https://doi.org/10.1590/1678-4499.20230181

PLANT PROTECTION
Article

Evaluation of virulence among Pakistani isolates of *Ralstonia solanacearum* inducing bacterial wilt in chilies across different agro-ecological zones

Muhammad Naveed Aslam[1], Tariq Mukhtar[2,*]

1. Islamia University of Bahawalpur – Faculty of Agriculture and Environment – Department of Plant Pathology – Bahawalpur, Pakistan.
2. Pir Mehr Ali Shah Arid Agriculture University – Department of Plant Pathology – Rwalpindi, Pakistan.

Received: Aug. 29, 2023 | **Accepted:** Oct. 17, 2023

Section Editor: Gabriel Constantino Blain

***Corresponding author:** drtmukhtar@uaar.edu.pk

How to cite: Aslam, M. N. and Mukhtar T. (2024). Evaluation of virulence among Pakistani isolates of *Ralstonia solanacearum* inducing bacterial wilt in chilies across different agro-ecological zones. Bragantia, 83, e20230181. https://doi.org/10.1590/1678-4499.20230181

ABSTRACT: No information regarding pathogenic variability among different isolates of *Ralstonia solanacearum* infecting chili from different agro-ecological zones of Pakistan with varying climatic and edaphic factors is available. Therefore, in the present study, variations were observed in biovar distribution, hypersensitive response, growth and virulence among 114 isolates of *R. solanacearum* collected from eight agro-ecological zones of Pakistan. Out of 114 *R. solanacearum* isolates, 81% were identified as Biovar III, while the remaining 19% were recognized as Biovar IV. Of all the 114 isolates of *R. solanacearum*, 77% showed positive hypersensitive response and mucoid growth, while 23% isolates gave negative hypersensitive response with non-mucoid growth. Out of 114 isolates of *R. solanacearum* consisting of Biovar III and IV, 22.8% were found avirulent, 25.4% weakly virulent, 29.8% virulent, and the remaining 21.9% were highly virulent. Variations among 114 *R. solanacearum* isolates were also observed in four provinces of the country. Among 92 *R. solanacearum* Biovar III isolates, 21.7% were identified as avirulent, 25% weakly virulent, 34.4% virulent, and 22.8% were highly virulent in the eight agro-ecological zones of the country. Similarly, out of 22 *R. solanacearum* Biovar IV isolates, 27.3% were detected as avirulent, weakly virulent and virulent, while 18.2% isolates were found highly virulent. The isolates having non-mucoid growth and negative hypersensitive response were found avriulent, while those with mucoid growth and positive hypersensitive response were weakly virulent to highly virulent. The information will help design control strategies accordingly and develop resistant cultivars against the bacterium.

Key words: pathogenic virulence, Biovar III, bacterial wilt, hypersensitive response, avirulent.

INTRODUCTION

The solanaceous crops are seriously threatened by bacterial wilt incited by *Ralstonia solanacearum* in the warm temperate, in subtropical and tropical areas of the globe (Hayward 1991). The disease is distributed worldwide, with different magnitudes. Bacterial wilt has caused significant losses in various crops in different countries. In Bangladesh, the disease incidence on aubergine was about 30%. In Ethiopia, the main chili and potato growing areas reported disease incidences of 55 and 25%, respectively, on chili and potato (Bekele et al. 2011). Bacterial wilt also affected banana farms in the Amazon basin of Peru. The disease spread rapidly throughout the Peruvian Jungle, forcing the farmers to destroy their crops (French and Sequeira 1968).

Ralstonia solanacearum has a very wide host range, and more than 450 host plants belonging to 54 botanical families were attacked by the bacterium inflicting huge yield losses (Wicker et al. 2007). The pathogen caused huge damage and losses to tobacco, tomato, and potato crops in Brazil, Columbia, Indonesia, South Africa and the United States of America. In Philippines, average losses of 15% were observed on tomato, 10% on capsicum and aubergine, and 2–5% on tobacco

(Zehr 1969). In India, the disease reduced the yield of potato by 30–70% and of aubergine by up to 65%. In China, peanut production decreased by 30% due to the infection (Sitaramaiah and Sinha 1983). In some cases, the disease wiped out the entire crop of tomato in India. Similarly, bacterial wilt caused extensive losses to potato crops in Greece (Zachos 1957).

The bacterium has also been found widely prevalent in different agro-ecological zones of Pakistan and invaded many crops and vegetables (Aslam et al. 2017a, 2017b, 2019). Geddes (1989) for the first time reported the bacterium from Pakistan. Later surveys revealed an average incidence of about 6% on aubergine, 17% on hot peppers, 11% on potatoes, 22% on sweet pepper, and 13% on tomatoes in Pakistan (Begum et al. 2012; Shahbaz et al. 2015; Aslam and Mukhtar 2023b).

The bacterium has also been known to play a part in disease complexes. When *R. solanacearum* teams up with root-knot nematodes, the combined losses are significantly greater than when each one acts alone, making the plants highly susceptible to bacterial wilt (Ghosh et al. 2016; Asghar et al. 2020; Junaid et al. 2020; Mukhtar and Kayani 2019, 2020; Shahid et al. 2022, 2023; Saeed et al. 2023; Yaseen et al. 2023).

The bacterium has been found as a major hindrance for the lucrative cultivation of solanaceous crops in Pakistan including chili. Among the major chili growing countries, Pakistan is ranked fifth in cultivation and tenth in terms of production in the world (FAO 2012).

In Pakistan, the average per hectare yield of chili (2.54 tons/h) is fairly low than developed countries, which are getting many times higher yields. Among various biotic factors responsible for this low yield, *R. solanacearum* is considered as the major restriction.

Ralstonia solanacearum is ubiquitous in prevalence with highly variable species, which include five races and five biovars (Hayward 1991), and the three major races significantly differ in genetic variability (Cook et al. 1989). Race I is highly wide in host range, whereas race III infects only a small number of hosts mainly potatoes and has been found genetically more divergent in areas where potatoes were originated as compared to other regions of the globe (Smith et al. 1995). The populations of race III, obtained from potato plantations, exhibited high degree of genetic diversity in South America, whereas it comprised of a single major clone in Kenya. The strains of race I showed high diversity in several tropical parts of the world as it was confirmed on solanaceous crops in West Indies (Frey et al. 1996). In Malaysia, its confirmation was made on peanut (Smith et al. 1998) and on several hosts in Australia (Gillings and Fahy 1994).

Being complex in nature, *R. solanacearum* has shown great degree of diversity pathogenically, phenotypically, physiologically, genotypically and also in terms of host range (Genin and Boucher 2004). Pathogenicity and virulence of strains of *R. solanacearum* are governed by several factors like (EPS I) heterogeneous polymer of N-acetylated extracellular polysaccharide I (Orgambide et al. 1991; Denny 1995; McGarvey et al. 1999), swimming motility by means of flagella, type III (T3SS), type II secretion systems (T2SS) (Denny et al. 1990; Van Gijsegem et al. 1995; Liu et al. 2005), type IV pili-driven twitching motility (Tans-Kersten et al. 2001; Kang et al. 2002), and cell wall degrading enzymes (Denny et al. 1990; Liu et al. 2005).

In Pakistan, little research has been conducted on this pathogen (Aslam and Mukhtar 2023b). Information regarding pathogenic variability among various isolates of *R. solanacearum*, infecting chilies collected from eight different agro-ecological zones in Pakistan, each with distinct climatic conditions and edaphic factors, is scarce. Hence, the primary objective of the present study was to assess the pathogenic virulence of 114 isolates of the bacterium across eight different agro-ecological zones. The findings from this research will aid farmers in devising effective control strategies and assist breeders in developing cultivars resistant to the bacterium.

MATERIALS AND METHODS

Description of geographical zones

In toto, 114 isolates of *R. solanacearum* infecting chili were obtained from 14 main chili producing districts of the eight agro-ecological zones located in the four provinces of Pakistan. The country, located between latitude 30°00' N and longitude 70°00' E in the Asian subcontinent, experiences a dry and extreme climate, characterized by scorching summers and harsh winters with limited rainfall. The country's landscape varies from high mountains and valleys in the north to the Pothowar

region, followed by the vast Indus plain, which is 322-km wide and 1,287-km long, sloping at a 1% gradient from north to south. The western parts of the country border low to high mountains, including the Baluchistan plateau. Two sandy deserts, the Thar Desert in the lower region and the Thal Desert in the upper region, are found in the Indus basin. These diverse ecological regions classify Pakistan into distinct agroecological zones with varying climatic conditions and edaphic factors (Aslam and Mukhtar 2023a).

Collection of *Ralstonia solanacearum* isolates

Chili plants with typical symptoms of the disease were dug up cautiously with rhizospheric soil, and brought to the laboratory for further analyses. The bacterial infection with the diseased plants was confirmed serologically (Opina and Miller 2005). All the isolates were coded accordingly.

Isolation of *Ralstonia solanacearum*

The isolation of *R. solanacearum* was made primarily from the infected stems collected from surveyed fields of each district of eight agro-ecological zones. The infected stems from the collar region were cut into 10-cm lengthwise sections, surface sterilized with 70% ethanol, chopped into small pieces and placed in 5-mL sterile distilled water for 5 minutes with continuous shaking in a shaker at room temperature. The bacterial suspension (100 µL) from each sample was streaked separately on the triphenyle tetrazolium chloride (TTC) medium, spread uniformly and incubated at 28°C for 48 h for bacterial growth (Englerbrecht 1994).

Purification and confirmation of *Ralstonia solanacearum*

Pure cultures of the bacterium were procured from a single colony obtained from each bacterial culture by inoculating aseptically onto nutrient agar and TTC media. The individual colonies were again inoculated on semiselective medium from South Africa (SMSA) media amended with bacitracin, cyclohexamide, penicillin, and tripheny tetrazolium chloride (TTC or TZC) medium to keep from any contamination. Further confirmation of the pure cultures of 114 isolates of *R. solanacearum* was done serologically (Opina and Miller 2005) and by their hypersensitivity response (Khan, M. et al. 2023a; Khan, R. A. et al. 2023b; Mehmood et al. 2023).

Hypersensitive response

Serologically confirmed isolates were assessed for their hypersensitive response on *Nicotiana tabacum*. Bacterial culture (10^8 cfu/mL suspension) from each isolate in sterilized distilled water was made and injected into leaf mesophyll of *N. tabacum* plants with the help of sterilized syringe. For positive control, only distilled water was infiltrated. Each leaf of *N. tabacum* was inoculated twice, and, for each isolate, bacterial suspensions were infiltrated in the leaves of three plants by following the same method. Inoculations of tobacco plants were made at 28°C and assessed after 24 and 48 h for their hypersensitive response, i.e., development of necrosis on the leaves of inoculated plants. After confirmation, the isolates were assigned codes accordingly.

Characterization of *Ralstonia solanacearum*

The isolates of the bacterium were further characterized on the basis of morphology, i.e., by their growth patterns (mucoid and non-mucoid growth) and biochemical tests (Atiq et al. 2022; Khurshid et al. 2022) viz. gram reaction, catalase activity, levan production (Schaad 1988; Rahoo et al. 2022), KOH loop test (Suslow et al. 1982), oxidase activity (Kovacs 1956), lipase activity, pigment production (King et al. 1954), arginine dihydrolase reaction (Thornley 1960), gas production (Van den Mooter et al. 1987), oxidation, and fermentation activity (Hayward 1964).

Molecular confirmation

For molecular confirmation, the DNAs from the 114 purified isolates of *R. solanacearum* were isolated, quantified and amplified by using the primer pair JHFegl: 5'GACGATGCATGCCGCTGGTCGC 3' and JHRegl: 5'CACGAACACCACGTTGCTCGCATTGG 3'. The polymerase chain reaction (PCR) products electrophoresed through 1% agarose gel were visualized with ultraviolet light after ethidium bromide staining. All the isolates yielded a 750-bp band that corresponded to *R. solanacearum* (Anwar et al. 2022; Ashraf et al. 2022).

Identification of biovars

The isolates of *R. solanacearum* were categorized into biovars based on their consumption of different sugars (Hayward 1964; He et al. 1983).

Evaluation of virulence among *Ralstonia solanacearum* isolates

Variability among 114 isolates of *R. solanacearum* was studied by hypersensitivity response, growth of the bacterium on medium, and their pathogenicity.

Preparation of inoculum

Each isolate was grown on TTC medium for 24–48 h to get fresh cultures. The pure culture of each isolate was suspended in sterilized distilled water and adjusted to 10^8 cfu/mL through dilution series.

Virulence of *Ralstonia solanacearum* isolates

The virulence of 114 isolates of *R. solanacearum* consisting of Biovar III and IV was assessed on the highly susceptible variety of chili (Aruba) by using the method described by Klement et al. (1990). The experiment was carried out in the glasshouse of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. The seeds of highly susceptible variety of chili, i.e., Aruba, were soaked in water for 24 h for their proper germination. The soaked seeds were sown in germination trays containing sterilized peat mass. The trays were put in the glasshouse at the temperature of 25°C. Three weeks after emergence, the seedlings at 3–4 leaf stage were transferred to polythene bags measuring 12.75 × 10.15 cm containing sterilized soil (sand and compost at the ratio of 3:1:1).

The purified cultures of 114 isolates of *R. solanacearum* were individually prepared and adjusted to 10^8 cfu/mL. Three-week old chili seedlings were then separately inoculated with 50 mL of bacterial suspension from each isolate through soil drenching. One third part of each root system was injured prior to drenching to facilitate penetration of the bacterium. Each isolate was inoculated on four chili plants. Symptoms of bacterial wilt were observed 10 days after inoculation of the pathogen. The association of the bacterium with the symptom development was confirmed by immunostrips (Opina and Miller 2005). The levels of virulence of isolates were categorized by following the scale described in Table 1 (Shahbaz et al. 2015).

Table 1. Pathogenicity scale for virulence assessment.

Scale	Description	Response/Virulence
0	No symptoms	Avirulent
1	Partial symptoms	Weakly virulent
2	Complete wilting	Virulent
3	Plant collapsed and dead	Highly virulent

Source: Shahbaz et al. (2015).

RESULTS

Variability among different isolates of *Ralstonia solanacearum*

In the current findings, variations regarding Biovar distribution, hypersensitive response, growth pattern, and virulence were observed among 114 isolates of *R. solanacearum* procured from different sites of 14 districts located in the eight agro-ecological zones of Pakistan.

Variations in biovar distribution

Among the total 114 isolates of *R. solanacearum*, 81% were confirmed as Biovar III, whereas the rest 19% isolates were identified as Biovar IV. Likewise, Biovar III, which was found dominant, was reported from all the zones of the country. On the contrary, Biovar IV was reported from only four ecological zones.

Variability in hypersensitive response and growth

Out of all the 114 isolates of *R. solanacearum*, 77.2% were found positive in hypersensitive response and showed mucoid growth. On the other hand, 22.8% of the isolates showed negative hypersensitive response and non-mucoid growth.

Virulence of *Ralstonia solanacearum* isolates

Of the total 114 isolates of the bacterium comprising of Biovar III and IV, 23% were identified as non-virulent, and 25% showed weak virulence reaction. On the other hand, 30% of the isolates were found virulent, and the rest of the 21.9% isolates were recognized as highly virulent. Variations amongst 114 isolates of the bacterium were also observed in the four provinces of the country (Table 2). Out of 92 Biovar III isolates of the bacterium, 22 and 25% were found non-virulent and weakly virulent, respectively. Contrarily, 34% of the isolates were confirmed as virulent, and 23% showed highly virulence reaction from all the zones of the country. Likewise, of the 22 biovar IV isolates of the bacterium, 27% each showed non-virulence, weakly virulence, and virulence responses, whereas remaining 18.2% of the isolates were detected as highly virulent. The zone wise virulence of isolates of Biovar III and IV is given in Tables 3 and 4, respectively. The details of avirulent, weakly virulent, virulent and highly virulent isolates in all the zones have been given in Table 5.

Table 2. Province wise pathogenic variability among isolates of *Ralstonia solanacearum* in the country.

Province	Total isolates	A virulent	%age	Weakly virulent	%age	Virulent	%age	Highly virulent	%age
Sindh	47	5	10.6	13	27.7	14	29.8	15	31.9
Punjab	42	13	31.0	8	19.0	13	31.0	8	19.0
Khyber Pukhtunkhwa	13	4	30.8	5	38.4	2	15.4	2	15.4
Balochistan	12	4	33.3	3	25.0	5	41.7	0	0.0
Overall	**114**	**26**	**22.8**	**29**	**25.4**	**34**	**29.8**	**25**	**21.9**

Table 3. Pathogenic variability among isolates of *Ralstonia solanacearum* Biovar III in eight agro-ecological zones of the country.

Agro-ecological zone	Total isolates	Avirulent	%age	Weakly virulent	%age	Virulent	%age	Highly virulent	%age
Barani areas	8	2	25.0	1	13.5	3	37.5	2	25.0
Western dry mountains	12	4	33.0	2	16.6	5	41.6	1	8.3

Continue...

Table 3. Continuation...

Agro-ecological zone	Total isolates	Avirulent	%age	Weakly virulent	%age	Virulent	%age	Highly virulent	%age
Northern irrigated plains	19	5	26.3	4	21.0	6	31.5	4	21.0
Sandy deserts	14	3	21.0	5	35.7	3	21.0	3	21.0
Indus delta	13	1	7.7	3	23.0	5	38.0	4	30.7.0
Southern irrigated plains	13	0	00.0	3	23.0	4	30.7	6	46.0
Suleiman piedmont	6	3	50.0	2	33.0	1	16.6	0	00.0
Wet mountains	7	2	28.5	3	42.8	1	14.2	1	14.2
Overall	**92**	**20**	**21.7**	**23**	**25.0**	**28**	**30.4**	**21**	**22.8**

Table 4. Pathogenic variability among isolates of *Ralstonia solanacearum* Biovar IV in four agro-ecological zones of the country.

Agro-ecological zone	Total isolates	Avirulent	%age	Weakly virulent	%age	Virulent	%age	Highly virulent	%age
Northern irrigated plains	7	3	42.9	2	28.6	1	14.3	1	14.3
Sandy deserts	3	1	33.3	1	33.3	1	33.3	0	0.0
Indus delta	7	1	14.3	2	28.6	3	42.9	1	14.3
Southern irrigated plains	5	1	20.0	1	20.0	1	20.0	2	40.0
Overall	**22**	**6**	**27.3**	**6**	**27.3**	**6**	**27.3**	**4**	**18.2**

Table 5. Details of isolates showing variability among *Ralstonia solanacearum* isolates in different agro ecological zones of Pakistan.

Agro-ecological zone	Avirulent	Weakly virulent	Virulent	Highly virulent
Indus delta	RsTt7, RsBd10	RsTt1, RsTt3, RsTt5, RsBd1, RsBd7	RsTt2, RsTt6, RsTt8, RsTt9, RsBd2, RsBd4, RsBd5, RsBd9	RsTt4, RsTt10, RsBd3, RsBd6, RsBd8
Southern irrigated plain	RsMp1	RsMp3, RsMp8, RsUm2, RsUm5	RsMp2, RsMp4, RsMp9, RmUm1,RsUm8	RsMp5, RsMp6, RsMp7, RsMp10, RsUm3, RsUm4, RsUm6, RsUm7
Sandy deserts	RsBW4,RsBw6, RsBw8, RsSg3	RsBw2, RsSg2, RsSg4, RsSg6, RsSg8, RsSg9	RsBw1, RsBw5, RsBw7, RsSg7	RsBw3, RsSg1, RsSg5
Northern irrigated plain	RsKr1, RsKr3, RsKr6, RsPP1,RsPP7, RsPP8,RsMn1, RsMn4	RsKr4, RsKr9, RsPP3, RsPP5, RsPP9, RsMn8,	RsKr2, RsKr5. RsKr8, RsPP2, RsPP4, RsMn5, RsMn7	RsKr7, RsPP7, RsMn2, RsMn3, RsMn6
Barani areas	RsAK4, RsAK6	RsAK2	RsAk1, RsAk3, RsAk7	RsAk5, RsAk8
Wet mountains	RsNw2, RsNw4	RsNw1, RsNw3, RsNw7	RsNw6	RsNw5
Western dry mountains	RsKK3, RsKK6, RsLi3, RsLi5	RsKK1, RsKK4,	RsKK2, RsLi1, RsLi2, RsLi4, RsLi6	RsKK5
Sulaiman piedmont	RsBn2, RsBn3	RsBn1, RsBn4, RsBn5	RsBn6	-

DISCUSSION

The results reported in the current study showed differences among 114 isolates of the bacterium procured from different ecological zones of the country in their hypersensitive reaction, growth patterns, and virulence. Out of all the 114 isolates of *R. solanacearum*, 77.2% were found positive in hypersensitive response and showed mucoid growth. On the other hand, 22.8% of the isolates showed negative hypersensitive response and non-mucoid growth. The study also

established relationship between the growth and virulence among the bacterial isolates. The isolates showing non-mucoid growth were found non-virulent. On the other hand, the isolates with mucoid growth showed weakly virulence to highly virulence responses. In the same way, isolates with positive hypersensitive responses were found virulent, whereas isolates showing negative hypersensitive responses were non-virulent.

The differences in these parameters can be ascribed to differences in environmental and edaphic factors in various ecological zones of the country. Morphological variability in terms of growth has also been reported by many workers among different isolates of *R. solanacearum*, which corroborated the present findings. Two types of morphological colonies of the bacterium, fluidal or mucoid and afluidal or non-mucoid, can be found on agar media plates (Smith 1920; Kelman 1953; Denny and Hayward 2001; EPPO 2004).

The mucoid substance is produced by the accumulation of an exopolysaccharide (EPS), which causes these mucoid colonies to exhibit a typical irregularity of their surfaces (Smith 1920), often with characteristic whorls in the center. When the conditions become favorable, the bacterial colonies spontaneously pass through a morphological change from fluidal to afluidal, causing a great decrease in disease-inciting capability of these cells (Kelman 1954; Buddenhagen and Kelman 1964; Brumbley and Denny 1990). This process is referred to as phenotypic conversion (Denny et al. 1994; Poussier et al. 2003) and happens in most of the *R. solanacearum* strains (Kelman 1954). Such PC-type variants can be seen on agar media plates without difficulty by prolonged culture (Kelman 1954; Buddenhagen and Kelman 1964) and by growing the bacterium in a non-aerated liquid medium supplemented with glucose and an organic source of N (Kelman and Hruschka 1973). Mainly, the virulent strains of *R. solanacearum* have been found non-motile and non-flagellated, whereas non-virulent strains generally have 1–4 polar flagella and are very motile (Kelman and Hruschka 1973).

It is well documented that all the virulent strains (mucoid colonies) of *R. solanacearum* yield EPS (Kelman 1954; Buddenhagen and Kelman 1964; Boucher et al. 1992; Poussier et al. 2003), whereas EPS-deficient mutants (non-mucoid colonies) have been found non-virulent. The bacterial EPS seems to be much diversified because of its variable composition among strains (Drigues et al. 1985). Within plants, EPS might function by blocking the vascular tissues (xylem vessels), by direct intervention in the normal movement of the fluid of the plant, or by destroying the vessels as a result of hydrostatic overpressure (Schell 2000).

Contrariwise, colonization of the stem by the bacterium is also favored by EPS I, as EPS I-deficient mutants have been reported to reproduce at a very slow rate, and grow slowly on the stems of infected plants (Saile et al. 1997; Araud-Razou et al. 1998). Therefore, EPS I would contribute to minimize or avoid the recognition of bacterial surface structures such as pili and/or lipopolysaccharide by plant defense mechanisms (Araud-Razou et al. 1998; Young and Sequeira 1986). Since EPS-deficient mutants can cause infection and reproduce to a certain degree within plants without inciting wilt symptoms, therefore EPS may participate mostly at later stages of the process, regulating disease intensity instead of the infective capability of *R. solanacearum*. In *R. solanacearum*, EPS is considered as the major element in explaining the virulence of the bacterium (Schell 2000; Hikichi et al. 2007).

Pathogenic variability among different isolates might also be due to genetic and physiological differences. Pathogenesis and genetic diversity together do a specific part in host-plant resistance. Morphologically, similar isolates are not necessarily alike hereditarily, there must be certain variations. The varying genetic pattern causes variations in the morphology and pathogenesis, which has been proved by means of several molecular tools (Iqbal and Mukhtar 2014). Pathogenic variability might have been related to the phenomena of host specialization, as it has been observed in phytopathogenic fungus *Macrophomina phaseolina*. Su et al. (2001) reported host specialization in maize on the basis of pathogenic, genetic and physiological variances. Likewise, Cloud and Rupe (1988) studied host specialization in soybean. The mechanism of host specialization is established within a specific host taking a long time. *R. solanacearum* does not have uniform biology, host range and act as complex variants, as it does not behave as single bacterium, that is why it is described into biovars, races, groups, sub-races, and strains.

Differences in the morphology, pathogenicity and all are essential for the bacterium to adapt in a better way in response to diversified environmental conditions. It will also be helpful for host plant resistance, breeding resistant cultivars of various crops and vegetables to bacterial wilt, and designing novel disease management approaches.

AUTHORS' CONTRIBUTION

Conceptualization: Aslam, M. N. and Mukhtar T.; **Methodology:** Aslam, M. N. and Mukhtar T.; **Investigation:** Aslam, M. N.; **Writing – Original Draft:** Aslam, M. N.; **Writing – Review and Editing:** Mukhtar T.; **Funding Acquisition:** Aslam, M. N.; **Supervision:** Mukhtar T.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

FUNDING

Higher Education Commission of Pakistan
Project No. 20-1580/NRPU/R&D/HEC/10

ACKNOWLEDGMENTS

The authors highly acknowledge the assistance and cooperation rendered by the local farmers in the areas visited.

REFERENCES

Anwar, W., Javed, S., Ahmad, F., Akhter, A., Khan, H. A. A., Kalsoom, R. and Haider, M. S. (2022). *Boeremia exigua* leaf spot: A new emerging threat to *Gossypium hirsutum* L. in Pakistan. Plant Protection, 6, 167-174. https://doi.org/10.33804/pp.006.03.4275

Araud-Razou, I., Vasse, J., Montrozier, H., Etchebar, C. and Trigalet, A. (1998). Detection and visualization of the major acidic exopolysaccharide of *Ralstonia solanacearum* and its role in tomato root infection and vascular colonization. European Journal of Plant Pathology, 104, 795-809. https://doi.org/10.1023/A:1008690712318

Asghar, A., Mukhtar, T., Raja, M. U. and Gulzar, A. (2020). Interaction between *Meloidogyne javanica* and *Ralstonia solanacearum* in chili. Pakistan Journal of Zoology, 52, 1525-1530. https://doi.org/10.17582/journal.pjz/20190501030529

Ashraf, K., Nawaz, M., Yousaf, N. and Afshan, N. (2022). First report of leaf spot of *Chlorophytum comosum* caused by *Thielavia terrestris* from Pakistan. Plant Protection, 6, 247-252. https://doi.org/10.33804/pp.006.03.4313

Aslam, M. N. and Mukhtar, T. (2023a). Characterization of *Ralstonia solanacearum* causing bacterial wilt from major chili growing areas of Pakistan. Bragantia, 82, e20230001. https://doi.org/10.1590/1678-4499.20230001

Aslam, M. N. and Mukhtar, T. (2023b). Distributional spectrum of bacterial wilt of chili incited by *Ralstonia solanacearum* in Pakistan. Bragantia, 82, e20220196. https://doi.org/10.1590/1678-4499-2022-0196

Aslam, M. N., Mukhtar, T., Ashfaq, M. and Hussain, M. A. (2017a). Evaluation of chili germplasm for resistance to bacterial wilt caused by *Ralstonia solanacearum*. Australasian Plant Pathology, 46, 289-292. https://doi.org/10.1007/s13313-017-0491-2

Aslam, M. N., Mukhtar, T., Hussain, M. A. and Raheel, M. (2017b). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. Journal of Plant Diseases and Protection, 124, 585-590. https://doi.org/10.1007/s41348-017-0100-1

Aslam, M. N., Mukhtar, T., Jamil, M. and Nafees, M. (2019). Analysis of aubergine germplasm for resistance sources to bacterial wilt incited by *Ralstonia solanacearum*. Pakistan Journal of Agricultural Sciences, 56, 119-122. https://doi.org/10.21162/PAKJAS/19.6082

Atiq, M., Ashraf, M., Rajput, N. A., Sahi, S. T., Akram, A., Usman, M., Iqbal, S., Nawaz, A., Arif, A. M. and Hasnain, A. (2022). Determination of bactericidal potential of green based silver and zinc nanoparticles against bacterial canker of tomato. Plant Protection, 6, 193-199. https://doi.org/10.33804/pp.006.03.4318

Begum, N., Haque, M. I., Mukhtar, T., Naqvi, S. M. and Wang, J. F. (2012). Status of bacterial wilt caused by *Ralstonia solanacearum* in Pakistan. Pakistan Journal of Phytopathology, 24, 11-20.

Bekele, B., Hodgetts, J., Tomlinson, J., Boonham, N., Nikolic, P., Swarbrick, P. and Dickinson, M. (2011). Use of a real-time LAMP isothermal assay for detecting 16SrII and 16SrXII phytoplasmas in fruit and weeds of the Ethiopian Rift Valley. Plant Pathology, 60, 345-355. https://doi.org/10.1111/j.1365-3059.2010.02384.x

Boucher, C. A., Gough, C. and Arlat, M. F. (1992). Molecular genetics of pathogenicity determinants of *Pseudomonas solanacearum* with special emphasis on hrp genes. Annual Review of Phytopathology, 30, 443-461. https://doi.org/10.1146/annurev.py.30.090192.002303

Brumbley, S. M. and Denny, T. P. (1990). Cloning of phcA from wild-type *Pseudomonas solanacearum*, a gene that when mutated alters expression of multiple traits that contribute to virulence. Journal of Bacteriology, 172, 5677-5685. https://doi.org/10.1128/jb.172.10.5677-5685.1990

Buddenhagen, I. W. and Kelman, A. (1964). Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology, 2, 203-230. https://doi.org/10.1146/annurev.py.02.090164.001223

Cloud, G. L. and Rupe, J. C. (1988). Preferential host selection of isolates of *Macrophomina phaseolina*. Phytopathology, 78, 1563.

Cook, D., Barlow, E. and Sequeira, L. (1989). Genetic diversity of *Pseudomonas solanacearum*: detection of restriction fragment length polymorphism with DNA that specify virulence and the hypersensitive response. Molecular Plant-Microbe Interactions, 2, 113-121. https://doi.org/10.1094/MPMI-2-113

Denny, T. P. (1995). Involvement of bacterial polysaccharides in plant pathogenesis. Annual Review of Phytopathology, 33, 173-197. https://doi.org/10.1146/annurev.py.33.090195.001133

Denny, T. P. and Hayward, A. C. (2001). Gram–negative bacteria: *Ralstonia*. In N. W. Schaad, J. B. Jones and W. Chun (Ed.). Laboratory guide for identification of plant pathogenic bacteria (p. 151-174). 3rd ed. Saint Paul: American Phytopathological Society Press.

Denny, T. P., Brumbley, S. M., Carney, B. F., Clough, S. J. and Schell, M. A. (1994). Phenotype conversion of *Pseudomonas solanacearum*: its molecular basis and potential function. In A. C. Hayward and G. L. Hartman (Eds.). Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum* (p.137). Wallingford: CAB International.

Denny, T. P., Carney, B. F. and Schell, M. A. (1990). Inactivation of multiple virulence genes reduces the ability of *Pseudomonas solanacearum* to cause wilt symptoms. Molecular Plant-Microbe Interactions, 3, 293-300.

Drigues, P., Demery-Lafforgue, D., Trigalet, A., Dupin, P., Samain, D. and Asselineau, J. (1985). Comparative studies of lipopolysaccharide and exopolysaccharide from a virulent strain of *Pseudomonas solanacearum* and from 3 avirulent mutants. Journal of Bacteriology, 162, 504-509. https://doi.org/10.1128%2Fjb.162.2.504-509.1985

Englerbrecht, M. C. (1994). Modification of a semi–selective medium for the isolation and quantification of *Pseudomonas solanacearum*. ACIAR, Bacterial Wilt Newsletter, 10, 3-5.

[EPPO] Empresa Paranaense de Projetos e Obras. (2004). Diagnostic protocols for regulated pests: *Ralstonia solanacearum*. EPPO Bulletin, 34, 173-178.

[FAO] Food and Agriculture Organization of the United Nations. (2012). The State of Food Insecurity in the World 2004: Monitoring progress towards the World Food Summit and Millennium Development Goals. Rome: FAO.

French, E. R. and Sequeira, L. (1968). Bacterial wilt or moko of plantain in Peru. Fitopatologia, 3, 27-38.

Frey, P., Smith, J. J., Albar, L., Prior, P., Saddler, G. S., Trigalet-Demery, D. and Trigalet, A. (1996). Bacteriocin typing of *Burkholderia* (*Pseudomonas*) *solanacearum* race 1 of the French West Indies and correlation with genomic variation of the pathogen. Applied and Environmental Microbiology, 62, 473-479. https://doi.org/10.1128/aem.62.2.473-479.1996

Geddes, A. M. W. (1989). Potato Atlas of Pakistan: Information of potato production by agro ecological zones. In Pak-Swiss Potato Development Project (Ed.). Reconnaissance survey of potato production and marketing in South Waziristan Agency and D. I. Khan (N. W. F. P.) (p. 76-77). Islamabad: Pakistan Agricultural Research Council.

Genin, S. and Boucher, C. (2004). Lessons learned from the genome analysis of *Ralstonia solanacearum*. Annual Review of Phytopathology, 42, 107-134. https://doi.org/10.1146/annurev.phyto.42.011204.104301

Ghosh, P. P., Dutta, S., Khan, M. R. and Chattopadhyay, A. (2016). Role of *Meloidogyne javanica* on severity of vascular bacterial wilt of eggplant. Indian Phytopathology, 69, 237-241.

Gillings, M. R. and Fahy, P. (1994). Genomic fingerprint: Towards a unified view of *Pseudomonas solanacearum* species complex. In G. L. Hartman and A. C. Hayward (Eds.). Bacterial Wilt: the disease and its causative agent, *Pseudomonas solanacearum* (p. 95-113). Wallingford: CAB International.

Hayward, A. C. (1964). Characteristics of Pseudomonas solanacearum. Journal of Applied Microbiology, 27, 265-277. https://doi.org/10.1111/j.1365-2672.1964.tb04912.x

Hayward, A. C. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology, 29, 67-87. https://doi.org/10.1146/annurev.py.29.090191.000433

He, L. Y., Sequeira, L. and Kelman, A. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Disease, 67, 1357-1361.

Hikichi, Y., Yoshimochi, T., Tsujimoto, S., Shinohara, R., Nakaho, K., Kanda, A. and Ohnishi, K. (2007). Global regulation of pathogenicity mechanism of *Ralstonia solanacearum*. Plant Biotechnology, 24, 149-154. https://doi.org/10.5511/plantbiotechnology.24.149

Iqbal, U. and Mukhtar, T. (2014). Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. The Scientific World Journal, 2014, 950175. https://doi.org/10.1155/2014/950175

Junaid, M., Ahmad, M. and Saifullah (2020). Investigating the impact of root-knot nematode double infection on bacterial wilt of tomato. Pesquisa Agropecuária Brasileira, 9, 1347-1353. https://doi.org/10.19045/bspab.2020.90141

Kang, Y. W., Liu, H. L., Genin, S., Schell, M. A. and Denny, T. P. (2002). *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Molecular Microbiology, 46, 427-437. https://doi.org/10.1046/j.1365-2958.2002.03187.x

Kelman, A. (1953). The bacterial wilt caused by *Pseudomonas solanacearum*. A literary review and bibliography. Technical bulletin of North Carolina Agricultural Experiment Station, 99, 194.

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

Kelman, A. and Hruschka, J. (1973). Role of motility and aerotaxis in selective increase of avirulent bacteria in still broth cultures of *Pseudomonas solanacearum*. Journal of General Microbiology, 76, 177-188. https://doi.org/10.1099/00221287-76-1-177

Khan, M., Ahmad, I., Atiq, M., Asif, M., Rashid, M. H. U., Ahmad, S., Shaheen, H. M. F. and Adil, Z. (2023a). Comparative assessment of various antibiotics for controlling bacterial blight in *Eucalyptus camaldulensis*. Plant Protection, 7, 311-319. https://doi.org/10.33804/pp.007.02.4747

Khan, R. A., Atiq, M., Rajput, N. A., Ahmad, I., Husnain, A., Nawaz, A., Mehtab, M. and Ahmad, W. (2023b). Assessment of phytoextracts and synthetic chemicals for controlling leaf blight of *Syzygium cumini*. Plant Protection, 7, 193-205. https://doi.org/10.33804/pp.007.02.4689

Khurshid, M. A., Mehmood, M. A., Ashfaq, M., Ahmed, M. M., Ahmed, N., Ishtiaq, M., Hameed, A. and Rauf, A. (2022). Characterization of *Bacillus thuringiensis* from cotton fields and its effectiveness against *Spodoptera litura*. Plant Protection, 6, 209-218. https://doi.org/10.33804/pp.006.03.4375

King, E. O., Ward, M. K. and Raney, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory and Clinical Medicine, 44, 301-307.

Klement, Z., Rudolph, K. and Sands, D. C. (1990). Methods in Phytobacteriology. Budapest: Akad Miai Kiad.

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature, 178, 703. https://doi.org/10.1038/178703a0

Liu, H. L., Zhang, S. P., Schell, M. A. and Denny, T. P. (2005). Pyramiding, unmarked deletions in Ralstonia solanacearum shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. Molecular Plant-Microbe Interactions, 18, 1296-1305. https://doi.org/10.1094/mpmi-18-1296

McGarvey, J., Denny, T. P. and Schell, M. A. (1999). Spatial-temporal and quantitative analysis of growth and EPS production by *Ralstonia solanacearum* in resistant and susceptible tomato cultivars. Phytopathology, 89, 1233-1239. https://doi.org/10.1094/phyto.1999.89.12.1233

Mehmood, B., Jamil, M., Shafique, S., Khan, M. R., Zafar, T., Bhatti, R. M., Qayyum, H., Tasneem, K., Younas, M. T. and Bakar, M. A. (2023). Evaluating the biocontrol efficacy of selected botanical extracts against bacterial spot of tomato. Plant Protection, 7, 255-261. https://doi.org/10.33804/pp.007.02.4726

Mukhtar, T. and Kayani, M. Z. (2019). Growth and yield responses of fifteen cucumber cultivars to root-knot nematode (*Meloidogyne incognita*). Acta Scientiarum Polonorum Hortorum Cultus, 18, 45-52. http://dx.doi.org/10.24326/asphc.2019.3.5

Mukhtar, T. and Kayani, M. Z. (2020). Comparison of the damaging effects of Meloidogyne incognita on a resistant and susceptible cultivar of cucumber. Bragantia, 79, 83-93. https://doi.org/10.1590/1678-4499.20190359

Opina, N. L. and Miller, S. A. (2005). Evaluation of immunoassays for detection of *Ralstonia solanacearum*, causal agent of bacterial wilt of tomato and eggplant in the Philippines. Acta Horticulturae, 695, 353-356. https://doi.org/10.17660/ActaHortic.2005.695.43

Orgambide, G., Montrozier, H., Servin, P., Roussel, J., Trigalet-Demery, D. and Trigalet, A. (1991). High heterogeneity of the exopolysaccharides of *Pseudomonas solanacearum* strain GMI 1000 and the complete structure of the major polysaccharide. Journal of Biological Chemistry, 266, 8312-8321.

Poussier, S., Thoquet, P. and Trigalet-Demery, D. (2003). Host plant–dependent phenotypic reversion of *Ralstonia solanacearum* from non–pathogenic to pathogenic forms via alterations in the *phc*A gene. Molecular Microbiology, 49, 991-1003. https://doi.org/10.1046/j.1365-2958.2003.03605.x

Rahoo, A. M., Rahoo, R. K., Saeed, M., Burhan, M. and Keerio, N. (2022). Molecular identification and growth of *Xenorhabdus* and *Photorhabdus* symbionts of entomopathogenic nematodes. Plant Protection, 6, 91-100. https://doi.org/10.33804/pp.006.02.4211

Saeed, M., Mukhtar, T., Ahmed, R., Ahmad, T. and Iqbal, M. A. (2023). Suppression of *Meloidogyne javanica* infection in peach (*Prunus persica* (L.) Batsch) using fungal biocontrol agents. Sustainability, 15, 13833. https://doi.org/10.3390/su151813833

Saile, E., McGarvey, J. A., Schell, M. A. and Denny, T. P. (1997). Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by *Ralstonia solanacearum*. Phytopathology, 87, 1264-1271. https://doi.org/10.1094/phyto.1997.87.12.1264

Schaad, N. W. (1988). Laboratory guide for the identification of plant pathogenic bacteria. Saint Paul: American Phytopathological Society.

Schell, M. A. (2000). Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. Annual Review of Phytopathology, 38, 263-292. https://doi.org/10.1146/annurev.phyto.38.1.263

Shahbaz, M. U., Mukhtar, T., Irfan-ul-Haque, M. and Begum, N. (2015). Biochemical and serological characterization of *Ralstonia solanacearum* associated with chilli seeds from Pakistan. International Journal of Agriculture and Biology, 17, 31-40.

Shahid, M., Gowen, S. R. and Burhan, M. (2022). Studies on the possible role of plant host on the development of root-knot nematode, *Meloidogyne javanica* and *Pasteuria penetrans* as affected by different harvesting dates. Plant Protection, 6, 133-141. https://doi.org/10.33804/pp.006.02.4207

Shahid, M., Gowen, S. R., Burhan, M., Niaz, Z. and Haq, A. (2023). Studies on the efficacy of heterogeneously produced *Pasteuria penetrans* (PP3) isolate over individual Pasteuria isolates in the spore attachment, and pathogenic potential on three *Meloidogyne* species. Plant Protection, 7, 9-16. https://doi.org/10.33804/pp.007.01.4529

Sitaramaiah, K. and Sinha, S. K. (1983). Relative efficacy of some selected antibiotics on bacterial wilt (*Pseudomonas solanacearum* biotype 3) of Brinjal. Indian Journal of Mycology and Plant Pathology, 13, 277-281.

Smith, E. F. (1920). An introduction to the bacterial diseases of plants. United States: W. B. Saunders Company.

Smith, J. J., Kibata, G. N., Murimi, Z. K., Lum, K. Y., Fernandez-Northcote, E., Offord, L. C. and Saddler, G. S. (1998). Biogeographic studies on *Ralstonia solanacearum* race 1 and 3 by genomic fingerprinting. In P. Prior, C. Allen, and J. Elphinstone (Eds.). Bacterial Wilt Disease: Molecular and Ecological Aspects (pp. 50-55). Berlin: Springer-Verlag.

Smith, J. J., Offord, L. C., Holderness, M. and Saddler, G. S. (1995). Genetic diversity of *Burkholderia solanacearum* (synonym *Pseudomonas solanacearum*) race 3 in Kenya. Applied and Environmental Microbiology, 61, 4263-4268. https://doi.org/10.1128/aem.61.12.4263-4268.1995

Su, G., Suh, S. O., Schneider, R. W. and Russin, J. S. (2001). Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology, 91, 120-126. https://doi.org/10.1094/phyto.2001.91.2.120

Suslow, T. V., Schroth, M. N. and Isaka, M. (1982). Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology, 72, 917-918.

Tans-Kersten, J., Huang, H. Y. and Allen, C. (2001). *Ralstonia solanacearum* needs motility for invasive virulence on tomato. Journal of Bacteriology, 183, 3597-3605. https://doi.org/10.1128/jb.183.12.3597-3605.2001

Thornley, M. J. (1960). The differentiation of *Pseuodomonas* from other Gram-negative bacteria on the basis of arginine metabolism. Journal of Applied Bacteriology, 23, 37-52. https://doi.org/10.1111/j.1365-2672.1960.tb00178.x

Van den Mooter, M., Maraite, H., Meiresonne, L., Swings, J., Gillis, M., Kersters, K. and De Ley, J. (1987). Comparison between *Xanthomonas campestris* pv. *manihotis* and *X. campestris* pv. *cassava* by means of phenotypic, protein electrophoretic, DNA hybridization and phytopathological techniques. Journal of General Microbiology, 133, 57-71. https://doi.org/10.1099/00221287-133-1-57

Van Gijsegem, F., Gough, C., Zischek, C., Niqueux, E., Arlat, M. F. and Boucher, C. A. (1995). The hrp locus of *Pseudomonas solanacearum*, which controls the production of a type III secretion system, encodes eight proteins related to components of the bacterial flagellar biogenesis complex. Molecular Microbiology, 15, 1095-1114. https://doi.org/10.1111/j.1365-2958.1995.tb02284.x

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M. and Prior, P. (2007). *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. Applied and Environmental Microbiology, 73, 6790-6801. https://doi.org/10.1128/AEM.00841-07

Yaseen, I., Mukhtar, T., Kim, H. T. and Arshad, B. (2023). Quantification of resistance to *Melidogyne incognita* in okra cultivars using linear and nonlinear analyses of growth parameters and nematode infestations. Bragantia, 82, e20230114. https://doi.org/10.1590/1678-4499.20230114

Young, D. H. and Sequeira, L. (1986). Binding of *Pseudomonas solanacearum* fimbriae to tobacco leaf cell-walls and its inhibition by bacterial extracellular polysaccharides. Physiological and Molecular Plant Pathology, 28, 393-402. https://doi.org/10.1016/S0048-4059(86)80081-9

Zachos, D. G. (1957). The brown rot of potatoes in Greece. Annales de l'Institut Phytopathologique Benaki.

Zehr, E. I. (1969). Studies of the distribution and economic importance of *Pseudomonas solanacearum* EF Smith in certain crops in the Philippines. Philippine Agricultural Scientist, 53, 218-223.