EFFECTS OF SOIL AMENDMENT ON BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACERUM* AND TOMATO YIELDS IN ETHIOPIA

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Abstract: Abstract: Field experiments were conducted in Ethiopia to evaluate the effect of silicon fertilizer and sugarcane bagasse on tomato bacterial wilt (*Ralstonia solanacearum*). Silicon fertilizer significantly reduced the bacterial population, mean wilt incidence, percent severity index, and corresponding areas of disease incidence, and severity progress curves in the moderately resistant tomato cultivar (King Kong 2). Similarly, sugarcane bagasse resulted in a significant reduction of mean wilt and percent severity index, the corresponding areas under disease incidence and severity progress curves and the bacterial population at 5 days post inoculation, compared to the control, in cultivar King Kong 2. However, neither silicon fertilizer nor sugarcane bagasse resulted in any significant reduction of all disease parameters in the moderately susceptible cultivar Marglobe. Silicon fertilizer and sugarcane bagasse amendments also increased fruit yield for cultivar King Kong 2, but not for cultivar Marglobe. The total silicon content was also significantly increased in silicon fertilizer amendment, followed by sugarcane bagasse amended plants. The study recommends use of silicon fertilizer as a soil amendment under field conditions to augment resistance in moderately resistant cultivars where bacterial wilt disease problems prevail. However, a silicon fertilizer or silicon source was not found to substantiate or improve a susceptible cultivar. Sugarcane bagasse was demonstrated to possess a potential as an alternative soil amendment material and as an alternative silicon source.

Key words: Bacterial wilt, *Ralstonia solanacearum*, sugarcane bagasse, soil amendment, tomato, silicon

INTRODUCTION

Diseases caused by different fungal and bacterial pathogens (Jones *et al*. 1991) are among the major constraints of tomato production. Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi *et al*. 1995) is one of the most important diseases that limit tomato production worldwide in general, and in Ethiopia in particular. The disease also constitutes a serious damage to the cultivation of many other Solanaceous crops (potato, tobacco, pepper and eggplant) in tropical, sub-tropical and temperate regions (Hayward 1991). Different management strategies of bacterial wilt disease have been studied in many parts of the world with special focus on host plant resistance, cultural practices, and soil amendments (Saddler 2005). Soil amendments that enhance host plant resistance have been given due consideration (Datnoff *et al*. 2001). However, no effective universal disease management strategies have been identified so far. Therefore, a search for alternative bacterial wilt disease management options is important.

Silicon (Si) has been reported for having a significant effect in reducing bacterial wilt incidence in tomato in a hydroponics culture system, and peat substrate (Dannon and and Wydra 2004). Diogo and Wydra (2007) also reported an induced basal resistance on cell wall level, in silicon treated tomato plants compared to non-treated plants. Thus, an elucidation of the effect of silicon amendment under field conditions, for the management of bacterial wilt on tomato is necessitated. Furthermore, a search for an alternative silicon source from locally available materials, specifically a plant residue that possess a considerable silicon concentration is essential (Gascho 2001). In this regard, plant residues from sugarcane (*Saccharum* spp.) bagasse have been evaluated. This study was initiated to confirm the beneficial effect of silicon application in managing of tomato bacterial wilt under field conditions, using silicon fertilizer and sugarcane bagasse. as the alternative organic silicon source,

MATERIALS AND METHODS

Study Location and Site Characteristics

The experiments were carried out in Ethiopia at Melkassa Agriculture Research Center (MARC) located at 8°24’985 N latitude and 39°19’529 E longitudes, with an altitude of 1 550 meter above sea level. The average

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annual rainfall in the area is 763 mm, about 70% of which is received during the main rainy season from June to September. The annual average maximum temperature is 28.4°C, the minimum average temperature 14°C. The soil texture of the field plot was a loamy type with 32% sand, 42% silt and 26% clay, pH = 7.9.

Treatments and experimental design

A factorial combination of a 3x2x2 treatment levels, consisting of two silicon sources [silicon fertilizer supplied as (Agrosil LR ® COMPO GmbH & Co. KG, Muenster, Germany)], sugarcane bagasse, and the source without external silicon, two tomato cultivars Marglobe (moderately susceptible) and King Kong 2 (moderately resistant), and artificial inoculation of *R. solacearum* and non-inoculated combinations were arranged in a Randomized complete block design (RCBD) with four replications. The plot size of 4 m x 3 m was separated by two-meters of weed-free, bare ground, from other plots and blocks. The experiment was repeated twice with one month planting date differences, during March–August and April–September 2007. Silicon fertilizer (Agrosil LR ®), was applied to the field plots according to the average manufacture's recommendation rate of 15 kg per 100 m^2. Sugarcane bagasse was applied at a rate of 1 t/ha, two weeks before transplanting. All other cultural practices for growing tomato under field conditions were followed according to recommended practices in Ethiopia. For the control of late and early blights, prophylactic fungicide sprays (Ridomil® Gold MZ 68 WP) at 7–10 days intervals were applied one month after transplanting. For the control of fruit worm, insecticides (Karate 5% EC) were sprayed at early flowering and fruit setting.

Bacterial strain and inoculation

A highly virulent *R. solanacearum* strain TomNa3 isolated from tomato in Ethiopia was used for artificial field inoculation. The pathogen was cultivated on triphenyl tetrazolium chloride (TTC) agar medium (French *et al*. 1995) and further multiplied on Nutrient glucose agar (NGA). The inoculum suspension was prepared with an Optical density (OD) of 0.06 at 620 nm corresponding to approximately 10^8 colony forming unit per milliliter (CFU/ml). The inoculum suspension was inoculated as a soil drench to the field transplanted seedlings, at a rate of 1 liter/m^2 as described by Michel *et al*. (1997), on same day of transplanting.

Quantification of bacterial population

Quantification of *R. solanacerum* population was performed on randomly selected plants per treatment as described by Diogo and Wydra (2007), at 5 and 12 days post inoculation. Average colony counts were multiplied by the dilution factor and the results were transferred to Log_{10}(CFU) +1) before analysis of variance.

Disease measurements

Symptom development was evaluated daily until the fourth day, then at 7, 10, 14, 21, 30 and 45 days after transplanting. A six point rating scale (0–5) modified from Winstead and Kelman (1952) was used, where: 0 = no wilt symptoms, 1 = one leaf wilted, 2 = two or more leaves wilted, 3 = all leaves except the tip wilted, 4 = whole plant wilted and 5 = death (collapse) of the whole plant. Disease incidence was assessed as percentage of wilted plants within each treatment. Disease incidence was calculated according to the formula, (I = NPSWS/NPPT × 100), where: I – wilt incidence, NPSWS – number plants showing wilt symptoms, NPPT – Number of plants per treatment.

Percent severity index (PSI) was calculated using the method described by Cooke (2006). PSI = ∑ (scores × 100)/(number of plants rated × maximum scale of the scores) for each scoring date.

Area under disease incidence progress curve (AUDiPC) and Area under percent severity index progress curve (AUPSiPC) for each treatment was calculated using the formula adopted from Jerger and Vijanen-Rollinson (2001).

Assessment of fruit yield and silicon content

Tomato fruits were periodically harvested from two central rows. The periodically harvested fruits were weighed and the weights are summed up. Then, yield per plot was converted to tone per hectare. Total silicon content of tomato at the collar region was determined by spectrophotometeric method as used by Dannon and Wydra (2004), and Diogo and Wydra, (2007).

Data analyses

Data were analyzed using the statistical analysis system (SAS for Windows, 1999–2001, SAS Institute, carry, USA) for analysis of variance (ANOVA) followed by means separation based on least significant difference at 5% using the Waller-Duncan K-ratio t test.

RESULTS AND DISCUSSION

Effect on *R. solanacerum* population and wilt development

Population of *R. solanacerum* was significantly reduced ($p < 0.05$) in silicon fertilizer treated plants both at 5 and 12 days post inoculation (dpi) compared to non-amended for moderately resistant cultivar (Table 1) with an average of 29.2 and 17.6% reduction, respectively. Similarly, in sugarcane bagasse treatment, the bacterial population was also significantly reduced compared to the control treatment at 5 dpi but not at 12 dpi in moderately resistant cultivar. However, no significant reduction was obtained in bacterial populations in the moderately susceptible cultivar for all treatments.

Mean wilt incidence and percent severity index were significantly lower for silicon fertilizer treated moderately resistant cultivar with a final mean wilt incidence reduction of 51.6 and 57.8% respectively (Fig. 1). Sugarcane bagasse treatment also resulted in a significant reduction of mean wilt incidence and percent severity index (Fig. 1) in moderately resistant cultivars compared to the non-amended. In moderately susceptible cultivar, however, there was no significant difference in mean wilt incidence and percent severity index (Fig. 1).

Disease severity and wilt incidence expressed as AUPSiPC, and AUDiPC were significantly reduced by 36.5 and 40.3%, respectively, for silicon treated moder-

Table 1. Effects of soil amendments with silicon fertilizer and sugarcane bagasse on population of *R. solanacerum* at 5 and 12 dpi under field conditions, mean Area under wilt incidence progress curve (AUDiPC) and Area under percent severity index progress curves (AUPSiPC) on two tomato cultivars King Kong 2 and Marglobe

Cultivar	Treatment[1]	*R. solanacerum* population [Log_{10} (CFU)/g FW[2]]				AUDiPC[2]	AUPSiPC[2]
		experiment I		experiment II			
		5 dpi	12 dpi	5 dpi	12 dpi		
King Kong 2	+Si+Rs	5.6±0.29 C	7.4±0.12 B	5.5±0.19 C	7.3±0.21 B	557.3±15.37 D	378.6±13.66 C
	+Sc+Rs	7.6±0.14 B	8.9±0.05 A	7.5±0.28 B	8.9±0.12 A	824.6±14.02 C	616.0±32.10 B
	-Si/-Sc+Rs	7.9±0.081 A	8.9±0.05 A	7.7±0.31 B	8.9±0.046 A	877.8±15.59 B	634.1±25.47 B
Marglobe	+Si+Rs	7.66±0.25 B	8.9±0.01 A	7.7±0.19 B	8.8±0.14 A	1209.1±42.05 A	942.5±10.67 A
	+Sc+Rs	7.9±0.035 A	8.9±0.04 A	8.2±0.66 A	8.9±0.12 A	1214.9±38.56 A	943.,0±24.33 A
	-Si/-Sc+Rs	7.9±0.085 A	8.9±0.04 A	7.7±0.16 B	8.9±0.037 A	1224.1±32.66 A	950.4±17.94 A

[1] in treatment combination (-Si/-SC/-Rs), [+Si+ (-Rs)] and [+Sc+ (-Rs)], where no bacteria were inoculated no natural infection occurred and no bacteria population was recorded, therefore, zero values have been omitted

[2] mean ±SE from two experiments and four replications; means followed with the same letter in same column are not significantly different from each other based on Waller-Duncan K-ratio test ($p < 0.05$)

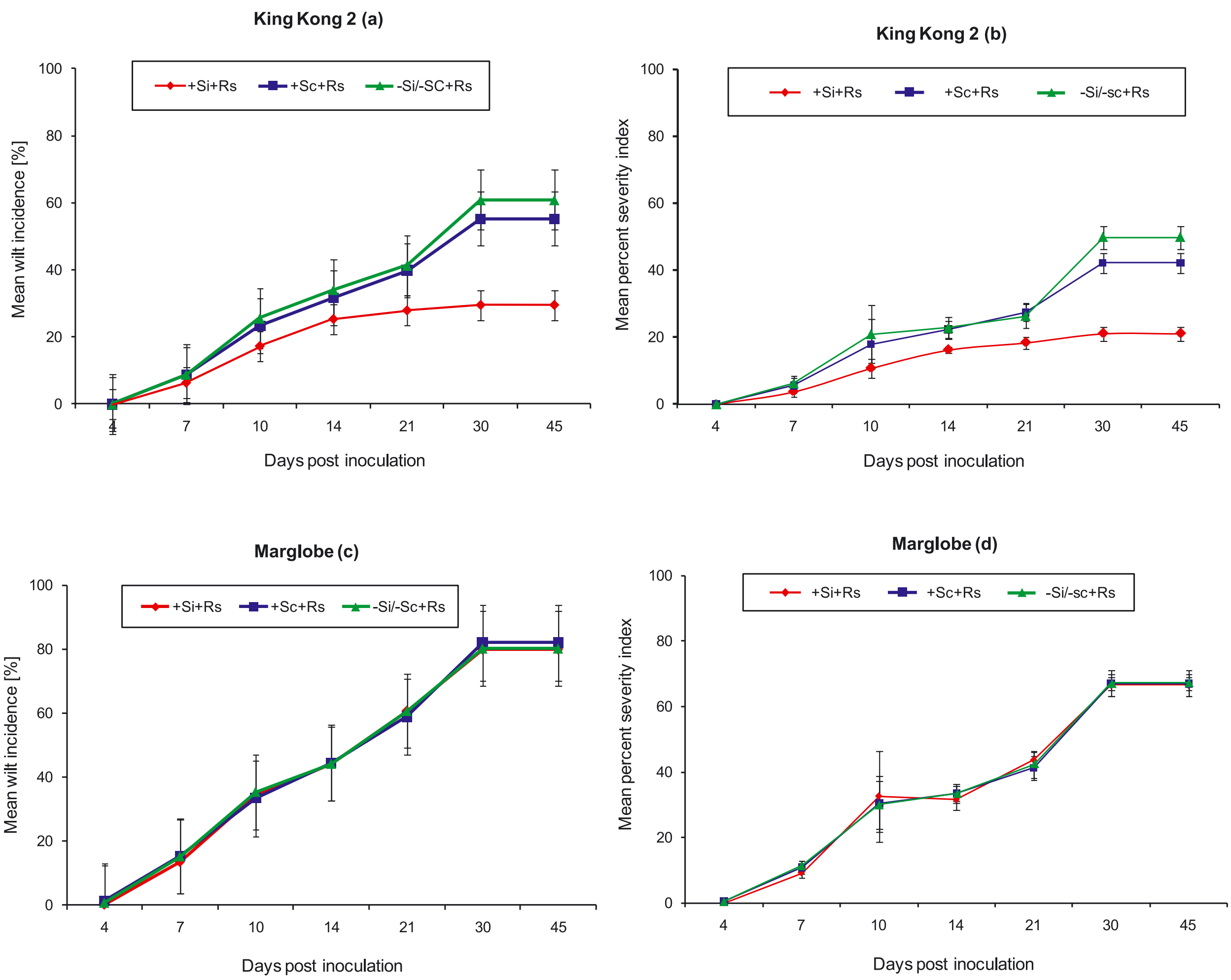

Fig. 1. Bacterial wilt development expressed as mean wilt incidence and percent severity index on two tomato cultivars, King Kong 2 and Marglobe with and without silicon and sugarcane bagasse treatment under field conditions

ately resistant, cultivar (Table 1). In a similarly way, the same parameters were significantly reduced with sugarcane bagasse amendment compared to non-amendments by 6 and 2.85%. On moderately susceptible cultivar no significant differences were found for all treatments (Table 1). The beneficial effect of silicon amendment in the moderately resistant tomato genotype complements the findings of Diogo and Wydra (2007). The beneficial effect of sugarcane bagasse is related to the report of Gascho (2001) where it indicated considerable silicon concentration for sugarcane bagasse and rice hulls.

Effect on fruit yield and silicon content

Application of silicon fertilizer and sugarcane bagasse amendments significantly increased marketable fruit yields in moderately resistant cultivars compared to non-amendment, where higher fruit yield was obtained with silicon fertilizer treatment than with sugarcane baggasse. However, marketable fruit yield was not significantly influenced by silicon fertilizer treatments in both tomato cultivars in non-inoculated plants. The results complement the reports of Statamatakis *et al*. (2003). However, in absence of disease, fruit yield of moderately susceptible cultivar was significantly higher than fruit yield of moderately resistant cultivars. Silicon content of the two tomato cultivars was significantly increased at 30 dpi and at harvest stage, for silicon and sugarcane bagasse treatments. An increase of 48.2 and 63.6% silicon content was observed in moderately resistant cultivar due to silicon fertilizer, compared to sugarcane bagasse and control treatment, respectively. Amendment with sugarcane bagasse also significantly increased the concentration of silicon content in both tomato cultivars by 24.8 and 29.8%, compared to non-amended treatments. An increase in silicon content is related to the report of Lewin and Reimann (1969) which indicated the amount of silica present in the plants (as percent of dry weight) increases in direct proportion to the amount of silicic acid dissolved in the soil solution or culture medium.

In conclusion, the study confirms the silicon resistance inducing effect in moderately resistant tomato cultivars to minimize bacterial wilt under a control environment. The study recommends use of silicon fertilizer as a soil amendment under field conditions to augment moderately resistant cultivars where bacterial wilt diseases problem prevail. However, a silicon fertilizer or silicon source was not found to substantiate or improve a susceptible cultivar. Furthermore, sugarcane bagasse demonstrated potential significance as an alternative soil amendment and alternative silicon source. The beneficial effects of sugarcane bagasse require further investigations.

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POLISH SUMMARY

WPŁYW POLEPSZENIA GLEBY NA WYSTĘPOWANIE BAKTERYJNEGO UWIĄDU POWODOWANEGO PRZEZ *RALSTONIA SOLANACEARUM* ORAZ PLON POMIDORA W ETIOPII

W warunkach polowych prowadzono doświadczenia w celu oceny wpływu nawozu krzemowego oraz wytłoków trzciny cukrowej na występowanie bakteryjnego uwiądu pomidora (*Ralstonia solanacearum*). Nawóz krzemowy istotnie wpływał na ograniczenie populacji bakterii, średnie występowanie uwiądu pomidora, procentowy indeks nasilenia choroby jak też areał jej występowania oraz krzywą wzrostu nasilenia choroby u średnio odpornej odmiany pomidora (King Kong 2). Podobnie do nawozu krzemowego, wytłoki trzciny cukrowej w istotnym stopniu ograniczały występowanie uwiądu, procentowy indeks nasilenia choroby, zasięg jej występowania, krzywą wzrostu nasilenia choroby i populację bakterii już po 5 dniach od momentu inokulacji w porównaniu do kontroli z średnio odporną odmianą pomidora. Jednakże, żaden z zastosowanych nawozów, a więc nawóz krzemowy i wytłoki trzciny cukrowej nie wpływały istotnie na zmniejszenie parametrów dotyczących występowania choroby na średnio podatnej odmianie pomidora. Wzbogacenie gleby zarówno nawozem krzemowym jak i wytłokami trzciny cukrowej powodowało wzrost plonu owoców u średnio odpornych odmian pomidora w przeciwieństwie do odmian podatnych na chorobę. Po zastosowaniu nawozu krzemowego jak też wytłoków trzciny cukrowej wzrosła całkowita zawartość krzemu w badanych roślinach. Na podstawie uzyskanych wyników badań można by zalecić stosowanie nawozu krzemowego w warunkach polowych jako czynnika wzmacniającego kondycję zdrowotną średnio odpornych odmian zwłaszcza w rejonach silnego występowania choroby pomimo, że nie stwierdzono dobrych efektów działania nawozu krzemowego oraz wytłoków trzciny cukrowej u średnio podatnych odmian. Wytłoki trzciny cukrowej okazały się dobrym alternatywnym źródłem krzemu oraz materiałem wpływającym na polepszenie gleby.