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Study on the Response of some Solanaceous Plants to *Ralstonia solanacearum* Biovars 2A and 2T

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A B S T R A C T

Ralstonia solanacearum is a very destructive bacterial plant pathogen that causes wilt disease in solanaceae crops. To study the response of potato, tomato, eggplant and petunia to bacterial wilt disease, two isolates representing biovars 2A and 2T of *R. solanacearum* were evaluated for their pathogenicity aggressiveness and tobacco hypersensitivity response (HR) at two different temperature regimes. The response of plants was estimated by appearance of wilting symptoms and bacterial density in the xylems of inoculated plants over a four weeks period. The results indicated that isolates representing biovar 2T caused less disease in all the species and cultivars compared to isolates biovar 2A, at both temperature conditions and also, there were significant differences in susceptibility to biovars 2A and 2T of *R. solanacearum* among tomato, eggplant and petunia and potato cultivars.

Introduction

Ralstonia solanacerum is a bacterial pathogen that causes wilting and death of several hundred plant species and distributed in more than 50 families (Hayward, 1991). Many economically important crops such as solanaceous plants like potato and tomato affected in most tropical and subtropical and some warm temperate areas (Hayward 1991; Grimault et al., 1994).The disease can also occur in cool temperate areas (French and Martin, 1985; Janse, 1996).The pathogen can be transmitted through soil, contaminated irrigation water, equipment or personnel. It also spreads very easily by transplanting infected plants and propagative materials. Strains of *R. solanacearum* are differentiated into five races according to host range (Buddenhagen et al., 1962; He et al., 1983) and six biovars based on the utilization of three disaccharides (maltose, lactose, and cellobiose) and three hexose alcohols (mannitol, sorbitol, and dulcitol) (Hayward, 1964; Hayward et al., 1992). Although there is no general correlation between races and biovars, strains of biovar 2 almost always belong to race 3. Race 3 biovar 2 strains based on morphological, physiological and biochemical characteristics devided on two biovars 2A and 2T (Hayward et al., 1992). Biovar 2A is the causal bacterial wilt of potato in highlands and cool climates and has a wide distribution (Hayward, 1994; Janse, 1996). Hayward et al. (1992) identified a tropical variant of biovar 2 (N2 or 2T). Biovar 2T is found in tropical lowland like the Brazilian and Peruvian belt of South America, Africa, Japan and Indonesia (Marin and El-Nashaar, 1993; Hayward, 1994) and has also been reported from Iran (Nouri et al., 2006; Irandoust et al., 2008). The purpose of this study was to investigation on the pathological characteristics of *R. solanacearum* biovars 2A and 2T.

Materials and Methods

Bacterial strains and growth conditions

R. solanacearum strains PS3 and AD4 were included in the study. Strain PS3, originally isolated from potato in Isfahan province, identified as biovar 2A and AD4 collected from the potato-growing areas of Khuzestan province, identified as biovar 2T (Nouri et al., 2006). Each bacterial strain was streaked on triphenyl tetrazolium chloride (TZC) medium (Kelman, 1954) and incubated at 28°C for 48 h. For preparation of bacterial suspension, a single colony was suspended in sterile distilled water, and adjusted to OD_{600} nm 0.5 equivalent to 10^6-10^8 colony forming units (CFU ml^{-1}).

Plant material

Potato (cvs. Agria, Arinda, Labida, Sante, Marfona and Ramus), tomato (cv. Early urbana VF), eggplant (cv.

Depressum), geranium (*Pelargonium x hortorum*), petunia (*Petunia hybrida*) and tobacco (*Nicotiana tabacum* cv. White Burley) seedlings were transplanted in plastic pots (8 cm diameter) containing a soil-vermiculite mixture, and grown in a greenhouse under two different temperature conditions, i.e. 25-31°C and 39-45°C. Relative humidity (RH) was 70%.

Hypersensivity reaction test

The ability of strains to induce hypersensivite reaction (HR) on tobacco leaves (*Nicotiana tabacum* cv. White Burley), was tested according to Lozano and Sequeira (1970). The fully expanded leaves were infiltrated by injecting a bacterial suspension into the intercellular spaces with a hypodermic syringe fitted with a fine needle. Reactions were recorded after 8, 12, 24, 48 and 72h.

Pathogenicity tests

For pathogenicity tests, six potato cultivars, tomato, eggplant, geranium and petunia plants at the fourth to fifth true-leaf stage were inoculated by puncturing the basal part of the stem with a needle dipped in bacterial suspension. Four plants of each host were inoculated with each strain and, together with controls inoculated with water, were placed in a greenhouse at 25-31°C and 39-45°C, under natural light conditions and 70% RH. Pots were arranged in a completely randomized way and the onset of wilting symptoms considering the Discharge time of bacterial ooze and disease severity of plants scoring the percentage of wilted leaves with an arbitrary scale where: 0 = no leaves wilted (healthy plant), 1 = 1-25% wilted (tolerant plant), 2 = 26-50% wilted (moderately tolerant plant), 3 = 51-75% wilted (susceptible plant) and 4 = all leaves wilted or dead (highly susceptible) (Swanson *et al*., 2005), were evaluated every second day up till the 28th day post inoculation. The disease progress curves of bacterial biovars in six potato cultivars, tomato, petunia, geranium and eggplant till 28 days post inoculation were obtained and the results of 21 and 28 days post inoculation with biovars 2A and 2T of *R. solanacearum*, were analyzed.

Determination of bacterial density in the xylem

To determine bacterial density in the xylem, a piece of stem 2-3 cm long was cut from the base and placed in a glass container with 9 ml sterile water. Within 18h, 10-fold serial dilutions were made, streaked on TZC plates in triplicate and incubated at 28°C for 48h. Typical colonies of *R. solanacearum* were counted and total CFUs were calculated per gram of fresh matter and were log-transformed (Grimault et al., 1994).

Statistical analysis.

The Statistic Analysis System program (SAS/IML, version 6.10) was used for analysis of variance (ANOVA) followed by LSD test at 5% for mean separation.

Results and Discussion

Hypersensivity reaction

Biovar 2T strains induced a rapid HR on tobacco leaves after 24h and the lesion be-came progressively darker and visible after 48h while biovar 2A strains elicited a yellowish discoloration of infiltrated tissues after 24h and caused slow spreading necrosis after 48h. Tobacco leaves infiltrated with sterile water were unaffected.

Pathogenicity tests

To determine the pathological characteristics of *R. solanacearum* biovars 2A and 2T, bacterial suspension of PS3 (representative strain of biovar 2A) and AD4 (representative strain of biovar 2T) were inoculated to the stems of plants separately and the observations were evaluated up till the 28th day post inoculation. Symptoms usually consisted of wilting of the leaves in potato, tomato, eggplant, geranium and petunia. Infection induced a considerable ooze and decay of the pith surrounding the point of inoculation after which the plants wilted and died.

According to ANOVA, there was significant three-way interaction between biovar, temperature and cultivar on the onset of wilting symptoms and disease severity in 21 and 28 days post inoculation on the susceptibility of test plants to biovars 2A and 2T strains ($P<0.05$). In fact, the onset of wilting symptoms and the disease severity caused by biovars 2A and 2T in potato cultivars under two different cool (25-31°C) and warm (39-45°C) conditions and tomato, eggplant, geranium and petunia plants were significantly different. The pathogenicity experiment showed that potato cultivars were significantly different from each other in susceptibility to biovars 2A and 2T.

Accordingly, in potato cultivars, the disease severity of cv. Sante was scored as 1 (tolerant), cv. Marfona 2 (semi tolerant), cvs Agria and Arinda 3 (susceptible) and cvs Ramus and Labida 4 (very susceptible) to both biovars (Fig. 1). Also, in other plants inoculated with biovar 2A strain, the disease severity was scored as 4 (very susceptible) and in plants inoculated with biovar 2T strain, the disease severity in tomato, geranium and petunia scored as 1 (tolerant) and in eggplant was 2 (semi tolerant) (Fig. 2). As compared with biovar 2T strain, biovar 2A was more pathogenic and aggressive to all hosts at both temperature regimes (Figs. 3) and symptom appeared in a shorter time after inoculation (Figs. 1, 2). In fact, isolates of biovar 2T caused a milder disease in all species and cultivars inoculated, compared with isolates of biovar 2A, at both temperature regimes. Disease development was significantly reduced and started much later in plants inoculated with biovars 2A and 2T under cool conditions (Fig. 1, 3).

Determination of bacterial density in the xylem

According to ANOVA, the effect of bacterial density in xylem on the susceptibility of potato, tomato, eggplant, geranium and petunia plants to biovars 2A and 2T strains was significant ($P<0.05$). The bacterial density in inoculated plants xylem was generally positively correlated with the severity of wilting. On the basis of Grimault et al. (1994) findings, restriction of *R. solanacearum* invasiveness in the vascular tissues of the stem is associated with resistance properties i.e. the more resistant, the lower the stem colonization so the bacterial density in the xylem in all hosts inoculated with biovar 2A strain was higher than biovar 2T at both temperature regimes (Fig. 4).

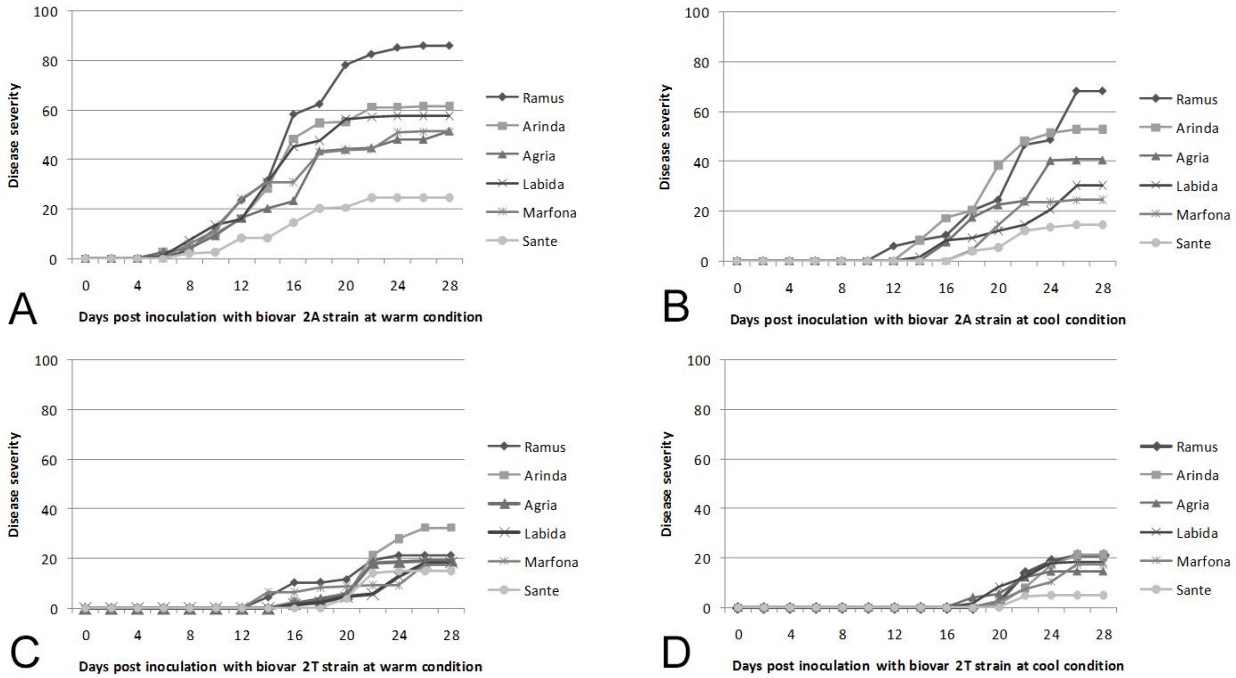

Fig 1 Disease progress of potato cultivars inoculated with biovar 2A and 2T strains at (A,C) warm (39-45°C) and (B,D) cool (25-31°C) conditions, 28 days post inoculation.

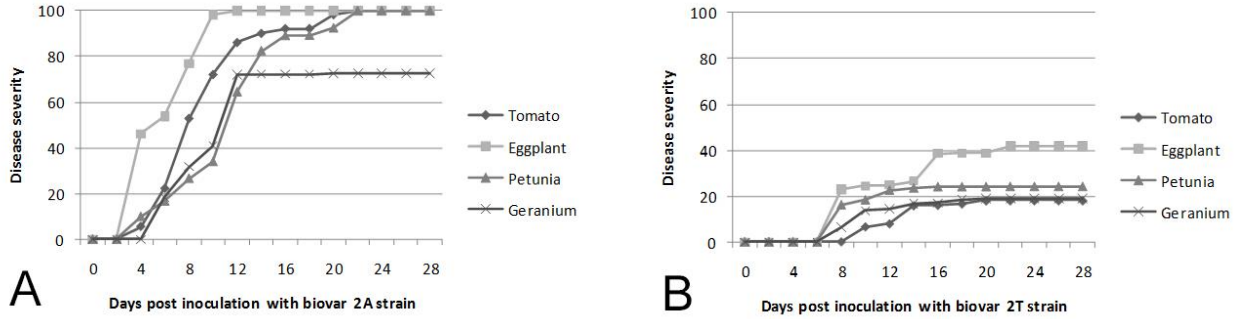

Fig 2 Disease progress of tomato, petunia and geranium inoculated (A) biovar 2A and (B) 2T strains, 28 days post inoculation.

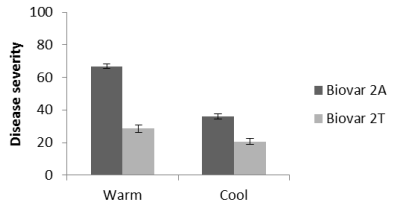

Fig 3 Disease severity of biovars 2A and 2T atwarm (39-45°C) and cool (25-31°C) conditions.

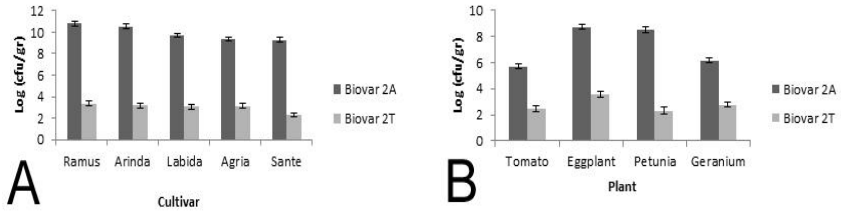

Fig 4 Bacterial density of biovars 2A and 2T in (A) potato cultivars (B) tomato, eggplant, petunia and geranium.

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