

## ENTRY, MOVEMENT AND INFECTION OF $^{32}\text{P}$ LABELLED *RALSTONIA SOLANACEARUM* (SMITH) YABUUCHI *ET AL.* IN CHILLI (*CAPSICUM ANNUUM* L.)

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**Abstract:** Autoradiography and radioassay techniques were utilized to study entry, movement, growth and multiplication, colonisation and infection of *Ralstonia solanacearum* in chilli. Radioassay of the inoculated resistant and susceptible varieties of chilli revealed that radioactivity counts were more in susceptible variety than in the resistant variety labelled with  $^{32}\text{P}$  at 1 h as well as 24 h of inoculation. This radiotracer technique using  $^{32}\text{P}$  labelled bacteria in chilli is a potential tool in such plant pathological studies.

**Keywords:** Autoradiography,  $^{32}\text{P}$ , radioactivity, radioassay, *Ralstonia solanacearum*

### INTRODUCTION

Chilli production in India is always threatened by bacterial wilt disease caused by *Ralstonia solanacearum*. Only limited information is available on defence mechanism against bacterial wilt of chilli. Radioisotopes are used to study complex dynamic biological system, which includes pathological aspects (Singh, 1989). Use of radioisotopes in bacteria especially *R. solanacearum* to study etiology of infection is novel. The present investigation deals with entry, movement, growth and multiplication, colonization and infection of *R. solanacearum* employing radiotracer techniques in chilli plant system.

### MATERIALS AND METHODS

The bacterial wilt resistant variety Ujwala and the susceptible variety Pusa Jwala were used to determine the pattern of distribution of bacteria in the chilli host tissues. Sixty days old chilli seedlings were used for inoculation. The radionuclide  $^{32}\text{P}$  was procured from the Board of Radiation and Isotope Technology, Bombay. Radioactivity concentration of 44.4 kBq  $\text{ml}^{-1}$  ( $1.2 \mu\text{Ci ml}^{-1}$ ) of  $^{32}\text{P}$  was chosen for plant inoculation since this amount of radiation did not affect growth of *Ralstonia solanacearum*. Virulent strain of *Ralstonia solanacearum* from the pure culture was grown in peptone casamino acid broth.  $^{32}\text{P}$  at 44.4 kBq ml was added to a conical flask containing 50 ml sterilized broth. One ml of 48 hour-old bacterial inoculum was added to the above broth and incubated for 48 hours. This culture was transferred to sterile centrifuge tubes aseptically and centrifuged at 7000 rpm. The clear supernatant was poured off, and the cells were re-suspended in sterile medium and re-centrifuged at 8000 rpm. Again two more

centrifugations were done, at 9000 and 10000 rpm by re-suspending the pellets in sterile medium. Centrifugations were done for 10 min at 30°C. All the above procedures were conducted in aseptic condition.

Plant inoculation was done by inserting root system of chilli seedlings into a bottle containing the above labelled bacteria, after giving root injuries and kept in position by plugging with cotton. The inoculated plants were placed in greenhouse. After the prescribed period of absorption (1h and 24h), the aerial plant parts were detached and arranged on an absorbent paper in their original position. The specimen sandwiched between absorbent sheets were then pressed in herbarium press and allowed to dry under room temperature.

One loopful of inoculum from the glass bottle was taken and streaked on tetrazolium chloride (TZC) medium (Kelman, 1954) to confirm viability and virulence of the bacteria.

Autoradiography of the dried pressed plant samples was done. The x-ray films were exposed for a period of two days in dark. The plant parts were then removed and the film developed using a commercial x-ray film developer solution.

Radioassay of plant samples was also done. Determination of radioactive bacteria ( $^{32}\text{P}$ ) in plant samples was done by Cerenkov counting method (Wahid *et al.*, 1985). The method consisted of wet digestion of dried and finely cut stem and leaves separately with 2 : 1 nitric : perchloric acid mixture ( $\text{HNO}_3 + \text{HClO}_4$ ). Radioactivity was determined in a computer controlled liquid scintillation system. The count rates (cpm) were corrected for background and decay.

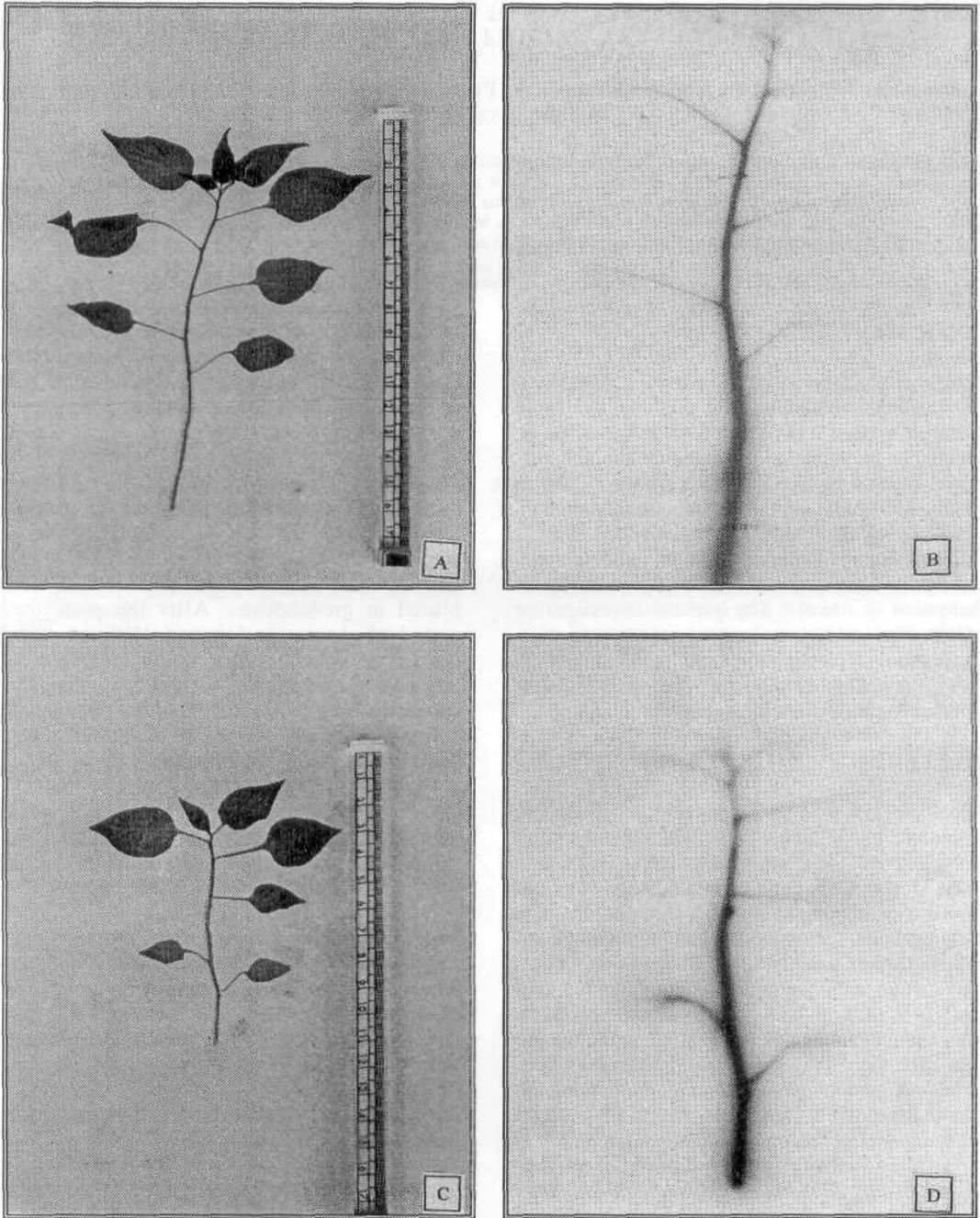


Plate 1. Distribution pattern of  $^{32}\text{P}$  tagged *R. solanacearum* in bacterial wilt resistant and susceptible varieties

- (A) Resistant variety inoculated with  $^{32}\text{P}$  tagged bacteria      (B) Radioautograph of resistant variety  
 (C) Susceptible variety inoculated with  $^{32}\text{P}$  tagged bacteria      (D) Radioautograph of susceptible variety

## RESULTS AND DISCUSSION

The radioactivity of  $^{32}\text{P}$  was taken as evidence for presence of bacteria in a particular tissue. The autoradiographs revealed that the bacteria entered the plant and moved to various tissues. Presence of radioactivity arising from  $^{32}\text{P}$  in stem, leaves and growing points is indicative of this. The autoradiograph revealed no appreciable difference in the extent of distribution of  $^{32}\text{P}$  tagged bacteria in resistant and susceptible varieties (Plates 1).

Table 1. Distribution pattern of  $^{32}\text{P}$  tagged *R. solanacearum* in resistant and susceptible genotypes

Variety	Time interval (h)	Plant parts digested	Radioactive counts (cpm g <sup>-1</sup> )
Ujwala	1	Stem	1347
Ujwala	1	Leaf	168
Ujwala	24	Stem	17259
Ujwala	24	Leaf	2291
Pusa Jwala	1	Stem	3933
Pusa Jwala	1	Leaf	1831
Pusa Jwala	24	Stem	49594
Pusa Jwala	24	Leaf	6644

Accumulation of tagged bacteria was more in the stem. The observations of Warren (1951) that the distribution or rate of movement of bacteria *Erwinia stewartii* within the susceptible or resistant varieties did not differ significantly in the autoradiographs is in line with the present findings. This indicated that even in resistant varieties, the bacteria were widely distributed and resistance does not depend only upon the localization of bacteria for infection.

The radioassay indicated that the resistant and susceptible genotypes differed in the accumulation of radiolabelled bacteria in different plant parts. The radiolabelled bacterial counts were lesser in the bacterial wilt resistant variety Ujwala when compared to the susceptible variety Pusa Jwala in both stem and leaves during 1 h and 24 h of inoculation (Table 1). The highest radioactivity was observed in the stem of Pusa Jwala (49594 cpm g<sup>-1</sup>). In Ujwala it was only 17259 cpm g<sup>-1</sup>. In leaf also, there was difference between resistant

(2291 cpm g<sup>-1</sup>) and susceptible (6644 cpm g<sup>-1</sup>) varieties. The absorbed  $^{32}\text{P}$  labelled bacteria accumulated mainly in the stem portions compared to leaves in both resistant and susceptible varieties.

Absorption time also showed marked difference in resistant and susceptible varieties. In the susceptible variety, after one hour of absorption, the radio labelled bacteria amounted to 3933 cpm g<sup>-1</sup> in stem and 1831 cpm g<sup>-1</sup> in leaves. In resistant variety, it was only 1347 cpm g<sup>-1</sup> and 168 cpm g<sup>-1</sup> respectively.

Radioactivity counts were more in susceptible than in resistant variety at both inoculation periods. This indicated that the variety Ujwala offered some amount of resistance to bacterial entry, mobility and colonization.

Re-isolation attempts were positive and very good growth of bacterium was observed in PCA slants and thus verified the recovery of the bacteria. Results of autoradiograph and radioassay confirmed presence of labelled bacteria in stem and leaves of both the varieties. The study highlighted possibilities of tracing bacterial entry movement and colonization in chilli.

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