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# Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against *Ralstonia solanacearum*

Leonard Muriithi Kiirika [a], Frank Stahl [b], Kerstin Wydra [a,*]

[a] Gottfried Wilhelm Leibniz Universität Hannover, Institute of Plant Diseases and Plant Protection, Herrenhäuser Straße 2, 30419 Hannover, Germany
[b] Institute of Technical Chemistry, Leibniz Universität Hannover, Schneiderberg, 30419 Hannover, Germany

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ABSTRACT

Silicon (Si) and chitosan (Chi) treatments induced resistance in tomato against bacterial wilt caused by *Ralstonia solanacearum*. Gene expression analysis conducted at 72 h post inoculation via TOM2 microarray revealed regulation of 204 and 126 genes in genotypes King Kong 2 and L390, respectively, with their majority classified into the categories defense-related, signal transduction and transcription. In the microarrays, translationally-controlled tumor protein homolog involved in stress reaction of plants, the defense genes chitinases and peroxidases were highly up-regulated in combined Si and Chi treatment. Bacterial wilt incidence was reduced by 40% and 56.6% in Si and Chi treatment, respectively, in King Kong 2, and by 26.6% and 33.3% in Si and Chi treatment, respectively, in L390, and by 74.7% in King Kong 2 and 46.6% in L390 after combined application of Si and Chi. Evidence of their synergistic effects is reported.


## 1. Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating diseases in tomato (*Solanum lycopersicum*) production. It is regarded as a species complex because of its wide host range expanding over more than 200 plant species and widely distributed in different environments worldwide [1], imposing a production problem with no reliable control measures available. Use of resistant varieties showed limited effects in managing the disease since no stable resistance has yet been found.

Enhancing host resistance using elicitors such as silicon in form of silicon dioxide and chitosan, a derivative of chitin, can be an effective control strategy. Si is known as a multifunctional element that significantly increases plant tolerance against biotic stresses such as pests and diseases as well as abiotic stresses including toxic metal concentrations, drought and frost [2–4]. Resistance effects of Si against various pathogens was reported in Si accumulator plants such as cucumber and vines against powdery mildew [5,6] rice against sheath blight and blast [7,8]. Concerning its different modes of action in disease resistance, Si was reported to play a mechanical role in cell wall reinforcement and accumulation at the infection sites [9,10]. Si is shown to act as a modulator influencing the expression of plant defense responses where it interacts with key components of plant stress signaling systems by binding to hydroxyl groups of proteins involved in signal transduction leading to resistance induction [5].

Chi is a natural, non-toxic homopolymer of β-1, 4-linked 2-amino-D-glucose units, containing only small amounts of 2-acetamido-D-glucose units. It is the main derivative of chitin found in cell walls of fungi and exoskeletons of crustaceans, mainly shrimps [11,12]. Chi is obtained from chitin via a deacetylation process involving modification of chitin structure by removal of acetyl groups in concentrated alkaline solution and is one of the most effective members of the oligosaccharin group shown to have plant resistance eliciting function [12]. Oligochitosan is shown to inhibit pathogen invasion, induce phytoalexin production and expression of defense-related genes in various plants including rice, tobacco, oilseed rape and grapevine [13–17]. At gene expression level, the up-regulation of jasmonic acid (JA)/ethylene (ET)

*Abbreviations:* AUDPC, area under disease progress curve; AVRDC, Asian Vegetable Research and Development Centre; Chi, chitosan; ChiIR, chitosan induced resistance; CFU, colony forming units; DW, dry weight; JA, jasmonic acid; kDa, kilo Dalton; ROS, reactive oxygen species; Rs, *Ralstonia solanacearum*; Si, silicon; SiIR, silicon-induced resistance; (TCTP), translationally controlled tumor protein; TTC, triphenyl tetrazolium chloride.

* Corresponding author. Present address: Centre for Tropical and Subtropical Agriculture and Forestry (CeTSAF) – Tropenzentrum, Georg-August Universität Göttingen, Büsgenweg 1, 37077 Göttingen, Germany. Tel.: +49 551 393909; fax: +49 551 394556.
*E-mail addresses:* kiirika@genetik.uni-hannover.de (L.M. Kiirika), stahl@iftc.uni-hannover.de (F. Stahl), wydra@ipp.uni-hannover.de, kwydra@uni-goettingen.de (K. Wydra).

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pathway defense marker gene *PDF1.2*, and the increase of activity of defense-related enzymes such as phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and JA biosynthesis enzyme lipoxygenase was observed after application of oligochitosan in oilseed rape leaves [16,18]. Accumulation of JA in tobacco leaves treated with oligochitosan was reported, where JA and methyl jasmonate play a key role in signal transduction and resistance to pathogens and insects [19,20]. Generation of reactive oxygen species molecules such as nitrogen dioxide (NO) and hydrogen peroxide ($H_2O_2$) is reported in *Brassica napus* epidermal cells and tobacco cell suspension cultures treated with oligochitosan [12]. The ROS molecules play a role as plant defense signaling molecules by diffusing through the cell membranes, initiating a secondary signal transduction cascade [21].

Combining more elicitors to synergistically induce a higher and more effective resistance forms a potential approach to managing pathogens. Though, also antagonistic effects may occur, as described in our previous studies on simultaneous application of Si and microbial antagonists [22,23]. Enhanced host resistance as synergistic effect after simultaneous application of Si and Chi was recently reported on apple (*Malus domestica*), resulting in the inhibition of brown rot caused by *Monilinia fructifolia* [24]. Induced resistance was also reported in spring barley against *Rhynchosporium secalis* and *Blumeria graminis* f. sp. *hordei* using combined elicitors acibenzolar-S-methyl, β-aminobutyric acid and *cis*-jasmone [25,26] However, only few aspects of the biochemical and molecular background of Si-induced resistance were to-date described from hydroponic culture system and in substrate grown tomatoes [27,28]. Recent transcriptome analysis of the Si-induced resistance (SiIR) in tomato revealed a priming effect with up-regulation of defense-related genes at 72 hpi [29]. Resistance to bacterial wilt in tomato was suggested to manifest in cell wall composition and structure of tomato vessels [30,31] and in changes of the proteome involving pathogenesis-related proteins [32].

Although the resistance inducing effects of Chi in plants against pathogens is reported, molecular mechanisms underlying the induced resistance remain unknown. Additionally, the molecular background of the possible synergistic or antagonistic interactions of these two elicitors has not been elucidated. Therefore, in the present study, a gene expression analysis by microarrays was employed to explore the global transcriptome changes during SiIR and Chi-induced resistance (ChiIR) in tomato against *R. solanacearum*.

## 2. Materials and methods

### 2.1. Plant materials and culture conditions

Tomato seeds of genotypes King Kong 2, moderately resistant and L390, susceptible to *R. solanacearum* received from KNOWN-YOU company, Taiwan and the Asian Vegetable Research and Development Centre (AVRDC), Taiwan, respectively, were sown in peat substrate (Klasmann, Lithuanian Peat Moss, Germany) supplemented with 4 g/l $CaCO_3$ and kept under greenhouse conditions (20 °C day/night, 14 h light per day/30K Lux and 70% RH). Thirty days after sowing, the seedlings were transplanted to individual pots containing 150 g of substrate and transferred to quarantined climate chamber with 30/27 °C day/night, 12 h light, 30K Lux and 85% relative humidity, and watered regularly to the soil field capacity.

### 2.2. Si and Chi supply

Si was applied as previously described by Diogo and Wydra [28]. Si-treated plants received Aerosil powder (pure form of silicon dioxide – Degussa, Germany) at a rate of 1 g/l substrate at seeding. Plants were watered with a nutrient solution amended with monosilicic acid in a concentration of 1.4 mM $Si(OH)_4$. The nutrient solution contained 2.5 M $Ca(NO_3)_2$; 2.5 M $K_2SO_4$; 2.5 M $MgSO_4$; 2.5 M $KH_2PO_4$; 50 mM $H_3BO_3$; 0.3 mM $CuSO_4$; 0.5 mM $ZnSO_4$; 5 mM $MnSO_4$; 0.5 mM $MoNa_2O_4$; 50 mM Fe-EDTA; 50 mM NaCl at pH = 5.8. Non Si-treated plants were cultivated in peat substrate without Aerosil-200 and received nutrient solution free of monosilicic acid. Monosilicic acid was obtained by ion exchange of potassium silicate solution $K_2SiO_4$ with cation exchangers (20 ml volume, Bio-rad Laboratories, Germany) [33].

Chi was obtained from ChiPro GmbH Germany, extracted from residues of deep-sea crabs, with a viscosity < 100 Mpa, 85% degree of deacetylation and soluble in water. After transplanting, Chi was applied as a drench by first dissolving the powder in $ddH_2O$ (33.3 $g^{-1}$) to receive a homogenous liquid suspension and pouring 54 ml of the suspension directly onto the substrate.

### 2.3. Experimental design

The experiment consisted of eight treatments arranged in a completely randomized design: (i) plants with application of Si and Chi, inoculated with *R. solanacearum* (Si, Chi, Rs), (ii) plants with Si, without Chi, inoculated with *R. solanacearum* (Si, Rs), (iii) plants with Chi, without Si, inoculated with *R. solanacearum* (Chi, Rs), (iv) plants with Si and Chi, without *R. solanacearum* inoculation (Si, Chi), (v) plants with Si, without Chi, without *R. solanacearum* inoculation (Si), (vi) plants with Chi, without Si, without *R. solanacearum* inoculation (Chi), (vii) plants without Si and Chi, inoculated with *R. solanacearum* (Rs) and (viii) plants without Si, without Chi and without *R. solanacearum* (control).

Three plants per treatment were randomly selected at 72 h post inoculation (hpi) for RNA extraction and gene expression profiling. RNA extraction for the microarray experiment was performed using 500 mg midstem tissue collected from 3 plants and considered as one biological replicate. Further three plants were selected for bacteria quantification and Si analysis. Ten plants were kept for assessing plant development and symptom evaluation over four weeks post inoculation. Three independent repetitions were carried out.

### 2.4. Inoculation and quantification of bacteria and symptom evaluation

Inoculation of plants with bacteria and bacteria quantification in midstems were performed as described in Wydra and Beri [31]. A highly virulent strain of *R. solanacearum*, To-udk2, race 1 biovar 3, phylotype I from Thailand was used for inoculation. Inoculum was obtained by cultivating bacteria colonies in Petri dishes on NGA agar medium containing: 5% Bacto peptone, 0.25% D-Glucose, 0.3% beef extract and 1.5% agar per liter double-distilled water ($ddH_2O$). Cell cultures were incubated at 30 °C for 48 h and then harvested from the agar plates by flooding Petri dishes with distilled water. Suspensions were adjusted to an optical density of 0.06 at 660 nm wavelength (Spectronic 20 Bausch and Lomb) corresponding to approximately $10^8$ colony-forming units per milliliter (CFU/ml) and diluted five times. Four-week old plants were inoculated by soil drenching with 30 ml of the bacterial suspension per pot.

*R. solanacearum* was quantified in mid-stems (5 cm sections) of inoculated plants by first weighing, sterilizing with 70% alcohol for 20 s, rinsing with $sdH_2O$ and macerated in 3 ml $sdH_2O$. The macerate was filtered through cotton tissue and the suspension centrifuged for 15 min at 1300 × g at room temperature. The obtained pellet was resuspended in 1 ml sterile demineralized water. Tenfold serial dilution was prepared and 0.1 ml of each dilution plated on two replicates of TTC medium and incubated for 48 h at 30 °C. Bacterial colonies were counted and calculated as colony forming units per gram fresh matter (CFU/g FW).

Disease severity was assessed daily using a disease score based on 10 plants per treatment. The evaluation started when the first symptoms were observed on the leaves and continued until 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 four leaves excluding the tip and 5 = wilting of the whole plant (death). Wilt incidence was recorded daily and calculated as a percentage of dead plants at the evaluation date out of the total plants in a treatment.

The area under disease progress curve (AUDPC) for each plant per treatment and experiment was calculated on the basis of disease severity and wilt incidence using the trapezoidal integration of the disease progress curve over time with the following formula [34]:

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

where $x_i$ and $x_{i-1}$ are the mean disease severity or wilt incidence at time $t_i$ corresponding to days post 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 ten plants in each treatment. The shoot dry weight including surviving and dead plants was determined one month after inoculation for inoculated and non-inoculated plants treated with Si and Chi by drying plant material at 65 °C over 5days.

### 2.5. Silicon analysis

Total silicon content in roots was determined at 3 dpi by spectrophotometry according to the method developed by Novozamsky et al. [35], modified by the Institute for Plant Nutrition, University of Hannover. Plant materials were dried at 80 °C and ground in a Mikro-Dismembrator S (Sartorius AG, Germany) at 2200 rpm for 90 s. Concentration of Si in the tissue sample was determined by digesting 10 mg of the grounded material with 500 μl mixture of 1 M HCl and 2.3 M HF at the rate 1:2 and then shaken overnight. Samples were centrifuged at 10,000 × g for 10 min and 20 μl of the supernatant retained. 250 μl of 3.2% $H_3BO_3$ was added and the mixture shaken again overnight. 250 μl of colour-reagent consisting of a mixture of 0.08 M $H_2SO_4$ and 20 g/l $(NH_4)_6Mo_7 \times 4H_2O$ at the rate 1:1 was added and incubated for 30 min. 250 μl of 33 g/l tartaric acid and 250 μl of 4 g/l ascorbic acid were added and sample absorbance measured at 811 nm in a spectrophotometer (Micro Quant, Biotech, USA).

### 2.6. RNA extraction

Total RNA was isolated from plant midstems by grinding them into powder in liquid nitrogen. 200 mg powder was homogenized in 1 ml TRIzol® reagent (Invitrogen) and the insoluble material removed from the homogenate by centrifugation at 12,000 × g for 10 min at 4 °C. The mixture separates into a lower red phenol-chloroform phase, an interphase and a colorless upper aqueous phase containing the RNA. RNA was precipitated from the aqueous phase by addition of 200 μl chloroform, then centrifuged again at 13,000 × g for 5 min at 4 °C. Resulting supernatant was discarded and the pellet briefly air-dried for 5 min followed by dissolving in 200 μl RNase free water. RNA concentration was measured using Nanodrop ND1000 spectrophotometer and quality verified by the Agilent 2100 bioanalyzer (Agilent).

### 2.7. Complementary DNA (cDNA) synthesis

The cDNA was synthesized and labeled according to MEN Micromax TSA labeling and detection kit (PerkinElmer). In 8 μg of total RNA, 2 μl dNTPs reaction mix, 1 μl biotin-nucleotide or fluorescein-nucleotide, 1 μl oligo dT (100 μM), 1 μl random hexamer (100 μM) were added and denatured at 65 °C for 10 min followed by incubation for 10 s on ice. For reverse transcription, 5 μl 5X reaction buffer, 3 μl dithiothreitol (DTT) and Superscript III (RNase inhibitor mix) were added and the mixture incubated at 42 °C for 2 h. 2.5 μl 0.5M EDTA (pH 8.0) and 2.5 μl 1 N NaOH was added and incubated at 65 °C for 30 min. 6.5 μl 1M Tris–HCl (pH 7.5) was added and the labeled cDNA purified with QIAquick PCR purification kit (QIAGEN).

### 2.8. Labeling for microarray

Transcriptome profiling was performed according to Heine et al. [36] using TOM2 microchip containing oligonucleotides (72 nucleotide length) corresponding to ~12,000 unigenes. TOM2 microchips were rehydrated over a 65 °C water bath for 2 min and treated with UV radiation at 65 mJ for 2 min. The mixture of Top-Block and labeled cDNAs from two different treatments each labeled with biotin or fluorescein nucleotide was applied to the pre-hybridized microchip, covered with a glass cover and shake-incubated at 42 °C and 650 rpm overnight. The microchips were uncovered, washed with saline sodium citrate buffer and sodium dodecyl sulfate and the spotted areas framed with ImmEdge™ pen. 300 μl TNB-G blocking buffer (0.1 M TrisHCl, 0.15 M NaCl, 0.5% blocking reagent, 10% Goat serum) was applied and incubated for 10 min followed by washing in TNT (0.1 M TrisHCl, 0.15 M NaCl, 0.05% Tween 20). The first and second conjugation steps were performed by adding 200 μl anti-Fl-HRP (horseradish peroxidase) conjugate solution (2 μl anti-Fl-HRP dissolved in 198 μl TNB-G) and 200 μl of streptavidin-HRP conjugate solution (2 μl streptavidin-HRP conjugate dissolved in 198 μl TNB-G), respectively, followed by 10 min incubation and washing in TNT buffer. In the detection steps, 250 μl cyanine-3-tyramide (0.5 μl Cy3 dissolved in 249.5 μl amplification diluent) and 250 μl cyanine-5-tyramide (0.5 μL Cy5 dissolved in 249.5 μl amplification diluent) were laid on in the first and second steps, respectively, followed by 10 min incubation and washing 3 times in TNT buffer for 5 min. HRP inactivation in the first step was carried out by applying 300 μl of inactivation solution (10 μl 3 M Sodium acetate, pH 5.2, 100 μl 35% $H_2O_2$ and 190 μl $ddH_2O$) and washing 3 times in TNT buffer. Microchips were washed in pre-heated (42 °C) saline-sodium citrate buffer and in $dH_2O$ for 1 min, dried by centrifugation at 1200 rpm and scanned at 635 and 532 nm wavelength with two different laser powers using the GenePix 4000B scanner (Axon Instruments, Inc., Union City, CA). The experiment was repeated three times from three independent trials and each replicate repeated twice in a dye-swap design to reduce technical error. Microchips images were extracted and quantified using GenePix Pro 6.0 software, normalized by the sum of all corresponding spot intensities and data generated from different scans for each individual spot averaged by the mean. A locally weighted linear regression was executed as a normalization method in order to account for intensity dependent effects. The $\log_2$ of expression ratio ($\log_2$ fold change) was calculated from the mean of data for each gene on two respective microarrays and gene regulation assessed using Student's *t*-test. Significantly regulated genes were annotated and functionally classified using TED (Tomato expression database), BLAST (Basic local alignment search tool), SGN (Solanaceae genomics network) and TAIR databases (The Arabidopsis information resource).

### 2.9. Statistical analysis

The Statistic Analysis System (SAS, for Windows, 1999–2001) program was used for analysis of variance (ANOVA) followed by Tukey test at 5% for means separation. Data of bacterial numbers

were log-transformed and significance difference was tested by Tukey test $\alpha = 0.05$.

## 3. Results

### 3.1. Symptom development

Bacterial wilt symptoms developed fast in genotypes King Kong 2 and L390 with a wilt incidence of 70% and 80%, respectively in plants without Si or Chi treatments at 14 days post inoculation (dpi). Plants amended with both Si and Chi showed a slower disease development and a final reduction in wilt incidence at 28 dpi by 74.7% in King Kong 2 and 46.6% in L390. Also single application of Si and Chi resulted in reduction of disease incidence and severity (Fig. 1, Fig. 2). Wilt symptom appearance in Si and Chi treated plants was delayed by three days and five days in King Kong 2 and L390, respectively, as compared to non-treated plants inoculated with *R. solanacearum*. The effects of Si and Chi treatment against *R. solanacearum* were significantly higher ($p \leq 0.001$) in combined than of single treatments (Fig. 1).

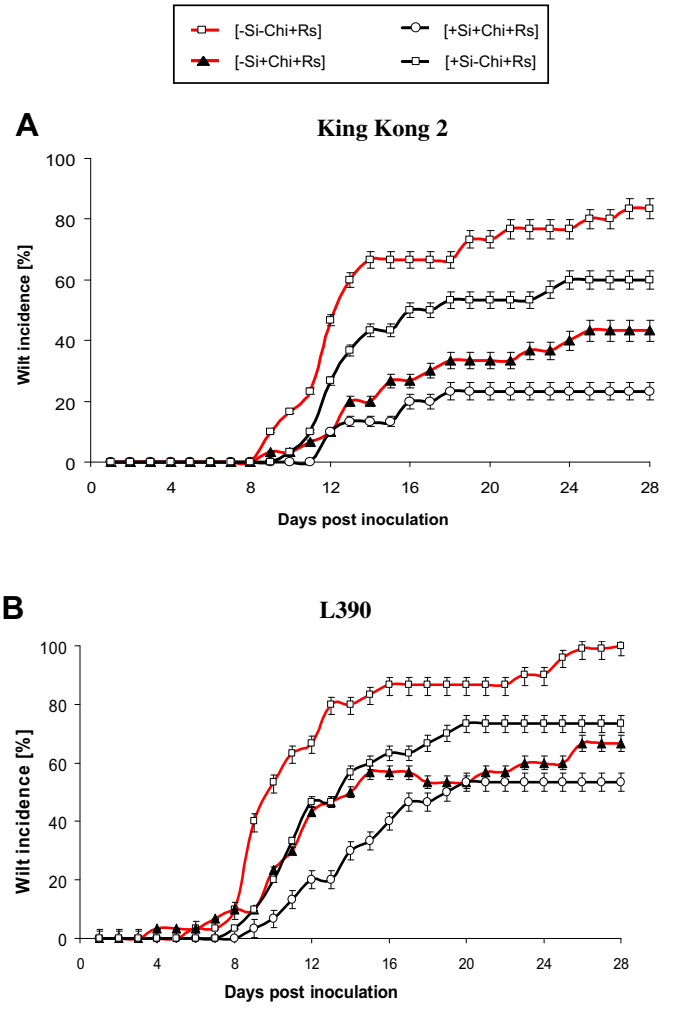


**Fig. 1.** Bacterial wilt symptom development expressed as wilt incidence. (A) King Kong 2 (moderately resistant) and (B) L390 (susceptible) over 28 days after inoculation in Si and Chi-treated [+Si+Chi+Rs], Si-treated [+Si−Chi+Rs], Chi-treated [−Si+Chi+Rs] and non-treated [−Si−Chi+Rs] plants inoculated with *R. solanacearum*. No further changes occurred after 28 days. Data points are means of 10 plants per treatment for three independent trials. Wilt incidence was calculated as percentage of dead plants (disease class 5) in a treatment.

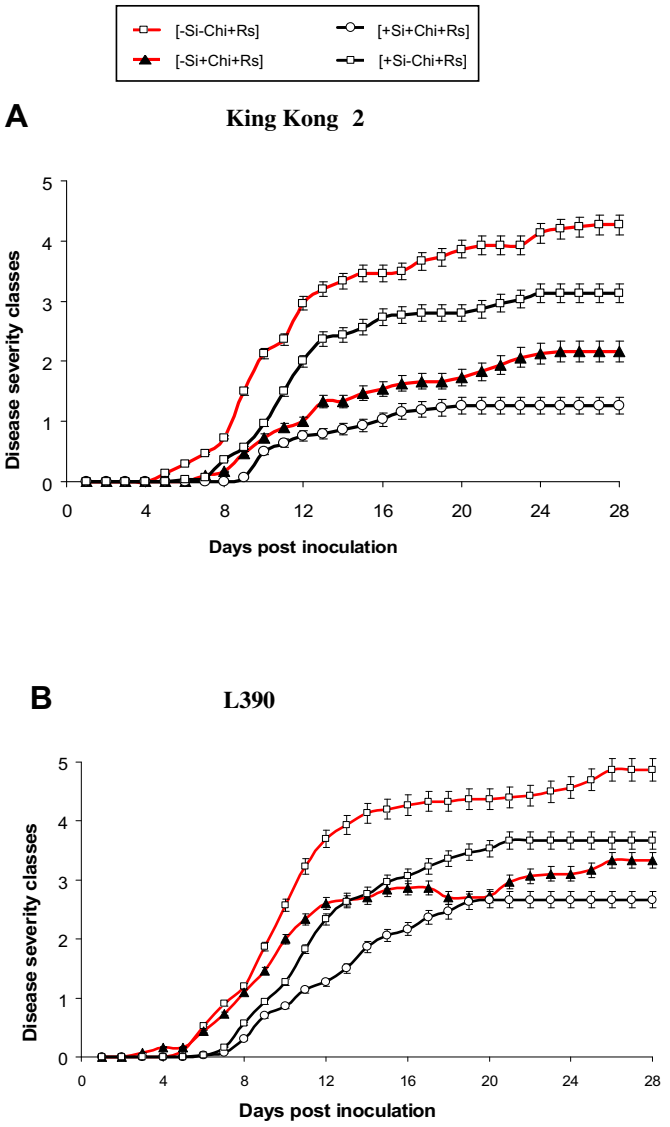


**Fig. 2.** Bacterial wilt symptom development expressed as disease severity. (A) King Kong 2 (moderately resistant) and (B) L390 (susceptible) over 28 days after inoculation in Si and Chi-treated [+Si+Chi+Rs], Si-treated [+Si−Chi+Rs], Chi-treated [−Si+Chi+Rs] and non-treated [−Si−Chi+Rs] plants inoculated with *R. solanacearum*. No further changes occurred after 28 days. Data points are means of 10 plants per treatment for three independent trials. Disease severity is defined as the average of disease classes of all plants in a treatment at a given assessment date.

At 28 dpi, the majority of plants (80%) of genotype King Kong 2 without treatment, inoculated with *R. solanacearum* reached the severity class 4.2, while only few plants (23%) with combined Si and Chi treatment reached severity class 1.2. In genotype L390, plants treated with neither Si nor Chi, inoculated with *R. solanacearum* reached severity class 4.9 (100% of plants) at 28 dpi, while with combined application the severity class of 2.6 (53% of the plants) was lower than in single applications (Fig. 2).

### 3.2. Bacterial population in stems

The bacterial number in midstems at 3 dpi calculated and expressed as log CFU/per gram of fresh matter was significantly reduced in combined application of Si and Chi in both genotypes King Kong 2 and L390 in comparison to non-treated, *R. solanacearum* inoculated plants (Table 1). A non-significant reduction of bacteria numbers was observed in single application

**Table 1**
Bacterial population in midstems in CFU/g fresh weight at 3 dpi in Chi and Si-treated [+Si+Chi+Rs], Si-treated [+Si−Chi+Rs], Chi-treated [−Si+Chi+Rs] and non-treated [−Si−Chi+Rs] plants in tomato genotypes King Kong 2 and L390 inoculated with *R. solanacearum*.

| Genotype | Treatment | Bacterial numbers log(CFU)/g][a] at 3 dpi in midstems |
|---|---|---|
| King Kong 2 | [+Si+Chi+Rs] | 4.8 ± 1.16 aA[b] |
| | [+Si−Chi+Rs] | 7.1 ± 1.48 bA |
| | [−Si+Chi+Rs] | 7.9 ± 0.61 bA |
| | [−Si−Chi+Rs] | 8.7 ± 1.31 bA |
| L390 | [+Si+Chi+Rs] | 6.0 ± 0.47 aB |
| | [+Si−Chi+Rs] | 7.7 ± 1.50 aA |
| | [−Si+Chi+Rs] | 8.2 ± 0.35 aA |
| | [−Si−Chi+Rs] | 9.9 ± 0.46 bA |

[a] Bacterial numbers quantified as colony forming units (CFU/g fresh weight) of the plant organ (midstems) following log transformation.
[b] Means followed by same letter are not significantly different based on Tukey test ($p \leq 0.05$). Small letters refer to comparison between treatments for the same genotype whereas capital letters refer to comparison between genotypes for the same treatment. Values are means of three independent trials ± standard errors.

of Si and Chi. The observed variation in bacteria counts between single plants reflected the typical expression of partial resistance against *R. solanacearum*, with some plants showing high colonization and severe symptoms including plant death, and others only low colonization without symptoms.

### 3.3. Silicon content in roots

Si treatment led to an increase in Si accumulation in plant roots by up to 0.71 mg/g dry weight in genotype King Kong 2 and 0.98 mg/g dry weight in genotype L390 at 3 dpi in comparison to Si non-amended plants, which only accumulated a Si concentration of 0.13–0.24 mg/g dry weight in King Kong 2 and 0.14–0.25 mg/g dry weight in genotype L390 (Table 2).

### 3.4. Shoot weight

Shoot dry matter was significantly higher in combined Si and Chi-treated, *R solanacearum*-inoculated plants in comparison to non-treated plants in genotypes King Kong 2 by 72.8% and in L390 by 57.7% (data not shown). In single applications, only Si-treated plants showed a higher dry weight.

**Table 2**
Si concentration (mg/g dry weight) in tomato genotypes King Kong 2 (moderately resistant) and L390 (susceptible) cultivated in peat substrate amended with Si 3 days after inoculation with *R. solanacearum*.

| Genotype | Treatment | Silicon concentration [mg/g dry weight] 3 dpi in roots |
|---|---|---|
| King Kong 2 | [+Si+Chi+Rs] | 0.55 ± 0.21 aA[a] |
| | [−Si+Chi+Rs] | 0.13 ± 0.02 aA |
| | [+Si−Chi+Rs] | 0.71 ± 0.28 bA |
| | [−Si−Chi+Rs] | 0.24 ± 0.12 aA |
| | [+Si+Chi−Rs] | 0.27 ± 0.10 aA |
| | [−Si+Chi−Rs] | 0.19 ± 0.02 aA |
| | [+Si−Chi−Rs] | 0.36 ± 0.17 aA |
| | [−Si−Chi−Rs] | 0.13 ± 0.05 aA |
| L390 | [+Si+Chi+Rs] | 0.98 ± 0.18 bA |
| | [−Si+Chi+Rs] | 0.25 ± 0.12 aA |
| | [+Si−Chi+Rs] | 0.79 ± 0.22 bA |
| | [−Si−Chi+Rs] | 0.14 ± 0.02 aA |
| | [+Si+Chi−Rs] | 0.47 ± 0.35 aA |
| | [−Si+Chi−Rs] | 0.13 ± 0.02 aA |
| | [+Si−Chi−Rs] | 0.50 ± 0.23 aA |
| | [−Si−Chi−Rs] | 0.11 ± 0.01 aA |

[a] Means followed by same letter are not significantly different based on Tukey test ($p < 0.05$) for comparison of means. Small letters vertically refer to the comparison between treatments for the same genotype. Capital letters horizontally refer to comparison between genotypes for the same treatment. Values are means of three independent trials ± standard errors. Increase in Si accumulation in plant organ (roots) per treatment, per genotype compared to non-treated plants.

### 3.5. Gene expression profiling at 72 hpi

Transcriptome profiling performed in stems at 72 hpi in Si and Chi-treated and non-treated tomato genotypes King Kong 2 and L390, inoculated with *R. solanacearum* revealed differential gene regulation with majority of highly regulated genes in King Kong 2, a moderately resistant genotype. In response to Si and Chi treatments, 204 genes were significantly regulated in King Kong 2 compared to non-amended plants challenged with *R. solanacearum*. (Supplementary Table 1). The up- and down-regulated genes in single and combined application of Si and Chi were functionally categorized as defense-related genes, signal transduction, transcription, stress related, metabolism and binding protein (Figs. 4 and 5). In genotype King Kong, 154 genes (75.5%) were up-regulated and 50 genes (24.5%) down-regulated. Comparing between treatments, 104 genes were regulated in combined

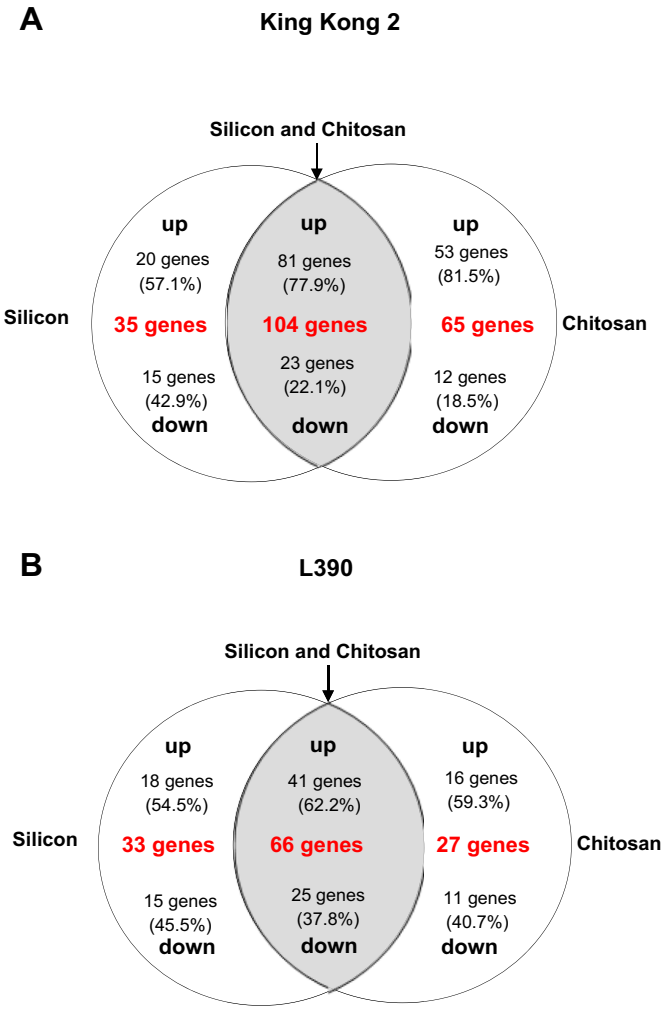


**Fig. 3.** Venn diagram showing the number of commonly up- or down-regulated genes with statistically significant gene expression level changes. (A) Genotypes King Kong 2 and (B) genotype L390 regulated genes in response to single (non-overlapping regions) or combined application of Si and Chi (overlapping regions) relative to non-treated, *inoculated* with *R. solanacearum*. Figures in brackets shows the up- or down-regulated genes expressed as a percentage of total genes regulated in each treatment category.

application of Si and Chi with 81 genes (77.9%) up-regulated and 23 genes (22.1%) down-regulated in this category. The total number of regulated genes in combined application of Si and Chi was higher than in single applications (Fig. 3A).

In susceptible genotype L390, 126 genes were significantly regulated with 75 genes (59.5%) up-regulated and 51 genes (40.5%) down-regulated. Combined application of Si and Chi resulted in regulation of 66 genes, with 41 genes (62.2%) up-regulated and 25 genes (37.8%) down-regulated. Single application of Si and Chi resulted in significant regulation of 33 and 27 genes, respectively (Fig. 3B).

In single application of Si, genes up-regulated upon inoculation with *R. solanacearum* include pathogenesis-related protein 10, disease resistance protein 1, chitinase, disease resistance protein (NBS-LRR class), peroxidase, Jasmonate ZIM domain 3 and zinc finger protein. In single application of Chi, defense-related genes up-regulated include CC-NBS-LRR putative disease resistance protein, chitinases, transducin family protein and xyloglucan-specific fungal endoglucanase inhibitor protein precursor (Tables 3 and 4).

In combined application of Si and Chi, genes up-regulated include translationally-controlled tumor protein homolog (highest up-regulated), pathogenesis-related protein 6, jasmonate ZIM domain 3, Disease resistance protein 1, TIR-NBS-resistance protein, zinc finger protein, etc. Genes involved in transcription and signal transduction such as homeodomain protein containing 'homeobox' domain signature, zinc finger protein, WRKY transcription factor, DNA binding protein, receptor-related serine/threonine kinase and leucine rich repeat protein family were up-regulated in Si and Chi-treated plants.

Interestingly, genes such as ethylene-responsive protein, ubiquitin-protein ligase putative.

F-box family protein, PHD finger transcription factor and cytochrome P450 were up-regulated only in combined application of Si and Chi. Leucin rich repeat protein, homeobox protein, serine/threonine protein kinase were up-regulated only in single application of Si while UDP-glucose 4-epimerase and two beta-D-glucan exohydrolases in single application of Chi. Translationally-controlled tumor protein homolog and WRKY transcription factor were up-regulated in combined application and in single application of Si but not in Chi application.

The gene (SGN-U213440) translationally-controlled tumor protein homolog showed the highest level of up-regulation 13.9-fold observed in Si and Chi-amended plants (Table 3). Jasmonate ZIM-domain protein 3 gene (SGN-U213679) was up-regulated 8.8-fold in treatment with Si. This gene is similar to jasmonate tify (ZIM)-domain containing proteins. Chitinase class 3 protein was up-regulated 13.6-fold and glycosyl hydrolase family 17 was up-regulated 6.3-fold. Both genes are characterized as defense-related genes. Down-regulation of a thioredoxin −8.9-fold and an up-regulation of a thioredoxin peroxidase 7.1-fold were observed, which play an important role in production of reactive oxygen species (ROS). Other gene products regulated variably include pathogenesis-related protein 10 (PR10) and cell wall-related gene beta-galactosidase/glycosyl hydrolase. Combined application of Si and Chi resulted in synergistic up-regulation of various genes including zinc finger protein [11.9-fold], pathogenesis-related leaf protein 6 [8.2-fold], peroxidase [7.9-fold], disease resistance protein 1 [4.6-fold], Jasmonate ZIM-domain protein 3[10.8-fold] and transducin family protein [5.5-fold] (Table 4).

## 4. Discussion

Single and combined application of Si and Chi effectively reduced bacterial wilt in tomato caused by *R. solanacearum*. In our previous studies, the molecular mechanisms underlying silicon-induced resistance (SiIR) was described, revealing the role of Si in priming the plant defense as demonstrated by gene activation in Si-treated plants challenged with *R. solanacearum* [37]. In our present studies, we have shown that the effect of combining two elicitors was greater than in single application. Some mechanisms which may be involved in the synergistic effects of combined Si and Chi application in controlling bacterial wilt were elucidated via gene expression analyses. In this study, defined Chi compound of high molecular weight was utilized as its properties are of paramount importance in determining the characteristics which vary depending mainly on the level of deacetylation and viscosity [38]. For example, You-Jin Jeon et al. [39] reported growth inhibition of Gram-negative and Gram-positive bacteria using a high molecular weight Chi.

In the present study, bacterial wilt development was reduced in genotypes King Kong 2 and L390 in response to Si and Chi application. Similar results were reported for King Kong 2 in Si treated plants in our previous studies [27–29], suggesting the role of Si in enhancing plant tolerance and in resistance induction against *R. solanacearum*. The effects were higher in combined than in single treatments with Si and Chi. Results on bacterial quantification in stems at 3 dpi prior to appearance of the symptoms revealed a higher reduction of bacteria in King Kong 2 treated with combined Si and Chi than in single applications. However, Si amendment in L390 resulted in non-significant reduction in bacterial numbers, but a tendency of increased tolerance was observed manifested in less symptom development in spite of a similar bacterial number, as earlier on reported [27].

The higher effects observed in combined application indicate possible synergistic actions, enabling the plant to react faster and more efficiently to pathogen invasion than after single application. Priming of plant defense in tomato against bacterial wilt by Si has previously been suggested [28,37]. Antibacterial activity of Chi depicted as growth inhibition of bacterial leafspot (*Xanthomonas axonopodis* pv. *poinsettiicola*), crown gall (*Agrobacterium tumefaciens*), and soft rot (*Erwinia carotovora*, now *Pectobacterium carotovorum*) was reported by Mohammed and Badawy [40], and antifungal activity against grey mold (*Botrytis cinerea*), root rot (*Fusarium oxysporum*) and damping off disease (*Pythium debaryanum*) was reported [41,42].

The gene expression analysis at 72 hpi revealed differential up- and down-regulation of genes mainly involved in plant defense signal transduction, transcription and metabolism. This time point was chosen following the results of Ghareeb et al. [37] who determined in time course studies the highest up-regulation at 72 hpi. In the current study, the majority of genes up-regulated in King Kong 2 and L390 treated either with single or combined application of Si and Chi inoculated with *R. solanacearum* were genes involved in plant defense and signal transduction.

Translationally controlled tumor protein [up-regulated 13.9-fold] is reported to have a calcium binding site and interacts with $Na^+$, $K^+$-ATPase as its cytoplasmic repressor [43,44]. TCTP is highly regulated at transcription and translational levels and by a wide range of extracellular signals [45,46]. The expression in plants is not widely reported, but its regulation was observed in rice roots and soybeans exposed to abiotic stress [47]. Functional characterization of TCTP revealed its role in growth and defense response in cabbage (*Brassica oleraceae*) [48]. Databases show that the TCTP gene in *Arabidopsis thaliana* is ubiquitously expressed with high transcript levels found in most tissues [49]. Ubiquitous expression of TCTP is described in eukaryotes, functioning as an essential factor for survival and cell protection under stress conditions, cell cycle progression and apoptosis [45].

The up-regulation of jasmonate ZIM-domain protein 3 (JAZ) in Si-treated genotype King Kong 2 and in L390 confirms our previous results [37]. JAZ is similar to jasmonate tify (ZIM)-domain

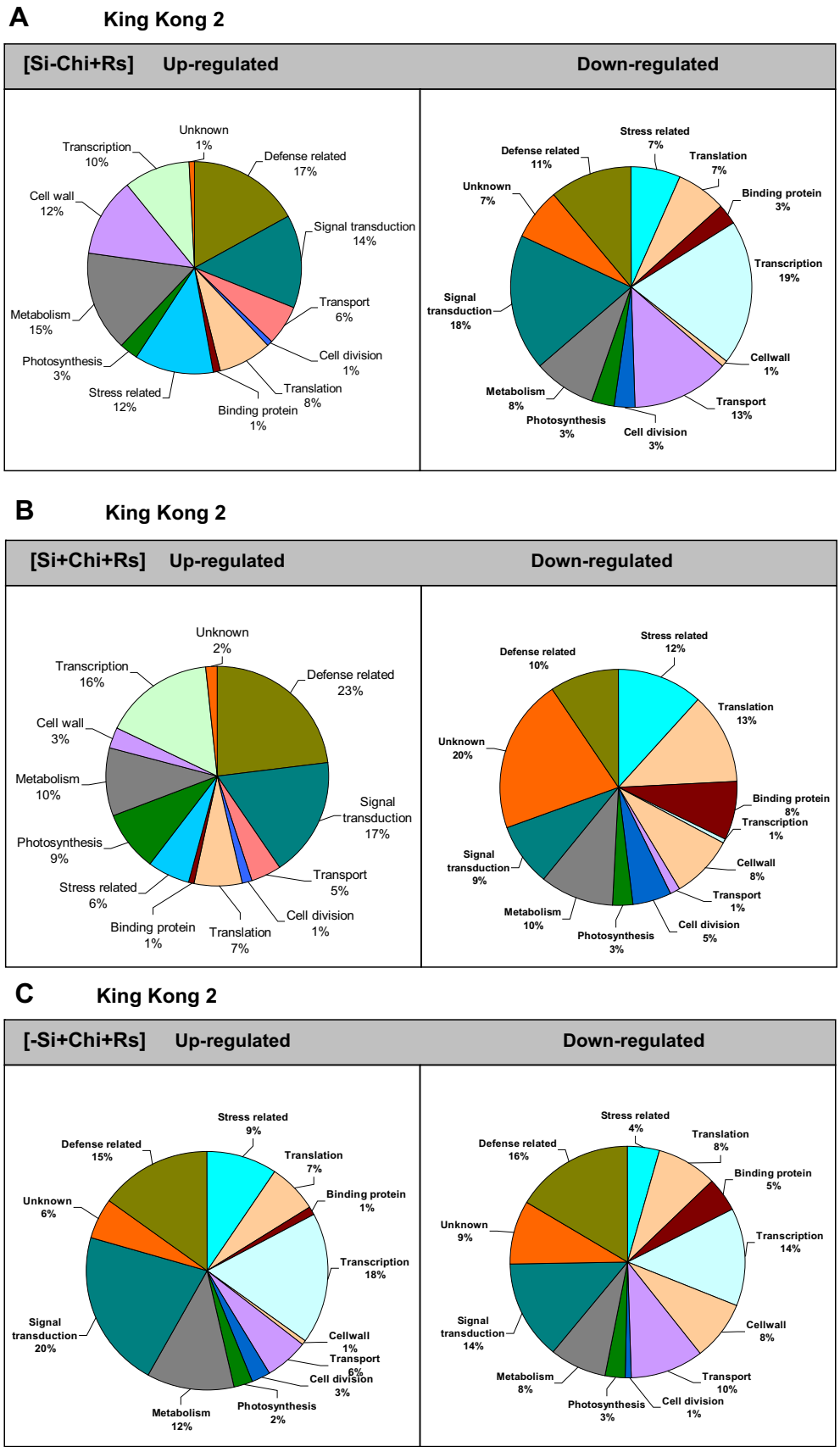


**Fig. 4.** Classification of up- and down-regulated genes in genotype King Kong 2 at 72 hpi. (A) Single application of Si (Si−Chi+Rs), (B) combined Si and Chi application (Si+Chi+Rs) and single application of Chi (−Si+Chi+Rs) challenged with *R. solanacearum*.

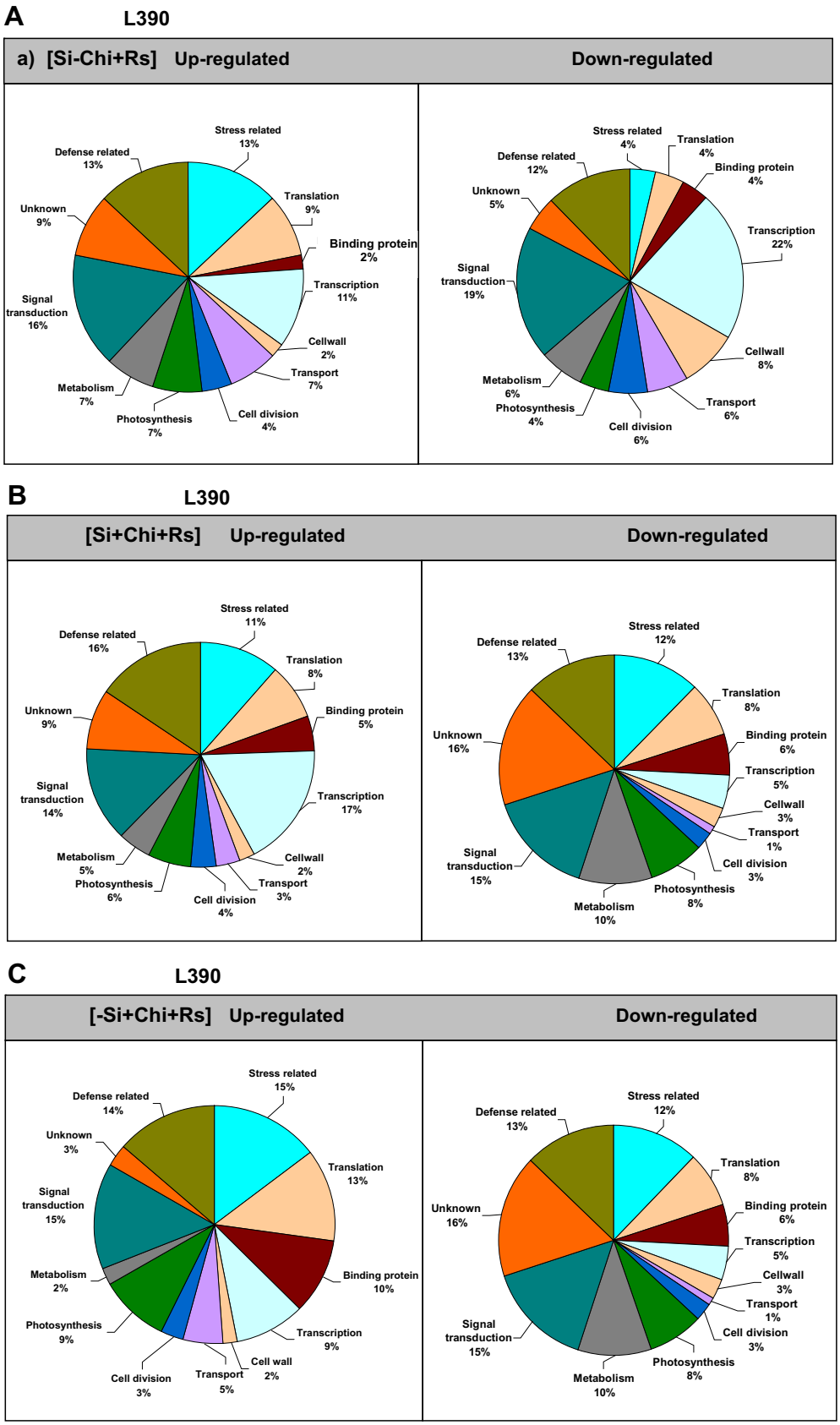


**Fig. 5.** Classification of up- and down-regulated genes in genotype L390 at 72 hpi. (A) Single application of Si (Si−Chi+Rs), (B) combined Si and Chi application (Si+Chi+Rs) and (C) single application of Chi (−Si+Chi+Rs) challenged with *R. solanacearum*.

**Table 3**
Functional classification of significantly up- or down-regulated genes at 72 hpi in genotypes King Kong 2 and L390 treated with Si and Chi compared to non-treated plants challenged with *R. solanacearum*.

| Gene ID | Fold change ($log_2$) | Locus | Annotation | Function | $e^{-value}$[a] |
|---|---|---|---|---|---|
| SGN-U225377 | −8.9 | AAY42864 | Thioredoxin | Defense | $2e^{-48}$ |
| SGN-U225149 | 4.6 | A49332 | Disease resistance protein 1 | Defense | $2e^{-084}$ |
| SGN-U212989 | 6.4 | AAU00066 | Pathogenesis-related protein 10 & 6 | Defense | $1e^{-74}$ |
| SGN-U226627 | 6.3 | NP_190284 | Glycosyl hydrolase family 19 | Defense | $2e^{-140}$ |
| SGN-U222205 | 5.5 | XP002329457 | TIR-NBS-LRR resistance protein | Defense | $4e^{-73}$ |
| SGN-U218657 | 8.3 | AAB08443 | Chitinase class II | Defense | $3e^{-135}$ |
| SGN-U226064 | 7.1 | BAE16559 | Thioredoxin peroxidase 1 | Defense | $2e^{-012}$ |
| SGN-U215595 | 5.1 | BAF44533 | Chitinases class IV | Defense | $3e^{-136}$ |
| SGN-U217904 | 13.6 | AAB08443 | Chitinases class III | Defense | $3e^{-135}$ |
| SGN-U216406 | 5.8 | BAA33065 | Beta-D-glucan exohydrolase | Defense | $1e^{-194}$ |
| SGN-U213764 | 6.3 | NP_200656 | Glycosyl hydrolase family 17 | Metabolism | $4e^{-15}$ |
| SGN-U215711 | 4.8 | XP_002521428 | Beta-galactosidase, putative | Metabolism | 00 |
| SGN-U214589 | 7.8 | AAN87262 | xyloglucan-specific fungal endoglucanase inhibitor protein precursor | Defense | $3e^{-52}$ |
| SGN-U213191 | 5.2 | XP002298678 | Serine hydroxymethyltransferase 1 | Metabolism | $3e^{-252}$ |
| SGN-U235936 | 6.9 | BAG80552 | UDP-glucose:glucosyltransferase | Cell wall | $4e^{-104}$ |
| SGN-U219345 | 6.2 | XP002513688 | Calmodulin-binding heat-shock protein | Stress | $2e^{-65}$ |
| SGN-U215130 | 4.7 | XP002277399 | Zinc finger, RING-type | Signaling | $8e^{-74}$ |
| SGN-U237910 | −5.4 | ABX38720 | ABA 8′-hydroxylase | Signaling | $1e^{-106}$ |
| SGN-U228980 | 7.5 | BAI49996 | 42 KDa chitin-binding protein | Signaling | $3e^{-27}$ |
| SGN-U220746 | 4.9 | NP179618 | Zinc finger (CCCH-type) | Signaling | $5e^{-86}$ |
| SGN-U241645 | 11.9 | NP_565365 | Zinc finger (B-box type) family protein | Signaling | $1e^{-121}$ |
| SGN-U238276 | 5.5 | NP_199205 | Transducin family protein | Signaling | $4e^{-012}$ |
| SGN-U226308 | 10.8 | NP566590 | Jasmonate Zim Domain Protein 3 | Signaling | $4e^{-13}$ |
| SGN-U213440 | 13.9 | Q6DUX3 | Translationally-controlled tumor protein | Signaling | $3e^{-48}$ |
| SGN-U228361 | 10.1 | NP_176918 | Leucine-rich repeat family protein | Signaling | $3e^{-101}$ |
| SGN-U214620 | 7.0 | XP_002520146 | Homeobox protein, putative | Transcription | $1e^{-078}$ |
| SGN-U232642 | 6.7 | NP_191922 | UDP-glucose 4-epimerase | Metabolism | $1e^{-045}$ |
| SGN-U219304 | −3.0 | XP_002519733 | WRKY transcription factor | Transcription | $2e^{-018}$ |

[a] $e^{-value}$ (expected value) expressing number of hits expected when searching a database of a particular size.

containing proteins, which use key regulators (repressors) of the JA-signaling pathway [50]. JAZ family proteins show domain similarity with transcription factors ZIM (Zinc-finger Inflorescence Meristem) which have recently been annotated as tify proteins [49], and are described as a target of the Skp/Cullin/F-box complex ($SCF^{COI1}$), a type of E3 ubiquitin ligase, which forms the COI1-JAZ complex with COI1. JAZ proteins are associated to repression of JA-responsive genes and interact with MYC2, the key transcription activator of JA-regulated gene expression. Therefore, its high expression at 72 hpi might be due to early events in up-regulation of the JA-signaling pathway and a regulation in form of a pulsed response to jasmonate [50]. Regulation of cellular responses to stimuli could also occur at the posttranslational level implying that up-regulation of ubiquitin-protein ligase following Si amendment may also contribute to signaling of defense responses in plant. It was reported to be involved in fine-tuning of the JA-related response through activation of the COI1-JAZ complex [50,51].

Reactive Oxygen Species (ROS), a product of redox reactions in plants are controlled mainly by the thioredoxins and glutaredoxin protein families shown to have a strong similarity [52]. Thioredoxin plays a role in detoxification of ROS through peroxiredoxins [53] and glutathione peroxidases [54]. In our study, down-regulation of a thioredoxin, glutaredoxin and up-regulation of thioredoxin peroxidase were observed. Thioredoxins are small proteins with a redox-active disulfide bridge, which regulate a large number of cellular processes through their redox property. Thioredoxins are reduced by NADP-linked thioredoxin reductase (NTR) [55] and can inhibit programmed cell-death by acting as endogenous regulator. These proteins are antioxidants that function as peroxidases when coupled to the sulfhydryl reducing system [56].

Genes such as glycosyl hydrolase family 3 protein and glycosyl hydrolase family 17 were up-regulated in Chi-treated plants. These genes are widely characterized as defense genes and divided into two families exhibiting different hydrolytic mechanisms. Glycosyl hydrolases (GH) encompass class III and V chitinases belonging to GH family 18 with class I, II and IV chitinases comprising the largest part of the plant chitinases belonging to GH family 19. Chitinases with lysozyme activity utilize bacterial peptidoglycan as an alternative substrate [57]. Pathogenesis related induced chitinases play a role in the active defense mechanisms by hydrolyzing the invading pathogen through disruption of cells or during the hypersensitive response (HR) [57].

The cell wall related genes beta-galactosidase/glycosyl hydrolase, xyloglucan-specific fungal endoglucanase inhibitor protein precursor, UDP-glucose, glucosyltransferase and two beta-D-glucan exohydrolases were up-regulated. Our previous studies on histochemical analysis of the cell wall structure revealed changes in the pectic polysaccharide structures in the resistance reaction and due to Si treatment [28,30,31]. Pathogenesis-related protein 10 (PR10), up-regulated by 6.4-fold is reported to be induced in plants exposed to biotic or abiotic stress conditions, including pathogen infections. PR 10 proteins are also associated to fungal disease resistance [58]. The strongly induced PR10 gene in Chi-treated plants suggests that Chi caused distinct changes in the plant defense response patterns under the present experimental conditions. Increased expression of the WRKY transcription factor culminating in formation of hypersensitive response-like cell death as reported in tobacco by Menke et al. [59] points at involvement of Si in triggering host plant defense. Synergistic gene regulation was observed with combined application of Si and Chi for Jasmonate ZIM-domain protein 3, pathogenesis-related leaf protein 6, peroxidases, transducin family protein and zinc finger protein. The molecular mechanisms underlying the synergistic gene regulation by the two elicitors is not clearly understood. An intense abiotic stimulus from the two elicitors which is recognized by the host cell may lead to an actively orchestrated host plant response involving regulation of defense genes that underlie the synergistic induced resistance. Interestingly, genes such as cytochrome C oxidase,

**Table 4**
Highly up-regulated genes indicative of synergistic effects following combined application of Si and Chi in King Kong 2 ☐ and L 390 ☐ compared to single applications.

| Gene ID | Locus | Fold change [$log_2$] | | | Annotation | $e^{-value*}$ |
|---|---|---|---|---|---|---|
| | | Silicon | Silicon Chitosan | Chitosan | | |
| SGN-U213440 | Q6DUX3 | 3.4 | 13.9 | 0.0 | Translationally-controlled tumour protein | $3e^{-148}$ |
| SGN-U241645 | NP_565365 | 3.6 | 11.9 | 0.7 | Zinc finger (B-box type) family protein | $1e^{-121}$ |
| SGN-U227924 | XP_002510438 | 0 | 7.9 | 0 | NAD dependent epimerase/dehydratase, putative | $1e^{-109}$ |
| SGN-U213679 | ABY58971 | 8.8 | 10.8 | 1.4 | Jasmonate ZIM-domain protein 3 | $9e^{-172}$ |
| SGN-U213371 | Q03663 | 0.5 | 6.8 | 2.1 | Glutathione S-transferase | $8e^{-76}$ |
| SGN-U214589 | ABO36637 | 0 | 5.8 | 2.2 | Defensin | $6e^{-017}$ |
| SGN-U229584 | XP_002524515 | 2.9 | 3.8 | 2.5 | Polynucleotide kinase- 3'-phosphatase, putative | $6e^{-040}$ |
| SGN-U215054 | XP_002518214 | -1.0 | 5.7 | 0 | 6-phosphogluconolactonase, putative | $7e^{-109}$ |
| SGN-U213173 | NP_416134 | 0 | 3.7 | 2.4 | Beta-D-glucuronidase | 0 |
| SGN-U217131 | XP_002529843 | 0 | 6.6 | 0 | Casein kinase, putative | $4e^{-132}$ |
| SGN-U219237 | AAO32065 | 0 | 4.6 | 0 | Erwinia induced protein 1 | $1e^{-182}$ |
| SGN-U217556 | AAF34804 | 0.4 | 3.6 | 0.5 | CDK-activating kinase | $1e^{-155}$ |
| SGN-U238276 | NP_199205 | 0.7 | 5.5 | 1.6 | Transducin family protein | $4e^{-012}$ |
| SGN-U240396 | AAL78821 | 1.7 | 3.5 | 1.8 | Phospholipase D beta 2 | $2e^{-011}$ |
| SGN-U220166 | XP_002515338 | 2.6 | 6.4 | 0.7 | Ring finger protein, putative | $2e^{-64}$ |
| SGN-U218657 | AAB08443 | 3 | 8.3 | 0 | Chitinase, class II | $3e^{-135}$ |
| SGN-U215687 | XP_002511095 | 0 | 6.3 | 0 | Signal recognition particle 68 kDa protein, putative | $4e^{-198}$ |
| SGN-U242331 | ABW74566 | 0.5 | 5.3 | -0.7 | Integrase | $7e^{-023}$ |
| SGN-U221960 | XP_002517716 | 3.4 | 0.0 | -3.4 | serine/threonine-protein kinase | $3e^{-056}$ |
| SGN-U213234 | AAM15775 | 3.0 | 4.3 | 2.4 | MADS-box transcription factor MADS-RIN | $3e^{-105}$ |
| SGN-U213569 | P04284 | 6.4 | 8.2 | 7.1 | Pathogenesis-related leaf protein 6 | $2e^{-093}$ |
| SGN-U217017 | NP_186929 | 1.5 | 4.2 | 1.1 | Phosphatise/ protein tyrosine phosphatise | $2e^{-067}$ |
| SGN-U225855 | XP_002513039 | 0 | 7.1 | 0 | Ubiquitin-protein ligase, putative | $2e^{-055}$ |
| AF385366 | ACM92036 | 3.0 | 3.1 | 1.8 | Violaxanthin de-epoxidase | $6e^{-235}$ |
| SGN-U231278 | XP_002526040 | -1.1 | 6.0 | 1.9 | Hydrolase, putative | $3e^{-088}$ |
| SGN-U231419 | XP_002524999 | 2.0 | 5.0 | 1.4 | Aminoadipate-semialdehyde dehydrogenase, putative | $1e^{-078}$ |
| SGN-218137 | ACB70404 | 6.3 | 5.0 | 7.3 | CC-NBS-LRR putative disease resistance protein | $1e^{-152}$ |
| SGN-U217525 | XP_002529455 | 0.9 | 5.0 | -2.8 | Proline synthetase associated protein, putative | $1e^{-107}$ |
| SGN-U220325 | XP_002308717 | 0 | 5.0 | 0 | f-box family protein | $6e^{-087}$ |
| SGN-U225149 | A49332 | 1.2 | 4.6 | 0 | Disease resistance protein 1 | $2e^{-084}$ |
| SGN-U222205 | XP002329457 | 0.5 | 5.5 | 1.3 | TIR-NBS-LRR resistance protein | $4e^{-73}$ |
| SGN-U219304 | XP_002519733 | 4.8 | 6.2 | -3.0 | WRKY transcription putative | $2e^{-043}$ |
| SGN-U235280 | AAP34571 | 1.9 | 7.1 | 6.9 | Thioredoxin peroxidase 1 | $2e^{-018}$ |
| SGN-U218019 | P20076 | -0.9 | 4.3 | -4.5 | Ethylene-responsive proteinase inhibitor 1 | $1e^{-059}$ |
| SGN-U221397 | NP_188116 | -1.7 | 5.3 | 0.0 | PHD finger transcription factor, putative | $8e^{-041}$ |
| SGN-U222205 | NP_97441 | 0 | 6.9 | 0 | Pseudouridine synthase family protein | $3e^{-095}$ |
| SGN-U213360 | XP_002528199 | 3.2 | 4.9 | 0.9 | Heat shock 70 kDa protein, putative | $2e^{-071}$ |
| SGN-U218650 | ABG29323 | 0 | 4.8 | 0 | Receptor protein kinase, putative | $2e^{-105}$ |
| SGN-U232642 | NP_191922 | 0.0 | -0.8 | 6.8 | UDP-glucose 4-epimerase, putative | $6e^{-064}$ |
| SGN-U213676 | XP_002327136 | -1.3 | 3.6 | -1.3 | Light-harvesting complex I protein Lhca5 | $6e^{-062}$ |
| SGN-U213855 | XP_002298678 | 0 | 5.2 | 1.0 | Serine hydroxymethyltransferase 1 | $3e^{-252}$ |
| SGN-U239075 | XP_002521404 | 0.7 | 4.4 | 3.7 | DNA repair helicase rad5,16, putative | $2e^{-72}$ |
| SGN-U221176 | AAX69063 | 1.7 | 4.4 | 2.9 | Monoterpene synthase 1 | $3e^{-078}$ |
| SGN-U232973 | NP_565711 | 0 | 9.3 | 0 | ATP synthase protein I | $4e^{-007}$ |
| SGN-U213391 | AAG21691 | 0 | 3.3 | 1.1 | Lipoxygenase | 0 |
| SGN-U220329 | XP_002267948 | -0.4 | 5.1 | 3.7 | Similar to phosphatidylserine decarboxylase | $4e^{-155}$ |
| SGN-U215723 | P08216 | 2.6 | 4.0 | 2.4 | Malate synthase, glyoxysomal | $6e^{-285}$ |
| AF263101 | P93841 | 0.9 | 3.0 | 0 | 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase | $5e^{-215}$ |
| SGN-U223537 | NP_564948 | 2.6 | 7.9 | 0.5 | Peroxidase, putative | $1e^{-123}$ |
| SGN-U219622 | CAA71003 | 0.8 | 4.0 | 0.4 | Aldehyde dehydrogenase (NAD+) | $7e^{-011}$ |

[a] $e^{-value}$ (expected value) expressing number of hits expected when searching a database of a particular size.

thioredoxin, pseudouridine synthase family protein and Anthocyanin-O acyltransferase were down-regulated suggesting possible subtractive effects when Si and Chi are applied together. The up-regulation of genes such as *JAZ3* and thioredoxin peroxidase (involved in ROS generation) may give an indication of a prior induction or fine tuning of jasmonic acid and/or reactive oxygen species signaling pathways upon plant infection with the pathogen. Moreover, the majority of up-regulated genes and transcripts classified into defense-related products belong to salicylic acid dependent pathway.

In conclusion, Si and Chi-induced resistance was manifested as high gene regulation after challenging the plants with *R. solanacearum*, indicating priming effects. Phenotypic effects in the plant following induced resistance were manifested in bacterial reduction in plant tissue, reduced wilt incidence and increased shoot dry weight. The observed changes in gene expression pattern may partially explain the reduction in symptom development. Although Si is not recognized as an essential plant mineral element, its beneficial role in plants was often reported, such as conferring protective effects against pests and diseases. Our current study reports for the first time the synergistic effects of Si and Chi against a bacterial disease.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pmpp.2012.11.002.

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