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Effects of silicon on resistance to bacterial fruit blotch and growth of melon

Hailson Alves Ferreira [a, 1], Clístenes Williams Araújo do Nascimento [a], Lawrence Elliott Datnoff [b], Glauber Henrique de Sousa Nunes [c], Welka Preston [a], Elineide Barbosa de Souza [a], Rosa de Lima Ramos Mariano [a, *]

[a] Federal Rural University of Pernambuco, Department of Agronomy, R. Manuel de Medeiros, s/n, Dois Irmãos, Recife, Pernambuco, 52171-900, Brazil
[b] Louisiana State University Agricultural Center, Department of Plant Pathology and Crop Physiology, 302 Life Science Bldg., Baton Rouge, LA, 70803, USA
[c] Federal Rural University of the Semi-Arid, Department of Plant Sciences, Av. Francisco Mota, 572, Costa e Silva, Mossoró, Rio Grande do Norte, 59625-900, Brazil

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ABSTRACT

Melon bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* is responsible for substantial yield losses in Northeastern Brazil, and management options for this disease are limited. Since silicon has been shown to suppress a number of plant diseases including some caused by bacteria, a study was conducted to determine if this element might reduce BFB development. Yellow melon hybrid AF 4945 plants were grown in plastic pots containing soil supplemented with calcium silicate (0.00, 0.12, 0.24, 0.71 and 1.41 g Si kg^{-1} of soil) and inoculated with *A. citrulli* 20 days after plant emergence. Incidence (INC) and severity were evaluated every 4 days up to 20 days after inoculation. In addition, the incubation period (IP), disease index (DI), and area under the disease progress curve (AUDPC) were determined. Plant growth along with macro- and micro-nutrient accumulation also were determined from 45-day-old plants. The highest Si dose significantly reduced INC (50%), DI (89%), and AUDPC (85%) and increased the IP (192%) in comparison to the control. This dose also elevated soil Si levels, soil pH and Ca + Mg respectively by 62, 17 and 29%, whereas H + Al was reduced by 62%. Plant height (9%), shoot (24%) and root (49%) fresh weights as well as shoot dry weights (33%) were also significantly increased by 1.41 g Si kg^{-1} of soil. At this dose, Ca and Mg shoot concentrations reached 124 and 59%, respectively, and Si content increased up to the 0.24 g Si (39%) in comparison to the control. Supplying Si to melon plants clearly enhanced resistance to BFB but also elevated Ca and Mg levels in the plant tissue. Improved plant nutrition in melon appears to play an important role in helping to suppress BFB development.

1. Introduction

In Brazil, 94% of melon (*Cucumis melo* L.) production is found in the Northeastern region where bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* (Schaad et al.) Schaad et al. is the main bacterial disease. Despite the damage caused by BFB in Brazilian melons, few studies have focused on managing this disease using chemicals (Sales Junior et al., 2005) or biological control strategies (Oliveira et al., 2006; Santos et al., 2006; Medeiros et al., 2009; Conceição et al., 2014; Melo et al., 2015). Since no known resistant or tolerant melon cultivars are available, the major BFB control measure is the use of healthy and/or treated seeds. However, treated seeds will not completely eradicate this pathogen (Burdman and Walcott, 2012). In the field, copper fungicides and/or antibiotics have been used to manage BFB but this approach has had limited success in reducing the disease and subsequent yield losses (Sales Junior et al., 2005). Therefore, other strategies are needed to combat this disease and a practical alternative could be the use of mineral nutrition in order to increase disease resistance in melons (Marschner, 1995). Among the mineral elements studied, silicon (Si) stands out in its ability to reduce the severity of some

* Corresponding author.
E-mail addresses: hailson_alves@hotmail.com (H.A. Ferreira), clistenes@depa.ufrpe.br (C.W.A. Nascimento), ldatnoff@agcenter.lsu.edu (L.E. Datnoff), glauber@ufersa.edu.br (G.H.S. Nunes), welkapreston@hotmail.com (W. Preston), elineidebs@yahoo.com.br (E.B. Souza), rrmbac@gmail.com (R.L.R. Mariano).
[1] Present address: Federal Rural University of the Semi-Arid, Department of Plant Sciences, Av. Francisco Mota, 572, Costa e Silva, Mossoró, Rio Grande do Norte, 59625-900, Brazil.

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important plant diseases (Epstein, 1999; Datnoff et al., 2007).

Silicon has significantly contributed to the reduction in intensity of various economically important diseases in monocotyledon and dicotyledon species (Bélanger et al., 1995; Datnoff et al., 1997; Rodrigues and Datnoff, 2005; Datnoff et al., 2007; Resende et al., 2009; Silva et al., 2010; Kiirika et al., 2013). However, the beneficial effects of Si have been primarily reported for resistance against fungal plant pathogens (Chérif et al., 1994; Rodrigues et al., 2003; Silva et al., 2010; Tesfagiorgis and Annegarn, 2013; Tesfagiorgis et al., 2014). In pathosystems involving plant pathogenic bacteria, the effects of Si have not been as widely studied. Although the effect of Si on plant disease control is still poorly understood, plant resistance to disease is considered to be due either to an accumulation of absorbed Si in the epidermal tissue, or expression of pathogenesis-induced host defense responses. Accumulated monosilicic acid polymerizes into polysilicic acid and then transforms to amorphous silica, which forms a thickened Si-cellulose membrane (Hayasaka et al., 2008). By this means, a double cuticular Si layer protects and mechanically strengthens plants. Silicon also might form complexes with organic compounds in the cell walls of epidermal cells, therefore increasing their resistance to degradation by enzymes. Research also points to the role of Si *in planta* as being active since phenolic compounds, phytolexins, glucanases, peroxidases and PR-1 transcripts were all found to be associated with limited colonization by a number of fungal plant pathogens (Bélanger et al., 2003; Bélanger and Menzies, 2003; Rodrigues et al., 2003). Recently, a number of pathogenicity or stress-related genes were found to be either up- or down-regulated by Si (Brunings et al., 2009; Chain et al., 2009). These responses at both the physiological and molecular level suggest that Si might be mediating defense reactions to plant diseases.

Recent research in other pathosystems has assessed the associated effect of Si with the use of other treatments such as yeasts with *A. citrulli* in melon (Conceição et al., 2014); chitosan for management of *Ralstonia solanacearum* in tomato (Kiirika et al., 2013); adjuvants for management of *Podosphaera xantii* in melon (Tesfagiorgis and Annegarn, 2013); and biocontrol agents for management of *Podosphaera xantii* in zucchini (Tesfagiorgis et al., 2014).

Currently, very little information exists on using Si for enhancing resistance of melons to BFB (Conceição et al., 2014). Consequently, the purpose of this study was to investigate the effects of Si on BFB disease progress on melon, as well as any changes in chemical attributes of the soil, and plant growth and mineral nutrition.

2. Materials and methods

2.1. Silicon effects on the components of melon resistance to BFB

Two identical experiments were carried out in a greenhouse with an air temperature varying from 26 to 38 °C and air humidity between 36 and 77%. The soil type used in the experiments was a Si-deficient typical Spodosol collected at the Instituto de Pesquisa Agropecuária – IPA in the city of Goiana, Pernambuco, Brazil. The physicochemical soil characteristics were as follows: pH in water = 5.7; H + Al = 2.04 $cmol_c\ dm^{-3}$; Al^{3+} = 0.46 $cmol_c\ dm^{-3}$; Na^+ = 0.36 $cmol_c\ dm^{-3}$; K^+ = 0.04 $cmol_c\ dm^{-3}$; Ca^{2+} = 0.64 $cmol_c\ dm^{-3}$; Mg^{2+} = 0.16 $cmol_c\ dm^{-3}$; P = 2 mg dm^{-3}; N = 0.39 g kg^{-1}; CO = 6.39 g kg^{-1}; clay = 49 g kg^{-1}; silt = 36 g kg^{-1} and sand = 915 g kg^{-1}. The concentration of available Si (extraction in CH_3COOH) was 10.36 mg dm^{-3}. The Si source used was calcium silicate (64% SiO_2 and 16% CaO; Ipiranga Chemical, Brazil) and doses of Si were soil incorporated at the rates of 0.00, 0.12, 0.24, 0.71 and 1.41 g kg^{-1} of soil that are equivalent to 0.0, 240, 480, 1410 and 2820 kg Si ha^{-1}, respectively. Other macronutrients and micronutrients were applied to meet the nutritional requirements of the plants (Nascimento et al., 2006). Calcium concentration was adjusted with calcium carbonate (40% Ca; Sigma–Aldrich, USA) so that the Ca equivalents were the same across all treatments. For each pot, 5 dm^3 of soil was incubated for 20 days with a humidity of about 80%.

Seeds, yellow melon hybrid AF 4945 (Sakata®), were sown in plastic trays (128 cells, 68 × 34 cm) containing Basaplant® commercial substrate (BaseAgro, São Paulo, Brazil). Five days after emergence, seedlings were transplanted into the pots after soil incubation (two seedlings per pot). The *A. citrulli* isolate, Aac1, used in this study was obtained from Baraúna (RN) via the Culture Collection of the Phytobacteriology Laboratory of the Federal Rural University of Pernambuco, Recife, Brazil and identified using a polyphasic approach (Silva, 2010). This isolate also was characterized in relation to other isolates obtained from cucurbit hosts by Silva (2010) and Walcott et al. (2004) (=AAC201-21) and belongs to the group I of Walcott. This strain was previously preserved in cryogenic vials containing sterilized tap water at room temperature 25 ± 2 °C. The pathogen was grown in Petri dishes containing nutrient-yeast extract-dextrose agar (NYDA) (dextrose 10 g, meat extract 3 g, yeast extract 5 g, agar 18 g L^{-1}) at 30 °C for 48 h. After this period, a suspension was prepared in distilled water and adjusted using a spectrophotometer (Metronic M3) to an A_{580} = 0.25 that corresponded to a concentration of 3.4 × 10^7 colony forming units (CFU) mL^{-1}. The bacterial suspension was amended with Tween 20 (0.05%). Twenty days after emergence, plants were inoculated with 20 mL of this suspension by spraying the leaves until runoff using a hand sprayer. Plants sprayed only with sterile distilled water served as the control.

The plants were covered with pre-moistened plastic bags, 60 × 40 cm, and were held upright by circular wooden stakes 24 h before and after inoculation. During the trial, pots were kept at 80% water retention capacity through daily weighing and irrigation to make up for water lost by evapotranspiration. The experimental design was completely randomized in a factorial arrangement (5 × 2) representing five Si doses and with (+Ac) and without (−Ac) pathogen inoculation. Each treatment consisted of five replications, each replication made up of a pot with two plants. After inoculation, five leaves from each plant were evaluated daily for incidence and for 20 days at 4-day intervals for severity. The following components of resistance to BFB development on melon were determined: a) incubation period, scored by the number of days between inoculation and the appearance of disease symptoms, characterized by small water-soaked lesions that became necrotic and often surrounded by a chlorotic halo; b) disease incidence, calculated by percentage of leaves with symptoms per treatment 20 days after inoculation; c) disease severity, estimated based on a scale of 0–6 adapted from the diagrammatic scale used to determine severity of cucumber net spot caused by *Leandria momordicae* Rangel (Azevedo, 1997), where 0 = no symptoms, 1 = 1–5% of leaf area infected, 2 = 6–12% of leaf area infected, 3 = 13–37% of leaf area infected, 4 = 38–62% of leaf area infected, 5 = 63–87% of leaf area infected and 6 = 88–100% of leaf area infected; d) disease index 20 days after inoculation, calculated according to McKinney (1923) and e) area under the disease progress curve, calculated based on five evaluations of disease severity according to Shaner and Finney (1977).

2.2. Silicon effects on soil chemical attributes

In the above experiment, after Si incubation and before the melon seedlings were transplanted, soil samples were taken from each treatment and chemical analyses were carried out. The