# Silicon-Mediated Tomato Resistance Against *Ralstonia solanacearum* is Associated with Modification of Soil Microbial Community Structure and Activity

Lei Wang · Kunzheng Cai · Yuting Chen · Guoping Wang

Received: 14 December 2012 / Accepted: 14 January 2013 / Published online: 1 February 2013 © Springer Science+Business Media New York 2013

Abstract Bacterial wilt caused by Ralstonia solanacearum is a serious soil-borne disease of Solanaceae crops. In this study, the soil microbial effects of silicon-induced tomato resistance against R. solanacearum were investigated through pot experiment. The results showed that exogenous 2.0 mM Si treatment reduced the disease index of bacterial wilt by 19.18 % to 52.7 % compared with non-Si-treated plants. The uptake of Si was significantly increased in the Si-treated tomato plants, where the Si content was higher in the roots than that in the shoots. R. solanacearum inoculation resulted in a significant increase of soil urease activity and reduction of soil sucrase activity, but had no effects on soil acid phosphatase activity. Si supply significantly increased soil urease and soil acid phosphatase activity under pathogen-inoculated conditions. Compared with the non-inoculated treatment, R. solanacearum infection significantly reduced the amount of soil bacteria and actinomycetes by 52.5 % and 16.5 %, respectively, but increased the ratio of soil fungi/soil bacteria by 93.6 %. After R. solanacearum inoculation, Si amendments significantly increased the amount of soil bacteria and actinomycetes and reduced soil fungi/soil bacteria ratio by 53.6 %. The results suggested that Si amendment is an effective approach to control R. solanacearum. Moreover, Si-mediated resistance in tomato against R. solanacearum is associated with the changes of soil microorganism amount and soil enzyme activity.

L. Wang • K. Cai (⊠) • Y. Chen Key Laboratory of Tropical Agro-environment, Ministry of Agriculture, South China Agricultural University, Guangzhou 510642, China e-mail: kzcai@scau.edu.cn

G. Wang

College of Horticulture, South China Agricultural University, Guangzhou 510642, China

**Keywords** Silicon · Tomato · *Ralstonia solanacearum* · Soil enzyme activity · Soil microorganism

Bacterial wilt caused by Ralstonia solanacearum is a serious soil-borne disease widely distributed in tropical, subtropical, and some warm temperate regions of the world [28]. The pathogen generally enters a plant through the roots, penetrates the xylem, systemically colonizes the stem, and causes wilt symptoms [33]. Traditional controlling methods including resistant varieties, chemical agents, and crop rotation were used to control this pathogen. However, host resistance is easy to lose because of the rapid variation of pathogenic bacteria. Chemical application has limited effects on this pathogen, and may cause negative effects on food safety and the environment. Rational organic amendments may provide a practical, environmentally sound, and economical control strategy [12, 15, 52, 59]. However, some organic composts, such as city compost and livestock and poultry organic fertilizer, may contain heavy metal, antibiotics, and hormone, which restrict their practical applications.

Silicon is the second most abundant mineral element in the earth's crust [17]. A number of studies have indicated that Si can enhance the resistance of plants to various diseases [7, 12, 15, 20, 39]. Si-treated plants show higher resistance to pathogen penetration of host tissue because of the specific accumulation and polymerization of Si(OH)<sub>4</sub> in the cell walls [8, 29]. Si may also activate a series of biochemical defense responses to increase host resistance, including the increased antioxidant enzyme activities and the production of antifungal compounds such as phenolic metabolism product and phytoalexins, etc. [7, 20]. In tomato, the beneficial effects of silicon in prohibiting *R. solanacearum* development were also studied [12, 15]. Recent studies showed that Si could induce the production of resistance signal molecules, defense, signal transduction, and resistance-related genes and housekeeping genes in *R. sol-anacearum*-infected treatments [22, 23].

Pathogen infection may alter soil microbial community structure and influence the component and amount of soil microorganisms, transferring the soil from high-fertility "bacteria type" to low-fertility "fungi type" [34, 55]. The sensitivity of soil microorganisms to changes in soil conditions can reflect soil health status and act as indicators of soil quality [32]. Previous studies of Si-mediated pathogen resistance have mostly concentrated on aboveground-induced resistance [12, 15, 22, 23]. To our knowledge, no related studies focusing on the soil microbial effects of Si supply on bacterial wilt resistance have been reported. The objectives of this study were to investigate the impacts of Si amendment on soil microbial population densities and microbial activity in *R. solanacearum*-infected treatments in tomato.

## **Materials and Methods**

# Plant Materials and Growth Conditions

Tomato genotype Taiwan Red cherry (susceptible to R. solanacearum) was used throughout the experiment. Tomato seeds were surface-sterilized in water at 50 °C for 15 min, germinated on moist filter paper for 2 days in Petri dishes, and then sown in nursery soil (with nutrition soil and organic fertilizer ratio of 3:1). Tomato seedlings were grown in a growth chamber at 30 °C/25 °C (day/night) with a photoperiod of 14 h and a light intensity of 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. After 5 weeks of germination, the seedlings were transplanted to a polyethylene plastic pot (170 mm diameter×165 mm height) filled with 2 kg of soil. The soil was collected from a field with continuous cropping cultivation of tomato in Zhucun village, Zengcheng city, Guangdong province, China. The content of soil organic matter and soil-available N, P, K, and Si were 16.04 g kg<sup>-1</sup>, 67.24 mg kg<sup>-1</sup>, 110.8 mg kg<sup>-1</sup>, 49.94 mg  $kg^{-1}$ , and 31.02 mg  $kg^{-1}$ , respectively. Approximately 95.7 mg of urea, 235.7 mg of superphosphate, and 48 mg of potassium chloride were added per kilogram of soil before transplanting to meet the nutrient demand for tomato plant growth.

#### Experimental Design

The following four treatments were used in this experiment: no Si addition and no *R. solanacearum* inoculation (CK), Si addition (Si), *R. solanacearum* inoculation (Rs), and Si addition and *R. solanacearum* inoculation (Rs+Si). The experiment was arranged in a completely randomized design with 10 replications. Our preliminary experiment using different Si concentrations showed that 2.0 mM of Si exhibited the best effects in inhibiting bacterial wilt. Thus, 2.0 mM of Si was used in this experiment. Si was added as potassium silicate ( $K_2SiO_3$ ) to the soil before seedling transplantation. In the Si-deficient treatment, potassium chloride (KCl) was used to replenish potassium. After 15 days of *R*. *solanacearum* inoculation, all treated plants were harvested, and then divided into shoots and roots to measure Si content. Soil from different treatments were also collected to determine soil-available Si content, soil urease activity, soil acid phosphatase activity, soil sucrase activity, soil microbial population densities including soil bacteria, fungi, and actinomycetes, as well as the *R. solanacearum* content in soil.

#### R. solanacearum Inoculation

A highly aggressive strain of *R. solanacearum*, which was kept in our laboratory and has been determined to be race 1 biovar 3, was used to inoculate tomato plants. The bacteria were grown on CPG medium [33] for 48 h at 30 °C. The cells were harvested from agar plates by water flushing [15] and adjusted to  $OD_{600}=0.3$  (about  $3 \times 10^8$  CFU mL<sup>-1</sup>). Tomato plants were inoculated with *R. solanacearum* by cutting their roots and injecting the inoculum suspension (about  $3 \times 10^8$  CFU mL<sup>-1</sup>, 15 mL per pot). The non-inoculated plant roots were also cut and injected with the same volume of distilled water.

### Pathogen Symptom Evaluation

Disease development was evaluated every 2 days after pathogen infection using a disease score based on 10 plants per treatment according to the method [18]. The evaluation started when the first symptoms appeared on the leaves, and was continued until the symptoms were stable. The following scoring was used: 0=no symptom, 1=one leaf wilted, 3=two or three leaves wilted, 5=all except the top leaves wilted, 7=all leaves wilted, and 9=stems collapsed or plants died.

Disease index(%) = 
$$\left[\sum{(r \times N_r)/(R \times n)}\right] \times 100\%$$

where r is the mean disease severity,  $N_r$  is the number of infected plants with a rating of r, R is the value of the most serious disease severity in each treatment, and n is the total number of plants tested.

Determination of Si Content in Roots, Leaves, and Soil

Si content in tomato roots and leaves was determined according to the method described by van der Vorm [53]. Briefly, 0.1 g of leaf (or root) samples was ashed in porcelain crucibles for 3 h at 550 °C. The ash was dissolved in 1.3 % hydrogen fluoride, and the Si content in the solutions

was measured through colorimetric molybdenum blue method at 811 nm with a spectrophotometer (PGENERAL TU-1901 UV–VIS, Beijing, China).

Soil-available Si was extracted by citric acid with minor modifications [2]. About 10 g of air-dried, finely sifted soil (passed through a 2-mm sieve) was added to a plastic bottle with a volume of 250 mL. Approximately 100 mL citric acid was added, and then the plastic bottle was shaken and incubated (GXZ Intelligent; JiangNan Instrument Plant, China) at  $30\pm0.1$  °C for 5 h. Afterward, 5 mL of filtered fluid was taken to determine the Si concentration in the solutions by colorimetric molybdenum blue method at 811 nm with a spectrophotometer [UV-2501(pc)s 220 V; Shimadzu, China].

# Determination of Soil Enzyme Activity

Soil urease, soil phosphatase enzyme, and soil sucrase activities were selected to determine soil microbial activity.

Soil urease activity was determined using the method described by Yao and Huang [57], with minor modification. About 5 g of air-dried, finely sifted soil (passed through a 1mm sieve) was added to a 25-mL volumetric flask. Approximately 1 mL of toluene was added to the flask after 15 min, and then 10 mL of 10 % urea solution and 10 mL of citrate buffer (pH6.7) were added. The flask was shaken and then placed in an incubator (GXZ Intelligent; JiangNan Instrument Plant) at 37±0.1 °C for 24 h. After incubation, the sample was filtered through a quantitative filter paper. Subsequently, 3 mL of filtrate, 17 mL of deionized water, 4 mL of sodium phenate solution, and 3 mL of sodium hypochlorite solution were added to a 50-mL volumetric flask. After 20 min, deionized water was added to the flask to reach 50 mL volume in the test tube. Finally, soil urease activity was colorimetrically determined at 578 nm with a UV spectrophotometer [UV-2501(pc)s 220 V; Shimadzu] within 1 h.

Only acid phosphatase enzyme activity was measured in this study because the soil used was acidic, and acid phosphatase is the main phosphatase enzyme [16]. The activities of soil acid phosphatase were assayed on 1 g of oven-dry equivalents of buffered soil solutions incubated for 1 h at 37 °C after the addition of the enzyme-specific substrate solution. The product of all reactions, *p*-nitrophenyl phosphate, was colorimetrically measured at 412 nm on a UV spectrophotometer [51].

Soil sucrase activity was assayed according the method described by Guan et al. [26]. Briefly, 5 g of air-dried soil (sieved to <1 mm), 15 mL of 8 % glucose solution, 5 mL of 0.2 M phosphate buffer (pH5.5), and five drops of toluene were added to a 25-mL volumetric flask. After incubation for 24 h at 37 °C, the soil solution was filtered and a 1-mL aliquot was transferred to a volumetric flask with 3 mL of 3, 5-dinitrylsalicylate, and then heated for 5 min. After the solution reached room temperature, the product was

colorimetrically quantified at 508 nm using a spectrophotometer [UV-2501(pc)s 220 V; Shimadzu].

## Determination of the Densities of Soil Microbial Population

The amount of soil bacteria, fungi, and actinomycetes were determined via dilution plate method [25, 41], with minor modification. The media for soil bacteria, fungi, and actinomycetes were beef-protein medium, potato sucrose agar medium, and Gause 1 culture medium, respectively. About 5 g of air-dried soil was added to a flask with 50 mL of sterile water, and then the flask was shaken using a shaking table (ASH-202P shaker; Abbot Corporation, USA) for 20 min. About 0.5 mL of supernatant fluid was added to a tube equipped with 4.5 mL of sterile water. The solution was diluted to  $10^{-6}$ , and 0.1 mL of the diluted solution was taken and coated in the corresponding medium, which was then placed in an incubator (GXZ Intelligent; JiangNan Instrument Plant) at  $28 \pm 0.1$  °C. The bacteria, fungi, and actinomycetes were cultured for 2, 4, and 6 days, respectively. After incubation, the colony number was recorded to count the densities of different microbial populations.

The amount of *R. solanacearum* in the soil was determined using the plate method, with some modifications [38]. About 5 g of soil was collected at 15 days after pathogen inoculation, and then diluted to  $10^{-4}$  using 1:10 gradient dilution method. The soil-suspending liquid was coated by 100 µL TTC, and then incubated for 48 h at  $30\pm$ 0.1 °C using thermostatic cultivation (GXZ Intelligent; JiangNan Instrument Plant). The method of plate culture count is used to record the amount of soil bacterial wilt.

#### Statistical Analysis

All the data in the figures were expressed as the means  $\pm$  standard error of four replicates and analyzed by ANOVA using SPSS13.0 (Statistical Analysis Systems Institute, version 13.0; SPSS Inc., Chicago, IL, USA). Statistical differences among treatments were determined by Duncan's test (P < 0.05) and t test ( $P \le 0.05$ ).

#### Result

## Disease Index

Bacterial wilt symptoms developed fast and were observed at 5 to 6 days post-inoculation (dpi). Tomato plants treated with 2 mM Si had significantly lower disease indexes compared with the no-Si-treated control (Pi-1) lines (Fig. 1). Si application reduced the disease index of bacterial wilt by 52.7 %, 19.18 %, 32.10 %, and 39.2 % at 7, 9, 11, and 13 days post-inoculation, respectively. **Fig. 1** Effects of silicon supply and *R. solanacearum* inoculation on the disease index (%) of bacterial wilt in tomato plants. *Rs: R. solanacearum* inoculation only, Rs+Si: *R. solanacearum* inoculation and Si 2.0 mM application. The values are means±standard error with four replicates. Two *asterisks* on a column denote a significant difference at P < 0.01by using *t* test



## Silicon Concentration in Soil, Roots, and Shoots

Soil-available Si content in Si-treated tomato plants was significantly higher regardless of pathogen inoculation (Fig. 2). Si treatment increased soil available Si content by 15.18 % in the non-inoculated treatments and by 18.0 % in the inoculated treatments. Si concentration in tomato roots and shoots were also significantly increased in the Si-treated treatments. Si application increased the Si content by 23.87 % and 199.62 % in the roots and shoots of the non-inoculated treatments, and by 9.58 % and 344.82 % in roots and shoots of the inoculated treatments (Fig. 3). Si content was about five to 15 times higher in roots than that in shoots, regardless of Si supply. However, *R. solanacearum* did not have impacts in silicon uptake or distribution.

# Soil Enzyme Activity

Soil sucrase activity was significantly inhibited by *R. solana-cearum* infection. Compared with the non-inoculated treatment, *R. solanacearum* inoculation dramatically decreased



**Fig. 2** Effects of Si and *R. solanacearum* inoculation on soil available Si content. *CK*, *Si*, *Rs*, and *Rs*+*Si* indicate no Si and no inoculation, Si application only, *R. solanacearum* inoculation only, and *R. solanacearum* inoculation and Si application, respectively. The values are the means±standard error with four replicates. Significant differences (P < 0.05, using Duncan's new multiple range tests) among all treatments are indicated by different *letters* above the bars

soil sucrase activity by 76.8 %. Si supply had no effects on soil sucrase activity regardless of pathogen inoculation (Fig. 4a).

In the non-inoculated treatments, Si amendment did not influence soil acid phosphatase activity (Fig. 4b). However, Si application significantly increased acid phosphatase activity by 15.3 % in *R. solanacearum*-inoculated treatments.



**Fig. 3** Effects of Si and *R. solanacearum* inoculation on Si content in roots (**a**) and shoots (**b**). *CK*, *Si*, *Rs*, and Rs+Si indicate no Si and no inoculation, Si application only, *R. solanacearum* inoculation only, and *R. solanacearum* inoculation and Si application, respectively. The values are the means±standard error with four replicates. Significant differences (P<0.05, using Duncan's new multiple range tests) among all treatments are indicated by different *letters* above the bars



**Fig. 4** Effects of Si and *R. solanacearum* inoculation on the activity of soil sucrase (**a**), acid phosphatase (**b**), and urease (**c**). *CK*, *Si*, *Rs*, and Rs+Si indicate no Si and no inoculation, Si application only, *R. solanacearum* inoculation only, and *R. solanacearum* inoculation and Si application, respectively. The values are the means±standard error with four replicates. Significant differences (P<0.05, using Duncan's new multiple range tests) among all treatments are indicated by different *letters* above the bars

Compared with non-inoculated treatment, *R. solanacearum* infection significantly increased soil urease activity by 16.6 % (Fig. 4c). Si supply resulted in a significant increase of soil urease activity, which increased by 14.6 % and 18.5 % under non-inoculated and inoculated treatments, respectively.

#### Amount of R. solanacearum in Soil

*R. solanacearum* infection significantly increased the amount of *R. solanacearum* in soil by 51.41 % (Fig. 5). However, the soil treated with 2 mM Si had significantly lower amount of *R. solanacearum* compared with the non-Si-treated control. The amount of *R. solanacearum* in soil was decreased by 23.2 % because of Si application, which was a value close to the control level.

#### The Densities of Soil Microbial Population

Si supply and *R. solanacearum* inoculation significantly influenced the amount of soil microbial population (Fig. 6a–c). Compared with non-infected soil, the amounts of soil bacteria, fungi, and actinomycetes in *R. solanacearum*-infected treatments significantly were decreased by 52.5 %, 8.5 %, and 16.5 %, respectively. Soil bacteria had the highest reduced amplitude. However, simultaneous Si application and *R. solanacearum* increased the amount of soil bacteria and actinomycetes by 124.9 % and 22.4 %, respectively, compared with the non-Si-treated but inoculated soil. Soil fungi/bacteria ratio in the inoculated treatments was greatly increased by 93.6 %. However, Si application significantly reduced soil fungi/bacteria ratio by 53.8 % in the infected soil (Fig. 7).

# Discussion

Si-mediated plant resistance against different pathogens has been broadly studied in several pathosystems such as blast



**Fig. 5** Effects of Si and *R. solanacearum* inoculation on the population of *R. Solanacearum* in soil. *CK, Si, Rs*, and Rs+Si indicate no Si and no inoculation, Si application only, *R. solanacearum* inoculation only, and *R. solanacearum* inoculation and Si application, respectively. The values are the means±standard error with four replicates. Significant differences (P<0.05, using Duncan's new multiple range tests) among all treatments are indicated by different *letters* above the bars



**Fig. 6** Effects of Si and *R. solanacearum* inoculation on the amount of soil bacteria (**a**), fungi (**b**), and actinomycetes (**c**). *CK*, *Si*, *Rs*, and *Rs*+ *Si* indicate no Si and no inoculation, Si application only, *R. solanacearum* inoculation only, and *R. solanacearum* inoculation and Si application, respectively. The values are the means±standard error with four replicates. Significant differences (P<0.05, using Duncan's new multiple range tests) among all treatments are indicated by different *letters* above the bars

and sheath blight in rice [13, 46], powdery mildew in wheat, cucumber, and *Arabidopsis*, and rust in cowpea [3, 21, 29, 36]. In the current study, the application of 2.0 mM Si significantly reduced the *R. solanacearum* development of tomato plant (Fig. 1). This inhibited effect has also been



**Fig.** 7 Effects of Si and *R. solanacearum* inoculation on component ratio of soil microorganisms. *CK*, *Si*, *Rs*, and *Rs*+*Si* indicate no Si and no inoculation, Si application only, *R. solanacearum* inoculation only, and *R. solanacearum* inoculation and Si application, respectively. The values are the means±standard error with four replicates. Significant differences (P<0.05, using Duncan's new multiple range tests) among all treatments are indicated by different *letters* above the bars

reported by other studies [12, 15]. The mechanism by which Si reduces the incidence of R. solanacearum is still unclear. The current study also found that the lower disease severity in Si-treated tomato plants was in line with the lower amount of R. solanacearum in soil and higher Si content in tomato roots and shoots (Figs. 3 and 5). Therefore, Si uptake and accumulation in roots possibly have an important role in enhancing plant resistance to pathogen. Evidence showed that Si-mediated resistance to pathogen was associated with higher deposition of Si in plants which acted as a mechanical role [5, 27, 48]. More studies have shown that Si can induce host defense response by increasing the level of antifungal phenolic compounds (such as lignin, phytoalexins, chlorogenic acid, and rutin) [19, 45, 46, 49] and the activity of protective enzymes (such as peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase) [7, 10, 36]. Limited information about possible resistance mechanisms in tomato to R. solanacearum mediated by Si is available. Earlier studies suggested that Si had an indirect effect on wilt bacterial growth through induced resistance interacting with resistance factors of the plants [12, 15]. Recent studies have shown that Si-enhanced resistance to R. solanacearum in tomato was associated with the up-regulated expression of defense marker genes (such as jasmonic acid/ethylene marker genes) and housekeeping genes, including phosphoglycerate kinase genes, alpha-tubulin, and actin [22, 23].

However, all these explanations on Si-mediated pathogen resistance mechanism mostly focus on the inducing resistance of aboveground parts. Unlike blast or power mildew, bacterial wilt caused by *R. solanacearum* is a soil-borne disease. The pathogen generally enters a plant through the roots from the soil. Soil is the key issue to be considered in this problem. To our knowledge, no reports have investigated the inhibited function of Si to pathogen from the soil microbial perspective.

Soil microbial activity has an important function in quantifying soil function, such as the C and N cycle and organic matter decomposition [14, 40, 43]. As an integral part of nutrient cycling in soil, soil-specific enzyme activity including dehydrogenase and phosphatase can also be used to estimate soil microbial activity and evaluate soil health [1, 24, 31]. Our study provides evidence that the application of 2.0 mM Si significantly increases soil urease and soil acid phosphatase activity in response to R. solanacearum infection (Fig. 4). An essential aspect of soil health is the ability of the soil to resist soil-borne plant pathogens. This phenomenon is known as general disease suppression and is attributed to the total microbial activity [4, 58]. Hydrolytic enzymes including soil sucrase, urease, and phosphatase can characterize soil carbon, nitrogen, phosphorus, and nutrient cycle condition [54]. Sucrase activity has a good relationship with soil organic matter, nitrogen, phosphorus, microorganism quantity, and soil respiration intensity. The higher the fertility of the soil is, the stronger the sucrase activity [25]. Rasmussen et al. [44] found that activities of  $\beta$ glucosidase and cellobiohydrolase were positively correlated with soil pathogen suppressiveness to Fusarium culmorum in barley seedling. Similarly, Leon et al. [35] found that arylsulfatase activity had good correlation with the suppression of common root rot of snap bean. Organic amendments are often used to improve soil quality, notably by contributing to general suppressiveness through enhanced soil microbial biomass and activity [31]. Ros et al. [47] found that compost-induced defense response to fusarium wilt resistance led to an increase of soil phosphatase and urease activity. Compost prepared from waste onion peelings is more effective in reducing the viability of the sclerotia of Sclerotium cepivorum than that prepared from Brassica or carrot wastes [11]. Our study indicated that Si is a beneficial supplement that increases tomato resistance against R. solanacearum through increasing soil-specific enzyme activities (Figs. 1 and 4a, b).

A good relation exists between soil fertility and soil microorganism. The plant, soil, and soil microbes all work together to mediate and influence the various exchanges that contribute to plant health and productivity [9]. Beneficial microbiota can compete with pathogens for space and nutrients, or produce microbial agents, thereby improving plant health [37]. Our study found that the amounts of soil bacteria, fungi, and actinomycetes were significantly reduced, whereas soil fungi/bacteria ratio greatly increased, transforming the soil from "bacteria type" into "fungi type" after pathogen inoculation (Figs. 6 and 7). These results are similar to those of other studies regarding the change of soil microbial population resulted from pathogen infection [6,

38]. However, Si treatment significantly increased soil bacteria, actinomycetes, and the total amount of soil microorganism in R. solanacearum-infected soil; soil fungi/bacteria ratio greatly decreased (Figs. 6 and 7). Larkin [34] reported that continuous potato cropping resulted in the decline of the amount of soil bacteria and actinomycetes and increases the amount of soil fungi. Evidence showed that increased bacterial densities were associated with increased suppressiveness of amended soils toward southern blight (Sclerotium rolfsii) of processing tomatoes, the phytophthora root rot of alfalfa, and potato scab [6, 56]. Compost can increase the soil microbial quantity and improve soil quality, thereby reducing pathogen incidence [30, 42]. Sun et al. [50] suggested that soil bacteria and soil actinomycetes could improve the soil alkaline phosphatase, sucrose, and urease activity. Our study found that Si and R. solanacearum inoculation significantly reversed the reduction of the amount of soil bacteria and actinomycetes that resulted from pathogen infection (Fig. 6). Our results suggested that Si could improve soil fertility, increase the amount of soil antagonistic bacteria, and suppress R. solanacearum expansion to maintain a healthy soil ecosystem.

In conclusion, our study found that Si supply had an important role in suppressing bacterial wilt caused by *R*. *solanacearum*. The pathogen resistance mediated by Si was associated with the alteration of soil microbial activity and soil microbial community structure. However, further studies from molecular and proteome aspects are needed to elucidate the soil microbial mechanism, which will be helpful to provide a theoretical basis for Si fertilizer applications in vegetable production.

**Acknowledgments** This study was financially supported by the National Natural Science Foundation of China and the Natural Science Foundation of Guangdong Province (S2012010010331).

## References

- Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31:1471–1479
- Bao SD (2000) Soil agrochemical analysis. Third edition. China Agriculture Press, Beijing 234–235
- Bélanger RR, Benhamou N, Menzies JG (2003) Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis f. sp. tritici*). J Phytopathol 93:402–12
- Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486
- Bowen P, Menzies J, Ehret D, Samuels L, Glass ADM (1992) Soluble silicon sprays inhibit powdery mildew development on grape leaves. J Am Soc Hortic Sci 117:906–912
- Bulluck LR III, Ristaino JB (2002) Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities and yield of processing tomatoes. Phytopathology 92:181–189

- Cai KZ, Gao D, Luo SM, Zeng RS, Yang JY, Zhu XY (2008) Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. Physiol Plant 134:324–333
- Carver TLW, Zeyen RJ, Ahlstrand GG (1987) The relationship between insoluble silicon and success or failure of at tempted primary penetration by powdery mildew (*Erysiphe graminis*) germlings on barley. Physiol Mol Plant P 31:133–148
- Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fert Soils 48:489–499
- Chérif M, Asselin A, Bélanger RR (1994) Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. Mol Plant Pathol 84:236–242
- Coventry E, Noble R, Mead A, Whipps JM (2005) Suppression of Allium white rot (*Sclerotium cepivorum*) in different soils using vegetable wastes. Eur J Plant Pathol 111:101–112
- Dannon EA, Wydra K (2004) Interaction between silicon amendment, bacterial wilt development and phenotype of *Ralstonia solanacearum* in tomato genotypes. Physiol Mol Plant P 64:233–243
- Datnoff LE, Deren CW, Snyder GH (1997) Silicon fertilization for disease management of rice in Florida. Crop Prot 16:525–31
- Deng SP, Tabatabai MA (1994) Cellulase activity of soils. Soil Biol Biochem 26:347–354
- Diogo RVC, Wydra K (2007) Silicon-induced basal resistance in tomato against *Ralstonia solanacearum* is related to modification of pectic cell wall polysaccharide structure. Physiol Mol Plant P 70:120–129
- Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 9:167–172
- Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci USA 91:11–17
- Fang ZD (1998) Plant pathology research methods (third edition). China Agriculture Press, Beijing, 388
- Fawe A, Abou-Zaid M, Menzies JG, Bélanger RR (1998) Siliconmediated accumulation of flavonoid phytoalexins in cucumber. Phytopathology 88:396–401
- Fauteux F, Rémus-Borel W, Menzies JG, Bélanger RR (2005) Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiol Lett 249:1–6
- Fauteux F, Chain F, Belzile F, Menzies JG, Bélanger RR (2006) The protective role silicon in the *Arabidopsis*-powdery mildew pathosystem. Proc Natl Acad Sci USA 103:17554–17559
- 22. Ghareeb H, Bozsó Z, Ott PG, Repenning C, Stahl F, Wydra K (2011) Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato implicates priming effect. Physiol Mol Plant P 75:83–9
- Ghareeb H, Bozsób Z, Ottb PG, Wydra K (2011) Silicon and Ralstonia solanacearum modulate expression stability of housekeeping genes in tomato. Physiol Mol Plant P 75:176–179
- 24. Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ 341:265–279
- 25. Guan SY (1986) Soil enzymes and their research methods. Agriculture Press, Beijing
- Guan SY, Zhang DS, Zhang ZM (1991) Methods of soil enzyme activities analysis. Agriculture Press, Beijing, pp. 263–271
- Hayasaka T, Fujii H, Ishiguro K (2008) The role of silicon in preventing appressorial penetration by the rice blast fungus. Phytopathology 98:1038–1044
- Hayward AC (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu Rev Phytopathol 29:65–87
- 29. Heath MC, Stumpf MA (1986) Ultrastructural observations of penetration sites of the cowpea rust fungus in untreated and silicon-depleted French bean cells. Physiol Mol Plant P 29:27–39

- Hoitink HAJ, Fahy PC (1986) Basis for the control of soilborne plant pathogens with composts. Annu Rev Phytopathol 24:93–114
- Janvier C, Villeneuve F, Alabouvette C, Edel-Hermannb V, Mateille T, Steinbergb C (2007) Soil health through soil disease suppression: which strategy from descriptors to indicators? Soil Biol Biochem 39:1–23
- 32. Johnson NN, Lisa LBA, Kathy K, Carl G (2002) Soil microbial and chemical indicators of soil health response to agricultural intensification practices on black cracking clay soils. 17th WCSS, Thailand 14–21
- Kelman A (1954) The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance in a tetrazoli-um medium. Phytopathology 44:693–695
- 34. Larkin RP (2003) Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. Soil Biol Biochem 35:1451–1466
- Leon MCC, Stone A, Dick RP (2006) Organic soil amendments: impacts on snap bean common root rot (*Aphanomyces euteiches*) and soil quality. Appl Soil Ecol 31:199–210
- Liang YC, Sun WC, Si J, Römheld V (2005) Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. Plant Pathol 54:678–685
- Lindow SE, Leveau JH (2002) Phyllosphere microbiology. Curr Opin Biotech 13:238–243
- Liu QG, Yang Y (2006) The relationship between tomato resistance and the quantity of *Ralstonia solanacearum* and rhizosphere microbes. J Zhongkai Univ Agr Tech 19:31–34
- Ma JF, Yamaji NK (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11:392–397
- Makoi JHJR, Ndakidemi PA (2008) Selected soil enzymes: examples of their potential roles in the ecosystem. Afr J Biotechnol 7:181–191
- Martin JP (1950) Use of acid, rose Bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci 69:215–232
- Nelson EB, Craft CM (1992) Suppression of dollar spot on creeping bentgrass and annual bluegrass turf with compost-amended topdressings. Plant Dis 76:954–958
- Pavel R, Doyle J, Steinberger Y (2004) Seasonal pattern of cellulase concentration in desert soil. Soil Biol Biochem 36:549–554
- 44. Rasmussen PH, Knudsen IMB, Elmholt S, Jensen DF (2002) Relationship between soil cellulolytic activity and suppression of seedling blight of barley in arable soils. Appl Soil Ecol 19:91–96
- Rémus-Borel W, Menzies JG, Bélanger RR (2005) Silicon induces antifungal compounds in powdery mildew-infected wheat. Physiol Mol Plant P 66:108–115
- Rodrigues FÅ, Benhamou N, Datnoff LE, Jones JB, Bélanger RR (2003) Ultrastructural and cytochemical aspects of siliconmediated rice blast resistance. Phytopathology 93:535–546
- Ros M, Hernandez MT, Garcia C, Bernal A, Pascual JA (2005) Biopesticide effect of green compost against fusarium wilt on melon plants. J Appl Microbiol 98:845–854
- Samuels AL, Glass ADM, Ehret DL, Menzies JG (1991) Distribution of silicon in cucumber leaves during infection by powdery mildew fungus (*Sphaerotheca fuliginea*). Can J Bot 69:140–146
- Shetty R, Jensen B, Shetty NP, Hansen M, Hansen CW, Starkey KR, Jørgensen HJL (2012) Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. Plant Pathol 61:120–131
- 50. Sun XS, Feng HS, Wan SB, Zuo XQ (2001) Changes of main microbial strains and enzymes activities in peanut continuous cropping soil and their interactions. Acta Agron Sin 27:617–621
- Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angel GS, Bottomley PS (eds.) Methods of soil analysis. Soil Science Society of America, Madison, pp. 775–833

- 52. Overbeek LSV, Cassidy M, Kozdroj J, Trevors JT, Elsas JDV (2002) A polyphasic approach for studying the interaction between *Ralstonia solanacearum* and potential and potential control agent in the tomato phytosphere. J Microbiol Meth 48:69–86
- Van der Vorm PDJ (1987) Dry ashing of plant material and dissolution of the ash in HF for the colorimetric determination of silicon. Commun Soil Sci Plant 18:1181–1189
- 54. Visser S, Parkinson D (1992) Soil biological criteria as indicators of soil quality: soil microorganisms. AJAA 7:33–37
- 55. Wang RH, Zhou BL, Zhang QF, Zhang FL, Fu YW (2005) Effects of grafting on rhizosphere microbial populations of eggplants. Acta Hortic Sin 32:124–126
- Wiggins BE, Kinkel LL (2005) Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes. Plant Soil 268:271–283
- 57. Yao HY, Huang CY (2006) Microbial ecology and experimental techniques. Sciences Press, Beijing
- Yogev A, Laor Y, Katan J, Hadar Y, Cohen R, Medina S, Raviv M (2011) Does organic farming increase soil suppression against Fusarium wilt of melon? Org Agr 1:203–216
- Zhao N, Cai KZ, Wang GP, Wang Y (2008) Induced resistance of tomato plants to bacterial wilt by livestock wastes compost and its physiological mechanisms. J Agro-Environ Sci 27:2058– 2063