

Ralstonia solanacearum Virulence in Cotyledon Stage Tomato Seedlings

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Abstract

Ralstonia solanacearum causes a lethal bacterial wilt in more than 400 plant species that includes several agronomically important crops such as tomato, eggplant, potato, etc. Usually grown up tomato plants are used as model host to study its virulence functions. Recently we have demonstrated that cotyledon stage seedlings of tomato as well as eggplant can be used to study *R. solanacearum* virulence functions. In this review we are summarizing our recent understanding of *R. solanacearum* pathogenicity by using seedlings as model host.

Keywords: *Ralstonia solanacearum*; Bacterial wilt; Plant pathogenic bacteria; Seedlings

Introduction

Bacterial wilt, caused by *Ralstonia solanacearum*, is a threat to many economically important crop plants such as tomato, potato, eggplant, olive, banana, peanut, ginger, etc. [1,2]. Moreover, it can also infect tree plants such as cashew, custard apple, sunflower, eucalyptus etc [3-6]. Owing to its severity, it has been ranked the second most devastating phytopathogenic bacterium in the world [7]. The bacterium is exceptionally versatile as it can survive either as saprophyte in soil for a long period or as a pathogen in susceptible host; also, it can inhabit plants as endophytes without causing disease [2,8]. Thus, the pathogen acquires a tight regulation of its metabolic and pathogenicity factors through two-component sensory and regulatory network maintaining a balance between its various regulators at different niches [8-10].

Much of the virulence functions of this bacterium have been characterized by inoculating in grown up tomato plants [11]. In spite of our much progress in understanding the pathogenomics of this model plant pathogenic bacterium, several interesting facts regarding its pathogenicity could not be revealed using the grown-up tomato plants as model host. Some of the questions are as follows:

- (i) it is well known that *R. solanacearum* is a systemic pathogen that enter the host through the root and colonizes the entire plant before wilting it. It is interesting that the disease symptoms is prominent in the shoot but not in the root.
- (ii) Though the infection starts from the root, wilting symptom initiates at the shoot apical region and then gradually move downwards.
- (iii) The pathogen has a wide host range. How does the pathogen survive inside so many wide varieties of plants?
- (iv) It is a vascular pathogen. The host xylem also houses several other endophytes. Do these endophytes influence the disease caused by *R. solanacearum*.

Cotyledon stage seedling is a promising system to study the virulence of *R. solanacearum*. The advantage of using seedlings is the minimalistic interaction with other micro-organisms. The use of seedlings allows scoring the disease in a more accurate form as a large number of seedlings can be recruited for virulence study. The seedlings of tomato and eggplant can be inoculated by the pathogen either in the leaf [12,13] or in the root [14] to study its pathogenicity. The disease phenotype in seedling is observed within three days after inoculation. The

whole experiment starting from seed germination, inoculation and disease scoring can be completed in two weeks period (Figure 1). The pathogen localization in the host can be studied by appropriate staining of the entire seedling [15]. Recently, bio control against *R. solanacearum* infection has been done using seedlings [16,17].

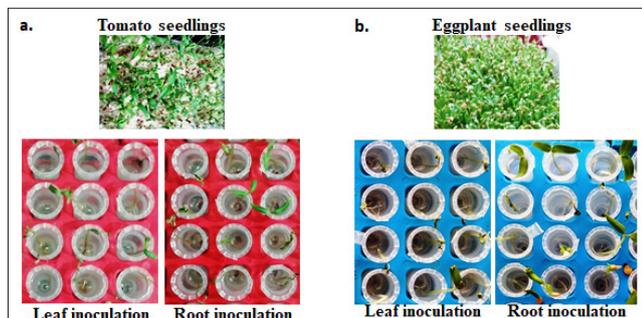


Figure 1: *R. solanacearum* pathogenicity in tomato and eggplant seedlings.

- a) Tomato seedlings is used as a host to study virulence of *R. solanacearum* by both leaf and root inoculations.
- b) Eggplant seedlings used as a host to study virulence of *R. solanacearum* by both leaf and root inoculations.

Discussion

Seedlings as model to study *R. solanacearum* virulence have several unique advantages. First, in both leaf [12] and root inoculation [14] studies, the seedlings are kept in sterile water which ensures minimal interaction with other micro-organisms. This has facilitated to study *R. solanacearum* infection under gnotobiotic condition [12-14,18]. It is evident that *R. solanacearum* infects and cause disease in seedlings without addition of some other bacteria from external sources such as soil. Secondly, the pathogen localization and disease progress in seedlings. Due to small size, the entire seedling could be stained at regular intervals after the infection. By gus staining, it is observed that *R. solanacearum* colonizes the shoot apical region of the seedlings within 24h of inoculation either in root or in the leaf [15]. This provides explanation for two important observations i.e.

- (i) The initiation of the disease from the apical region,
- (ii) Why the disease magnitude is higher by leaf inoculation than by root inoculation [15].

This finding has given a new insight to the disease mechanism. Thirdly, virulence comparison of *R. solanacearum* in different hosts. One of the difficulties to study *R. solanacearum* different hosts is the disease reproducibility. There are instances when the pathogen can live inside susceptible host without causing disease. The reason for this mysterious behavior of the pathogen is not known. This may a feature of the pathogen because of its ability to do systemic infection in the host plant. Considering it, infection in seedlings has a greater chance of disease reproducibility than grown up

plants. It is evident that under the same condition of infection, *R. solanacearum* is more aggressive eggplant seedlings than tomato seedlings. *R. solanacearum* mutants deficient in the same virulence factor exhibit significance difference with regard to their virulence between the two hosts. Apart from the above advantages, the small size and cost efficiency of the seedlings allows to perform large-scale screening of virulence deficient mutants [15].

Conclusion

Seedlings can serve as a potential model for studying virulence of a pathogen in a gnotobiotic condition. The reproducibility, low cost and the short duration for getting results ensure its utility in many host-pathogen interaction studies. It is a promising system to understand the mechanisms underlying the pathogenicity and host adaptability.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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