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Silicon application enhances resistance to xanthomonas wilt disease in banana

K. Mburu[ab], R. Oduor[b], A. Mgutu[b] and L. Tripathi[a]*

[a]*International Institute of Tropical Agriculture, Nairobi; and* [b]*Kenyatta University, Nairobi, Kenya*

Silicon (Si) has been reported to be a beneficial element and shown to enhance disease resistance in many crops, although it is not regarded to be critical for plant growth and development. In this study, the potential effect of Si supplementation on resistance to banana xanthomonas wilt (BXW) disease was evaluated using various banana cultivars. Si application at a concentration of 200 mg per plant per week was found to be optimal in enhancing resistance to BXW without any detrimental effects on plant growth. The effect of varying the duration of Si application showed continuous supply of Si before and after pathogen inoculation led to a significantly higher level of resistance to BXW in all the banana cultivars tested in comparison to non-Si-treated inoculated plants. Banana plants treated with Si before pathogen inoculation only, also exhibited high protection against BXW similar to plants treated continuously with Si. The total Si content in leaves increased significantly in Si-treated plants in comparison to non-Si-treated control plants. The amount of Si accumulation was directly correlated to the duration of application; plants treated with Si continuously showed significantly higher amounts of Si accumulation in leaves than plants where Si application was terminated following bacterial inoculation or when Si application started immediately after pathogen inoculation. The Si-treated plants also showed higher activity of the peroxidase enzyme in comparison to non-Si-treated control plants. This study confirms that application of Si enhances resistance to BXW and may provide an alternative disease management strategy.

Keywords: banana xanthomonas wilt, peroxidase, resistance, silicon, *Xanthomonas campestris* pv. *musacearum*

Introduction

Banana and plantain (*Musa* spp.) are major staple and cash crops for millions of people in the tropics and subtropics, with an annual world production of around 144 million tonnes (FAOSTAT, 2013). Approximately one-third of global *Musa* production is from Africa, of which more than 50% is produced in East Africa (FAOSTAT, 2013). Its production is plagued by a number of pests and diseases, particularly the banana xanthomonas wilt (BXW) disease caused by *Xanthomonas campestris* pv. *musacearum* (Xcm), which severely hampers banana cultivation in East Africa, leading to massive on-farm losses (Tripathi *et al.*, 2009). Xcm is mainly transmitted through insect vectors from the male buds of infected plants to those of healthy plants, use of contaminated farming tools and infected planting materials (Tushemereirwe *et al.*, 2004). The symptoms of infected plants include progressive yellowing and wilting of leaves, uneven and premature ripening of fruits with sections showing unique yellowish blotches in the pulp and dark brown placental scars and eventually, withering and rotting of infected plants (Tripathi *et al.*, 2009). The pathogen is highly contagious, and its spread has endangered the livelihood of millions of farmers who rely on banana for food and income. The disease has caused estimated economic losses of about $2–8 billion over the last decade, and substantial reductions in production have resulted in major price increases (Tripathi *et al.*, 2009). The disease is very destructive, infecting all banana varieties, including both East African Highland bananas (EAHBs) and exotic dessert and beer bananas. The economic impact of the disease is potentially disastrous because it destroys whole plants leading to complete yield loss. Currently, the disease is controlled through cultural on-farm disease management practices such as removal of the male flower (debudding), use of clean sterile farming tools and clean planting material. However, the adoption rate of these practices is low and inconsistent, as they are considered tedious. Additionally, farmers have associated debudding with poor fruit quality and hence are reluctant to adopt the practice.

There are also reports of biotechnological approaches towards developing transgenic bananas resistant to BXW; however, such efforts are still years away from releasing resistant lines (Tripathi *et al.*, 2014). Therefore, it is necessary to develop alternative technologies for managing BXW disease in order to maintain high productivity. One possible alternative is the application of silicon (Si), as this element has decreased the intensity of several diseases in crops of great economic importance. Si is beneficial to crops, but is not classified as an essential element required for plant growth (Epstein, 1999). It

*E-mail: l.tripathi@cgiar.org

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is the second most dominant element in the soil and its concentration in soil varies from 0·1 to 0·6 mM (Guntzer *et al.*, 2012). The accumulation of Si in plant species varies from 0·1 to 10% in dry weight. Plants are considered as Si accumulators if their Si content is higher than 1% of dry weight; examples include plants such as rice, wheat, sugarcane, sugar beet, barley, soybeans and tomatoes that are able to accumulate high amounts of the element in their tissues (Guntzer *et al.*, 2012). High deposition of Si in tissues forms a physical barrier that enhances the strength and rigidity of the tissues (Ma *et al.*, 2006). Plant species that are known to accumulate Si have evolved mechanisms to transport Si against its chemical gradient through both an active and passive process. The Si uptake mechanism has been most intensively studied in rice, as it is a well-known silicon accumulator (Van Bockhaven *et al.*, 2013). In rice, two silicon transporters with different modes of action are responsible for the transport of silicic acid past the Casparian strips in exo- and endodermal cells. Specific Si transporter genes (*Low silicon rice 1* (*Lsi1*) and *Low silicon rice 2* (*Lsi2*) genes), which mediate Si accumulation in rice, have been identified (Ma *et al.*, 2006). The uptake of Si by the Lsi1 transporter is a passive process, while the transport via the Lsi2 transporters is actively driven. In rice, once Si is taken up by Lsi1 in the exodermis and released by Lsi2, it diffuses through the apoplast of the aerenchyma. It is then taken up from the aerenchyma by the Lsi1 transporters located in the endodermis and Lsi2 transporters load it into the stele. The transporter gene responsible for xylem loading has not yet been characterized; however, once in the xylem, Si is taken up by leaf cells by means of a Lsi1-like transporter and Lsi6 polymerizes it (Ma *et al.*, 2006; Van Bockhaven *et al.*, 2013). Orthologues of rice *Lsi1* and *Lsi2* have been shown to be involved in Si absorption in other crops such as maize, barley, pumpkin and wheat (Van Bockhaven *et al.*, 2013). However, the uptake of silicon in these crops differs from rice due to differences in root structure.

There have been several reports on the ability of Si to improve tolerance to abiotic stresses such as drought, salinity, freezing, heavy metal toxicity and biotic stresses such as disease and pests, especially in crops that actively accumulate the element (Epstein, 1999; Heine *et al.*, 2006; Liang *et al.*, 2008). In particular, several studies have shown that adequate uptake of Si may substantially increase tolerance to both abiotic and biotic stresses in rice, sugarcane, sunflower, tomato, wheat and other crops (Heine *et al.*, 2006; Cai *et al.*, 2008; Kamenidou *et al.*, 2008; Liang *et al.*, 2008). Plants with low Si accumulation are known to be more susceptible to fungal disease and insect feeding, as well as other biotic and abiotic stresses that adversely affect crop production. There have been a few reports of Si-induced resistance against bacterial pathogens, with the most notable report being Si-induced resistance in tomatoes against *Ralstonia solanacearum* (Diogo & Wydra, 2007; Kurabachew & Wydra, 2014). The beneficial role of Si application for banana has been demonstrated only against fungal diseases such as black sigatoka and root-rot caused by *Mycosphaerella fijiensis* and *Cylindrocladium spathiphylli*, respectively (Vermeire *et al.*, 2011; Kablan *et al.*, 2012). In addition, Fortunato *et al.* (2012) showed that supplying Si to banana plants had a great potential to reduce the intensity of fusarium wilt. However, the role of Si in enhancing plant resistance against pathogen colonization is poorly understood; it is hypothesized to be through a combination of activation of defence genes and/or formation of a mechanical or physical barrier that restricts penetration of the pathogens (Fauteux *et al.*, 2005). Si-induced resistance is associated with increased activity of pathogenesis-related proteins and elevation of inhibitory phenolic compounds and phytolexins (Fauteux *et al.*, 2005). Inoculation of Si-treated rice plants with *Magnaporthe oryzae* significantly increased the activities of defence-related enzymes such as peroxidase (POD), catalase, super peroxidase dismutase, polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), which are involved in the hypersensitive response (Cai *et al.*, 2008). In addition, studies have shown that induction of basal resistance mechanisms at the cell wall level and at pit membranes may limit the spread of *R. solanacearum* in plant tissues, leading to resistance (Diogo & Wydra, 2007). This suggests interplay between activation of local and systemic defence responses as well as basal resistance mechanisms.

Because bananas are very efficient in Si uptake, this may be used as a possible method to enhance resistance to plant pathogens in this crop (Henriet *et al.*, 2006). Currently, there is no information available on the potential positive effects of Si application in banana for providing resistance to BXW. Therefore, the aims of this study were to investigate whether Si supplementation might augment existing banana disease control measures and offer a method of integrated pathogen management in bananas for BXW disease.

Materials and methods

Plant materials

Four banana cultivars were tested in this study: the East African Highland banana (EAHB) cultivars Ngombe and Uganda Green, and dessert cultivars Gros Michel and Giant Cavendish. *In vitro* banana plantlets were multiplied on proliferation medium (MS basal salts and vitamins (Murashige & Skoog, 1962), 10 mg L^{-1} ascorbic acid, 5 mg L^{-1} benzyl aminopurine (BAP), 30 g L^{-1} sucrose, 3 g L^{-1} Gelrite gellan gum, pH 5·8), with monthly subculture onto fresh medium, as described by Tripathi *et al.* (2008). The cultures were incubated in a growth room at 28 ± 2°C with a photoperiod of 16 h. Well-developed shoots with 3–4 leaves were transferred to rooting medium (MS basal salts and vitamins, 10 mg L^{-1} ascorbic acid, 1 mg L^{-1} indole-3-butyric acid (IBA), 30 g L^{-1} sucrose, 3 g L^{-1} Gelrite gellan gum, pH 5·8). Rooted plantlets were then transferred to soil in 8 cm-diameter plastic cups (Kenpoly) and acclimatized under small plastic tents to maintain high humidity for 1 month with weekly watering. Hardened plants were subsequently transferred

to a mixture of loamy soil and organic manure (1:1) in larger pots of 30 cm diameter (Kenpoly) and maintained in the glasshouse for 2 months.

Pathogen and inoculum preparation

The isolate of Xcm used for inoculation of banana cultivars was collected from diseased banana plants in a farmer's field in Uganda and confirmed through PCR using Xcm-specific primers (Adikini *et al.*, 2011). The bacterium was maintained on solid YTSA medium (1% (w/v) yeast extract, 1% (w/v) tryptone, 1% (w/v) sucrose and 1·5% (w/v) agar) supplemented with 50 mg L^{-1} cephalexin and stored at 4°C. For preparation of inoculum, the bacteria were cultured in liquid YTS medium supplemented with 50 mg L^{-1} cephalexin at 28°C and grown until an optical density (OD_{600}) of 1·0 corresponding to 10^8 colony-forming units (cfu) was attained. The bacterial culture was centrifuged at 3200 ***g*** for 5 min and the resulting pellet was resuspended in sterile double-distilled water. The OD_{600} of the bacterial suspension was adjusted to 1 with sterile water. The fresh inoculum was used for artificial inoculation of potted plants.

Determination of the optimal concentration of Si for enhancement of resistance to Xcm

Two-month-old banana plantlets of cultivars Ngombe and Gros Michel were watered weekly with 500 mL distilled water containing 100, 200, 500 or 1000 mg Si (in the form of metasilicate, Na_2SiO_3) per plant per pot for a period of 2 months; control plants were watered with distilled water only. After 2 months of Si application, the plants were inoculated by injecting 100 μL of the bacterial suspension (10^8 cfu mL^{-1}) into the lower side of the petiole of the second fully opened leaf using a syringe fitted with a 28-gauge needle; the control plants were inoculated with sterile water. The experiment consisted of three treatments with 10 replicates each: (i) plants with no Si application and not inoculated with Xcm (non-treated and non-inoculated control); (ii) plants with no Si application but inoculated with Xcm (non-treated and inoculated control); and (iii) plants treated weekly with Si concentrations of 100, 200, 500 or 1000 mg per plant for 2 months and then inoculated with Xcm followed by continuous weekly application of the respective amounts of Si thereafter for 60 days post- inoculation (dpi). The plantlets were kept in the screen house and observed for development of disease symptoms and severity. The number of days for development of initial BXW disease symptoms, number of days for complete wilting of plants and number of leaves wilted at 60 dpi were recorded and resistance (%) calculated as:

$$\left(\frac{\text{Reduction in wilting in leaves of Si-treated plants}}{\text{Total number of leaves wilted in non-Si-treated control plants}}\right) \times 100$$

Plant growth was also assessed during this period by recording plant height, pseudostem diameter, and length and width of the leaves at the end of 60 dpi. Total leaf area was calculated as:

$$\text{Total leaf area} = l \times w \times n \times \text{K}$$

where l is the length of the leaf, w is the width of the leaf, n is the number of leaves, and K is the constant factor (0·83) (Hewitt, 1955).

Determination of effect of varying duration of Si application on BXW disease

Following determination of the optimal Si concentration for inducing disease resistance, 2-month-old potted plants of the banana cultivars Ngombe and Gros Michel were irrigated with 500 mL distilled water containing 200 mg of Si per plant weekly for 60 days. The plants were inoculated with Xcm as previously described. The experiment consisted of five treatments with 10 replicates each. Treatments were: (i) plants with no Si application and no inoculation with Xcm (non-treated and non-inoculated control); (ii) plants with no Si application but inoculated with Xcm (non-treated and inoculated control); (iii) plants treated with 200 mg of Si per week continuously (before and after inoculation with Xcm) throughout the experiment; (iv) plants treated with 200 mg of Si per week only until artificial inoculation with Xcm and then Si application terminated after inoculation; and (v) plants treated with 200 mg of Si per week only after inoculation of the plants with Xcm. Data on development of disease symptoms, complete wilting of plants and total number of leaves wilted at 60 dpi was collected.

Effect of Si application on different cultivars of banana

Two-month-old potted plants of banana cultivars Ngombe, Gros Michel, Giant Cavendish and Uganda Green were irrigated weekly with 500 mL distilled water containing 200 mg of Si per plant for 60 days. The plants were inoculated with Xcm, as previously described, followed by weekly application of 200 mg of Si per plant for 60 days. The experiment consisted of three treatments with 10 replicates each. Treatments were: (i) plants with no Si application and no inoculation with Xcm (non-treated and non-inoculated control); (ii) plants with no Si application but inoculated with Xcm (non-treated and inoculated control); and (iii) plants treated with 200 mg of Si per week continuously (before and after inoculation with Xcm) throughout the experiment. The development of disease symptoms, complete wilting of plants and total number of leaves wilted at 60 dpi was recorded.

Effect of Si application on plant growth

Plant growth was assessed for all the plants with various treatments after 60 dpi. In particular, plant height, pseudostem diameter, and length and width of the leaves were recorded.

Enzyme activity assays

Plant leaf tissues were sampled from the inoculated leaf 7 days after inoculation. The leaf extracts for enzymatic assay were prepared following the method by Cai *et al.* (2008). POD activity was assayed by adding 0·1 mL of the plant tissue extract to a substrate mixture containing 50 mM sodium acetate buffer (pH 7), 25 mM guaiacol and 25 mM H_2O_2. The change in absorbance of the brown guaiacol at 470 nm was recorded for calculating enzyme activity. One unit of POD activity (U) was defined as an absorbance increase by 1 unit min^{-1} in 1 mg plant tissue extract.

Quantification of Si accumulation in leaf tissue

Leaf samples were collected from banana plantlets at 60 dpi, wrapped with aluminium foil and immediately immersed in

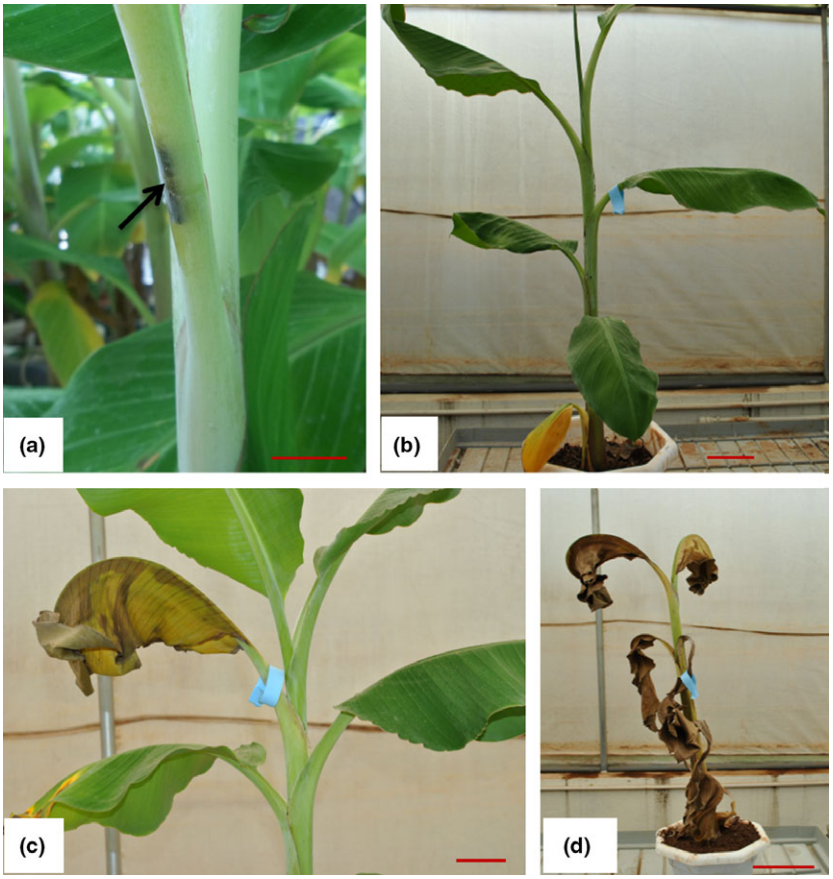

Figure 1 Symptoms of banana xanthomonas wilt on banana plants inoculated with *Xanthomonas campestris* pv. *musacearum*. (a) Necrosis at the point of inoculation of leaf of a silicon (Si)-treated plant, (b) Si-treated plant showing no disease symptoms after inoculation, (c) wilting of inoculated leaf of Si-treated plant, (d) non-Si-treated control plant showing complete wilting. Photographs were taken at 60 days post-inoculation. Bar = 10 cm.

liquid nitrogen. Thereafter samples (100 mg) were ground with a mortar and pestle, transferred into a 50 mL polypropylene screw-cap Falcon tube and 5 mL of 50% (w/v) sodium hydroxide (NaOH) added. The mixture was autoclaved for 1 h at 138 kPa, left to cool for 30 min and 2 mL of 50% (v/v) hydrogen peroxide was added, followed by autoclaving for 1 h at 138 kPa. The solution was diluted to 15 mL with distilled water and analysed for Si content using the molybdenum blue colourimetric (MBC) method (Hallmark *et al.*, 1982). Briefly, a 1 mL aliquot of diluted sample digest was mixed with 5 mL of 20% (v/v) acetic acid for 10 s and 2 mL of 0·3 M ammonium molybdate was added. After 5 min, 1 mL of 20% tartaric acid (w/v) was added, the mixture was vortexed and 1 mL of sodium bisulphite solution 12·5% (w/v) solution was added. Finally, the samples were diluted to a final volume of 15 mL with 20% acetic acid, and the blue colour was allowed to develop for 30 min. The tubes were shaken vigorously prior to determining absorbance. The Si standards were prepared by serial dilution of a 100 mg L^{-1} Si standard solution and the absorbance reading of the samples (standard and unknown) was taken using a spectrophotometer (Spectronic 20; Genesys) calibrated at 650 nm as described by Hallmark *et al.* (1982).

Data analysis

The experiments were arranged in completely randomized designs, with 10 replicates for each treatment. Data on number of days for development of BXW disease symptoms and complete wilting of the inoculated plants, number of leaves wilted and plant growth indicators such as plant height, pseudostem diameter and total leaf area were analysed by analysis of variance (ANOVA) and treatment mean comparisons by Tukey's test ($P \leq 0{\cdot}05$) using the software R (R Core Team, 2012).

Results

Determination of the optimal concentration of Si for enhancement of resistance to Xcm

To determine the optimal concentration of Si for enhancing resistance to BXW disease, the banana plantlets were treated with five different concentrations of Si ranging from 0 to 1000 mg of Si per plant per week for 2 months. After artificial inoculation with Xcm, the Si application continued for 60 dpi. Post-inoculation, the initial development of symptoms was observed as necrosis at the point of inoculation followed by wilting of the inoculated leaf that spread to other parts of the plant, leading, eventually, to complete wilting of susceptible plants (Fig. 1). Symptoms appeared early in the non-Si-treated control plants, with necrosis of the inoculated leaf starting at 15–19 dpi, whereas plants treated with different concentrations of Si showed delayed appearance of symptoms (Table 1). However, significant variations in the number of days to appearance of symptoms were observed among the plants treated with different Si

Table 1 Appearance of banana xanthomonas wilt disease symptoms in silicon (Si)-treated banana plants after artificial inoculation with *Xanthomonas campestris* pv. *musacearum*

Concentration of Si applied (mg/plant/ week)	Mean no. of days to appearance of disease symptoms (dpi)		Mean no. of plants showing appearance of disease symptoms (%)		Mean no. of days to complete wilting of inoculated plants (dpi)		Mean no. of plants showing complete wilting (%)	
	Ngombe	Gros Michel	Ngombe	Gros Michel	Ngombe	Gros Michel	Ngombe	Gros Michel
0	15·2 ± 0·7 a	19·0 ± 0·4 a	100·0 ± 0·0 a	100·0 ± 0·0 a	30·2 ± 3·1 a	34·67 ± 1·0 a	100·0 ± 0·0 a	100·0 ± 0·0 a
100	20·4 ± 1·5 a	35·3 ± 0·3 b	60·0 ± 10·0 b	20·0 ± 10·0 b	43·0 ± 8·9 b	53·3 ± 1·7 b	17·5 ± 2·5 b	20·0 ± 14·1 b
200	28·3 ± 0·8 b	38·3 ± 1·2 b	30·0 ± 0·0 c	15·0 ± 5·0 b	56·0 ± 1·6 b	59·0 ± 0·8 b	15·0 ± 5·0 b	10·0 ± 0·0 b
500	26·3 ± 2·1 b	31·3 ± 2·2 b	35·0 ± 5·0 c	35·0 ± 5·0 c	42·0 ± 1·0 b	44·5 ± 3·2 c	25·0 ± 5·0 b	20·0 ± 0·0 b
1000	24·8 ± 3·3 b	27·3 ± 1·4 c	50·0 ± 0·0 c	55·0 ± 5·0 c	38·3 ± 3·3 c	40·0 ± 1·1 c	30·0 ± 0·0 b	20·0 ± 0·0 b

The data presented are means of 10 replicates (mean ± SE).
Values marked with the same letter within a column are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$).

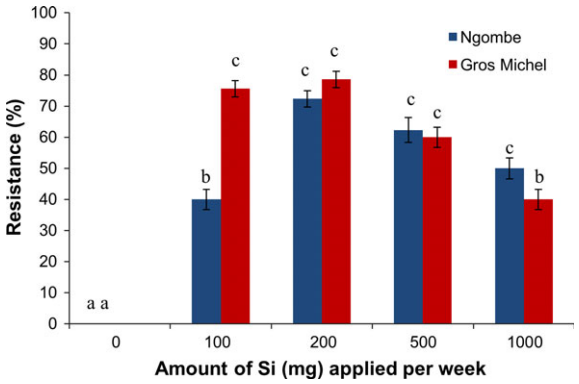

Figure 2 Effect of silicon (Si) application on resistance against *Xanthomonas campestris* pv. *musacearum* in the banana cultivars Ngombe and Gros Michel. The values are means of 10 replicates (mean ± SE). Mean values followed by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$). Resistance (%) was calculated as [(reduction in wilting in leaves of Si-treated plants/total number of leaves wilted in non-Si-treated control plants) × 100].

concentrations. Plants treated with 200 mg of Si per week exhibited the longest delay in appearance of symptoms in comparison to plants treated with other Si concentrations, with wilting of the inoculated leaf occurring at 28·3 ± 0·8 dpi for Ngombe and 38·3 ± 1·2 dpi for Gros Michel (Table 1). All the inoculated non-Si-treated control plants showed symptoms and eventually died. However, only 30–60% of Ngombe and 15–55% of Gros Michel plants treated with various Si concentrations showed appearance of disease symptoms. Although these Si-treated plants showed initial symptom development, in the majority of plants the symptoms did not progress to other plant parts.

All the inoculated, non-Si-treated control plants of Ngombe and Gros Michel completely wilted within 30–34 dpi, whereas the majority of the Si-treated plants did not wilt completely by 60 dpi. Only 10–30% of the Si-treated plants completely wilted in 38–56 dpi and 40–59 dpi for Ngombe and Gros Michel plants, respectively.

Si-induced resistance was significantly greater ($P < 0{\cdot}05$) for all the Si treatments compared to the non-Si-treated control plants (Fig. 2). At Si concentrations of 100 mg per plant per week, the level of Si-induced resistance against Xcm was found to be greater for Gros Michel than Ngombe. Both cultivars treated with concentrations of 200 or 500 mg of Si per week showed similar resistance, but Ngombe recorded a higher level of resistance in comparison to Gros Michel at 1000 mg Si per plant per week. The level of disease resistance in the cultivars increased with increasing Si concentration up to the application of 200 mg of Si per week and, thereafter, remained the same for Ngombe while for Gros Michel it decreased. Based on these results, weekly application of 200 mg of Si per plant was found to be the optimum concentration for enhancing resistance against Xcm and was used in subsequent experiments.

Effect of varying the duration of Si application on resistance to Xcm

The optimum Si concentration of 200 mg per plant per week was used to determine the effect of varying the duration of Si application on enhancing resistance to BXW disease. Both Ngombe and Gros Michel plants treated with Si showed delayed development of initial BXW symptoms compared to the non-Si-treated control plants. Disease symptoms appeared within 16 dpi in all the non-Si-treated control plants whereas plants treated with 200 mg per week of Si showed symptoms after 24–32 dpi (Table 2). BXW disease symptoms were observed in 100% of the non-Si-treated control inoculated plants. However, only 30% of the Ngombe and Gros Michel plants continuously treated with Si before and after pathogen inoculation and those that had Si treatment terminated upon Xcm inoculation had developed BXW disease symptoms. However, 55% of Ngombe and 40% of Gros Michel plants treated with Si after Xcm inoculation showed BXW symptoms.

Silicon-enhanced resistance to BXW disease in plants was significantly greater for the two cultivars tested in comparison to the inoculated non-Si-treated controls. In

Table 2 Effect of varying duration of silicon (Si) application on resistance to *Xanthomonas campestris* pv. *musacearum* (Xcm) on cv. Ngombe and Gros Michel

Treatment	Mean no. of days to appearance of disease symptoms (dpi)		Mean no. of plants showing appearance of disease symptoms (%)		Mean no. of days to complete wilting of inoculated plants (dpi)		Mean no. of plants showing complete wilting (%)	
	Ngombe	Gros Michel	Ngombe	Gros Michel	Ngombe	Gros Michel	Ngombe	Gros Michel
No Si treatment	16·9 ± 0·7 a	16·2 ± 0·7 a	100·0 ± 0·0 a	100·0 ± 0·0 a	34·6 ± 0·5 a	32·6 ± 0·3 a	100·0 ± 0·0 a	100·0 ± 0·0 a
Continuous Si treatment	27·0 ± 4·0 b	32·0 ± 0·4 b	30·0 ± 0·0 b	30·0 ± 0·0 b	53·5 ± 0·5 b	56·5 ± 1·5 b	20·0 ± 0·0 b	15·0 ± 2·5 b
Si treatment terminated after Xcm inoculation	25·0 ± 2·6 b	32·0 ± 1·5 b	30·0 ± 0·0 b	30·0 ± 0·0 b	44·7 ± 1·3 c	49·0 ± 0·0 c	20·0 ± 0·0 b	25·0 ± 5·0 b
Si treatment started immediately after Xcm inoculation	24·3 ± 1·1 b	30·3 ± 1·9 b	55·0 ± 5·0 c	40·0 ± 0·0 c	41·5 ± 0·6 d	42·3 ± 1·0 c	35·0 ± 5·0 c	40·0 ± 0·0 c

The data presented are means of 10 replicates (mean ± SE).
Values marked with the same letter within a column are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$).

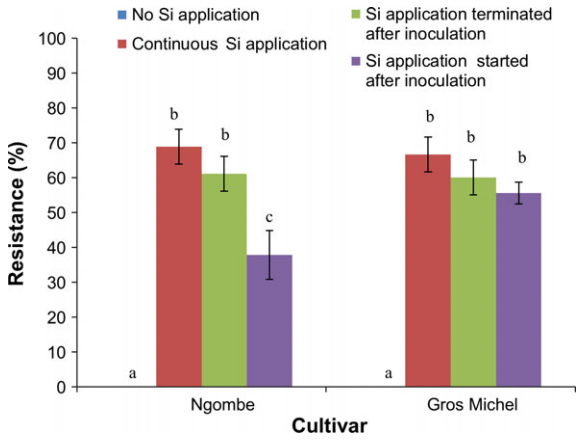

Figure 3 Evaluation of the level of resistance against *Xanthomonas campestris* pv. *musacearum* (Xcm) with varying duration of silicon (Si) applied to the banana cultivars Gros Michel and Ngombe. The values are means of 10 replicates. Mean values followed by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$). Resistance (%) was calculated as [(reduction in wilting in leaves of Si-treated plants/total number of leaves wilted in non-Si-treated control plants) × 100].

particular, continuous application of Si before and after inoculation resulted in 68·5 and 66·7% disease resistance for Ngombe and Gros Michel, respectively (Fig. 3). The level of disease resistance resulting from continuous Si treatment was not significantly different from plants that had Si supply terminated after pathogen inoculation. However, Ngombe plants in which Si treatment was started immediately after Xcm inoculation showed significantly lower resistance to BXW disease (37%) in comparison to the plants treated continuously with Si (Fig. 3).

Effect of Si application on different cultivars of banana

The effect of Si application on disease resistance was evaluated for different cultivars of bananas. The plants were continuously supplied with 200 mg of Si/plant/week before and after inoculation with Xcm. Similar to Ngombe and Gros Michel, the plants of Giant Cavendish and Uganda Green treated with Si showed delayed development of BXW symptoms compared to the control non-Si-treated plants (Tables 2 & 3). Disease symptoms appeared within 16–17 dpi in all the non-Si-treated control plants whereas plants treated with 200 mg Si per week showed symptoms after 23–32 dpi in all the cultivars tested (Tables 2 & 3). All the non-Si-treated plants wilted completely and died in 30–35 dpi, whereas only 30% of Giant Cavendish, 15% of Gros Michel, 20% of Ngombe and 35% of Uganda Green plants treated continuously with Si wilted completely (Tables 2 & 3) in 41–56 dpi.

Continuous application of Si to all cultivars tested resulted in significantly ($P \leq 0{\cdot}05$) higher levels of resistance in comparison to the non-Si-treated control plants (Fig. 4). However, the Si-enhanced resistance against Xcm in Giant Cavendish and Uganda Green plants continuously treated with Si was slightly lower (54–55%) in comparison to resistance in the cultivars Ngombe (68·5%) and Gros Michel (66·7%), suggesting that Si-enhanced resistance may vary according to cultivar (Fig. 4).

Effect of Si on growth of banana plants

Silicon application at concentrations of 200 mg per plant per week significantly ($P \leq 0{\cdot}05$) increased the plant height in Gros Michel and the pseudostem diameter in Ngombe plants compared to the non-Si-treated control plants (Fig. 5). In addition, both cultivars treated with 200 mg of Si per week had significant increases in total leaf area compared with the non-Si-treated control plants. Increase in plant height of Gros Michel and leaf area of Ngombe was also observed at a Si concentration of 100 mg per plant per week. In contrast, plants treated with 1000 mg of Si per plant per week had significantly

Table 3 Effect of continuous silicon (Si) application on development and spread of banana xanthomonas wilt symptoms in banana plants of cultivars Giant Cavendish and Uganda Green after inoculation with *Xanthomonas campestris* pv. *musacearum* (Xcm)

	Mean no. of days to appearance of disease symptoms (dpi)		Mean no. of plants showing appearance of disease symptoms (%)		Mean no. of days to complete wilting of inoculated plants (dpi)		Mean no. of plants showing complete wilting (%)	
Treatment	Giant Cavendish	Uganda Green	Giant Cavendish	Uganda Green	Giant Cavendish	Uganda Green	Giant Cavendish	Uganda Green
No Si treatment	17·5 ± 0·2 a	16·0 ± 0·36 a	100·0 ± 0·0 a	100·0 ± 0·0 a	35·4 ± 0·3 a	30·2 ± 0·3 a	100·0 ± 0·0 a	100·0 ± 0·0 a
Continuous Si treatment	23·8 ± 0·7 b	25·0 ± 0·6 b	60·0 ± 0·0 b	70·0 ± 0·0 b	41·7 ± 0·9 b	44·3 ± 0·9 b	30·0 ± 0·0 b	35·0 ± 5·0 b

The data presented are means of 10 replicates (mean ± SE).
Values marked with the same letter within a column are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$).

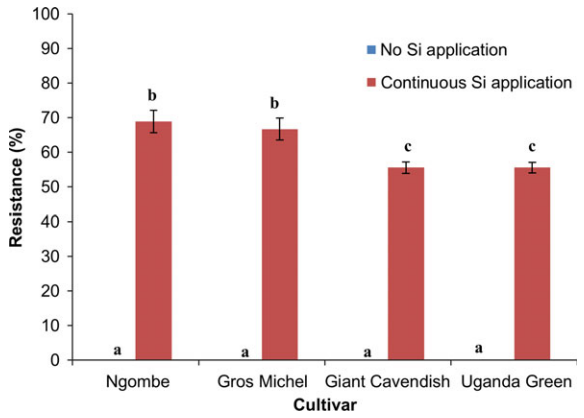

Figure 4 Evaluation of the level of resistance against *Xanthomonas campestris* pv. *musacearum* (Xcm) with continuous silicon (Si) application in the banana cultivars Gros Michel, Ngombe, Giant Cavendish and Uganda Green. The values are means of 10 replicates. Mean values followed by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$). Resistance (%) was calculated as [(reduction in wilting in leaves of Si-treated plants/total number of leaves wilted in non-Si-treated control plants) × 100].

($P \leq 0{\cdot}05$) reduced plant heights and total leaf area. In addition, discolouration was observed on the leaf edges of plants treated with 500 and 1000 mg of Si. These negative effects on growth parameters were more pronounced in plants treated with 1000 mg of Si per week.

Varying the duration of Si application (200 mg) resulted in improved growth parameters of the banana cultivars tested in comparison to the non-Si-treated control plants (Fig. 6). In particular, continuous Si application led to a significant ($P \leq 0{\cdot}05$) increase in plant height for the cultivars Gros Michel and Giant Cavendish in comparison to non-Si-treated plants. However, the increase in these parameters was not significantly different in Ngombe and Uganda Green. The improvement in plant height for plants of Gros Michel treated continuously with Si was also significantly ($P \leq 0{\cdot}05$) greater than for plants where the Si application was terminated after Xcm inoculation or Si application started after Xcm inoculation.

Continuous application of Si to Ngombe and Giant Cavendish resulted in a significant ($P \leq 0{\cdot}05$) increase in the pseudostem diameter in comparison to the non-Si-treated control plants (Fig. 6). However, the pseudostem diameter was not significantly increased for Gros Michel or Uganda Green, irrespective of the timing of Si application. In addition, continuous application of Si resulted in a significant ($P \leq 0{\cdot}05$) increase in the total leaf area of all the cultivars in comparison with their non-Si-treated controls, with the exception of Uganda Green (Fig. 6). However, the increase in total leaf area for plants that had Si application terminated after Xcm inoculation was not significantly different from plants that had Si application started after Xcm inoculation.

Quantification of Si accumulation in leaf tissue

Silicon content in leaf tissue was significantly ($P \leq 0{\cdot}05$) higher in Si-treated plants compared to the non-Si-treated control plants. The level of Si accumulation was dependent on the duration of Si supply (Table 4); continuous application of Si resulted in significantly higher amounts of Si accumulated in leaves compared to plants where Si application was terminated following Xcm inoculation or when application of Si started after Xcm inoculation.

POD enzyme analysis

Peroxidase activity in leaves of Xcm infected plants was significantly higher in plants that had been treated with Si in comparison to the non-Si-treated control plants, regardless of the duration of Si application (Fig. 7). The plants treated continuously with Si showed significantly higher POD activity than other treatments. However, POD activity in Ngombe and Gros Michel plants that had Si treatment terminated after Xcm inoculation was not significantly different from plants that had Si treatment starting immediately after Xcm inoculation.

Discussion

The important role of Si in increasing resistance to both biotic (pests and diseases) and abiotic stresses (drought,

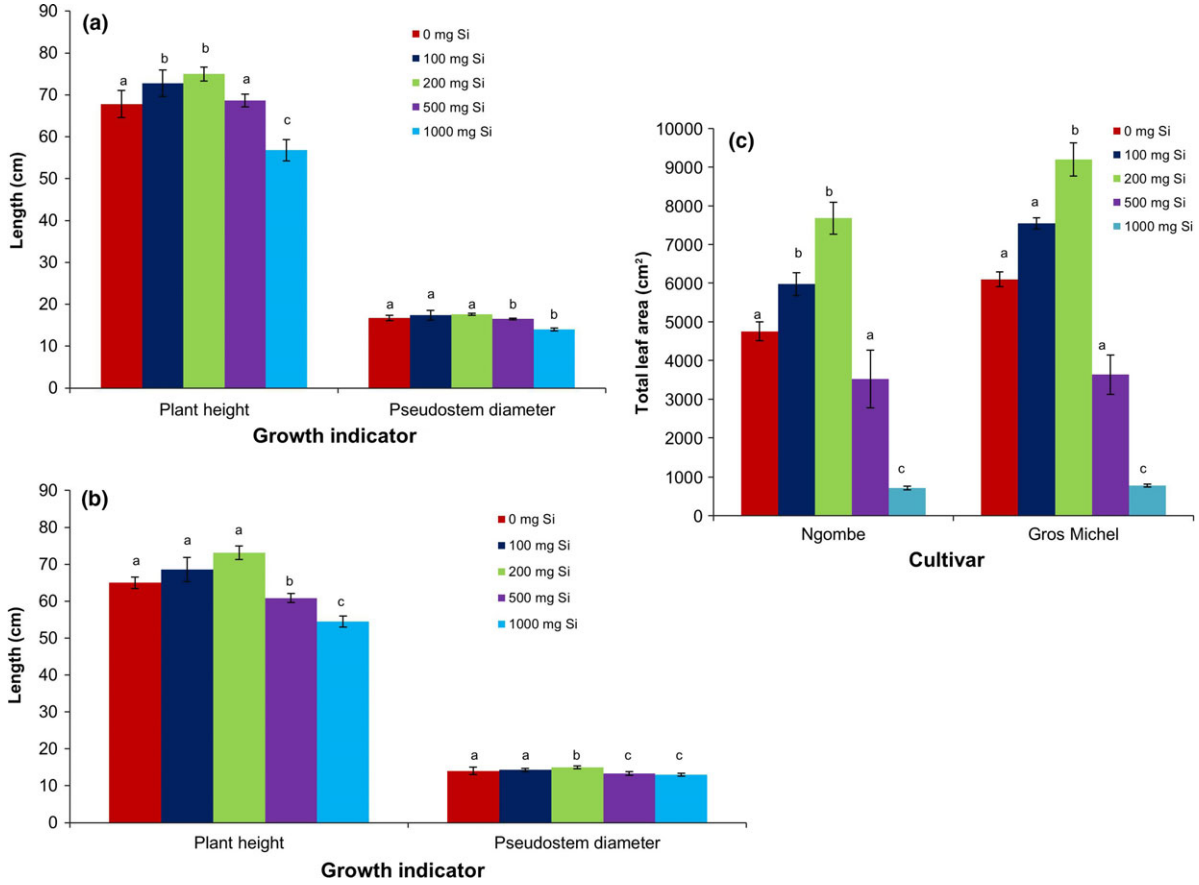

Figure 5 Effect of different silicon (Si) concentrations on growth parameters of different banana cultivars. (a) Plant height and pseudostem diameter of Gros Michel, (b) plant height and pseudostem diameter of Ngombe, (c) total leaf area of Ngombe and Gros Michel. The values are means of 10 replicates (mean ± SE). Mean values marked with the same letter within a parameter are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$).

salinity and heavy metals) in crops has been widely reported (Guntzer *et al.*, 2012). In this study, the effect of Si application to BXW disease was assessed using potted plants under screen house conditions. Banana plants treated with Si exhibited delayed onset of BXW disease symptoms compared to the non-Si-treated control plants. The uptake of Si and its deposition in banana tissues may have limited Xcm systemic spread and consequently increased the duration of time required for pathogen colonization. Previous studies on Si-induced disease resistance in other crops have indicated that besides forming a mechanical barrier, Si application also activates defence-related pathways upon infection (Fauteux *et al.*, 2005; Cai *et al.*, 2009). Si-induced resistance to bacterial pathogens has been reported in tomatoes (Wydra & Beri, 2006, 2007; Dahal *et al.*, 2010). In particular, tomato plants treated with Si showed enhanced resistance against bacterial wilt caused by *R. solanacearum* (Kiirika *et al.*, 2013). The resistance to bacterial wilt in tomato was effected through changes in the cell wall and up-regulation of genes involved in plant defence signal transduction, transcription and metabolism, indicating a priming effect (Wydra & Beri, 2006). This priming effect may be mediated via ethylene, jasmonic acid and/or reactive oxygen species signalling pathways (Ghareeb *et al.*, 2011). The silicon in tomatoes was shown to reduce the spread of the *R. solanacearum* in xylem vessels, through induction of basal resistance mechanisms at cell wall level and at pit membranes, which are the preferred route of spread of the bacteria in plant tissues (Diogo & Wydra, 2007). In particular, Si-treated plants showed an increased branching of rhamnogalacturonan I in cell walls. This may contribute to strengthening of the pit membranes of the vessels and of the walls of parenchyma cells, making them less easily degradable and consequently limiting the movement of bacteria from vessel to vessel (Diogo & Wydra, 2007). In addition application of Si against bacterial and fungal pathogens has also been reported to stimulate other biochemical host defence responses such as up-regulation of defence genes and an increase in inhibitory phenolic compounds (Cai *et al.*, 2009; Kiirika *et al.*, 2013). Thus, the protective role of Si is mediated by a combination of changes in mechanical properties and induction of plant defence

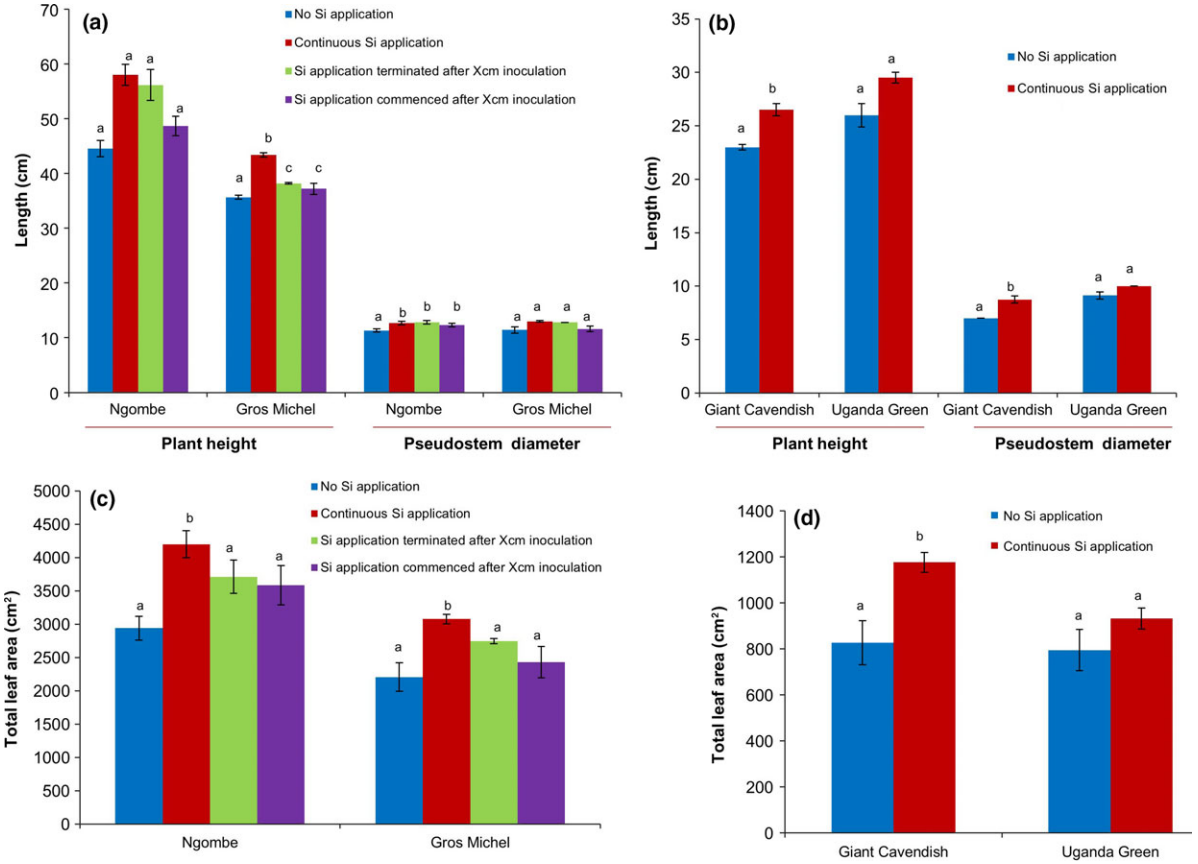

Figure 6 Effect of different duration and time of application of silicon (Si; 200 mg/plant/week) on growth parameters of banana cultivars inoculated with *Xanthomonas campestris* pv. *musacearum* (Xcm). (a) Plant height and pseudostem diameter of Ngombe and Gros Michel, (b) plant height and pseudostem diameter of Giant Cavendish and Uganda Green, (c) total leaf area of Ngombe and Gros Michel, (d) total leaf area of Giant Cavendish and Uganda Green. The values are means of 10 replicates. Mean values followed by the same letter within a given parameter are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$) means ± SE.

Table 4 Silicon (Si) accumulation in leaf tissues of banana plants of different cultivars treated with 200 mg Si per plant per week

	Si content (mg kg^{-1} fresh weight) in leaf tissues[a]			
Si treatment	Ngombe	Gros Michel	Giant Cavendish	Uganda Green
No Si treatment	29·9 ± 0·1 a	29·9 ± 0·4 a	24·4 ± 0·1 a	24·6 ± 0·2 a
Continuous Si treatment	41·9 ± 0·1 b	41·0 ± 0·1 b	37·3 ± 0·2 b	36·3 ± 0·2 b
Si treatment terminated after Xcm inoculation	35·2 ± 2·3 c	34·1 ± 1·1 c		
Si treatment started immediately after Xcm inoculation	31·2 ± 0·1 c	31·2 ± 0·1 c		

Means followed by the same letter within a column are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$).
[a]Means of three replicates (mean ± SE).

responses. Treatment of plants with Si increases its accumulation and deposition in leaves to form a cuticle-Si double layer that can impede penetration by a pathogen. Si can also induce the biochemical defence responses of the host and even prime the defence capacity of the plant to reduce disease incidence (Cai *et al.*, 2009).

The level of resistance to BXW disease increased as the amount of Si applied was elevated to concentrations of 200 mg of Si per week. Thereafter, a decrease or no change was observed in the level of resistance in response to increasing Si concentration, suggesting that there is a threshold at which Si optimally induces resistance to BXW disease. Consequently, Si concentration of 200 mg per plant per week was used in the evaluation of the effect of varying time points of Si application on disease resistance. In all the cultivars tested, continuous supply of Si or when Si application was terminated upon inoculation with Xcm resulted in delayed appearance of

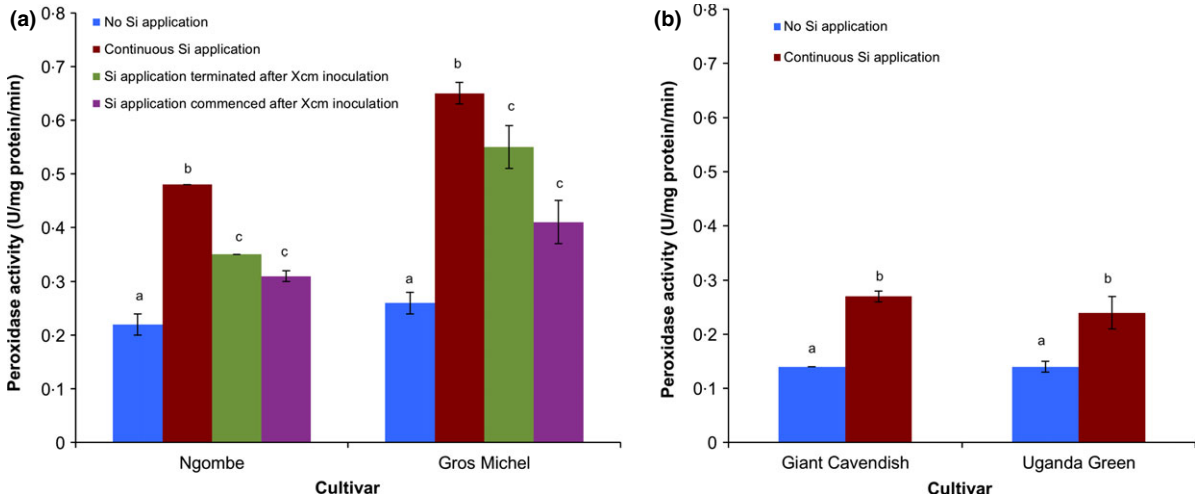

Figure 7 Peroxidase (POD) activity in leaves of banana plants treated with silicon (Si) and inoculated with *Xanthomonas campestris* pv. *musacearum* (Xcm). (a) Peroxidase activity in Ngombe and Gros Michel plants, (b) peroxidase activity in Giant Cavendish and Uganda Green plants. Values are means of three replicates. Means followed by the same letter within a given cultivar are not significantly different from each other by Tukey's HSD test ($P \leq 0{\cdot}05$) means ± SE.

disease symptoms and a higher level of BXW disease resistance compared to non-Si-treated control plants. It is noteworthy that even plants in which Si application terminated after inoculation with Xcm showed resistance to BXW, as previous reports have demonstrated that continuous application of Si both before and after pathogen inoculation was most effective in suppressing disease (Heine *et al.*, 2006).

The amount of Si in the plant tissue may have played a role in determining the level of pathogen resistance. In treatments where the plants had continuous supply of Si either only before, or both before and after inoculation with Xcm, the resistance observed against the Xcm pathogen may have been due to accumulated Si in the tissues acting as a mechanical barrier and preventing systemic spread of the pathogen and activation of other molecular systemic plant defence responses. Kim *et al.* (2002) showed that treatment of rice plants with Si resulted in cell wall fortification of rice leaves and may be closely associated with enhanced host resistance to blast. The Si was accumulated mainly in epidermal cell walls, middle lamellae and intercellular spaces within subepidermal tissues, with relatively small deposition on stomata guard cells (Kim *et al.*, 2002). However, in the present study, in plants that had Si application only from the point of inoculation, the level of resistance towards the pathogen might have largely been a result of activation of systemic defence responses because the plants had not previously accumulated Si in their tissues. Application of Si led to increased activity of the POD enzyme, suggesting activation of molecular plant defence responses. Several studies have reported the ability of Si to enhance plant defence responses against necrotic pathogens through activation of protective enzymes such as PAL, POD and PPO (Cai *et al.*, 2009). Thus, results of the present study indicate that both the concentration and time of application of Si may influence the level of plant resistance to BXW. Therefore it is possible that, depending on the status of Si in the plant tissue, the defence response may be either primarily through the physical barrier or/and activation of systemic defence responses.

Banana growth parameters were significantly enhanced in some of the cultivars treated with 200 mg of Si per week. However, the growth parameters were negatively impacted in plants treated with 1000 mg of Si per week, in comparison to the non-Si-treated controls. The differential effects of Si on growth parameters depending on the concentration may be attributed to chemical induced hormesis. Hormesis is a term used to describe the dose–response relationships of treatments, whereby at low concentration a chemical is able to exert beneficial effect (low-dose stimulation), while at high-dose there is inhibition or detrimental effects (Calabrese *et al.*, 1999). Treatment of the plant with 200 mg of Si per week might have resulted in a stimulatory effect leading to the beneficial growth attributes; however, when dosage increased to 500 and 1000 mg per week the inhibitory phase was triggered, resulting in detrimental effects evidenced by stunting and discolouration of the leaf edges. Negative effects such as stunting, deformed flowers and delay in flowering were observed in ornamental sunflowers at high concentrations of Si (Kamenidou *et al.*, 2008), thus suggesting Si application can vary from beneficial to detrimental depending on the concentration. Stimulatory effects of other compounds such as copper, iron nitrate and zinc sulphate on the growth of wheat have also been reported (Calabrese & Baldwin, 2000). The application of copper, iron nitrate and zinc sulphate to greenhouse-grown wheat plants resulted in a typical hormetic dose–

response curve in all growth indicators such as plant height, transpiration, fresh and dry weight (Calabrese & Baldwin, 2000). In addition low quantities of potassium iodide have been shown to moderately stimulate the growth (12–50%) in soil of peas, oats and radish (Calabrese & Baldwin, 2000).

The positive effects of Si on growth parameters were observed for all plants treated with Si regardless of the timing in relation to inoculation or the duration of Si supply. Enhancement of these growth parameters was most notable for plants that had a continuous supply of Si. Although Si has not been recognized as an essential element for plant growth, the beneficial effects of Si have been observed in a wide variety of plant species, particularly graminaceous plants such as rice and sugarcane as well as some cyperaceous plants (Kamenidou *et al.*, 2008). Si may be involved in cell elongation and/or cell division and this may explain the larger plant height and pseudostem diameter observed in Si-treated plants. The accumulated Si in leaf tissues has been shown to increase the photosynthetic rate through chlorophyll enhancement and increased mesophyll conductance (Detmann *et al.*, 2012). Such an effect on photosynthetic efficiency, which is the most basic and critical physiological process directly related to plant development, may lead to more yield, plant growth and production.

The presence of Si in plants seems to impact a number of plant molecular functions involved in defence responses, photosynthesis and respiration (Cai *et al.*, 2009; Detmann *et al.*, 2012). These effects have the potential of enhancing resistance to disease and improving agronomic traits. The use of Si as a regular farm treatment, either directly or in combination with other ingredients such as fertilizers, would be advantageous due to the relative ease of its application and it would greatly augment existing BXW disease management strategies. Ayana *et al.* (2011) have shown the potential of using Si fertilizer against tomato bacterial wilt (*R. solanacearum*). In their report, Si fertilizer significantly reduced the bacterial population, wilt incidence, severity index, and corresponding areas of disease incidence and severity progress curves in the moderately resistant tomato cultivar King Kong 2. In addition, Si fertilizer amendments also increased the tomato fruit yield, thus emphasizing the possibility of using Si not only to enhance disease resistance, but also for increased production. Already there are various commercially available formulations of Si fertilizers available in the market, thus offering the potential of using Si to improve the quality and quantity of agricultural products. Results from this study indicate that Si application has the potential to not only confer disease resistance to banana against Xcm, but also enhance a number of growth parameters. However, further studies are required to confirm if the results can be repeated under field conditions. In addition, it is essential to conduct further tests to ascertain the effect of applying Si in the field on yield and other important agronomic traits of mature banana plants.

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