Original Research Article

Races and biovar characterization of *Ralstonia solanacearum* causing brown rot disease of Potato

Abstract

Ralstonia solanacearum, the causative agent of brown rot/bacterial wilt, is a major cause of severe potato illnesses in many impoverished nations located in tropical and subtropical regions of the world. When it occurs, the disease is to blame for significant losses to the potato industry. The illness has the potential to completely destroy a crop and stop using a piece of land for potato cultivation for a number of years. A survey was carried out to investigate the extent of *Ralstonia solanacearum*-caused bacterial wilt of potato crops in important potato-growing talukas in the Banaskantha district of Gujarat, India, including Deesa, Dantiwada, Palanpur, and Amirgadh. The frequency of bacterial wilt disease in potatoes is rather low in this region. Four talukas were utilised to identify ten isolates (RsSt1 to RsSt10) of *R. solanacearum*, which were then used to determine the biovar and race. Gram-negative rod-shaped cells were present in the isolates of *R. solanacearum*. On TZC agar medium, these isolates developed colonies that were creamy or dull white in colour with a hint of pink or red in the centre. The oxidation of disaccharides (sucrose, lactose, maltose) and sugar alcohols (manitol, sorbitol, and dulcitol) by *R. solanacearum* isolates was used to determine the biovar characteristics. This revealed that, of the ten isolates, eight were classified as bv2, and the two remaining isolates (RsSt5 and RsSt7) belonged to bv2T. The pathogenicity test conducted on tobacco, tomato, and brinjal allowed for the race identification of *R. solanacearum* isolates, which were classified as race 3 due to their limited host range and ability to exclusively elicit wilt symptoms in potatoes and tomatoes. As a result, Races 3 and bv2 and bv2T were represented among the *R. solanacearum* isolates that caused bacterial wilt of potatoes in the Banaskantha district.

Key words: Brown rot/Bacterial wilt, *Ralstonia solanacearum*, Biovar, Races, Potato

Introduction

Ralstonia solanacearum is a chemoorganotroph, rod-shaped, strictly aerobic, Gram-negative bacterium that ranges in size from 0.8 to 2.0 μm [1-2]. One of the world's most devastating plant infections in tropical, subtropical, and mild temperate climates is *R. solanacearum*, which has been documented from six of the seven continents (Antarctica excepted). It affects around 450 plant species spread over 54 botanical families, including potatoes, and has a wide host range [3].

In the past, *R. solanacearum* strains were divided into six biovars and five races according to their varying capacities to generate acid from a panel of carbohydrates and host range, respectively. The geographic ranges and host ranges of the five races of *R. solanacearum* vary. Race 1 is an ill-defined group that is native to Asia, Africa, and South America in addition to the southern United States. Its host range is very broad. Race 2 affects bananas and is mainly found in Southeast Asia and Central America. Race 3 is found all over the world and is mostly connected to potatoes. In much of Asia and Hawaii, race 4 affects ginger, and race 5 affects mulberries in China [4].

The brown rot/bacterial wilt disease that affects solanaceous crops is endemic in parts of India's west coast, ranging from Thiruvananthpuram in Kerala to Khera in Gujarat, Karnataka, western Maharashtra, Madhya Pradesh, and Uttarakhand, as well as in the eastern plains of Assam, Odisha, and West Bengal, the Chhota Nagpur plateau, the Andaman and Nicobar Islands, and more. In Gujarat, the land area is more than half used for agriculture, accounting for the state's agricultural industry. The primary driver of employment and development in rural areas is agriculture. As per 3rd

advance estimate of DA&FW (2021-22), Gujarat stands fourth in the total potato production in India with 3.70 million tonnes of potatoes from 0.13 million ha area with average productivity of 28.46 t/ha. In Gujarat out of their total 33 districts Banaskantha has become one of the leading district in potato production. As per final forecast reports of AFW&CD, Govt. of Gujarat (2020-21), Banaskantha produces around 1.85 million tonnes of potatoes from 0.06 million ha area with average productivity of 30.84 t/ha.

Potato (Solanum tuberosum L.), a member of the Solanaceae family, is a vitally important starchy food crop around the world, popularly known as the "Poor Man's Friend" or "King of Vegetables" because of its high protein content and digestible calories. Around one billion people worldwide eat potatoes (FAO, 2008). Compared to cereals like rice and wheat, it produces more dry matter and edible energy in a shorter amount of time because it is a short-duration crop. As a superior vegetable and food crop, it can be found in over 100 different culinary forms. Potato protein is considered to be better than milk protein and has a higher biological value than cereal protein. Potatoes thereby reduce energy consumption and food expenses, making them an excellent addition to meat and dairy products. It is now a common meal for the masses to have for breakfast, lunch, and dinner. The cultivar, growth environment, and fertilisation schedule all affect the potato's makeup [5].

Methodology

Examining and gathering wilted potato plants

A survey was conducted to determine the extent of potato bacterial wilt in Banaskantha district of Gujarat in terms of per cent incidence in some selected talukas/tehsil viz, Deesa, Dantiwada, Palanpur and Amirgadh during winter 2021. To determine the percentage of bacterial wilt occurrence, at least five villages in each taluka and five farmer's fields from each village were surveyed. For a prompt field diagnosis, the streaming of milky white masses of bacterial cells (ooze) indicated the disease is bacterial wilt caused by *R. solanacearum*. Every examined hamlet provided at least ten samples of sick plants, which were then taken to the departmental laboratory to isolate several *R. solanacearum* isolates.

Evaluation of the percentage of disease occurrence

The percentage of the disease that is affected by potato bacterial wilt was surveyed. For every village and taluka, data on the incidence of wilt was collected at least three times from five different farmer's fields. Next, the wilt incidence as a percentage was determined using the following formula:

$$\% \text{ Wilt incidence} = \frac{\text{Number of wilted plants in each field}}{\text{Total number of plants in each field}} \times 100$$

Sterilization of samples and isolation of Ralstonia solanacearum

Samples of wilted potato plants were taken from the farmer's fields, and they were carefully cleaned using running tap water for 10-15 minutes to remove sand and soil particles. After five minutes of washing in 0.1% mercuric chloride (HgCl2) and rinsing in sterile distilled water, these plant pieces were surface sterilised. The plant components were then treated with 70% ethanol for two to three minutes, and then they were cleaned with sterile distilled water.

Using a flame-sterilized scalpel, the cut ends of the surface-sterilized segments were removed and put on petriplates containing triphenyl tetrazolium chloride (TZC). For 48–72 hours, the petriplates were incubated at 28±1°C to allow the bacteria to grow on the medium. After being selected, the lone colony of bacteria with a fluid, asymmetric, creamy white centre and a pink centre was placed onto the Nutrient Agar slants. For later usage, the cultures were kept on the Nutrient Agar slant and kept in storage at 4^{o}C.

Identifying the biovar

The ability of each Ralstonia solanacearum isolate to utilise the disaccharides (sucrose, lactose, maltose) and sugar alcohols (manitol, sorbitol, and dulcitol) generated by the HicarbohydrateTM kit (KB009, HiMedia Laboratories, Mumbai) (Table 1) in accordance with standard protocol was used to separate the biovars [6-7].

Table 1. Carbohydrates Utilization patterns of *R. solanacearum*.

Utilization of	Biovar of *R. solanacearum*					
	1	2	2T	3	4	5
Mannitol	-	-	-	+	+	+
Lactose	-	+	+	+	-	+
Dextrose	+	+	+	+	+	+
Trehalose	+	-	+	+	+	+
Maltose	-	+	+	+	-	+
D (+) Cellobiose	-	+	+	+	-	+
Dulcitol	-	-	-	+	+	-
Sorbitol	-	-	-	+	+	-

Races characterization

According to [8], the races of *R. solanacearum* isolated from potatoes were identified using the differential host of solanaceous crops, including tobacco, tomato, and brinjal. According to [9], the plants were grown in a mist house, and 48-hour-old *R. solanacearum* cultures containing 10^8 cfu/ml were injected into one-month-old tomato, brinjal, and tobacco plants (stem, leaf infiltration, and stem inoculation). The wilting of tomato, brinjal and tobacco plants was observed at regular interval.

Results and Discussion

Disease incidence of brown rot/bacterial wilt

A survey was conducted in four important potato-growing talukas in the Banaskantha district of Gujarat to determine the prevalence rate of bacterial wilt in potatoes. On the other hand, mild cases of bacterial wilt infection were observed in Deesa, Dantiwada, Palanpur, and Amirgadh talukas. The survey results showed that the largest wilt incidence was reported in Deesa taluka, i.e., 3-5 per cent, followed by Dantiwada, Palanpur taluka (1-5 per cent) and lowest bacterial wilt of potato incidence detected in potato fields of Amirgadh taluka (0-3 per cent). In this study, the data on wilt disease incidence was recorded from those fields having wilt incidence. It was also noted that some fields are totally free from the wilt disease were not considered in this study. Furthermore, the diversity of *R. solanacearum* isolates and the differences in soil characteristics present in the various locations investigated may be to blame for these variances in the incidence of bacterial wilt disease. The large diversity of host plants affected by this pathogen, the phenotype and genotype of *Ralstonia solanacearum*, its extensive geographical distribution, and the range of environmental circumstances conducive to bacterial wilt have all been linked to variations in wilt incidence in potatoes [10–13].

Isolation of plant pathogenic Ralstonia solanacearum

From the samples of wilted potato plants, a total of ten isolates of *R. solanacearum* was isolated from different locations: five from Deesa, two from Dantiwada, two from Palanpur, and one from the taluka of Amirgadh. These isolates were retained and marked sequentially as RsSt1 to RsSt10. The failure to isolate the bacteria from every affected plant resulted in a different number of isolates even though the same number of samples were taken from each study location. After being incubated at 28^{o}C for 24 to 48 hours, all of the isolates displayed fluidal, irregular, creamy white colonies with pink centres on TZC agar medium petriplates. The bacterial growth results on TZC medium that are reported here are comparable to those that have been reported by [14]. Based on

colony features on TZC media, an isolate's virulence can be ascertained. Avirulent mutant colonies were butyrous, deep red, frequently with a bluish border, whereas virulent wild type colonies are typically big, raised, fluidal, and either totally white or with a pale red centre.

Biovar determination of Ralstonia solanacearum

Using the HicarbohydrateTM kit, all ten *R. solanacearum* isolates were classified into biovars based on the results of oxidised disaccharides (lactose, maltose, and sucrose) and sugar alcohols (manitol, sorbitol, and dulcitol). The well's hue change was an indication of the oxidation reaction. Four of the six biovars known to exist in the world—bv1, bv2, bv2T, and bv3—have been shown to infect potato plants in India [15]. In present study out of ten isolates of *R. solanacearum* eight isolates belonged to bv2; only two isolates (RsSt5 and RsSt7) belonged to bv2T. A recent work [16] described the differentiation of *R. solanacearum* biovars based on their utilisation of carbohydrates. Additionally, they found that biovar II only oxidises disaccharides, biovar I only oxidises hexose alcohols, and biovar IV only oxidises alcohols. Biovar III oxidises both disaccharides and hexose alcohols.

Table 2: Result of carbohydrate utilization of *R. solanacearum* isolates

Utilization of	Isolates of *R. solanacearum*									
	RsSt1	RsSt2	RsSt3	RsSt4	RsSt5	RsSt6	RsSt7	RsSt8	RsSt9	RsSt10
Mannitol	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+	+	+	+	+
Trehalose	-	-	-	-	+	-	+	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+
D (+) Cellobiose	+	+	+	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-

Characterization of races

Ralstonia solanacearum isolates from potatoes were characterised based on their race using pathogenicity tests in a variety of hosts, including tobacco, tomato, brinjal, and potatoes (Kufri pukhraj). Ten Ralstonia solanacearum isolates were able to induce wilt symptoms in tomato and potato plants, but none of the isolates could induce wilt disease in tobacco or brinjal (Table 2 and Fig 1). After one week of inoculation, all isolates were classified as race 3 because they failed to cause the tobacco wilt symptom and merely displayed chlorosis on the inoculated leaf of tobacco plants. Out of the 10 isolates used in the current investigation, all of the *R. solanacearum* isolates belonged to race 3.

Hypersensitivity reaction

After 48 hours of inoculation, all isolates of *R. solanacearum* culture injected on tobacco leaves generate a yellowish discoloration of the infiltrating tobacco tissue, which progressively advances and is classified as race 3 since it is not pathogenic to tobacco.

There are five biovars and five pathogenic races of *R. solanacearum* known [17]. Race-1 affects tobacco, a number of other solanaceous crops, and numerous hosts in other plant families. It is widespread in tropical regions of the world. Like races 2, 4, and 5, it has a high temperature optimum of 35°C [18]. Race-2 is primarily found in South America's tropical regions and targets Heliconia and

bananas. Race-3 affects potatoes, tomatoes, and, on rare occasions, aubergine, capsicum, Pelargonium zonale, and higher altitudes in the tropics, subtropics, and temperate regions. It also affects various solanaceous weeds, including *Solanum nigrum* and *Solanum dulcamara* [19–20].

Table 3. Determination of *Ralstonia solanacearum* races based on host range

Isolates	**Appearance of symptom**					**Biovar**
	Potato	**Tomato**	**Tobacco (Wilt/Necrosis)**	**Tobacco (Chlorosis)**	**Brinjal**	
RsSt1	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt2	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt3	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt4	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt5	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2T
RsSt6	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt7	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2T
RsSt8	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt9	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
RsSt10	Wilting	Wilting	No reaction	Chlorosis	No reaction	bv2
Note : Races 4 and 5, which are solely harmful to mulberries and are not mentioned here, are pathogenic to ginger and a few other hosts [21].						

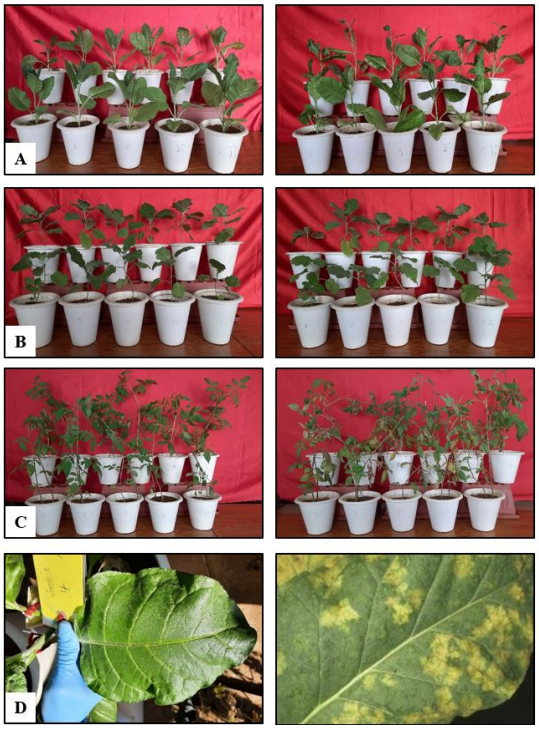

Fig 1:Characterization of races

A- Tobacco plants; B- Brinjal plants; C- Tomato plants; D- Hypersensitivity reaction on tobacco leaves

Conclusion:

The plant pathogen *Ralstonia solanacearum* exist in the soil of Banaskantha district and has potentiality to cause disease in potato and develop characteristic disease symptoms of brown rot/bacterial wilt. The species complex of *R. solanacearum*, the pathogen, and a number of soil variables may be responsible for the variance in the incidence of bacterial wilt in the main potato growing regions. Only all of the potato-growing regions in Gujarat's Banaskantha district had high concentrations of Biovars 2, bv2T, and Race 3 of *R. solanacearum*. The results of this work will be helpful in developing a molecular methods study of *R. solanacearum* population structures with an integrated management focus.

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