*World Journal of Agricultural Research, 2017, Vol. 5, No. 1, 42-51*
Available online at http://pubs.sciepub.com/wjar/5/1/6
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DOI:10.12691/wjar-5-1-6

# Silicon Induces Resistance to Bacterial Blight by Altering the Physiology and Antioxidant Enzyme Activities in Cassava

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**Abstract** Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is a devastating disease limiting cassava production. The potential effect of Si application on the physiological and biochemical mechanisms attributed to Si-mediated resistance of cassava to *Xam* was evaluated. The optimal concentration of Si in enhancing resistance to CBB without detrimental effects on plant growth was determined using cultivars TME14 and TMS60444 known for their susceptibility to *Xam*. Varied concentrations of Si (0.7 to 2.1 mM) were administered by watering the plants three times per week before and after *Xam* inoculation. The optimized Si concentration was used to evaluate the effect of Si supplementation on resistance to CBB disease using eight farmer-preferred cassava cultivars. The population of *Xam*, cultivar resistance, chlorophyll content, lipid peroxidation, $H_2O_2$ content, activity of antioxidant enzymes and total Si content in cassava cultivars were quantified 21 days post inoculation. Silicon concentration of 1.4 mM was optimal in enhancing cassava resistance to *Xam*. Silicon-treated plants of all cassava cultivars showed significantly ($P \leq 0.05$) lower *Xam* population ranging from 5% to 26.7% compared to non-Si treated control plants. Activities of antioxidant enzymes, malondialdehyde, $H_2O_2$ and chlorophyll contents were significantly ($P \leq 0.05$) higher in Si treated plants than non-Si treated plants. Silicon accumulation in leaves of Si treated plants was higher compared to non-Si treated control plants.

***Keywords:*** *cassava, silicon, bacterial blight disease, xanthomonas axonopodis pv. axonopodis, resistance*

**Cite This Article:** K. W. Njenga, E. Nyaboga, J. M. Wagacha, and F. B. Mwaura, "Silicon Induces Resistance to Bacterial Blight by Altering the Physiology and Antioxidant Enzyme Activities in Cassava." *World Journal of Agricultural Research*, vol. 5, no. 1 (2017): 42-51. doi: 10.12691/wjar-5-1-6.

## 1. Introduction

Cassava is ranked as the most important source of carbohydrates after sugarcane and maize in the tropics and subtropics [1]. Productivity of cassava in marginal soils, its ability to withstand harsh environmental conditions like drought and flexible harvesting times makes it an ideal crop in regions prone to drought, poverty and malnutrition [2]. However, biotic constraints such as pests and diseases have led to a steep decline in cassava yields. Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is one of the bacterial diseases with high occurrence in cassava growing regions of the world [3,4]. The bacterium is a foliar and vascular pathogen which manifests a wide range of symptoms including blight, wilting of the shoots, dieback, stem cankers, production of exudates and necrosis [4,5]. Penetration of *Xam* is through stomatal openings or through wounds on the leaf surfaces. The bacterium multiplies in the intercellular spaces and migrates in a systematic way through the xylem, resulting in wilting of the shoots [3]. The economic impact of the disease is potentially devastating because it destroys whole plants leading to complete yield loss.

The disease can be controlled by cultural on-farm disease management practices such as crop rotation, cutting and burying or burning of infected plants, intercropping cassava and cereals such as maize, restricting the movement of cassava planting materials from CBB affected to disease free areas, and the use of sterilized farm tools [5,6,7]. However, the adoption rate of these practices is low and inconsistent, as they are considered tedious and therefore have not been effective in control of the disease. Use of resistant cultivars has been exploited; however this resistance is sporadic and has not been characterised into the cultivars that are best adapted in the different cassava growing zones [8]. The lack of known genetic resistance in cassava against Xam, the difficulties associated with conventional breeding because of the sterility of most cultivars coupled with long generation time favor the development of alternative approaches to control the CBB pandemic. Use of chemical elements such as silicon has been reported widely in the induction of resistance against fungal and bacterial pathogens in many economically important crops [9,10]. Si amendment may therefore offer a promising alternative in the control of CBB.

Silicon (Si) is a commonly available element in many soils and is absorbed by plant roots in the form of non

charged silicic acid ($Si(OH)_4$). Despite silicon possessing immense benefits to crops, it has not been classified as an essential element required for primary plant growth. Several studies indicate that increased uptake of Si may boost tolerance to both abiotic and biotic stresses in rice, sugarcane, banana and tomato [11,12,13,14]. The ability of Si to induce plant resistance against phytopathogens is premised on two mechanisms: activation of plant related defense genes and formation of physical barriers thus restricting penetration and colonization of the host by the pathogen [15,16].

Two mechanisms of Si in inducing disease resistance in plants have been proposed [15]. The first was accumulation of silica in the plant cell walls which inhibits pathogen penetration by forming a mechanical barrier. The second was that the soluble form of Si stimulates the expression of natural plant defense responses hence strengthening plant disease resistance. In the presence of Si as an amendment, most terrestrial plants undergo the process of silicification leading to stimulation of lignin biosynthesis which strengthens the cell walls and xylem vessels [17]. Silicon application in rice plants increased resistance to rice blast, caused by *Magnaporthe grisea*, by production of phenolic compounds and phytoalexins [18]. Rice plants treated with Si displayed a rapid hypersensitive reaction [19] and an earlier epidermal cell death in response to *M. grisea* infection compared to non-Si treated rice plants. These findings clearly indicated that Si had a wider role in induction of resistance to rice blast than the provision of a mechanical barrier to the pathogen.

Various biotic stresses such as insect feeding, bacterial, viral and fungal diseases result in overproduction of harmful reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), superoxide ($O^{2-}$) and hydroxyl (OH) radicals in plants [20,21]. Inoculation of *Magnaporthe grisea* in Si-treated rice plants significantly increased the activities of antioxidative enzymes such as peroxidase, catalase, polyphenol oxidase, superoxide dismutase and phenylalanine ammonia-lyase [22].

There is no documented information available on the physiological and biochemical role of Si on elicitation of cassava plant resistance against bacterial blight. Furthermore, there is no information on Si and bacterial blight disease interaction in cassava. Therefore, this study aimed to: i) evaluate the role of Si uptake in enhancing cassava resistance to bacterial blight and ii) examine the influence of Si on physiological and biochemical reactions and their relevance to bacterial blight resistance. Such information may help provide evidence of Si-mediated resistance to cassava bacterial blight and improve management of the disease in cassava production.

# 2. Materials and Methods

## 2.1. Preparation of Plant Material

Stem cuttings of ten cassava cultivars (Albert, 98/0505, 92/0326, Namikonga, TME7, TME419, 01/1371, AR40-6, TME14 and TMS60444) were cut into smaller nodal sections using a sharp sterile surgical blade ensuring that each section contained at least two nodes; one for rooting and the other for formation of shoots. The nodal sections were planted in soil in 4 cm diameter plastic pots and maintained in a glasshouse with weekly watering for four weeks.

## 2.2. Pathogen Isolation and Inoculum Preparation

The pathogen *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) was isolated from diseased cassava plants sampled from a farmer's field in Kakamega County, Western Kenya. Characterization of the bacterium isolate through polymerase chain reaction (PCR) using *Xam*-specific primers confirmed it was *Xanthomonas axonopodis* pv. *manihotis*. The isolate was maintained in Yeast Peptone Glucose Agar (YPGA) medium (0.5% w/v yeast extract, 0.5% (w/v) peptone, 1% (w/v) glucose and 12 g $L^{-1}$ bacteriological agar) supplemented with 10 mg $L^{-1}$ cyclohexamide and 50 mg $L^{-1}$ cephalexin and stored at 4 °C. For preparation of inoculum, a single colony of *Xam* was cultured in Yeast Peptone Glucose (YPG) medium supplemented with 50 mg $L^{-1}$ cephalexin and incubated at 28 °C for 48 hours. Growth of the culture was monitored by measuring and ensuring that the optical density value of 1.0 which corresponds to $10^8$ colony forming units (CFU/ml) was attained. The bacterial suspension was centrifuged at 3,500 g for 5 minutes and the bacterial pellet suspended in sterile double-distilled water. The $OD_{600}$ of the bacterial suspension was adjusted to 1.0 before artificial inoculation.

## 2.3. Determination of Optimal Concentration of Si for Elicitation of Cassava Plant Resistance to *Xam*

Clean plantlets of cassava cultivars TMS60444 and TME14 susceptible to *Xam* were watered thrice a week with 1 litre of distilled water containing 0.7, 1.4 and 2.1 mM silicon (in the form of Sodium Metasilicate, $Na_2SiO_3$) for a period of one month prior to artificial inoculation with *Xam*. The control plants were watered with distilled water. Artificial inoculation with *Xam* was done by injecting 200 µl of the bacterial suspension ($10^8$ CFU/ml) into the stem of the plantlets using a 1 ml syringe fitted with a 28-gauge needle. Sterile double-distilled water was used to mock-inoculate the control plants. The experiment consisted of two combinations with four replicates each: (1) plants with no Si application but inoculated with *Xam* to act as inoculated control; and (2) plants treated with different Si concentrations for one month and then inoculated with *Xam* followed by continuous application of the respective amounts of Si for 21 days post inoculation (dpi). The plantlets were kept in the glass house and development of disease symptoms was assessed daily for 21 dpi. The average number of days for initial development of CBB symptoms and the number of wilted leaves were recorded at 21 dpi and the resistance (%) determined as;

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

Cassava leaf samples were harvested from plants watered with different concentrations of Si at 21 days post-inoculation for quantification of *Xam* population.

## 2.4. Quantification of *Xam* Population in Cassava Leaves after Inoculation

Twenty one days post-inoculation, leaf samples were harvested from all the plants and stored at -20°C. Exactly 1 g of each leaf sample was cut into smaller pieces using a sterile blade and aseptically introduced in 100 ml of Yeast Peptone Glucose (YPG) medium and incubated at 28°C at 150 rpm for 48 hours. Serial dilutions of the bacterial suspensions were performed upto $10^5$ dilution and 20 µl of each of the dilutions was cultured on Yeast Peptone Glucose Agar (YPGA) medium and incubated at 28°C for 48 hours. Colony counts were performed for leaf samples in all the treatment combinations.

## 2.5. Effect of Si Application on Plant Growth

Growth parameters such as number of leaves and the plant height of the various treatments were recorded 21 days post inoculation.

## 2.6. Evaluation of the Optimized Si Concentration in Selected Farmer-Preferred Cassava Cultivars for Bacterial Blight Disease Resistance

Following determination of the optimal Si concentration for inducing CBB disease resistance, two-month-old cassava cultivars (Albert, TME7, 01/1371, AR40-6, 92/0326, 98/0505, TME419 and Namikonga [NMK]) were watered with 1.4 mM of Si weekly for one month prior to artificial inoculation with *Xam* ($10^8$ CFU/ml) followed by continuous weekly applications. The development of CBB symptoms and total number of leaves wilted at 21 dpi were recorded. Leaf samples were harvested from plants at 21 days post-inoculation for quantification of *Xam* population and the determination of resistance (%).

## 2.7. Determination of Chlorophyll Content

The amounts of photosynthetic pigments (chlorophyll a [Chl a], chlorophyll b [Chl b], total [Chl T]) were assayed spectrophotometrically according to [23]. Leaf samples (100mg) from each treatment were weighed and homogenized in 1 ml of 80% (v/v) acetone, with a pestle and mortar, and incubated in the dark for 30 minutes. The extract was centrifuged at 3,500 × *g* for 5 minutes and the supernatant's absorbance reading was recorded at wavelengths of 647 and 664 nm. The chlorophyll content was expressed in µg $g^{-1}$ FW. Chl a, Chl b and Chl T were calculated according to [23] using the equations:

$$\text{Chl a} = 11.94A_{664} - 1.93A_{647},$$

$$\text{Chl b} = 20.36A_{647} - 5.50A_{664},$$

$$\text{Chl T} = \text{Chl a} + \text{Chl b}.$$

## 2.8. Determination of Malondialdehyde (MDA) Content

Malondialdehyde (MDA) which is an indicator of the level of lipid peroxidation in plants was estimated using the thiobarbituric acid (TBA) method as described by [24,25]. Briefly, 0.2 g of each leaf sample from both treatments was homogenized in 4 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution on ice using a mortar and pestle. The suspension was transferred into an Eppendorf tube and an additional 1 ml of TCA was added. The homogenate was centrifuged at 3,500 × *g* for 5 minutes and 0.5 ml of the supernatant transferred in to a fresh tube. One milliliter of 20% (w/v) TCA containing 0.5% (w/v) TBA was added and the mixture was incubated in a water bath (95 °C) for 30 minutes and then cooled in an ice bath. Finally the homogenate was centrifuged at 3,500 × *g* for 10 minutes and the absorbance of the supernatant was read at 532 and 600 nm. The level of lipid peroxidation was expressed as nmol malondialdehyde $g^{-1}$ fresh weight (FW) using the formula:

$$\begin{aligned}&\text{MDA content}\\&= \text{Amount of extraction buffer}\,(\text{ml})\\&\times \text{Amount of supernatant}\,(\text{ml})\\&\times\left[(\text{Abs } 532 - \text{Abs } 600)/155\right]\times 1000/\text{Amount of sample}\end{aligned}$$

Where:

532 nm represents maximum absorbance of the TBA-MDA complex; 600 is the correction for non-specific turbidity and 155 $mM^{-1}cm^{-1}$ is the specific molar extinction coefficient for MDA. All measurements were repeated three times from extracts of three batches of plants.

## 2.9. Measurement of Hydrogen Peroxide ($H_2O_2$) Content

Hydrogen peroxide content was determined spectrophotometrically using potassium iodide (KI) [26]. Briefly 0.5 g of the leaf samples were immersed in ice and ground with a mortar and pestle to a suspension then homogenized with 2 ml ice cold 0.1% (w/v) TCA. The homogenate was centrifuged at 3,500 × *g* for 30 min at 4°C. Finally, 0.5 ml of the supernatant was dispensed into a clean Eppendorf tube and 0.5 ml 10 mM potassium phosphate buffer (pH 7.0), 1 ml of 1 M potassium iodide (KI) was added and the absorbance read at 390 nm. Hydrogen peroxide content was calculated using the extinction coefficient 0.28/mM/cm and expressed as µmol/g FW.

## 2.10. Extraction and Determination of Antioxidant Enzyme Activities

Antioxidant enzymes peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT) were extracted according to [27] with slight modifications. Exactly 0.5 g of each leaf sample was homogenized with a mortar and pestle in 2 ml of homogenate buffer containing 100 mM potassium phosphate buffer pH 6.8, 0.2 mM EDTA and

1% w/v polyvinylpyrolydone (PVP) in an ice bath. The homogenates were centrifuged at 3,500 × *g* for 20 minutes at 4 °C and the supernatants were used immediately to assay for enzyme activities.

Peroxidase (POD) activity (1.1.11.1.7) was determined by measuring the increase of absorbance at 470nm because of formation of tetra guaiacol (extinction coefficient of 26.6 $mM^{-1}$ $cm^{-1}$) as described by [28]. Exactly 50 µL of the leaf homogenate was added to 2 ml of the reaction mix which contained 50 mM sodium acetate buffer (pH 7), 25 mM guaiacol and 25 mM $H_2O_2$.

Ascorbate peroxidase (APX) activity (EC1.11.1.11) was assayed spectrophotometrically by following the oxidation of ascorbic acid and measuring the change in absorbance at 290 nm. Exactly 10 µL of the leaf extract was added to 1 ml of the reaction mix containing 0.2 mM Tris/HCL buffer (pH 7.8), 0.25 mM ascorbic acid and 0.5 mM $H_2O_2$. The activity was calculated from the extinction coefficient (2.8 $mM^{-1}$ $cm^{-1}$) for the ascorbate [29].

Catalase (CAT) activity (EC 1.11.1.6) was determined as the decrease in absorbance at 240 nm for 1 minute following the decomposition of $H_2O_2$ [30]. The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.0), 15 mM $H_2O_2$ and 50 µl of crude enzyme extract at 25 °C. The activity was calculated from the extinction coefficient (40 $mM^{-1}$ $cm^{-1}$) for $H_2O_2$.

### 2.11. Determination of Si Content in Cassava Plant Leaves

The total silicon content in the plant samples was determined spectrophotometrically according to [13] with modifications. Leaf samples were collected at 28 dpi, wrapped in aluminium foil and quickly immersed in an ice bath. Briefly 0.1g of the leaf samples was weighed and ground with mortar and pestle and suspension transferred into Falcon tubes containing 5 ml of 50% (w/v) NaOH. The mixture was autoclaved at 138 kPa for 60 minutes and cooled for 30 minutes after which 2 ml of 50% (v/v) $H_2O_2$ was added. The mixture was autoclaved for another hour and the solution was diluted with distilled water to 15 ml. Analysis of Si content was done using the Molybdenum Blue Colorimetric (MBC) method described by [31]. The diluted sample solution (1 ml) was homogenized with 5 ml of 20% (v/v) acetic acid for 20 seconds and then 2 ml of 0.3 M ammonium molybdate was added. Five minutes later, 1 ml of 20% tartaric acid (w/v) was added and the mixture was vortexed. Lastly 1 ml of 12.5% sodium bisulphite solution (w/v) solution was added to the mixture and the samples diluted to a final volume of 15 ml with 20% acetic acid. The blue colour was allowed to develop for 1 hour. Before reading of the absorbance the sample tubes were shaken vigorously. Serial dilution of a 100 mg $L^{-1}$ Si solution was used to prepare Si standards and the absorbance was determined at a wavelength of 650 nm as described by [31].

### 2.12. Data Analysis

Data on *Xam* population (CFU/ml), physiological parameters and antioxidant enzymes activities were subjected to Analysis of Variance (ANOVA) using R statistical software version 3.2.5 (Lucent Technologies). Comparisons of treatment means were performed according to Tukey's HSD test ($P \leq 0.05$). Data on *Xam* population was log transformed before analysis.

## 3. Results

### 3.1. Optimisation of Si Concentration for Induction of CBB Disease Resistance

After artificial inoculation with *Xam*, characteristic symptoms of bacterial blight appeared earlier in non-Si treated control plants with wilting of the young leaves whereas Si-treated plants showed delay in manifestation of symptoms (Table 1). Comparison of *Xam* population isolated from all the experimental plants indicated that plants treated with 1.4 mM of Si per week had significantly ($P \leq 0.05$) reduced bacterial population (Table 2). Silicon-induced resistance to *Xam* was significantly ($P \leq 0.05$) higher for all Si concentrations compared to the non-Si treated plants for both cultivars TMS60444 and TME14 (Figure 1). At Si concentrations of 1.4 mM per plant per week, the level of Si-induced resistance was significantly ($P \leq 0.05$) greater compared to the other Si concentrations (Figure 1). Cassava plants treated with 1.4 mM Si resulted in 12.7% and 26.7% induced CBB disease resistance for TMS60444 and TME14, respectively (Figure 1). Growth parameters such as the number of leaves and plant height were measured in cassava plants treated with different Si concentrations (Table 3). Plants treated with 1.4 mM Si had the highest number of leaves at 21dpi. There were significant differences ($P \leq 0.05$) in the total number of leaves in the two cultivars treated with different Si concentrations. In both cultivars, plants treated with 1.4 mM Si resulted in the highest number of leaves compared with the other treatments. However, there were no significant differences ($P \geq 0.05$) between the two cultivars treated with the same Si concentration. Likewise, there were no significant differences ($P \geq 0.05$) in height of Si treated and the non-Si treated control TME14 cultivar. However, there were significant differences ($P \leq 0.05$) in between 1.4 mM Si, 0.7 mM and the non-Si treated control for TMS60444. Based on silicon's effect on the induction of different levels of systemic resistance, growth parameters and reduction in *Xam* population for the two cultivars, weekly application of 1.4 mM of Si was determined to be the optimal concentration for inducing cassava plant resistance against *Xam* and was therefore used in the subsequent experiment.

**Table 1. Mean number of days post inoculation (dpi) when wilting of the leaves of inoculated cassava plants occurred**

| Concentration of Si applied (mM) | Cultivars | |
|---|---|---|
| | TME14 | TMS60444 |
| 0 | 8.5±0.64$^{d}$ | 5.7±0.4$^{e}$ |
| 0.7 | 13.5±0.65$^{c}$ | 10.8±0.5$^{d}$ |
| 1.4 | 20.5±0.29$^{a}$ | 18.8±0.4$^{ab}$ |
| 2.1 | 17.5±0.65$^{b}$ | 17.7±0.75$^{b}$ |

Data are mean±SE of five plants per treatment. Mean values followed by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$).

**Table 2. The effect of different Si concentrations on population (CFU/ml) of *Xam* in cassava leaves at 21 days post inoculation**

| Concentration of Si (mM) | Cassava cultivars | |
|---|---|---|
| | TMS60444 | TME14 |
| 0 | 9.155±0.01[a] | 9.306±0.02[a] |
| 0.7 | 8.386±0.05[b] | 7.721±0.04[d] |
| 1.4 | 7.995±0.07[cd] | 6.868±0.13[e] |
| 2.1 | 8.059±0.01[c] | 8.660±0.006[b] |

Data are means ±SE of five plants per treatment. Mean values followed by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$).

**Table 3. Effect of different Si treatments on growth indicators on cassava cultivars TMS60444 and TME14**

| Si application (mM) | Mean no. of leaves | | Mean height (cm) | |
|---|---|---|---|---|
| | TMS60444 | TME14 | TMS60444 | TME14 |
| 0 | 21.5±0.65[e] | 22±0.7[de] | 18±0.71[b] | 19±0.41[ab] |
| 0.7 | 25.8±0.48[cd] | 29.5±0.65[bc] | 18.5±0.65[b] | 20±0.4[ab] |
| 1.4 | 33.5±1.55[ab] | 35.8±1.1[a] | 21.5±0.65[a] | 20.25±0.48[ab] |
| 2.1 | 31.3±0.8[b] | 26.8±0.86[c] | 19.5±1.04[ab] | 19±0.41[ab] |

Data are means ±SE of five plants per treatment. Mean values followed by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$).

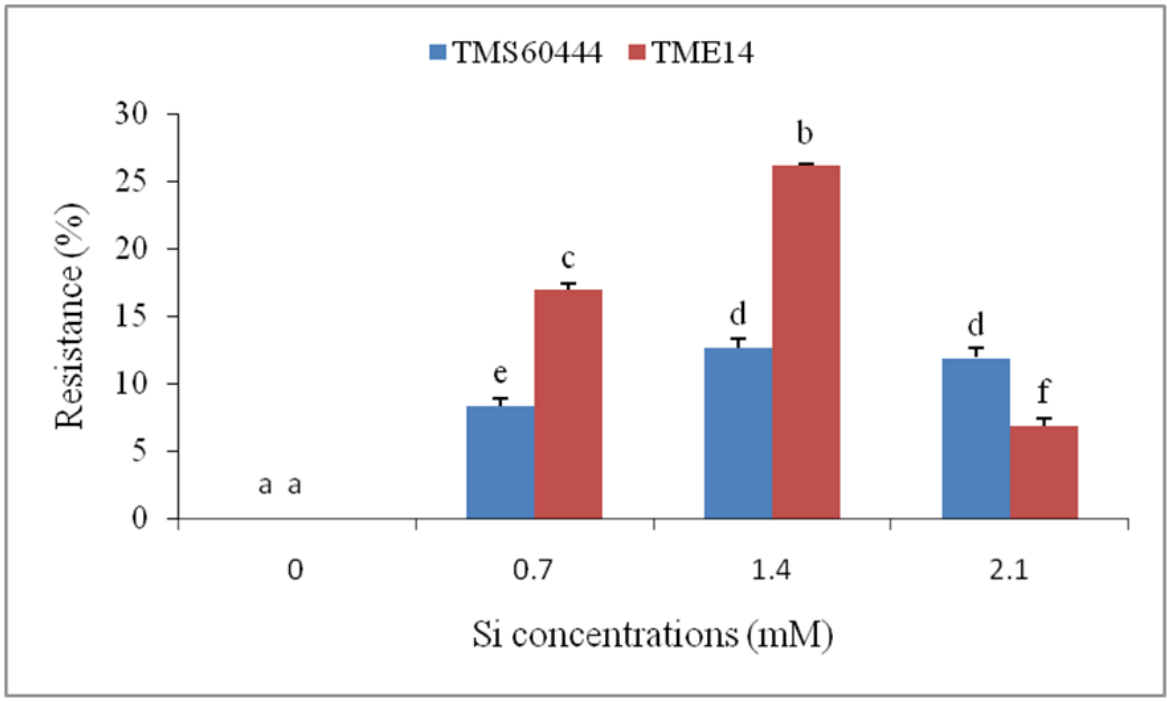


**Figure 1.** Level of resistance against *Xam* with varying concentrations of Si applied to cassava cultivars TME14 and TMS60444. Bars represent means ± SE of five replicates. Bars accompanied by the same letter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$)

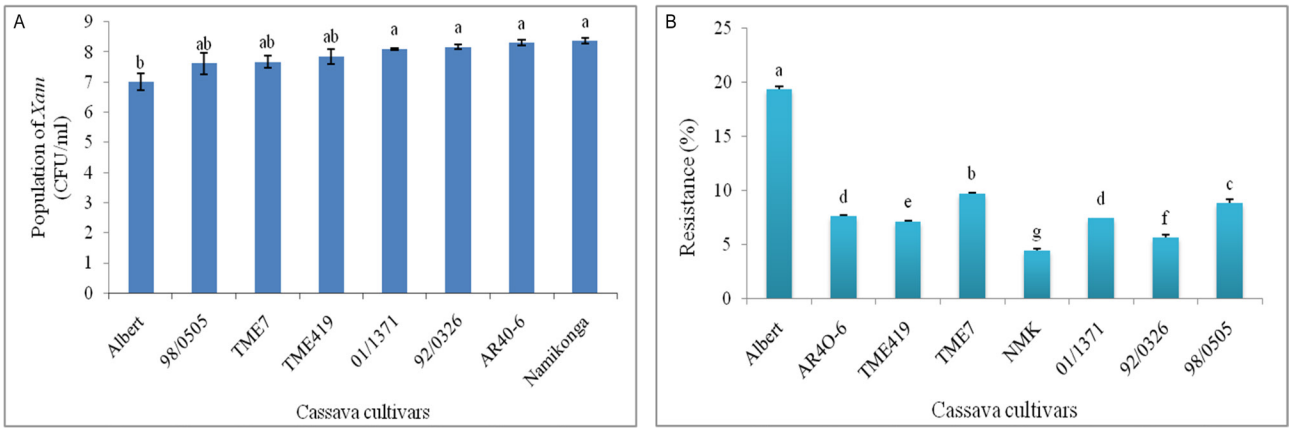


**Figure 2.** Effect of optimized Si treatment in selected cassava cultivars. (A) Reduction of *Xam* population in selected cultivars. (B) Level of resistance against *Xam* with 1.4mM Si treatment in selected cultivars. Bars represent means± SE of five replicates. Bars accompanied by the same letter within each figure are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$).

## 3.2. Effect of Optimal Si Application on *Xam* Population in Selected Cassava Cultivars

Application of 1.4 mM Si in selected farmer preferred cassava cultivars resulted in significant variation ($P \leq 0.05$) in the population of *Xam* (Figure 2). Cultivar Albert had the lowest *Xam* population while Namikonga had the highest. There were no significant differences in the population of *Xam* among cultivars Namikonga (NMK), AR40-6, 92/0326, TME419, 98/0505, TME7 and 01/1371 (Figure 2). The cultivars exhibited different levels of resistance to *Xam* with Albert having the highest (19.9%)

while resistance level for the other cultivars ranged between 5 and 10% (Figure 2).

### 3.3. Chlorophyll Content

There was a dramatic reduction in total chlorophyll content in the non-Si treated control plants for all the cultivars as compared to Si-treated plants (Figure 3). There were significant differences in the chlorophyll content ($P \leq 0.05$) between Si treated plants and non-Si treated control plants. The different cultivars tested also exhibited significant variations in the amounts of chlorophyll in their leaves.

### 3.4. Malondialdehyde (MDA) Content

Malondialdehyde (MDA) content was significantly ($P \leq 0.05$) higher in the non-Si treated control plants as compared to plants that were treated with 1.4 mM Si at 21 days post inoculation (Figure 4). This rise in MDA content in control plants indicated significant differences with the Si treated plants. There was an average 40% rise in MDA content in the non-Si treated control cultivars compared to the Si treated cultivars. Significant differences ($P \leq 0.05$) in the levels of MDA were observed among the selected cultivars treated with 1.4 mM Si.

### 3.5. Hydrogen Peroxide Content

Hydrogen peroxide was significantly ($P \leq 0.05$) higher in non-Si treated control plants compared to Si-treated plants (Figure 4). Hydrogen peroxide content was significantly ($P \leq 0.05$) reduced among Si treated plants in some cultivars while in others there were no significant differences.

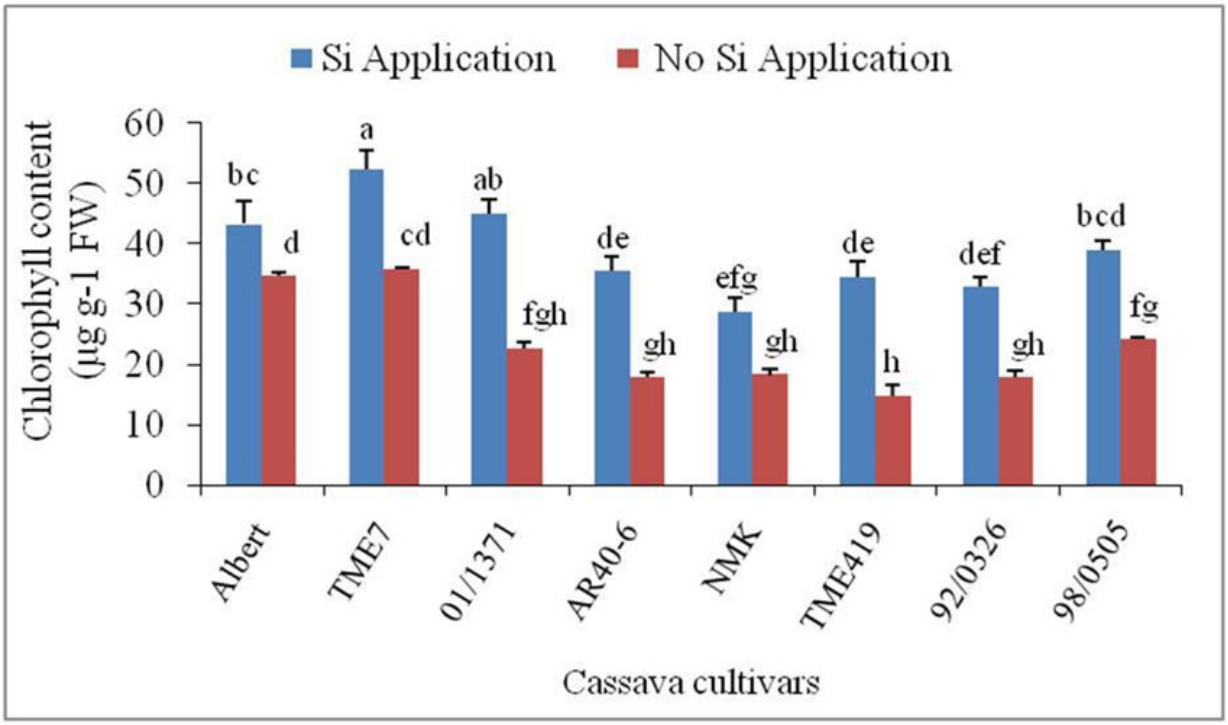


**Figure 3.** Total chlorophyll content in the selected cassava cultivars after 21dpi. Bars represent means ± SE of five replicates. Bars accompanied by the same letter within a given parameter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$)

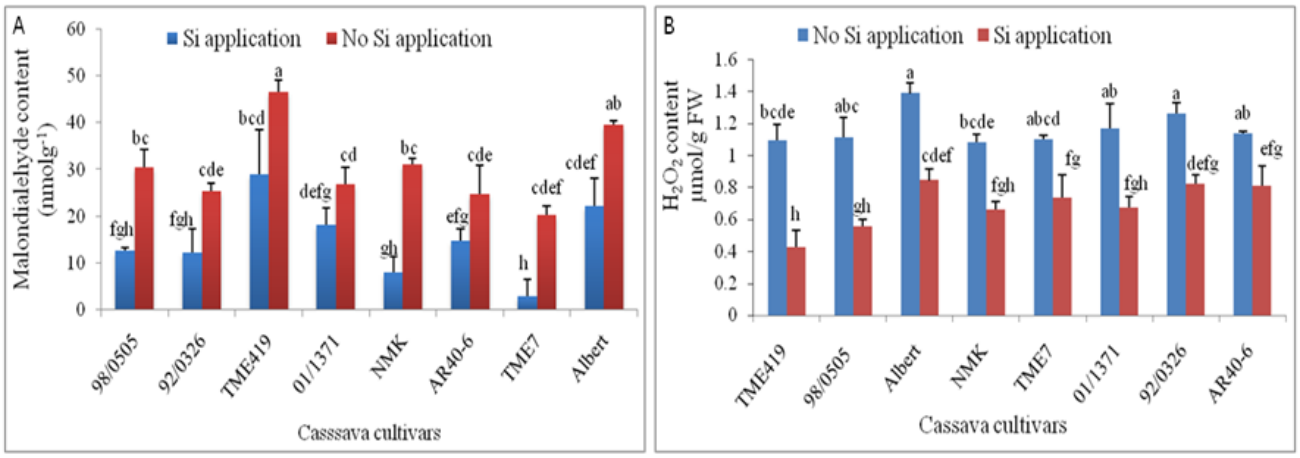


**Figure 4.** Physiological parameters attributed to Si-induced resistance in the selected cassava cultivars. (A) MDA content and (B) $H_2O_2$ content; Bars represent means ± SE of five replicates. Bars accompanied by the same letter within a given parameter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$)

### 3.6. Antioxidant Enzyme Activities

Enzyme activities of POD, APX and CAT increased significantly ($P \leq 0.05$) in the Si treated plants compared to the non-Si treated control plants (Figure 5). The different cultivars exhibited significant differences ($P \leq 0.05$) in the activity of different antioxidative enzymes.

### 3.7. Silicon Accumulation in Leaf Tissues

Silicon content in leaf tissues of the selected cassava cultivars was significantly ($P \leq 0.05$) higher in plants treated with Si compared to the non-Si treated control plants (Figure 6). Accumulation of Si was significantly higher among Si treated plants in some cultivars while in others there were no significant differences.

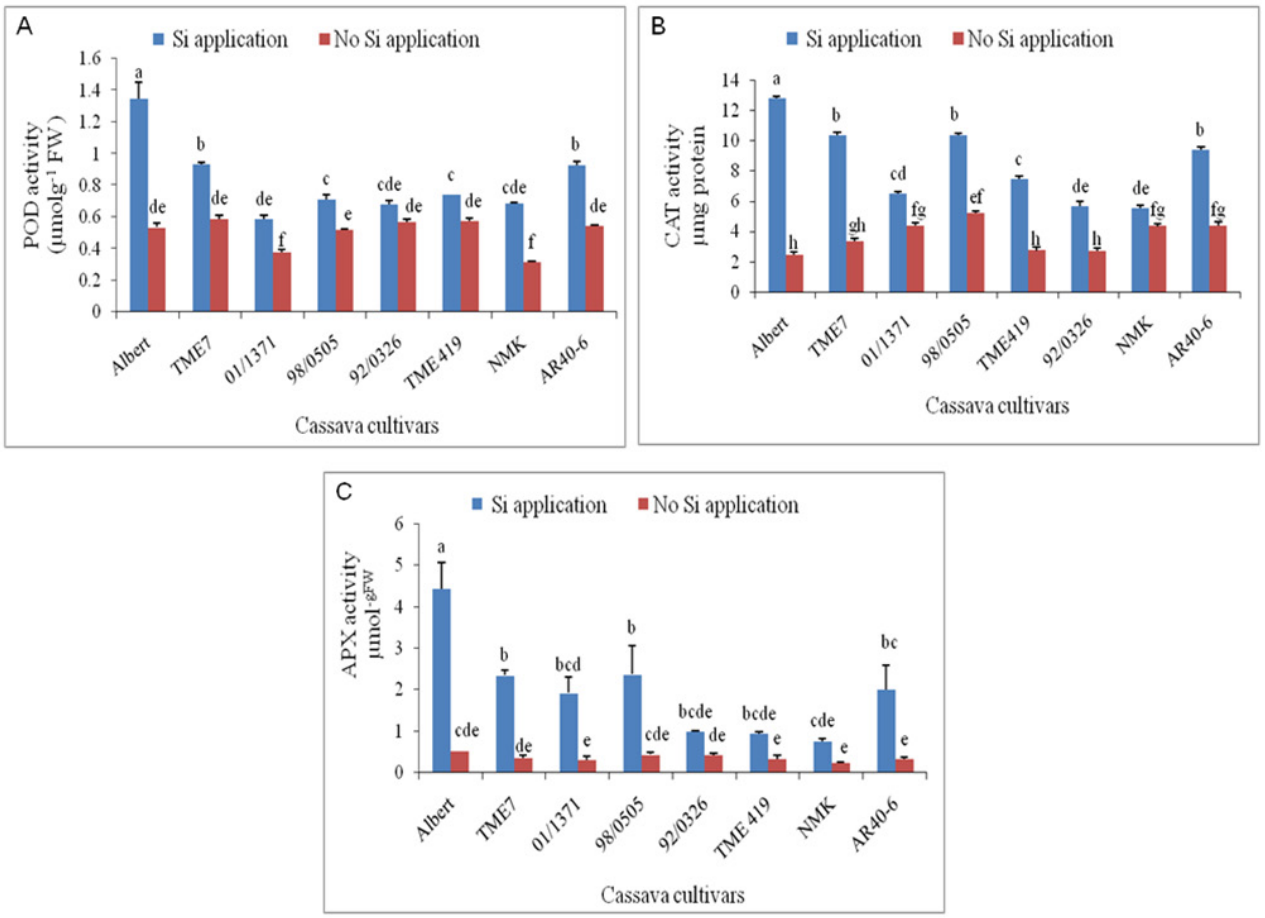


**Figure 5.** Biochemical parameters attributed to Si-induced resistance in the selected cassava cultivars. (A) POD activity, (B) APX activity, and (C) CAT activity; Bars represent means ± SE of five replicates. Bars accompanied by the same letter within a given parameter are not significantly different from each other by Tukey's HSD test ($P \leq 0.05$)

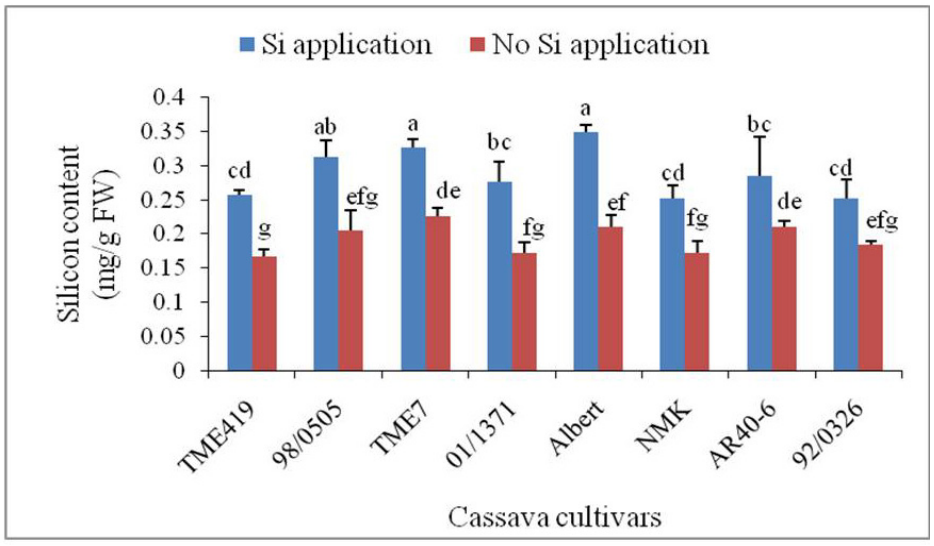


**Figure 6.** Silicon content (mg/g FW) in leaf tissues of different cassava cultivars at 21 days post inoculation with *Xam*. Bars represent means ± SE of five replicates. Bars accompanied by same letters are not significantly different according to Tukey's HSD test ($P \leq 0.05$)

## 4. Discussion

Several studies have documented the important role of Si in enhancing resistance to both biotic and abiotic stresses in many agriculturally important crops [9,19,32]. Despite this wealth of knowledge, the mechanisms by which Si exerts its beneficial effects are still poorly understood, a situation that has hindered the application of Si for agricultural purposes. In an attempt to unravel the physiological and biochemical mechanisms underlying Si-induced disease resistance, we investigated the physiological and biochemical events governing Si-mediated resistance against cassava bacterial blight. The results from this study not only demonstrate that Si can enhance plant resistance against bacterial pathogens but also provide physiological and biochemical evidence associated with an increase in the resistance against *Xam* infection in cassava plants supplied with Si. All the three Si concentrations tested significantly reduced the population of *Xam* in both cultivars TMS60444 and TME14 with 1.4 mM resulting in the highest reduction. However, as the concentration of Si increased to 2.1 mM, the level of resistance decreased to 11.2% and 6.9% for cultivars TMS60444 and TME14, respectively, suggesting that there is a threshold at which Si optimally induces cassava plant resistance to *Xam*. The two cultivars, TMS60444 and TME14, exhibited differences in the levels of Si induced resistance, days before onset of CBB symptoms, *Xam* population and growth parameters such as plant height and number of leaves, following Si application. These varied responses may be attributed to differences in the genotype characteristics.

Silicon application reduced bacterial blight development in all the eight farmer-preferred cassava cultivars tested. These findings are in agreement with [13] who reported that banana plants treated with 200 mg of Si per plant per week showed delayed development of *Xanthomonas* wilt disease as compared to non-Si treated control plants. Tomato plants treated with 1.4 mM Si [14] in the form of Monosilicic acid reduced the population of *R. solanacearum* in two tomato genotypes. In the present study, manifestation of CBB symptoms were delayed in Si treated plants. This suggests that Si uptake and accumulation in cassava tissues may have limited *Xam* penetration and spread hence prolonging the process of pathogen colonization. This is in agreement with [15] who reported that one of the primary mechanisms of Si in inducing resistance in plants is by provision of a mechanical barrier to the plants by the process of accumulation of amorphous silica in the cell walls therefore making penetration and spread of the pathogen difficult.

In terrestrial plants, one of the most important indicators of physiological activity is the rate of photosynthesis, which is determined by the chlorophyll content of plants. The total chlorophyll content in the leaves of Si treated plants was significantly higher than in the non-Si treated control plants. Reduction in total chlorophyll in cassava leaves as a result of bacterial blight disease may be as a result of the pathogen's effect on the release of transported toxins which leads to liberation of reactive oxygen species (ROS). The high chlorophyll content in leaves of Si treated plants was an indication that there was reduced damage due to yellowing and subsequent wilting which is a primary symptom of CBB during infection by *Xam*. The reduced damage in Si treated plants could be as a result of accumulation of Si in the leaf tissues and hence formation of a mechanical barrier resulting in obstruction of pathogen spread and colonization in the xylem vessels. These results indicated that Si treatment markedly affected the efficiency of photosynthetic apparatus in infected cassava leaves with a better potential for resistance. In addition, Si treatment resulted in mild symptoms of CBB and consequently reduced yellowing of the leaves. The current findings are in agreement with [33] who reported presence of high pigment concentrations in the leaves of banana plants due to reduced indirect root damage during *Fusarium* wilt infection. This reduction was due to obstruction of xylem vessels by the fungal mycelia resulting in mild symptoms of the disease.

Lipid peroxidation activity may be altered under biotic stresses [21,34]. It is measured by the formation of malondialdehyde (MDA) which is a byproduct of the decomposition of polyunsaturated fatty acids and has been used as an indicator for oxidative stress in plants [35]. Treatment of cassava cultivars with Si resulted in a significant reduction in the MDA levels compared to the non-Si treated control plants. Non-Si treated control plants exhibited a higher level of disease occurrence hence the increased levels of MDA. This could be due to the effect of *Xam* penetration that causes dysfunction of cell membranes leading to excess permeability of ions and electrolytes, which tend to increase the oxidative burst in cells. The application of Si mitigated the oxidative damage by decreasing the MDA content in plants. These results were in agreement with [14] who reported that Si reduced levels of lipid peroxidation by provision of an ameliorative effect of Si on the membrane integrity. These results suggest that MDA could be used as a useful index for screening CBB-tolerant cassava cultivars.

Various biotic stresses such as bacterial, viral and fungal diseases lead to overproduction of reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), superoxide ($O^{2-}$) and hydroxyl (OH) radicals in plants [20,21]. Hydrogen peroxide can play two roles in plant metabolism: at low concentrations it acts as a signal molecule for the induction of protective mechanisms triggering tolerance to various biotic stresses while at high concentrations it accelerates cellular damage leading to programmed cell death [36]. To monitor different roles, cellular ROS levels are tightly controlled. In this study, the $H_2O_2$ content in non-Si treated plants was higher compared to the Si treated plants. High levels of $H_2O_2$ in the non-Si treated plants could be attributed to *Xam* inoculation and spread of the pathogen in plant tissues. Inoculation of Si treated plants with *Xam* may have increased $H_2O_2$ levels but Si application induced the activation of a vast network of plant antioxidative enzymes especially catalase which is the major scavenging enzyme for $H_2O_2$ and degrades it to water and oxygen maintaining the appropriate cell levels.

Antioxidant enzymes play important roles in adaptation to biotic stress conditions. Peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT) are the major protective enzymes in plants and they can be affected by bacterial infections [21]. In the present study, Si

application resulted in a significant increase in the activity of POD, APX and CAT compared to non-Si treated control plants. The enhancement of antioxidant enzyme activities by Si is to protect plants from oxidative stress and has been considered as one of the primary mechanisms of induction of plant defense mechanism upon Si supplementation [13,33]. Non-Si treated control cassava plants displayed the lowest antioxidant enzyme activities, denoting a perturbation of antioxidant enzyme metabolism. In non-Si treated plants, higher accumulation of $H_2O_2$ following *Xam* inoculation impaired the defense of antioxidant metabolism, leading to an imbalance between the production and scavenging of ROS. Supplementation of Si controlled the generation of $H_2O_2$ and restored the balance between ROS production and its scavenging by enhancing the activities of antioxidant enzymes.

Treatment of cassava cultivars with Si increased the activity of POD in *Xam* inoculated plants. Peroxidase plays two main roles; scavenging for high levels of $H_2O_2$ and the provision of an abrupt defense response against plant pathogens [37]. Peroxidase also plays a role in the host defense response through the production of antimicrobial quantities of $H_2O_2$ in cell wall lignifications and triggers mechanisms of polymerization of phenolic compounds leading to the formation of suberin and lignin in plant cell walls [38,39]. Therefore, higher activity of POD leads to initiation of the lignification process which is considered as a resistance mechanism against pathogen attack. Activities of APX and CAT help in $H_2O_2$ scavenging during situations leading to oxidative stress in plants. Ascorbate peroxidase is one of the constituents of the ascorbate–glutathione pathway, whose main role is in scavenging of harmful ROS thereby protecting plant cells [40]. Catalase has a low affinity for $H_2O_2$ thus eliminates it if high levels are present by breaking it down to water and oxygen. The activities of APX and CAT were significantly higher in Si treated plants of all cultivars inoculated with *Xam*. This could probably be attributed to the rapid antioxidant response triggered by the peak levels of $H_2O_2$ within the cells. The increase in activities of POD, APX, and CAT in *Xam* inoculated cassava plants as a result of Si application could be correlated with defense mechanisms associated to a $H_2O_2$-regulated stress response signaling pathway. These results are in agreement with those reported by [13] on the application of Si that resulted to increased activity of POD enzyme in banana plants which suggested the activation of plant defense responses at a molecular level.

Accumulation of Si in the Si treated plants was much higher compared to the non-Si treated control plants. Although both non-Si and Si treated plants were able t o accumulate Si, the level of Si accumulated from artificial application was more available to the plant than in the non-Si treated plants. There was a direct correlation between the amount of Si accumulated in the tissues of the various cultivars and the level of induced resistance. These results are in agreement with [17] who reported that Si is present in tissues of all terrestrial plants and Si application increased the levels of accumulated Si. Accumulation of different amounts of Si in the different cassava cultivars could be genotype dependent. Findings from the present study indicate that cassava is not a silicon accumulator as compared to most plants in the graminaceae family like rice and wheat which are well known for accumulating larger amounts of Si in their tissues [11,41,42].

In conclusion, exogenous application of Si significantly increased the resistance of cassava plants to bacterial blight disease. The uptake of Si led to reduced $H_2O_2$ and MDA accumulation, and increased activities of APX, POD and CAT to induce host defense mechanisms. Silicic acid played an active physiological and biochemical role in enhancing resistance to bacterial blight of cassava. Therefore, application of Si fertilizers might be an effective option for enhanced resistance to cassava bacterial blight.

## Acknowledgements

This study was financially supported by Dr. Evans Nyaboga from the Department of Biochemistry, University of Nairobi, Kenya. This study contributed to the award of Master of Science degree in Microbiology to the first author.

## Competing Interests

The authors declare no conflict of interests.

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