Stellaria media	Pradhanang *et al*., 2000
***Solanum dulcamara* (bittersweet)**	Elphinstone *et al*., 1998
Urtica dioica	Wenneker *et al.*, 1999
***Ipomoea batatas* (China)**	He *et al*., 1983
***Eucalyptus* (Brazil, China)**	Dianese *et al*., 1990
Cassava (Indonesia)	Nishiyama *et al*., 1980
Peanut (China)	Middleton and Hayward, 1990

Table.2 Host range, Geographical distribution, Characteristics of races and their relationship with biovar of *Ralstonia solanacearum* (EPPO, 2004)

Race	Natural host range	Geographical distribution	Biovars
1	Wide variety: Ginger, olive, chili pepper, peanut, *Solanum* spp., and tobacco	Asia, Africa, Australia, North America, and South America	I, III, IV
2	*Musa* spp. (banana and plantain), peanut, *Heliconia*, and tomato	Caribbean, Asia, Central America, South America, and Hawaii	I
3	Solanaceous, and *Pelargonium* spp. (Geranium)	Worldwide (except Canada and United States)	II
4	Ginger	Australia, India, Asia, and Hawaii	III, IV
5	*Morus* spp. (Mulberry)	china	V

Table.3 Pathogenicity test for determination of race of *Ralstonia solanacearum* (*Rs*) isolates through different host range

Name of Plants	Bacterial wilt incidence at different days after transplanting (%)																			
	Isolate *Rs*3				Isolate *Rs*7				Isolate *Rs*8				Isolate *Rs*11				Isolate *Rs*12			
	30	45	60	75	30	45	60	75	30	45	60	75	30	45	60	75	30	45	60	75
Brinjal	0	0	18	30	0	0	12	15	0	0	16	20	0	0	0	10	10	10	25	25
Tomato	0	0	24	32	0	0	10	10	0	12	20	22	0	0	0	5	5	15	15	15
Chilli	0	20	32	38	0	0	0	14	0	12	30	36	0	0	0	0	0	20	20	20
Potato	0	6	6	6	0	0	0	0	0	5	10	10	0	0	0	0	5	10	15	15
Ginger	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Banana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Geranium	0	0	50	50	0	0	0	20	0	0	30	60	0	0	0	0	0	0	25	25
Rose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Soyabean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cucurbit	10	10	15	25	20	60	90	90	15	60	90	100	20	20	20	30	10	10	35	60
Cucumber	10	10	35	60	15	50	80	95	25	30	60	90	25	25	25	40	10	10	30	40
Sweet potato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mulberry	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table.4 Determination of race and biovar of different isolates of *Ralstonia solanacearum* (*Rs*) through different host range

Host range	Bacterial wilt incidence at different days after transplanting (%)					Race	Biovar
	Isolate *Rs*3	Isolate *Rs*7	Isolate *Rs*8	Isolate *Rs*11	Isolate *Rs*12		
Brinjal	+	+	+	+	+	1,3	I,II,III,IV
Tomato	+	+	+	+	+	1,2,3	I,II,III,IV
Chilli	+	+	+	+	+	1,3	I,II,III,IV
Potato	+	+	+	+	+	1,3	I,II,III,IV
Ginger	-	-	-	-	-	-	-
Banana	-	-	-	-	-	-	-
Geranium	+	+	+	-	+	3	II
Rose	-	-	-	-	-	-	-
Soyabean	-	-	-	-	-	-	-
Cucurbit	+	+	+	+	+	1	I
Cucumber	+	+	+	+	+	1	I
Sweet potato	-	-	-	-	-	-	-
Mulberry	-	-	-	-	-	-	-

Notes: + = positive reaction (wilt infected/wilt symptoms developed), - = negative reaction (not infected/no symptoms developed)

Fig.1 Determination of races of *Ralstonia solanacearum* by pathogenicity test in a.Cucurbit, b.Geranium, c.Potato, d.Mulberry, e.Chili, f.Brinjal and g.Tomato plant

In addition to race 2 that causes a wilt of triploid banana (*Musa* spp.) and *Heliconia* spp., while race 3 affects potato and tomato but it is weakly virulent on other solanaceous crops. The results also support the finding of Chandrashekara *et al.,* (2012) studied on 57 isolates and found that the ability to cause wilt in solanaceous and non solanaceous plants were designated as race 1 and biovar 3 on the basis of pathogenicity test and ELISA results. The findings of the present study are also supported by Ahmed *et al.,* (2013) they reported that the isolates of *R. solanacearum* obtained from wilted brinjal plant inducing wilt symptom in tomato, chili and brinjal was belonging to race 1 whereas other groups of *R. solanacearum* isolates causing bacterial wilt of potato collected from three selected growing areas Bangladesh was belong to race 3. The present results are in agreement with the results of Antony *et al.,* (2015) that the strains of *R. solanacearum* inoculated from bacterial wilt of brinjal crop from Tamil Nadu, Southern India was identified as race I biovar after the pathogenicity test. Similarly, Anitha *et al.,* (2018) reported that the strains of *R. solanacearum* causing bacterial wilt of brinjal were confirmed to belong to race 1 and biovar III based on symptom production on different hosts and hypersensitive response on tobacco plants. In the present investigation, the *Ralstonia solanacearum* bacteria causing bacterial wilt in brinjal can be included in race I followed by race III based on the findings of the previous workers.

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