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Effect of Silicate Fertilizer on Growth, Physiology and Abiotic Stress Tolerance of Chinese Cabbage Seedlings

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Abstract. The objective of this study was to evaluate the effect of silicate fertilizer on growth, physiology and abiotic stress tolerance of Chinese cabbage seedlings. Five silicate concentrations (8, 16, 32, 64, and 128mM) and control (non-treatment) were applied to Chinese cabbage seedlings twice a week. Three weeks after application of silicate treatment, seedlings were used for treating abiotic stresses and were assessed for growth and physiological characteristics. Growth parameters significantly increased in 8, 16, and 32mM treatments except 64 and 128mM. Total root surface area, total root length, and number of root tips increased in 8, 16 and 32mM treatments, but they decreased in treated seedlings with 64 and 128mM of silicate. The highest growth parameters and root morphology were observed in 8mM treatment. As for the effect on the seedling physiology, transpiration rates decreased while stomatal diffusive resistance increased to increasing silicate concentration. The application of silicate reduced the electrical conductivity, heating and chilling injury index at high and low temperatures. Silicate enhanced drought tolerance of Chinese seedlings by delaying the starting time of wilting point. The starting time of wilting point in the control was 3 days after discontinuation of irrigation, while in the 8, 64 and 128mM of silicate treatments were 4 days, and the 16 and 32mM treatments were 5 days. All plants were wilted after 5 days in control without irrigation whereas it showed in 8mM treatment after 6 days, in 16, 32, 64, 128mM treatments after 7 days.

Additional key words: growth characteristics, ion leakage, stomatal diffusive resistance, transpiration rate, root morphology.

Introduction

Although silicon is the second most abundant element both on the surface of the Earth's crust and in soils, but it has not yet been listed among the essential elements for higher plants. However, the beneficial role of silicon in stimulating the growth and development of many plant species has been generally recognized (Epstein, 1999; Liang, 1999; Ma, 2004; Liang et al., 2005). Silicon is also known to effectively mitigate various biotic and abiotic stresses. Silicon enhances resistance of plants to diseases and suppresses insect pests (Seebold et al., 2001; Fauteux et al., 2005). Silicon plays an important role in alleviating various abiotic stresses such as salt stress, metal toxicity, drought stress, radiation damage, nutrient imbalance, high temperature, freezing (Ma, 2004).

Chinese cabbage is one of the most important vegetable crops with world-wide production (Lee et al., 2010). In Korea its estimate annual production was 2.93 millions of tons (Cheigh and Park, 1994). Chinese cabbage is using as major raw material for Kimchi as well as preparing various foods. Chinese cabbage can be grown year-round by using the different varieties. Unfortunately, many environmental factors affect plant growth and development. Plants are able to modify their growth and physiology according to surrounding environment. The ability of plants to do this plays a key role in determining their tolerance to stress and their maintaining efficient growth (Murchie and Horton, 1997; Walters et al., 2003). Therefore, this study was conducted to investigate the effect of silicate fertilizer on growth, physiology and abiotic stresses tolerance of Chinese cabbage seedlings.

Materials and Methods

1. Plant material and growing conditions

Chinese cabbage (*Brassica campestris* L.spp. *Pekinensis* Pupr.) seeds 'Asia Alpine F1' (Asia seed Co., Ltd., Korea) were sown in the 128-cell plug trays (Bumnong. Co., Ltd, Korea) that were filled with commercial growing substrate

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(BM 2, Berger Group Ltd, Canada). Twenty-five days after sowing, the seedlings were transplanted to plastic pots (with top and bottom diameters of 10cm and 6.5cm, depth of 9.5cm and 8 bottom perforation) which filled with the commercial growing substrate. One week after sowing, seedlings were fertilized at overhead irrigation twice a week with Wonder Grow fertilizers (Chobi Co., Ltd, Korea). The experiment was carried out in a glasshouse at Kangwon National University from July to September 2014.

2. Silicate application and abiotic stress treatment

Effect of silicate concentrations on growth and physiology of Chinese cabbage seedlings was studied. Five silicate concentrations (8, 16, 32, 64, and 128mM, as 'Keunson' silicate acid fertilizer (Saturn Bio Tech Co., Korea) with the control (non-treatment) were applied to Chinese cabbage seedlings twice a week for 3 weeks. After the end of last treatment, seedlings were evaluated for physiology and growth characteristics. For abiotic stresses, seedlings were treated with high (50±1^{o}C) and low (0±1^{o}C) temperature. One day after the imposition of high and low temperatures, the leaf of cabbage seedlings were cut for analysis of the cell membrane integrity estimated by relative electrolyte leakage. Three days after imposition of stresses, seedlings were estimated heating and chilling injury index. On the other hand, for drought stress, twenty pots per treatment were irrigated with full water before transfer to the growth chamber, and then the irrigation was withheld until temporary wilting point was observed. Growth chamber conditions: relative humidity was maintained in 40-50%; light intensity was approximately 100µmol·m^{-2}·s^{-1} provided by fluorescent lamps; temperature were set up 25±1^{o}C.

3. Data collection and analysis

The number of leaf, leaf area, and leaf chlorophyll content were measured, leaf area (cm^2) was measured by leaf area meter (Area meter, Delta-T, UK), leaf chlorophyll content was measured by using a chlorophyll meter (Minolta, SPAD-502, Japan). Fresh and dry weights of shoot and root were measured. Dry weight of shoot and root were taken through oven-dry method at 80^{o}C for 72 hrs.

For the root morphology, the WinRHIZO Pro 2009c (Regent Instruments, Inc, Quebec, Canada) images analysis system was used, coupled with professional scanner Epson 10000XL (Seiko Epson Corporation, Nagano, Japan) according to Arsenault et al. (1995). The roots were detached from shoots and then placed in a tray (30x15x2 cm) with water and placed on the scanner. Scanned images were analyzed by the WinRHIZO program for the total root surface area, total root length, average root diameter, and number of root tips.

For physiological characteristics, stomatal diffusive resistance and transpiration rate were assessed with an LI-1600, steady state porometer (LIP) (LI-COR, Lincoln, Nebraska, USA) at 4th leaf from the top of 5 plants of each treatment. Data were collected between 11-13 hrs.

Relative ion leakage was also assessed by the electrolytes leakage from the leaf of ten plants for each treatment with similar sizes. Leakage of electrolytes was determined using conductivity meter (Mettle Toledo AG). The leaf segments (disks of leaves with d = 1 cm) were strictly washed, blotted dry, weighted and put in stopped vials filled with the exact volume of deionized water. The vials were then incubated for 2 hrs in the dark with continuously shaking and were then measured conduction (C_1). The vials were heated at 80^{o}C for 2 hrs and were measured conduction again (C_2). The electrolyte leakage was expressed as a percentage of relative ion leakage, which was calculated according to the following equation (Zhao et al., 2007): Relative electrolyte leakage (%) = $C_1/C_2 \times 100$.

Chilling and heating injury was assessed after treating 3 days at low and high temperatures, using the following scale: (1) 0~20% of leaf area damage; (2) 21~40% of leaf area damage, (3) 41~60% leaf area damage, (4) 61~80% of leaf area damage, and (5) 81~100% of leaf area damage.

Percentage of wilted plant in drought stress were calculated when 75% of leaves per seedling withered.

The experiment was arranged in completely randomized design. For the statistical analysis of growth and physiology parameters, ten seedlings per treatment were randomly selected. Data were analyzed using SAS v.9.3 software (SAS Institute Inc., Cary, NC, USA). Mean separations were calculated using Duncan's multiple range tests at $P \leq 0.05$.

Results

1. Effect of silicate on growth and physiology characteristics of Chinese cabbage seedlings

Plant growth parameter responses to different concentrations of silicate are given in Table 1. Compared to control, the growth parameters such as number of leaves, leaf area

Table 1. Effect of silicate concentrations on growth characteristics of Chinese cabbage seedlings.

Silicate concentrations (mM)	Number of leaf	Leaf chlorophyll value (SPAD)	Leaf area (cm^2)	Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root
Control	11.2 b[z]	26.8 c	422.3 b	22.65 c	1.18 c	1.42 b	0.15 b
8	12.3 a	29.0 b	477.0 a	26.38 a	1.30 b	1.61 a	0.17 a
16	11.5 b	30.2 b	449.3 b	24.85 b	1.50 a	1.45 b	0.17 a
32	11.3 b	29.6 b	433.8 b	23.53 b	1.47 a	1.46 b	0.17 a
64	11.3 b	30.5 b	419.0 b	22.18 c	1.12 c	1.44 b	0.15 b
128	10.3 c	32.0 a	375.0 c	18.89 d	1.03 d	1.20 c	0.14 c

[z]Mean separation within columns by Duncan's multiple range test at P = 0.05.

Table 2. Effect of silicate concentrations on root morphology of Chinese cabbage seedlings.

Silicate concentrations (mM)	Total root area (cm^2)	Total root length (cm)	Average root diameter (mm)	Number of root tips
Control	92.8 c[z]	446.2 cd	0.67 ab	2575.5 bc
8	112.2 a	535.5 a	0.71 a	3141.3 a
16	108.0 ab	509.1 ab	0.69 ab	2866.5 ab
32	100.3 bc	472.5 bc	0.67 ab	2589.0 bc
64	82.2 d	392.7 d	0.64 ab	2254.7 c
128	73.9 e	318.9 e	0.61 b	2198.8 c

[z]Mean separation within columns by Duncan's multiple range test at P = 0.05.

significantly increased in 8mM treatment, but they were not statistically different with 16, 32 and 64mM treatments. The unsatisfactory value of growth parameters was performed in 128mM treatment. Leaf chlorophyll value was advancement in all treatments. Silicate treatments notably improved fresh and dry weight of shoot and root compared with control. The fresh weight of shoot and root significantly increased in 8, 16 and 32mM treatment, but it obviously decreased in 128mM treatment. There was a similar result of shoot dry weight in 16, 32, 64mM treatments except 8mM. Dry weight root remarkably increased in 8, 16, and 32mM of silicate treatments. The lowest value of fresh and dry weight of shoot and root were obtained in 128mM of silicate treatment.

There was an expanded value of the total root surface area, total root length, and number of root tips in 8, 16 and 32mM treatments, but it did not show in 64 and 128mM treatments. The highest of total root surface area, total root length, and number of root tips was also obtained in 8mM of silicate treatment (Table 2).

Transpiration rate and stomatal diffusive resistance had a vice versa relationship to treatments application including control. Transpiration rate was higher in control treatment compared to silicate treatments and it had decreased trends with increasing silicate concentration (Fig. 1). On the other hand, stomatal diffusive resistance increased with increasing silicate concentration (Fig. 2).

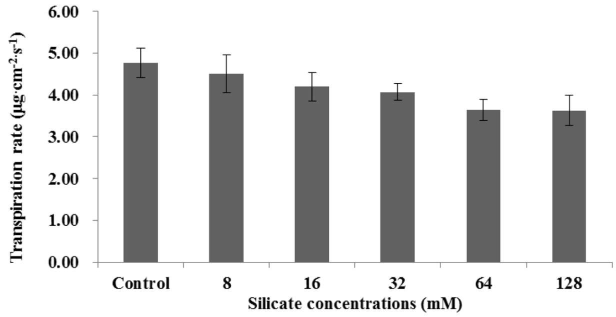

Fig. 1. Effect of silicate concentrations on transpiration rate of Chinese cabbage seedlings. Vertical bars represent ± SD, n = 5.

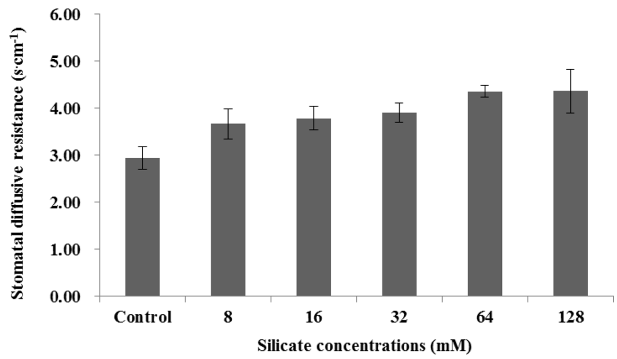

Fig. 2. Effect of silicate concentrations on stomatal diffusive resistance of Chinese cabbage seedlings. Vertical bars represent ± SD, n = 5.

2. Effect of silicate on abiotic stress of Chinese cabbage seedlings

Electrolyte leakage is another component connected with

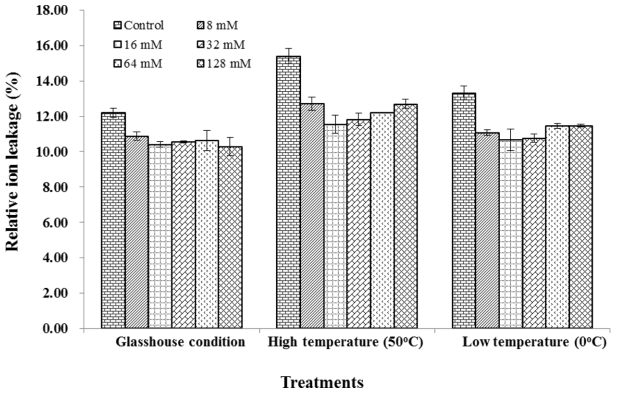

Fig. 3. Effect of silicate concentrations on relative ion leakage of Chinese cabbage seedlings in different temperatures (measured on 1 day after treating). The temperature in glasshouse was set 28±2°C for day time and 15±2°C for night time. Vertical bars represent ± SD, n = 3.

different stresses. In this study we found that silicate treatments led to significantly decrease in the electrical conductivity under all conditions (glasshouse, high and low temperature) compared to control. The values of relative ion leakage of all treatments in high temperature were higher than those in low temperature condition. Similar values of relative ion leakage were observed in all silicon treatments in the glasshouse condition, but they were found significantly different among silicate treatments at high and low temperature conditions. The lowest value of relative ion leakage was observed in treated seedlings with 16mM of silicate at both high and low temperatures. However, relative ion leakage increased with increasing silicate concentration over 32mM (Fig. 3). The application of silicate also reduced the heating and chilling injury index at high and low temperatures by reducing the damage to the leaf. The low values of heating or chilling injury were observed in treated seedlings with 16 and 32mM of silicate concentration (Table 3).

Silicate enhanced drought tolerance of Chinese seedlings by delaying the starting time of wilting point. The starting time of wilting point in the control was observed 3 days after discontinuation of irrigation, while in the 8, 64 and 128mM of silicate treatments the starting times were 4 days, and the 16 and 32mM of silicate treatments the starting times were 5 days. In the control, 100% of wilted plants were observed after 5 days without irrigation, while in the 8mM of silicate treatment after 6 days, in the 16, 32, 64, 128mM of silicate treatments after 7 days (Fig. 4).

Table 3. Effect of silicate concentrations on heating and chilling injury index of Chinese cabbage seedlings (measured at 3 days after treating in high (50±1°C) and low (0±1°C) temperatures).

Silicate concentrations (mM)	Heating injury index	Chilling injury index
Control	3.3 a	3.5 a
8	3.0 b	2.5 c
16	2.0 d	1.2 d
32	2.2 d	1.5 d
64	2.5 c	2.4 c
128	2.6 c	2.7 b

0: non-chilling or heating injury, 1: 0~20%, 2: 21~40%, 3: 41~60%, 4: 61~80%, 5: 81~100% of leaf area damage.

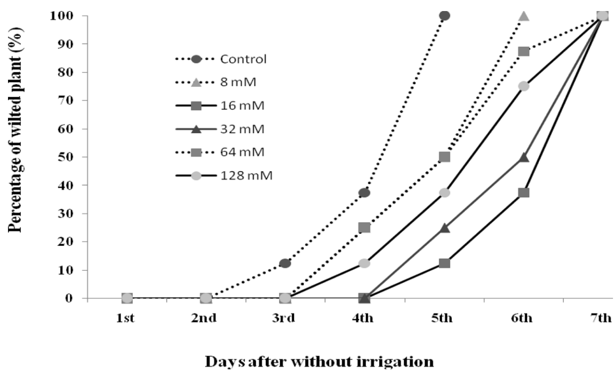

Fig. 4. Effect of silicate concentrations on percentage of wilted plant after without irrigation.

Discussion

In this experiment growth parameters significantly increased in treated seedlings with 8, 16 and 32mM of silicate compared to control. The highest growth parameters were observed in treated seedlings with 8mM of silicate. Although silicon has not been considered as an essential element for higher plants, nevertheless, application of silicon can improve the growth and yield of various crops (Adatia and Besford, 1986; Anderson et al., 1987; Edward et al., 1982). Gong et al. (2003) observed that silicon increased plant height, leaf area and dry mass of wheat even under drought. Singh et al. (2006) also suggested that silicon application increased dry matter and yield in rice. In addition, silicon has also been proved to be beneficial for the healthy growth and development of many plant species, particularly graminaceous plants such as rice and sugarcane and some cyperaceous plants (Epstein, 1999; Liang et al., 2005). However, growth parameters of Chinese cabbage seedlings decreased in 128mM of silicate treatment.

Transpiration rate decreased and stomatal diffusive resistance increased with increasing silicate concentrations. This result agreed with results of Lu and Cao, (2001) who reported that melon (*Cucumis melo* L.) showed benefits of Si supplementation with higher chlorophyll levels and reduced transpiration rates compare to untreated plants. Gao et al. (2006) found that silicon application of 2mM significantly decreased transpiration rate and conductance for both adaxial and abaxial leaf surface, but had no effect on transpiration rate and conductance from the cuticle in corn subjected to polyethylene glycol osmotic stress in solution culture. However, in our study transpiration rate had declined trends with increasing silicate concentration. This finding may have been due to an excessive silicon supply, causing the formation of silicate polymers on root surfaces.

Marschner et al, (1990) also pointed out that silicon enhances freezing tolerance of many crops. In this study, we found silicate treatments led to significantly decrease in the electrical conductivity under glasshouse, high and low temperature conditions. Silicate could migrate to reduce heating and chilling injury index during high and low temperature by reducing damage of leaf. At two levels of temperature, chilling injury index values of seedlings in silicate treatments were lower than those in non- silicate treatment. The low value of heating or chilling injury was observed in treated seedlings with 16 and 32mM of silicate. On the other hand, silicate enhanced drought tolerance of Chinese seedlings by delaying the starting time of wilting point. This result agreed with results of Trenholm et al. (2004) who reported that under severe drought stress, silicon-amended plants had better responses than non-amended plants and little improvement was seen under moderate drought stress.

In conclusion, low concentration of silicate stimulated the growth of Chinese cabbage seedlings by increasing growth parameters and root morphology. Transpiration rate decreased and stomatal diffusive resistance increased with increasing silicate concentrations. Silicate could migrate to reduce heating and chilling injury index during high and low temperature by reducing relative ion leakage and damage of leaf. On the other hand, silicate enhanced drought tolerance of Chinese seedlings by delaying the starting time of wilting point.

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규산비료 시용이 배추 묘의 생장과 환경내성에 미치는 영향

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적 요. 규산은 작물의 필수원소에는 포함되어있지 않으나, 화본과 작물을 중심으로 내도복성과 병충해 저항성의 향상 , 군락구조 개선에 의한 광합성 능력의 향상 등에서 폭 넓게 그 유용성이 알려져 왔으며, 최근에는 원예작물에서도 규산질 비료의 시용이 수량이나 병충해저항성을 향상시키는 효과가 입증되고 있어 친환경농업 관점에서도 주목을 받고 있다. 본 실험은 배추 육묘 중 규산질 비료의 시용이 묘소질과 저온, 고온, 건조 등 환경내성에 미치는 영향을 검토하기 위하여 수행하였다. 규산염 처리농도를 8, 16, 32, 64 및 128mM로 설계하여 주 2회 관주 처리 하고, 처리 3주 후에 생육조사 및 스트레스 내성에 대해 평가하였다. 생육조사 결과, 8, 16 및 32m의 농도에서는 대부분의 생육지표가 대조구에 비해 약간 증가하는 경향을 보였으나 8mM처리만 제외하고 통계적 유의차는 나타나지 않았다. 고농도인 128mM의 규산 처리구에서는 모든 생육 지표가 감소하였다. 총 뿌리 면적, 뿌리 길이 및 근단 수는 8, 16 및 32mM의 농도에서 증가했지만 64 및 128mM의 처리구에서는 감소하였다. 규산 처리 농도가 증가함에 따라 증산 속도는 감소한 반면 기공확산 저항은 증가하는 경향을 보였다. 상대적 이온 누출율도 대조구에 비해 규산염 처리구에서 감소되었으나, 처리 농도간 유의차는 나타나지 않았다. 규산처리에 의해 고온과 저온 장해 지표도 감소되었으며, 농도간에는16과 32mM이 가장 효과적이었다. 규산처리에 따라 건조내성도 증가하여 대조구는 단수 후 3일째부터 위조되기 시작하여 5일째는 전개체가 위조하였으나, 규산처리구는 4일(8, 64, 128 mM) 또는 5일(16과 32mM) 부터 위조가 시작되어 6일(8mM)이나 7일(16, 32 ,64및 128 mM)이 지나서야 모든 공시 개체가 위조되었다.

추가 주제어: 생육지표, 이온누출, 기공확산저항, 증산율, 뿌리형태