

IDENTIFICATION OF BIOVARS OF *Ralstonia (Pseudomonas) solanacearum* E.F.SMITH IN THE UPCOUNTRY OF SRI LANKA.

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ABSTRACT

Field surveys of the wilt causing bacterium *Ralstonia (Pseudomonas) solanacearum* were carried out at 28 locations in two agro-ecological regions (upcountry wet zone and upcountry intermediate zone) in the upcountry of Sri Lanka to ascertain the biovar-types and their distribution pattern in solanaceous crops such as potato, tomato, brinjal and capsicum. The survey and identification were carried out during the November 1991-January 1993 period. The isolates from plant samples were examined for the biovar-types at the Regional Agricultural Research and Development Centre, Bandarawela.

Three of the four biovars described by Hayward were found in upcountry Sri Lanka. Biovar 3 was isolated from potato in the upcountry wet zone. Biovar 2 was also isolated from potato at Pidurutalagala and Nuwara Eliya where the occurrence of biovar 3 is not a common feature under the cool climate. Biovar 3 was isolated from the potato, tomato, brinjal and capsicum fields in the upcountry intermediate zone. In addition, biovar 4 was isolated from potato at Boralanda area which is situated in the same agroecological region.

KEY WORDS :- Biovar-types, Field survey, Isolation, Oxidize

INTRODUCTION

Bacterial wilt caused by *Ralstonia (Pseudomonas) solanacearum* E.F.Smith affects many crops in tropical and subtropical regions of the world. The pathogen has a wide host range and survives in soil for extended periods (Kelman, 1953; Buddenhagen and Kelman, 1964; Hayward, 1991).

Ralstonia (Pseudomonas) solanacearum isolates have been grouped into five biovars on the basis of the ability to utilize and/or oxidize three hexose alcohols and three disaccharides (Hayward, 1964; He *et al.*, 1983). The biovar classification is a special purpose classification which is primarily of application in the context of

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epidemiology rather than taxonomy (Hayward and Hartman, 1994). Hayward (1964) grouped *Ralstonia (Pseudomonas) solanacearum* into four biovars. Seneviratne (1969) reported the occurrence of biovar 2,3 and 4 in the upcountry of Sri Lanka. Biovar 2 was obtained from potato cultivated above 1890m and in virgin lands in the upcountry wet zone (UCWZ). Biovar 3 was isolated from tomato, potato, capsicum and brinjal in the upcountry intermediate zone (UCIZ). Biovar 4 was found in potatoes grown at Boralanda area (UCIZ). Bandara (1983) reported that biovar 2 isolates from potato grown in the central highlands was delimited to isotherm 16°C. Biovar 3 was found to be the most commonly distributed type throughout the island.

The investigation reported in this paper was primarily concerned with ascertaining whether the biovar types and their distribution had changed over the years throughout the major agricultural areas in upcountry Sri Lanka.

MATERIALS AND METHODS

a) Field Survey:

The surveying and sampling were carried out at 28 locations (Fig. 1) in two different agro-ecological regions in the upcountry during the November 1991-1993 period. Plant samples of solanaceous crops such as potato, tomato, capsicum and brinjal which showed typical symptoms of bacterial wilt were collected from the farmer fields in these locations.

b) Isolation and growth of bacterium

Isolations were made from plants that exhibited typical symptoms of wilt in the field. Infected plant stems were thoroughly washed, surface sterilized and vascular tissues dissected-out. The bacterial ooze from these tissues was allowed to stream into sterile water. The bacterial suspension in sterile water was plated on medium consisting of K_2HPO_4 0.5g; $MgSO_4 \cdot 7H_2O$ 0.25g; Peptone, 5.0g; Sucrose, 20.0g; Agar, 20.0g and distilled water, 1 L. The pH was adjusted to 7.2 with 40% NaOH prior to autoclaving at 121°C for 20 minutes. The isolates were multiplied in a medium consisting of $MgSO_4 \cdot 7H_2O$ 0.3g; K_2HPO_4 2.0g; Yeast extract, 4.0g; Casein Hydrolysate, 8.0g; Sucrose, 10.0g; Agar, 18.0g in 1 L distilled water.

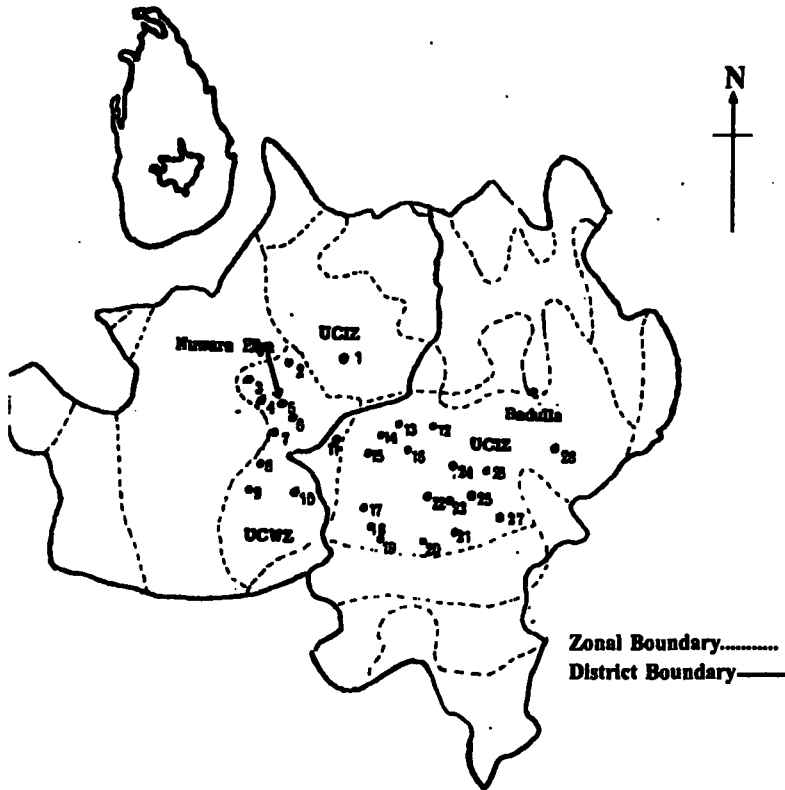


Fig. 1 Sampling locations of bacterial wilt in the upcountry of Sri Lanka

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|--------------------|--------------------|-------------------|
| 1. Ragala | 11. Boragas | 21. Bandarawela |
| 2. Kandapola | 12. Maspenne | 22. Mathatilla |
| 3. Piduruthalagala | 13. Uva Paranagama | 23. Neluwa |
| 4. Udaradella | 14. Ambagasdowa | 24. Attampitiya |
| 5. Nuwara Eliya | 15. Keppetipola | 25. Kumbalwela |
| 6. Sita Eliya | 16. Welimada | 26. Helahalpe |
| 7. Nanu Oya | 17. Boralanda | 27. Liyangahawela |
| 8. Blakpool | 18. Rathkarawwa | 28. Ballaketuwa |
| 9. Meepilimana | 19. Haputalegama | |
| 10. Ambewela | 20. Kahagolla | |

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c) Bio-chemical reaction and observation

The basal medium was made up of $\text{NH}_4\text{H}_2\text{PO}_4$, 10.0g; KCl, 0.2g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2g; Peptone, 1.0g; 1% Bromothymol blue, 0.3ml; Agar (Difco), 18.0g at pH 7.1. The carbon compounds (mannitol, sorbitol, dulcitol, lactose, maltose, cellobiose) were added to the basal medium to obtain a final concentration of 1% (w.v) as described by Hayward (1964) and He *et al.* (1983). Agar slopes were inoculated with one day old cultures of the different isolates and incubated at 34°C for 24hrs and then maintained at room temperature (approximately 24°C) for the remaining period of the observation. Observations were made on the presence or absence of yellow colouration, 14 days after inoculation.

d) Pathogenicity test

All isolates were tested for pathogenicity using triphenyl tetrazolium chloride (TZC) agar medium (peptone, 10g; Casein Hydrolysate, 1g; Glucose, 5g; Agar, 17g) in 1 litre of distilled water. After autoclaving, 5 ml of 1% triphenyl tetrazolium chloride (aqueous solution) was added. Cultural characteristics of colonies produced on TZC-agar medium (Kelman, 1954) have been used in the identification of *Ralstonia (Pseudomonas) solanacearum*. In addition to the above tests, all isolates were tested for pathogenicity by using the susceptible tomato variety 'Marglobe'. The tomato plants were raised in sterilized compost - soil mixture in clay pots (15 cm diameter). A sharp toothpick coated with bacterial slime from TZC-medium (one day old cultures) was forced into the stem in the axil of the second or third expanded leaf below the stem apex. Inoculated plants were placed in the greenhouse (18°C night, 28°C-35°C day) for the development of wilt symptoms. Plants were observed for wilt and yellowing 3-4 days after inoculation.

RESULTS AND DISCUSSION

The location characteristics and the biovar type are summarised in Table 1. Greenhouse pathogenicity tests showed positive symptoms of wilt development on the inoculated tomato seedlings within 3-5 days. In the bio-chemical tests, the development of a yellow colour in the medium indicated the production of acid from the oxidation of carbon sources. Cultures which failed to produce a colour change within this period were observed for two weeks before being discarded.

Table 1. Distribution of biovars of *Ralstonia (Pseudomonas) solanacearum* in upcountry Sri Lanka

Location	Elevation (m)	Agro-climatic zone	Site of collection	Host*	Biovar
Pidurutalagala	2160	UCWZ	Highland	P (15)	3
			Highland	P (04)	2
Uderadella	2130	UCWZ	Highland	P (12)	3
Nanu Oya	1950	UCWZ	Highland	P (19)	3
Meepilimana	1860	UCWZ	Highland	P (22)	3
Nuwara Eliya	1860	UCWZ	Highland	P (40)	3
			Highland	P (04)	2
Sita Eliya	1860	UCWZ	Highland	P (15)	3
Black pool	1800	UCWZ	Highland	P (29)	3
Ambewela	1800	UCWZ	Highland	P (13)	3
Kandapola	1800	UCWZ	Highland	P (18)	3
Ragala	1500	UCIZ	Highland	P (17)	3
Boragas	1350	UCWZ	Highland	P (18)	3
Liyangahawela	1290	UCIZ	Highland	P (09)	3
			Low land	P (05)	3
			Highland	T (12)	3
Boralanda	1260	UCIZ	Highland	P (14)	3
			Low land	P (03)	4
			Low land	T (10)	3
Kappetipola	1260	UCIZ	Highland	P (13)	3
			Low land	T (06)	3
			Low land	C (05)	3
Rathkarawwa	1260	UCIZ	Highland	P (05)	3
			Low land	P (07)	3
			Highland	B (03)	3
			Highland	C (05)	3
			Highland	T (07)	3
Haputalegama	1260	UCIZ	Highland	P (29)	3
			Low land	T (05)	3
			Low land	B (05)	3
Neluwa	1260	UCIZ	Highland	P (10)	3
			Low land	T (08)	3
			Low land	B (05)	3
Uva Paranagama	1230	UCIZ	Highland	P (36)	3
			Low land	P (05)	3
			Low land	T (15)	3

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Location	Elevation (m)	Agro-climatic zone	Site of collection	Host*	Biovar
Maspanna	1230	UCIZ	Highland	P (05)	3
			Highland	P (10)	3
Kahagolla	1200	UCIZ	Highland	P (05)	3
			Low land	P (05)	3
			Low land	T (17)	3
			Low land	B(05)	3
Bandarawela	1950	UCWZ	Highland	P (10)	3
			Low land	P (05)	3
			Low land	T (10)	3
			Low land	B (11)	3
			Low land	C (08)	3
Etampitiya	1860	UCWZ	Highland	P (10)	3
			Highland	T (05)	3
Ambagasdowa	1860	UCWZ	Highland	P (05)	3
			Low land	T (08)	3
			Highland	C (05)	2
Matatilla	1860	UCWZ	Highland	P (10)	3
			Highland	T (15)	3
			Low land	T (10)	3
Welimada	1800	UCWZ	Highland	P (08)	3
			Low land	P (10)	3
			Highland	T (05)	3
			Low land	B (09)	3
Helahalpe	1800	UCWZ	Low land	P (04)	3
			Low land	T (05)	3
			Low land	B (33)	3
			Low land	C (05)	3
Kumbalwela	1800	UCWZ	Low land	B (25)	3
			Low land	T (10)	3
Ballaketuwa	1500	UCIZ	Low land	P (05)	3
			Low land	T (15)	3

* - Hosts : P-Potato; T-Tomato; B-Brinjal; C-Capsicum;

The number of isolates from each host is given within brackets

UCWZ - Upcountry Wet Zone, UCIZ- Upcountry Intermediate Zone

Highland - Rainfed hill regions in the Up country.

Lowland - Rainfed and irrigated flat lands in the Up country

All the isolates from the UCWZ except Pidurutalagala (four potato samples) and Nuwara Eliya (four potato samples), confirmed the presence of biovar 3. Biovar 2 was obtained only from potato at Pidurutalagala and Nuwara Eliya in the UCWZ. Isolates from potato, tomato, brinjal and capsicum from UCIZ were of biovar 3. Biovar 4 was detected from potato isolates (three samples) collected from Boralanda (UCIZ), where Biovar 3 was also isolated.

Race 3/biovar 2 of *Ralstonia (Pseudomonas) solanacearum* is primarily pathogenic to potato and persists only under cool humid conditions while race 1/biovar 3 and 4 have a wide host range and survive in warm areas (Shekhawat *et al.*, 1992). There is considerable evidence to prove the existence of cool temperature adapted strains of biovar-2 in Sri Lankan conditions. (Seneviratne, 1969). Indian literature revealed the occurrence of race 1 and 3 and biovar 3,4 and 2. The isolates from cool humid hilly areas contained race 3/ biovar 2. In other areas like Panchagani hills and Palani hills of Tamil Nadu race 1/biovar 3 was also reported (Shekhawat *et al.*, 1978).

Seneviratne (1969) classified the isolates of the pathogen in Sri Lanka into three biovars using Hayward's (1964) biochemical classification. Biovar 2 was obtained from potato cultivars above 1890m and in virgin lands in the UCWZ; biovar 3 was obtained from tomato and potato in several locations previously cropped with cultivated species in the warmer dry zone; and biovar 4 was found in potatoes grown at Gorandiyatenna (Boralanda area-UCIZ).

According to Bandara (1983), Biovar 2 was isolated from potato grown in the central highlands and it was found to be delimited to isotherm 16°C. Biovar 3 which was pathogenic to all solanaceous crops, was isolated from almost all the sampling sites throughout the island.

In this study, the biovar 3 was found in potato from the UCWZ. But, Seneviratne (1969) had found only biovar 2 in this area. In 1960 and 1970, seed stocks used in potato farms were imported from Holland, Germany and Scotland, where bacterial wilt does not occur. In the UCWZ, solanaceous crops had not been grown before, potato being the first susceptible crop to be cultivated in these fields. Outbreak of bacterial wilt in these cultivations was caused by biovar 2 in every case investigated (Seneviratne 1969). The potato enterprise in Sri Lanka has had a turbulent cultivation

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history. At certain points of time, seed potatoes were brought to Rahangala from Sita Eliya for storage and subsequently transported back to the Nuwara Eliya district. Also, some farmers from UCWZ (e.g. Nuwara Eliya) had brought their seed potato which originated from UCIZ for their cultivation purposes. These facts may be associated with the dispersal of biovar-3 to upcountry wet zone areas as well. Further, Seneviratna (1978) studied the survival of *Pseudomonas solanacearum* biovars 2 and 3. Biovar 3 survived at Sita-Eliya for 24 months when the soil was kept free from other hosts between subsequent plantings of potatoes. Shekhawat *et al.*, (1992) indicated the movement of seed potatoes in India has always been from hills to the plains and never from plains to the hills. The reversal of this process may result in introduction of race 1/ biovar 3 in hills.

Biovars of *Ralstonia (Pseudomonas) solanacearum* which affect potato, tomato, capsicum and brinjal and their distribution pattern are summarized in Table 2. Biovar 3 was the most commonly distributed type in the upcountry. Biovar 2 and 4 were not very common and are found in certain locations in the Nuwara Eliya (UCWZ) and Boaralanda areas (UCIZ), respectively.

Table 2. Biovar types of *Ralstonia (Pseudomonas) solanacearum* and their distribution pattern in the upcountry of Sri Lanka.

Biovar type	Crop	Distribution area
Biovar-1	-	-
Biovar-2	Potato	Nuwara Eliya district (UCWZ)
Biovar-3	Potato, Tomato, Brinjal and Capsicum (Pepper)	Upcountry (UCWZ, UCIZ)
Biovar-4	Potato	Boralanda areas (UCIZ)

CONCLUSIONS

It appears that Biovar 3 is the most commonly distributed biovar-type in the upcountry of Sri Lanka. Sporadic occurrence of Biovar 2 and 4 are also observed in the UCWZ and UCIZ, respectively.

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REFERENCES

- Bandara, J.M.R.S. 1983. Biotype distribution of vascular wilt pathogen *Pseudomonas solanacearum* in Sri Lanka. J. Natl. Sci. Coun. Sri Lanka 11(1): 65-76.
- Buddenhagen, I.W. and A. Kelman, 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2: 203-230.
- Hayward, A.C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 27:265-277.
- Hayward, A.C., H.M. El-Nashaar, U. Nydegger and L. De Lindo. 1990. Variation in nitrate metabolisms in biovars of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 69: 269-280.
- Hayward, A.C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29: 65-87.
- Hayward, A.C. and G.L Hartman. 1994. Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum*. p259. CAB International, Wallingford, Oxon OX10 8DE, UK.
- He, L.Y.,L. Sequeira and A. Kelman. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis. 67: 1357-1361.
- Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*: A literature review and bibliography. p194. North Carolina Agric. Exp. St. Tech. Bull. 99.
- Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathol. 44: 693-705.
- Seneviratne, S.N. de S. 1969. On the occurrence of *Pseudomonas solanacearum* in the hill country of Ceylon. J. Hort. Sci. 44: 393-402.
- Seneviratne, S.N.de. S. 1978. Survival in the soil of *Pseudomonas solanacearum*. pathogens and pests of the potato in the tropics. pp47-55. Proceedings of the second regional symposium on potato production in South East Asia and the Pacific.

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Shekhawat, G.S., R. Singh and V. Kishore. 1978. Distribution of bacterial wilt and races and biotypes of the pathogen in India. J.Indian Potato Assoc. 5: 155-165.

Shekhawat, G.S., S.K Chakrabarti and A.V. Gadewar. 1992. Potato Bacterial Wilt in India. p52 Tech. Bull. 38.