Plant and Soil **148**: 107–113, 1993.

PLSO 9663

Interaction between calcium and silicon in water-cultured rice plants

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Received 22 June 1992. Accepted in revised form 8 October 1992

Key words: calcium, rice, silica body, silicon, soft X-ray irradiation

Abstract

The interaction between Ca and Si in water-cultured rice plants (*Oryza sativa* L. cv. Akebono) was investigated in terms of uptake. The effect of Ca levels in the solution on Si chemical forms and on the formation of silica bodies in the leaf blades was also examined using soft X-ray irradiation for detection of silica bodies. Si addition (1.66 m*M* Si) decreased both Ca content of the shoot and uptake at each Ca level. This might mainly result from a decreased transpiration rate caused by Si. Si uptake was not affected when the Ca levels were increased. The results of Si forms showed that silica sol constituted more than 90% of the total Si in the leaf blades regardless of Si and Ca levels, and soluble silica and/or polysilicic acid seems to gel physically over 8.0 m*M* Si within the plants. Significant difference in the numbers of silica bodies on the third leaf blade was not found between different Ca levels at the same Si level. The content of Si in the leaf blade seems to be a determining factor for the formation of silica bodies.

Introduction

Silicon (Si) has been demonstrated to be necessary for healthy growth of rice plants. In 1955, Si was first recognized as a fertilizer in Japan, 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 to 15 percent increase in rice yield has been reported (Takahashi et al., 1990). Nowadays silicate fertilizers are also applied in South Korea, Taiwan, Hawaii and more recently in China.

Slags, which are by-products of the iron manufacturing industry, are the main silicate fertilizers used now. Their main composition is calcium silicate. When they are applied to soils, not only Si, but also Ca is supplemented as an accompanying element. However, the effect of Ca is not often considered.

In 1984, Tsuno and Higashi (1984a,b), Tsuno and Kashahara (1984) indicated that the application of silicate fertilizer caused rice plants to be attacked by blast disease, based on their investigation in field. They analyzed that this resulted from accompanying Ca because they found that leaf Ca content has a negative correlation coefficient with leaf Si content, and when the Ca content became high at the same Si content, the number of silica bodies decreased. From these facts, they concluded that Ca in the plants reduced the formation of silica bodies, and antagonism occurred between Si and Ca.

However, Miyake et al. (1987), and Miyake and Takahashi (1992) conducted a field experiment under the treatment of different Ca and Si levels. They found that the Si content in the rice shoot hardly decreased even when Ca application increased to 6.5 times more than Si. The number of the dumbbell-shaped silica cells on the epidermal tissues of the flag leaf could also not be regarded as significant difference among Si or Ca added plants (Miyake and Takahashi, 1992).

In the study presented in this paper, we examined the interaction between Ca and Si in terms of uptake using water culture. The effect of Ca levels in the solution on Si forms and on the formation of silica bodies in the leaf blades is also discussed.

Materials and methods

Effect of Si addition on Ca uptake

Rice seeds (*Oryza sativa* L. cv. Akebono) were soaked in distilled water for one day and then sown in a plastic container. To avoid Si contamination, acid washed polyethylene beads were used as rooting media. After 10 days, the seedlings were transplanted into 10 liter plastic container for preculture, and 1/4 strength of Kimura B nutrient solution was supplied as shown in previous paper (Ma and Takahashi, 1990). The solution was renewed once a week.

After 2 more weeks, three selected seedlings each were transplanted into 3 liter pots with the Kimura B solution containing no Si or 1.66 m*M* Si as silicic acid prepared by the Okuda and Takahashi method (1961). The pots were arranged on greenhouse tables in a completely randomized design in three replicates. The strength of the nutrient solution was modified with the progression of the growth from 1/4 to 2 times, and the pot sizes were also changed correspondingly (Ma et al., 1989). Water distilled with a copper still was used for the experiment. The solution was renewed once a week in the earlier growth stages, and once every three days in the later stages. The pH of the solution was adjusted to about 5.5 with KOH. Transpiration rate was measured by weighing the pots before and after the solution renewal throughout the whole growth period. The plants were harvested at maturity, and were separated into roots and shoots. The content of Ca in the shoots and roots was determined by atomic absorption spectrophotometry after HNO_3-$HClO_4$ digestion. To avoid Si interference, La was added as 1000 mg kg^{-1}. The total Si content was done by the colorimetric molybdenum blue method after the samples were melted with anhydrous Na_2CO_3 (Okuda and Takahashi, 1961).

Effect of Ca levels on the formation of silica bodies

Rice seedlings were prepared by the method described above. Three selected seedlings of 20-day-old each were transplanted into 3 liter pots contained Kimura B solution with different Ca and Si levels. The pots were arranged on greenhouse tables in a complete randomized design with 0.33, 1.66 m*M* Si as main treatments and 0.37, 1.25, 2.50 m*M* Ca as subtreatments in three replicates. Si treatments were supplied as silicic acid and Ca treatments as $CaCl_2$. The culture method was the same as described above. Two months after treatment, the third leaf blade numbered from the top was taken for silica body detection, and another plant was sampled for Si fractionation of leaf blades. The other plants were harvested at the same time for Ca and Si analysis using the method described above.

The silica bodies were detected by soft X-ray irradiation. This method was developed by Takeoka et al. (1983). It was based on a fact that amorphous silica gel absorbs X-ray energy more markedly than epidermal tissues do, causing more clear transparency on the developed X-ray film. After sampling, the leaf blades were ironed immediately to keep them in expanding state. Irradiation on enclosed soft X-ray film by the microsource of X-rays, CMR, emitted by the SOFTEX machine (Model M1005NA) was then performed, using under 8 KeV, 5 mA for one minute. Four leaf blades were irradiated for each treatment and silica bodies per 1 mm^2 around 10 cm from tip were counted 10 times on both sides of lamina midrib after the films were developed and made into photographs.

Si fractionation in the leaf blades was conducted as follows. Five grams of fresh leaf blades were homogenized with 50 mL of distilled water for 3 minutes. The homogenate was filtered through gauze into a 100 mL flask to which 1 mL of 0.2 *N* HCl was added to minimize the rate of possible transformation of silicic acid by both polymerization and depolymerization. After filled with distilled water to the mark, the content was transferred to a centrifugal tube and centrifuged at 5000 r.p.m. for 10 minutes, and then filtered through a dry filter paper. A 5 mL of the filtrate was used for direct colorimetric determination of Si immediately. This Si form is regarded as "soluble silica". A 20 mL of the filtrate was placed in a nikel crucible and evaporated to dryness on a hot plate. After the completion of evaporation, the content was melted with anhydrous Na_2CO_3. The Si was then determined by colorimetric method. This Si form represent "soluble silica plus polysilicic acid". Total Si of the same sample was also determined, and the "silica sol" was calculated from total Si and other two Si forms. The content of Ca in the leaf blade was also determined using the same samples.

Table 1. Dry weight, Ca, Si content, and transpiration rate of rice plants cultured in the solution with or without Si. Values are means ± SD (n = 3)

Treatment	Silicon	
	+	−
Dry weight (g)		
Shoot	114.9 ± 2.11	75.9 ± 0.50
Root	10.1 ± 0.97	9.34 ± 0.08
Si content (%)		
Shoot	5.34 ± 0.08	0.05 ± 0.01
Root	0.43 ± 0.01	0.02 ± 0.01
Ca content (%)		
Shoot	0.13 ± 0.01	0.26 ± 0.01
Root	0.02 ± 0.00	0.01 ± 0.00
Ca uptake (mg/pot)	146.9 ± 11.1	196.9 ± 6.7
Transpiration rate (H_2O g/g dry wt.)	506.8	662.9

Results

Si addition increased shoot dry weight (Table 1). The plants treated with or without Si contained 5.34, 0.05% Si in the shoot, and 0.43, 0.02% Si in the root, respectively. The Ca content of the shoots with Si was about half of that without Si. The Ca uptake was decreased by 20% due to Si addition. The transpiration rate was 30% more in the plants without Si than those with Si.

Shoot Ca content increased with increasing Ca levels in the solution at the same Si level, and decreased when Si level increased from 0.33 to 1.66 m*M* Si (Table 2). The effect of increasing Si and Ca levels on the Ca uptake showed the same trends as on the Ca content.

Shoot Si content, as well as Si uptake were hardly affected by increasing Ca levels in the solution (Table 2). The numbers of silica bodies on the third leaf blade did not change among

Table 2. The concentration and uptake of Ca and Si in rice shoots, Si bodies in the third leaf blade grown on nutrient solution with different Ca and Si levels. Values are means with SD (n = 3). Si1, Si2 represent Si levels of 0.33, 1.66 m*M* Si which were supplied as silicic acid, and Ca1, Ca2 and Ca3 represent 0.37, 1.25 and 2.50 m*M* Ca supplied as $CaCl_2$

Treatment	Si1			Si2		
	Ca1	Ca2	Ca3	Ca1	Ca2	Ca3
	Concentration (% dry wt.)					
Ca	0.20	0.38	0.47	0.16	0.28	0.36
	(0.01)	(0.02)	(0.00)	(0.00)	(0.01)	(0.03)
Si	1.49	1.44	1.57	5.30	5.34	5.31
	(0.02)	(0.03)	(0.19)	(0.17)	(0.13)	(0.24)
	Uptake (mg/pot)					
Ca	20.9	38.4	47.0	15.4	31.7	39.7
	(0.83)	(0.58)	(1.54)	(0.53)	(1.51)	(3.47)
Si	153.4	144.9	157.2	526.1	596.6	589.0
	(1.49)	(6.74)	(13.7)	(41.5)	(3.23)	(20.2)
	Si body (nos./1 mm^2)					
	4.7	4.6	2.5	61.1	69.2	62.3
	(4.6)	(4.9)	(2.5)	(12.3)	(9.8)	(10.4)

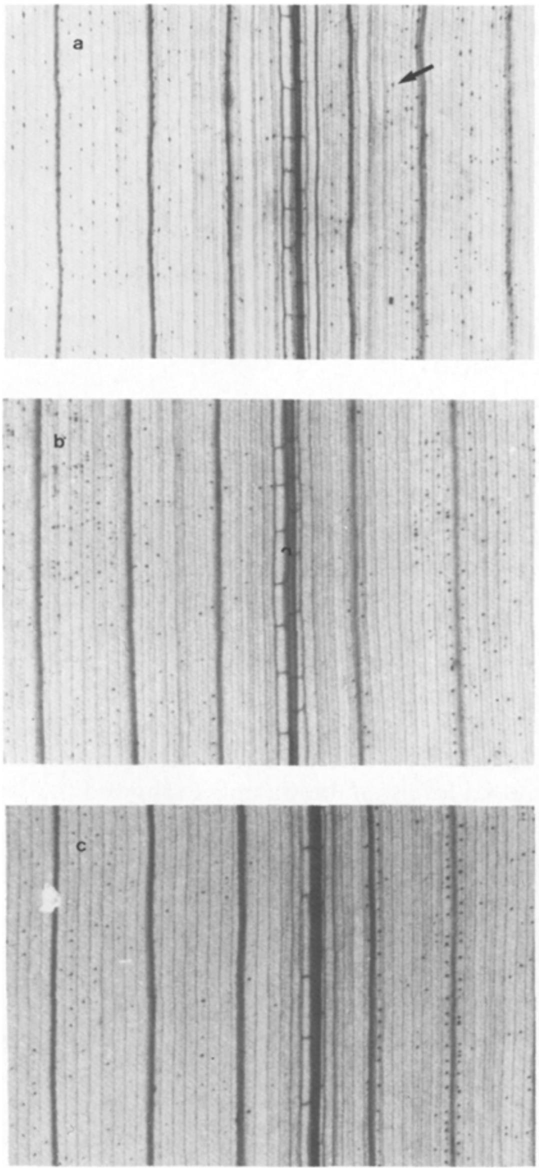

Fig. 1. Microphotograph of a selected portion (around 10 cm from tip) of the third leaf blade of rice plant obtained by soft X-ray irradiation. **a**, **b**, and **c** show the Si bodies (*arrow*) of the leaf blade treated with 0.37, 1.25, 2.5 m*M* Ca at 0.33 m*M* Si, respectively. (Magnification: 15×)

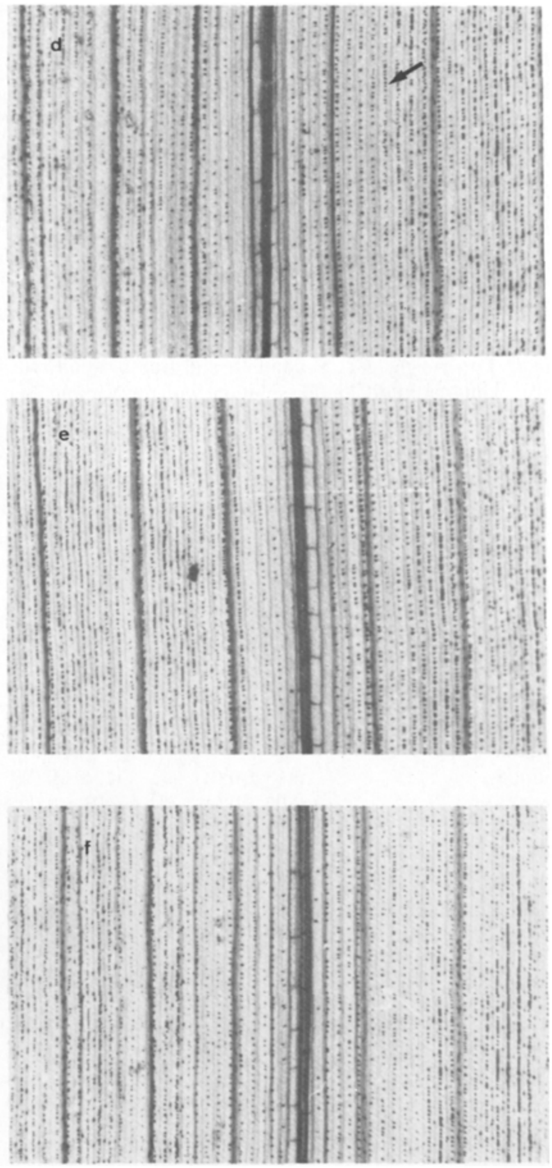

Fig. 2. Microphotograph of a selected portion (around 10 cm from tip) of the third leaf blade of rice plant obtained by soft X-ray irradiation. **d**, **e**, and **f** show the Si bodies (*arrow*) of the leaf blade treated with 0.37, 1.25, 2.5 m*M* Ca at 1.66 m*M* Si, respectively. (Magnification: 15×)

different Ca levels of the same Si level (Table 2, Figs. 1, 2), however, the numbers significantly increased in the plants cultured in 1.66 m*M* Si solution compared to those in 0.33 m*M* Si solution. A significant correlation coefficient ($r = 0.98$ at 5% level) was obtained between the Si content of the leaf blades and the Si body numbers. However, there was not a significant negative correlation coefficient between the Ca content and either the Si content of the leaf blades ($r = -0.48$) or Si body numbers ($r = -0.51$).

More than 90% of Si was present in the form of silica sol in the leaf blades regardless of Si and

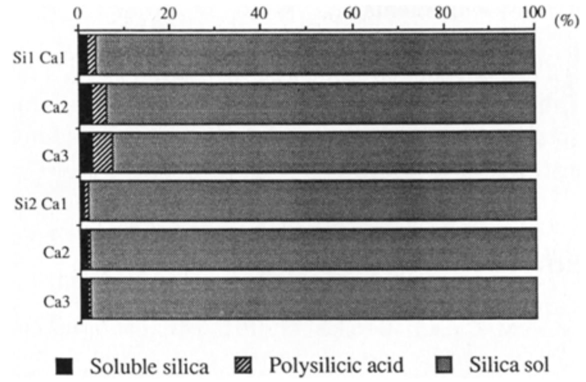

Fig. 3. Percentage of three Si forms in the leaf blade treated with different Ca and Si levels. Si1, Si2 represent Si levels of 0.33, 1.66 m*M* Si which were supplied as silicic acid, and Ca1, Ca2, and Ca3 represent 0.37, 1.25, and 2.50 m*M* Ca supplied as $CaCl_2$.

Ca levels (Fig. 3). The concentration of soluble silica and polysilicic acid was below 8.0 m*M*, and changes in the Si forms were not found at any Si and Ca levels.

Discussion

In water-cultured rice plants, not only the Ca content, but also the Ca uptake was decreased by the Si addition (Table 1). This fact suggests that the decrease in the Ca content was not only caused by dilution via Si promoted growth. Although it is still a controversial question whether Ca uptake by plant roots is active or passive, most investigators assumed Ca uptake to be a passive, mass flow-regulated process (Lazaroff and Pitman, 1966; Michael and Marschner, 1962). Drew and Biddulph (1971) reported that an increase in transpiration resulted in an increased translocation of Ca to the bean shoot provided the Ca concentration of the nutrient solution was high. These facts suggest that Ca uptake and translocation are closely related to the transpiration. In the present study, the transpiration rate was significantly decreased by the Si addition (Table 1) and the decreased Ca uptake could have resulted from this.

In addition, Si also deposits in the free space of the root (Sangster, 1978). This may also affect Ca uptake through decreasing apoplastic flow by precipitation with Ca, although the mechanism is not clear.

Takahashi et al. (1990) ascribed the beneficial effects of Si on rice plants to prevent lodging, fungal, and insect attack, to improve posture for light interception and decrease mutual shading in the community, to promote photosynthesis and so on. All these effects can be attributed to the deposition of Si in the epidermal layer, that is, to the formation of silica bodies. If high Ca levels reduced the formation of Si bodies as reported by Tsuno et al. (1984), three possibilities could be considered, that is, high Ca levels may decrease Si uptake, affect the transformation of Si chemical forms within the plants, or inhibit the formation of Si bodies. In the present study, each possibility has been examined respectively.

Table 2 shows that when the Ca levels increased from 0.37 to 2.5 m*M* Ca, neither Si content nor Si uptake was affected at either Si level. This fact suggests that it is not possible that high Ca affects the formation of Si bodies by decreasing Si uptake.

Okuda and Takahashi (1962), and Takahashi and Hino (1978) indicated that rice plants absorb Si in the form of monosilicic acid. After uptake, Si is transported to the shoot with the transpiration stream. With water loss, the Si is gradually concentrated and polymerized within the plant. The final product is silica sol which deposits on the motor cells of epidermal tissues, forming silicified cells (Si bodies). If higher Ca levels affect the formation of Si bodies, Ca may inhibit the transformation to silica sol. Because Si chemical forms in the plant have remained quite uncertain, we separated the Si in the leaf blades into three forms; soluble silica, polysilicic acid, and silica sol according to their reaction with molybdate and solubility in water. The term "soluble silica" refers to a group of silicic acids that react easily with molybdate. Orthosilicic acid is probably a predominant member of this group. The term "polysilicic acid" is used to designate a group of silicic acids which is dissolved or dispersed in water, but does not give a color reaction with molybdate. The term "silica sol" refers to polymerization products of orthosilicic acid that are water-insoluble. As shown in Figure 3, silica sol is the most prevalent form at any Ca levels, and the soluble silica and polysilicic acid retained below 8.0 m*M* Si regardless of Ca and Si levels. These facts suggest that when the concentration of soluble silica and polysilicic acid is over 8.0 m*M* Si in the plants, the Si will gel physically, while Ca does not show any influence during the transformation of Si forms.

With increasing Ca levels, the Ca content increased correspondingly (Table 2). However, the number of Si bodies was not affected by increasing Ca content (Table 2, Figs. 1, 2). A big difference in numbers of Si bodies was only shown in the plants containing different Si content. When Si content was low, few Si bodies were observed (Fig. 1), while when Si content was increased to 5.3% Si, many Si bodies arranged between veins regardless of the Ca contents (Fig. 2). This result suggests that the Si content is a determining factor for the formation of Si bodies.

It is clear that in the water-cultured rice plants, Si decreases Ca uptake, while Ca does not affect Si uptake, Si forms or the formation of silica bodies. The discrepancies between our results and the investigation in the field by Tsuno (1984a, b) may result from following two possible reasons.

The first one may be attributable to a pH effect. The application of calcium silicate to soil often cause soil pH to increase. This effect results in stimulating the ammonification of organic nitrogen (Ma and Takahashi, 1991; Miyake and Takahashi, 1992). Extra nitrogen may dilute the Si content via promoted growth. Thus it is possible that the application of silicate fertilizer affect the formation of Si bodies indirectly through nitrogen nutrition.

The second one may be ascribable to the detection method for Si bodies. Si bodies do not distribute uniformly in the leaf blade. The methods used until now such as histochemistry method can not allow one to observe extensive areas of tissues or organs. Thus these methods may result in an error in calculating the numbers of Si bodies because of the variations. However, the soft X-ray irradiation method used in the present study improved these shortage. This method let one to compare the silica bodies in the extensive areas although we can not present all of them in this paper.

Acknowledgements

The authors would like to thank Dr. Yoji Takeoka, Faculty of Agriculture, Nagoya University for his valuable discussion and kind instruction in using SOFTEX.

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Section editor: H. Marschner