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The Effect of Silicon on the Growth Traits and Resistance of Zinnia (*Zinnia elegans* Jacq.) to Powdery Mildew Disease

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Abstract

To investigate the effects of silicon on the growth characteristics and the reduction of the powdery mildew damage to Zinnia (*Zinnia elegans* Jacq. 'Magellan'), an experiment was carried out as a factorial experiment in the form of a randomized complete block design with two factors including eight treatments and three replications. The first factor included silicon concentrations at four levels (0, 50, 100, 150 mg L^{-1} Si) and the second factor consisted of the Si application methods (foliar spray and medium drenching). Depending on the concentration and method of silicon supplied, several horticultural traits were improved as a result of Si supplementation. Silicon content of Si-treated plants (100 and 150 mg L^{-1} Si foliar spray) significantly increased as compared with untreated controls. Disease severity of powdery mildew was reduced by sodium silicate. The results of our study demonstrated that foliar spray treatments were more effective in managing disease than drenches treatments.

Keywords: Powdery mildew, Silicon (Si), Sodium silicate, Zinnia (*Zinnia elegans* Jacq.).

INTRODUCTION

Silicon (Si) is the second most abundant element in soil. In soil solution, Si occurs mainly as monosilicic acid (H_4SiO_4) at concentrations ranging from 0.1 to 0.6 mM and is taken up by plants in this form (Epstein, 1994; Ma and Takahashi, 2002). Several beneficial effects of Si have been reported including increased photosynthetic activity, increased insect and disease resistance, reduced mineral toxicity, improvement of nutrient imbalance, and enhanced drought and frost tolerance (Ma, 2004). The ability of plants to accumulate Si varies widely between species. Plant species belonging to the families of Poaceae and Cyperaceae absorb Si at concentration levels which is equal to or greater than that of some essential nutrients like N and K (Savant *et al*., 1997). The difference in Si accumulation was attributed to the ability of the roots to take up Si. Although Si is abundant in soil, most plants especially dicots are unable to take up a large amount of Si from soil (Ma, 2004). *Zinnia elegans* Jacq. is a dicotyledon belonging to the Asteraceae family that accumulates silicon in relatively high concentrations as compared with other dicots (Frantz *et al*., 2008). The objective of this study was to evaluate the efficacy of Si for the management of powdery mildew and growth traits in zinnia.

MATERIALS AND METHODS

Zinnia elegans 'Magellan' seeds (Graines Voltz Company, France) were sown into plug trays using a cocopeat media under greenhouse conditions and transplanted to plastic pots (16 cm top diameter) when 4-6 true leaves were present. The media was prepared using cocopeat: perlite 2:1 (v/v) and fertilized weekly with 200 ml/pot (prepared by dissolving 2 grams in 1 liter of water) Rosasol® 18-18-18 (Rosier Company, Belgium).

The experiment was performed as a 4×2 factorial experiment in randomized complete block design with three replications, each of which had 10 flowers as experimental units. A week after transplanting seedlings to pots, sodium silicate (Na_2SiO_3) was applied weekly at the rates 0, 50, 100, 150 mg/l Si as medium drench (200 ml/ plant) and foliar spray (until runoff). The pH of the silicon solutions was adjusted to 6 with diluted HCl. Days to anthesis (first flower) from sowing the seed, height (measured from pot rim to the tallest point), first flower and lateral flowers diameter (measured at the widest point), number of lateral shoots, display life [flowers that showed a light yellowing, browning and dryness of ray florets petals (ligules) edges], stem diameter (basal stem), number of flowers, and fresh and dry weights of shoot and root were recorded for all flowers.

Powdery mildew developed from natural inoculum. Disease evaluations were made at 3-day intervals after the first appearance of powdery mildew symptoms (4 times). The disease severity was rated using a 0 to 4 scale (Lebeda, 1984), where 0= no powdery mildew present, 1= 1 to 25%, 2= 25 to 50%, 3= 50 to 75%, 4= 75 to 100% of upper leaf surface covered with powdery mildew (Fig.1). All leaves were rated for each plant and an average of disease calculated. Disease ratings were used to calculate the area under disease progress curve (AUDPC) for each treatment by Campbell and Madden (1990) formula as follows:

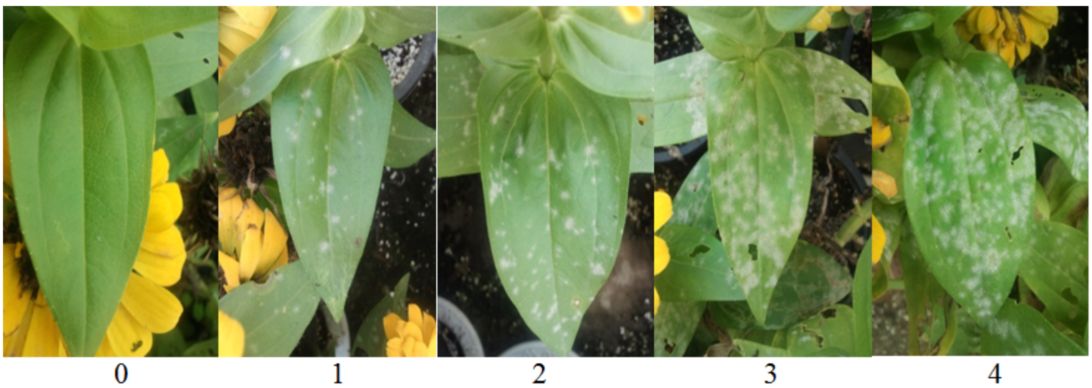

Fig. 1. A 0-4 scale was used for disease rating

$AUDPC = \sum_{i=1}^{n-1}[(y_i + y_{i+1})/2](t_{i+1} - t_i)$ where n is number of disease assessment times, y is the disease severity, and t is the time duration of the epidemic. Six leaves of upper, middle and lower zinnia plants were oven-dried at 70˚C for two days. Silicon content was determined by Elliott and Snyder's method (1991). Analysis of variance (ANOVA) was done using SAS statistical software package and means were compared using Tukey's multiple range test. Non-parametric Friedman rank test was used for the analysis of disease severity ratings.

RESULTS AND DISCUSSION

Effects of silicon on growth traits of zinnia flowers

A significant difference was found between the concentration of Si applied and the number of days to anthesis, first flower and lateral flowers diameters, flower life, the average number of flowers, and dry and fresh weight of roots ($P < 0.01$). Stem diameter, number of flowers, plant height, and shoot dry weight was not related to application methods. Likewise, the interaction between concentration and method of Si application was significant for the number of days to anthesis, first flower and lateral flowers diameters, flower life, number of lateral shoots, number of flowers, and fresh weight of roots and shoots (Table 1).

An promoted anthesis was observed when the media was drenched with 100 and 150 mg L^{-1} Si drenches (Table 2). It is unclear how these Si treatments promoted anthesis, but it may have been the result of an overall speed growth of the whole plant.

In our study, the highest number of flowers was obtained when plants were supplied with 150 mg L^{-1} Si drenches and 50 mg L^{-1} foliar sprays. The highest number of lateral shoots was observed in 150 mg L^{-1} Si drenches which was not significant with the control. Conversely, a decrease in number of lateral shoots was observed in plants sprayed weekly with sodium silicate (100 mg L^{-1} Si) (Table 2). One of the most significant factors stimulating lateral bud development is the cytokinin level in shoots. It was detected that silicon could increase the level of cytokinin in wheat plants (Hanafy Ahmed *et al*., 2008). Some of the Si treatments affected first and lateral flower diameter of zinnia as compared with the untreated control. First flower diameters were increased by 7.05 mm and 4.36 mm with weekly drenches of 100 and 150 mg L^{-1} Si, respectively. Lateral flower diameters were significantly increased in all treatments, except for foliar spray of 150 mg L^{-1} Si (Table 2). Similar results were also observed in other ornamental plant species (Kamenidou *et al.*, 2008, 2010; Mattson and Leatherwood, 2010; Sivanesan *et al*, 2013). Although not measured in our study, Si application has been found to reduce evapotraspiration (Lu and Cao, 2001; McAvoy and Bernard, 1996), which could have contributed to increased turgor pressure within the flower, resulting in cell swelling and thus larger flower diameters.

The highest display life of our zinnia flowers were obtained when plants were treated with silicon (100 and 150 mg L^{-1} Si drenches) (Table 2). Sevvas *et al*. (2002) stated that Si improved gerbera flower quality by providing mechanical strength to the stems. Hwang *et al*. (2005) also reported that the application of potassium silicate improved the growth and quality of cut flower miniature rose in rockwool culture system. The reason responsible for this might be the interaction of this element with cellular membrane, as Hodson and Sangster (1988) reported that the accumulated monosilicic acid was polymerized into polysilicic acid and then was transformed into amorphous silica, which formed a thickened silicon-cellulose membrane, by which a double cuticular layer protected and mechanically strengthened the plants. Si might also form complexes with organic compounds in the cell walls of epidermal cells, therefore increasing their resistance to degrading enzymes (Snyder *et al*., 2007).

The effects on growth characteristics in Si-treated plants may be due to altered levels of plant growth regulators in zinnia. Hwang *et al*. (2007) reported that Si application increased the average GA_1 and GA_{20} levels in rice cultivars. Thus, beneficial effects of Si may be due to altered

Table 1. Analysis of variance of experimental factors on the zinnia traits

S.o.V	df	Days to anthesis	First flower diam.	Lateral flowers diam.	Display life	No. of lateral shoot	Stem diam.	No. flower	Height	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root
Block	2	5.94^{ns}	0.02^{ns}	3.97^{ns}	0.62^{ns}	0.32^{ns}	0.05^{ns}	1.9^{ns}	18.7*	19.4^{ns}	1.48^{ns}	0.65^{ns}	0.67*
Concentration (C)	3	36.21**	6.82**	45.03**	37.7**	0.22^{ns}	0.2^{ns}	3.2**	6.8^{ns}	125.19^{ns}	22.05**	3.40^{ns}	1.08**
Method (M)	1	77.76**	92.43**	18.83**	11.7**	3.5**	0.37^{ns}	0.6^{ns}	0.09^{ns}	209.68*	26.22*	0.16^{ns}	1.19*
C×M	3	20.61*	25.40**	7.18*	5.7*	1.39**	0.1^{ns}	1.8*	4.7^{ns}	232.46*	16.04*	4.30^{ns}	0.40^{ns}
Error	14	3.74	0.90	1.30	1.25	0.12	0.10	0.51	3.9	43.25	3.2	2.24	0.13
CV (%)	-	2.61	1.29	1.60	3.99	5.54	4.17	6.67	7.05	7	13.5	12.79	21.21

ns: not significant,* significant at the 5% level probality, **significant at the 1% level probality, *** significant at the 0.1% level probality.

Table 2. The interaction of concentration and method of Si application for the zinnia traits

Concentration (mg L^{-1})	Method	Days to anthesis	First flower diam. (mm)	Lateral flowers diam. (mm)	Display life (day)	No. of lateral shoot	No. flower	Fresh weight of shoot (g)	Fresh weight of root (g)	Leaf Si concentration (mg kg^{-1})
0	Spray	77.43^{a}	72.03^{cd}	67.43^{c}	24.73^{c}	6.53^{abc}	9.60^{b}	87.15^{b}	11.05^{ab}	80.57^{c}
0	drench	77.43^{a}	72.03^{cd}	67.43^{c}	24.73^{a}	6.53^{abc}	9.60^{b}	87.15^{b}	11.05^{ab}	80.57^{c}
50	spray	74.10^{ab}	72.22^{bcd}	72.35^{ab}	27.66^{bc}	6.26^{abc}	11.73^{a}	100.63^{ab}	14.66^{ab}	102.63^{abc}
50	drench	73.03^{ab}	74.61^{abc}	72.18^{ab}	27.20^{bc}	6.13^{abc}	10.73^{ab}	93.66^{ab}	15.04^{ab}	90.98^{bc}
100	spray	74.50^{ab}	69.91^{d}	72.24^{ab}	28.20^{bc}	5.40^{c}	10.86^{ab}	90.72^{ab}	16.13^{a}	121.64^{a}
100	drench	69.06^{b}	79.08^{a}	75.31^{a}	30.60^{ab}	7.06^{ab}	11.53^{ab}	99.42^{ab}	14.13^{ab}	88.45^{bc}
150	spray	76.83^{a}	71.25^{cd}	69.93^{bc}	28.60^{abc}	5.80^{bc}	10.06^{ab}	85.22^{b}	16.13^{a}	114^{ab}
150	drench	68.93^{b}	76.39^{ab}	74.11^{a}	32.26^{a}	7.30^{a}	11.73^{a}	107.14^{a}	9.39b	92.47^{bc}

* In each column, values with same letter(s) are not significantly different at the 5% probability level, according to Duncan Multiple Range Test

Fig. 2. Flower deformation observed in zinnia supplemented with weekly Na_2SiO_3 foliar sprays of 150 mg L^{-1} Si.

endogenous level of GA. However, further studies are required to confirm this.

Deformed flower heads were also observed in plants supplemented with weekly Na_2SiO_3- spray of 100 and 150 mg L^{-1} Si (Fig. 2). The flower deformations may have been the result of Si-treated mechanical strength, which decreased the plasticity of the cell walls of the floral meristems, resulting in uneven tissue expansion. The indirect effects of Si supplements on soil pH and other plant nutrients were investigated as potential causes of these symptoms. Because Si concentrations vary in solubility with concentration of OH^-, and consequently with pH, uniform pH was maintained across the treatments. The treatments pH values were adjusted to 6, indicating that the observed flowering abnormalities in the silicon foliar sprays were not likely to have been the result of a pH imbalance caused by Si supplementation.

Several combinations of Si method and concentration increased fresh weight of shoots and roots (Table 2). These results are in agreement with previous findings (Moon *et al*., 2008). As noted previously, reduced evapotranspiration may have contributed to the increased cell turgor pressure.

Effects of silicon on powdery mildew development in zinnia plants

Disease severity and AUDPC were related to the concentration of Si application at all evaluation dates except the first symptoms observing of date. Likewise, disease severity at all times and AUDPC were related to application methods. Interaction between concentration and Si application method was not significant for disease severity and AUDPC (Table 3). All sodium silicate treatmentsreduced disease significantly in comparison to the control plants (Table 4). In our study, the best method of Si application for controlling powdery mildew was foliar spray (Table 5).

Foliar and root applications of Si reduced the number of colonies of powdery mildew developing in cucurbits such as cucumber, muskmelon and zucchini squash (Menzies *et al*., 1992).

Table 3. Analysis of variance of the experimental factors on development of powdery mildew disease and leaf Si concentration in zinnia

S.o.V	df	Disease ratings in 0, 3, 6, 9 days after the first appearance of powdery mildew symptomsa				AUDPC	Leaf Si concentration
		0	3	6	9		
Block	2	0.11^{ns}	0.02^{ns}	0.03^{ns}	0.01^{ns}	1.2^{ns}	8.30^{ns}
Concentration (C)	3	0.14^{ns}	0.19**	0.26**	1.07**	24.2**	744.3**
Method (M)	1	0.56*	0.18*	0.34*	0.77**	30.03**	1651.86**
C×M	3	0.19^{ns}	0.02^{ns}	0.13^{ns}	0.17^{ns}	6.7^{ns}	299.76*
Error	14	0.07	0.02	0.04	0.06	2.01	81.73
CV (%)	-	34.18	10.67	10.92	9.57	9.43	9.37

ns: not significant,* significant at the 5% level probality, **significant at the 1% level probality, *** significant at the 0.1% level probality

Table 4. Simple effect of Si concentrations on dry weight of root and the development of powdery mildew disease in zinnia flowers

Concentration (mg L^{-1})	Dry weight of root (g)	Disease ratings in 0, 3, 6, 9 days after the first appearance of powdery mildew symptoms				AUDPC
		0	3	6	9	
0	2.13^{a}	1^{a}	1.68^{a}	2.21^{a}	3.14^{a}	17.88^{a}
50	2.06^{a}	0.89^{a}	1.37^{b}	$1.86a^{b}$	2.60^{b}	14.95^{b}
100	$1.54a^{b}$	0.72^{a}	1.34^{b}	1.78^{b}	2.27^{b}	13.88^{b}
150	1.28^{b}	0.66^{a}	1.27^{b}	1.74^{b}	2.22^{b}	13.39^{b}

* In each column, values with same letter(s) are not significantly different at the 5% probability level, according to Duncan Multiple Range Test

Table 5. Simple effect of methods Si applied on dry weight of root and development powdery mildew disease of zinnia flowers

Method	Dry weight of root (g)	Disease ratings in 0, 3, 6, 9 days after the first appearance of powdery mildew symptoms				AUDPC
		0	3	6	9	
Spray	1.97^{a}	0.66^{b}	1.33^{b}	1.78^{b}	2.38^{b}	13.91^{b}
Drench	1.52^{b}	0.97^{a}	1.50^{a}	2.02^{a}	2.74^{a}	16.15^{a}

* In each column, values with same letter are not significantly different at the 5% probability level, according to Duncan Multiple Range Test

Powdery mildew colony number in grape leaves was reduced to 11% of the control leaves when foliar Si sprays were used (Bowen *et al.*, 1992). Powdery mildew development in *Arabidopsis thaliana* was observed rarely when plants were watered with a nutrient solution containing soluble Si (Ghanmi *et al*., 2004). Application of Si to soil or hydroponic cultivation resulted in suppression of powdery mildew in the highly susceptible 'Toyonoka' strawberry cultivar (Kanto *et al*., 2004; 2006). Belanger *et al*. (2003) found that Si amendments to the soil mix or added to the nutrient solution protected wheat from powdery mildew.

The protective role of Si has been attributed to the accumulation of Si in the leaves, which creates a physical barrier to pathogens (Adatia and Besford, 1986; Samuels *et al.*, 1991a, 1991b). Alternatively, Si may have a more active role by inducing the plant's own defense mechanisms (Fauteux *et al.*, 2006; Remus-Borel *et al.*, 2005; Rodrigues *et al*., 2004; 2005). For instance, Fawe *et al*. (1998) demonstrated that the addition of Si to cucumber plants enhanced resistance to powdery mildew by increasing antifungal activity in the plant. Similarly, Liang *et al*. (2005) and Rodrigues *et al.* (2005) found that Si enhanced the activity of pathogenesis-related proteins and thus increased resistance to pathogen attack on cucumber and rice plants, respectively.

Evaluation of silicon accumulation

Many of the beneficial effects of Si on agricultural crops are associated with silica gel deposition on leaves and stems, resulting in reduced transpiration and increased stem strength (Ma *et al.,* 2001). Similarly, Si uptake and deposition in zinnia tissue may be responsible for the improved quality observed in some of the Si treatments of this study. Thus, the Si concentrations deposited in leaves were determined.

Si concentrations and methods of application had a significant effect on silicon content of leaves ($P < 0.01$) and also the interaction of Si concentration and application method was significant ($P < 0.05$) (Table 3). The highest Si content in leaves was observed in 100 and 150 mg L^{-1} Si as they showed significant differences with control (Table 2). However, treatments increasing leaf Si concentrations above 102.63 mg kg^{-1} (100 and 150 mg L^{-1} Si foliar sprays) were associated with flower deformations, resulting in unmarketable flowers.

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