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Silicon-induced basal resistance in tomato against *Ralstonia solanacearum* is related to modification of pectic cell wall polysaccharide structure

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Abstract

Bacterial wilt incidence was reduced by 38.1% and 100% in silicon-treated plants of the moderately resistant tomato genotype King Kong 2 and the resistant genotype Hawaii 7998 grown in peat substrate. At 5 days post inoculation the bacterial population was significantly reduced in stems and roots of genotype Hawaii 7998, and in stems of King Kong 2 in silicon-treated plants compared to non-treated plants, indicating a silicon-induced resistance, since silicon accumulated in roots, but not in stems, while a tolerance effect was observed in the susceptible genotype L390. Characterization of possible molecular mechanisms involved in silicon-mediated resistance by immuno-histochemical analysis of stem cell walls indicated silicon-induced changes in the pectic polysaccharide structure. After infection homogalacturonan with non-blockwise degradation of methyl-esters was increased in vessel walls in non-silicon-treated plants, but not in silicon-treated plants, possibly indicating the action of pathogen pectinmethylesterase. Also the staining of vessel walls for arabinogalactan-protein in infected, non-silicon-treated plants was not observed in silicon-treated plants. In inoculated, silicon-treated plants, staining for arabinan side chains of rhamnogalacturonan I (RG I) was increased in some vessel walls, and fluorescence of antibodies for galactan side chains of RG I overall increased in the xylem parenchyma compared to non-silicon-amended plants. These observations suggest an induced basal resistance on cell wall level after silicon treatment, while the yellow or brown autofluorescence occurring in inoculated, non-silicon-treated plants disappeared.

Keywords: Bacterial wilt; Basal resistance; Induced resistance; Pectic polysaccharides; Plant cell wall structure; *Ralstonia solanacearum*; Silicon; Tolerance; Tomato (*Solanum lycopersicum*)

1. Introduction

Bacterial wilt caused by the soilborne, vascular pathogen *Ralstonia solanacearum* [1] is a devastating disease limiting production of tomato as well as a wide range of other crops [2]. The pathogen also attacks hosts without causing symptoms and survives latently in plant tissues, thus contributing to its widespread dispersal and subsequent establishment in different environments worldwide.

Infection of the plant is favored by injured roots at lateral root emergence sites and stem wounds, or it may occur through stomata. Within the plant, the bacteria invade the intercellular space of the root cortex and subsequently colonize the vascular parenchyma. The cell walls are disrupted, facilitating the spread of the bacteria through the vascular system [3]. The typical symptom on tomatoes is a flabby appearance of the youngest leaves usually at the warmest time of the day 5–6 days after inoculation with *R. solanacearum*. Depending on the environmental conditions, wilting of the whole plant may follow rapidly due to reduced sap flow caused by the presence of large amounts of *R. solanacearum* cells and their exopolysaccharide slime in xylem vessels. Plants later collapse and die from further degradation of vessels and surrounding tissues. The bacterium returns to the soil after plant death, shifting between two different physiological states, one adapted for saprophytic survival, the

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phenotypic conversion (PC)-type, and the other for pathogenesis (wild-type) [4]. Another intriguing aspect of *R. solanacearum* biology is the entry into the viable but non-culturable (VBNC) state, in which cells are unable to divide sufficiently on growth medium to yield visible colonies, but they maintain viability [5]. The long-term survival of the bacterium could therefore be due to its ability to enter the dormant-like VBNC state [6]. Because of the complex nature of this pathogen, several management strategies were advocated for the control of the disease with limited success. Chemical control is nearly impossible and antibiotics such as streptomycine, ampicillin, tetracycline and penicillin have hardly any effect [7]. Biological control is still in its research stage [8,9]. Thus, the use of resistant varieties is the simplest and most effective method for controlling the disease. Unfortunately, resistance is broken down by the genetic diversity of the strain as well as local environmental conditions. Therefore, increasing varietal resistance in the framework of an integrated approach may be the most suitable means to eradicate the disease. A beneficial effect of silicon by increasing resistance has been reported only against fungal pathogens and in silicon accumulator plants such as cucumber, oat, rice, wheat, barley and sugarcane [10–12]. In non-accumulator plants, such as tomato, the effect of silicon on a bacterial disease has been investigated so far in a hydroponic culture system [13], although not in detail. To elucidate the influence of silicon on tomato challenged with *R. solanacearum*, phenotypic and immunohistochemical studies were undertaken to follow the development of bacterial wilt in tomato (*Solanum lycopersicum*) grown in substrate amended with silicon and to analyse interactions between silicon, the plant and the pathogen.

2. Materials and methods

2.1. Plant materials and silicon supply

Tomato genotypes L390 and Hawaii 7998, susceptible and resistant to *R. solanacearum*, respectively, received from the Genetic Resources and Seeds Unit of the Asian Vegetable Research and Development Center (AVRDC, Taiwan), and King Kong 2, moderately resistant, from KnownYou Company, Taiwan, were used. Seeds of each genotype were sown in peat substrate (Klasmann, Lithuanian Peat Moss, Germany) kept under greenhouse conditions (20 °C day/night temperature, 14 h of light per day/30K lx, and 70% relative humidity), and transplanted after 3 weeks to individual pots with 300 g of the same substrate. At sowing, silicon-treated plants received Aerosil powder (Degussa, Germany) (pure form of silicon dioxide) at the rate of 1 g/l substrate, and were additionally daily supplied with a nutrient solution amended with monosilicic acid [$Si(OH)_4$]. Five litres of the nutrient solution [2.5 M Ca $(NO_3)_2$, 1 M NH_4NO_3, 2.5 M K_2SO_4, 2.5 M $MgSO_4$, 2.5 M KH_2PO_4, 50 mM H_3BO_3, 0.5 mM $ZnSO_4$, 0.3 mM $CuSO_4$, 5 mM $MnSO_4$, 0.5 mM $MoNaO_4$, 50 mM NaCl and 50 mM FeDTA] were prepared and monosilicic acid was added to achieve a concentration of 1.4 mM $Si(OH)_4$. Non-silicon-treated plants were watered with nutrient solution without silicon and separated from silicon-treated plants. Monosilicic acid was obtained after exchange of potassium silicate solution (K_2SiO_2) with cation exchangers (20 ml volume, Biorad Laboratories, Germany) [14]. The experiments were conducted in a climate chamber with 30/27 °C day/night temperature, 85% relative humidity, 14 h light/day (photonflux of 350 μmol/m^2 s).

2.2. Experimental design

A $2 \times 3 \times 2$ factorial experiment consisting of two levels of silicon (Si), three tomato genotypes and inoculated and non-inoculated treatments was arranged in a completely randomized design with four replications: (i) plants with silicon, inoculated with *R. solanacearum* (+Si, +Rs), (ii) plants without silicon, inoculated with *R. solanacearum* (−Si, +Rs), (iii) plants with silicon, without *R. solanacearum* inoculation (+Si, −Rs), (iv) plants without silicon, without *R. solanacearum* inoculation (−Si, −Rs). Three plants were randomly selected per treatment and per sampling date at 5 and 12 days post inoculation (dpi) for bacterial and silicon quantifications, while 8 plants/treatment were kept for symptom evaluation. Stem and root samples were subdivided into two parts, one for bacterial population assessment and the other for silicon analysis. The experiments were repeated three times under the same conditions.

2.3. Inoculation and bacterial population assessment

A highly virulent strain of *R. solanacearum,* To-udk2, race 1 biovar 3 from Thailand was used to inoculate plants. Inoculum was obtained as follows: the isolate was grown on tetrazolium chloride (TTC) medium [15] [per l: peptone 20 g, casein hydrolysate 1 g, D-glucose 5 g, agar 15 g; pH adjusted to 7.2; 2,3,5-Triphenyl TTC (Sigma, Deisenhofen) as a 0.5% (w/v) solution was filtered separately and 10 ml were added to the cooled agar medium before pouring]. After incubation for 2 days at 30 °C, cells were harvested from agar plates by flooding with sterile, distilled water and adjusted to an optical density of 0.06 at 660 nm wavelength (Spectrotonic 20 Bausch and Lomb) corresponding to about 10^8 colony-forming units per millilitre (CFU/ml). Three-week old plants were transplanted in individual pots and inoculated subsequently by soil drenching with 30 ml of bacterial suspension per pot, corresponding to about 10^7 CFU/g of substrate, around the base of the plants. After inoculation, plants were watered up to the soil field capacity, avoiding water surplus, and kept in a climate chamber. *R. solanacearum* was quantified in the mid-stems (5 cm sections) and roots of selected plants at 5 and 12 dpi. Stem pieces were weighed, surface sterilized with 70% alcohol for 20 s, and washed and macerated in sterile water. The suspension was centrifuged for 20 min at 5000*g* at