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Studies on the Effect of Silicon Nutrition on Plant Growth, Mineral Contents and Endogenous Gibberellins of Three Rice Cultivars

Soo-Won Jang[1], Muhammad Hamayun[2], Eun-Young Sohn[2], Dong-Hyun Shin[2], Kil-Ung Kim[2] and In-Jung Lee[2*]

[1]*KT & G Central Research Institute, Chungbuk, Republic of Korea*
[2]*Division of Plant Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Republic of Korea*

Abstract

Silicon is one of the key elements for healthy growth and development in rice crops. We analyzed the effect of silicon (Si) on some growth parameters, plant mineral contents, and bioactive gibberellins in three rice cultivars. Silicon was applied at the rates of 0 kg/0.1ha (control), 40 kg/0.1ha, and 80 kg/0.1ha throughout the course of experiment. Plant growth parameters were enhanced by the application of elevated Si, though plant height and culm length were more favorably affected than the respective dry weights. The plant mineral contents analyzed also increased except potassium, in all treatments where Si was applied, demonstrating that Si application promotes the absorption of these minerals in rice crops. The endogenous gibberellins measured in our study showed that GA_1 is the only bioactive GA form present in rice seedlings. The endogenous GA_1 and its precursor GA_{20} contents increased after Si application. However, this increase in endogenous GA_1 and GA_{20} contents, and plant growth parameters were different according to the rice cultivars. Our results indicate that Si is a beneficial element in rice nutrition and that different cultivars of *Oryza sativa* show differential responses to Si nutrition in terms of their growth and development.

Key words: silicon nutrition, growth parameters, mineral contents, GAs analysis, GC-MS-SIM

Introduction

The phytohormones play a vital role in the growth and development of plants. They act at micromolar or even lower concentrations to regulate physiological and developmental processes including seed germination, leaf expansion, stem elongation, flowering, and seed formation. Bioactive gibberellins play an essential role in many aspects of plant growth and development, such as stem elongation, flower and fruit development, and seed germination (Ross *et al.* 1997). GAs are synthesized from isopentenyl pyrophosphate via geranylgeranyl pyrophosphate (Graebe 1987; Sponsel 1995; Hedden and Kamiya 1997; Lange 1998). The first committed step of GA biosynthesis is the formation of *ent*-kaurene from geranylgeranyl pyrophosphate, with copalyl pyrophosphate as an intermediary. This reaction is catalyzed by the enzymes *ent*-copalyl diphosphate synthase and ent-kaurene synthase, which have been cloned from various plant species (Sun and Kamiya 1997). *ent*-Kaurene is metabolized to GAs by membrane-associated monooxygenases and soluble, 2-oxoglutarate-dependent dioxygenases (Graebe 1987).

* To whom correspondence should be addressed
In-Jung Lee
E-mail: ijlee@knu.ac.kr
Tel: +82-53-950-5708

Silicon is the second most abundant element present in the earth's crust and, quantitatively, it is the major mineral inorganic constituent of higher plants, though it had not been considered an essential element (Epstein 1994) and is thus omitted from any of the numerous formulations of nutrient solutions widely used in plant physiological research, such as the Hoagland solution. However, silicon deprived plants are often structurally weaker, have abnormal growth and development, and are more susceptible to abiotic and biotic stresses than silicon-replete plants (Epstein 1994; Rafi *et al.* 1997). Silicon needed to be included among the elements that play an indispensable role in plant life (Epstein 1999). The increased vegetative and reproductive growth of some members of family Poaceae in the presence of silicon has led to the suggestion that silicon may be essential for plant growth and development (Cheng 1982).

Past investigations have revealed the importance of silicate material in rice farming systems. Silicon was first used as fertilizer in Japan in 1955 and since then, 1.5 to 2.0 tons per ha of silicate fertilizer have been applied to silicate deficient paddy soils. As a result, a 5-15% increase in rice yield has been reported (Takahashi *et al.* 1990). Presently, silicate fertilizers are also applied in South Korea, Taiwan, Hawaii, and more recently, in China. Slags, byproducts of the iron manufacturing industry, are the main silicate fertilizers now used and

contain more than 25% Si. Due to the increasing importance of Si in rice crops, an understanding of the effects of silicon nutrition on different growth parameters and endogenous gibberellin content in rice plants needs special attention as growth responses to silicon nutrition may be regulated by plant growth hormones. The status of bioactive gibberellins in response to silicate fertilizer application has never been investigated in rice plants, though considerable attention has been focused on the role of gibberellins in controlling shoot elongation.

In the present study, an effort was made to investigate silicon content in rice and how it affects the biosynthesis of bioactive GAs along with some pertinent growth parameters.

Materials and Methods

A complete randomized block design (CRBD) experiment was designed and conducted at the Crop Physiology Laboratory, Kyungpook National University, to investigate the effects of elevated Si application on endogenous bioactive gibberellins and some growth parameters in three rice cultivars. There were three treatment levels, six replications per treatment and 24 plants per replication.

General procedures

Seeds of three rice cultivars viz. Daesanbyeo, Dongjinbyeo, and Junambyeo were obtained from the Yeongnam Agricultural Research Institute, Milyang. The seeds were surface sterilized in 5% NaOCl for 10 min, rinsed with deionized water, left to imbibe in aerated deionized water and incubated in nursing beds for five days. The germinated seeds were transplanted on day five to plastic pots (22×15×7cm) filled with vermiculite. All the plants were grown in a controlled environment chamber with a 16 h at 30°C day and 8 h at 20°C night regimen and light intensity of 1000 μmol $m^{-2}s^{-1}$. The plants were provided twice with half strength Yoshida nutrient solution. The silicate fertilizer was applied as silicon source on day five after sowing at the rates of 100 kg/0.1ha and 200 kg/0.1ha, while one treatment was silicate fertilizer free (control). The concentration of Si was 40% in the silicate fertilizer used for this experiment.

The endogenous bioactive GA_1 and its precursor GA_{20} contents were quantified on day 12 of the application of silicate fertilizer.

Growth parameters

The plant height, plant dry weight, culm length, and culm dry weight were measured on day seven after Si application to the plants. Dry weight was measured after drying samples at 70°C for 48 h in an oven (Bohm 1979).

Analysis of endogenous minerals

The plant samples were dried at minus 80°C, ground and then passed through a 2 mm mesh sieve. For nitrogen content determination, the sample was eluted in a sulfate-salicylic acid solvent and then analyzed through Kjeltec system, Foss Inc. The P, K, $CaCO_3$, and MgO contents were determined by digesting a 0.5 g of sample in 10 ml HNO_3 through Microwave digestion system (Mars 5, CEM) and then analyzed by Inductivity coupled plasma (Optima 3200RL, Perkin-Elmer). In case of Si, 1.0 g sample was extracted in 30 ml ternary solution and the weights were measured.

Extraction and quantification of endogenous bioactive GAs

The plants were harvested and the immediately frozen in liquid nitrogen and stored at -80°C. When all the required material for GA analysis had been collected, the samples were lyophilized for 24 h. The extraction method used for extraction and quantification of endogenous gibberellins was based on the already established procedure by Lee *et al.* (1998). The lyophilized samples were ground to a fine powder in a mortar and pestle. The powdered tissue (0.5 g) was extracted with 80% methanol (MeOH). The 80% MeOH was removed by filtration, and the tissue was then extracted with 100% MeOH until the extract was clear. The volume of the 80% and 100% extracts were recorded, the two extracts were combined, and water was added to bring the combined MeOH extract concentration to 60% and 20 ng [$^2H_2$]GAs internal standards were added. This solution was chilled for 1 h at -70°C and precipitated chlorophyll was removed by filtration through a GF/A filter. The extract was adjusted to pH 8.0 to 8.3 using 2M NH_4OH and passed through a 3 g of Davisil C_{18} column (90-130μm, Alltech, Deerfield, IL, USA). The eluant was reduced to near dryness at 40°C in vacuum. The sample was then dried onto 1g celite and then loaded onto 4 g SiO_2 partitioning column (deactivated with 20% water) to separate the gibberellins as a group from more polar impurities. Gibberellins were eluted with 80 ml of 95:5 ethyl acetate (EtOAc): hexane saturated with formic acid. This solution was dried at 40°C in vacuum, redissolved in 4 ml of EtOAc and partitioned three times against 4 ml of 0.1 M phosphate buffer (pH 8.0). Drop wise addition of 2M NaOH was required during the first partitioning to neutralize residual formic acid. Polyvinylpolypyrrolidone (PVPP) 1 g was added to the combined aqueous phases, and this mixture was slurried for 1 h. Following the removal of the PVPP by filtration, 6M HCl was added to reduce the pH 2.5. The extract was partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction was dried in vacuum, and the residue was dissolved in 3 ml of 100% MeOH. This solution was dried on a Savant Automatic Environmental Speedvac (*AES* 2000, Madrid, Spain). The dried sample was subjected to reverse-phase C_{18}-HPLC. Radioactivity for each concentrated GA fraction was counted for 3 min in a liquid scintillation counter. Each GA fraction was redissolved in 100% methanol, transferred to a 1 ml vial, and dried under N_2 at 40°C. The sample was dissolved in 35 mm^3 of methanol, and the GA methyl ester was prepared with ethereal diazomethane. The sample was dried under N_2, re-dissolved in methanol and methylated one more time. The sample was dissolved in 35 $\mu\ell$ pyridine, and silylated for 30 min at 65°C with the same amount of N, O-Bis (trimethylsilyl)-trifluoroacetamide (BSTFA) with % trimethylchlorosilane (TMCS) (Pierce Chemical Co., Rockford, IL, USA). The sample was then reduced to dryness with N_2 and solublized in anhydrous dichloromethane. 1 $\mu\ell$ of each sample was injected on-column on a 30m×0.25mm (i.d.), 0.2 μm film thickness DB-1 capillary column (J & W Co., Folson, USA). The GC oven temperature was programmed for 1 min at 60°C, then increased at a rate of 15°C min^{-1} to 200°C followed by 5°C min^{-1} to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector with an interface and source temperature of 280°C, an ionizing voltage of 70 eV, and a dwell time of 100 ms. Full scan mode (the first trial) and three major ions of the supplemented [$^2H_2$]GAs internal standards (the second trial) and the endogenous gibberellins were monitored simultaneously. Retention

time was determined by using the hydrocarbon standards to calculate the KRI (Kovats retention indices) value.

Statistical analysis

The data was analyzed statistically for standard deviation by using sigma plot 2004 software.

Results

Effect of elevated silicate levels on growth parameters

The data recorded for growth parameters showed that silicate fertilizer had resulted in an enhanced growth of all rice cultivars. The plant height of rice cultivars showed a significant increased at basic (40 kg/0.1ha) and double silicate applied treatments (80 kg/0.1ha) compared with the control. The plant height was highest at double silicate treatments in all cultivars. The plant dry weight also showed an increase in response to silicate application, though the increase recorded in this parameter was not pronounced. The culm length and culm dry weight were also affected by elevated silicate nutrition. All the silicate applied treatments recorded a steady increase in culm length and dry weight with increased silicate nutrition as compared to the control but the increase measured after applying a double quantity of silicate was not as pronounced as compared to basic silicate applied treatments (Table 1).

Table 1. Effect of elevated silicon levels on the plant height, plant dry weight, culm length and culm dry weight of three rice cultivars.

Rice cultivar	Silicon level (kg/0.1ha)	Plant		Culm	
		Height(cm)	Dry weight(g)	Length(cm)	Dry weight(g)
Daesanbyeo	0	32.7±1.7	6.3±0.4	14.3±0.6	2.29±0.3
	40	39.5±2.2	7.1±0.1	17.0±0.8	2.64±0.1
	80	42.5±2.6	8.7±0.6	18.0±0.7	2.71±0.1
Dongjinbyeo	0	34.9±2.4	7.0±0.4	14.6±1.2	2.28±0.4
	40	42.6±2.1	8.5±0.7	17.4±0.6	2.67±0.2
	80	44.2±2.0	9.1±0.5	18.1±0.7	2.70±0.2
Junambyeo	0	39.2±2.1	6.9±0.3	15.3±0.7	2.35±0.1
	40	41.0±0.9	7.9±0.5	17.1±0.8	2.57±0.3
	80	42.8±2.2	8.8±0.4	18.4±1.3	2.55±0.1

Mineral contents

The application of Si has also affected the uptake of different macro and micronutrients. The macronutrients (NP) have increased while K has decreased insignificantly with the application of elevated Si amounts. The micronutrients i.e. Fe, Mn, Cu, Zn, Mg, Ca and Si contents have markedly increased with the application of elevated Si levels (Table 2).

Table 2. Analysis of macro and micro elements in rice in response to elevated Si application.

Silicate fertilizer level (kg/0.1 ha)	T-N (%)	P_2O_5 (%)	K_2O (%)	CaO (%)	MgO (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Si (ppm)
0	2.68	1.48	5.40	0.12	0.08	716.0	675.0	11.6	53.7	53.7
40	2.70	1.58	5.35	0.16	0.09	1216.2	835.3	12.5	76.2	76.2
80	2.72	1.66	5.28	0.17	0.11	685.2	985.2	12.6	82.6	82.6

Effect of elevated silicate levels on endogenous bioactive GAs (GA_1 and GA_{20})

During the present investigation, bioactive endogenous gibberellins for three rice cultivars were analyzed in order to establish the effect of elevated Si levels on these growth regulators. In bioactive GAs, only GA_1 was observed in seedlings of three rice cultivars. A significant increase in the endogenous bioactive GA_1 and its precursor GA_{20} content was recorded in response to elevated Si levels.

In cv. Daesanbyeo, GA_1 content increased 1.5-fold at basic Si (40 kg/0.1ha) and 4.3-fold at double Si (80 kg/0.1ha) levels compared to the control treatment (1.5 ng/g). The endogenous GA_{20} content also showed a significant increase i.e. 2.4 folds at basic while 4.1-fold at double Si levels compared to the control (2.3 ng/g). In cv. Dongjinbyeo, there was a 1.4-fold increase in GA_1 contents at basic and a 2.0-fold increase at double silicon nutrition compared to control treatments (4.0 ng/g) while the endogenous GA_{20} content increased 1.2 times at basic and 2.6 times at elevated silicon as compared to the control (2.7 ng/g). In cv. Junambyeo, endogenous GA_1 contents increased 1.7-fold at basic silicon application and 2.1-fold at double silicon levels compared to the control (4.3). The GA_{20} contents also increased 2.0 times at basic and 2.5 times at double silicon fertilizer application compared to control treatments (2.0 ng/g) (Fig. 1).

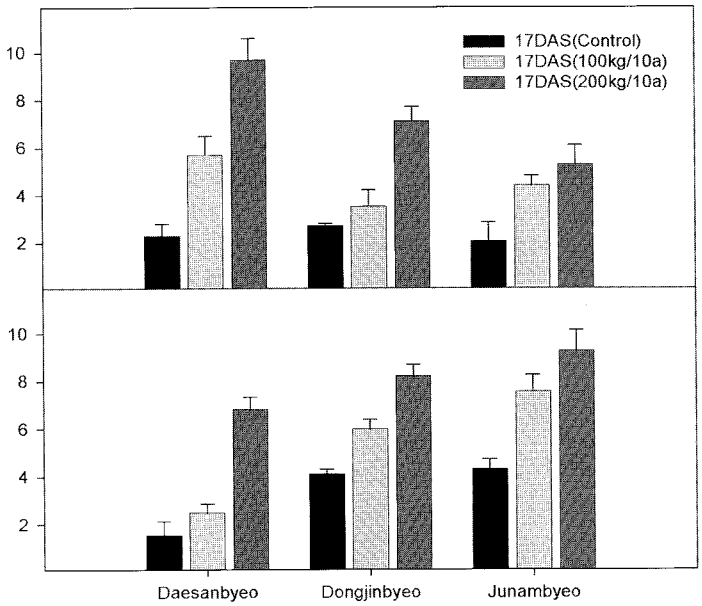

Fig. 1. Effect of silicon on bioactive GA contents(GA_1, GA_{20}) of three rice cultivars at 12[th] days after silicate fertilizer application.

Discussion

There is ample evidence that Si, when readily available to plants, plays a favorable role in their growth, mineral nutrition, mechanical strength, and resistance to fungal diseases and herbivory in a wide variety of plant species. In general, Poaceae plants contain the highest levels of Si, 1- 10% on a dry weight basis, and the effect of silicon on growth is well studied in this plant group. It has been shown that silicon increases the dry weight of rice, oat, barley, wheat, and annual brome by 2-20% (Lewin and Reiman 1969; Gali and Smith 1992). Promotion

of growth by silicon was also reported in other species such as cucumber (Adatia and Besford 1986), loblolly pine (Emadian and Newton 1989), cotton (Li *et al.* 1989), and poinsettia (McAvoy and Bible 1996). In many instances, growth promotion is due to the improvement of plant resistance to abiotic stresses such as aluminum and other metal toxicities, salinity or water stress, and biotic stresses such as pathogens and herbivores (Epstein 1999). Whether silicon stimulates plant growth under conditions without stress is still debated. Our study confirmed that plant height, plant dry weight, culm length, and culm dry weight were significantly affected by basic (40 kg/ 0.1ha) and double (80 kg/ 0.1ha) silicon applications. The plant height and culm length increased in all cultivars but the difference in height at basic and double silicon applied treatments were insignificant, indicating that high doses of silicon above a critical level may be less effective in maintaining the same growth rate in these parameters. Similarly, Richter *et al.* (1989) reported that silicon application nearly doubled the yield of rice over a 2-year period (mean for all eight genotypes in the study). The higher yields due to Si applications were correlated with higher flag leaf Si concentrations. Although marked improvement of yields due to Si applications has been noted mainly for rice and sugarcane, other Gramineae such as barley may also benefit from such applications. Fujii *et al.* (1999) and Saigusa *et al.* (2003) studied the effect of silicon fertilizer in nursery bed soil on the growth of rice seedlings, suggesting that silicon application increased dry matter production by increasing the photosynthetic rate of individual leaves and by improving the canopy structure.

The endogenous mineral contents differed according to the silicate levels and a steady increase in the concentration of these macro and micro nutrients was observed with increased Si applications. This clearly demonstrated that the uptake of different macro and micro nutrients were enhanced by elevated Si doses. In case of K only, the increased Si application has resulted in a reduced uptake thus indicating a negative correlation. Our results coincide with that of Roy *et al.* (1971), who observed that silicate applications tended to increase the P concentration in the green tops of sugarcane (metabolically more active tissue) and decrease P in the stalk (metabolically less active tissue) when P nutrition was low. This tendency did not occur when nutrition was high. These observations suggest that P utilization in the plant may be improved by the addition of Si when available P is low. Certain facts suggest that P availability may be controlled by the levels of Mn and Fe in plants when P is low. P is translocated and redistributed in plants as inorganic P, and since a strong affinity exists between P and Fe or Mn, the relationship of these elements may affect P nutrition. Biddulph (1953) found that P precipitated with Fe in plants, and Bortner (1935) reported that P might combine with Mn into an inactive form. Thus, plant P/Fe and P/Mn ratios may be more indicative of P nutrition than P concentration. Okuda and Takahashi (1962) reported that Si promoted the oxidation power of roots and thus decreased the solubility and uptake of Fe and Mn. However, in our experiment Fe and Mn uptake was favored by the addition of Si.

The present study showed that bioactive endogenous gibberellins were significantly affected by silicon and its application in elevated amounts had resulted in an increased GA_1 and GA_{20} contents in the three rice cultivars. However, the increase in concentrations of endogenous GA_1 and GA_{20} contents were different in these rice cultivars showing that the response potential of different cultivars to silicate fertilizer application may vary with in the same species or subspecies. Our results are in keeping with those of Winslow (1992), who reported that different rice genotypes responded differentially to Si applications. The indica group of genotypes was more responsive to Si applications than were the japonica, both in terms of yield and in terms of the total amount of Si in the shoot.

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