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Silicon Application on Standard Chrysanthemum Alleviates Damages Induced by Disease and Aphid Insect

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Abstract. To elucidate the role of silicon in biotic stress such as pests and diseases, standard chrysanthemum was grown in pots filled with soil without application of pesticide and fungicide. Si treatment was largely composed of three groups: K_2SiO_3 (50, 100, and 200 $mg \cdot L^{-1}$), three brands of silicate fertilizer (SiF1, SiF2, and SiF3) and tap water as a control. Si sources were constantly drenched into pots for 14 weeks. Application high concentration K_2SiO_3 (200 $mg \cdot L^{-1}$) and three commercial Si fertilizers for 14 weeks improved growth parameters such as plant height and the number of leaves. In the assessment of disease after 4 weeks of Si treatment, percentage of infected leaves was not significantly different from that of control. After 14 weeks of Si treatment, however, the infected leaves were significantly reduced with a 20-50% decrease in high concentration (200 $mg \cdot L^{-1}$) of potassium silicate and all commercial silicate fertilizers. Colonies of aphid insect (*Macrosiphoniellas anborni*) were also reduced in Si-treated chrysanthemum, showing 40-57% lower than those of control plants. Accumulation of silicon (approximately 5.4-7.1 $mg \cdot g^{-1}$ dry weight) in shoots of the plants was higher in Si-supplemented chrysanthemum compared to control plants (3.3 $mg \cdot g^{-1}$ dry weight). These results indicate that using potassium silicate or silicate fertilizer may be a useful for management of disease and aphid insect in soil-cultivated chrysanthemum.

***Additional key words*:** beneficial effects, biotic stress, potassium silicate, silicate fertilizer, soil cultivations

Introduction

Silicon (Si) is one of the most beneficial elements for several plants although it is not considered as an essential plant nutrient. Silicon deposits in the leaves, stems, and hulls in the form of amorphous silica gel ($SiO_2 \cdot nH_2O$) and soluble silicic acid ($SiOH_4$) (Epstein, 1999). In monocotyledon, several plants were demonstrated to be improved in production, disease resistance and abiotic stress tolerance by Si supplement (Ma et al., 2001; Savant et al., 1996). Especially, rice (*Oryza sativa* L.) is well known to most effectively accumulate Si due to the presence of a transporter for xylem loading (Ma and Yamaji, 2008). Accordingly, high concentration of silicon in rice tissues contributes to enhance resistance to disease (Cai et al., 2008), reduce sodium uptake under salt stress (Gong et al., 2006), and reduces the toxicity of heavy metals (Zhang et al., 2008). Today, silicon fertilization has become a routine practice in rice production. Also, the beneficial effects of Si on plant growth, biotic and abiotic stresses have been observed in a wide variety of plant species (Ma et al., 2001; Richmond and Sussman, 2003).

Greenhouse production of floricultural crops mainly uses soilless substrates, which have limited amounts of plant-available Si compared to mineral soils (Voogta and Sonnevelda, 2001). With the change to soilless growing media in the horticultural industry, the role of Si in horticultural crops became apparent. Generally, hydroponics using water or soilless substrates has been used as a valuable technique for the studies on the effect of Si on plant, which makes it possible to produce silicon-deprived plants because they contain little soluble Si. Thus, previous researches on Si supplement in floricultural crops have focused on water solution or soilless substrates. Floricultural crops in soilless substrates have exhibited a variety of responses related to the morphological traits depending on Si source and rate supplemented, and plant species (Kamenidou et al., 2008, 2009; Mattson and Leatherwood, 2010). The inclusion of Si supplements has proven beneficial effects for several crops

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like tomato (Diogo and Wydra, 2007), rose (*Rosa hybrida* 'Meipelta') (Gillman et al., 2003) and chrysanthemum ('Backwang') (Moon et al., 2008). Meanwhile in the studies related to species from dicots, Si supplement particularly in soil has not been recommended with focused on the technique supplying Si under the silicon-deprived condition. Effects of Si on floricultural crops grown in soil also need to be confirmed because some floricultural crops are actually cultivated in soil system supplied with conventional nutrition solution. Chrysanthemum is commonly cultivated in soil, and thus the study on such potential of Si as a supplement or integrated pest management (IPM) tool is required.

To elucidate the role of silicon in soil cultivation, standard chrysanthemum (*Dendranthema grandiflorum* Kitamura cv. Shinro, bred in Republic of Korea) was obtained from vegetative cuttings and grown in pots without application of pesticide and fungicide during the period of cultivation. The present study was to evaluate the effect of Si supplement on growth trait and biotic stresses (disease and aphid insect) in the soil system of greenhouse. The goals of this study were to evaluate the effects of different Si sources and concentrations on the growth traits and biotic stresses, with estimation of capacity accumulating Si in chrysanthemum.

Materials and Methods

Plant Material and Si Supplement

The rooted cuttings of *Dendranthema grandiflorum* cv. Shinro (obtained from chrysanthemum farm in Changwon-si) were transplanted into round pots (12 cm in diameter) containing soil (upland soil:leaf mold:river sand = 3:3:4, v/v/v) and granule fertilizer (3 g/pot) (Green Coat, DHC Co., Ltd., Korea) (on 18 June, 2010), and grown with weekly application (200 mL/pot) of 1,000 time diluted standard nutrient solution (Hyponex, HYPONEX, Japan) in a greenhouse under ambient light and temperature. Total 7 treatments were prepared to investigate Si supplemental effect on chrysanthemum (Table 1). Potassium silicate (K_2SiO_3) and commercial Si fertilizers were used as Si source: three levels of concentration (14, 28, or 56 mg·L^{-1} Si) of K_2SiO_3 and three silicate fertilizers of commercial products such as SiF1 (Kyunsantech®, Taehung F&G Co., Ltd., Korea), SiF2 (Silamol®, HUBAS Korea, Korea), and SiF3 (Kunson®, Saturn Biotech. Co., Ltd., Korea). Commercial Si fertilizers were supplied with the concentration recommended by an instruction of each product (Table 1). Si drench (200 mL/pot) was started 7 days after transplanting and continued weekly until August, 2010. During 2 months of Sep. to Oct. 2010, Si drenches were conducted biweekly. Chrysanthemum plants received total 12 Si-drenches during the experiment.

Data Collection and Analysis

Recording plant height and the number of leaves was performed at 4 and 14 weeks after beginning of Si drench. The assessment of leaf disease naturally infected was evaluated as percentage of infected leaves by counting the number of total leaves and infected leaves at 4 and 14 weeks after Si treatment. Statistical analyses were conducted for 20 plants per treatment, which was randomly selected from three blocks. To estimate effect of Si on aphid insect resistance of chrysanthemum, in the middle of November, 2010 when chrysanthemum was naturally infested with

Table 1. Si sources and total Si used in the experiment and their abbreviations in Figs.

Si sources			Supplemented Si			Abbreviation
Type: solution	Concn.	Si content (%)	Concn. of Si (mg·L^{-1})	mg Si/pot per drench	Total mg Si	
Potassium silicate (mg·L^{-1})						
	50		14	2.8	33.6	Si14
K_2SiO_3	100	28.0^z	28	5.6	67.2	Si28
	200		56	11.2	134.4	Si56
Commercial Si fertilizer [% (v/v)]						
Kyusantech®	0.10	18.0	180^x	36.0	432.0	SiF1
Silamol®	0.02	2.5^y	5	1.0	12.0	SiF2
Si22®	0.03	26.0^z	78	15.6	187.2	SiF3
Control						
Tap water				None		TW

^{z}Means percentage of SiO_2 in the solution of Si source.
^{y}Means percentage of SiO_4 in the solution of Si source.
^{x}Calculated under assumption that 1 mL of fertilizer solution is equal to 1 g of that.

aphids (*Macrosiphoniellas anborni*), total number of aphid colonies was counted from 10 plants per treatment in the experiment designed to three blocks. Statistical analysis with SPSS (Ver. 12.0, SPSS Inc., IL, U.S.A.) was subjected to one-way analysis variance (ANOVA) followed by Duncan's multiple range test at $P \leq 0.05$. Effect of interaction between aphid population and block was estimated by two-way ANOVA.

Si analysis in plant tissue was conducted with shoots collected at 20 weeks after commencing Si treatment. The silicon concentration was determined by the colorimetric molybdenum blue method as described by Elliot and Snyder (1991). Data from each Si treatment were statistically analyzed from three blocks (n = 3).

Results and Discussion

Effect of Si Application on Plant Growth: The Number of Leaves and Plant Height of Chrysanthemum

Si treatment using potassium silicate and different Si fertilizers were evaluated to determine which ones showed a beneficial effect on the growth of the chrysanthemum. Weekly drenches of potassium silicate and three silicate fertilizers for 14 weeks produced a significant difference in the number of leaves of chrysanthemum plants compared to that of control (Fig. 1A). Drenches with potassium silicate of 200 $mg \cdot L^{-1}$ (56 $mg \cdot L^{-1}$ Si) and three kinds of Si fertilizer (SiF1, SiF2, and SiF3) increased plant height (+ 20-23%) as well as the number of leaves (+ 7-20%). However, the plant growth was not affected by low concentration of K_2SiO_3 (14 $mg \cdot L^{-1}$ Si) (Figs. 1B, C, and D). During the short period of Si supplement (4 weeks), significant differences in the number of leaves were not observed. Weekly supply of 28 $mg \cdot L^{-1}$ Si (total 67 mg Si, Table 1) started improving the plant height (Table 1, Fig. 1B, and C). Three Si fertilizers showed the responses comparable to high concentration of K_2SiO_3 (56 $mg \cdot L^{-1}$ Si) in plant height, but no difference among the Si fertilizers although total amount of Si in those fertilizers are quite different (Table 1 and Fig 1C). Unlike morphological trait in the plant height, the number of leaves

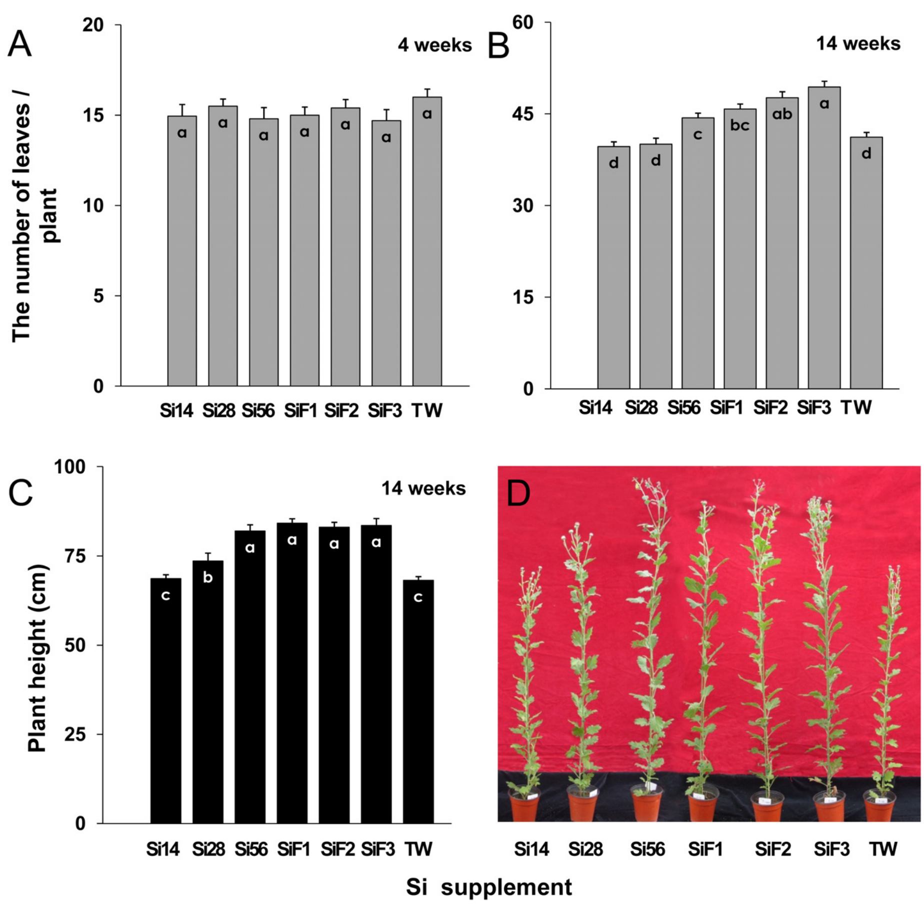

Fig. 1. The number of leaves per pot plant (A and B) and plant height (C) of chrysanthemums grown in soil pots for 4 weeks (A) and 14 weeks (B, C, and D) after commencing Si amendment (on 24 June, 2010). Potassium silicate and commercial Si fertilizer were used as Si source: Si supplement abbreviations as in Table 1. Values are the means ± SE of 20 pots/treatment randomly selected from 3 blocks. Means with different letters are significantly different ($p < 0.05$ by Duncan's multiple range test).

between them showed significant difference (Fig. 1B). It is likely that Si can be differentially absorbed by the plant depending on Si form supplemented (Kamenidou et al., 2009; Ma and Takahashi, 2002), or other ingredients of the fertilizers could influence on the growth traits or Si uptake of roots (Paik, 1975).

The effect of Si treatment on the growth traits were reported to vary in plant species and growth parameter measured. Si supplementation of new guinea impatiens and lobelia resulted in increased plant height (Mattson and Leatherwood, 2010), whereas *Bracteantha bracteata* (Vent.) Anderb. & Haegi and zinnia had a reduced height (Kamenidou et al., 2009; Mattson and Leatherwood, 2010). Here, standard chrysanthemum 'Shinro' showed a positive response of maximally 20% higher plant height than that of control plant. Plant height and stem diameter in chrysanthemum 'Backwang' grown with silicate in medium, were greater than those in the control (Moon et al., 2008). Chrysanthemum 'Baekma' grown in medium was also reported to be increased in diameter, strength and hardness of the stem by foliar treatment of Si (Choi et al., 2009).

Effects of Si Supplement on Biotic Stresses in Chrysanthemum 'Shinro'

The effect of Si on disease tolerance in chrysanthemum was determined by counting the total and infected leaves. Four weeks after Si treatment, most of the chrysanthemum showed visual symptoms of infection to white rust (*Puccinia horiana* Henn) on leaves, but significant difference was not observed as compared to control plant (Fig. 2A). Around the end of growth stage (14 weeks after Si treatment), the predominant lesions, naturally formed on the leaves of chrysanthemum, were not due to white rust but other disease considered as leaf rot, brown leaf spot and soft rot. Incidence of white rust stayed under the 5% level. Percent infected leaves were obtained from leaves with such lesions as shown in Fig. 2B. Excluding the lowest rate of Si (Si14), all the other rates of K_2SiO_3 and Si fertilizers decreased the rate of infected leaves (Fig. 2B). K_2SiO_3 with 28 or 56 mg·L^{-1} Si resulted in 17% to 20% decrease compared to that of control plants, and all brands of fertilizer, SiF1, SiF2, and SiF3, had a more decrease than K_2SiO_3 (-44~52%). Disease tolerance was significantly increased with Si concentration of K_2SiO_3. Commercial silicate fertilizers were more effective in blocking the occurrence of leaf disease than potassium silicate. It is possible to explain this difference observed between potassium silicate and silicate fertilizers that additional nutrients, exist in the commercial fertilizers, may cooperate with Si in increasing the tolerance to disease. With periodical Si supplement for 4 weeks there was no beneficial effect

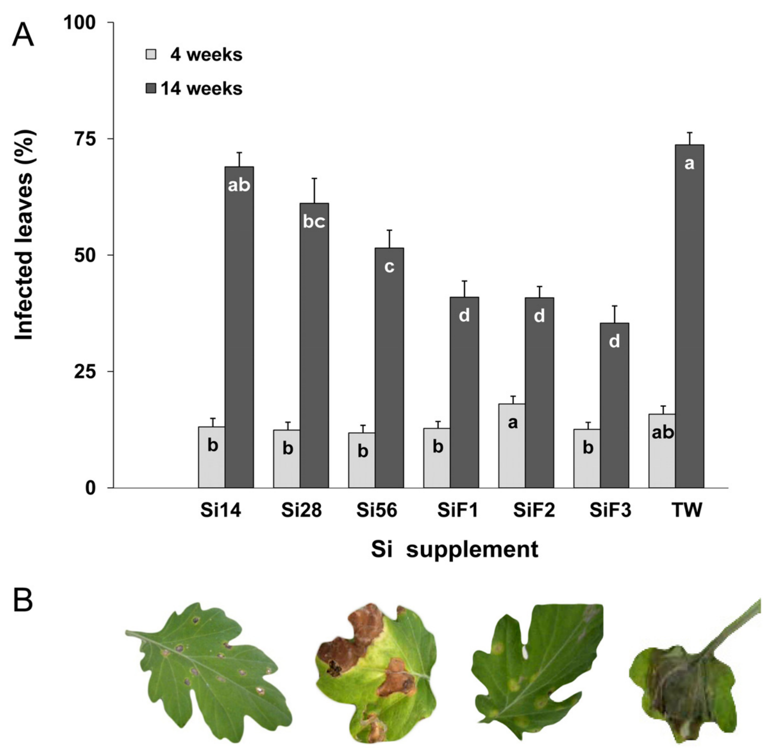

Fig. 2. Infected leaves (%) of chrysanthemum per pot at 4 weeks and 14 weeks after commencing Si treatment (A). Lesions of leaves infected naturally after 14 weeks of treatment: symptoms due to disease that were considered as black leaf spot, leaf rot, white rust and soft rot from the left (B). Values are the means ± SE of 20 pots/treatment randomly selected from 3 blocks.

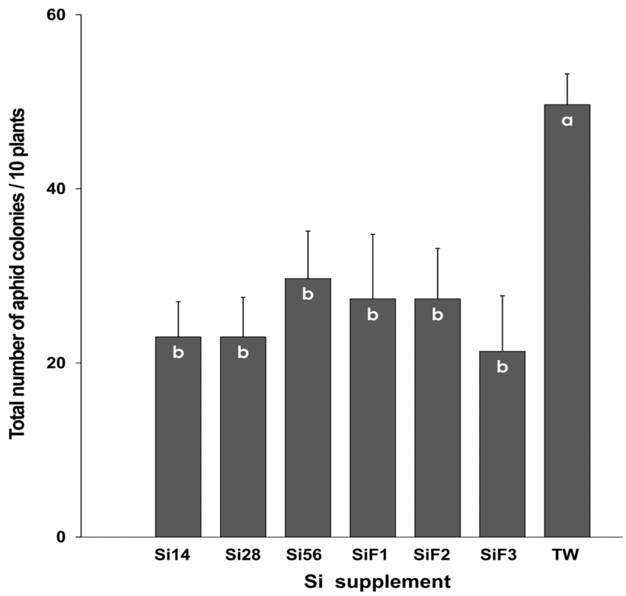

Fig. 3. Populations of chrysanthemum aphids per 10 pot plants at black cluster 20 weeks after commencing Si treatment (on 5 Nov. 2010) resulting from constant Si amendment for 14 weeks. Vertical bars represent standard errors from 3 blocks (n = 3).

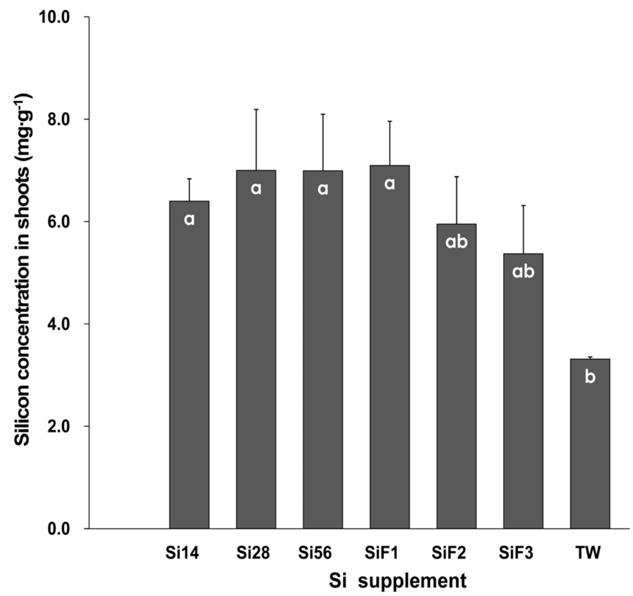

Fig. 4. Silicon concentration in shoots of Si-supplemented plants or Si-unsupplemented plants (control, tap water). Supplemented chrysanthemum received 12 drenches from potassium silicate or commercial Si fertilizer; shoots of the plants were collected 20 weeks after commencing Si supplement to determine Si content by the colorimetric molybdenum blue method. Each bar represents a mean of 3 replicates.

on the growth traits and disease tolerance. But as the supplement was lasted for 14 weeks, Si supplement had positive effects on the growth and disease tolerance of chrysanthemum. The results support that Si should be accumulated in chrysanthemum tissues at a high level enough to have such effects (Ma and Yamaji, 2006).

Additionally, all Si treatments significantly reduced the population of aphids with 40-57% lower colonies than that of untreated chrysanthemum, but significant difference between all Si sources was not observed in suppressing the population of aphids (Fig. 3). Statistic analysis resulted in having no effect of interaction between population of aphid and block (aphid population × block, p = 0.19). This excludes the possibility the other environmental factor with Si supplement can affect aphid population in the experiment. Also, any detrimental effects caused by Si supplement were not observed in chrysanthemum plants for the entire period of experiment.

Si content in shoots of chrysanthemum was increased with Si supplement; Si contents of Si-supplemented plants ranged from 5.4 $mg \cdot g^{-1}$ to 7.1 $mg \cdot g^{-1}$, which were 62% to 114% higher than that of control plant (Fig. 4). However, there was no significant difference between different Si sources or total amount of Si supplemented. These contents indicate Si uptake ability and deposition capacity of chrysanthemum since plant silica is deposited in any plant part and, once deposited, is not relocated (Ma and Yamaji, 2006). There was an inverse relationship between Si content of the plant shoots (Fig. 4) and population of aphid on the plants (Fig. 3). This correlation supports that the beneficial effects of Si are directly associated with Si accumulated in tissues of chrysanthemum shoots. Plants differ greatly in their ability to accumulate Si, ranging from 0.1% to 10.0% of dry weight (Ma and Takahashi, 2002), and particularly dicots are unable to accumulate high levels of Si in their shoots (0.5-4.5 $mg \cdot g^{-1}$ DW) (Mattson and Leatherwood, 2010). Comparing our results with these, Si content in chrysanthemum shoots (5.4-7.1 $mg \cdot g^{-1}$ DW) demonstrated that the plant has a high capacity to accumulate Si in the tissues. This high deposition could help chrysanthemum have positive effects of Si supplement on biotic stress of chrysanthemum even in soil cultivation. Si deposition in shoots was not directly related to total amount of Si supplemented (Table 1). Form of Si supplemented varies with Si sources, and ability of Si uptake can be different depending on their Si forms. This possibility may be involved in the previous report that the beneficial effects of Si supplement depend on the form of Si used in zinnia (Kamenidou et al., 2009). With 12 drenches for Si supplement, chrysanthemum accumulated Si in the tissues at the same level regardless of rate of potassium silicate supplied. This may have resulted from a chance that maximally deposited the inside of the plant, Si is not adsorbed by chrysanthemum plant any more.

In this study, such a high Si accumulation in the tissues resulted in enhancing the resistance to disease and insect pest (aphids) in chrysanthemum plants. Although these beneficial effects on biotic stress have been reported in many crops, rice (Nakata et al., 2008), zucchini (Savvas et al., 2009), oriental melon (Buttaro et al., 2009), arabidopsis (Fauteux et al., 2006) and cut rose (Fauteux et al., 2005), including chrysanthemum (Parrella et al., 2007), this may be the first report on biotic stress for floriculture crop grown in soil.

Recently, it has been reported that many floricultural crops exhibited differentials in morphological response and Si accumulation (Mattson and Leatherwood, 2010) depending on plant species or cultivar. In soil cultivation, silicon supply may lead to unaffected or distorted results in growth or responses to environmental stress (Epstein, 1994). However, chrysanthemum accumulated Si in the plant tissues enough to give positive responses even in soil cultivation according to this experimental result. In addition, this result suggests that using potassium silicate or silicate fertilizer in soil-cultivated chrysanthemum may be ecologically useful tool in the IPM program to improve resistance to disease and pest insect.

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