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Comparison of root and foliar applications of potassium silicate in potentiating post-infection defences of melon against powdery mildew

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The application of silicon to the roots or leaves reduces the severity of powdery mildew (*Podosphaera xanthii*) in melon but the latter treatment is less effective. This study compared key biochemical defence responses of melon triggered by *P. xanthii* after root or foliar treatment with potassium silicate (PS). Treatments consisted of pathogen-inoculated or mock-inoculated plants supplied with PS via roots or foliarly, as well as a non-treated control. The activity of defence enzymes and the concentration of phenolic compounds, lignin and malondialdehyde were determined from leaf samples at different time points after inoculation. Pathogen-inoculated plants irrigated with PS showed both an accumulation of silicon and primed defence responses in leaves that were not observed in pathogen-inoculated plants either sprayed with PS or not treated. These responses included the anticipated activity of peroxidase and accumulation of soluble phenols, the activation of chitinase and repression of catalase, and the stronger activation of superoxide dismutase, peroxidase and β-1,3-glucanase. Moreover, the lignin concentration increased in response to inoculation, whereas the malondialdehyde concentration decreased. For the foliar treatment, however, only an increase in lignin deposition was observed compared with the control plants. The results show that silicon strongly plays an active role in modulating the defence responses of melon against *P. xanthii* when supplied to the roots as opposed to the foliage.

Keywords: biochemical defences, *Cucumis melo*, induced resistance, oxidative stress, *Podosphaera xanthii*, priming effect

Introduction

Melon (*Cucumis melo*) is the main fresh fruit export of Brazil, and the value of this crop reached $122 million in 2010 (IBRAF, 2012). Powdery mildew caused by *Podosphaera xanthii* is a serious threat to both the yield and quality of the crop worldwide (Stadnik *et al.*, 2001). The disease is characterized by white colonies composed of mycelia and conidia that develop on leaf surfaces, petioles and stems. Under favourable environmental conditions, the colonies coalesce, and the host tissue becomes chlorotic and usually senesces earlier than in healthy tissue (Zitter *et al.*, 1996).

The disease can be controlled using cultivars carrying race-specific resistance genes and by fungicide application. However, genetic resistance is not durable (Hosoya *et al.*, 2000), and there are several reports of resistance or insensitivity of *P. xanthii* to various groups of fungicides (McGrath, 2001). These limitations and the increasing environmental concerns related to the widespread use of pesticides, especially in crops that are consumed unprocessed, have led to the development of alternative control strategies. An attractive approach is to use non-toxic chemicals that induce the physiological state of priming, characterized by a faster and/or stronger activation of the plant defence responses (Conrath *et al.*, 2006). As this priming effect occurs when the plant is challenged by the pathogen, the metabolic cost of defence is alleviated (Bolton, 2009). The supply of silicon to plants provides satisfactory control of powdery mildew in many species, including melon (Dallagnol *et al.*, 2012). However, in some cases the mechanisms of disease control have been shown to differ depending on whether silicon is applied to roots or to leaves (Liang *et al.*, 2005; Guével *et al.*, 2007; Lemes *et al.*, 2011). When supplied to the leaves as a salt (e.g. potassium silicate, PS), silicon is thought to act directly on the pathogen by forming a physical barrier after its deposition and/or by an ionic or pH effect on germinating conidia (Liang *et al.*, 2005; Guével *et al.*, 2007). However, when supplied through the roots, it reinforces the walls of epidermal cells and enhances various defence responses, including the increased formation of papillae and deposition of callose at the penetration sites and the

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increased production of pathogenesis-related proteins (Kim *et al.*, 2002; Liang *et al.*, 2005; Shetty *et al.*, 2012). Silicon also modulates the expression of several other genes of various metabolic functions. Interestingly, in most cases this modulation occurs only in the presence of the pathogen, which is compatible with the view of silicon as a priming agent (Van Bockhaven *et al.*, 2013).

A previous study reported that the application of foliar or root amendments of PS to melon plants effectively reduced the area under the powdery mildew progress curve by 65 or 73%, respectively (Dallagnol *et al.*, 2012). However, root amendment was more effective than foliar spraying in reducing other epidemic components, such as the colony expansion rate, colony area, conidial production and disease progress rate. These results correlated with higher foliar silicon concentrations, suggesting that the effect on the disease could result in part from metabolic changes in host defence due to the accumulation of the element within the tissues.

The present study investigated the priming-inducing effects of silicon on biochemical defence mechanisms of melon plants after inoculation with *P. xanthii*. The activities of oxidoreductases (superoxide dismutase, catalase, polyphenol oxidase and peroxidases) and glycosylases (chitinase and β-1,3-glucanase) were quantified at various time points after inoculation in mock-inoculated and pathogen-inoculated plants that had previously received root or foliar applications of PS. Additionally, the concentrations of total soluble phenols and lignin, which are known to accumulate as a response against biotic stresses, and the concentration of malondialdehyde (MDA), an indicator of cell membrane damage resulting from lipid peroxidation, were evaluated.

Materials and methods

Plant material and growth

Seeds of a commercial melon hybrid (cv. Jangada; Sakata Seeds Sudamerica) were sown in 8-L plastic pots (Nutriplan) containing 2 kg of Plantmax substrate (Eucatex); the physicochemical characteristics of this substrate have been reported elsewhere (Dallagnol *et al.*, 2012). The concentration of available Si in this substrate (extracted in 0·01 M $CaCl_2$) corresponded to 25·2 mg dm^{-3}. After emergence, the plants were maintained in a greenhouse during the experiments and were fertilized weekly with 50 mL of a nutrient solution prepared according to Dallagnol *et al.* (2012). The photon flux density (PFD) on sunny days at noon inside the greenhouse was measured using a LI-250A light meter (LI-COR) at approximately 800 μmol photons m^{-2} s^{-1}. The daily mean temperatures ranged from 20 to 28°C.

Silicon amendments

Sili-K (Unaprosil), which is composed of 15% K_2O (210 g L^{-1}) and 12·2% soluble Si (171 g L^{-1}), was used as the source of silicon. The product was applied foliarly (foliar PS treatment) or via irrigation water (root PS treatment). The control (−PS) consisted of spraying the plants with water and irrigating them daily with 300 mL of a KCl solution as described below. The plants of the foliar treatment were irrigated in the same manner and were sprayed, 24 h before inoculation, on the upper leaf surface with PS at 30 mL L^{-1} (pH 10·2) until run-off using a handheld manual sprayer (model 0417.02.00; Guarany Ind. & Com. Ltd). For the root treatment, the pots were irrigated daily with 300 mL of a PS solution (0·34 mL PS in 1 L deionized water; final Si concentration 2 mM, pH 5·5–6·5 adjusted using 1 M NaOH or 1 M HCl as necessary). To standardize the amount of potassium supplied to the plants in the root treatment, plants of the foliar and control treatments were irrigated daily with 300 mL of a 1·53 mM KCl (Sigma-Aldrich) solution with the pH adjusted in the same manner as that described above.

Experimental design

The experiment consisted of the combination of the three PS treatments (root application, foliar application and non-treated control) and two inoculation conditions (inoculated with *P. xanthii* or mock-inoculated with water) over six or four sampling times, for the combinations that involved the inoculated or the mock-inoculated conditions, respectively. Treatments were replicated three times, each replication represented by a single plant. The plants of each PS treatment were grown in 13 pots containing three plants each. The plants in nine pots were inoculated with *P. xanthii* while the plants in the remaining four pots were mock-inoculated with water. Inoculated and mock-inoculated plants were kept in separate compartments in the greenhouse and exposed to the same PFD, temperature and humidity conditions. Two pathogen-inoculated plants in each pot (18 plants in total) were used in the biochemical analysis and the remaining one (nine plants in total) was used to assess disease severity as described below. Mock-inoculated plants were sampled in the same manner as the inoculated ones, totalling 12 plants for the biochemical analysis. For the biochemical analysis, leaf samples were taken at 24, 48, 72, 96, 120 and 144 h after inoculation (hai) from three pathogen-inoculated plants and at 24, 72, 120 and 144 hai from three mock-inoculated ones. Sampled plants were chosen at random and were not resampled. The experiments were performed twice.

Pathogen inoculation and disease assessments

An isolate of *P. xanthii* was obtained from a commercial melon crop and maintained on plants of cv. Jangada. For inoculation, a conidial suspension (2×10^4 conidia mL^{-1}) was prepared according to Dallagnol *et al.* (2012) and sprayed onto melon leaves as a fine mist using a handheld manual sprayer 35 days after sowing, when plants had 6–7 leaves.

The following variables were evaluated on the fourth and fifth leaves: number of powdery mildew colonies per leaf cm^2, colony area and disease severity. The number of colonies was counted 7 days after inoculation (dai) in three distinct leaf regions that were chosen at random. Colony area was estimated as the mean area of five colonies randomly chosen on each leaf 10 dai, which corresponded to the time when the colonies of the –PS treatment began to coalesce. Disease severity was evaluated on individual leaves 20 dai and expressed as the percentage of leaf area covered by powdery mildew (Dallagnol *et al.*, 2012).

Biochemical analyses

Biochemical analyses were performed using the fourth leaf of each plant. The leaves were flash-frozen and pulverized with a

mortar and pestle, and the resultant fine powder was used in the analyses.

Enzyme activity

Enzyme activities were measured using 0·5 g of ground leaf tissue. The tissue was homogenized in tubes containing 4 mL of a solution of 100 mM potassium phosphate buffer (pH 6·5), 1 mM phenylmethylsulphonyl fluoride and 40 mg polyvinylpolypyrrolidone, which were placed in an ice bath. The homogenate was centrifuged at 12 000 ***g*** for 15 min at 4°C, and the supernatant was used as the crude enzyme extract. The soluble protein content of the extract was measured as described in Dallagnol *et al.* (2011).

The superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated based on the colourimetric quantification of the photoreduction of nitroblue tetrazolium (NBT; Sigma-Aldrich) according to Gupta *et al.* (1993). One unit of SOD was defined as the amount required to inhibit the photoreduction of NBT by 50%. The specific activity of SOD was expressed in units of SOD mg^{-1} of protein. The activity of catalase (CAT, EC 1.11.1.6) was determined by quantifying the degradation of hydrogen peroxide (H_2O_2; Merck) using a spectrophotometer according to Azevedo *et al.* (1998). The CAT activity was expressed as μmol H_2O_2 degraded min^{-1} mg^{-1} of protein. The activities of peroxidase (POX, EC 1.11.1.7) and polyphenol oxidase (PPO, EC 1.10.3.1) were determined based on the colourimetric quantification of pyrogallol (Sigma-Aldrich) oxidation as described by Dallagnol *et al.* (2011) and expressed as mol purpurogallin produced min^{-1} mg^{-1} of protein using an extinction coefficient of 2·47 mM cm^{-1}. The activity of chitinase (CHI, EC 3.2.1.14) was determined based on the colourimetric quantification of *p*-nitrophenyl cleaved from the chitin substrate analogue *p*-nitrophenyl-*β*-D-*N*,*N*′-diacetylchitobiose (PNP; Sigma-Aldrich) and expressed as mmol *p*-nitrophenyl produced min^{-1} mg^{-1} of protein using an extinction coefficient of 7×10^3 mM cm^{-1} as described by Dallagnol *et al.* (2011). The activity of *β*-1,3-glucanase (GLU, EC 3.2.1.6) was determined colourimetrically using a 4 mg mL^{-1} solution of carboxymethyl-Curdlan-Remazol Brilliant Blue R as a substrate (CM-Curdlan-RBB; Loewe Biochemica) according to Guzzo & Martins (1996). The enzyme activity was expressed as the absorbance at 600 nm mg^{-1} of protein.

Lipid peroxidation assay

The oxidative damage to lipids was estimated as the content of total 2-thiobarbituric acid (TBA; Merck) reactive substances in 0·2 g of ground leaf sample, and was expressed as equivalents of MDA using the method described by Dallagnol *et al.* (2011) as modified from Cakmak & Horst (1991). The concentration of MDA in each sample was expressed in nmol MDA g^{-1} of fresh matter weight using the extinction coefficient of 155 mM cm^{-1}.

Determination of total soluble phenolics and lignin and lignin-like phenolic polymers

Total soluble phenolics (TSPs) and lignin and lignin-like phenolic polymers were extracted from 0·1 g leaf samples according to Dallagnol *et al.* (2011). The concentration of TSPs was determined using the Folin–Ciocalteu phenol reagent (Sigma-Aldrich), expressed as μg of phenolics (in terms of catechol) g^{-1} of fresh matter weight. The dried alcohol-insoluble residue from the TSP extraction was used to determine the lignin concentration using thioglycolic acid (Sigma-Aldrich) as described by Dallagnol *et al.* (2011). The concentration of lignin–thioglycolic acid (LTGA) derivatives was quantified colourimetrically and was expressed as $\mu g\ g^{-1}$ of fresh matter weight, using the alkali lignin 2-hydroxypropyl ether (Sigma-Aldrich) as a standard.

Determination of Si and K in the leaf tissues

The leaves used to determine the concentrations of Si and K were collected from plants used in each replication after the last disease evaluation. The leaves were rinsed with deionized water, dried for 72 h at 65°C and pulverized individually to a fine powder using a mortar and pestle. For the mock-inoculated plants, the fourth and fifth leaves of each plant were sampled at the same time as the leaves of the pathogen-inoculated plants and processed as described above. The concentration of Si was determined based on the colourimetric analysis of 0·1 g of alkali-digested leaf tissue (Dallagnol *et al.*, 2012). The concentration of K in leaf tissue was determined by assaying 0·2 g of dry tissue after nitric–perchloric acid digestion in a flame photometer (Dallagnol *et al.*, 2012).

Data analysis

Data from the disease assessments and the concentrations of Si and K in the leaf tissues were analysed using ANOVA, and the treatment means were compared using Tukey's test ($P \leq 0{\cdot}05$). For the biochemical variables, Student's *t*-tests were used to compare the means of pathogen-inoculated × mock-inoculated plants within PS treatments. The same test was used to compare the means of pathogen or mock-inoculated plants of the root PS and foliar PS plants with the –PS control plants. All tests were performed using SAS v. 8.0 (SAS Institute, Inc.).

Results

Effect of PS amendments on disease severity

Data from the disease assessments of the two experiments were pooled for statistical analysis after the homogeneity of variances was verified using Cochran's test (data not shown). No disease symptoms were observed in the mock-inoculated plants during the experiment. In the pathogen-inoculated ones, both the foliar PS and root PS treatments significantly reduced ($P \leq 0{\cdot}05$) all disease variables compared to the –PS control (Fig. 1). The number of colonies per cm^2 did not differ between the foliar and root treatments ($P = 0{\cdot}07$), but these treatments reduced the number of colonies per cm^2 by 57 and 68%, respectively, as compared to the –PS control (Fig. 1a). However, compared with the foliar treatment, the root treatment significantly reduced the colony area (Fig. 1b) and disease severity (Fig. 1c) by 43 and 53%, respectively.

Biochemical responses of plants treated with PS

Statistical tests were performed for each experiment separately; because the results were similar, only the results of one experiment are presented and discussed.

Oxidoreductase activities

Comparisons of mock-inoculated and pathogen-inoculated plants within the PS treatments indicated that in

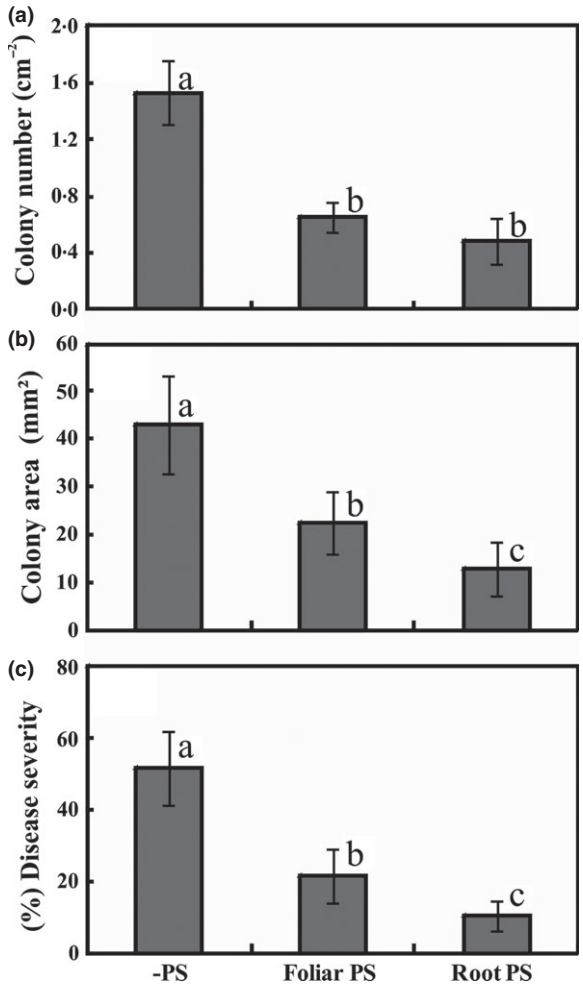

Figure 1 Colony number per cm^2 of leaf area (a), colony area (mm^2) (b), and disease severity (percentage of the leaf surface covered by powdery mildew) (c) of powdery mildew on melon leaves not supplied with potassium silicate (−PS) or supplied foliarly (Foliar PS) or via roots (Root PS). The bars represent the standard deviation of the means. The data represent the combination of two experiments (n = 18). The means followed by different letters were significantly different based on Tukey's test (P = 0·05).

the –PS control, the inoculation with *P. xanthii* significantly increased the activities of SOD (0·9–1·5 times) from 72 to 144 hai (Fig. 2a), of POX (0·5–1·6 times) at 120 and 144 hai (Fig. 2g), and of PPO (0·2 times) at 144 hai (Fig. 2j). The activity of CAT, however, did not change (Fig. 2d). Essentially the same results were observed in the foliar PS treatment (Fig. 2b,e,h,k), except that the rise in the activity of PPO was detected earlier (24 hai) in the pathogen-inoculated plants (Fig. 2k). In the root PS treatment, the activities of SOD also increased in the pathogen-inoculated plants compared to the mock-inoculated ones. However, higher values were observed in pathogen-inoculated plants, notably at 72 hai, when the activity was 2·5 higher than in the mock-inoculated plants (Fig. 2c). The activity of POX was similar to the –PS and foliar PS treatments, except that an increase in pathogen-inoculated plants was also detected at 72 hai (Fig. 2i). However, the activity of CAT was markedly different in the root PS treatment, being reduced in activity by 1·3 and 2·2 times in pathogen-inoculated plants compared to the mock-inoculated plants at 24 and 72 hai, respectively (Fig. 2f). Finally, no differences in the activities of PPO were observed (Fig. 2l) between pathogen and mock-inoculated plants.

Comparisons between foliar PS and –PS treatments of plants inoculated with *P. xanthii* indicated that the activity of SOD decreased (0·3 times) at 96 hai (Fig. 2b) in the foliar PS treatment, whereas the activity of POX increased (0·6 to 1·1 times) during the 24–72 hai interval (Fig. 2h) compared with the −PS control. No differences were detected for the activities of CAT (Fig. 2e) and PPO (Fig. 2k). For the root PS treatment, however, the activity of SOD (Fig. 2c) significantly increased earlier (48 hai) than in the foliar PS or −PS control, persisted significantly higher at 72 hai and then decreased at 96 hai to the level observed for foliar PS. The activity of CAT was markedly different from that observed in the foliar PS versus −PS comparison, as up to 1·8-fold reductions were observed during the 24–96 hai period (Fig. 2f). The activity of POX (Fig. 2i) increased (0·32–0·55 times) during 48–96 hai and the activity of PPO (Fig. 2l) remained unchanged, as in the foliar PS treatment.

Comparisons between the root PS and –PS treatments for the mock-inoculated plants did not detect any differences in oxidoreductase activities. Interestingly, however, the activity of POX increased significantly during 24–72 hai in the foliar PS treatment in comparison to the –PS control (Fig. 2h).

Glycolytic enzyme activities

No significant differences were detected in the activity of CHI between pathogen- and mock-inoculated plants within the −PS control and foliar PS treatments (Fig. 3a, b). However, the activity of GLU increased at 72, 120 and 144 hai with the pathogen in the −PS and foliar PS treatments to similar extents (Fig. 3d,e). For the pathogen-inoculated plants of the root PS treatment the activities of CHI (Fig. 3c) and GLU (Fig. 3f) significantly increased 0·48-fold at 72 hai and up to 0·6-fold at 72 and 120 hai compared to the mock-inoculated plants, respectively.

Significant differences were detected in the activities of CHI and GLU between the root PS and –PS treatments for pathogen-inoculated plants only. The activity of CHI increased 0·24 to 0·65 times at 48–96 hai (Fig. 3c) and the activity of the GLU increased 0·56 and 0·22 times at 48 and 96 hai, respectively (Fig. 3f).

Significant differences in enzyme activities were not detected for mock-inoculated plants among any of the PS treatments.

Lipid peroxidation

The concentration of MDA increased significantly from 120 to 144 hai upon infection by the pathogen for all PS treatments (Fig. 4). The largest difference was observed

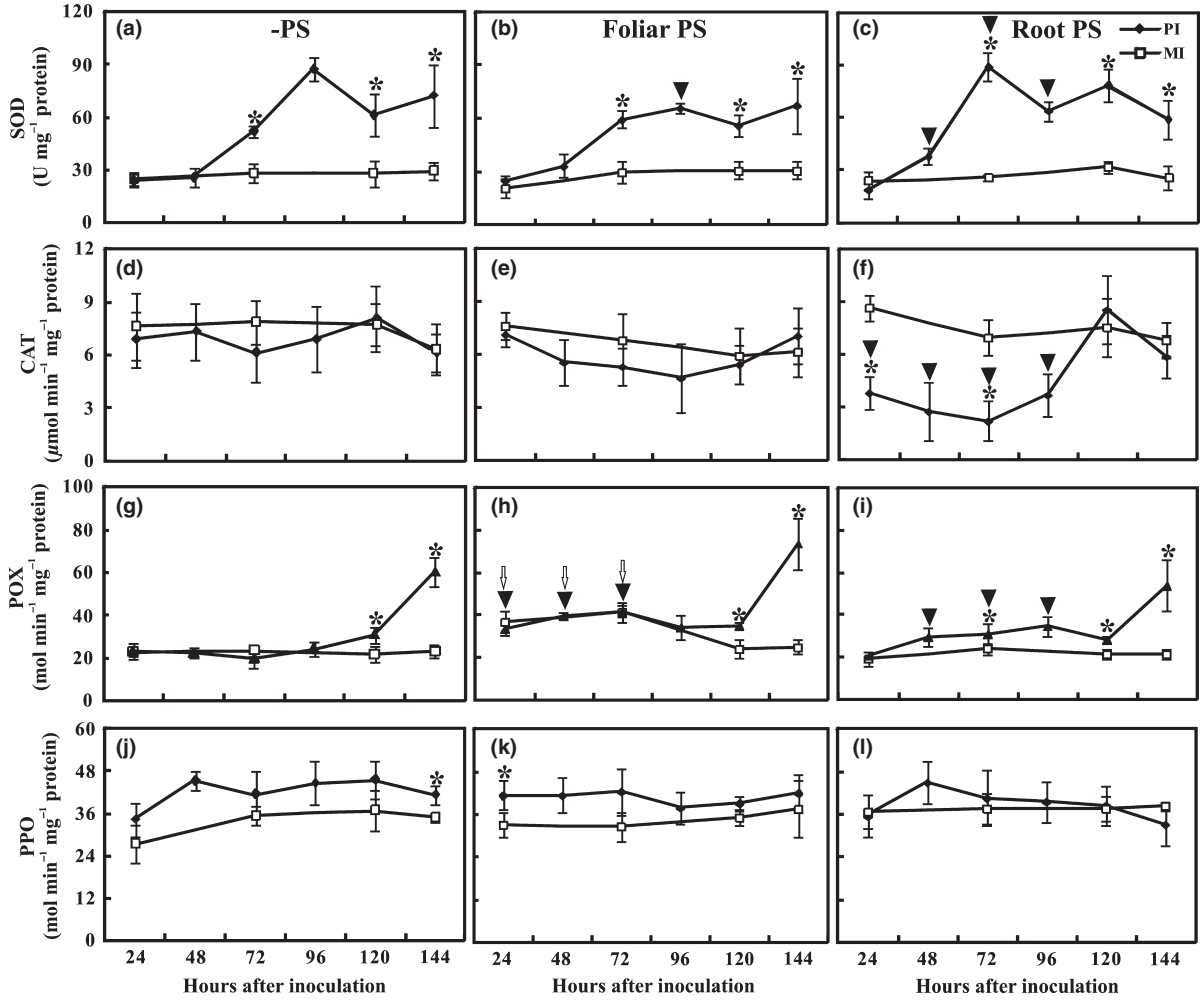

Figure 2 Activities of superoxide dismutase (SOD) (a, b, c), catalase (CAT) (d, e, f), peroxidase (POX) (g, h, i) and polyphenol oxidase (PPO) (j, k, l) in melon leaves not supplied with potassium silicate (–PS) (a, d, g, j), supplied foliarly (Foliar PS) (b, e, h, k), or via roots (Root PS) (c, f, i, l) that were pathogen- (PI) or mock-inoculated (MI). The bars represent the standard deviation of the means (n = 3). The means of pathogen-inoculated plants marked by an asterisk (*) differ significantly from those of mock-inoculated plants within the same PS treatment based on the t-test ($P \leq 0{\cdot}05$). The means of mock-inoculated foliar PS plants marked by a white arrow (⇩) differ from the mock-inoculated ones of both the –PS and root PS treatments based on the t-test ($P \leq 0{\cdot}05$). The means of pathogen-inoculated plants of the root or foliar PS treatments marked by an inverted triangle (▼) differ from the means of pathogen-inoculated plants of the –PS treatment at the same time interval based on the t-test ($P \leq 0{\cdot}05$).

at 144 hai for the –PS control, where MDA levels increased by 1·3 times (Fig. 4a). However, the root PS treatment for pathogen-inoculated plants decreased the concentration of MDA by 0·2 and 0·3 times at 120 and 144 hai, respectively, compared to the –PS control (Fig. 4c). Differences were not detected between the foliar PS and the –PS treatments (Fig. 4a,b).

Significant differences were not detected among the mock-inoculated plants of any treatments.

Concentrations of TSPs and LTGA derivatives

The concentration of TSPs increased significantly in response to pathogen inoculation in all treatments, but at different times. For the –PS control treatment (Fig. 5a), the concentration in the pathogen-inoculated plants was 0·2 times higher than in the mock-inoculated ones only at 72 hai. For the foliar PS treatment (Fig. 5b), concentrations were also higher in pathogen-inoculated plants, but differences were detected later, at 120 and 144 hai. A different response was observed for the root PS treatment, where the levels of TSPs in the pathogen-inoculated plants were consistently higher (0·2–0·4 times) than in the mock-inoculated ones throughout the sampling times (Fig. 5c). The concentration of LTGA derivatives in plants did not change in response to inoculation with *P. xanthii* for the –PS control (Fig. 5d), but did increase up to 0·2 times at 72 and 120 hai for the foliar PS treatment (Fig. 5e) and 0·2–0·3 times at 72, 120 and 144 hai for the root PS treatment (Fig. 5f).

A significant difference between the root PS and –PS treatments in the concentration of TSPs for pathogen-inoculated plants occurred only at 48 hai (Fig. 5c).

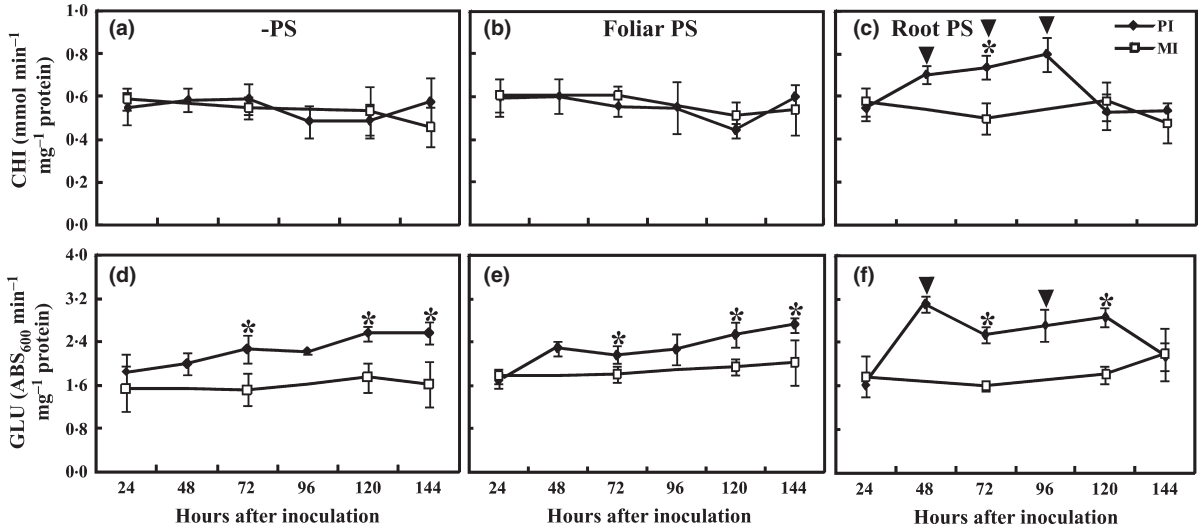

Figure 3 Chitinase (CHI) (a, b, c) and β-1,3-glucanase (GLU) (d, e, f) activities in melon leaves not supplied with potassium silicate (–PS) (a, d), supplied foliarly (Foliar PS) (b, e), or via roots (Root PS) (c, f) that were pathogen- (PI) or mock-inoculated (MI). Bars represent the standard deviation of the means (n = 3). The means of pathogen-inoculated plants followed by an asterisk (*) differ significantly from those of mock-inoculated plants of the same PS treatment based on the t-test ($P \leq 0{\cdot}05$). The means of pathogen-inoculated plants of the root or foliar PS treatments followed by an inverted triangle (▼) differ from the means of pathogen-inoculated plants of the –PS treatment based on the t-test ($P \leq 0{\cdot}05$).

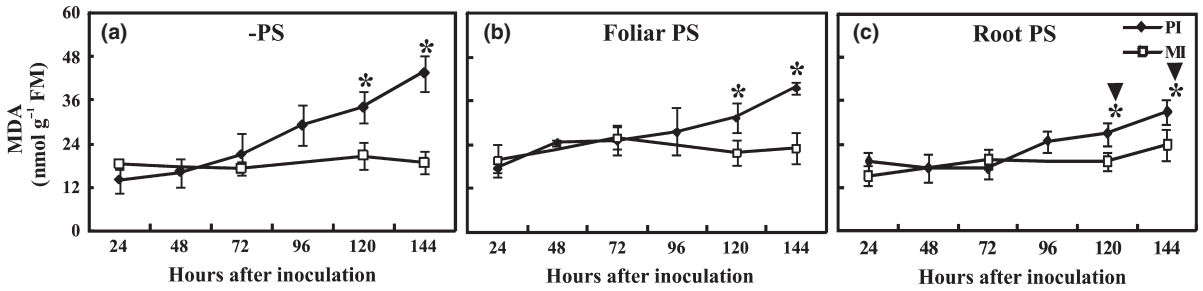

Figure 4 Concentration of malondialdehyde (MDA) in melon leaves not supplied with potassium silicate (–PS) (a), supplied foliarly (Foliar PS) (b) or via roots (Root PS) (c) that were pathogen- (PI) or mock-inoculated (MI). The bars represent the standard deviation of the means (n = 3). The means of pathogen-inoculated plants followed by an asterisk (*) differ from those of mock-inoculated plants of the same PS treatment based on the t-test ($P \leq 0{\cdot}05$). The means of pathogen-inoculated plants of the root or foliar PS treatments followed by an inverted triangle (▼) differ from the means of pathogen-inoculated plants of the –PS treatment based on the t-test ($P \leq 0{\cdot}05$). FM = fresh matter.

Differences were not detected in the concentration of LTGA among these treatments (Fig. 5d,e,f). Finally, differences between PS treatments were not found in the concentrations of TSPs and LTGA when comparing mock-inoculated plants.

Concentration of Si and K in leaf tissues

Data related to the concentrations of Si and K in the two experiments were pooled for statistical analysis after the homogeneity of variances was verified using Cochran's test (data not shown). Because significant differences were not observed between the pathogen- and mock-inoculated plants within treatments (data not shown), only data of the pathogen-inoculated plants are presented (Fig. 6). The concentration of Si in the leaf tissue was 1·3 and 1·2 times higher for the root PS treatment compared to the –PS and foliar PS treatments, respectively; significant differences were not detected between the –PS and the foliar PS treatments (P = 0·72; Fig. 6a). No difference in the concentration of K was detected among the treatments (P = 0·79; Fig. 6b).

Discussion

In a previous study, Dallagnol *et al.* (2012) demonstrated that PS reduced several epidemic components of powdery mildew in melon plants when it was supplied either to the roots or leaves. However, the reduction in disease severity was always more pronounced in the former case, suggesting that the accumulation of silicon in the internal tissues controlled powdery mildew in a manner distinct from that when PS was applied foliarly. In the current study, the effects of root and foliar

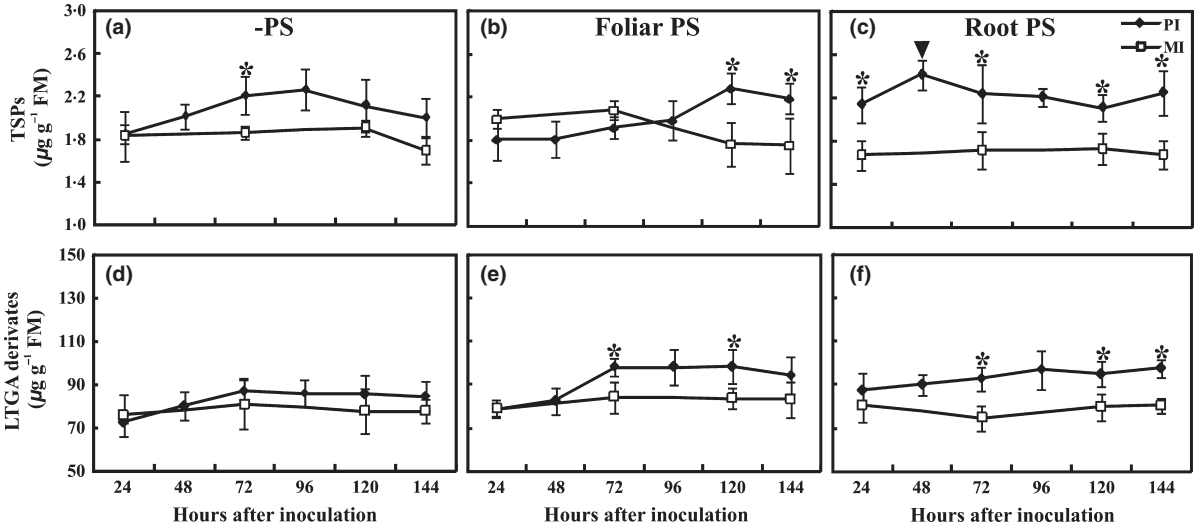

Figure 5 Concentrations of total soluble phenols (TSPs) (a, b, c) and lignin-thioglycolic acid (LTGA) derivatives (d, e, f) in melon leaves not supplied with potassium silicate (−PS) (a, d), supplied foliarly (Foliar PS) (b, e), or via roots (Root PS) (c, f) that were pathogen- (PI) or mock-inoculated (MI). The bars represent the standard deviation of the means (n = 3). The means of pathogen-inoculated plants followed by an asterisk (*) differ from those of mock-inoculated plants of the same PS treatment based on the t-test ($P \leq 0{\cdot}05$). The means of pathogen-inoculated plants of the root or foliar PS treatments followed by an inverted triangle (▼) differ from the means of pathogen-inoculated plants of the –PS treatment based on the t-test ($P \leq 0{\cdot}05$). FM = fresh matter.

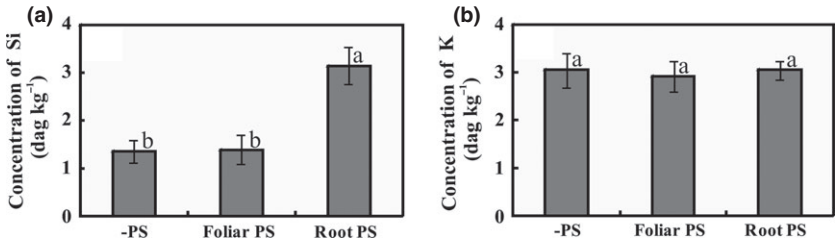

Figure 6 Concentrations of silicon (Si) (a) and potassium (K) (b) in melon leaves not supplied with potassium silicate (−PS), supplied foliarly (Foliar PS), or via roots (Root PS). The bars represent the standard deviation of the means based on the combination of two experiments (n = 18). The means followed by different letters differ significantly based on Tukey's test ($P \leq 0{\cdot}05$).

PS treatments on the biochemical responses of melon plants were compared at various time points after inoculation with *P. xanthii*. Comparisons were made between pathogen-inoculated and mock-inoculated plants within treatments and among treatments, and revealed significant differences between these two types of PS application. These differences coincided with a lower disease severity in the plants that received PS through the roots, expressed as smaller instead of fewer fungal colonies on the leaf surface. The activation of silicon-mediated defences against *P. xanthii* was previously reported in cucumber (Liang *et al.*, 2005), where root applications of silicon were more effective than foliar applications in activating four enzymes (POX, PPO, CHI and PAL) 7 dai. In the present study, the understanding of this phenomenon in cucurbits was extended by analysing an increased number of biochemical responses. In addition, these responses were assessed from the early stages of infection up to the time of symptom appearance by using a time course experimental design.

Comparisons within the −PS control indicated that infection of a susceptible melon plant with *P. xanthii* induced biochemical alterations that corresponded to those previously reported in other interactions involving powdery mildews (El-Zahaby *et al.*, 1995; Kovács *et al.*, 2011). These included the activation of enzymes involved in the antioxidant pathway, represented first by SOD, followed by POX and PPO, in an attempt to alleviate cell oxidative stress (El-Zahaby *et al.*, 1995). However, even after activation of this protective system, damage to the cell membranes still occurred, as deduced from the increased concentration of MDA. This damage could result from the asynchronous production and removal of H_2O_2, leading to the accumulation of reactive oxygen species (ROS) and the peroxidation of lipids (Tománková *et al.*, 2006). Consistent with this and similar to that observed in wheat and barley lines infected

with *Blumeria graminis* (Harrach *et al.*, 2008), no alteration in CAT activity was observed, indicating that the scavenging activity of this enzyme was not activated. GLU activity increased upon infection, but the activity of CHI did not differ between the pathogen- and mock-inoculated plants. Other studies have reported increases in GLU activity in response to *Erysiphe pisi* infection (Rakshit *et al.*, 2000). Furthermore, no significant alterations in phenolic compounds and lignin (LTGA derivatives) were observed, indicating that cellular structural defences were not activated in a compatible interaction between melon and *P. xanthii*.

Comparisons between inoculated and mock-inoculated plants within the PS treatments indicated that inoculation with the pathogen led to increased concentrations of LTGA derivatives at 72 hai in both PS treatments. As this increase was not observed in inoculated plants of the –PS treatment, it is concluded that the application of PS possibly restricts the pathogen colonization by promoting both the thickening and the reinforcement of the cell walls, in a manner similar to that described in rose plants amended with silicon in response to infection by *Podosphaera pannosa* (Shetty *et al.*, 2012). Apart from this, the responses to infection observed in the plants of the foliar PS treatment were very similar to those of the –PS treatment. By contrast, some relevant differences were observed in the root PS treatment. An increase in the activity of POX as a result of infection was observed earlier (72 hai) than in the –PS treatment (120 hai) and the total soluble phenols started to accumulate at 24 hai as a result of infection, whereas in the –PS and foliar PS treatments differences in TSP concentrations between mock-inoculated and pathogen-inoculated plants were detected later at 72 and 120 hai, respectively. In addition, the increase in the activity of CHI and the decrease in the activity of CAT as a result of infection were detected only in this treatment. Taken together, these results suggest that, upon infection, prior amendment with silicon to the roots causes plants not only to anticipate some common responses but also to trigger some unique ones.

Comparisons across PS treatments revealed other interesting features of the action of silicon. For instance, by comparing mock-inoculated plants, it became evident that, regardless of the mode of application, the responses of the plants treated with PS were very similar to those of the ones not supplied with the compound, the only exception being for POX in the foliar PS treatment. The activation of POX in this case could reflect stress related to the accumulation of salt in the epidermis. Thus, it is concluded that, as in other species such as tomato, wheat, *Arabidopsis* and rice (reviewed in Van Bockhaven *et al.*, 2013), silicon does not trigger the defence mechanisms in melon in the absence of the pathogen. By comparing pathogen-inoculated plants, however, it became apparent that silicon also promotes a stronger activation of some responses when supplied to the roots. This was observed for SOD, POX and GLU, where the activities of these enzymes were generally higher in the pathogen-inoculated plants of the root PS treatment compared to the –PS treatment.

The results of the current study indicated that silicon controls *P. xanthii* by different mechanisms depending on whether it is applied to the roots or to the leaves. When foliarly applied, PS did not induce significant alterations in the biochemical profile of plants when compared to control plants that did not receive PS. As Si did not accumulate in the leaf tissues, the antifungal action observed in this study could have resulted from changes in the ionic potential or pH on the leaf surface that interfered with conidial germination, as suggested previously (Liang *et al.*, 2005). In this case, as diverse salt sprays have a positive effect on disease control as well, it cannot be concluded that this mechanism is exclusive to Si (Guével *et al.*, 2007).

When applied to the roots, PS triggered responses that were anticipated or stronger than those observed in plants not supplied with PS. In addition, the compound induced responses that were not observed in the other treatments. As the concentrations of K in the leaf tissues did not differ among treatments, these effects can be attributed to Si. These effects, coupled with the little impact on the defence responses of mock-inoculated plants treated with Si, are hallmarks of the priming effect (Conrath *et al.*, 2006); hence, the results indicate that Si stimulates priming in melon plants when supplied to the roots. The decreased activity of CAT in the early stages of fungal infection, coupled with the stronger activity of SOD, most probably led to the accumulation of H_2O_2 at the infection sites, which contributed to reducing the number and size of the fungal colonies. The accumulation of H_2O_2 at the penetration sites and association with arrest of powdery mildew growth in plants supplied with Si through the roots has been reported in roses infected with *P. pannosa* (Shetty *et al.*, 2012). The modulated activities of POX, CHI and GLU probably also contributed to suppressing the fungal attack. POX is involved in protecting cells from oxidative stress, as well as in the reinforcement of the cell wall, through the use of H_2O_2 as a substrate for lignin polymerization (Hiraga *et al.*, 2001), which is consistent with the observed reduced sizes of the fungal colonies of this treatment. The protective role of POX could reflect the lower concentration of MDA compared to the –PS treatment, because this compound is a biomarker for oxidative stress. The activation of CHI and GLU, which are enzymes that degrade the cell wall of true fungi, and the early accumulation of total soluble phenols most probably also contributed to suppressing fungal attack. The accumulation of phenolic compounds as a result of supplying the roots with silicon has been reported in other pathosystems, such as cucumber–*P. xanthii*, rice–*Bipolaris oryzae*, wheat–*B. graminis* f. sp. *tritici*, *Arabidopsis*–*Erysiphe cichoracearum* and rose–*P. pannosa* (Menzies *et al.*, 1991; Bélanger *et al.*, 2003; Ghanmi *et al.*, 2004; Rodrigues *et al.*, 2005; Dallagnol *et al.*, 2011; Shetty *et al.*, 2011). In addition to the antimicrobial effects of these compounds, phenols can be oxidized by PPO, leading to the formation of quinones, which are more toxic to microorganisms than the phenols themselves (Mayer,

2006). However, the involvement of quinones in plant defence was not evident in the root PS treatment as there was no difference in PPO activity between the pathogen-inoculated and mock-inoculated plants.

As previously reported (Dallagnol *et al.*, 2012), PS was more effective in reducing the severity of *P. xanthii* in melon when applied to the roots. Thus, irrigation with PS is a viable control strategy of this pathogen, especially in Brazil where melon crops are grown mainly in semi-arid areas of the northeastern region and drip irrigation is recommended to maximize yields. Moreover, as irrigation with PS induces cell priming, additional gains might be expected from the control of other pests because this physiological state confers protection over a wide variety of biotic stresses (Conrath *et al.*, 2006).

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