

Original Research Article

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In-vitro Evaluation of *Arabidopsis thaliana* Ecotypes against *Ralstonia solanacearum* Race4

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ABSTRACT

Keywords

R. solanacearum, race 4, *Arabidopsis thaliana*, ecotype, wilting, Resistant.

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Bacterial wilt caused by the soil-borne bacterium *R. solanacearum* race 4 is a lethal disease of several vegetables and spices in the family Solanaceae and Zingiberaceae respectively. In order to understand the phenotypic response of six *A. thaliana* ecotypes [Landsberg Erecta (Ler) Columbia-0 (Col-0), ABH1, Hc160, Sc1 and SIC] to *R. solanacearum* isolate, CaRs-Mep, different dilution of bacterial suspension were inoculated on roots of plants. The bacterium typically wilted the plantlets in a density dependent manner where the yellowing and wilting was observed within 7 days and 14 days at 10 and 1 OD unit respectively. All ecotypes were highly susceptible to race 4 strain of *R. solanacearum*, except ecotype Ler, where no yellowing and changes in root and shoot length was observed. Ler ecotype showed complete resistant phenotype and hence can be used in resistance programme against the *R. solanacearum* Race 4.

Introduction

Bacterial wilt disease caused by *Ralstonia solanacearum* (Smith, 1896; Yabuuchi *et al.*, 1995) is one of the most important constraints in production of vegetables in the tropical and sub-tropical regions. The bacterial pathogen belonging to β -Proteobacteria is aerobic non-spore forming, Gram-negative, soil borne, motile with a polar flagellar tuft and has broad host range in over 50 plant family with more than 400 reported host plants (Hayward, 2000). The high incidence of plant mortality and lack of effective control methods make *R. solanacearum* as one of the world's most destructive plant pathogens (Prior *et al.*, 1998). The bacterial species are highly

complex with five races, equal number of biovars and four phlotypes. The species divided into 5 different races (race 1-5) based on host range, five biovars (biovar 1-5) based on carbon utilization/oxidation and four phlotypes (phlotype 1-4) based on conserved nucleotide sequences in the intergenic regions of ribosomal DNA. In India, the predominant races responsible for crop loss are race 1 and race 4 with limited occurrence of race 3. While race 1 affects solanaceous vegetables, the race 4 is known to infect several plants in the family Zingiberaceae. Race 4 strains of *R. solanacearum* is known to cause wilting in Zingiberaceae plants such as edible ginger in

many small and marginal farming communities in India and other South East Asian countries who depends on this crop for their livelihood (Sarma and Kumar, 2004). In India, the disease is found in Kerala, Karnataka, Himachal Pradesh, Sikkim, West Bengal, Assam and other North Eastern states. Occurrence of highly aggressive, genetically identical and single virulent lineage of race 4 is found to cause severe wilt in India (Kumar *et al.*, 2014). An integrative approach, which incorporates several methods of control, has been recognized as most successful in curbing disease incidence. Progress towards producing resistant or tolerant plant varieties has been accelerated by the availability of genomic tools in particular, the adoption of *A. thaliana* as a model plant. Various plant pathogens are also virulent on *Arabidopsis*, providing a model to conduct pathogenicity tests (Naidoo, 2008). Many ecotype of *Arabidopsis* are available that shows

different phenotype against same pathogen because of the presence or absence of different resistance gene. To better understand the behaviour of *R. solanacearum* race4 / biovar3, we used six different ecotypes of *A. thaliana* in order to evaluate and understand the behaviour of the model plant to race 4 strain of *R. solanacearum*.

Materials and Methods

Bacterial strain

The bacterium *R. solanacearum* Race 4/ Biovar 3 strain CaRs-Mep isolated from bacterial wilt affected small cardamom plants. The milky bacterial ooze, so obtained, was streaked onto CPG agar amended with 2, 3, 5-triphenyltetrazolium chloride (50 µg ml⁻¹) and incubated at 28°C for two to three days. Strain resistant to rifamycin at 50 µg ml⁻¹ was used in the experiment.

Table.1 Effect of root inoculated *R. solanacearum* race 4 on growth of *Arabidopsis* ecotypes

| Ecotype | Root length (cm) | Number of roots | Shoot Length (cm) | Number of Leaf |
|---------|------------------|-----------------|-------------------|----------------|
| Col-0 | 4.26 | 9 | 2.10 | 7 |
| Mock | 4.86 | 9 | 2.71 | 7 |
| Ler | 4.56 | 14 | 2.4 | 9 |
| Mock | 4.65 | 13 | 2.5 | 9 |
| ABH | 1.75 | 3 | 1.57 | 6 |
| Mock | 2.02 | 4 | 1.8 | 6 |
| SIC1 | 1.8 | 4 | 1.3 | 6 |
| Mock | 2.2 | 5 | 1.5 | 6 |
| Sc1 | .8 | 3 | 1.2 | 6 |
| Mock | 1.2 | 4 | 1.5 | 6 |
| Hc160 | 1.6 | 4 | 1.3 | 7 |
| Mock | 2.1 | 6 | 1.7 | 7 |

@ Bacterial dilution 10⁹ cfu/ml

Fig.1 Phenotypic changes in other ecotypes of *Arabidopsis* due to colonization by *R. solanacearum*

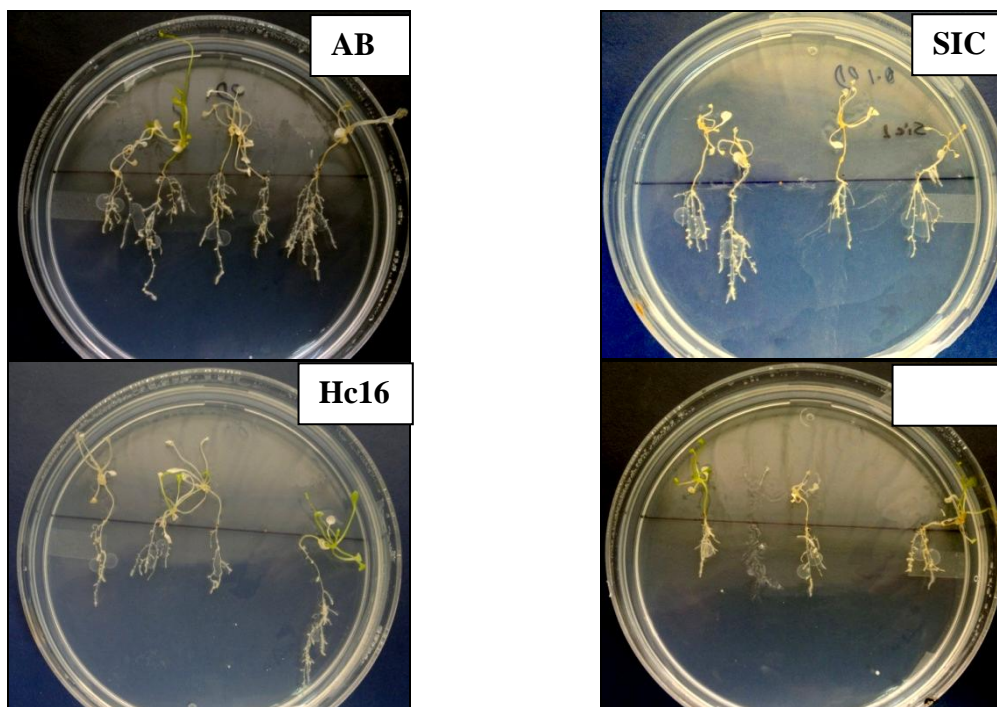
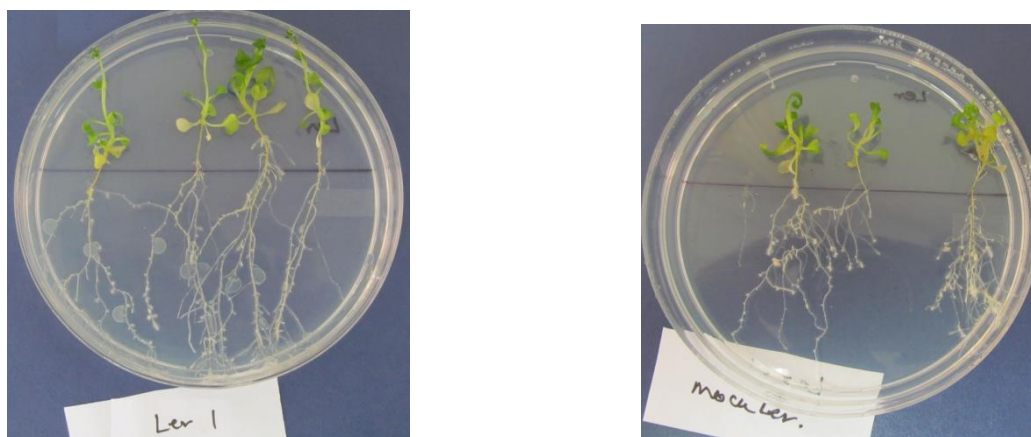


Fig.2 Neither of phenotypic changes in Ler ecotype of *Arabidopsis* is present



Plant Material and growth condition

Six ecotypes of *A. thaliana* namely; Landsberg Erecta (Ler) and Columbia-0 (Col-0), ABH1, Hc160, Sc1 and SIC were used for experimental study. Sterilization of *A. thaliana* seeds was performed by using protocol of Sauer & Burroughs 1986. Sterilized seeds were placed on Murashige

and Skoog medium and different dilution of bacterial suspension (5µl per seed) were spot inoculated on seed and on roots of 21 days old plantlets. The seeds were arranged in line at two third of petri plate and transferred to growth chamber, after 48 hours of cold treatment at 4°C. The Growth conditions were 22/20°C (day/night) temperature, 24 hours light period and 40 % relative humidity.

Inoculum preparation and Inoculation

Ralstonia solanacearum race 4 biovar 3 (CaRs-Mep) were cultured on CPG agar supplemented with rifamycin antibiotic incubated for 72 hours at 28°C. 10^9 and 10^{10} cfu/ml bacterial suspensions were prepared by using serial dilution method. Fourteen days old plants were taken for inoculation and middle of the root was inoculated with 5µl of bacterial suspension per plants. Separate MS plates were used for varying concentration of bacteria.

Results and Discussion

During our study *Arabidopsis* indicated that five ecotypes screened were susceptible to *Ralstonia solanacearum* race 4 and showed a varying degree of infection percentage. The pathogen attacks primarily on the roots. The severity of symptoms was depended on concentration of bacteria inoculated on roots. Different types of symptoms were observed in five susceptible ecotypes; symptom like yellowing, decay, reduced numbers of roots. *R. solanacearum*-inoculated plants had no symptoms by 2 dpi (Days post inoculation), then started to wilt at 3 or 4 dpi and had wilted completely by 7 dpi (Fig 1). Interestingly, Ler ecotype doesn't showed any changes in phenotype for all tested concentrations and even no wilting symptoms were observed at 30 dpi with 10^9 cfu/ml of bacterial suspensions, so we again inoculated the higher dose of bacterial suspension that was 10^{10} cfu/ml, but plants showed no wilting symptoms (Fig 2) indicating that, it is resistant to bacterium *R. solanacearum*. The effect of root inoculated *R. solanacearum* race 4 on growth of *Arabidopsis* ecotypes is shown in Table 1. In earlier reports, Race 1 strains of the bacterium were used and induced changes have been documented in *Arabidopsis* (Hu *et al.*, 2008). There are no reports on *A. thaliana*-*R. solanacearum* race 4 interaction

published or available still. Landsberg erecta ecotype showed completely resistant phenotype as no yellowing was observed and also no changes in root and shoot length colonization irrespective of bacterial concentration as indicated by normal growth behavior of the plantlets even after prolonged incubation. Being an important race for India and other ginger growing regions, it becomes essential to find the resistance source against the race. Hence Ler Ecotype can be used in breeding programmes to engineer durable resistance against bacterial wilt pathogen *Ralstonia solanacearum* Race 4 as there are no reports of resistance source against the bacterial pathogen.

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