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Silicon induces resistance to postharvest rot of carrot caused by *Sclerotinia sclerotiorum* and the possible of defense mechanisms

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ABSTRACT

The inhibitory potential of silicon (Si) against *Sclerotinia sclerotiorum*, the causal agent of postharvest carrot rot was investigated under in vitro and in vivo conditions, and possible modes of action were evaluated. Silicon at the concentration of 10 mM strongly decreased the mycelial growth of *S. sclerotiorum* by 92.2% with inhibition of sclerotia formation by 76.3%. Myceliogenic and carpogenic germination of *S. sclerotiorum* sclerotia were inhibited by 89.1 and 78.9%, respectively, at the same concentration. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were 12 and 30 mM, respectively. Cell membrane permeability and lipid peroxidation of *S. sclerotiorum* mycelia were increased with Si treatment. Silicon at 10 mM significantly decrease the content of oxalic acid in *S. sclerotiorum* mycelia compared to the control. Application of silicon at 10 mM reduced the incidence of sclerotinia rot in carrot roots by 60.6% with 40 mm lesion length. Silicon treatment enhanced the activities of peroxidase (POD), polyphenoloxidase (PPO) and phenylalanine ammonia-lyase (PAL) in carrot roots inoculated with *S. sclerotiorum*. These results confirm that silicon might applied as an alternative tool to chemical fungicides for controlling sclerotinia rot of carrot during storage and to promote the defense response in carrot.

1. Introduction

Sclerotinia rot of carrot is a destructive disease, both in the field and during storage, throughout carrot production areas of the world (Rubatzky et al., 1999; Kora et al., 2003). The disease causes severe losses by 50–100% during long-term storage (Finlayson et al., 1989). *Sclerotinia sclerotiorum*, the causal agent of the disease, is a necrotrophic homotallic fungus which infects 408 different plant species among 278 genera from 75 plant families (Boland and Hall, 1994). The fungus can survive for up to 10 years in soil as dormant sclerotia depending on environmental conditions including various biological and physical factors (Harvey et al., 1995; Liu et al., 2017). Mature sclerotia may germinate myceliogenically to produce mycelia or carpogenically to produce apothecia (Huang and Kozub, 1993; Liang et al., 2010). Recently, there is no resistant cultivars to *S. sclerotiorum* among all cultivated carrot cultivars (Subbarao, 2002; Kora et al., 2005). Despite synthetic chemical fungicides are the most commonly strategy to control sclerotinia rot of carrot in the field, no commercial fungicide is available to control the storage phase of this disease (Hildebrand et al., 2008). However, the use of chemical fungicides for long time increase the development of resistant strains of *S. sclerotiorum* (Chen et al., 2011). Also, many of these synthetic fungicides have toxicity and carcinogenic properties as well as long degradation period. Furthermore, the increasing concerns over pesticide residues on storage products have promoted the exploration of new alternative tools that reduce disease incidence for postharvest diseases management (Bautista-Banos et al., 2006).

One promising alternative tool for controlling postharvest diseases is the application of the abiotic elicitor, silicon, (Si). Numerous studies have reported the efficacy of Si on postharvest diseases, for example, sodium metasilicate significantly reduced decay incidence and lesion diameter of blue mold and brown rot in sweet cherry fruit caused by *Penicillium expansum* and *Monilinia fructicola* (Qin and Tian, 2005). In a similar study, sodium silicate at 100 mM was effective in reducing the incidence and severity of postharvest decay on Hami melons caused by three fungal pathogens, *Alternaria alternata, Fusarium semitectum* and *Trichothecium roseum* (Bi et al., 2006). Li et al. (2009) indicated that the sodium silicate treatment at 100 and 200 mM decreased the diameter of dry rot lesions in potato tubers inoculated with *F. sulphureum*. Also, both silicon oxide and sodium silicate reduced the infection rate of postharvest Fusarium rot on Chinese cantaloupe (Liu et al., 2009). In this context, silicon in the form of sodium silicate decreased disease

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incidence and lesion diameter of green mold in citrus fruits caused by *P. digitatum* (Liu et al., 2010). Sodium silicate at 100 mM reduced the pink rot disease of muskmelon which caused by *T. roseum* during storage according to Li et al. (2012).

Silicon confers resistance to plants through different mechanisms 1) via formation of mechanical or physical barriers preventing fungal penetration (Fauteux et al., 2005; Van et al., 2013), 2) by enhancing the activity of defence-related enzymes such as phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO), peroxidase (POD), chitinase (CHI) and β-1,3- glucanase (GLU) (Liang et al., 2005; Cai et al., 2008; Fortunato et al., 2012), 3) through production and accumulation of antifungal compounds such as phytoalexins, phenols, flavonoids and proteins in plants during pathogen infection (Rodrigues et al., 2004; Xavier Filha et al., 2011) and 4) by activating multiple signaling pathways and transcription of genes related to plant resistance (Brunings et al., 2009; Vivancos et al., 2015).

Currently, there are no reports in the literature on using silicon for controlling the postharvest rot of carrot caused by *S. sclerotiorum*. Therefore, the present study evaluated the effects of silicon on *S. sclerotiorum* under in vitro and in vivo conditions. Besides, the activity of three important enzymes related to the systemic acquired resistance (SAR) was investigated after application of silicon.

2. Materials and methods

2.1. Pathogen

Sclerotinia sclerotiorum was isolated by picking off mycelium or sclerotia from infected carrot roots with the typical symptoms of *Sclerotinia* carrot rot, and maintained on potato dextrose agar (PDA) at 4 °C. To confirm the identity of the fungus and its virulence, the pathogen was inoculated into carrot roots and re-isolated onto PDA. The fungal pathogen was identified using morphological characteristics of mycelia and sclerotia according to the description of Saharan and Mehta (2008).

2.2. Effect on mycelial growth and formation of sclerotia

Silicon (Si) in the form of sodium metasilicate (Sigma -Aldrich Chemical, USA) was firstly dissolved in sterile distilled water and then added to the PDA plates (90 mm in diameter) to obtain final concentrations of 4, 6, 8 and 10 mM. PDA plates without Si were used as a control. The mycelial disks (5 mm in diameter), taken from the edge of 3 d cultures of *S. sclerotiorum*, were placed in the centre of plates. The plates were incubated at 20 °C for 6 days. There were three PDA plates, replicates, for each treatment. The percent inhibition of mycelial growth in each treatment was calculated by the formula: Inhibitory of mycelia growth (%) = [(C − T)/C] × 100, where C and T represented the mycelial growth (mm) in the control and treated plates, respectively. The percent inhibition of sclerotia formation was calculated after 15 days according to the following formula: Inhibition of sclerotia formation (%) = 100 − (T × 100/C), where T and C are the number of sclerotia in the treatment and control, respectively. The experiment was conducted twice.

2.3. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Mycelial discs of *S. sclerotiorum* (5 mm diameter) were removed from 3 d old cultures and placed in the center of PDA plates (90 mm in diameter) containing serial concentrations of Si (12, 14, 16, 18, 20 to 40 mM). The plates were incubated at 20 °C. The MIC was defined as the lowest concentration of complete inhibition of the visible mycelial growth. For the MFC, the mycelial discs of *S. sclerotiorum*, which failed to grow, were transferred onto fresh PDA media without Si and incubated at 20 °C. The MFC was determined by the incubation of the lowest concentration of Si, after which no revival of pathogen was observed. There were three replicates for each treatment, and the experiment was repeated two times.

2.4. Effect on myceliogenic germination of sclerotia

S. sclerotiorum was grown on PDA in Petri dishes (90 mm in diameter) at 20 °C. After 30 days, sclerotia were gently harvested and air-dried at room temperature. Sclerotia were surface disinfected in 70% ethanol for 2 min. Then, the sclerotia were submerged in each concentration of Si (4, 6, 8 and 10 mM) or in sterile distilled water as a control for 24 h. The sclerotia in each treatment were collected, blotted dry on sterile filter paper, surface-sterilized in 2% sodium hypochlorite for 2 min, rinsed in sterile distilled water three times, and individually inoculated on PDA in Petri dishes (90 mm in diameter), 5 sclerotia per dish. The number of germinated sclerotia was recorded after incubation at 20 °C for 6 d. Three replicate plates were used for each treatment and the experiment was performed twice. The percent inhibition of myceliogenic germination of sclerotia was measured using the formula: Inhibition of myceliogenic germination (%) = 100 − (G × 100/C), where G and C are the number of myceliogenically germinated sclerotia in treatment and control, respectively.

2.5. Effect on carpogenic germination of sclerotia

Sclerotia produced from 30 d old fungal growth on PDA were gently harvested and air dried on sterile filter paper. The sclerotia with diameter more than 5–6 mm were used in this study to prevent experimental errors resulting from the small size of sclerotia. One hundred grams of steam-sterilised natural sandy soil was placed in sterilised glass jars. Five sclerotia were placed at 0.5 cm depth by pressing gently over the soil surface in the glass jars. Different concentrations of Si (4, 6, 8 and 10 mM) were sprayed on the sclerotia into the jars using sterile medical syringes twice a week. Treatment without Si was used as a control. The glass jars were incubated at 20 °C for 12 weeks. The sclerotia with visible stipes were considered as carpogenically germinated. The experiment was repeated twice and there were three jars for each treatment. The percent inhibition of carpogenic germination of sclerotia was calculated according to the formula: Inhibition of carpogenic germination (%) = 100 − (G × 100/C), where G and C are the number of carpogenically germinated sclerotia in treatment and control, respectively.

2.6. Effect on cell membrane permeability

The mycelial disks of *S. sclerotiorum* (5 mm in diameter), taken from the margins of 3 d cultures, were inoculated into 250 ml conical flasks containing 100 ml of potato dextrose broth (PDB) and incubated with shaking at 175 rpm and 25 °C. After 36 h, the flasks were amended with Si at the concentration of 10 mM, and the flasks without Si were used as a control. After the flasks were shaken for additional 36 h, the mycelia were collected and washed twice with double distilled water and filtrated in vacuum for 10 min. 0.5 g of mycelia was suspended in 20 ml of double distilled water. The electrical conductivity of the double distilled water was measured after 0, 5, 10, 20, 40, 60, 80, 100, 120, 140, 160 and 180 min with a Jenco 3173 conductivity meter (Jenco Technologies, San Diego, CA, USA). After 180 min, the mycelia were boiled for 5 min, and the final conductivity was measured. The relative conductivity of mycelia was calculated by the following formula: Relative conductivity (%) = conductivity/final conductivity × 100. The experiment was conducted two times with four replicates for test.

2.7. Effect on lipid peroxidation

Mycelia from 3 d cultures of *S. sclerotiorum* growing on PDA were collected, washed and dehydrated on filter paper. Three grams of