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Interaction between silicon amendment, bacterial wilt development and phenotype of *Ralstonia solanacearum* in tomato genotypes

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Abstract

Silicon amendment significantly reduced bacterial wilt incidence expressed as area under disease progress curve for tomato genotypes L390 (susceptible) by 26.8% and King Kong2 (moderately resistant) by 56.1% compared to non-treated plants grown in hydroponic culture. However, wilt incidence in silicon-treated plants of genotype L390 reached 100% at 13 days post-inoculation (dpi), while in genotype King Kong2, plant death was retarded by 6 days, with 20% reduction of final wilt incidence. Bacterial numbers were significantly lower in silicon-treated compared to non-treated plants in King Kong2 at 2 dpi in midstems and in all organs at 5 dpi, and in Hawaii 7998 (resistant) in all organs at 2 dpi. Differences between genotypes were obvious on midstem level (5 dpi), where bacterial populations were generally significantly lower compared to roots. Increased tolerance was observed in genotypes L390 and King Kong2 with silicon treatment.

Silicon accumulated in roots and was low in stems and leaves. Inoculation with *Ralstonia solanacearum* did not significantly affect silicon uptake and distribution. Negative correlations between root silicon content and bacterial numbers of midstems in genotypes Hawaii 7998 and King Kong2 suggested an induced resistance. Indications for an influence of host genotype and silicon treatment on the phenotypic conversion of *R. solanacearum* strain To-udk2-sb from fluidal to non-fluidal colonies in planta were observed.

This is the first report on the effect of silicon on a bacterial disease and in a silicon-non-accumulator plant.

Keywords: Induced resistance; Mineral nutrition; Phenotypic conversion (PC); Tolerance

1. Introduction

Bacterial wilt is widely distributed in tropical, subtropical and some temperate regions of the world. The disease ranks as one of the most important if not the most important disease of bacterial origin in the world [22], causing sometimes total losses in tomato crops [33]. Causal agent is *Ralstonia solanacearum* (Smith) Yabuuchi et al. (1995) [45], a highly diverse and adaptive bacterium, that differs in host range, geographical distribution, pathogenicity, epidemiological interactions and physiological properties [4,18]. On the basis of host range, *R. solanacearum* strains have been traditionally divided into races [17], while physiological and genetic characterization resulted in the formation of biovars and divisions [13,17]. The bacterium invades the plant vascular tissues through wounded roots or natural openings, which occur after the emergence of secondary roots. It progresses through intercellular spaces into the xylem. Colonization of stems results in browning of the xylem, foliar epinasty and lethal generalized wilt [3].

Control of *R. solanacearum* is difficult due to its wide host range and its survival capacity in various environments such as irrigation water and soil. Host plant resistance breakdown due to high genotype x environment interactions was frequently observed [42]. Therefore, only integrated control combining host plant resistance and cultural and biological measures seems promising.

Resistance against this pathogen is described as quantitative or polygenic [43]. In quantitative resistance, cell wall characteristics or modifications play a key role, directly as barriers against pathogens or indirectly through their effect on the increase of other active compounds [31]. Many soil elements found in cell walls have been reported to influence the susceptibility or resistance of plants to pathogen

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infections, among them silicon as part of the cell wall, which is considered to be a 'beneficial' element for plants and higher animals [10]. In nature, silicon is found in the form of silica (SiO_2), aluminium silicates, or iron or calcium silicates and is absorbed by the plant as mono-silicic acid ($Si(OH)_4$) [6]. Ma et al. [26] classified plant species into silicon accumulator species, intermediate types and silicon non-accumulators. The uptake mode is active for the first group, passive for the second and rejective for the third.

Application of silicon has been shown to increase leaf chlorophyll and plant metabolism, enhance plant tolerance to environmental stresses such as cold, heat and drought, mitigate nutrient imbalance and metal toxicity in the plant, reinforce cell walls and increase plant resistance against pathogens and insects [10]. In rice, a silicon-accumulator plant, silicon occurs in the wall of epidermic cells or extracellular locations such as storage and vascular tissues, intercellular spaces and cuticular layers [36] and has been reported as important component of papilla [12]. Following uptake and transport of silicon, the process of silicification, the polymerisation of silicic acid into silica gel SiO_2H_2O, begins, whereby silicon together with lignin contributes to the rigidification of cell walls in leaves and xylem vessels [26]. Therefore, it was proposed that silicon plays a role in the formation of mechanical barriers restricting the penetration of pathogens [1,9]. Silicon enhanced resistance against fungal diseases such as *Pythium* and *Sphaeroteca fuliginea* in cucumber [12,35] and blast and sheath blight diseases in rice [9]. The role of a mechanical barrier could not be confirmed, but induced resistance was suggested to be involved [7,27,40]. The effect of silicon on bacterial diseases and on pathogens in non-accumulator plants remains so far unknown.

R. solanacearum possesses the ability to react to changes in its environment with a wide range of physiological states. Among them, the phenomenon of phenotypic conversion (PC), has long been known [21]. The high variability of the pathogen in phenotypic and genetic features is expressed in response to varying environmental conditions. *R. solanacearum* shows a spontaneous shift from the mucoid (fluidal) to the non-mucoid type under certain growth conditions [23], whereby bacteria become avirulent, lose their ability to produce exopolysaccharides, reduce their endoglucanase activity, and increase motility and endo-polygalacturonase activity [2]. Only recently the involvement of the host plant in the reversion of the PC phenomenon has been shown with a susceptible genotype [29]. A possible interaction between host genotype and PC has not been described, nor is the direct or indirect effect of silicon treatment on the state of the pathogen known.

The goal of the study was to find out if silicon amendment enhances the degree of resistance of tomato to *R. solanacearum*, to elucidate whether direct or indirect mechanisms are involved and to determine a possible effect of silicon or genotype on PC of the pathogen.

2. Materials and methods

2.1. Plant materials and culture

Tomato genotypes L390 (susceptible to *R. solanacearum*), King Kong2 (moderately resistant) and Hawaii 7998 (resistant) were provided by the Genetic Resources and Seeds Unit of the Asian Vegetable Research and Development Center (AVRDC, Taiwan). Experiments were performed in hydroponic culture. Tomato seeds were pre-germinated in Petri dishes at 30 °C and transferred into a plate system containing wet filter papers. About one week old seedlings were transferred in black pots with lids containing 4.5 L hydroponic solution: 0.5 M Ca $(NO_3)_2$, 0.2 M NH_4NO_3, 0.5 M K_2SO_4, 0.5 M $MgSO_4$, 0.5 M KH_2PO_4, 10 mM H_3BO_3, 0.1 M $ZnSO_4$, 0.06 mM $CuSO_4$, 1 mM $MnSO_4$, 0.1 mM $MoNaO_4$, 10 mM NaCl and 10 mM FeEDTA; 20 ml monosilicic acid [1.4 mM $Si(OH)_4$] was added to 5 L of final nutrient solution; monosilicic acid was obtained after exchange of potassium silicate solution K_2SiO_2 with cation exchangers (20 ml volume, Biorad Laboratories, Germany) (Hochmuth, 1999 [19], modified by Institute for Plant Nutrition, University of Hannover). The hydroponic solution was aerated through a tube system connected to a pump (Hagen, Germany) and changed at the day of inoculation of *R. solanacearum*. Plants were grown in a climate chamber with 30/26 °C day/night temperature, 80% RH and light for 12 h/day (photonflux of 350 μmol/m^2s).

2.2. Experimental design

Four treatments were arranged in a completely randomi sed block design: (i) plants with silicon, inoculated with *R. solanacearum* (*Rs*+Si), (ii) plants without silicon, inoculated with *R. solanacearum* (*Rs*−Si), (iii) plants with silicon, without *R. solanacearum* inoculation (−*Rs*+Si, control) and (iv) plants without silicon, without *R. solanacearum* inoculation (−*Rs*−Si, control), with 16 plants per genotype and treatment. Four plants were randomly selected per treatment and sampling date at two and five days post-inoculation (dpi) for bacterial and silicon quantification, while eight plants were kept for symptom evaluation. Leaves, stems and roots of sampled plants were subdivided in two parts, one for bacterial quantification and the other for silicon analysis. Experiments were repeated three times.

2.3. Inoculation and quantification of bacteria

The highly virulent *R. solanacearum* strain To-udk2-sb from Thai origin belonging to race 1 biovar 3 (Leykun, 2003; Thaveechai, unpublished) was used for inoculation of the tomato plants. Bacteria were grown on TTC medium [21] for 48 h at 30 °C. Five hundred millilitre of inoculum suspension ($OD_{660\ nm}$ 0.06 corresponding to approximately

10^8 CFU/ml) were poured in each pot to obtain a final inoculum concentration of approximately 10^7 CFU/ml.

R. solanacearum was quantified in leaves, midstems and roots of four randomly selected plants two and five days post-inoculation. Leaves, midstems (5 cm) and roots were weighed, treated with 70% alcohol for 10 s and rinsed and macerated with sterile demineralised water. The suspension was centrifuged for 20 min at 5500×*g* at room temperature and the pellet resuspended in 1.5 ml of sterile demineralised water. Tenfold serial dilutions were prepared and 0.1 ml of each dilution was plated with two replicates on TTC medium. Bacterial colonies were counted after 48 h incubation at 30 °C. Fluidal (>1 mm diameter) and non-fluidal (<1 mm diameter) colony types were differentiated.

2.4. Symptom evaluation

Evaluation began with the appearance of leaf symptoms and was continued daily until all plants had died or symptoms became stable. Five disease severity classes (modified after [44]) were 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. Wilt incidence was calculated as the percentage of dead plants (class 5) at the evaluation date out of the total number of plants in the treatment. The area under disease progress curve (AUDPC) was calculated on the basis of disease severity and of wilt incidence using the following formula [20,39]:

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

where x_i and x_{i-1} are disease severity or wilt incidence and t_i and t_{i-1} are consecutive evaluation dates ($t_i - t_{i-1}$ was equal to 1).

2.5. Silicon quantification

Total silicon content of leaves, stems and roots of four randomly selected plants was determined for organs of the same plants which were used for bacterial quantification at two and five days post-inoculation, following the method developed by Novozamsky et al. [28], modified by the Institute for Plant Nutrition, University of Hannover. Plant materials were dried at 65 °C for 3 days and grounded in a mill. Five hundred microlitres of the mixture of 1 M HCl and 2.3 M HF (1:2) were added to 10 mg of grounded plant material and shaken overnight. After centrifugation at 10,000×*g* for 10 min, 20 µl of the supernatant were retained. A volume of 0.25 ml of 3.2% of boric acid (H_3BO_3) was added to the supernatant and shaken overnight. Then, 0.25 ml of colour reagents [1:1 mixture of 0.08 M H_2SO_4 and 20 g/l (NH_4) $6Mo_7$ $4H_2O$] were added, the mixture was incubated for 30 min and 0.25 ml of 33 g/l tartaric acid and 0.25 ml of 4 g/l ascorbic acid were added successively. A volume of 0.3 ml of each sample was read in a micro-plate at 811 nm (Micro Quant, Biotech, USA). A series of standard silicon contents and blanks were included in the readings. Silicon content of the samples (in ppm) was calculated using regression equation of the standard silicon contents, corrected by subtracting the blanks means and expressed in mg/g dry weight.

2.6. Statistical analysis

The Statistic Analysis System (SAS For Windows, 1999–2001, SAS Institute Carry, USA) program was used for analysis of variance (ANOVA) followed by Tukey test at 5% for means separation. Data of bacterial numbers were log-transformed. Regression analysis was used to determine the relation between silicon content and bacterial numbers of plant organs.

3. Results

3.1. Symptom development

Disease severity and wilt incidence expressed as AUDPC of silicon-treated plants were significantly lower compared to non-treated plants of genotypes L390 with 16.1 and 26.8%, respectively, and King Kong2 with 41.3 and 56.2%, respectively (Table 1), whereby the values of genotype King

Table 1
Area under disease progress curves (AUDPC) for disease severity and wilt incidence in treatments with silicon (*Rs*+Si) and treatments without silicon (*Rs*−Si) in genotypes L390 (susceptible) and King Kong2 (moderately resistant) after inoculation with *R. solanacearum* strain To-udk2-sb

Treatments	AUDPC			
	Disease severity		Wilt incidence	
	L390[a]	King Kong2	L390	King Kong2
Rs+Si[b]	33.87±1.69aA[c]	16.97±2.45aB	704.16±46.40aA	135.42±36.32aB
Rs−Si	40.40±1.71bA	28.91±3.16bB	962.50±62.50bA	308.25±14.57bB

[a] Means of three repeated trials±SE.

[b] *Rs*+Si=silicon-treated plants inoculated with *R. solanacearum*, *Rs*−Si=non-silicon-treated plants inoculated with *R. solanacearum*. Disease severity was defined as the average of disease classes of all plants of a treatment (−Si or +Si) at an evaluation date, while wilt incidence was the proportion of dead plants (disease class 5) out of the total number of plants in the treatment.

[c] Means followed by the same letter are not significantly different with Tukey test at 5%. Small letters vertically refer to comparison between treatments for the same genotype and capital letters horizontally to comparison between genotypes for the same treatment.

Table 2
Fresh and dry weights of aerial parts of 2 months old plants of genotype Hawaii 7998 (resistant) in treatments with (*Rs*+Si) and without silicon (*Rs*−Si) three weeks after inoculation with *R. solanacearum* strain To-udk2-sb

Treatment	Fresh weight (g)[a]	Dry weight (g)
Rs+Si[b]	27.78±2.81a[c]	2.16±0.23a
−*Rs*+Si	30.11±2.67a	2.21±0.23a
Rs−Si	19.59±1.89b	1.66±0.18a
Control	20.37±1.87ab	1. 64±0.15a

[a] Means of three repeated trials±SE.
[b] *Rs*+Si=silicon-treated plants inoculated with *R. solanacearum*, −*Rs*+Si=plants inoculated with *R. solanacearum* without silicon treatment, *Rs*−Si=non-silicon-treated plants inoculated with *R. solanacearum*, control=plants without inoculation of *R. solanacearum* and without silicon treatment.
[c] Means followed by the same letter are not significantly different with Tukey test at 5%.

Kong2 were significantly lower compared to L390. Though genotype Hawaii 7998 did not show symptoms, the fresh weight of inoculated plants was significantly higher in treatments with silicon compared to treatments without silicon (Table 2).

In genotype L390 disease severity and wilt incidence were retarded in 'Si+' plants by about two days and reached the same level as in 'Si−' plants at day 12 and 13, respectively (Fig. 1). In genotype King Kong2, disease severity increased slower in silicon-treated than in non-treated plants, and wilt incidence development was retarded by 6 days. Plant death did not occur until 12 dpi in silicon-treated plants, while at 11 dpi already 62.5% of non-silicon-treated plants had died. At the end of the trials, 46% of plants had survived in silicon treatments and 33% in treatments without silicon (Fig. 1B). No further change in the mortality rate occurred after 16 dpi. No symptom appeared in genotype Hawaii 7998 until the end of the trial in both treatments.

3.2. Quantification of bacteria

Silicon application did not affect bacterial numbers in roots, midstems and leaves of genotype L390, while significantly lower bacterial numbers were found in silicon-treated midstems, and in all organs of genotype King Kong2 at 2 and 5 dpi, respectively (Table 3). In genotype Hawaii 7998, significantly lower bacterial numbers were observed in all organs of silicon-treated plants compared to non-treated plants at 2 dpi, but not at 5 dpi. Comparing plant organs, bacterial numbers were significantly higher in roots than in midstems and leaves of the three genotypes at 2 and 5 dpi.

Comparing genotypes, bacterial numbers were significantly lower in midstems and leaves of genotype Hawaii 7998 compared to genotype L390 in silicon-treated and non-silicon-treated plants at 5 dpi, and in midstems and leaves of silicon-treated plants at 2 dpi.

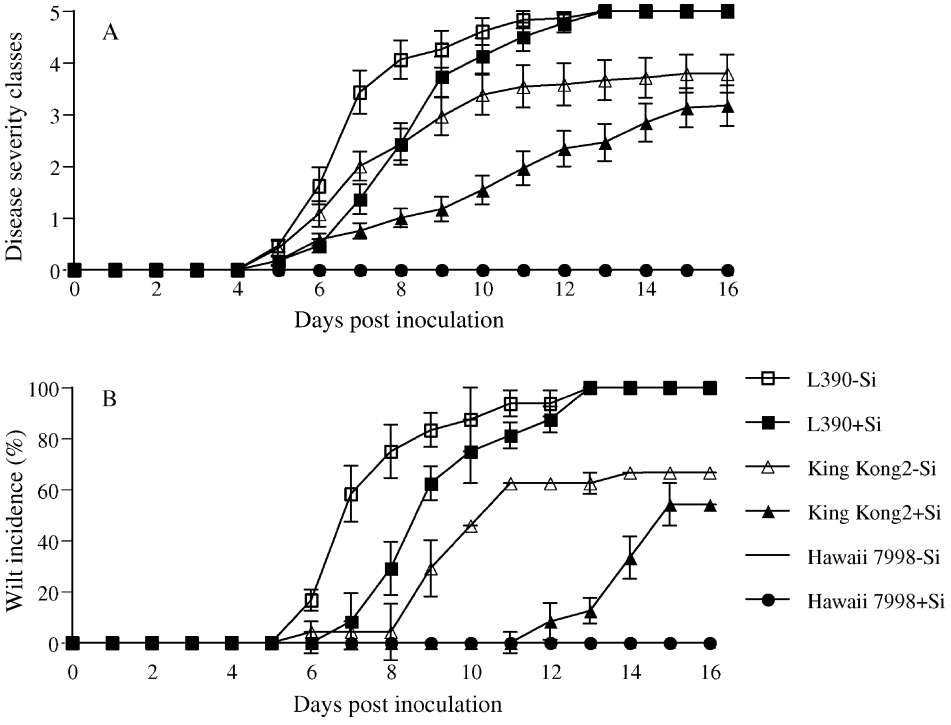

Fig. 1. Bacterial wilt symptom development expressed in disease severity classes (A) and wilt incidence (B) in silicon-treated and non-treated plants inoculated with *R. solanacearum* strain To-udk2-sb in tomato genotypes L390 (susceptible), King Kong2 (moderately resistant) and Hawaii 7998 (resistant). −Si=plants without silicon supply, +Si=plants supplied with silicon. V1=genotype L390, V2=genotype King Kong2 and V3=Hawaii 7998. Plants were grown in hydroponic culture. Disease severity was defined as the average of disease classes of all plants of a treatment (−Si or +Si) at an evaluation date, while wilt incidence was the percentage of dead plants (class 5) in the treatment. No further changes occurred after 16 dpi. Data are means of three repeated trials±SE.

Table 3
Bacterial numbers of roots, midstems and leaves of silicon-treated and non-treated plants of tomato genotypes L390 (susceptible), King Kong2 (moderately resistant) and Hawaii 7998 (resistant) at 2 and 5 days post-inoculation with *R. solanacearum* strain To-udk2-sb

Genotype	Treatment	Bacterial number [$\log_{10}$ (CFU/g)] 2 dpi			Bacterial number [log (CFU/g)] 5 dpi		
		Roots[a]	Midstems	Leaves	Roots	Midstems	Leaves
L390	*Rs*+Si[b]	6.90±0.21aBα[c]	4.18±0.19aAα	4.15±0.13aAα[d]	7.92±0.20aBα	6.64±0.41aAα	6.21±0.40aAα
	Rs−Si	7.53±0.24aBα	4.61±0.26aAα	4.55±0.21aAα	7.89±0.09aBα	6.71±0.36 aABα	6.44±0.45aAα
King Kong2	*Rs*+Si	7.42±0.18aBα	3.87±0.14aAα	3.69±0.26aAαβ	7.88±0.15aBα	5.14±0.40aAαβ	4.60±0.29 aAβ
	Rs−Si	7.49±0.20aBα	4.56±0.18bAα	4.05±0.17aAα	8.91±0.49 bCβ	7.69±0.07 bBβ	6.14±0.46bAαβ
Hawaii 7998	*Rs*+Si	6.27±0.43aBα	3.25±0.17 aAβ	3.16±0.18 aAβ	7.52±0.25aBα	4.69±0.21 aAβ	4.38±0.24 aAβ
	Rs−Si	7.66±0.29bBα	4.12±0.12bAα	4.28±0.09bAα	7.90±0.12aBα	4.67±0.22 aAλ	4.82±0.24 aAβ

[a] Means of three repeated trials±SE. Counts of fluidal and non-fluidal colonies combined.
[b] *Rs*+Si=silicon-treated plants inoculated with *R. solanacearum*, *Rs*−Si=non-silicon-treated plants inoculated with *R. solanacearum*.
[c] Means followed by the same letter are not significantly different with Tukey test at 5%. Small letters vertically refer to comparison between treatments for the same organ, capital letters horizontally refer to comparison between organs for the same treatment and genotype and Greek letters vertically refer to comparison between genotypes for the same organ and treatment.
[d] Early high level of bacterial numbers in leaves may be due to fast access to roots in hydroponic culture and high transpiration, and, thus, passive transport of bacteria, under conditions in the climatic chamber.

Comparing genotype Hawaii 7998 to genotype King Kong2, bacterial numbers in Hawaii 7998 were significantly lower in roots and midstems in non-silicon-treated plants at 5 dpi and in midstems of silicon-treated plants at 2 dpi.

Comparing genotype King Kong2 to L 390 in treatments without silicon, no differences were observed at 2 dpi, but bacterial numbers were higher in roots and stems of King Kong2 than in L390 at 5 dpi. In silicon treatments, these differences were not observed. Similar bacterial numbers were generally observed in the roots of the three tomato genotypes across treatments and evaluation dates. No differences were found between bacterial numbers in the nutrient solution with and without silicon (at 2 dpi: 7.70±0.13a and 7.67±0.11a, respectively; at 5 dpi 8.04±0.21a and 7.58±0.10a $\log_{10}$ CFU/ml, respectively), and compared to distilled water (at 2 dpi: 7.51±0.10a; at 5 dpi: 7.94±0.12a) (data not shown).

Comparing symptom development (Table 1) and bacterial populations (Table 3), symptom development (AUDPC of disease severity and wilt incidence) and bacterial numbers at 5 dpi were reduced in all organs of genotype King Kong2 in silicon treatments. Although in genotype L390 bacterial numbers were not reduced in silicon treatments, symptom development was significantly reduced. Bacterial numbers in roots and stems of silicon-treated plants of genotypes L390 and King Kong2 were similar at 5 dpi, but symptom development expressed as AUDPC of disease severity and wilt incidence was significantly reduced in genotype King Kong2 compared to genotype L390 by 50 and 81%, respectively, in silicon treatments.

Plotting wilt incidence against bacterial numbers at 5 dpi, the effect of silicon application on the reduction of bacterial numbers in genotype King Kong2 is demonstrated, whereas a disease reducing effect occurred in both genotypes L390 and King Kong2 (Fig. 2).

3.3. Bacterial colony types

Dark-red colonies of *R. solanacearum* of the fluidal (>1 mm diameter after 48 h incubation) and non-fluidal type (<1 mm diameter) were isolated from the three tomato genotypes at 2 and 5 dpi on TTC agar medium. Their identity was confirmed by nitrocellulose membrane enzyme-linked immuno-sorbent assay (NCM-ELISA). Sub-culturing the non-fluidal colony type resulted in non-fludial and fluidal colonies. Colonies similar to *R. solanacearum* were never detected in non-inoculated plants.

Bacterial numbers in nutrient solution with and without silicon at 2 and 5 dpi were similar (see above), while the percentage of non-fluidal colonies increased in nutrient solutions and in water from 2 to 5 dpi (Fig. 3). In the original inoculum, the percentage of fluidal colonies (78.0±7.3b) was significantly higher compared to non-fluidal colonies (22.0±7.3a) (data not shown).

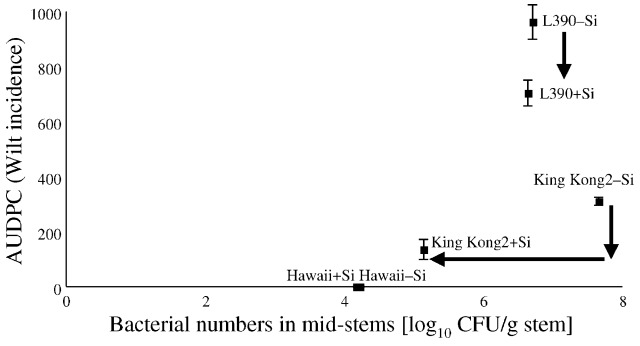

Fig. 2. Distance between the three tomato genotypes L390 (susceptible), King Kong2 (moderately resistant) and Hawaii 7998 (resistant) with regard to the AUDPC based on wilt incidence and bacterial numbers in the midstems at 5 dpi. Data are from means of the AUDPC based on wilt incidence (see Table 1) and of bacterial numbers in the midstems at 5 dpi. Wilt incidence=percentage of dead plants (class 5) in a treatment at an evaluation date.

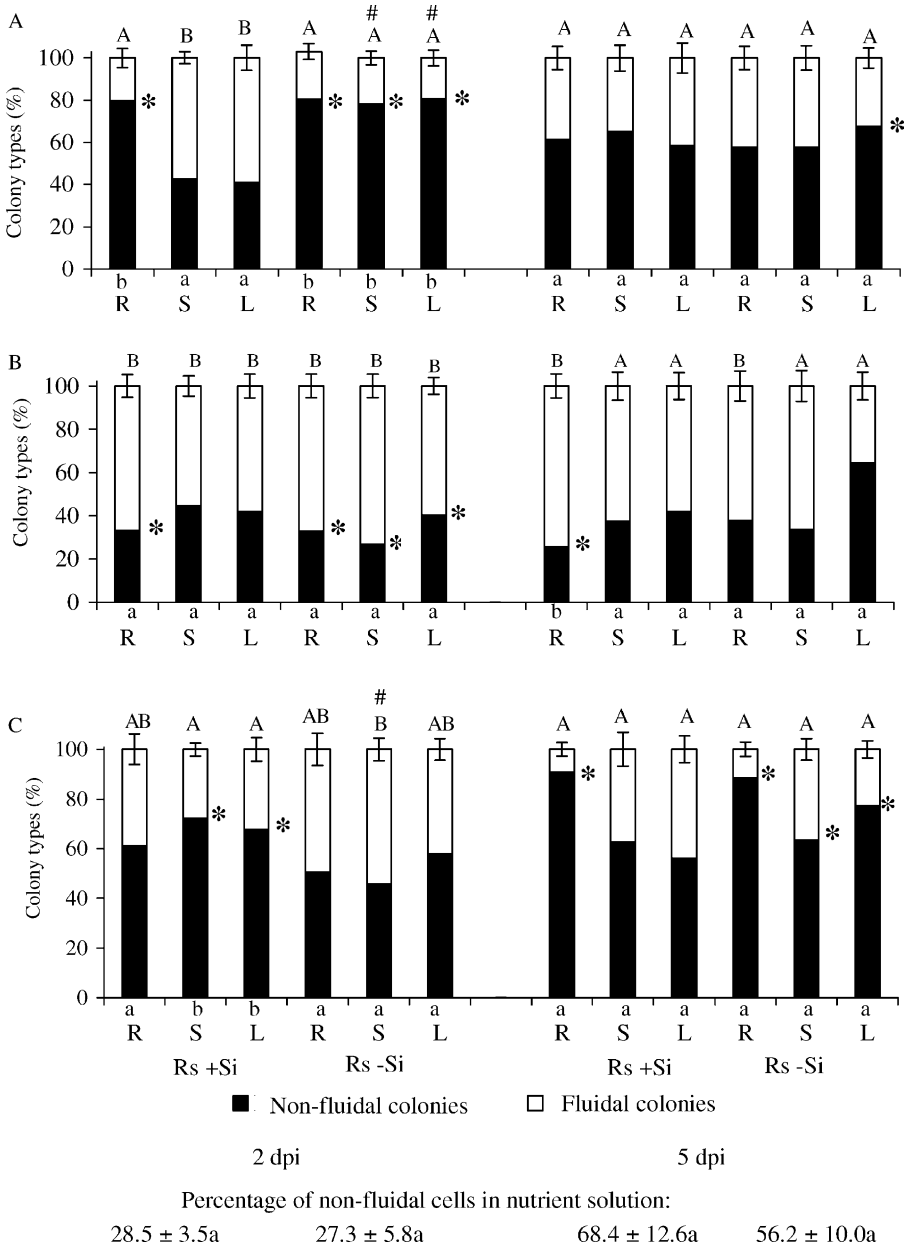

Fig. 3. Percentages of fluidal (>1 mm diameter) and non-fluidal (<1 mm diameter) colony types of *R. solanacearum* strain To-udk2-sb in roots (R), midstems (S) and leaves (L) of silicon-treated (*Rs*+Si) and non-treated (*Rs*−Si) plants of tomato genotypes L390 (susceptible) (A), King Kong2 (moderately resistant) (B) and Hawaii 7998 (resistant) (C) at 2 and 5 dpi on TTC medium. Additionally, the percentage of non-fluidal colonies in nutrient solution with and without silicon at 2 and 5 dpi is given. Means from three repeated trials ± SE. Stars indicate significant differences between colony types. Double crosses indicate significant differences between treatments for the same organs and genotype. Capital letters (A, B, AB) refer to comparison between genotypes for the same treatment and organ and small letters to comparison between nutrient solution and genotypes for the same treatment and organ. Tukey test at 5% probability. Percentage of non-fluidal colonies in water: 36.6% at 2 dpi, 66.7% at 5 dpi.

In genotype L390, the percentage of non-fluidal colonies was significantly lower in silicon-treated stems and leaves than in non-silicon treated ones at 2 dpi, while in genotype Hawaii 7998 the percentage of non-fluidal colonies was higher in silicon-treated stems than in non-silicon treated ones.

Differences between genotypes occurred (i) in non-silicon-treated plants with higher percentages of non-fluidal colonies in all organs of genotype L390 compared to King Kong2, and in stems compared to Hawaii 7998 at 2 dpi, and (ii) in silicon-treated plants with higher percentage of non-fluidal colonies in stems and leaves of genotype Hawaii 7998 compared to the other genotypes. In genotype King Kong2 a lower percentage of non-fluidal colonies was observed in roots of both treatments at 2 and 5 dpi compared to genotype L390, and at 5 dpi compared to genotype Hawaii 7998.

Comparing colony types in plants and nutrient solution at 2 dpi, a higher percentage of non-fluidal colonies occurred

in all non-silicon-treated organs and in silicon-treated roots of genotype L390, and in stems and leaves of silicon-treated plants of genotype Hawaii 7998. No influence of plants on colony type was observed across genotypes and treatments at 5 dpi except in roots of genotype King Kong2, where a lower percentage of non-fluidal colonies occurred in silicon-treated plants.

3.4. Silicon distribution

Silicon treatment increased highly the silicon content in roots of the three tomato genotypes at 2 and 5 dpi (Fig. 4), but only slightly in leaves and stems at 2 dpi and only in leaves at 5 dpi of genotype L390, and in leaves of genotype Hawaii 7998 at 2 dpi. In leaves, silicon was generally slightly but significantly higher than in stems across genotypes at both evaluation dates. In the non-silicon-treated control, generally no differences between the silicon content of the three plant organs occurred. A difference between genotypes in their ability to accumulate silicon was not observed and *R. solanacearum* did not influence silicon uptake or distribution.

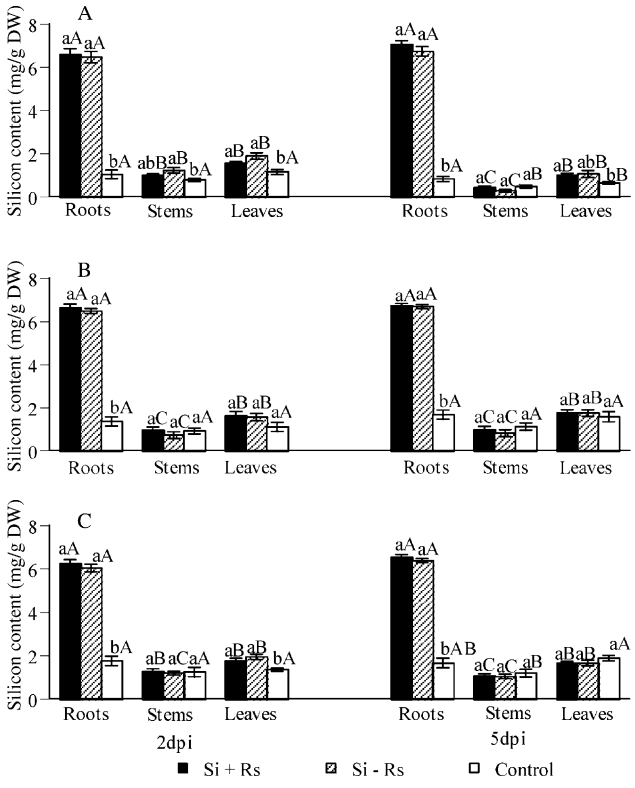

Fig. 4. Silicon content [mg/g dry weight (DW)] of roots, stems and leaves of silicon-treated plants inoculated with *R. solanacearum* strain To-udk2-sb (Si+*Rs*), of non-inoculated plants (Si−*Rs*) and of non-silicon-treated plants (control) of tomato genotypes L390 (susceptible) (A), King Kong2 (moderately resistant) (B) and Hawaii 7998 (resistant) (C) at 2 and 5 dpi. Means from three repeated trials ± SE. Means followed by the same letters are not significantly different with Tukey test at 5%. Small letters refer to comparison between treatments and capital letters to comparison between plants organs for the same treatment. Comparison of the three genotypes by the silicon content of the roots did not show any differences.

Silicon was always found in plants grown in silicon-free solution. Tomato seeds and the environment were probably the main sources of silicon. However, the silicon content of the seeds of the three genotypes was low and similar comparing genotypes, with silicon contents in L390 of 0.38 ± 0.001, in King Kong2 0.30 ± 0.08 and Hawaii 7998 with 0.47 ± 0.07 mg/g dry weight (data not shown).

3.5. Relation between bacterial numbers and silicon content

Negative correlations were found between the silicon content in roots and bacterial numbers in stems of genotypes King Kong2 and Hawaii 7998 with determination coefficients of 0.18 and 0.33, respectively, and between roots' silicon and bacterial numbers of roots and leaves of genotype Hawaii 7998, with determination coefficients of 0.30 and 0.50, respectively, at 2 dpi (Table 4). At 5 dpi negative correlations were found in genotype King Kong2 between silicon content of the roots and bacterial numbers of roots, stems and leaves, with 24, 37 and 28%, respectively, of the variations in bacterial populations explained by the variations in roots' silicon. In leaves of genotypes L390, King Kong2 and Hawaii 7998 20, 18 and 36%, respectively, of the variation in bacterial numbers could be explained by the variation of silicon content at 2 dpi. No correlation was found between the bacterial numbers of the different organs and their silicon content in L390 and Hawaii 7998 at 5 dpi.

4. Discussion

Silicon application to hydroponic-grown tomato plants reduced bacterial wilt development (AUDPC) in the susceptible genotype L390 and in the moderately resistant genotype King Kong2. Evaluation of wilt incidence revealed differences between treatments more clearly than severity classes. Nevertheless, all plants of genotype L390 had died at 13 dpi, while the final wilt incidence of King Kong2 was reduced by 20%. Silicon may have increased the effect of resistance factors present in genotype King Kong2, while in genotype L390, which lacks effective resistance factors, disease development could only be delayed, and in the symptomless, resistant genotype Hawaii 7998 silicon treatment caused an increase in fresh weight (significant) and dry weight (non-significant).

R. solanacearum was found in all organs of the tested genotypes, including the resistant genotype Hawaii 7998. Also Yamazaki [46] and Leykun [25] reported high latent infection in stems of Hawaii 7998 and Hawaii 7996. Comparing genotypes, bacterial numbers in midstems and leaves, but not in roots, were significantly lower in genotype Hawaii 7998 than in genotype L390 across treatments (5 dpi) and in silicon treatments at 2 dpi, suggesting

Table 4
Relationship between bacterial numbers [$\log_{10}$ (CFU/g)] of roots, midstems and leaves and total silicon content of roots (mg/g dry weight) of genotypes L390 (susceptible) and King Kong2 (moderately resistant) and Hawaii 7998 (resistant), 2 and 5 days post-inoculation with *R. solanacearum* strain To-udk2-sb

Silicon content		Coefficients					
		Bacterial number					
Genotype	Organ	Root		Midstem		Leaf	
		R^{2a}	Slope	R^2	Slope	R^2	Slope
2 dpi							
L390	Leaf[b]	na[c]	na	na	na	0.20*	−0.80*
King Kong2	Root	–[d]	–	0.18*[e]	−0.09*	–	–
	Leaf	Na	na	na	na	0.18*	−0.15*
Hawaii 7998	Root	0.30*	−0.28*	0.33**	−0.15**	0.50**	−022**
	Leaf	Na	na	na	na	0.36**	−1.22**
5 dpi							
King Kong2	Root	0.24*	−0.14*	0.37**	−0.39**	0.28**	−0.32**

[a] R^2 = determination coefficient; as bacterial growth was exponential, the real regression equation would be 10^{ax+b}. Data from three repeated trials were analysed.
[b] Only organs which showed at least one significant correlation are included in the table.
[c] na = not analysed.
[d] – = no correlation.
[e] *Significant at $P<0.05$; **significant at $P<0.001$.

the effect of resistance mechanisms in stems of genotype Hawaii 7998. The dates of 2 and 5 dpi were chosen because in pre-trials first wilting occurred at 6 dpi, and the action of resistance factors must have onset before symptom appearance in the susceptible genotype. Similar bacterial numbers in roots of tomato genotypes with different degree of resistance were also observed by Prior et al. [32], who concluded that resistance did not result from a limitation of bacterial penetration in roots, but they localized resistance mechanisms in the midstem. Thus, a decrease of bacterial density in midstems compared to the collar was also reported by Grimault et al. [15] and Vasse et al. [41], with a significant correlation between the bacterial population at midstem level and the degree of resistance [14].

In treatments without silicon, bacterial numbers in all organs of genotype King Kong2 were equal to L390 or higher, although symptom development was significantly reduced. These observations point at a mechanism of tolerance in King Kong2, which increased after silicon treatment, when disease severity was further reduced, and of an induced tolerance in genotype L390, where silicon-treated plants showed less symptoms with similar bacterial numbers compared to non-treated plants.

In silicon-treated tomato plants, the highest level of silicon was found in roots, as it is typical for silicon non-accumulator plants [27]. Accumulation of silicon depends on the ability of the plant to continue taking up silicon after the process of silicification begins in older tissues. However, silicon accumulator plants like rice, wheat and barley continue to actively take up silicon after the beginning of silicification and, thus, the shoot accumulates more silicon than the roots [26]. Unequal distribution of total silicon between plant organs is therefore a common phenomenon for most crops. Differences may also appear inside the same organ as reported for wheat and barley leaves [36]. Infection with *R. solanacearum* did not influence silicon accumulation by tomato. In contrast, cucumber plants were reported to accumulate more silicon around penetration sites after infection by *Pythium* spp. or by *Sphaerotheca fulginea* [7,27].

Silicon amendment reduced bacterial numbers in the moderately resistant genotype King Kong2 in midstems (2 dpi) and in all organs (5 dpi), and in the resistant genotype Hawaii 7998 in all organs (2 dpi). Regression analysis revealed significant direct effects of silicon in roots on the bacterial population in roots of genotypes Hawaii 7998 (2 dpi) and King Kong2 (5 dpi), and of silicon in leaves on bacterial numbers in leaves for the three genotypes (2 dpi), suggesting a direct influence of silicon on colonization of these organs. But, the direct effect of silicon in leaves should not be over-emphasized considering the low increase of silicon in these organs and the non-significant decrease in bacterial numbers in genotypes L390 and King Kong2 after silicon treatment. Nevertheless, rigidification of cell walls in roots may hinder cell wall degradation by the pectic enzymes, various polygalacturonases and pectin methyl esterase of *R. solanacearum* [2] and contribute to a reduced bacterial multiplication. A barrier effect of silicon has been reported at early steps in resistance of crops such as cucumber, oat, rice, wheat, barley and sugarcane against fungal diseases [7,24,34,35,37].

A significant negative correlation of silicon in roots and bacterial numbers in midstems and leaves of genotypes Hawaii 7998 (2 dpi) and midstems (2 dpi) and midstems and leaves (5 dpi) of King Kong2 indicated an indirect effect of silicon. Thus, silicon in roots explained 18–37% of the variation of bacterial populations in midstems, indicating that silicon induced resistance to the pathogen in midstems, where mechanisms of resistance were found to be located in tomato [32]. It was suggested that silicon in roots plays

a role in the signalling network and can induce resistance systemically in other organs [12]. Silicon has been reported to stimulate host defence mechanisms in crops such as cucumber and barley by increasing the level of inhibitory phenolic compounds and the activity of chitinases, β-1,3-glucanases, peroxides, phenylalanine ammonia-lyase and polyphenoloxidase [5,11,27]. Furthermore, silicon was involved in the increased resistance of cucumber by enhancing the antifungal activity attributed to a phytoalexin [11]. Information on possible resistance mechanisms in tomato to *R. solanacearum* is limited, but bacterial spread was suggested to be limited by production of tyloses forming physical barriers, and gums and other deposits [14].

The PC of *R. solanacearum* was frequently reported in stored cultures [30,38], but reasons for the transformation are unknown. Non-fluidal colonies, present in low percentage in the inoculum suspension, were increased in isolations from genotype L390 (2 dpi). Quorum sensing was found to determine the PC, directed by the phcA confinement system [8]. PC was recently reported to be reversible in presence of a susceptible host plant [29]. Interactions with genotypes of different resistance were to date not investigated.

Grimault and Prior [16] also found that root invasion was not a limiting factor for bacterial multiplication, when high numbers of bacteria occurred in tomato stems without producing wilt symptoms and suggested that in planta plant–bacteria interactions are involved in reduced bacterial multiplication, and that resistance in tomato is expressed on stem level. They speculated that the resistant host might be involved in impairing exopolysaccharide production of *R. solanacearum* in planta resulting in higher numbers of non-fluidal cells in the resistant plant. Thus, our results indicated an influence of the plant, comparing the percentage of non-fluidal cells in planta in the susceptible genotype with the cells in nutrient solution, where the silicon amendment did not have an effect on the phenotype. But, we observed significantly higher numbers of non-fluidal cells in stems of untreated plants of the susceptible genotype, compared to the nutrient solution and to King Kong2 and Hawaii 7998 at 2 dpi, while bacterial numbers in stems were similar across genotypes. After silicon treatment, the number of non-fluidal cells was reduced in the susceptible genotype and increased in the resistant genotype at 2 dpi.

Thus, plant factors might have an influence on the loss of the ability to produce high quantities of extracellular polysaccharides, without or prior to a suppressing effect on bacterial multiplication. Before a difference in bacterial number in stems between non-silicon-treated genotypes occurred, an interaction between the plant and the pathogen seems to take place and becomes obvious in the PC of *R. solanacearum*. Although the PC phenomenon has been generally described for *R. solanacearum* strains in culture, it may be that our strain has a higher tendency to change its phenotype than other strains. Also Thaveechaii (personal communication) confirms, that after subculturing each colony type of strain To-udk2-sb, both types may occur again. The non-fluidal type was observed to be still virulent, and inoculation of fluidal and non-fluidal types each in a susceptible tomato plant resulted in both types after re-isolation. Also Poussier et al. [29] described a host plant-dependent PC of *R. solanacearum* from non-pathogenic forms. Nevertheless, in an ongoing trial performed in substrate culture, strain To-udk2-sb did not show the two colony types in culture nor after re-isolation, indicating the high variability of the strain after some months of storage in sterile water at room temperature (unpublished data).

On the reasons for the PC in planta it can only be speculated that in the susceptible plant extracellular polysaccharides as a protection from resistance factors are not yet needed at 2 dpi and bacterial cells lose their fluidal character for some time. Under silicon treatment, which might induce resistance factors, bacteria in stems of the susceptible genotype showed lower percentages of non-fluidal colonies and behaved similar as in the untreated resistant genotypes. The reasons for the higher number of non-fluidal colonies in stems of genotype Hawaii 7998 after silicon treatment at 2 dpi compared to non-treated plants and to the nutrient solution remain unclear. Further investigations with more genotypes and strains are ongoing.

Resistance of tomato against *R. solanacearum* was enhanced by silicon treatment in the three genotypes, and accumulated silicon in roots was negatively correlated to bacterial numbers in other organs. Therefore, silicon is suggested to be involved in induced resistance and increased tolerance, interacting with resistance factors of the plants, with an indirect effect on bacterial growth and the physiological status of the bacteria. The role of the genotype and the molecular basis of resistance induction as well as the interaction between silicon, host and pathogen are being studied. The silicon effect should be verified in various substrates and soils, and integrated in a bacterial wilt management system.

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