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RESEARCH ARTICLE

Soil application of potassium silicate reduces the intensity of downy mildew in bitter gourd (*Momordica charantia* L.) leaves

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Abstract: Effect of soluble silicon (Si) on downy mildew in bitter gourd (*Momordica charantia* L.) leaves caused by *Pseudoperonospora cubensis* was tested using three concentrations of liquid potassium silicate (100, 200, 300 mg Si/kg) added to the growing medium containing top soil, compost and sand. Disease severity, plant growth parameters, silicon accumulation on leaves and cuticle-epidermal layer thickness of transverse sections of leaves were measured. Si treated plants exhibited less disease severity than that of control plants and 200 mg Si/kg treatment resulted in highly significant disease suppression (37 - 53% reduction against controls). The suppression of the disease strongly correlated with the Si accumulation in leaves. Cuticle-epidermis thickness was higher in Si- treated leaves (17.2 - 21.3 µm) against non-treated (10.3 - 13.81 µm). Numbers of leaves, flowers or fruits in Si treated plants were significantly higher ($p < 0.05$) than control plants except in 300 ppm Si level. Structural changes created by Si may at least be partly responsible for reducing the fungal disease severity in bitter gourd leaves.

Keywords: Bitter gourd, disease resistance, downy mildew, potassium silicate, silicon.

INTRODUCTION

Plants are constantly threatened by a wide array of pathogens. Plants defend against microbial pathogens through complex resistance mechanisms by either providing constitutive or induced resistance (Van Bockheven *et al*., 2013). However, activation of defenses against one type of pathogen often enhances susceptibility against others (De Vleesschauwer *et al*., 2006). However, the application of silicon (Si) has been proven to protect plants against a broad range of pathogens (Ma *et al*., 2006; Guntzer *et al*., 2012). Although Si is the second most abundant element (46 %) in the earth crust, the plant available silicon in soil occurs mainly as monosilicic acid (H_4SiO_4) only at concentrations ranging from 0.1 to 0.6 mM (Epstein 1994). The silicon content in plant tissues varies greatly among species and can range from 0.1- 10 % on dry weight basis. Accordingly, plants can be identified as silicon accumulators, intermediate accumulators and non- accumulators (Ma and Takahashi, 2002).

Application of silicon has shown to reduce the severity of fungal diseases in many plants (Datnoff *et al*., 1997; Cai *et al*., 2008). Two hypotheses for the Si-enhanced resistance to diseases have been proposed; (i) Si deposited on the tissue surface acts as a physical barrier, preventing physical penetration and / or making the plant cells less susceptible to enzymatic degradation by fungal pathogens (Heine *et al*., 2007), and this hypothesis is supported by the positive correlation between the Si content and the degree of suppression of diseases and pests, and (ii) Si functions as a signal to induce the production of various chemical defenses against pathogens. Cherif *et al*. (1994) reported that soluble Si activates defense mechanisms in cucumber against *Pythium* by showing enhanced activity of chitinases, peroxidases and polyphenoloxidases, and increased accumulation of phenolic compounds.

Bitter gourd/bitter melon (*Momordica charantia* L.; Family Cucurbitaceae) is a popular vegetable in tropical countries due to its unique

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taste and medicinal properties (Robinson and Decker-Walters, 1996). Extensive investigations have shown that the extracts of many parts of this plant have marked diuretic and hypoglycemic properties both in animals and humans (Chaturvedi, 2012; Matheka and Alkizim, 2012). However, bitter gourd plants in the field are susceptible to many fungal diseases leading to loss of yield and product quality. Among these, downy mildew is considered as the most serious foliar disease of bitter gourd in Sri Lanka (Anon, 1990). At present, application of fungicides is the main control method. The exact nature of protective effects by Si against fungal pathogens in bitter gourd is not well understood. Relatively few published work related to silicon- bitter gourd system indicates that this crop is a moderate-silicon accumulator (Heine *et al*., 2007). Therefore, better understanding of protective effects of Si in bitter gourd will open up a new avenue as an alternative method to application of fungicides. The objective of the present study was to test the effectiveness of soil amendment with Si on downy mildew severity in bitter gourd leaves at field level and to determine if any cytological changes were associated with fungal restriction.

EXPERIMENTAL

Plant material and growth medium

Momordica charantia L. cultivar Matale green was grown in plastic pots containing sand: top soil: compost at 2:1:1 ratio as the growing medium. The pots were arranged in a plant house at the Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Sri Lanka (7.0 -9.0º N; 81 - 82º E) with 1.5 m x1.5 m plant spacing. Three seeds were planted per pot and thinning to one seed was done at four leaf stage. Potassium silicate (20% SiO_2; 80% K_2O; Daejung, Korea) in three concentrations, 100 ppm Si (100 mg/ kg soil), 200 ppm Si (200 mg/kg soil) and 300 ppm Si (300 mg Si/kg soil) were added to the growing medium at the 4-leaf stage and continued up to 28 days at seven day intervals. Media without silicon but added with potassium fertilizer in appropriate amounts to compensate the effect of added potassium in the form of K_2SiO_3 were used as the control; Control 1 = No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 100 ppm Si level, Control 2 = No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 200 ppm Si level and Control 3 = No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 300 ppm Si level. Basal dressings and top dressings of fertilizer as well as irrigation, pesticide application, staking, pruning and trellising were done according to the recommendations of the Department of Agriculture, Sri Lanka (Anon, 1990).

Plant- available Si and pH in growth medium

Potting media with added fertilizer were analyzed for plant-available Si and pH before adding silicon. Soil mixture (0.1 g) was added to 50 g of Na_2CO_3 solution (10 g dm^{-3}) plus 50 g of NH_4NO_3 solution (16 g dm^{-3}) and agitated for one hour. The samples were kept undisturbed for 5 days before determining Si (Buck *et al*., 2010). Plant available Si level was determined by colorimetric analysis (Snyder, 2001) for 25 sub samples of the soil mixture. Soil pH in 25 sub samples was measured as described by Dharmakeerthi *et al*. (2007).

Assessment of downy mildew disease incidence

Downy mildew disease severity in bitter gourd leaves was rated through external observations on weekly basis using a self-prepared scale (0 = no disease, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, and 5 = 81 to 100% of upper leaf surface covered with disease). Disease ratings were used to calculate area under disease progress curve (AUDPC) for each treatment by the midpoint rule method (Campbell and Madden, 1990):

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{(y_i + y_{i+1})}{2}\right] \times (t_{i+1} - t_i)$$

(n = number of disease assessment times, y = disease severity, t = time duration of the epidemic).

Evaluation of silicon accumulation in bitter gourd leaves over time

Leaves were analyzed for silicon accumulation starting at 35 days after the first Si application and continued up to 77 days at seven day intervals. Four to five leaves per bitter gourd plant were collected from 60 cm to 90 cm above the upper level of the potting medium and oven dried at 70 ºC for three days. Dried tissue was ground finely to pass through a 0.45 mm mesh. Silicon content was determined with slight modification to the procedure of autoclave-induced digestion (Elliot and Snyder, 1991). A 100 mg sample of leaf powder was placed in polypropylene tubes with 2 ml of 50% hydrogen peroxide (H_2O_2) and 3 ml of 50% sodium hydroxide (NaOH). Each tube was shaken gently and covered with plastic caps and then placed into an autoclave at 103 kPa for 30 min. Tubes were removed and the volume was increased to 50 ml with distilled water. Si content of leaves was determined colorimetrically as follows: a 1 ml aliquot was taken from the digested plant tissue and mixed in 10 ml of distilled water. Then, 0.25 ml of 1:1 hydrochloric acid, 0.5 ml of ammonium molybdate solution (100 g/l, pH 7.0), 0.5 ml tartaric acid (200 g/l), and 0.7 ml of a reducing solution were added. The reducing solution was prepared by dissolving 4 g sodium sulfite (Na_2SO_3), 0.8 g 1-amino-2-naphthol-4-sulphonic acid, and 50 g sodium bisulfite ($NaHSO_3$) in 500 ml water. Five minutes elapsed between the addition of the ammonium molybdate and the tartaric acid. After 10 min, the absorbance was measured at 815 nm with a spectrophotometer (ChromTech CT6200, Taiwan). A series of Si standard (from 0 to 5 ppm) prepared from a Si standard solution (1000 mg /l, Fluka, Sigma Aldrich) was used to generate a regression equation for determining final Si content (mg/kg dry weight) in leaves.

Effect of silicon on the growth of the bitter gourd plant

Number of leaves, flowers, and fruits per plant were counted at seven day intervals starting from 35 days after the first Si treatment application.

Effect of silicon application on structural changes in leaves

Transverse sections (TS) of leaves obtained from the plants treated with 200 ppm Si and control plants were observed under light microscope and cuticle–epidermal layer thickness was measured in tissues obtained from 10 replicate leaves to determine silicon deposition in leaves after staining with silver amine chromate (Kaufman *et al*., 1985).

Data analysis

The experiment was laid out in complete randomized design (n = 30). Data gathered were analyzed using Analysis of Variance (ANOVA) and by linear regression by Statistical Analysis System (SAS) for windows version 9.0. Differences between treatment means were obtained by Duncan's multiple range test at 5% significance level ($p<0.05$).

RESULTS

Plant- available Si and pH in the growth medium

The potting medium contained an average of 2.87 mg dm^{-3} Si (0.47 mM). pH of the soil mixture before amendment with Si was 7.2.

Downy mildew disease incidence

Downy mildew disease was identified by its typical symptoms; angular chlorotic lesions in the infected leaves, eventually, turned necrotic and curled upwards. Further, Gray-brown to purplish-black fungal growth was observed on the lower surface of the leaf. Symptoms initially appeared in the field 8 weeks after seeding and gradually increased until the end of the experiment (77 days after seeding). Disease severity of silicon treated plants was lower than that of control plants during the entire period tested (Figure 1). However, regression analysis did not indicate a significant relationship [regression coefficient (R^2) = 0.25] between disease severity (AUDPC) and Si application rates (Figure 2). Among the three silicon treatments 200 ppm Si showed a highly significant effect ($p<0.001$) on disease suppression where 37% -53% reduction of disease severity was evident against non-Si treated controls over the study period.

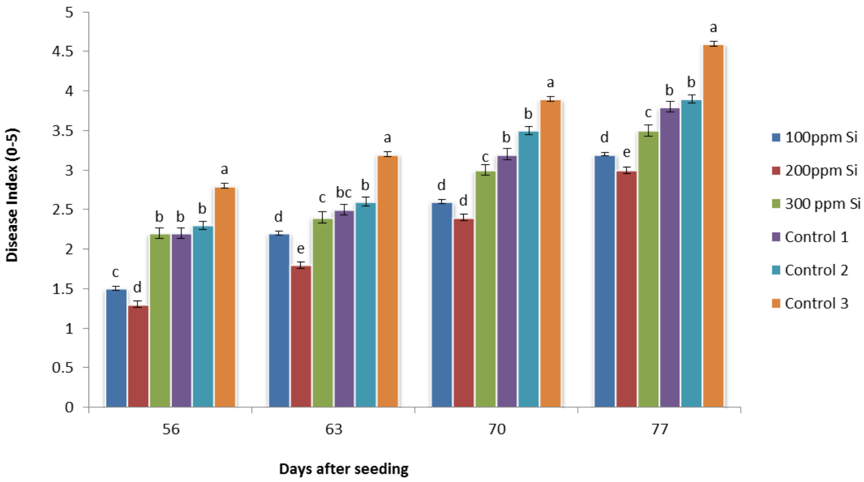

Figure 1: Variation of the downy mildew disease severity on bitter gourd grown in media amended with different silicon concentrations (Disease index 0 = no disease symptoms; 5 = 81 to 100% of upper leaf surface covered with disease symptoms of downy mildew.

Control 1= No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 100 ppm Si level, Control 2 = No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 200 ppm Si level and Control 3= No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 300 ppm Si level.)

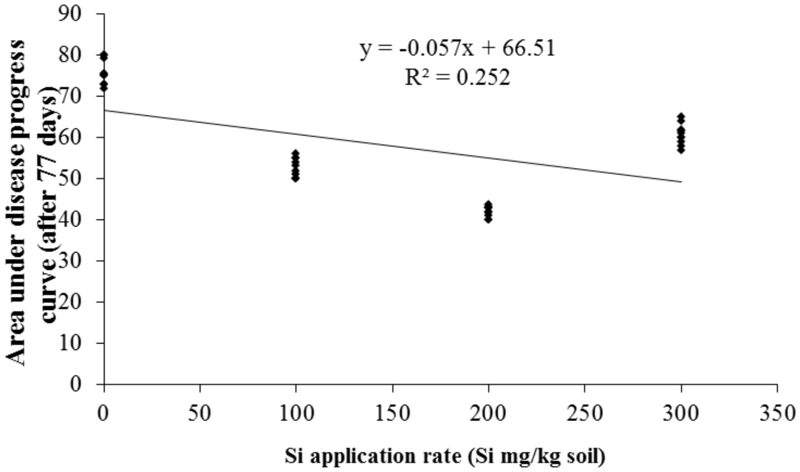

Figure 2: Relationship between Potassium silicate rates added to the growth medium and disease severity of downy mildew in bitter gourd leaves expressed as area under disease progress curve (AUDPC).

Silicon accumulation in leaves over time

All Si treated plants exhibited significantly higher Si levels in leaves compared to non-treated plants. The highest Si content was detected in 200 ppm Si treated plants throughout the period of investigation where as the lowest Si content was shown by the plants that did not receive Si (control 1-3). There was a strong relationship (R^2 = 0.95) between the tissue accumulated Si and disease suppression in leaves (Figure 3). It was also noted that Si content accumulated in leaves in 300 ppm Si rate was less than 200 ppm and 100 ppm Si applied plants. When the silicon application was terminated at 28 days after first Si application, accumulated Si content in leaves went up to the maximum levels (range 2.5-3.5 % dry wt.) by 42 days and then decreased gradually up to 0.5-0.8 % dry wt. level over time (Figure 4).

Plant growth

Effect of Si on plant growth parameters including number of leaves, flowers and fruits per plant was significantly higher than those in non-Si treated plants and the highest growth rate was associated with 200 ppm application. However, effect of 300 ppm on plant growth was less pronounced (Table 1).

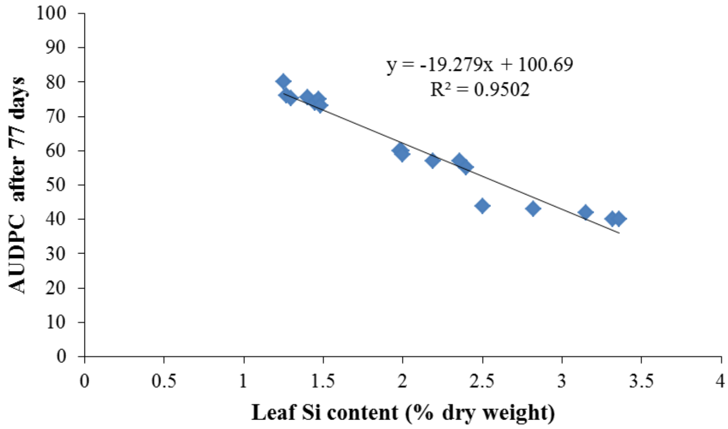

Figure 3: Relationship between accumulated Si level in bitter gourd leaves and downy mildew disease severity expressed as area under disease progress curve (AUDPC)

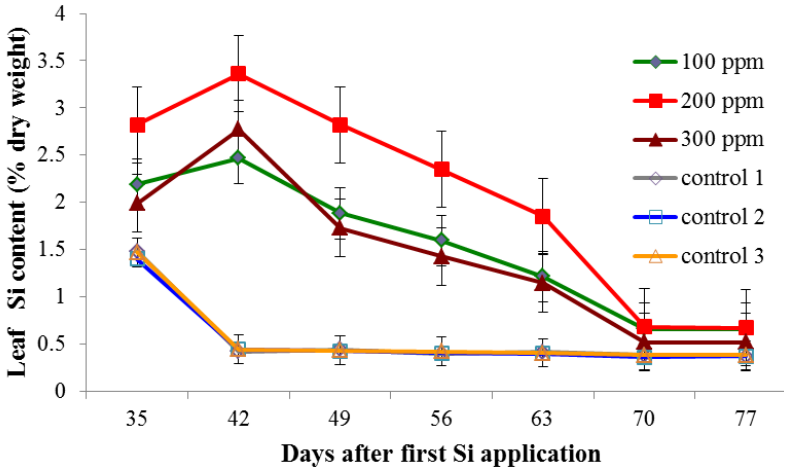

Figure 4: Changes in Si accumulation in bitter gourd leaves over time. Note: Si application was started at 4-leaf stage and continued up to 28 days with seven days interval.

Table 1: Variation in leaf, flower and fruit number per bitter gourd plant with different silicon treatments

Si level	Leaf Number x (no/plant)	Flower Number x (no/plant)	Fruit Number x (no/plant)
100ppm Si	98.5 b	7.4 ab	3.7 b
200ppm Si	114.4 a	8.0 a	4.9 a
300ppm Si	99.3 b	6.6 abc	1.7 e
Control 1	77.6 c	2.8 d	1.4 f
Control2	82.2 c	4.0 bcd	1.9 d
Control 3	84.3 c	4.7 bc	1.7 e

[x] Observations made 70 days after seeding.

Note. Control 1= No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 100 ppm Si level, Control 2= No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 200 ppm Si level and Control 3 = No addition of silicon but K fertilizer was added to compensate the effect of added K in the form of potassium silicate in 300 ppm Si level.

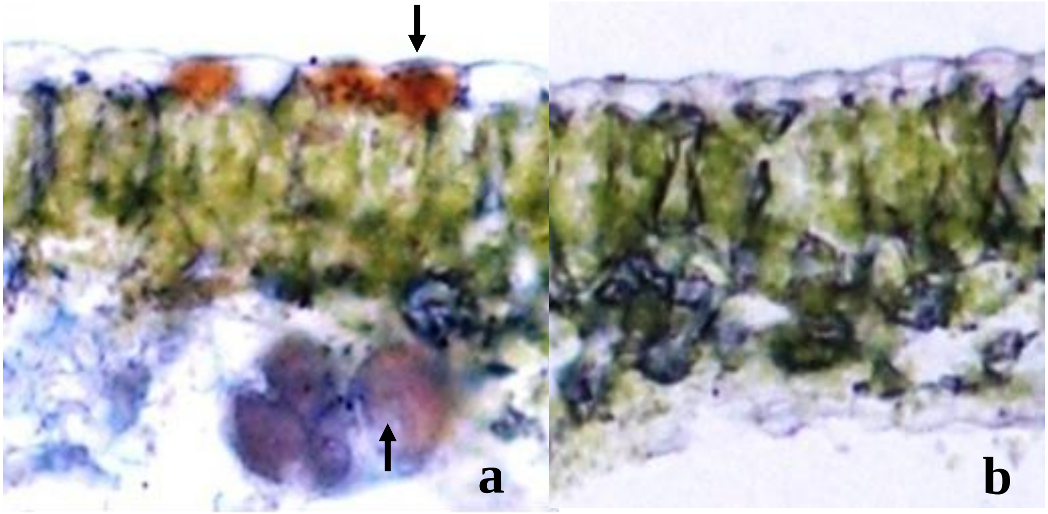

Figure 5: Transverse sections of bitter gourd leaves after Si application where a) treated b) un-treated (magnification x 400). Arrows indicate Si deposited cells stained with reddish brown colour with silver amine chromate.

Si application and structural changes in leaves

In transverse sections of bitter gourd leaves, the cuticle - epidermal layer thickness in Si treated plants was significantly higher (60 %) than that of control plants. The average cuticle - epidermal layer thickness in Si treated plants was 19.3 µm whereas in control plants it was 12.1 µm. Further, it was noted that the cuticle thickness decreased with the progress of downy mildew over time regardless of the Si treatment (data not presented). When bitter gourd plants were diseased, cuticle - epidermal layer thickness decreased to an average of 15.8 µm where in the control plants it was 10.2 µm. Staining of silica bodies with silver amine chromate indicated the presence of silica deposits in some of the epidermal cells (Figure 5).

DISCUSSION

Suppressive effect of silicon against plant pathogens has been known for many years (Cherif *et al*., 1994; Kanto *et al*., 2004) especially for diseases in rice plants (Datnoff *et al*., 1997). Present study revealed that soluble silicon has a suppressive effect on downy mildew in bitter gourd leaves. Soil analysis before Si amendment revealed that the soluble Si level in the potting mix was low and pH was nearly neutral. In general, minerals are bound to soil particles when pH levels are too high or too low thus making unavailable to plants. Si is found in the soil solution as solubilized monosilicic acid $[Si(OH)_4]$ at a pH < 9, and as the silicic anion SiO_3^{2-} at a pH>9 (Jones and Handreck, 1967; Lewin and Reimann, 1969; Raven, 1983;). Therefore, the soil used for the current study was suitable for Si application.

It was clear that disease severity of Si treated plants was lower than that of control plants during the entire study period. These findings are in agreement with the current understanding that Si amendments reduce disease severity of crops that are grown in Si-deficient soils (Liang *et al*., 2005). It was noted that disease suppression was not related to the rate of Si application at any of the evaluation dates, although it was strongly affected by soluble silicon accumulated in the leaf tissue. In this study, no soil pH adjustments were made thus continuous application of increasing amount of potassium silicate to the soil may have increased soil pH leading to reduction of Si absorption. The concentration of Si in the soil solution is controlled by a pH dependant reaction and due to the adsorption of monosilicic acid at high pH by sesquioxides, the amount of soluble Si decreases as the pH increases. Si accumulation also varies considerably with the plant species, and the difference in Si accumulation has been attributed to the ability of the roots to take up Si (Ma *et al*., 2001; Ma and Takahashi 2002). Contrasting observations have been reported with studies on Si effectiveness against diseases in different crops. According to Mathai *et al*. (1977), Si reduced the intensity of sheath blight in rice but there was no significant difference between the high and low rates of Si applied where as Datnoff and Snyder (1994) have shown that as the rate of Si in the soil increased the intensity of sheath blight in rice was significantly reduced. Further, the silicon accumulation in the plant tissue was negatively correlated with blast severity in rice (Datnoff and Snyder, 1994) and powdery mildew on cucumber (Miyaki and Takahashi, 1983).

The present research showed that soluble Si level in leaves decrease over time after the Si application is terminated. It has been reported that continuous Si application is necessary in disease management because once it is deposited in the plant tissue it is not retranslocated (Datnoff *et al*., 2007).

The changes in cuticle- epidermal layer thickness during the experimental period may have resulted by deposition of silica as indicated by staining in brown colour with silver amine chromate in some of the epidermal cells. Presence of silica deposits was also observed at the sites of downy mildew infection. Thus it was likely that downy mildew infection was controlled by the increased silicification of the epidermal cells. On Si uptake into the plant, phytoliths (silica bodies) are likely to form in cell walls, cell lumen, and intercellular spaces and in subcuticular layer (Sangster *et al*., 2001). In the leaf blades of rice, silica is deposited as a 2.5-mm layer immediately beneath the cuticle layer as a Si–cuticle double layer. However, the silicification of cells is not restricted to the leaf blades and silicified cells are also found within the epidermis and vascular tissue of the stem, leaf sheath and hull (Prychid *et al*., 2004). Nevertheless, further observations under electron microscope are necessary for a better understanding on Si deposition in bitter gourd.

When considering growth characteristics, Si treatment had positive effects on number of leaves, flowers and pods in bitter gourd plants. Research findings from rice, sugarcane, sorghum, pearl millet and strawberry have also shown a positive correlation between Si application and growth and yield of plants (Miyaki and Takahashi, 1983; Ma, 2004). Silicon may be involved in cell elongation and/or cell division. In sugarcane, plant height was quadratically related to the rate of silicon applied. Emardian and Newton (1989) observed increased plant growth in Si applied strawberry and they described it as the enhanced tissue elasticity and symplastic water volume which were associated with cell expansion and plant growth. Therefore, observation of the present study was similar to the findings with many other crops. However, the question why 300 ppm Si level deviated from expected positive effect warrants further investigation.

Protective role of Si can at least be partly due to the accumulation of Si in the bitter gourd leaves, enhancing their strength and rigidity creating a physical barrier to pathogens as described by many researchers (Adatia and Besford, 1986; Samuels *et al*., 1991). Alternatively, Si may have a more active role by inducing the plant's own defense mechanisms by producing compounds such as flavanoid (Fawe *et al*.,1998), diterpenoids (Rodrigues *et al*., 2004) and pathogenesis-related (PR) proteins (Liang *et al*., 2005; Rodrigues *et al*., 2005). Further investigations are currently under way with bitter gourd to elucidate other mechanisms linked with Si application and enhanced disease resistance against downy mildew.

CONCLUSIONS

These results provide the first evidence that silicon can decrease downy mildew in bitter gourd leaves, which is a valuable option that may be used as an integrated disease management strategy, especially when the fungicide application needs to be reduced to obtain quality plant product.

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