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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

room temperature and the pellet was resuspended in 1 ml of sterile demineralized water. Ten-fold serial dilutions were prepared and 100 µl of each dilution was plated with two replicates on TTC medium and incubated for 48 h at 30 °C.

2.4. Symptom evaluation and shoot dry weight

Disease severity was assessed daily using a disease score based on 8 plants/treatment. The evaluation started when the first symptoms were observed on the leaves and was continued until the symptoms became stable. The following scoring was used: 0 = no symptom, 1 = one leaf wilted, 2 = two leaves wilted, 3 = three leaves wilted, 4 = wilting of all leaves without the tip, 5 = wilting of the whole plant (death). Wilt incidence was calculated as the percentage of dead plants at the evaluation date out of the total number of plants in the treatment.

The area under disease progress curve (AUDPC) for each plant in each treatment and experiment was calculated on the basis of disease severity and wilt incidence using the trapezoid integration of the disease progress curve over time with the following formula [16,17]:

$$\mathrm{AUDPC} = \sum[(x_i + x_{i-1})/2](t_i - t_{i-1})$$

where x_i and x_{i-1} are the mean disease severity or wilt incidence at time t_i, corresponding to days after inoculation; t_i and t_{i-1} are consecutive evaluation dates, and $t_i - t_{i-1}$ is equal to 1. The total AUDPC represents the sum of AUDPC for all eight plants in each treatment. The AUDsPC and AUDiPC were calculated for disease severity and disease incidence, respectively. The shoot dry weight including surviving and dead plants was determined 1 month after inoculation for inoculated and non-inoculated plants by drying plant material at 65 °C over 3–5 days.

2.5. Silicon analysis

Total silicon content of stems and roots of the same samples also used for bacterial quantification was determined at 5 and 12 dpi by spectrophotometry, using the method developed by Novozamsky et al. [18], modified by the Institute for Plant Nutrition of the University of Hannover. Plant materials were dried in an oven (Heraeus, Germany) at 65 °C for 3 days and ground in a mill (Janke & Kunkel IKA Labortechnik, Germany). The concentration of silicon in tissue samples was determined by digesting 10 mg of grounded plant materials in 500 µl of the mixture 1 M HCl and 2.3 M HF (1:2) and shaken overnight. After centrifugation of the samples at 10,000*g* for 10 min, 20 µl of the supernatant was retained in Eppendorf tubes and 0.25 ml of 3.2% boric acid (H_3BO_3) was added and shaken overnight. Then 0.25 ml of colour reagents [1:1mixture of 0.08 M H_2SO_4 and 20 g/l (NH_4) $6Mo_7 \cdot 4H_2O$)] was added, and incubated for 30 min, followed by addition of 0.25 ml of 33 g/l tartaric acid and 0.25 ml of 4 g/l ascorbic acid. The sample absorbance was measured at 811 nm in a spectrophotometer (Micro Quant, Biotech, USA).

2.6. Immuno-histochemical analysis of tomato cell walls

Stem samples from the moderately resistant genotype King Kong 2 inoculated and non-inoculated with *R. solanacearum* and amended or not amended with silicon were prepared for immuno-histochemical analysis. Monoclonal, primary antibodies raised against pectic polysaccharide epitopes were LM2 specific for arabinogalactan-proteins (AGPs), LM5 specific for (1→4)-*β*-D-galactan side chains and LM6 specific for (1→6)-*α*-L-arabinan side chains of rhamnogalacturonan I (RG I), and LM7 specific for non-blockwise de-esterified homogalacturonans (HG) (Plant Probes, c/o P. Knox, University of Leeds, UK). Fresh sections of the mid-stems were cut in thin slices of <0.5 mm by free-hand sectioning and fixed in PIPES buffer [50 mM piperazin-*N*, *N*-bis (PIPES); 5 mM $MgSO_4$; 5 mM ethylene glycol bis (EGTA), pH = 6.9] containing 4% paraformaldehyde (MercK, Darmstadt, Germany) and kept at 4 °C overnight. The next day the slices were washed in PBS + Tween 0.1% (phosphate buffered saline) followed by blocking in PBS + milk powder 5% (Fluka, GmbH, Buchs, Switzerland) for 1 h at room temperature. Incubation with the primary antibodies (LM2, LM5, LM6 and LM7) was performed overnight at 4 °C at 1:10 dilution in TBS + 0.05% Tween 20 (tris buffered saline) (Carl Roth GmbH & Co., Karlsruhe, Germany). The slices were washed with PBS + Tween 0.1% 3–5 times for 5 min each followed by dH_2O. Incubation with the secondary antibody anti-rat IgG FITC (fluoroisothiocyanat, green fluorescence) (Sigma-Aldrich, Steinheim, Germany) at 1:100 dilution in TBS + 0.05% Tween 20 was then performed overnight at 4 °C. Subsequently, the slices were washed again with PBS + Tween 0.1% 3–5 times for 5 min each followed by dH_2O. Finally, the sections were mounted in Citifluor (AF1) antifade (Plano, Wetzlar, Germany) on glass slides and observed under a photomicroscope (Axioskop 2 plus, Carl Zeiss, Göttingen, Germany) equipped with epifluorescence illumination with a filter system appropriate for FITC (fluorescein florescence excitation: 450–490 nm, beamsplitter: 500 nm, emission: 510–576 nm) (Carl, Zeiss, Göttingen, Germany). Control samples were mid-stem sections inoculated or not treated with *R. solanacearum* observed with addition of Citifluor only, and samples treated similarly as described above, but with secondary antibody only, omitting the primary antibody, to determine unspecific fluorescence [19].

2.7. Data analysis

Data were analysed using the statistical analysis system (SAS for windows, 1999−2001, SAS Institute, Carry, USA) for analysis of variance (ANOVA) followed by means separation at 5% (Tukey test). Data of bacterial numbers were log-transformed.

3. Results

3.1. Symptom and bacterial population development

Bacterial wilt symptom severity development was significantly reduced in silicon-treated (+Si) plants of genotypes King Kong 2 and Hawaii 7998 compared to plants without silicon (−Si) donation grown in peat substrate (Fig. 1A). In genotype L390, disease development was delayed by about 1 day in +Si plants, but reached a similar level as in non-treated plants at 16 dpi. Evaluating disease incidence, no dead plant was observed in genotype Hawaii 7998 amended with silicon up to 20 dpi compared to 33.3% plant death in non-silicon-amended plants (Fig. 1B). However, 88.3% plants died in L390 amended with silicon compared to 95.8% in non-amended plants, while in King Kong 2 the final disease incidence was reduced by 38% and in Hawaii 7998 by 100%. The area under the disease severity progress curve (AUDsPC) in King Kong 2 and Hawaii 7998 were reduced by 33.8% and 81.2%, respectively, in silicon treatments, while the reduction by 11.4% in L390 was not significant (Table 1). The AUDiPC of disease incidence was reduced by 57.3% and 100% in King Kong 2 and Hawaii 7998, respectively, and, non-significantly, by 13% in L390 by silicon treatment.

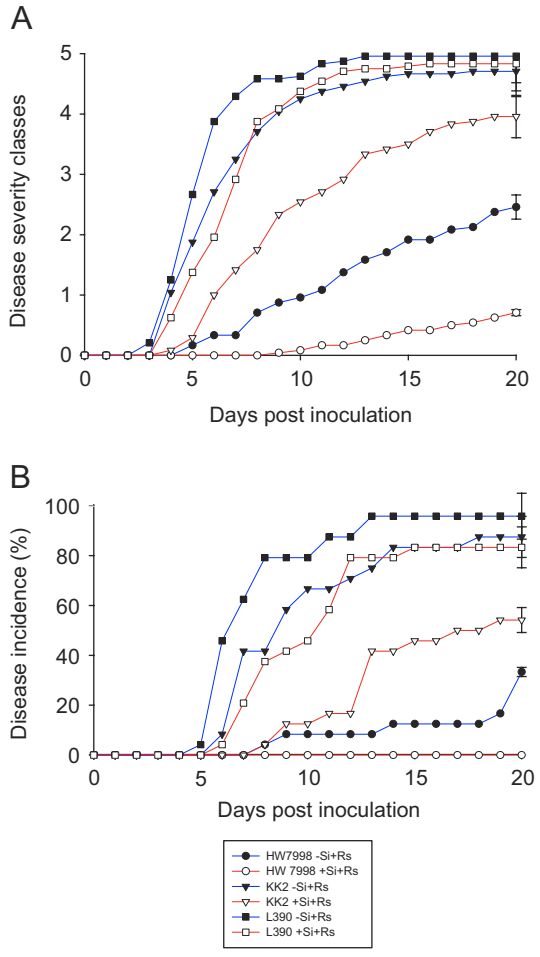

Fig. 1. Bacterial wilt symptom development expressed as disease severity (A) and wilt incidence (B) on tomato genotypes L390 (susceptible), King Kong 2 (moderately resistant) and Hawaii 7998 (resistant) over 20 days after inoculation. No further change occurred after 20 days. Data from three repeated trials are presented. Disease severity is defined as the average of disease classes of all plants of the treatment at the given assessment date. Wilt incidence is calculated as the number of dead plants out of the total number of plants in the treatment.

Silicon amendment significantly reduced bacterial populations in mid-stems of genotypes King Kong 2 and Hawaii 7998 by 46.2% and 37%, respectively, at 5 dpi, and by 33.5% and 37.3%, respectively, at 12 dpi (Table 2), while in root organs, bacterial numbers were reduced only in the silicon-amended Hawaii 7998 by 27.6% and 35.6% at 5 and 12 dpi, respectively. In contrast, no significant difference was found in treatments of genotype L390, although a slightly lower bacterial population was recorded in both roots and stems treated with silicon at 5 dpi. Comparing populations in stems and roots, significantly reduced bacterial numbers were found in stems compared to roots of silicon-treated plants of genotypes Hawaii 7998 by 39% and 17.9% and King Kong 2 by 32.9% and 32.7% at 5 and 12 dpi, respectively. This difference was also observed in silicon non-amended plants of Hawaii 7998 with 30% and 15.7% reduction at 5 and 12 dpi, respectively. No difference between tissues occurred in genotype L390.

3.2. Shoot weight

Shoot dry matter was significantly higher in silicon-treated, *R. solanacearum*-inoculated plants in comparison to non-silicon-treated plants in genotypes King Kong 2 and Hawaii 7998 (Table 3). Also in genotype L390, silicon treatment led to an increase in dry weight by 243% in infected plants, though this difference was not significant due to the high variability in reactions between plants, where some plants survived and others died. Silicon treatment also significantly increased the dry weight of non-infected plants of genotype King Kong 2.

3.3. Relation between bacterial numbers and disease development

In genotype L390, disease severity and incidence were reduced at similar bacterial numbers in stems in silicon-treated plants (Fig. 2). This effect was accompanied by the non-significant plant dry weight increase by 243%, indicating a tolerance effect, while in Hawaii 7998 and King Kong 2 reductions in both symptom development and bacterial numbers were observed.

3.4. Silicon concentration in tomato genotypes

Silicon treatment led to an increase of silicon in roots by 80–86% across genotypes at 5 dpi, while no significant increase was observed in stems (Table 4). At 12 dpi silicon content was about 2–3 times higher in roots than in stems

Table 1
Area under wilt severity and incidence progress curves (AUDsPC, AUDiPC) in treatments with (+Si) and without (−Si) silicon of genotypes L390 (susceptible), King Kong 2 (moderately resistant) and Hawaii 7998 (resistant), over 20 days after inoculation

Treatments	AUDPC disease severity			AUDPC disease incidence		
	L390	KK2	Hw7998	L390	KK2	Hw7998
+Si+Rs	64.3±2.3 aA	42.6±4.9 bB	3.9±1.5 bC	83.3±0.3 aA	54.2±0.4 bB	0.0±0.0 bC
−Si+Rs	72.6±1.8 aA	64.3±3.6 aA	20.8±4.6 aB	95.8±0.2 aA	87.5±0.3 aA	33.3±0.4 aB

AUDPC = total area under disease progress curve based on disease severity and incidence. Total AUDPC was obtained by summing the area under the disease progress for all 8 plants in each treatment. Data points represent the means of three repeated trials±standard errors.
Means followed by same letter are not significantly different based on Tukey's ($P = 0.05$). Small letters vertically refer to the comparison between treatments for the same genotypes and capital letters horizontally refer to the comparison between genotypes for the same treatment.

Table 2
Influence of silicon and tomato genotype on the *R. solanacearum* population in roots and stems of genotypes L390 (susceptible to *R. solanacearum*), King Kong 2 (moderately resistant) and Hawaii 7998 (resistant) amended (+Si) and non-amended (−Si) with silicon at 5 and 12 days post inoculation

Genotype	Treatment	Bacterial number [log(CFU)/g]			
		5 dpi		12 dpi	
		Root	Stem	Root	Stem
L390	+Si+Rs	7.5±0.5 aA	7.4±0.7 aA	8.8±0.4 aA	9.2±0.2 aA
	−Si+Rs	7.8±0.5 aA	8.6±0.4 aA	8.9±0.5 aA	9.3±0.3 aA
KK2	+Si+Rs	6.8±0.7 aA	4.5±0.1 aB	7.8±0.3 aA	5.3±0.3 aB
	−Si+Rs	7.2±0.5 aA	8.4±0.4 bB	8.6±0.2 aA	7.9±0.2 bA
Hw7998	+Si+Rs	5.6±0.3 aA	3.4±0.2 aB	4.6±0.3 aA	3.8±0.3 aB
	−Si+Rs	7.8±0.3 bA	5.4±0.5 bB	7.2±0.1 bA	6.1±0.1 bB

Means followed by same letter are not significantly different based on Tukey's ($P = 0.05$). Small letters vertically refer to the comparison between treatments for the same genotypes and capital letters horizontally refer to the comparison between genotypes for the same treatment.

Table 3
Influence of silicon and tomato genotype on shoot dry matter 1 month after inoculation of L390 (susceptible to *R. solanacearum*), King Kong 2 (moderately resistant) and Hawaii 7998 (resistant) amended (+Si) and non-amended (−Si)

Genotypes	Treatments	Dry weight (g)
L390	+Si+Rs	1.48±0.50 bA[2]
	−Si+Rs	0.61±0.14 bA
	+Si–Rs	5.73±0.73 aA
	−Si–Rs	4.13±0.64 aA
KK2	+Si+Rs	3.62±1.03 aB
	−Si+Rs	0.76±0.12 bA
	+Si–Rs	7.32±0.81 cB
	−Si–Rs	5.02±0.93 aA
Hw7998	+Si+Rs	7.34±1.17 aC
	−Si+Rs	3.88±1.03 bB
	+Si–Rs	7.55±0.91 aB
	−Si–Rs	5.74±0.58 abA

Means of three repeated trials±standard error.
Means followed by same letters are not significantly different with Tukey ($P = 0.05$). Small letters refer to the comparison between treatments for the same genotype and capital letters refer to the comparison between genotypes for the same treatments.

in silicon-amended plants, and had generally increased in all genotypes and tissues including the control plants not treated with silicon. Comparing genotypes, silicon in stems in silicon-treated plants was by 3 times higher, though not significant, in genotype Hawaii 7996 than in L390 at 12 dpi. Silicon content in seeds of −Si plants and in peat substrate was low or negligible, respectively. Seed content was 0.38 mg/g dry weight in L390, 0.32 mg/g in King Kong 2, and 0.47 mg/g in Hawaii 7998, with no significant difference between genotypes, while the peat substrate contained 0.002 mg/g dry weight.

3.5. Immuno-histochemical analysis of tomato cell walls

Changes in stem cell wall composition after silicon treatment and pathogen inoculation were investigated by immuno-histochemical analysis of stem sections from genotype King Kong 2 at 20 dpi, stained with monoclonal antibodies specific for pectin polysaccharide epitopes. Clear differences in green fluorescent staining with the four antibodies were observed in reaction to inoculation in the xylem parenchyma and specifically in and around vessel walls in stem sections of infected plants (Fig. 3E, M, I,

and Q) compared to healthy plants (Fig. 3C, D, K, L, G, H, O, and P) (Table 5).

Staining sections with antibody LM7, detecting non-blockwise de-esterification of homogalacturonan (HG), a strong green fluorescence of vessel walls and surrounding tissue was observed after infection in non-silicon-treated plants (Fig. 3E), which did not occur in silicon-treated plants (Fig. 3F). Also in sections stained with LM2 for AGP a green fluorescence of xylem parenchyma and a strong yellow-green fluorescence of vessel walls and around vessels was seen after infection in −Si plants (Fig. 3I), while in +Si plants the fluorescent reaction around vessels was not observed (Fig. 3J). Similarly, staining with LM6 for (1→6)-α-L-arabinan side chains of RG I showed an increased yellow-greenish fluorescence of tissue around vessels in −Si plants after infection (Fig. 3M), while in +Si plants a strong green fluorescence was observed in some vessel walls, but no yellow fluorescence around vessels and in the xylem parenchyma (Fig. 3N). Staining with antibody LM5 specific for (1→4)-β-D-galactan side chains of RG I after infection revealed a yellow and brown fluorescence reaction around vessels and in some areas of the xylem parenchyma in −Si plants, which was not found in +Si treated-plants, whereas in the latter, the overall green fluorescence of the xylem parenchyma seemed to have increased (Fig. 3Q and R).

Comparing antibody-stained sections of non-inoculated plants with or without silicon treatment, generally no clear reaction in response to silicon treatment was observed. Control sections without antibody staining (Fig. 3A and B) or with only secondary antibody staining [20, this volume] non-treated with silicon showed a weak yellow autofluorescence specifically in areas around vessels in infected plants.

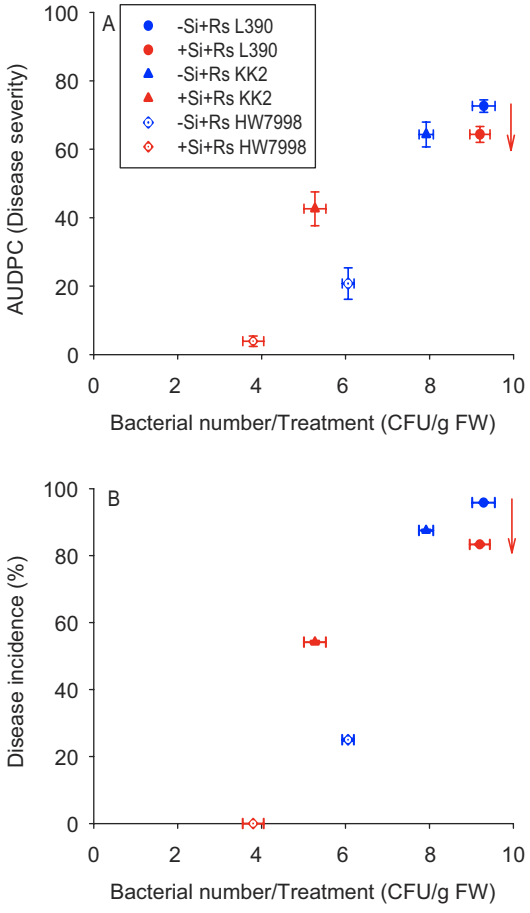

Fig. 2. Relation between bacterial wilt disease expressed as the area under the disease progress curve (AUDPC) based on disease severity (A) or wilt incidence (B) and the bacterial population in mid-stems of tomato genotypes L390, King Kong 2 and Hawaii 7998 amended and non-amended with silicon inoculated with *R. solanacearum* 12 days after inoculation.

4. Discussion

Silicon amendment reduced symptom development in the moderately resistant genotype King Kong 2 and the resistant Hawaii 7998. Bacterial populations in stems and roots were reduced in these genotypes, while in the susceptible genotype L390, no significant difference occurred between treatments. This observation indicates that important resistance mechanisms are located in the

Table 4
Silicon concentration (mg/g dry weight) in tomato genotypes L390 (susceptible), King Kong 2 (moderately resistant) and Hawaii 7998 (resistant) grown in pots non-amended and amended with Si 5 and 12 days after inoculation with *R. solanacearum*

Treatments	L390		KK2		Hw7998	
	Stem	Root	Stem	Root	Stem	Root
5 dpi						
+Si+Rs	0.18±0.08 aB	1.74±0.79 aA	0.14±0.04 aB	1.58±0.69 aA	0.17±0.04 aB	1.78±0.81 aA
−Si+Rs	0.11±0.02 aA	0.24±0.05 bA	0.11±0.03 aA	0.29±0.07 bA	0.10±0.02 aA	0.35±0.09 bA
12 dpi						
+Si+Rs	2.73±1.05 aB	8.26±1.67 aA	3.61±1.67 aB	9.67±1.32 aA	6.04±2.74 aB	10.88±1.10 aA
−Si+Rs	1.51±0.72 aA	3.07±0.47 bA	3.19±1.43 aA	4.06±1.23 bA	3.55±1.64 aA	2.81±0.98 bA

Means followed by the same letter are not significantly different based on Tukey's ($P = 0.05$). Small letters vertically refer to the comparison between treatments for the same genotypes and capital letters horizontally refer to the comparison between plant organs for the same treatments and genotypes. Data points represent means of three repeated trials±standard errors.

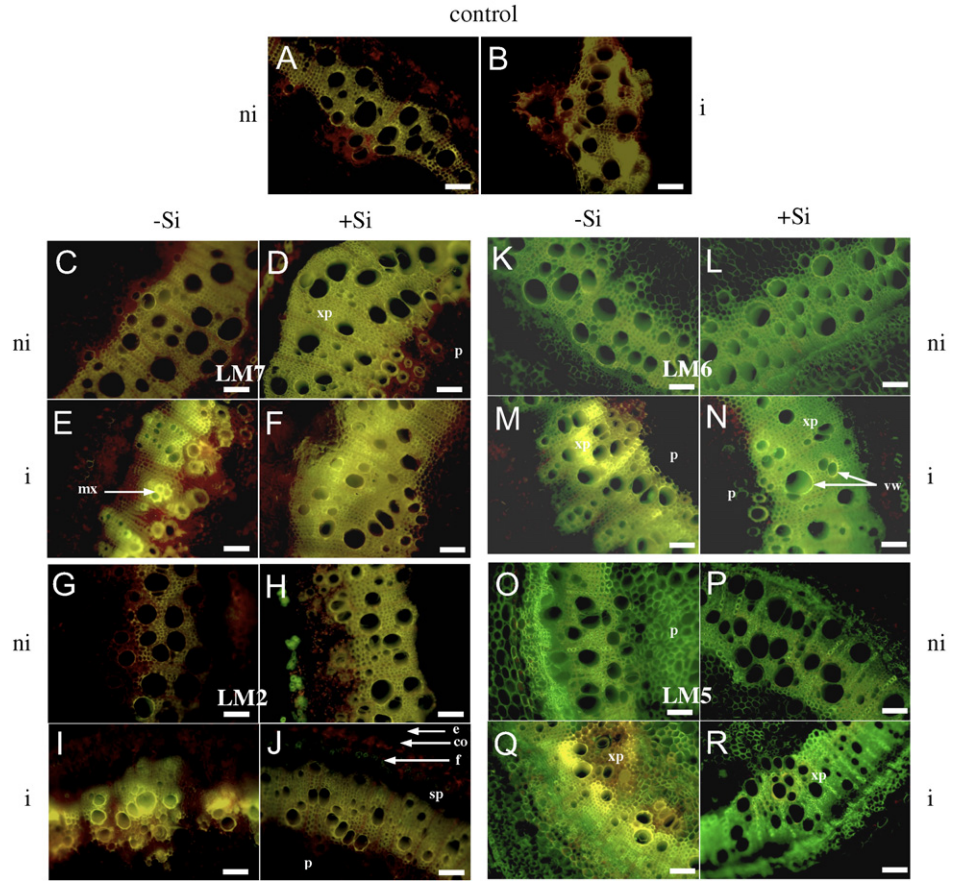

Fig. 3. Mid-stem cross sections of tomato genotype King Kong 2; non-amended with silicon non-inoculated (A) and inoculated (B) with *R. solanacearum* at 20 dpi, mounted in Citifluor (control); probed with the antibody LM7 for localization of non-blockwise de-esterification of homogalacturonan: non-inoculated (C, D) and inoculated (E, F) with *R. solanacearum*, and non-amended (C, E) and amended with silicon (D, F); probed with the antibody LM2 for localization of arabinogalactanprotein: non-inoculated (G,H) and inoculated (I, J) with *R. solanacearum*, and non-amended (G, I) and amended with silicon (H, J); probed with the antibody LM6 for localization of (1→6)-α-L-arabinan side chains of rhamnogalacturonan I: non-inoculated (K, L) and inoculated (M, N) with *R. solanacearum*, and non-amended (K, M) and amended with silicon (L, N); probed with the antibody LM5 for localization of (1→4)-β-D-galactan side chains of rhamnogalacturonan I: non-inoculated (O, P) and inoculated (Q,R) with *R. solanacearum*, and non-amended (O, Q) and amended with silicon (P, R). Co = collenchyma, e = epidermis, f = fibres, mx = metaxylem, p = pit region, vw = vessel walls, xp = xylem parenchyma; background was adjusted to standardize observations; all bars = 50 μm.

stems, which seem to be specifically triggered by the silicon treatment in genotype King Kong 2, while in Hawaii 7998 also in non-silicon-treated plants the massive colonization of stems is limited. Therefore it is suggested, that silicon, although primarily accumulating in the roots of all genotypes, induced resistance in stems, and may additionally increase resistance in roots of Hawaii 7998, but not of King Kong 2. Low bacterial numbers were also recorded in stems of tomato genotypes with different levels of resistance by Dannon and Wydra [13], Wydra et al. [9] and Leykun [21] who concluded that resistance did not result from a limitation of bacterial penetration in roots, but was due to resistance mechanisms located in stems. Similarly, Grimault et al. [22,23], Vasse et al. [8] and Leykun [21] also reported a decrease of bacterial density in stems as compared to collars, where Leykun established differences between resistant genotypes by determining the latent infection in stem sections. Moreover, Grimault et al. [24] found a significant correlation between the bacterial population in stems and the degree of resistance, and, therefore, concluded that resistance did not result from a physical barrier to root penetration, but to the capability of the plant to restrict the bacterial movement to the stems. Our results additionally indicate that in Hawaii 7998 important mechanisms of resistance on root level may be elicited.

In genotype L390, disease development was delayed and plant weight increased by 234% in silicon-treated plants in spite of similar bacterial numbers in stems found in +Si and −Si treatments, indicating a tolerance effect in silicon-treated plants. Also Dannon and Wydra [13] reported a tolerance effect in silicon-treated infected susceptible tomato plants in hydroponic culture.

The high standard errors which generally influence the statistical significance of the data of bacterial wilt inoculated plants are due to the fact that in *R. solanacearum*-inoculated plants the variability in individual plant reaction is generally high, with a high percentage of plants dying in susceptible genotypes, and a lower percentage in more resistant genotypes, but also a small or high number

Table 5
Reactions of stem tissues of genotype King Kong 2 on inoculation with *R. solanacearum* at 20 days post inoculation and treatment with or without silicon

Primary antibody	Treatment	Inoculated plants
Control[a]	−Si	Yellow fluorescence of large areas around vessels (Fig. 3B)
LM7	−Si	Strong green fluorescence of vw and around vessels (Fig. 3E)
	+Si	Weak green fluorescence around vessels (Fig. 3F)
LM2	−Si	Strong yellow-green fluorescence of vw and around vessels (Fig. 3I)
	+Si	No tissue fluorescence (Fig. 3J)
LM6	−Si	Yellow-green fluorescence around vessels (Fig. 3M)
	+Si	Strong green fluorescence of vw; no fluorescence of tissue around vessels (Fig. 3N)
LM5	−Si	Strong yellow fluorescence of vw and brown necrosis of tissue around vessels (Fig. 3Q)
	+Si	Green staining of xp; no fluorescence of vw and tissue around vessels (Fig. 3R)

Staining performed with antibodies LM7, LM2, LM6 and LM5 specific for pectic polysaccharide epitopes (see Fig. 3).
+Si = silicon-treated plants; −Si = non-silicon-treated plants; xp = xylem parenchyma; vw = vessel walls.
[a]Stem sections mounted in antifading reagent Citifluor; control sections omitting secondary antibody (see [19]).

of plants, respectively, are surviving without showing any symptoms. Therefore, generally, disease evaluation in bacterial wilt is recommended to be measured as incidence.

The higher concentration of silicon in the roots of Si-amended plants compared to the stems indicates, that within the root, particularly in the endodermis, silicon is rejected, preventing the transfer to the shoot. Also Ma et al. [25] and Dannon and Wydra [13], the latter in hydroponic culture, reported the highest accumulation of silicon in the root of silicon-treated tomato plants. Absorbed as silicic acid, silicon polymerizes into silica gel in the plant, leading to a great reduction in uptake. Silicon accumulator plants such as rice, wheat and barley continue actively to take up silicon after silicification, and, thus, shoots accumulate more silicon than roots [25]. Regarding the silicon content found in non-silicon-amended tomato plants, seeds and the environment, and, possibly, the demineralised water in the nutrient solution may be additional silicon sources. However, seed silicon content was similar in the tested genotypes [13].

Silicon is reported to play an important role in fungal disease suppression in many Si-accumulator crops [10,12]. This is explained by its strong affinity in the form of monosilicic acid for organic polyhydroxyl compounds, such as *ortho*-diphenols, which participate in the synthesis of lignin in the cell wall in maturation or during pathogen attacks. The stimulation of host defense mechanisms to fungal pathogens has been reported in crops such as cucumber and barley, where the level of inhibitory phenolic compounds and the activity of pathogenesis related (PR)-proteins and phytoalexins increased [26–28]. Non-accumulator plants were not studied.

To characterize possible resistance mechanisms on cell wall level in the interaction of tomato with *R. solanacearum*, immuno-histochemical analyses were performed. The increase in the non-blockwise de-esterification of HG observed after infection in −Si plants, but not in +Si plants is suggested to be due to the action of pathogen pectinmethyl-esterase that removed the methyl-esterification in a homogeneous way, while this effect was not observed in silicon-treated plants. Similar observations were made by Wydra and Beri [19] and Beri [29] in immuno-tissue prints and microscopical studies of tomato stems infected with *R. solanacearum*. The tissue prints confirm, that the fluorescence at least to a major part was due to staining with antibodies and not to autofluorescence. Also McMillan et al. [30] related changes in pectins to the onset of resistance mechanisms, though they only analysed the degree of methylesterification of extracted potato pectins.

The increased staining for AGPs in xylem vessel walls and of arabinan side chains of RG I around vessel walls and of galactan side chains of RG I in and around vessel walls of −Si plants after inoculation may be due to the interaction with the pathogen, while this reaction was not observed in +Si plants, and no necrotic tissue areas occurred. The increased arabinan detection in vessel walls in infected, Si-treated plants and the higher fluorescence of the xylem parenchyma of LM5 and LM6 stained sections suggests that silicon-induced increased branching or new deposition of RG I in cell walls. Nevertheless, it has to be considered that increased tissue staining with antibodies may also be due to an easier accessibility of these epitopes to the specific antibodies after degradation of other cell wall components due to infection [3], and increased staining in the xylem parenchyma might be caused by variations in section thickness after free-hand cutting.

Changes in cell wall structure after infection may strengthen xylem vessels limiting bacterial spread. An increased branching of RGI in cell walls of infected plants may contribute to strengthening the pit membranes of the vessels and of the cell walls of parenchyma cells [31] and make them less easily degradable, thereby inhibiting movement of bacteria from vessel to vessel. It may also be speculated that newly synthesized pectic polymers with changed composition are deposited in the cell walls, as a response to infection. Also Wydra and Beri [19] observed strong staining with these antibodies in stem sections of L390, but not of Hawaii 7996, and, in the latter, a significantly increased number of stained vessels—five- and nine-fold for arabinan and galactan epitopes of RGI, respectively—after inoculation.

A high content of branched RG I changes the physico-chemical characteristics of pectin, making it a less suitable gelling agent [32], which would create more unfavourable conditions for a bacterial pathogen. Additionally, highly branched RG I are less easily degradable by a pathogen. Therefore, induction of branching of RGI might contribute to basal resistance against *R. solanacearum*. It can be speculated, that silicon induces basal resistance factors at cell wall level after infection, which may subsequently lead to the elicitation of more specific resistance mechanisms in the plant.

In control samples without antibody staining, no fluorescence was observed before inoculation. However, the yellow fluorescence observed in the vessel walls and the xylem parenchyma after infection may be due to auto-fluorescent phytoalexins and phenolic substances released in reaction to infection. Nevertheless, in previous studies immuno-tissue prints showed that increased fluorescence in antibody-stained sections inoculated with *R. solanacearum* was due to the specific staining by antibodies and not to autofluorescence [20]. Silicon treatment reduced the yellow autofluorescence observed in inoculated tissues of King Kong 2, suggesting that silicon reduces tissue degradation by changes in plant cell wall structure. Other reactions observed in histological studies of xylem vessels by other authors [24] were tyloses which are thought to be involved in limiting bacterial spread in vessels. Also amino-acids and organic acids in the xylem fluid of tomato [33], were suggested to play a role in resistance of tomato against *R. solanacearum*.

In conclusion, our results indicate that silicon can reduce bacterial spread in xylem vessels, which may be due to induction of basal resistance mechanisms at cell wall level and at pit membranes, which are the privileged route of spread of *R. solanacearum* in plant tissues. Biochemical and molecular studies on silicon-induced resistance signalling pathways in infected plants are ongoing.

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