

Second

Silicon in Agriculture Conference



August 22-26, 2002

Tsuruoka, Yamagata, Japan



Second
Silicon in Agriculture
Conference

Organized by
Silicon in Agriculture Organizing Committee
and
Japanese Society of Soil Science and Plant Nutrition

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Welcome Message

Welcome to Tsuruoka and welcome to the Second Silicon in Agriculture Conference.

The first conference was held in Florida, three years ago. We are so pleased to have again the chance that many scientists of different disciplines who are interested in silicon meet together and discuss on common concerns.

In this conference, under six sessions, 30 oral and 55 poster presentations will be held. Besides scientific program, we plan a half-day field excursion to have a glance at Shonai plains, one of the most famous rice producing area in Japan, and also we plan two satellite symposia. The one is "Sustainable paddy farming systems in Shonai Plains" under joint auspices of the Shonai Paddy Farming Promotion Society, many farmers in the Shonai area will take in the symposium. The other is an open lecture meeting for Tsuruoka citizen titled "Agriculture and Environment".

In order to hold the conference, we were financially supported by the local government and contributions from companies and individual scientists in this country. We are very appreciative of these supports.

We will be amply rewarded, if you will feel that you are profiting by listening to the presentations of others and by an active exchange of ideas during the conference.

Again, we cordially welcome all delegates and accompanying persons who are kindly participating in the Second Silicon in Agriculture Conference in Tsuruoka, Japan.

A handwritten signature in black ink, reading "Eiichi Takahashi". The script is fluid and cursive, with the first name "Eiichi" and last name "Takahashi" clearly distinguishable.

Eiichi Takahashi

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Scientific Program

Plenary Lectures

- 1 **Silicon in Plant Nutrition** 1
Epstein E
- 2 **An Introduction to the Silicon Research in Japan** 6
Takahashi E

Session 1. Silicon and Plant Diseases (11-19)

- 11 **Silicon in Plant Cell Defenses Against Cereal Powdery Mildew Disease** 15
Zeyen RJ
- 12 **Research of Silicate for Improvement of Plant Defense against Pathogens in Japan** 22
Kanto T
- 13 **Relationship between Susceptibility of Rice Plants to Blast Disease and Leaf Silica Content under Different Atmospheric CO₂ Conditions** 27
Kobayashi T, Ishiguro K, Nakajima T, Kim HY, Okada M and Kobayashi K
- 14 **Silicon Induces a Defense Response to Rice Blast Infection** 29
Rodrigues FA, Benhamou N, Datnoff LE, Bélanger RR, Jones JB and Korndörfer GH
- 15 **Effect of Silicon Nutrient on Bacterial Blight Resistance of Rice (*Oryza sativa* L.)** 31
Chang SJ, Tzeng DDS and Li CC
- 16 **Accumulation of Silicon around Penetration Sites of *Magnaporthe grisea* and Silicon-dependent Promotion of Superoxide Generation after Inoculation on Rice Leaf Epidermis** 34
Maekawa K, Watanabe K, Kanto T, Aino M and Iwamoto Y
- 17 **Influence of Silicon Sources on Foliar Blast, Neck Blast, Grain Spots and Yield of Flooded Rice** 39
Santos GR, Korndörfer GH, Pelúzio JM, Didonet J, Reis Filho JCD and Cesar NS
- 18 **Effect of Silicon Rates on Some of the Most Important Diseases and Yield of Flooding Rice Crop** 40
Santos GE, Korndörfer GH and Reis Filho JCD
- 19 **Pre-treatment of Sargent Crabapple Leaf Discs with Potassium Silicate Reduce Feeding Damage by Adult Japanese Beetle** 41
Shirazi AM and Miller FD

Session 2. Silicon in Soils (21-38)

- 21 **Plant-Available Silicon in Paddy Soils** 43
Sumida H
- 22 **Silicon Status of Selected Louisiana Rice and Sugarcane Soils** 50
Bollich PK and Matichenkov VV
- 23 **Persistence and Availability of Rice Plant Si in Soils** 54
Itoh S and Prakash NB
- 24 **Effect of Ca-Silicate Amendments on Soil Chemical Properties under a Sugarcane Cropping System** 57
Berthelsen S, Noble A, Kingston G, Hurney A and Rudd A
- 25 **Comparison of Three Methods for Evaluation of Available Silicon in Paddy Soils** 58
Kato N, Kumagai K, Nakagawa F and Sumida H

26	Enhancement of Available Silicate in a Soil by Potassium Uptake of some Crop Species	62
	Sugiyama M and Ae N	
27	Silicon Behavior in Terrace Paddy Fields Located in Hilly and Mountainous Regions	65
	Saigusa M, Kobayashi N and Itoh T	
28	Soil Classification on Deficiency of Activated Si	68
	Bocharnikova EA, Calvert DV and Matichenkov VV	
29	Estimation of Critical Level of Available Silicic Acid and Application Rate of Silicate Fertilizer for Paddy Fields	69
	Sato Y, Nagasawa K, Nakagawa F, Morioka M, Kumagai K and Ueno M	
30	The Concentration of Silica in Rice Plant with Reference to the Silica Status in Paddy Field and River Water in Yamagata Prefecture	72
	Kumagai K, Nakagawa F, Morioka M, Nagasawa K, Sato Y, Konno Y and Ueno M	
31	Evaluation of Silicon Sources by Biological and Incubation Test	76
	Korndörfer GH, Pereira HS, Camargo MS and Silva MF	
32	Dynamics of Silicon in Paddy Field with the Reference of the Amount of Silicon in Irrigation Water	77
	Hanzawa K, Suzuki M, Yaginuma T and Nogi T	
33	Effects of Cultivated Conditions on Si Uptake from Subsoil by Rice Plant	80
	Sato N	
34	Enhancement of Phosphorus Leaching by Soybean Cultivation Especially in Brazilian Oxisol	83
	Murakami M and Ae N	
35	Prospectives of Si Fertilization for Reduction of P and N Leaching from Cultivated Areas	86
	Matichenkov VV, Calvert DV and Snyder GH	
36	Effect of Recycling of Plant Silicon on Phosphorus Utilization in Paddy Soils of Karnataka, South India	87
	Prakash NB, Nagaraj H, Vasuki N, Janardhana Gowda NA and Siddaramappa R	
37	Reaction of Phosphate and Silica-alumina Solutions	91
	Nanzyo M	
38	Adsorption of Phosphate onto Silica Gel under Ferric Ion Coexisting Condition	95
	Nagai M, Katayama Y and Hori T	

Session 3. Silicon in Plants (41-62)

41	Silicon and Abiotic Stress	99
	Hodson MJ and Sangster AG	
42	The Role of Silicon in Turfgrass Disease Management	105
	Datnoff LE, Brecht MO, Kucharek TA and Nagata RT	
43	Characterization of Silicon Uptake by Rice	111
	Ma JF and Tamai K	
44	Comparison of Silicon Uptake Characteristics between Two Cultivars of Pumpkin (<i>Curcubita moschata</i> Duch)	114
	Iwasaki K, Matsumura A, Sakai N, Takemoto K and Tanaka S	
45	Silicate Accumulation in Aging Leaves of Dicotyledonous Canopy Trees: Effects of Phylogeny, Climate and Soil Silicate Availability	118
	Kitajima K	
46	Silicon Stimulates Oat Leaf Growth by Modifying Cell Wall Properties	121
	Hossain MT, Soga K, Wakabayashi K, Fujii S, Yamamoto R and Hoson T	

47	The Aqueous Chemistry of Organosilicate Complexes	125
	Knight CTG, Gillson AME, Deguns EW and Kinrade SD	
48	Selective Uptake of Silicate Minerals by Plants	126
	Akagi T, Fu FF and Yabuki S	
49	Difference in Silicon Concentration in Leaves among Tree Species : Implication for Supplement of Mechanical Strength	130
	Nakanishi T, Onishi R and Akagi T	
50	Silicon Forms in Cell Wall of Rice and Tomato Plants	134
	Inanaga S and Chen NC	
51	Organosilicates in Nature	138
	Knight CTG, Gillson AME, Deguns EW and Kinrade SD	
52	Exogenous Silicon (Si) Increases Antioxidant Enzyme Activities and Reduces Lipid Peroxidation in Roots of Salt-Stressed Barley (<i>Hordeum vulgare</i> L.)	140
	Liang Y, Chen Q, Zhang W and Ding R	
53	Influence of Silicon on the Tolerance of the Upland Rice to the Soil Water Stress	152
	Korndörfer GH, Faria RJ and Datnoff LE	
54	Accumulated Silicon in Tropical Forage Species (<i>Brachiaria decumbens</i> and <i>Brachiaria brizantha</i>) and Tolerance to Hydric Deficit	153
	Melo SP, Korndörfer GH, Korndörfer CM, Lana RMQ and Santana DG	
55	Isolation and Characterization of a Rice Mutant Defective in Si Uptake	154
	Tamai K, Wu G, Ichii M and Ma JF	
56	RFLP Mapping of a Gene for Si Uptake in Rice (<i>Oryza sativa</i> L.)	157
	Arimura M	
57	Microarray Analysis of Transcript Profiles in Response to Si Nutrition	160
	Watanabe S, Ohkama N, Hayashi H, Yoneyama T, Yazaki J, Fujii F, Maho K, Yamamoto K, Sakata K, Sasaki T, Kishimoto N, Kikuchi S and Fujiwara T	
58	Fine Structure and Development of Rice Husk Accompanied with Silica Shell	163
	Fujita M and Kawamura K	
59	Some Industrial Utilizations of Rice Husks and Silica-Carbon Shells	168
	Kawamura K, Suzuki M and Fujita M	
60	Increased Co-accumulation of Iron and Silicon may be Responsible for Greener Leaves in Sugarcane Treated with Silicated Amendments	172
	Kingston G, Berthelsen S, Hurney AP, Rudd AV and Noble AD	
61	In Vitro Effects of Potassium Silicate Application and Nitrogen Fertilization on Shoot and Root Growth in <i>Tagetes erecta</i> ‘Lady First’	173
	Shirazi AM, Kwang F, Roberts KC and Jordan SL	
62	Effect of Silicon on Photosynthetic Rate, Chlorophyll Fluorescence and Chlorophyll Content of Tomato (<i>Lycopersicon esculentum</i> Mill.) Plants under Salt Stress	174
	Al-aghabary K and Zhu ZJ	

Session 4. Silicate Fertilizers and Crop Production (71-98)

71	Silicate Fertilizers in Japan	175
	Owa N	
72	Silicon Application in Nutrient Solutions for Horticultural Crops	181
	Voogt W	
73	Role of Silicon in “Potassium Silicate Fertilizer”	191
	Mizuochi T	
74	Si Fertilizers: Past, Present and Future	195
	Matichenkov VV, Bocharnikova EA and Calvert DV	

75	Solubilities of New Silicon Source, Fused Potassium Silicate Fertilizer, Produced from Steelmaking Slag	196
	Yao Y, Takahashi T and Akiyama T	
76	Effect of Silica Gel Application on Growth and Silicon Contents of Rice Seedlings in Nursery Beds with Different Available Silicon Contents	198
	Niizuma S, Kubo S and Morikuni H	
77	Effect of Acidified Porous Hydrate Calcium Silicate Applied in a Nursery Bed Soil on Growth and Nutrient Uptake of Rice Seedling	200
	Saigusa M, Heinai H, Shibuya K, Okazaki H and Yoshida K	
78	Effects of Porous Hydrated Calcium Silicate on Silicon and Nitrogen Concentration of a Young Leaf Blade in a Rice Plant (<i>Oryza sativa</i> L.) as Nutrients Influencing its Resistance to Rice Blast (<i>Magnaporthe grisea</i>)	205
	Yamamoto K, Sibuya K and Saigusa M	
79	Neutralized Autoclaved Aerated Concrete as Silicate Fertilizer for Rice Seedlings	208
	Yoshida K, Okazaki H and Saigusa M	
80	A New Determination Method for the Solubility of Silicate Fertilizers: Examination of Silicate Dissolution using Citrate Buffer Solutions	211
	Tomita M and Furukawa Y	
81	Evaluation of the Solubility of Silicate Fertilizers using an Ion Exchange Resin	215
	Furukawa Y and Tomita M	
82	Silicon in Fertilizers Evaluated by Sodium Carbonate Plus Ammonium Nitrate	219
	Pereira HS, Korndörfer GH, Reis CB and Correa GF	
83	Comparison of Silicon Methods for Fertilizers and Slag	220
	Pereira HS, Korndörfer GH, Moura WF and Correa GF	
84	Role of Silicon on the Production of Rice	221
	Saigusa M	
85	The Role of Silicon on Tropical Crops	227
	Korndörfer GH	
86	Effect of Root and Foliar Applications of Silicon on Growth and Quality of Five-Selected Vegetables in Deep Flow Technique	228
	Jaenaksorn T and Nokyoo W	
87	The Application Method of New Silicon Sources	240
	Mayumi H, Ando H, Fujii H, Hayasaka T, Yokoyama K, Ando T, Inoue T and Honda T	
88	How Dose Silicon Influence on Resistance of Rice Blast Disease?	243
	Hayasaka T, Fujii H, Mayumi H, Ando H and Namai T	
89	Categorization of Soil Type with Reference to Behavior of Silicon in Soil	247
	Ando T, Fujii H, Yokoyama K, Ando H and Mayumi H	
90	Early Growth of Rice Plants as Affected by Silica Gel Application to the Nursery Bed in Different Condition of Wind	249
	Yokoyama K, Fujii H, Hayasaka T, Ando H and Ando T	
91	Effect of Silicon on Growth of Hydroponically Grown Cotton Genotypes	252
	Aziz T, Gill MA, Irshad M, Ahmad I and Akhtar MS	
92	Effect of Si Fertilizers on Citrus	253
	Calvert DV, Matichenkov VV and Bocharnikova EA	
93	Yield Response of Sugarcane from Uptake of Applied Silicon in Australia	254
	Kingston G, Hurney AP, Berthelsen S, Rudd AV and Noble AD	
94	Silicon Content in the Native Brazilian Savanna's Fruits	255
	Korndörfer CM, Ribeiro KP, Salles DRM, Moura WF and Álvares TC	

95	Silicon Uptake by <i>Brachiaria decumbens</i> and its Influence on Rumen Dry Matter Degradability	256
	Korndörfer CM, Korndörfer GH, Abdallal AL and Bueno ICS	
96	The Silicon Role on <i>Brachiaria decumbens</i> Degraded Pasture	257
	Korndörfer CM, Korndörfer GH and Cardoso K	
97	Foliar Silicon Content, Extrafloral Nectaries, Ants and Herbivory at Brazilian Tropical Savannah	258
	Oliveira FR, Del-Claro K and Korndörfer GH	
98	Evaluation of Candidate Silicon Fertilizers	259
	Snyder GH, Rich DW, Barbosa-Filho MP and Elliott CL	

Section 5. Silicon Studies in Asian Countries (101-106)

101	Recent Research of Si-alleviated Stresses in Plants in China	261
	Liang Y, Sun W and Ding R	
102	Research on Agricultural Utilization of Silicon in Korea: Progress and Prospects	262
	Kang YS and Jung YT	
103	Status and Utilization of Silicon in Indian Rice Farming	266
	Prakash NB	
104	Fertilizer Application and Integrated Crop Nutrition Management in Vietnam	274
	Thanh LQ	
105	Soil Sciences Research in Thailand	275
	Sutigoolabud P	
106	Field Excursion Outline of the Yamagata Prefectural Agricultural Experiment Station Shonai Branch	279
	Fujii H	

Conference Schedule

Thursday August 22

- 14:00 — *Registration*
Poster Mounting
- 18:00 — *Welcome Reception*

Friday August 23

- 8:40 — 9:00 *Welcome and Opening Addresses*
Tomizuka Y, The Mayor, Tsuruoka City
Takahashi E, Chairperson,
Second Silicon in Agriculture Conference Organizing Committee

Plenary Lectures

- 9:00 — 9:50 **1** **Epstein E**
Silicon in Plant Nutrition 0.5 m M/L Si; nutr. soln.
- 9:50 — 10:40 **2** **Takahashi E**
An Introduction to the Silicon Research in Japan
- 10:40 — 11:00 Coffee Break

Session 1. Silicon and Plant Diseases

Chairperson **Ishiguro K**

- 11:00 — 11:40 **11** **Zeyen RJ**
Silicon in Plant Cell Defenses Against Cereal Powdery Mildew Disease
- 11:40 — 13:00 Lunch
- 13:00 — 13:40 **12** **Kanto T**
Research of Silicate for Improvement of Plant Defense against Pathogens in Japan
- 13:40 — 14:00 **13** **Kobayashi T**
Relationship between Susceptibility of Rice Plants to Blast Disease and Leaf Silica Content under Different Atmospheric CO₂ Conditions
- 14:00 — 14:20 **14** **Rodrigues FA**
Silicon Induces a Defense Response to Rice Blast Infection
- 14:20 — 14:40 **15** **Chang SJ**
Effect of Silicon Nutrient on Bacterial Blight Resistance of Rice (*Oryza sativa* L.)
- 14:40 — 15:00 Coffee Break

Session 2. Silicon in Soils

Chairperson Kingston G

15:00	—	15:40	21	Sumida H Plant-Available Silicon in Paddy Soils
15:40	—	16:00	22	Bollich PK Silicon Status of Selected Louisiana Rice and Sugarcane Soils
16:00	—	16:20	23	Ito S Persistence and Availability of Rice Plant Si in Soils
16:20	—	16:40	24	Berthelsen S Effect of Ca-Silicate Amendments on Soil Chemical Properties under a Sugarcane Cropping System
16:40	—	17:00		Poster Review (Session 1 and 2)
17:10	—			Poster Presentation

Saturday August 24

Session 3. Silicon in Plants

Chairperson Ma JF

8:30	—	9:10	41	Hodson MJ Silicon and Abiotic Stress
9:10	—	9:50	42	Datnoff LE The Role of Silicon in Turfgrass Disease Management
9:50	—	10:10	43	Ma JF Characterization of Silicon Uptake by Rice
10:10	—	10:30		Coffee Break
10:30	—	10:50	44	Iwasaki K Comparison of Silicon Uptake Characteristics between Two Cultivars of Pumpkin (<i>Curcubita moschata</i> Duch).
10:50	—	11:10	45	Kitajima K Silicate Accumulation in Aging Leaves of Dicotyledonous Canopy Trees: Effects of Phylogeny, Climate and Soil Silicate Availability
11:10	—	11:30	46	Hossain MT Silicon Stimulates Oat Leaf Growth by Modifying Cell Wall Properties
11:30	—	11:50	47	Knight CTG The Aqueous Chemistry of Organosilicate Complexes
11:50	—	13:00		Lunch

Session 4. Silicate Fertilizers and Crop Production

Chairperson Snyder GH

13:00	—	13:40	71	Owa N Silicate Fertilizers in Japan
13:40	—	14:20	72	Voogt W Silicon Application in Nutrient Solutions for Horticultural Crops
14:20	—	14:40	73	Mizuochi T Role of Silicon in "Potassium Silicate Fertilizer"
14:40	—	15:00		Coffee Break
15:00	—	15:40	84	Saigusa M Role of Silicon on the Production of Rice
15:40	—	16:20	85	Korndörfer GH The Role of Silicon on Tropical Crops
16:20	—	16:40	86	Jaenaksorn T Effect of Root and Foliar Application of Silicon on Growth and Quality of Five-Selected Vegetables in Deep Flow Technique
16:40	—	17:00	87	Honda T The Application Method of a New Silicon Sources
17:00	—	17:20		Poster Review (Session 3 and 4)
17:30	—			Poster Presentation

Sunday August 25

Section 5. Silicon Studies in Asian Countries

Chairperson Asanuma S

8:30	—	8:55	101	Liang Y Recent Research of Si-alleviated Stresses in Plants in China
8:55	—	9:20	102	Kang YS Research on Agricultural Utilization of Silicon in Korea: Progress and Prospects
9:20	—	9:45	103	Prakash NB Status and Utilization of Silicon in Indian Rice Farming
9:45	—	10:10		Coffee Break
10:10	—	10:35	104	Thanh LQ Fertilizer Application and Integrated Crop Nutrition Management in Vietnam
10:35	—	11:00	105	Sutigoolabud P Soil Sciences Research in Thailand
11:00	—			Field Excursion
18:00	—			Banquet

Monday August 26

Satellite Symposia

Plenary Lectures

Silicon in Plant Nutrition

Emanuel Epstein*

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The title of this chapter, silicon in plant nutrition, is a contradiction in terms for the great majority of plant nutritional and crop scientists. That is so because silicon is not considered a plant nutrient, or essential element. Yet this present Second Silicon in Agriculture Conference, well attended as was the first, clearly shows that a minority of us, those attending as well as colleagues we know of, holds a different view. When an element is essential for some plants, such as diatoms and Equisitaceae, and when many higher plants respond to its deprivation as to an environmental stress, then surely the near-universal dismissal of the element from consideration must be questioned. That dismissal is due to two factors, one specific, the other general. The specific one has to do with the acceptance by the plant scientific community of a definition of essentiality by which silicon fails to qualify as an essential element, or nutrient. The second, more general point is that for higher plants, any either-or distinction between essentiality of an element and its negation fails for silicon as well as for some other elements.

1. A NEW DEFINITION OF WHAT QUALIFIES AS AN ESSENTIAL ELEMENT, OR NUTRIENT

There did not exist until 1939 a formal definition of what is meant by an essential element, or nutrient (Epstein, 1999, 2000). In that year, Arnon and Stout (1939) published a paper dealing with copper as a micronutrient, and in it promulgated a formal definition of essentiality which has found almost universal acceptance. The considerations given below lead to the conclusion that that definition needs to be discarded.

The definition reads: "an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium."

The first part (a) is simply incorrect, unless the meaning of "deficiency" is intended to be entirely different from its common connotation in plant physiology, but the authors make no such claim for their use of the term. Plants obviously deficient in some nutrient may yet complete the vegetative or reproductive stage of their life cycle. For an example, the present author's Master's thesis dealt with manganese deficiency in fruit trees. The trees in question were beyond doubt deficient in manganese, the evidence being symptomology, manganese content of the leaves, and positive response to manganese application. The trees grew poorly, and fruit yields were low. Yet the trees had grown for years under this deficiency condition. Instances of similar observations could be given for just about any essential element, and for large numbers of both wild and crop species. Criterion (a) of the Arnon-Stout definition is invalid.

Criterion (b) specifies that the deficiency is specific to the element, i.e. no other element can substitute for it. But substitution of another element implies that the element in question is "deficient." Thus criterion (b) merely reiterates (a).

Finally, (c) states that for essentiality to be established, the element must be directly involved in the plant's

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metabolism, and not merely remedy or ameliorate some unfavorable condition of the medium. Yet an account of the discovery of the essential elements (Epstein, 2000) makes it amply clear that the essentiality of many of them was established by growing the experimental plants in solutions rigorously purged of the element, without the discoverers having any knowledge of the direct involvement of the element in the nutrition of the plant.

Thus none of these criteria of essentiality can be sustained, and use of this definition should be discontinued. It has had the untoward effect of relegating all elements not known to be "essential" to the status of nonentities in little need of attention in the study of mineral plant nutrition, and plant biology in general.

Epstein and Bloom (2003) therefore devised a new definition of elements that are essential, or nutrients: An element is essential if it fulfills either one or both of two criteria, viz. (1) the element is part of a molecule which is an intrinsic component of the structure or metabolism of the plant, and (2) the plant can be so severely deprived of the element that it exhibits abnormalities in its growth, development, or reproduction, i.e. its "performance," in comparison with plants not so deprived.

Taking the first of these criteria, no plants can grow without water, and therefore hydrogen and oxygen are essential elements, or nutrients. The same reasoning goes for many other elements: nitrogen (amino acids, etc.), phosphorus (ATP, Rubisco, etc.), sulfur (cysteine, methionine), magnesium (chlorophyll), etc.

The second criterion is to the effect that the plant can be so severely deprived of the element as to show abnormal features of growth, development, or reproduction, or its "performance." It is by this test that the essentiality of most known nutrient elements has been established. It is an operational definition in the sense that it involves experiments designed to minimize the availability of the element under consideration to the plant. Deprivation of the plant of an element does not necessarily imply that the supply of the element in the growth medium is minimized, though that is most often the cause of the deprivation. But deprivation of an element may also be brought about by excessively high concentrations of another element. Thus iron deficiency chlorosis may occur as the result of high concentrations of manganese. Examples abound in the literature of such "antagonisms" or "induced deficiencies" (Marschner, 1995; Epstein and Bloom, 2003).

Soils are not suitable as media in experiments on essentiality, containing as they do at least small amounts of most of the elements in the periodic table of elements. For that reason the technique of solution culture has been indispensable for the discovery of the essentiality of most of the elements now recognized as essential (Epstein, 2000). That is so because unlike that of soils, the composition of nutrient solutions can be precisely controlled by the researcher, who can apply rigorous chemical procedures to purge the solution of the element under consideration, or add an "antagonistic" element.

This discussion of essentiality and its definition was prompted by the failure of silicon to qualify as essential, or as a nutrient, by the 1939 Arnon-Stout definition. Let us now examine silicon in terms of the new definition given above. Silicon does not qualify as a nutrient by the first of the two criteria: it is not "part of a molecule which is an intrinsic component of the structure or metabolism of the plant." (The statement refers to higher plants, not to certain algae or Equisitaceae, whose requirement for silicon is well established.) We do not know, at this time, of any such molecule.

Things are different when we turn to the second criterion, i.e. that the plant can be so severely deprived of the element as to show abnormalities which fail to appear in plants not deprived of it. The volume edited by Datnoff et al. (2001), the proceedings of the first of these conferences on silicon in agriculture, is replete with instances of multifarious abnormalities in plants deprived of silicon to a greater or lesser extent, or conversely, having such abnormalities reversed once the supply of the element is augmented. In this author's contribution to that conference (Epstein, 2001) no less than 13 features of higher plants affected by silicon were listed; there no doubt are more. There is no need to belabor the point at this meeting, for it is attended by scientists who know full well that silicon has a major bearing on plant nutrition and crop performance.

On the basis of these considerations, can it then be concluded that silicon is an essential element, or nutrient, for higher plants generally? It is felt that it is not appropriate to give an unequivocal answer to this

question.

On the one hand, plants grown in conventional nutrient solutions in the formulation of which silicon was not included are in a sense experimental artifacts; there are no plants in either natural or agricultural ecosystems so severely deprived of silicon. On the other hand, there is ample evidence that even in natural and agricultural ecosystems addition of silicon may enhance the growth of plants. Instances range all the way from cucumber to pine (Epstein, 1999). Conversely, plants grown in "minus silicon" experimental solutions may not differ notably in their growth from those grown in "plus silicon" solutions (Ulrich and Ohki, 1956; Wutscher, 1989), even when differences in mineral nutrition are encountered.

If, then, keeping these considerations in mind, the question is asked whether silicon meets the second part of the proposed new definition of essentiality, i.e. whether plants can be so severely deprived of the element as to show abnormalities in their performance, then we are at a loss to come up with an unequivocal yes or no. That being the case, we must ask ourselves whether the very question we are grappling with (essential or not?) is meaningful. Are we really dealing with an either/or proposition? This brings us to the second main argument, which in this case is raised in the context of silicon in plant nutrition but has more general applicability.

2. FUZZY LOGIC OF SILICON IN PLANT NUTRITION

Since the 1960s "fuzzy logic" has entered the realm of science and technology. The awkward term does not mean that the logic is fuzzy but refers to the logic of fuzzy sets. The crucial point is that when it comes to the real world, as against abstractions made about it, we are often at a loss to assign a crisp black-or-white designation. Things often are not black or white, young or old, sufficient or insufficient, entirely distinct. Rather, time and again we are up against situations where such categorizations make little sense because everything in that context is a matter of degree. In fuzzy logic, things, phenomena, qualities, etc. are assigned to sets the elements of which belong to it partially, or to different degrees, ranging from 0 to 1. That degree is called a function, f . Thus in fuzzy logic, a given element or object or quality may not belong, or fail to belong, to a set but belong to it partially. McNeill and Freiburger (1993) consider as one among many examples the question whether Jill, who plays tennis twice a year, is a tennis player. Fuzzy logic does not demand a yes-or-no answer, but can assign a degree to her belonging to the set of tennis players. The book by these authors is a lively introduction to fuzzy logic. Other good accounts are by Kosko (1993), Kosko and Isaka (1993), and Terano et al. (1992). (The latter book was initially published in 1987 in Japanese; fuzzy logic, though developed in the U.S.A., has had much more of an impact in Japan.) Fuzzy logic has already been applied to biological problems, including mineral nutrition (of humans); see for example the papers by Lee et al. (1995) and Wirsam and Uthus (1996).

Let us now consider the essentiality of silicon in the light of fuzzy logic. As already pointed out, a fuzzy set is determined by a function f that assigns a real number ranging from 0 to 1 to each object in a certain domain. In inorganic plant nutrition, setting criteria for the assignment of degrees would amount to specifying such a function, f , whose domain would be the set of mineral elements.

The intent here is to foster the concept of fuzzy logic in the realm of plant nutrition, not to formulate a function f resulting in the assignment of a numerical "degree of essentiality" to silicon, i.e., a certain number between 0 (not known to be essential) and 1 (essential). The "degree of essentiality" of silicon will vary vastly with the genotype, ranging from 1 for the Equisitaceae to little above 0 in, say, the tomato, Lycopersicon esculentum. To describe the status of silicon, then, the term "quasi-essential" (Epstein, 1999) is suggested to signify that according to fuzzy logic, it occupies, for plants in general, a position in the range of 0 to 1.

This proposal complements the newly defined concept of essentiality, while indicating that silicon must be considered as a plant constituent. A quasi-essential element, then, is one not meeting the terms of that definition, but a low availability of which leads often to demonstrable abnormalities in structure or function. Should

silicon turn out to be essential as a micronutrient, the mere designation of it as "essential" would be inadequate, because of the normally substantial (macronutrient) levels of it in plant tissue. It is these quantitatively major amounts that bring about the features referred to above. It would therefore be necessary to designate silicon as an essential micronutrient and a quasi-essential macronutrient.

Is silicon the only element consideration of which gives rise to dissatisfaction with the present simplistic scheme of assigning each element to one of two sets: essential or not-known-to-be-essential? The answer is no. Unlike silicon, chlorine is a known micronutrient, but recent evidence (Engel et al., 2001, and references cited there) suggests that under some conditions some species or cultivars grow optimally only at tissue chlorine concentrations on the order of substantial fractions of 1% of the dry matter, i.e., concentrations characteristic of the macronutrients. For those conditions and genotypes, therefore, chlorine is not just essential as a micronutrient, but in addition, quasi-essential as a macronutrient. Similar considerations apply to sodium.

To sum up, it is now necessary to look upon the designation of an element as essential or not known to be so in a more circumspect way than has been the case. Both qualitative and quantitative considerations prompt a more sophisticated approach. For elements such as silicon, which do not meet clear-cut criteria of essentiality, yet are often necessary if the plant is to achieve normal biological competence, the term "quasi-essential" is fitting. It is urged, finally, that silicon be routinely included in the formulation of nutrient solutions, an ample supply of the element being the norm for most plants in both natural and agricultural ecosystems.

ACKNOWLEDGMENTS

I thank the organizers of this meeting for providing me with the opportunity to present these thoughts to the world's best forum to consider them, this Second Silicon in Agriculture Conference. I am indebted to Jeffrey C. King, of the Department of Philosophy of this campus, for introducing me to fuzzy logic. My colleagues, A. J. Bloom and D. W. Rains, critically read the manuscript. As always, the secretarial staff of my department spared no pains in assisting me in the preparation of this paper.

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An Introduction to the Silicon Research in Japan

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WHY SILICON BECAME A FERTILIZER ELEMENT IN JAPAN ?

The first paper suggested the relation of silicon and rice plants growth was published in 1917. This paper showed that the Si content of rice leaves infected with a blast disease was much less than that of healthy leaves. Following that, several papers reported that high Si rice cultivars were less susceptible to a blast disease and the application of Si gave rice plants resistance to fungal diseases. So the Si research in Japan was began with the study on blast disease. At the time when any fungicide was not available, fungal disease such a blast disease was a serious problem for rice cultivation. The involvement of Si in the disease attracted the interest of plant pathologists.

In 1930s, water culture experiment was applied to examine the effect of Si on the growth of rice plant. Si free water culture caused decreased growth of rice, especially yield was significantly reduced by increasing empty grains. The effect of Si application was more obvious under heavy dressing of nitrogen fertilizers and damage of brown spot and blast was alleviated by Si application.

As such it became clear in the late 1930s that Si is important for healthy growth and high grain yield of rice, but these research achievements from the laboratories were not applied in the field. This is because that lots of Si are present in the soil and it is gradually solubilized with weathering and that Si could also be supplied from irrigation water and compost. It was considered that additional Si was not necessary to be supplied to soils. Furthermore, appropriate Si materials for fertilizer use were not available at that time.

However, as the results of field experiments on "Akioiti" carried out in 1940s, it was recognized that Si in the soil is not always sufficient for the healthy growth of rice (Akioiti means autumn decline of rice plant growth which happens in degraded paddy fields).

Rice is the most important crop for Japan and rice has been cultivated successively on submerged soil from the past. Rice cultivation is characterized by continuous cropping without any injury. This cultivation system is different from that of Europe, in which upland cereals are main crops. Continuous cropping system fits to Japan which has small arable lands per person. In upland field rice cannot be cultivated continuously, but paddy field makes continuous cropping of rice possible.

Compared to upland field, paddy field gets a lot of irrigation water. Total amount of irrigation water during rice growth period reaches 14 thousands tons per hectare. Part of water was evaporated from the leaves and surface of paddy fields, the remaining water is percolated from soil with leaching of soluble components. During waterlogging period, paddy soil is always washed by water, resulting in leaching of both toxic compounds and nutrient bases such as Ca, K, Mg.

In addition, because soil surface is covered by water and subsequently shut off from the air, oxygen in soil is consumed and soil becomes strongly reductive condition. Iron in soil is then reduced and subsequently solubilized, resulting in eluviation of Fe to subsoil. Eluviation of Mn and Si occurs similarly. Sulphate ion in soil is also reduced to H_2S , which is converted to non-toxic FeS with Fe, but in case active Fe is not enough, rice roots will be damaged by H_2S .

Degraded paddy soils are such where nutrients such as bases, Fe, Mn, and Si are leached out and the damage of H_2S to rice roots is serious. In these soils, "Akioiti" of rice easily happens. "Akioiti" is a phenomenon that the growth of rice is good until the end of summer, but thereafter the lower leaves become wilted and

brown spots occur, finally resulting in low yield. These kind of paddy soils can also be used for continuous rice cropping, but the productivity becomes lower year after year.

At the time of food shortage after the 2nd World War, it was an important project to improve degraded paddy soils. Therefore lots of field trials were conducted. As a result, it was found that supplement of Si in addition to bases and Fe is also important for the improvement of the productivity in declined paddy soils. On the other hand, it was indicated that slags, a by-product from iron industry which was developed speedily, could be used as potential silicate fertilizers. From 1952, the Ministry of Agriculture, Forestry and Fisheries of Japan therefore launched out field trials of slag at nation wide agricultural experiment stations.

As a result, beneficial effects of slag were observed at various places. Then in 1955, the Ministry of Agriculture, Forestry and Fisheries decided to put Si in fertilizer list. Slag which has sufficient plant-available Si, but not contains toxic components, was recognized as "silicate fertilizer" and the standard of silicate fertilizer was decided. Slag was also utilized in European and American countries, but it was used as lime materials. Japan is the first country that uses slag as a Si source to crops.

The birth of silicate fertilizer in Japan can be attributed to following reasons:

- 1 Rice, the most important crop in Japan, is characterized by high accumulation of Si in the plant;
- 2 To increase rice yield per hectare in Japan, rice is usually cultivated with high density and heavy application of nitrogen fertilizers;
- 3 There are wide distributions of Si-deficient soils such as degraded paddy soils;
- 4 Cheaper silicate fertilizers like slag are provided from iron industry; and
- 5 Return of rice straw to paddy soils as compost, which was a main Si source, is gradually decreased because of labour shortage.

With general applications of silicate fertilizers, Si researches have been actively carried out in various fields of soil, fertilizer, and plant sciences, as will be introduced in the following items 1,2 and 3.

DEVELOPMENT OF THE SILICON RESEARCH AFTER THE BIRTH OF SILICON FERTILIZER

1 Soil related silicon research

1-1 Investigation of the natural supply amount of Si for rice plant.

Natural sources of Si for rice are from irrigation water and soil. The amount of supply depends on parent materials and geology of river basin.

As mentioned above, paddy soils are irrigated with water at average of 14,000 ton per hectare during the growth period of rice, therefore, Si in irrigation water gives important impact on rice.

Kobayashi(1954)collected 116 samples of rice straw from each part of the country and investigated the relationship between Si concentration in rice straw and Si concentration in irrigation water. A positive relationship between them was found.

He also collected river water used for irrigation all over the country and measured Si concentration in river water. Among 380 rivers investigated, the lowest concentration of SiO_2 is 4.1 ppm and the highest is 61.5 ppm with an average of 21.6 ppm. If 14,000 ton water per hectare is irrigated for rice, it is calculated that an average of 300 kg SiO_2 per hectare is supplied to rice from irrigation water annually.

As shown in Fig. 1, Si concentration in rivers of I, II and V regions is high. This is related to existence of volcanoes in these regions, while river water originated from aqueous rock usually has low Si concentration.

Si supply capacity of paddy soils varies greatly with soils. In 1955, Ministry of Agriculture, Forestry and Fisheries of Japan made a nationwide survey on Si content of flag leaf. According to the data from 37,949 samples, the SiO_2 content of the flag leaf varied widely depending on region. About 5% of samples examined showed the SiO_2 content less than 7.5%, while 9% of samples showed the content higher than 23%. The variation of SiO_2 content in the flag leaf reflect the Si supply power of paddy soil. In region II and V, the Si



Figure 1. Average SiO_2 concentration of river waters in five regions of Japan

Regions		No. of rivers	SiO_2 (ppm)		
			Maximum	Minimum	Average
I	Hokkaido	40	49.7	10.2	27.0
II	16 Prefectures	166	61.5	8.0	21.9
III	6 Prefectures	34	27.7	7.6	14.4
IV	16 Prefectures	76	31.7	4.1	13.6
V	8 Prefectures	64	54.6	10.9	30.9
		380	61.5	4.1	21.6

Average SiO_2 content in flag leaf in the five regions of Japan in 1955

Regions	No. of sampling sites	Average SiO_2 content (%)	Percentage of samples with a SiO_2 content (%) of	
			< 7.5 %	7.6 - 12.5 %
I	278	13.6	2.9	39.2
II	15,902	17.8	1.2	12.3
III	8,059	13.0	9.5	40.3
IV	9,102	13.2	9.6	36.3
V	4,608	17.5	1.2	13.0
Whole land	37,949	15.6	5.4	24.2

content is high, while the Si content in region III and IV is low (Fig.1). This trend is consistent with that for Si concentration in river water investigated by Kobayashi.

In the sites where the SiO_2 content of the flag leaf is lower than 12.5%, there is a possibility of Si-deficiency in soils. The application of Si fertilizers would be effective in these sites which account for nearly 30% of the total.

Imaizumi and Yoshida (1958) summarized Si supply capacity of soils with different parent materials, based

on the data from nationwide agricultural experiment stations. The results showed that volcanic ash and shale originated soils have high Si supply capacity and soils derived from quartz porphyry and granite, and peat have low capacity of Si supply. Furthermore, it was found that new volcanic ash soil is rich in water-soluble Si, but the Si supply capacity of volcanic ash soils decreased with aging because of desilicated process.

They calculated a balance sheet for Si taken up by rice and for Si supplied from irrigation water. The average amount of Si uptake by rice was estimated to be 950 kg SiO_2 per hectare, of which 260 kg SiO_2 was originated from irrigation water, in other words 27% Si in rice is supplied from irrigation water and the remaining Si from soil.

1-2 Evaluation of the Si availability in paddy soils

With appearance of silicate fertilizers, it became necessary to make a criterion for the application of silicate fertilizer in paddy fields.

Acetate-buffer soluble Si method

Imaizumi and Yoshida(1958) made an intensive investigation on Si supply power of paddy soils and Si requirement of rice. They collected various kinds of soil from different area of the country and extracted available Si using different extractants. Meanwhile, they grew rice in a pot filled with different soils. They found that among extractants tested, pH 4.0 acetate buffer solution was most fitted to Si uptake by rice.

Since 1958, the acetate buffer method has been widely used for determination of soil available Si. However, in the late 1970's, it was found that soil available Si extracted by acetate buffer could not reflect Si uptake by rice. In some soils applied with slags, extremely high available Si was detected and differed with kinds of slags. Acetate buffer method is originally developed for evaluation of availability of natural Si in soil and acetate buffer is strong enough to extract Al-bound Si in slags which is not available for rice plant. Therefore, a new method was necessary to be developed for evaluation of available Si of soils which had been applied with slags.

Improvements in the method of evaluation

Several new methods were proposed such as 'incubation under submerged condition method' (1986), 'supernatant method'(1988), 'easily soluble Si method'(1991), 'surface water dissolution method'(1992).

Available Si in paddy soils can be divided into two parts; one is Si solubilized without development of soil reduction and the other is Si solubilized with progress of soil reduction. Temperature and pH of soil also affects the availability of soil Si. Moreover, soil has a capacity keeping Si concentration in soil solution at a constant level when Si concentration in soil solution decreases with Si uptake by rice. Overall, all factors affecting the availability of soil Si should be taken into account for the evaluation of Si availability in paddy soil.

Among new methods listed above, 'incubation under submerged condition method' was adopted in 'Standard Method of Soil Analysis' published in 1986. The procedures of this method are as follows. Taken 10 g of air-dried soil into 100 ml polyethylen bottle containing 60 ml of distilled water. After shaking and degassing, the bottle is sealed and incubated at 40°C for 1 week without shaking. Finally, the Si concentration in the supernatant is determined. In contrast to acetate buffer method, a good correlation was obtained between the Si content of rice straw and soil available Si determined by this method, irrespective of slag application.

Although several methods developed to improve acetate buffer method, these methods including 'incubation under submerged condition method' need a long time for extraction process. A rapid method is requested to be developed for analysis of a large number of samples. Then, recently 'phosphate buffer method'(1996, modified 2000) was proposed as a rapid method.

In this method, it is postulated that rice plant absorbs not only the Si in soil solution but also the Si adsorbed by soil solid phase, and this adsorbed Si can be extracted by phosphate.

The extraction procedure is as follows. To 5g air-dried soil added 50 ml of 40 mM sodium phosphate buffer solution(pH 6.2) and shaking for 5 min. Extraction is conducted at 40°C for 24 hours, then shaking for 5 min.

and is filtrated. The available Si extracted by this method had a good correlation with the Si content of rice straw.

In 2001, this phosphate buffer method adopted as a standard method of soil analysis with incubation under submerged condition method.

2 Fertilizer related silicon research

2-1 Silicate slags

Before silicate fertilizers are applied, the main source of Si supply was rice straw compost. In 1955, when slag appeared as silicate fertilizers, compost was applied at an average rate of 6.5 t per hectare. However, from 1960, the rate of compost application gradually decreased, resulted in 4.5 t in 1970, and less than 2 t in 1990. The decrease of Si supply from compost was partially supplemented by silicate fertilizer.

As a silicate fertilizer, slag must have more than 20% of 0.5 N HCl soluble silica and more than 35% of alkali component, and the content of toxic metal must be under permissible limit. For the fertilizer use 10 kinds of slags are listed.

As an official method for quantitative analysis of slag, Si is extracted with 0.5 N HCl at a ratio of 1:150 with shaking. However, available Si measured by this method is sometimes not correlated with Si uptake by rice.

Takahashi(1981) used various kinds of slags with different chemical compositions and cooling methods and compared Si uptake of rice from slag-applied soils in pot experiment. The absorption percentage of Si from the slags differed widely with the kinds of slags, ranging from 73% to 26%. The value is lower in acidic slags and slags with coarse particles has lower absorption percentage of Si than those with fine particles and slowly cooling. After some investigations it was found that 4% ammonium citrate at pH 4.5 is more suitable for evaluation of available Si in slags. However, the problem is for slags from phosphorous production, which has high availability for rice, the solubility was not high using this method.

Kato and Owa(1996) investigated the dissolution process of slags under rice growing paddy field condition and proposed a new extraction method for the evaluation of Si availability in slags. In this method, the slags were dissolved in water with the addition of a weakly acidic cation exchange resin. The pH of the extraction was well controlled between 6 and 7 which is the pH of submerged paddy soil during the extraction, and the Si dissolution from the slags was enhanced by the addition of resin. The new method showed a good correlation with Si recovery by rice.

Numerous trials on application effects of calcium silicate were carried out systematically at agricultural experiment stations all over the country. The effects of application were generally larger in degraded paddy soils and peaty paddy soils. One important effect was that silicate fertilizer application raised the optimum level of nitrogen fertilizers.

The application rate of silicate fertilizers is based on Si removal by rice, usually 1.5 to 2.0 ton per hectare. At the end of 1960s, the annual consumption of calcium silicate reached 1.3 million tons. If these fertilizers were applied to degraded paddy fields estimated to 690 thousand hectares, the application rate was 1.9 tons per hectare.

However, owing to a change in the agricultural situation, consumption of silicate fertilizer also decreased from 1.3 million tons in 1968 to 0.28 million tons in 1998. A balance sheet for Si output and input was calculated by Calcium Silicate Fertilizer Association. The result shows that the revenue and expenditure of Si is in deficit in paddy soils of whole country, and the Si exhausted was as high as 0.99 million tons per year, which is equal to 465 kg SiO₂ per hectare in average (Fig.2).

This fact suggests that Si supply power of paddy soils is reducing year by year. From the data estimated for each prefecture, the deficit of Si in Yamagata, Akita and Niigata, the main rice producing prefectures, is higher than that in the other prefectures.

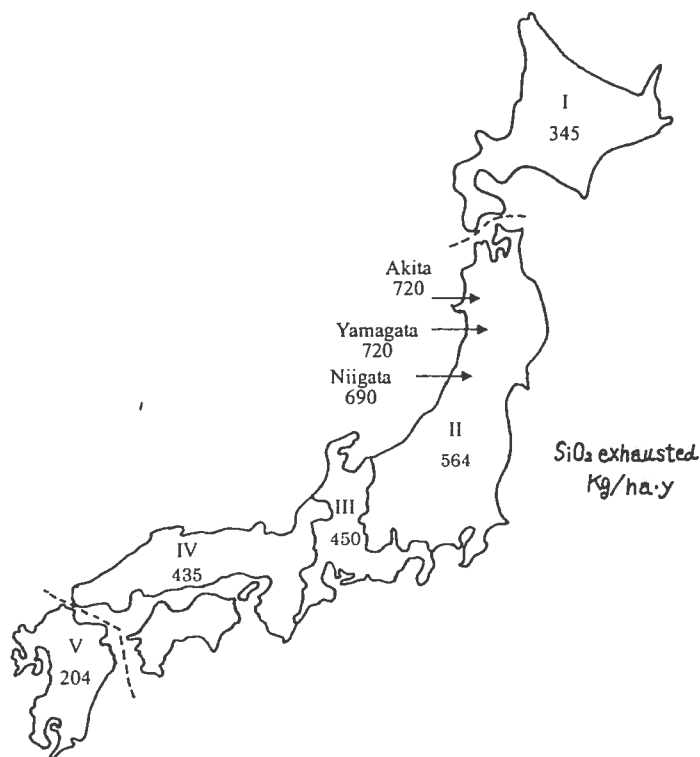


Figure 2. Balance sheet of SiO_2 in paddy field in 1987

Total area harvested (10^3ha)	Total Rice Yield (10^3t)	Total SiO_2 taken up by rice plant (10^3t)	SiO_2 (10^3t) supplied from				SiO_2 exhausted from soil (10^3t)
			Silicate Fertilizer	Compost	Irrigation water	Total	
2,122.8	10,570.9	2,114.2	297.6	212.3	618.3	1,128.2	986.0

2-2 Silicate fertilizers other than slags

Besides slags there are several kinds of fertilizers for Si supply. These are fused magnesium phosphate, potassium silicate, porous hydrate calcium silicate and silica gel.

Fused magnesium phosphate appeared in 1950 as a dried phosphate fertilizer and contains 16-26% acid soluble silica. Products containing more than 20% soluble silica are recognized as silicate fertilizer. Annual consumption at present is around 0.2 million tons.

Potassium silicate appeared as a slow-release potassium fertilizer in 1978. Fly ash which is produced from coal power plant is used as silicate material. According to the official standard of commercial fertilizers, the fertilizer must have more than 20% of citrate-soluble potash and 25% of 0.5 M HCl soluble silica. Silica in this fertilizer is proved to be effective in rice in increasing resistance to fungal disease and insect damage. At present annual demand of this fertilizer is 50,000 tons, and more than 90% is used for rice.

Porous hydrate calcium silicate produced from quick lime, quartz and cement which are reacted under 180°C , 10 atm pressure is used for wall material in construction. As the standard for wall material is very strict, quite high percentage of out of standard product is resulted. To utilize these waste wall materials, it was considered to be produced as a fertilizer. In 1993, "a light porous cement powder fertilizer" was recognized as a new silicate fertilizer and standardized that the fertilizer must have more than 15% of 0.5 N HCl soluble silica and more than 15% of base component.

Silica gel is usually used as a desiccating agent, while it was found that silica gel is suitable for a Si source in nursery bed. Different from usual silicate fertilizers, silica gel does not cause pH increase which brings diseases to rice seedlings. As it is fitted for nursery use, official standard for this fertilizer was decided in 1999. Silica gel fertilizer must have more than 80% of 0.5 N sodium hydroxide soluble silica because silica gel is not dissolved in hydrochloric acid.

3 Plant related silicon research

3-1 Characteristics of Si accumulators and their distribution in plant kingdom

It is already known that there is a group of plants with high Si content such as rice, bamboo, scouring rush etc and Si has some beneficial effects on these plants. Takahashi *et al* (1976-1981) tried to characterize Si accumulating plant species in plant kingdom. They collected nearly 500 species of plants ranging from Bryophyta to Angiospermae and analyzed their mineral composition.

The analytical data showed that there is much difference of Si content among the plants tested and plants with high Si content have a tendency to be low in Ca and B content. To discriminate Si-accumulator and non Si-accumulator, two criteria - Si content and Si/Ca ratio - were proposed.

If average Si concentration in soil solution is 10 ppm and average water requirement of plant is 500 ml, the Si content of plant which uptake Si passively will not exceed 0.5% on dry weight basis, and plants with Si content above 1% (twice the critical value) in addition to Si/Ca ratio above 1 (Ca content should not exceed Si content) are defined as Si accumulator.

Based on this criteria, distribution of Si accumulators in phylogenetic tree is investigated. High Si accumulation can be seen in Bryophyta, and Lycopside and Sphenopsida of Pteridophyta, but this accumulation initiates to be lost from Pteropsida in Pteridopyta to Gymnospermae and Angiospermae. However, high Si accumulation appears again in Cyperaceae and Gramineae in monocots. The distribution of Si accumulator is fitted well to the phylogenetic tree.

3-2 Difference in Si uptake mode among plant species

Solution culture experiments revealed that there are three kinds of Si uptake mode - active, passive, and rejective. For instance, rice plant decreases Si concentration in solution remarkably suggesting active uptake, while tomato plant increases it (rejective uptake) and cucumber does not change Si concentration so much (passive uptake). These differences in Si uptake are lost when the roots are removed, and all the excised tops uptake Si passively. These results indicate that the different Si uptake systems are operated in the root and these systems will cause a large variation in the Si content of the top.

3-3 Characteristics of Si uptake by rice

Several experiments using metabolic inhibitors suggested that the Si uptake by rice requires ATP and partially involves metabolism related to P uptake. Although rice roots play an important role in active uptake of Si, transpiration plays a certain role in translocation and accumulation of Si to the top of rice. It is also found that the chemical form of Si absorbed by rice is mainly nondissociated silicic acid molecule and it works favorably for Si uptake by rice. In the case of silicate anion, Si uptake by rice decreases and is depressed by phosphate and nitrate anions coexisting in solution.

3-4 Utilization of Ge for the study on Si uptake mechanism

Ge is a cognate element of Si and has similar chemical properties with Si. The response of plants to Ge was compared with that to Si in a series of studies. As the results, it is cleared that although physiological effects of Ge are different from those of Si within the plants, there is a similarity in the uptake by roots between Si and Ge. It is a powerful tool to use radioisotope to investigate the behavior of an element in plants. The radioisotope of Si only has a half life of 2.62 h, which is too short to be used, however, Ge has a radioisotope ^{68}Ge

with a very long half life of 282 days. Utilization of ^{68}Ge will be very useful for studies on Si uptake mechanism.

3-5 Functions of Si in plant growth

A lot of soil and water culture experiments have been carried out to examine the effects of Si on the growth of rice plants. Carefully conducted Si free culture revealed that rice plant can grow without Si, however, with increasing Si supply the growth, especially grain yield is improved. Moreover, the effect of Si becomes evident under various biotic and abiotic stresses. Si increases resistance to fungal diseases such as rice blast and suppresses insect pest such as stem borer. Si has functions in improving the resistance of rice to nutrient imbalance stresses such as N excess, P deficiency, and toxicities of Fe, Mn, Al etc. All of these effects are finally attributed to deposition of silica on the tissue.

Si deposited on the surface of leaf blade of rice keeps the leaf erect. Therefore, Si may stimulate canopy photosynthesis by improving light interception. This is particularly important in dense plant stands and when nitrogen fertilizers are heavily applied to minimize mutual shading.

Si deposited beneath the cuticle of the leaves decreases the transpiration from the cuticle. This function of Si contributes to improve the water economy of rice and also reduces water stress which causes stomata closure and therefore decreases the photosynthetic rate.

Under P deficient condition Si has the effect of improving grain yield by promoting translocation of absorbed P to the panicle. Si decreases Fe and Mn uptake by rice, thus P/Fe and P/Mn ratio in the plant increases which facilitates the mobility of P.

Si enhances the oxidizing power of the rice roots. It is probably due to promoting oxygen supply from the shoot to the root, resulting in oxidation of Fe from ferrous to ferric on the root surface, thereby suppressing excess uptake of Fe and translocation of Fe from the root to the shoot. Mn also seems to be oxidized on the root surface, which decreases the Mn content in the top.

The alleviative effect of Si on Al toxicity is also reported. Ionic Al inhibits root growth and nutrients uptake but silicic acid interacts with Al ion and converts it into a non-toxic form.

Besides rice plant, the effect of Si was observed in some upland crops. Si content in cucumber leaves is fairly high under a plenty of Si supply, and Si supply is very effective to prevent cucumber from powdery mildew infection.

Furthermore, it is revealed recently that Si alleviates Mn toxicity in cucumber plants. Cucumber in Japan has been produced by grafting onto pumpkin stocks. Grafting on the bloom-type stocks which produce a white powder of silica (blooms) on the fruits surface has been replaced by grafting on the new bloomless-type stocks, because cucumber fruits with blooms are not preferred by consumers in Japan. However, occurrence of Mn toxicity in cucumber increased by using bloomless-type stocks and this has been attributed to rejective uptake of Si by the new pumpkin stock.

Si supply can prevent Mn toxicity in the bloom-type cucumber but can not in the case of the bloomless-type cucumber. The results of experiments suggest that alleviative effect of Si on Mn toxicity is due to the ability of the bloom-type cucumber to better uptake Si and translocate it to the shoots and there Si converts Mn in a metabolically inactive form.

The effect of Si on the growth of tomato which is a rejective type was also reported. Abnormal symptoms appeared at the flowering stage of Si free cultured tomato. The symptoms begin with the depression of meristem tissue growth, deformation of young leaves near the top and bore malformed fruits or not fruits. Marschner et al argued that the symptoms may be caused by Si deficiency-induced Zn deficiency because more P was taken up in the absence of Si and excess P may precipitate with Zn, resulting decreased availability of internal Zn.

COCLUSION AND PERSPECTIVE

The Si content of plant is remarkably different among species and the cause of the difference is due to the characteristics of root. Si is not an essential element but Si is a typical beneficial element especially for Si accumulators. In plants, absorbed silicic acid polymerizes and deposits as silica gel outside the cell, and does not affect metabolic processes inside the cell. Therefore Si does not cause excess injury in plant. In this respect Si is an unique element.

Plants use Si as a protective material against biotic and abiotic stresses. Si plays an important role in maintaining healthy growth of plant. It can be expected that the application of non-toxic Si reduces the rate of pesticide and fungicide application, and subsequently reduces their environmental pollution.

To utilize more the beneficial effects of Si, we should try to promote Si fertility of soil which is now declining in Japan. Another interesting approach is to genetically modify the crops of Si-excluder to be able to utilize Si in the soil. One of the ways is probably to introduce Si transporter of rice into these crops.

The significance of Si in plant growth has been underestimated because Si is abundant in soil. However, with changes of global environment, the role of Si will become more and more important for healthy and sustainable production of crops in future.

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Session 1

Silicon and Plant Diseases

Silicon in Plant Cell Defenses Against Cereal Powdery Mildew Disease

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Many Gramineae need silicon (Si) to achieve maximum health and disease and pest resistance. This is especially true in cereals like barley and wheat. Elevating Si levels in deficient soils, or in hydroponic growth solutions, increases powdery mildew disease resistance. Conversely, Si deprivation in barley and wheat leads to poor growth habit and increased powdery mildew susceptibility [Germar, 1935; Jiang 1993]. During cell defense responses to powdery mildew attack, Si accumulates specifically in epidermal cells. This accumulation is temporally and spatially coincident with processes acting during penetration resistance, and with fungal-induced, programmed epidermal cell death [Kunoh and Ishizaki, 1976; Kunoh, 1990; Zeyen *et al.* 1983].

POWDERY MILDEW FUNGUS INFECTION PROCESS

The cereal powdery mildew fungus *Blumeria graminis* infects and colonizes living epidermal cells. Study of the infection process is aided by the fact that *B. graminis* develops in a synchronized manner. Within minutes of landing on a cereal plant's epidermis, a spore (conidium) produces a 'primary germ tube' (PGT) whose role is surface attachment, partial cell wall penetration, water uptake and environmental sensing (Fig. 1, A). When the PGT senses a favorable surface, a second germ tube forms (i.e., the appressorial germ tube - AGT). The AGT terminus flattens into a structure called an appressorium (8-9 h post inoculation - h p.i.) (Fig. 1, A). From its underside is produced a small penetration peg that attempts to penetrate the epidermal cell wall. If AGT penetration succeeds (10-14 h p.i.), the peg begins to differentiate into a nutrient-absorbing structure called a haustorium. *B. graminis* can only use nutrients from living epidermal cells to produce hyphal growth. Within 5-7 days *B. graminis* forms a powdery appearing mass that produces new conidia. Thus "powdery mildew" becomes visible to the naked eye on infected leaf and stem surfaces [Aist and Bushnell, 1991].

PLANT CELL DEFENSES

Epidermal cells have two active defenses against *B. graminis* attack. The first is penetration resistance. The second is 'hypersensitive' epidermal cell death (HR)—the programmed 'suicide' defense mechanism that may occur when penetration resistance fails (Fig. 3, A-D). Following HR, *B. graminis* stops growing, presumably from starvation because nutrient uptake requires participation of a living epidermal cell [Aist and Bushnell, 1991]. During both active cell defenses Si and other chemical elements are spatially and temporally accumulated.

PENETRATION RESISTANCE

Complex physiological processes are associated with epidermal cell penetration resistance. All these processes begin with signaling events caused by *B. graminis* germ tube contact, and include fungal-elicited transcription and expression of plant defense response genes. These induced genes encode for proteins needed

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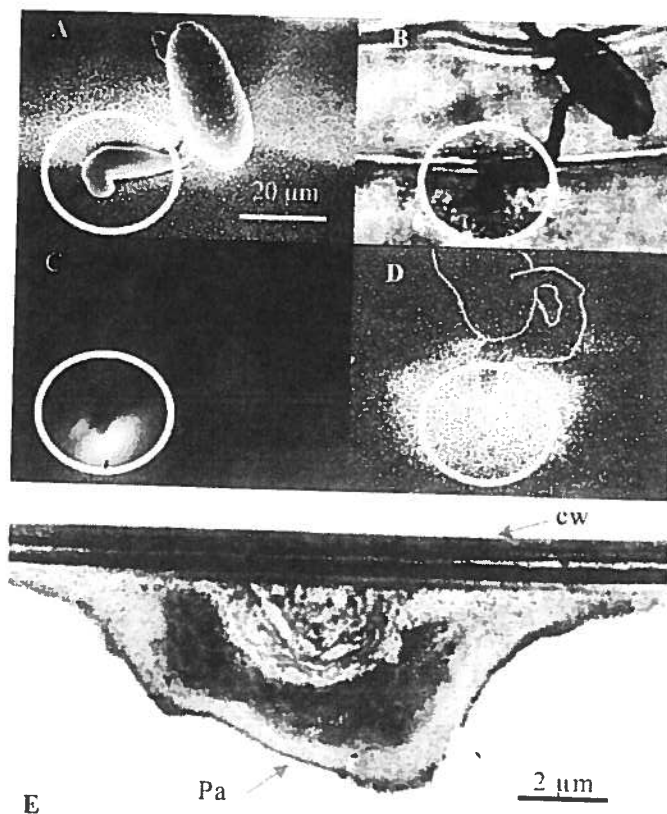


Figure 1. Germinated spores (conidia) of the powdery mildew fungus, *Blumeria graminis*, attacking barley epidermal cells. Circles are epidermal cell response zones where localized cell responses occur. (A) A scanning electron photomicrograph of a conidium of *B. graminis* attacking a barley leaf epidermal cell ((16 h post inoculation (p.i.); a frozen-hydrated specimen)). The short primary germ tube (upper left) emerged shortly after spore contact with the barley epidermal cell. The hook-shaped appressorium at the terminus of the second germ tube is seen inside the response zone circle. (B) A bright field light micrograph at 16 h p.i. showing the epidermal cell cytoplasmic aggregate which is depositing materials on the inside of the epidermal cell wall. (C) An incident fluorescent photomicrograph using UV light excitation (20 h p.i.) causing autofluorescence of phenylpropanoid-based compounds in the response zone and in a young papilla. (D) An X-ray dot map of insoluble Si accumulation in the response zone at 24 h p.i. (E) Transmission electron micrograph of a cross section through a papilla (Pa) deposited on the inside surface of an epidermal cell wall (cw) in the response zone (attacking appressorium not visible-24 h p.i.). The electron density of the cell wall (cw) is partially due to phenylpropanoid-based autofluorogenic compounds and amorphous Si. The papilla matrix is also infused with the phenylpropanoid compounds and with antimicrobial proteins. When the papilla has matured it contains insoluble Si.

for cell defenses and antifungal activity [Thordahl-Christensen *et al.* 2000].

During penetration resistance, microscopically visible events occur in a highly localized 'response zone' surrounding and subtending the attacking *B. graminis* appressorium (Fig. 1, A-D). The first occurs at 9-16 h p.i. and is a vigorously moving aggregation of epidermal cell cytoplasm, the 'cytoplasmic aggregate' (Fig. 1, B). Coincidentally, staining properties in the response zone of the epidermal cell wall change, from acidic to basic due to a localized change in cell wall pH, and at the same time localized phenylpropanoid autofluorogens are evident in the response zone (Fig. 1, C). There is also a localized accumulation of H_2O_2 , and rapid cross-linking of epidermal cell wall proteins in the response zone. Meanwhile, the cytoplasmic aggregate deposits callose (β 1-3 glucans) on the inside surface of the epidermal cell wall. The resultant dome-shaped deposit is called a papilla (Fig. 1, E), and its callose matrix is infused with phenylpropanoid autofluorogens (Fig. 1, C) and with antimicrobial enzymes and proteins [Thordahl-Christensen *et al.* 2000; Zeyen *et al.*, 2002].

SILICON AND OTHER ELEMENTS INVOLVED IN PENETRATION RESISTANCE

Silicon (Si^{++}). Research using scanning electron microscopes (SEM) equipped with X-ray microanalysis (XRMA) and cell sectioning or microsurgery established that Si, and to lesser extents other inorganic elements, are highly localized in epidermal cell response zones (Fig. 1, D). Zeyen *et al.* [1993] used SEM-based XRMA to determine the occurrence and timing of soluble and insoluble chemical elements in response zones of a genetically defined barley-*B. graminis* interaction. They used frozen-hydrated and freeze-dried leaf specimens to maintain *in situ* locations of soluble elements, and compared results to acetic acid-ethanol fixed and critical point dried leaf tissues where soluble elements were removed (Fig. 2). Soluble Cl, K, Mn, Ca, and Mg were localized in response zones during and after papilla deposition (16 h p.i. and 24 h p.i. respectively). Si was abundant in response zones (10 times normal cell levels) and remained soluble during papilla deposition (16 h p.i.), but Si was bound and insoluble in matured papillae (24 h p.i.). By 24 h p.i. insoluble Si was found at 100 times the levels found in unattacked cells, or outside the response zone of the attacked cell (Fig. 2). The abundance of insoluble Si in matured papilla at 24-48 h p.i. agreed with previous findings [Carver *et al.* 1987; Kunoh and Ishizaki, 1976; Kunoh, 1990].

Active processes in the cytoplasmic aggregate presumably transport and concentrate soluble Si specifically

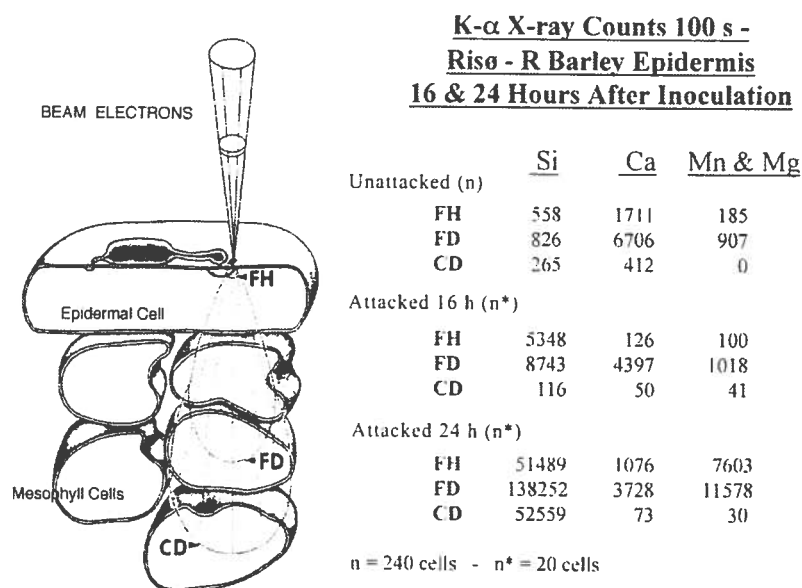


Figure 2. Drawing of a portion of a barley leaf with a powdery mildew fungus germling attempting to penetrate, and K- α X-ray counts for Si, Ca, and Mn-Mg at 16 and 24 h. Shown are maximum depths a 12 kV electron beam would penetrate and excite elemental specific X-rays, as a result of three different specimen preparation procedures. In frozen-hydrated preparations (FH) maximum penetration depth was 3.4 μ m, while in freeze-dried (FD) preparations it was 68.1 μ m. Both FH and FD preparation processes preserve soluble elements. The critical-point dried (CD) specimens were fixed in acetic acid-ethanol and then ethanol and liquid CO₂ substituted before high pressure sublimation. These processes cause loss of soluble elements and mass. Electron beam penetration was 102.2 μ m. Tear drop shaped areas represent spread of electrons during specimen interaction and where elemental-specific X-rays could have been generated.

The data for FH and FD preparations include both soluble and insoluble inorganic chemical elements, while that from CD preparation represents only insoluble elements not lost during the fixation and critical point drying processes [Zeyen *et al.*, 1993]. Data for unattacked, control cells are from cells adjacent to attacked cells. At 16 h p.i. epidermal cytoplasmic aggregates are depositing materials onto the inner surfaces of epidermal cell walls, and Si, Ca, Mn & Mg in the response zone are soluble. At 24 h p.i. a matured papilla exists beneath the fungal attack zone. At this time Ca, Mn & Mg continue to be soluble while most Si has become insoluble.

in the response zone. If so, these processes must prevent polymerisation of Si before transport across the plasma membrane, before transport into the epidermal cell wall and before transport into the papilla. Putative Si transport processes would likely involve microtubules, microfilaments and perhaps the endoplasmic reticulum, all of which are components of cytoplasmic aggregates [Jiang, 1993; Zeyen, 2002]. Evidence supporting this hypothesis was produced by Zeyen and coworkers and reviewed by Zeyen *et al.* (2002). The evidence included accumulation of electron-dense materials (perhaps lipid-Si complexes) in barley epidermal cell cytoplasmic aggregates subtending the response zone before papilla deposition, and a report by Akutsu *et al.* [1980] who found Si-rich vesicles in the cytoplasmic aggregate during papilla deposition.

Jiang [1993] experimentally interrupted papilla deposition in Si-supplied and Si-deprived barley leaves and determined that the main contribution of Si to enhanced penetration resistance occurred early, before papilla deposition was complete. Since accumulated Si is soluble at this time it was considered unlikely that Si acted simply by forming an insoluble, opaline silicate barrier in the response zone of the epidermal cell wall. These results agreed with a previous observation by Carver *et al.* [1987] who found a degree of penetration resistance without evidence of insoluble Si accumulation in the response zone. Presumably, in first-formed barley leaves, soluble Si and other processes produce an initial level of penetration resistance in the response zone, and this occurs before papilla deposition is completed. Matured papillae with their insoluble Si deposits then make a further, final contribution to overall penetration resistance [Jiang, 1993].

While Si is not biochemically inert [Epstein, 2000], the mechanism(s) by which Si acts and the biochemistry it affects are poorly understood. In oat, Si levels are not strongly associated with *B. graminis* penetration resistance, and insoluble Si accumulation was found to be independent from phenylpropanoid-based autofluorogen accumulation (Fig. 1,C) [Carver *et al.*, 1998b]. However, in oat Si deprivation affected levels of phenylpropanoid-based autofluorogens localized in the response zone. Silicon deprivation caused oat epidermal cells to increase levels of localized phenylpropanoid-based autofluorogens in response zones, and this increase was associated with higher frequencies of *B. graminis* penetration resistance. Further investigation determined that Si deprivation had caused a significant rise in phenylalanine ammonia-lyase activity, the gateway enzyme for phenylpropanoid biosynthesis. Thus in oat the potential negative effects of Si deprivation on penetration resistance were compensated for by the energy-expensive process of phenylpropanoid biosynthesis [Carver *et al.*, 1998a; Zeyen, 2002].

Calcium (Ca^{2+}). Soluble Ca also accumulates in epidermal cell response zones surrounding and subtending *B. graminis* appressoria (Fig. 2). Calcium is essential for plants and is vital to signal transduction and amplification, where it often acts as a second messenger. Indeed, Ca-modulated proteins regulate an astonishing variety of cell processes, including the cytoplasmic aggregate response that eventually deposits the papilla. In barley coleoptiles, excess Ca caused increased *B. graminis* penetration resistance while Ca deprivation delayed cytoplasmic aggregate formation and allowed for greater *B. graminis* penetration success [Aist and Bushnell, 1991; Kunoh, 1990]. In addition, Ca may more directly and specifically affect deposition of the papilla's callose matrix because Ca is required for Ca-dependent callose synthase [Zeyen *et al.*, 1993; Zeyen *et al.*, 2002].

Manganese (Mn^{2+}) and *Magnesium* (Mg^{2+}). Manganese and Mg also accumulate in epidermal cell response zones surrounding and subtending *B. graminis* appressoria (Fig. 2). They are essential for plants and have definite roles in cell defense. Manganese is a component of the defense-related enzymes superoxide dismutase, certain Mn-acid phosphatases and Mn-peroxidase. Furthermore, Mn stimulates H_2O_2 production, and activates and regulates portions of phenolic and lignin biosynthesis via dehydrogenases, transferases, hydroxylases, decarboxylases, and peroxidase. It is also needed for nucleic acid synthesis, and activates other crucial plant enzymes including indole acetic acid oxidase. In its role as an enzyme activator, Mn can often be substituted for *in vitro* by Mg. Magnesium's major role is as the chelated ion in ATP and other triphosphonu-

cleotides. The Mg/ATP complex is the sole biologically active form of ATP [Zeyen *et al.*, 1993]. Thus, finding Mg in cytoplasmic aggregates would be expected, because aggregates contain abundant mitochondria required for metabolic activity that is necessary, both before and during papilla deposition [Zeyen *et al.*, 2002].

HYPERSENSITIVE CELL DEATH AND THE ROLE OF SILICON

If *B. graminis* penetration succeeds, the second line of defense is HR cell death (Fig. 3, A-D). The HR response is considered a form of programmed cell death. In some, but not all respects, HR resembles apoptosis in animal cells. *B. graminis*-induced HR in barley may begin as early as 15-18 h p.i. The HR death process may require less than 30 min. During HR the epidermal cell's protoplasm disintegrates, its nucleus condenses and the cell loses turgor pressure and collapses (Fig. 3, A-B). Collapsed, HR dead epidermal cells are totally autofluorescent (Fig. 3, C) and by 24 h p.i. are filled with insoluble Si. (Fig. 3, D) [Zeyen *et al.*, 1983]. Neighboring unattacked epidermal cells are unaffected and are not filled with insoluble Si.

Although the details of Si accumulation during or following HR are unknown, the process superficially resembles differentiation of normal silica cells found in wheat and barley epidermis. As part of their differentiation silica cells are programmed to undergo protoplasmic disintegration and their nuclei collapse. Then their cell lumen becomes filled with solid, hydrated amorphous silica. In silica cells, Si accumulation is initiated in a zone next to the cell wall and proceeds inward, trapping cytoplasmic debris in vesicles at the center of the deposit [Blackman, 1969].

Koga *et al.* [1988] investigated the relationship between Si accumulation and HR in barley. They used liquid-fixed, critical point dried specimens and discovered that when HR began, at 15 and 18 h p.i., insoluble Si was not detectable. However, insoluble Si accumulation was associated with intense phenolic-based autofluorescence throughout the epidermal cell, indicating that cell death had occurred (Fig. 3, C-D). The fact that

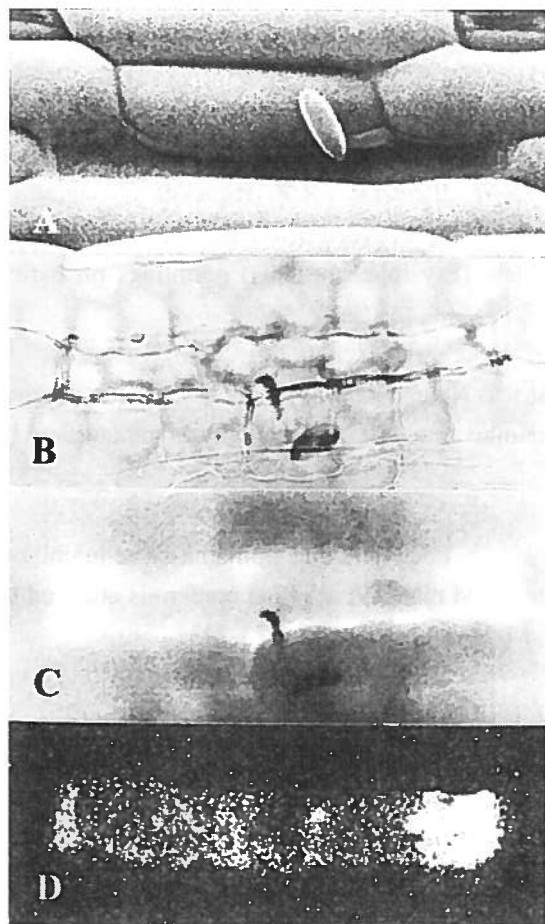


Figure 3. (A) A scanning electron micrograph of an epidermal cell that has undergone the hypersensitive cell death defense (HR) due to *B. graminis* attack. A frozen-hydrated specimen 24 h p.i. (B) A bright field light micrograph of the HR response at 24 h p.i. A fixed (acetic acid-ethanol) and stained (Trypan Blue) specimen. (C) An incident fluorescent photomicrograph using UV light excitation (24 h p.i.) of the same HR event as in B. Note, the whole-cell autofluorescence caused by accumulation of phenylpropanoid compounds. (D) An X-ray dot map of insoluble Si distribution in a HR dead epidermal cell at 24 h p.i. The cell was fixed and critical point dried.

insoluble Si was found after HR, suggests a passive or auto catalytic process for Si polymerization. Therefore, Zeyen *et al.* [1983] and Koga *et al.* [1988] suggested that Si is secondary to HR. Silicification of HR epidermal cells appears to be a way of encasing and thus neutralizing dead cell contents, while providing strength and integrity to the remaining epidermis.

IN CONCLUSION

At the cell level, Si is the most abundant chemical element found in response zones associated with penetration resistance. Its abundance exceeds even that of carbon. Presumably, active processes in the cytoplasmic aggregate beneath the response zone selectively accumulate and concentrate soluble Si while preventing polymerization prior to transport across the plasma membrane into the epidermal cell wall and later into the papilla. Accumulation of Si in whole cells that have undergone HR appears to be a method of encasing and isolating the contents of the dead cell while providing strength and ensuring the integrity of the epidermis.

Recent discoveries involving Si-containing proteins, Si transport proteins and Si responsive mRNAs [Epstein, 2000], could provide the tools and means for designing experiments to elucidate and perhaps manipulate the mechanism(s) involved in selective Si accumulation.

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stabilize protein conc. of def resp.
signaling pathways leading to greater
efficiency (transductions)
effect nuclear pores allowing signal molecules
to enter faster
effect transcription factors.

Research of Silicate for Improvement of Plant Defense against Pathogens in Japan

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INTRODUCTION

Silicon is not a necessary element for higher plants, but in Japan, many past researchers discovered that silicon is effective for defense against pathogens and insects. Suppressive effects of silicate have been investigated in Japan since the 1920s.

Perhaps the first report was Kawashima's original. It showed that silicate significantly suppressed rice blast. Rice plants can absorb large amounts of silicate and prevent blast disease. After World War II, research of silicates against pathogens made great progress. For rice disease, it is clear that silicate suppressed not only blast damage, but also brown spot, stem rot, and sheath brown rot. In the 1980s, Miyake and Takahashi reported that silicate was also effective against cucumber powdery mildew and fusarium wilt.

In 1991, Hazama *et al.* reported that silicate suppressed corynespora leaf spot on cucumbers. Finally, we discovered in the 1990s that silicate suppresses strawberry powdery mildew. Silicate is good for plant health. Silicate increases some plant yields.

This review shows how silicate has been used to prevent pathogens. We show, where possible, the mechanism of silicate protection against pathogens.

AGAINST RICE DISEASE

(1) Rice Blast (*Magnaporthe grisea*)

In 1927, as shown in Fig. 1, Kawashima reported that silicate suppressed neck rot significantly. In this

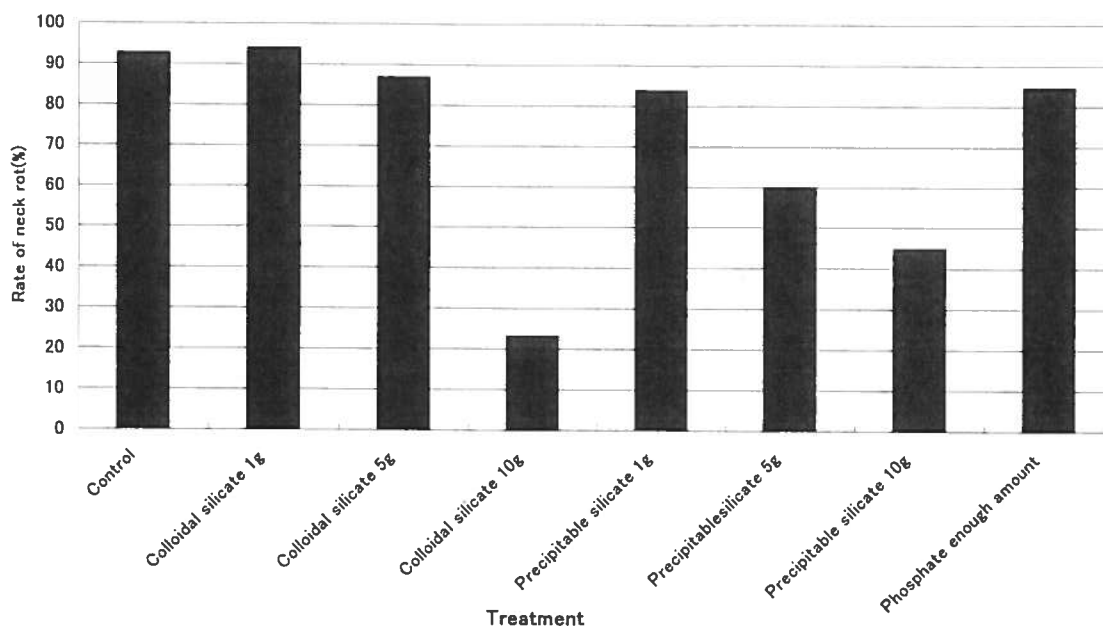


Fig. 1 Suppressive effects of silicates against neck rot (Kawashima, 1927)

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study, using the susceptible variety “Jinriki”. In a colloidal silicate plot, the neck rot rate was only 23.1%, but in a control plot it was 92.6%. Silicate suppressed neck rot to one-fourth of the control. In the silicate plot, about three-fold silicate content per dry weight accumulated in comparison with control.

In 1953, Akai also showed that colloidal silicate suppressed neck rot significantly; he also showed that increased nitrogen fertilization reduced suppressive effect of silicate. Akai explained that the silicate suppressed neck rot by generating physical change through silicification of motor cells at main blast fungus infection sites.

In 1985, Oyama showed that barnyard manure did not suppress blast disease, but that adding calcium silicate did, even years with the damage due to a cool summer. Oyama described that rice leaves with more than 5% silicate content at the panicle formation stage present effective leaf blast suppression. He also noted that greater than 8% of silicate content in rice leaves at heading suppressed neck rot.

In 1997, Takeuchi reported that adding calcium silicate reduced neck rot and rice protein contents in the field. It increased eating quality indirectly. Recently, rice products have been overabundant. Therefore, increasing eating quality is one necessary theme for rice product marketing.

In 2000, Hayasaka *et al.* reported that silica gel significantly suppressed rice seedling blast. That was probably the first report that described silicon and rice seedling blast. They examined effects of 250 g to 1000 g silica gel mixed into 3000 g of soil before sowing naturally infested rice seeds. Seedling blast incidence in boxes with 250 g silica gel was 80% lower than that in the control box without silica gel.

In 2001, Maekawa *et al.* reported that 250 g of silica gel reagent or 3 g (SiO_2 ingredient) of potassium silicate solution reagent per nursery box were most and second most effective of six materials for suppressing seedling blast. They were (silica gel, potassium silicate solution reagent, potassium silicate solution fertilizer, autoclaved light concrete, calcium silicate, and tricyclazole).

(2) Brown Spot (*Bipolaris oryzae*)

In 1953, Akai showed that colloidal silicate significantly suppressed brown rot, but the effect was reduced by adding double amounts of nitrogen. He explained how silicate suppressed brown rot. As with blast disease, silicification of epidermal cells, including motor cells, was able to prevent brown spot penetration (Fig. 2).

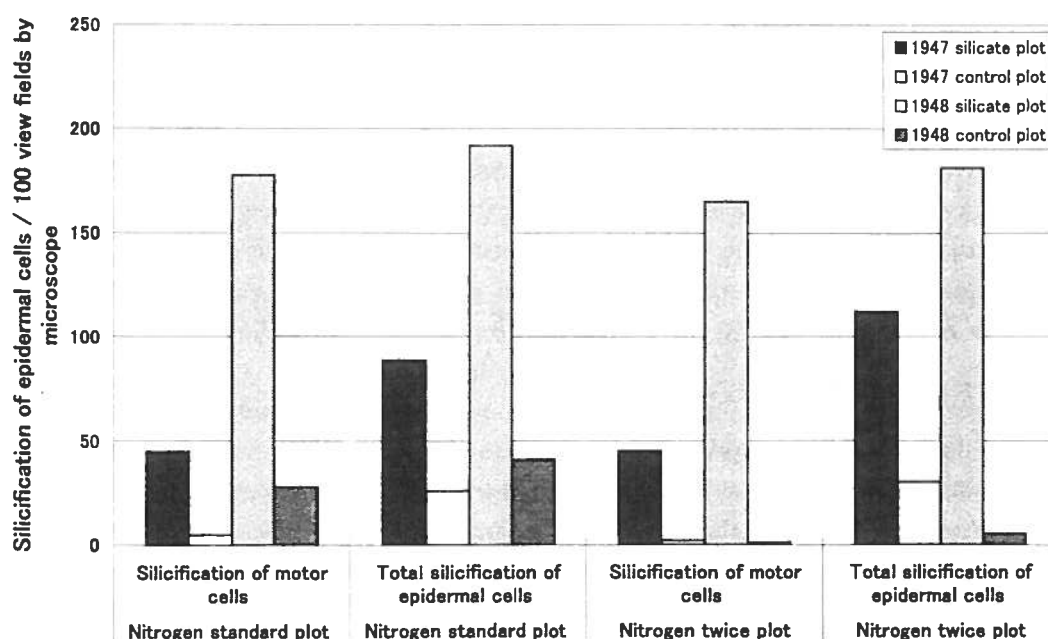


Fig. 2 Silicification of epidermal cells in matured rice leaves adding nitrogen fertilizer (Akai, 1953)

(3) Stem Rot (*Helminthosporium sigmoideum* var. *irregulare*)

In 1958, Yoshii *et al.* reported that silicate suppressed stem rot. They explained that adding silicate reduced soluble nitrogen in rice plants and increased the carbon/soluble nitrogen ratio.

(4) Sheath Brown Rot (*Pseudomonas fuscovaginae*)

In 1999, Shirai *et al.* reported that silicon fertilizer (silica gel and calcium silicate) suppressed sheath brown rot. That was probably the first report that silicate suppressed plant pathogenic bacteria. Silicate and nitrogen in rice leaves were negative correlation. Increased silicate content in rice leaves decreased sheath brown rot damage. They considered that damage (screenings or sterility) was avoided with silicate content exceeding 6% in rice leaves at heading and 10% at maturity.

AGAINST CUCUMBER DISEASE

(1) Powdery Mildew (*Sphaerotheca fuliginea*)

In 1983, Miyake and Takahashi reported that silicate suppressed powdery mildew significantly on solution-cultured cucumber. They showed that dry leaf silicate content increased proportionally with increased concentration of silicate in the culture solution; colonies of powdery mildew decreased. Marked suppressive effects of silicate appeared when silicate concentration was 50 to 100 ppm in solution. This report was valuable because it was probably the first report of suppressive effects against pathogens on dicotyledons.

In 1991, Motojima reported that rootstock for the so-called bloomless cucumber was able to absorb plant nutrients normally, except for silicate. Bloomless rootstock absorbed silicate only one-third the rate of rootstock; therefore, grafted cucumber became susceptible to powdery mildew. He showed that many blooms on cucumber fruit surface lessened powdery mildew occurrence.

(2) Fusarium wilt (*Fusarium oxysporum* f.sp. *cucumerinum*)

In 1983, Miyake and Takahashi also reported that silicate fertilizer application promoted growth and yield of cucumber plants; it further reduced damage caused by wilt disease in soil culture. They showed that both calcium and potassium silicate were effective against fusarium wilt. Application amounts of both fertilizers were 2000 to 4500 kg/ha. Occurrences of wilted plant in silicate treatment plots were one-third to one-half of the control plot.

(3) Corynespora Leaf Spot (*Corynespora cassiicola*)

In 1991, Hazama *et al.* reported that bloomless cucumber rootstock absorbed plant nutrients normally, but little silicate; therefore, the grafted cucumber became susceptible to powdery mildew and corynespora leaf spot. Higher silicate contents in cucumber leaves decreased powdery mildew and corynespora leaf spot. Incidence of corynespora leaf spot in the bloomless rootstock plot was about three times that of the fully blooming rootstock plot.

AGAINST STRAWBERRY DISEASE

(1) Powdery Mildew (*Sphaerotheca aphanis* var. *aphanis*)

In 1997, Kanto *et al.* reported that soluble potassium silicate suppressed powdery mildew on strawberry significantly in hydroponics. They showed that silicate content in dry leaves increased proportionally to increased silicate concentration in the culture solution; incidence of powdery mildew decreased. Markedly suppressive effects of silicate appeared with silicate concentrations of 50 to 100 ppm in solution. Moreover, they showed that strawberry leaf rigidity increased proportionally to increased silicate concentration in culture solution.

In 1999, Kanto *et al.* reported that soluble potassium silicate suppressed powdery mildew in soil culture by

foliar application or by soil drench. Effective silicate concentration was 500 ppm. They showed that suppressive effects of silicate were expressed differently between the varieties "Toyonoka" and "Sachinoka". They also examined silicate influence on fruit qualities like BRIX, acids, and vitamin C. Results were similar to the control group.

In 2001, Kanto *et al.* selected soluble potassium silicate fertilizers having the best suppressive effect (control value) against powdery mildew in severe disease condition. As shown in Table 1, the best fertilizer was "soluble potassium silicate fertilizer A".

Table 1. Suppressive effects of variety soluble potassium silicates against strawberry powdery mildew in severe disease condition

Materials ^{a)}	Proportion of disease leaves (%)	Disease severity ^{b)}	Control value ^{c)}
Soluble potassium silicate Fertilizer A solution	53.1	17.2*	55.6
Soluble potassium silicate Fertilizer B solution	75.2	26.6	31.2
Soluble potassium silicate Fertilizer C solution	69.5	21.7	43.8
Soluble potassium silicate Reagent solution	62.9	17.6*	54.4
Control	89.9	38.6	—

MECHANISMS

Silicate suppresses some plant pathogens including bacteria. Where and how does silicate suppress plant pathogens? There appears to be no specific site; still, some discoveries are illustrative.

On rice plants, Akai reported generating physical change by silicification of motor cells that were main infection sites by fungus in 1953. Silicification of motor cells was closely related with fungal penetration prevention, but less related with prevention of fungal hyphal extension in plant tissues after invasion (Ono, 1982).

There were no significant differences in germination percentage of blast fungus conidia, formation percentage of blast fungus appressorium, or the blast-disease lesion-area proportion with either high or low rice-leaf silicate content (Hayasaka *et al.*, 1999). Cultivating rice plants in a shade culture decreased the number of silicified cells, but blast disease did not develop. Therefore, silicate mechanisms in rice plants are supposed not only for prevention for fungal invasion mechanically, but other mechanisms. In 2002, Maekawa *et al.* observed by X-ray microanalysis that there were high accumulations of silicon near blast appressorium on inoculated rice leaves.

On cucumber plants, Hazama *et al.* showed in 1991 that silicate suppressed cuticle infection pathogens like powdery mildew and corynespora leaf spot, but not stomatal infection pathogens like downy mildew.

On strawberries, Kanto *et al.* showed in 1997 that strawberry leaf rigidity increased proportionally with increased silicate concentration in the culture solution; silicate increased physical resistance to penetration by plant pathogens. Kanto *et al.* observed that there were significant differences of germination percentages of powdery mildew conidia between leaves treated with silicate and non-treated leaves. Direct suppressive effects of soluble potassium silicate against powdery mildew conidia germination were not observed whether pH was adjusted or not.

CONCLUSIONS

Silicon is a very useful element for preventing many plant pathogens on rice, cucumbers, and strawberries.

This report shows possible applications for silicate not only for monocotyledons, but also for dicotyledons.

Recently, Integrated Pest Management (IPM) has become important worldwide. Silicon is one IPM method with promise for use in other plants. Research on silicates will become increasingly popular and will continue to elucidate silicon mechanisms on plants and pathogens.

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Relationship between Susceptibility of Rice Plants to Blast Disease and Leaf Silica Content under Different Atmospheric CO₂ Conditions

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INTRODUCTION

Rising atmospheric CO₂ concentrations are predicted to continue in the future. Consequently, concerns are increasing about physiological and anatomical changes in crop plants under elevated CO₂. So far, there have been a number of reports of increased stomatal resistance under elevated CO₂ in plant growth chambers. For rice plants, this increase possibly affects the uptake of silica from the soil and results in a reduction in leaf silica content. It is well known that the silica content of rice leaves is inversely related to the susceptibility of rice to blast disease, which is currently the most serious threat to rice production in the world and is caused by the fungus *Magnaporthe grisea*. For this reason, it is possible that the susceptibility of rice plants to blast disease could increase with rising atmospheric CO₂ concentrations. In this work, we examined the influence of twice-ambient CO₂ on leaf silica content and susceptibility to blast disease in rice grown in a FACE (Free-Air CO₂ Enrichment) field experiment.

MATERIALS AND METHODS

A full description of the Rice FACE facility is provided by Okada *et al.* (2001). The experiment was carried out in rice paddy fields in Shizukuishi, Japan (39°41'N, 140°58'E) over a three-year period from 1998 to 2000. Rice seedlings (cv. Akitakomachi) were raised in chambers for 30 days under ambient and elevated CO₂ conditions, and transplanted into paddy fields in the middle of May. The experimental design was a completely randomized block design with four replications. Rice plants growing in ambient and elevated CO₂ plots were inoculated with the blast fungus in early and late July. The number of leaf lesions per plant was assessed 14 days after each inoculation, and plants with higher numbers of lesions were considered to be more susceptible. Leaf samples collected just before each inoculation were analyzed to determine nitrogen and silica content.

RESULTS AND DISCUSSION

In 1998 and 2000, more lesions were observed on leaves of plants grown under elevated CO₂ than those under ambient CO₂ (Table 1). In both these years, leaf silica contents were significantly higher in ambient CO₂ than in elevated CO₂ (Table 2). In 1999, however, CO₂ concentration did not significantly affect lesion number (Table 1) or silica content (Table 2).

These results confirm that the silica content of rice leaves is closely related to susceptibility to blast disease. Silica in rice leaves probably plays some role in the defense mechanism against blast fungus infection. For two of the three seasons during this experiment (1998; 2000), elevated CO₂ decreased leaf silica content compared with ambient CO₂. During clear sunny conditions in July 1998, transpiration rate and surface tem-

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Table 1. Lesion number of leaf blast per hill under elevated and ambient CO₂ condition.

Year	Inoculated date			
	July 1-3		July 18-22	
	Ambient	Elevated	Ambient	Elevated
1998	86.88	142.88	24.25	33.75
1999	26.33	26.65	5.87	6.14
2000	17.81	24.94	7.06	9.31 ¹⁾

1) *: Significant at P=0.05 with the paired t-test between ambient and elevated conditions.

Table 2. Leaf silicon and nitrogen content under elevated and ambient CO₂ condition.

Sampling date	SiO ₂ (%)		N (%)	
	Ambient	Elevated	Ambient	Elevated
1998/7/1	4.77	4.04*	4.14	4.24
1998/7/22	4.87	4.23*	2.85	2.63
1999/7/7	4.27	4.49	4.47	4.27
1999/7/21	4.35	4.06	3.87	3.41*
2000/7/3	4.12	3.46*	3.99	3.7
2000/7/18	4.77	3.72*	2.78	2.85

1) *: Significant at P=0.05 with the paired t-test between ambient and elevated conditions.

perature of rice leaves were measured in both elevated and ambient CO₂. In elevated CO₂, transpiration rates were lower but surface temperatures were higher than those in ambient CO₂ (data not shown). This indicates that stomatal resistance of rice increases with increasing concentration of atmospheric CO₂ under field conditions. Lower transpiration rates in elevated CO₂ probably caused a lower silica uptake and consequently a lower silica content in leaves. It remains unclear why there was no significant difference in leaf silica content in 1999 according to CO₂ concentration; different weather conditions, such as increased solar radiation, may have affected the uptake and distribution of silica to leaves.

This is probably the first experiment to show that changes in atmospheric CO₂ in field conditions can decrease leaf silica content in rice and increase susceptibility of rice to blast disease. This study indicates that global climate change, including an elevation of atmospheric CO₂ concentration, could provoke changes in crop-pathogen pathosystems that decrease crop productivity.

Silicon Induces a Defense Response to Rice Blast Infection

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Application of silicon (Si) to soils deficient in this element reduces the epidemic rate of blast, caused by *Magnaporthe grisea* (Hebert) Barr, in both irrigated and upland rice. Recently, Seebold *et al.* (2001) studied the effects of Si on several components of resistance to blast using susceptible, partially resistant, and completely resistant rice cultivars. They observed that, regardless of cultivar resistance, incubation period was lengthened, and the number of sporulating lesions, lesion size, rate of lesion expansion, and number of spores per lesion were significantly reduced by Si. In the present study, we wanted to examine whether Si could induce rice plants to express any microscopically visible host defense reactions in order to prevent or restrict the process of infection of *M. grisea*. The rice cultivar M-201 was chosen for this study because it has no major or minor genes for resistance to race IB-49 of *M. grisea*. Plants were grown in Promix, a soil-less mix, and watered daily with nutrient solution amended with 1.7 mM (100 ppm) Si (+Si) in the form of potassium silicate or left unamended (-Si). Plants were inoculated at the fourth leaf stage with a conidial suspension (2.5×10^4 conidia ml⁻¹) obtained from sporulating cultures of isolate IB-49. Single lesions were collected from the third and fourth leaves on the main tiller from both treatments 96 hours after inoculation. This corresponded to the time when lesion size differences were visibly apparent. Samples were processed for light and electron microscopy observations according to Benhamou and Bélanger (1998). Colonization by *M. grisea* coincided with extensive mesophyll cell disorganization and was observed more markedly in thin sections examined from lesions collected from unamended plants (Fig. 1A). Furthermore, host cell walls were no longer discernible in massively invaded areas in the mesophyll; dead cells showed signs of strong contraction without limiting the spread of fungus through the cells; and the presence of an amorphous material accumulating in response to fungus invasion was not detected. Additionally, the vascular bundle was also profusely colonized by *M. grisea* (Fig. 1B). In contrast, in thin sections obtained from lesions collected from +Si-treated plants, we observed the accumulation of an amorphous material in response to the invading hyphae. This material was densely stained with toluidine blue resulting in a green color indicative of the presence of phenolic-like compounds. Most *M. grisea* hyphae colonizing both upper epidermal and mesophyll cells were trapped in this material and appeared structurally altered, and, in some areas, were reduced to empty hyphal shells (Fig. 1C and D). In conclusion, this study provides the first evidence that Si is probably inducing a chemical defense response to blast infection in rice.

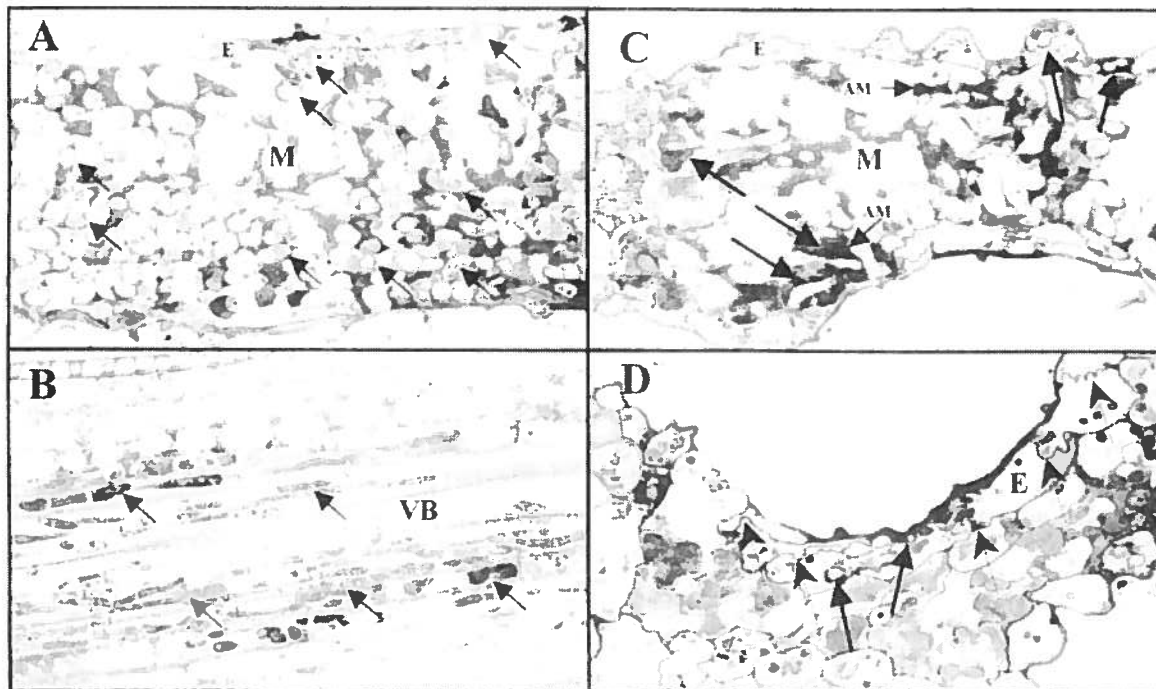


Figure 1. Light micrographs of rice leaves infected by *Magnaporthe grisea* collected 96 hours after inoculation. Thin resin-embedded sections (0.7 to 1 μ m) were stained with 1% aqueous toluidine blue prior examination. **A** and **B**, thin sections obtained from lesions collected from -Si-treated plants (1000x). *M. grisea* hyphae (small arrows) abundantly colonize the upper epidermal (E) and mesophyll (M) cells and also the vascular bundle (VB) with absence of any amorphous material accumulating in response to fungus invasion. Fungus hyphae infecting the mesophyll cells cause extensive cell damage and dead cells show signs of strong contraction in response to infection. **C** and **D**, thin sections obtained from lesions collected from +Si treated plants (1000x). Accumulation of amorphous material (AM) around invading hyphae is evidently found in both upper epidermal and mesophyll cells (large arrows). The amorphous material is specifically deposited along the epidermal cell wall (dashed arrows).

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Effect of Silicon Nutrient on Bacterial Blight Resistance of Rice (*Oryza sativa* L.)

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ABSTRACT

Rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a serious disease worldwide. In Taiwan, this disease affects about 20,000 ha of rice field or 5% of total rice area annually. In order to cope with this disease, many researchers tried to solve the problem through genetic improvement, pest management and cultural practices (Ou 1985).

Silicon is not an essential element for plant growth in many crop species, but it is known to be able to reduce the epidemics of plant diseases, especially in rice (Datnoff *et al.* 1991, 1992). The present studies are aimed to study the effects of different concentrations of silicon on the development of bacterial blight disease in rice plant cultured under the nutrient culture system as well as under the paddy field conditions. The bacterial leaf blight susceptible variety Taichung Native 1 (TN1) and resistant breeding line TSWY7, were grown with the nutrient culture system under the greenhouse condition. The cultivar TN1 which is susceptible to this disease, was found to have a lower silicon content in leaves than that of the resistant breeding line, TSWY7 under the nutrient cultural system. The result of experiment indicated, that the degree of disease resistance was increased in parallel with the increased amount of applied silicon (Table 1). Under the high concentration of silicon, the disease lesion became much shorter and smaller; thus the degree of disease resistance was greatly enhanced.

It was observed that the soluble sugar content in the leaves was positively correlated with the epidemics of bacterial leaf blight disease. The higher soluble sugar content in the leaves, the higher the disease incidence. Under the condition of higher rate of silicon application, the soluble sugar content in the leaves reduced considerably, thus create an internal environment not suitable for the growth of pathogen of the disease, which in turn to contribute to the field resistance of the disease. This interesting phenomenon was observed at various developmental stages of rice plant in the field.

Table 1. Effect of different silicon concentrations on silicon and soluble sugar contents at tillering stage of TSWY7 and TN1 rices in hydroponic culture in relation to their resistance against infection by XM42 isolate of *Xanthomonas oryzae* pv. *oryzae*.

Test plants	Si (ppm)	Leaf composition (% dry weight)		Lesion length (cm)
		Si	Soluble sugar	
TN1	0 ¹	1.20 ^{b2}	6.64 ^a	21.00 ^a
	50	4.74 ^a	5.94 ^b	16.00 ^a
	150	4.73 ^a	5.48 ^c	7.10 ^b
TSWY7	0	3.03 ^c	6.54 ^a	4.90 ^a
	50	5.25 ^b	5.78 ^b	3.90 ^b
	150	8.61 ^a	5.10 ^c	1.55 ^c

¹ Add silicon (50 ppm) once 28 days after transplanting.

² Data followed by the same letter in each column set indicate the difference was not significantly different at 5% level by the Duncan's Multiple Range Test.

In a separate field experiment, three levels of silicate slag (0, 2, 4 t/ha) were applied onto a plot where four varieties of rice with varying degrees of resistance to bacterial blight disease were grown. The result of this field experiment indicated that silicate slag application was able to reduce the lesion length of bacterial blight up to 40.2% in the first crop, and 13.7% in the second crop season (Table 2). The result of another field experiment also indicated, that the more silicate slag was applied, the more enhanced resistance to bacterial blight for rice. In this experiment, the decrease percentage of lesion length was found to range from 5% to 22% among the four rice varieties (Teaching negative 1, Taichung Sen 10, Tai-Keng 9, and Tainung 67) tested. The application of 4 t/ha of silicate slag was found to be especially effective to inhibit the epidemics of bacterial blight disease for susceptible variety, Teaching Seen 10 (Table 3).

It is concluded from the present study, that although silicon or silicate slag can not completely control the epidemics of bacterial blight disease, however it can enhance the field resistance of rice plant to the disease considerably. Today there is no effective pesticides available to control this disease, and very few genetic resources available for resistance of this disease. Under these circumstances, application of silicon will certainly provide an alternative way of solution to the disease. However, it is emphasized that breeding for disease resistance by using a new disease resistance genetic resource is far more important than the manipulation of silicon in the field. In this connection, the author is currently engaging the rice breeding work with special emphasis on the resistance to bacterial blight disease.

Table 2. The lesion length (cm) of rice leaves inoculated by *Xanthomona oryzae* pv. *oryzae* isolate XM42 between silicate slag application and check in the first and second crops of 1996.

Season crop	Silicate slag		Check Lesion length (cm)
	Lesion length (cm)	Decreasing percentage* (%)	
First crop	5.88 ^{b**}	-40.2	9.83 ^a
Second crop	5.63 ^b	-13.7	6.52 ^a

* Decreasing percentages of lesion length when the silicate application compared as non-silicate application.

** Means with the same letter between silicate and non-silicate applications or between cultivars in the same season crop are not significantly different at 5% level by Least Significant Difference Test.

Table 3. The lesion length (cm) of rice leaves inoculated by *Xanthomona oryzae* pv. *oryzae* isolate XM42 among the different amounts of silicate slag application and rice cultivars after 14-day inoculation in the first crop of 1997

Applied silicate (ton/ha)	Indica type		Japonica type		Mean
	Taichung native 1	Taichung sen 10	Tai-keng 9	Tainung 67	
4	6.31 ^{b*}	4.20 ^b	1.73 ^b	2.22 ^a	3.62 ^b
	(-22.1)	(-19.1)	(-10.8)	(-5.5)	(-17.7)
2	6.23 ^b	5.24 ^a	2.30 ^a	2.61 ^a	4.10 ^b
	(-23.1)	(-1.0)	(-18.6)	(-11.1)	(-6.8)
0 (Check)	8.10 ^a	5.19 ^a	1.94 ^{ab}	2.35 ^a	4.40 ^a
Mean	7.01 ^a	5.01 ^b	2.01 ^c	2.39 ^c	

Decreasing percentages of lesion length when the different silicate application compared as check (0 ton/ha), are given in the parentheses.

* Means with the same letter among different amounts of silicate applications or among different varieties are not significantly different at 5% level by Duncan's Multiple Test.

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Accumulation of Silicon around Penetration Sites of *Magnaporthe grisea* and Silicon-dependent Promotion of Superoxide Generation after Inoculation on Rice Leaf Epidermis

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INTRODUCTION

It is reported that rice seedling blast is suppressed by the treatment of silicon¹⁾. The mechanism by which silicon enhances blast resistance of rice is that silicon accumulates in epidermal cells, such as motor cells; there it may stop invasion and progress of hypha of *Magnaporthe grisea*. However, the use of too much nitrogen causes blast to increase, even if silicon is employed²⁾. Furthermore, when rice is cultivated in shade conditions, though the number of silicified cells decreases, the silicic acid content rate increases and rice leaf blast was suppressed³⁾. From these facts, it is anticipated that the mechanism, aside from prevention of penetration by silicified cells, also exists.

Though it is reported in the cucumber (*Cucumis ativus L.*), inoculated powdery mildew (*Sphaerotheca fuliginea*) gives an example for observing silicon distribution in infected areas when it is infected with pathogenic fungi⁴⁾; it is not reported silicon distribution in rice which was inoculated *Magnaporthe grisea*.

Then the mapping of elements around the penetration sites of *Magnaporthe grisea* on rice leaf blade epidermis was done by cool stage installation variable-pressure scanning electron microscope (VP-SEM) and energy dispersive X-ray microanalysis (EDX) without any pretreatment. In addition, in order to investigate whether there is the possibility of silicon promoting physiological resistance in rice, the generation of active oxygen was measured in rice treated silicon after *Magnaporthe grisea* was inoculated.

MATERIALS AND METHODS

(1) The accumulation of the silicon in the *Magnaporthe grisea* penetration site.

Rice seedlings (*Donntokoi*) 18 days after sowing (Day 18), were transplanted in a plastic case (12 × 8 × 6 cm), and were cultivated hydroponically in a glass house. The nutrient solution exchanged 2 times for the week. The concentration of each element of nutrient solution was set like the following. N : 30 P₂O₅ : 15 K₂O : 42 Fe : 3 Mn : 0.5 B : 0.2 Zn : 0.1 Cu : 0.02 and Mo : 0.01 (each mg kg⁻¹). On Day 33, SiO₂, as potassium silicate solution reagent (SiO₂ ingredient 197–202 g L⁻¹), was added to the nutrient solution in 100 mg kg⁻¹ concentration. PH of nutrient solution was adjusted in 5.4.

On Day 43, leaf blade of fifth leaf lightly added the injury by leaf punch of the 1.5 mm diameter, and inoculated the conidia suspension (conidia density 10⁵ per mL⁻¹) of compatible *Magnaporthe grisea* (race 007) by a drop. The position of inoculated leaf blade was cut off, and it was fixed on the sampling stage by the adhesive without any pretreatment. It would be observed and was analyzed just before inoculation and after inoculation 64 hours at VP-SEM (Hitachi, S-3500N) and EDX (Horiba, EMAX ENERGY). The pressure of the sample chamber was set 30 Pa, temperature of the sampling stage was -10°C, and acceleration voltage of 15 kV was selected.

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(2) The measurement of superoxide

A quantity of 3.3 g rice seed (*Koshihikari*) was sown in a plastic case (8 cm diameter, 4 cm depth) with packed soil; they were cultivated in a glass house. Ridging filled 100g per case using marketed nursing seedling molding (Ube baido toku 2 : N : P₂O₅ : K₂O = 0.2 : 0.2 : 0.33 g kg⁻¹). The cover soil was used the marketed cover soil (housaku). Potassium silicate solution reagent was diluted with about 15 mL of distilled water to produce a total 1.9 g (0.4 g of SiO₂ content) per case were poured on Days 12, 18, 21, 27, 32, and 36.

Yielded rice plants (3.1 leaf stage) were put in darkness at room temperature for the day preceding inoculation. Third leaf blades of rice plants (3.1 leaf stage) were inserted into a hole in a petri dish (glass base dish) with facing leaf blades surface down from head, and fixed with adhesive tape. One leaf was inserted into each petri dish. The 100 ul of conidia suspension (conidia density 10⁶ per mL⁻¹) of *Magnaporthe grisea* (race 007) was added 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo [1,2- α] pyrazin-3-one (MCLA) to produce a 100uM solution; it was inoculated by pipette injecting from the tip about 4 cm under the leaf blade portion.

Weak light emissions in inoculated position on epidermal cell were measured by video camera (C2400-47) for extremely faint luminescence measuring microscope fixed to invert microscope for 24 hours from the right after inoculation. The generation of the superoxide (O₂⁻) was measured by the two-dimensional photo counting system (ARUGAS-50, Hamamatsu Photonics) in every 15 minutes.

RESULTS AND DISCUSSION

Silicon distribution and the spectrum of main elements in the leaf blade cross-section mainly on motor cell of rice which used silicon and not inoculated *Magnaporthe grisea* are shown in Fig.1. Silicon was distributed at high-density in epidermal cells; it was low-density in the mesophyll cells. Though Yoshida⁵⁾ reported that silicon was abundantly deposited by HF-etching method in epidermal cells, his result was similar even in EDX using living samples.

In fifth leaf blade epidermis of rice plant which was treated with silicon, 64 hours after only injury, the

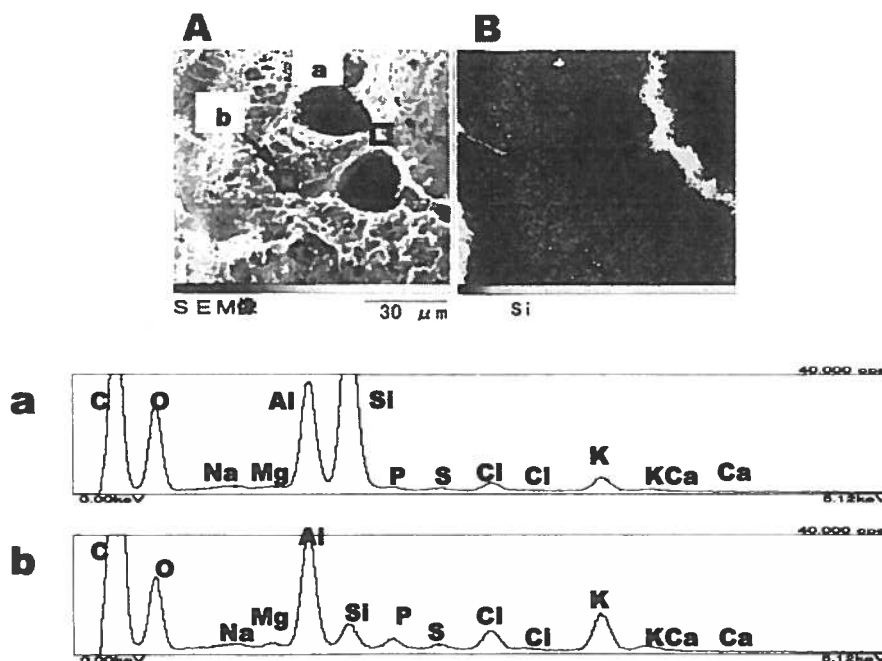


Fig. 1. SEM image and mapping of silicon on cross section of motor cells on rice leaf blade epidermis.

A : SEM image B : Mapping of silicon (dots show distributions of silicon)

a : An X-ray spectrums in the position of a in A.

b : An X-ray spectrums in the position of b in A.

silicon was visible in the epidermal cell knobs except for guard cells and accessory cells. Elements such as nitrogen, potassium, magnesium, chlorine, calcium were uniformly distributed (Fig. 2).

By that same time, inoculated conidia had sprouted and extended germ tubes, and hemispherical appressorium which were the penetration sites to leaf epidermis were formed at the tip of germ tubes and silicon had accumulated near the appressoria in high density (Fig. 3). However, elements such as nitrogen, potassium,

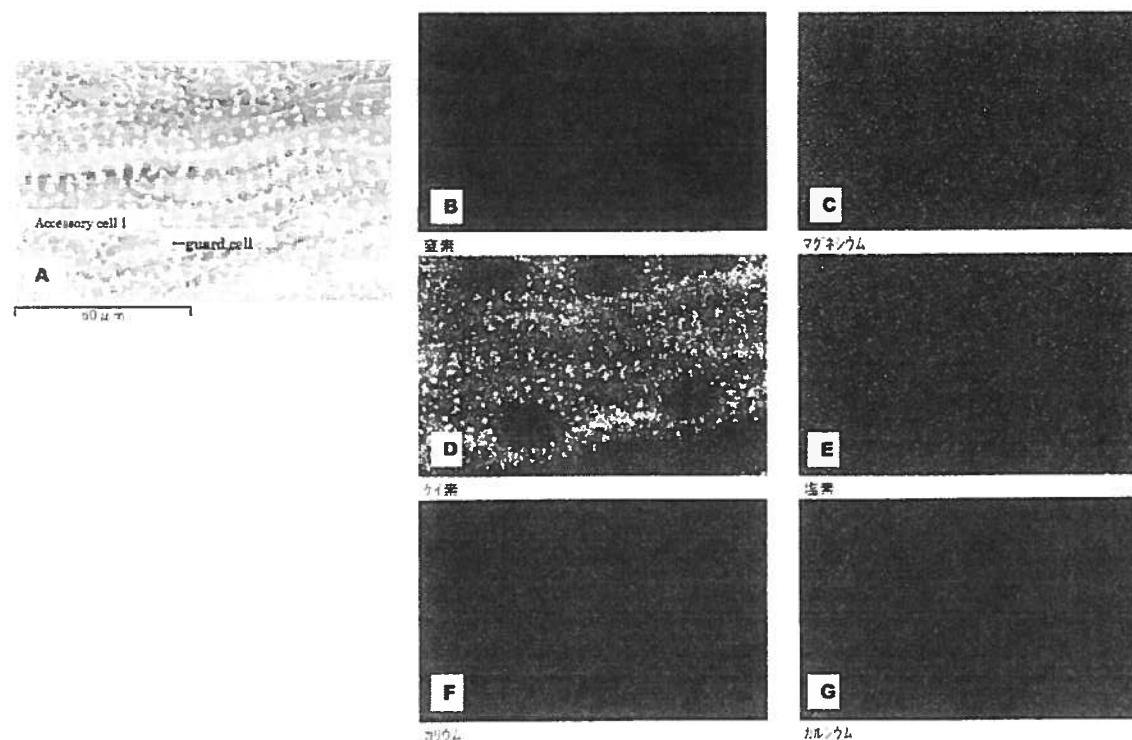


Fig. 2. Mapping of main elements on rice leaf blade epidermis which treated silicon. (64 hours after only injury).
A : SEM image B : Nitrogen C : Magnesium D : Silicon E : Chlorine F : Potassium G : Calcium

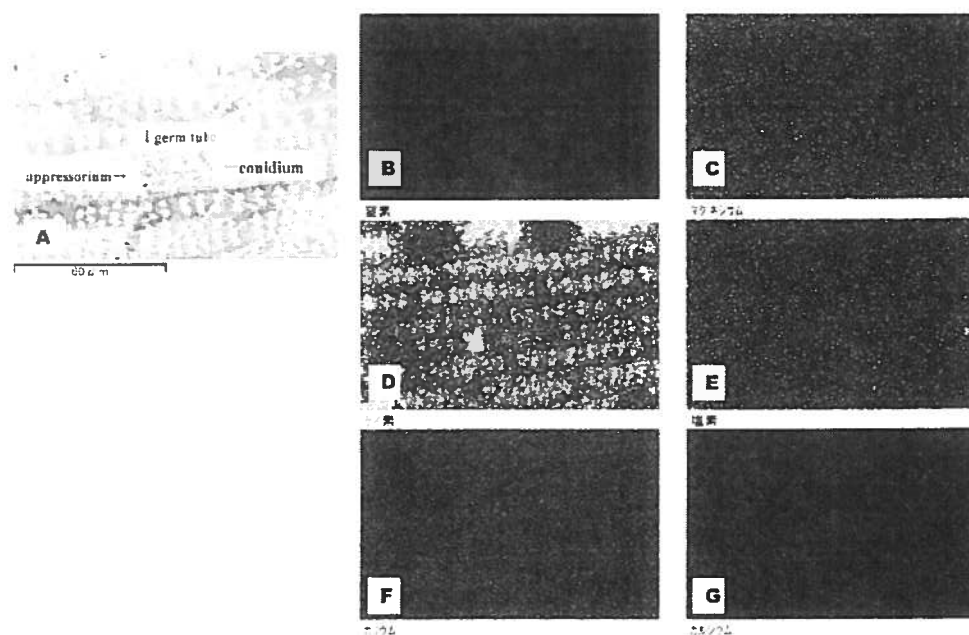


Fig. 3. Mapping of the main elements around the penetration site of *Magnaporthe grisea* on rice leaf blade epidermis which treated silicon (64 hours after inoculation). A : *Magnaporthe grisea* on epidermis B : Nitrogen C : Magnesium D : Silicon E : Chlorine F : Potassium G : Calcium

magnesium, calcium, chlorine were uniformly distributed in spite of the inoculation on epidermal cells (Fig. 3).

In conventional EDX and wavelength-dispersive X-ray analysis (WDX), there is failure of transfer and elimination of elements during dehydration, drying and washing, and further contamination of elements by fixatives used during sample pretreatment. Since living samples are analyzed in this report, silicon mapping is a more approximately natural form. That is to say, it was possible to observe that silicon accumulates near the appressorium of *Magnaporthe grisea* by VP-SEM and EDX on the silicon use rice leaf blade epidermis. This is the first report which showed the behavior of silicon on rice leaf blade epidermis while *Magnaporthe grisea* penetrated.

Silicon accumulated near the appressorium seems to be in a soluble condition before it accumulates because retranslocation of polymerized and accumulated silicon is not done in the epidermal cell apoplast region⁵). It is not clear what role that silicon accumulated near the appressorium fulfills in rice blast resistance. Though a specific role of accumulated silicon in the blast suppression mechanism is unknown, there exists the possibility of it not being a physical mechanism; this requires future examination.

As for rice plants that had been grown on soil, superoxide (O_2^-) was generated in higher levels in rice which had been treated with potassium silicate than in the non-treated rice at the inoculation after 0~0.25 hour (Fig. 4). Significance was shown by the most rigorous 1% significance level. After post-0.25 hour inoculation, O_2^- generation also rapidly decreased in both treatments; it changed with equal generated quantities afterwards. At 21.50~21.75 hours after inoculation, O_2^- generation in potassium silicate treatment slightly increased over the value shown for the non-inoculated case, and in hoop; except for it, a great difference could not be discerned between the two treatments.

A phenomenon (oxidative burst) whereby active oxygen forms rapidly is known in plant tissues which have received various environmental stress such as attack by pathogenic fungi and insects, high and low temperature, drying, ultraviolet rays, and injury⁶). This has a role as a signal intermediate which induces gene expression of resistance related enzymes, generation of phytoalexin, and systemic acquired resistance in plant defense reactions⁷). Furthermore, active oxygen promotes an oxidative bridging reaction of glycoprotein with rich hydroxyproline in cell walls⁸).

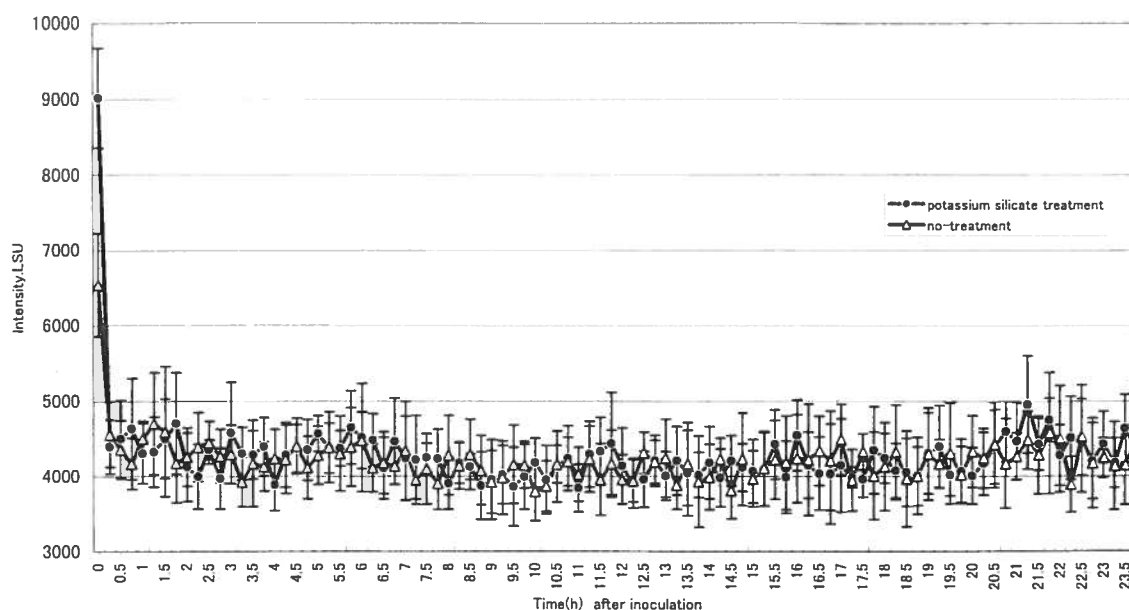


Fig. 4. The transition of the generation of superoxide (O_2^-) in rice which treated potassium silicate after inoculated with conidia of *Magnaporthe grisea*. The leaf blade surface was faced to the bottom, and the conidia suspension was inoculated from the bottom.

In present experiments, there was an O_2^- generation peak right after *Magnaporthe grisea* inoculation despite silicon treatment. By recognizing elicitor ingredients included in the conidia suspension, when the conidia suspension contacted leaf blade, rice seemed to cause rapid generation of active oxygen (oxidative burst). Here, O_2^- generation was promoted right after silicon inoculation; silicon treatment may amplify recognition signals in pathogenic fungus infection.

This is the first report that shows that silicon promotes the generation of active oxygen after the pathogenic fungus infection. In plants under stress condition, active oxygen has multiple functions. Future investigation must address which part of the defense reaction route is especially affected by the active oxygen generated with silicon promotion.

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Influence of Silicon Sources on Foliar Blast, Neck Blast, Grain Spots and Yield of Flooded Rice

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Several factors can affect the rice production, among them, the diseases are great concern to the growers, because it decrease yield and affect the grain quality. The silicon fertilization has been demonstrating efficiency in the control and or reduction of the incidence of several important rice diseases. An experiment was carried out in Formoso do Araguaia (GO) district using "Javaé" cultivar, susceptible to the main rice disease. The experimental was a randomized blocks design with 7 treatments and 5 replications. The treatments (silicon sources) were: check plot (without silicon application); imported calcium silicate (Albright & Wilson); MB4; slag (Silifertil); SAMA; talc friable; Anfibolito. The different sources were applied in the dose of 4000 kg ha⁻¹ and incorporated in the depth of approximately 10-15 cm. As a result it was observed that just the imported silicate (Albright & Wilson) was significantly different from the check plot (control) regarding foliar blast disease and incidence of grain spots. The Albright & Wilson silicate was the only one to provided significant increases on yield compared to the control, it was approximately 1880 kg ha⁻¹ or 84% higher compared to the control. The other Si sources did not differ of the control.

Effect of Silicon Rates on Some of the Most Important Diseases and Yield of Flooding Rice Crop

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This work had for main objective to verify the effect of silicon rates on some of the most important diseases and yield of flooding rice crop growth in the State of Tocantins/Brazil. In the 1999/2000 year crop, 30 days before planting, silicon fertilizer was incorporated into the soil. The experiment was installed on the field in a randomized blocks design with 5 treatments and 5 replications. The treatments consisted of five metasilicate rates with 42% of Si content, being: T1 = check plot, T2 = 1000 kg/ha, T3 = 2000 kg/ha, T4 = 4000 kg/ha and T5 = 6000 kg/ha. Larger foliar blast severity was observed in the treatment without silicon (check plot), which differed statistically from the T5 = 6000 kg/ha treatment that provided smaller blast severity in the leaves. The increase of silicon application didn't affect blast disease in the panicles. With relationship to the grain discoloration it was verified that, in spite of the lack of statistical difference, smaller severity levels were observed in the higher silicon rates. Higher productivity levels were verified with the application of 6000 and 4000 kg/ha of metasilicate, respectively

Pre-treatment of Sargent Crabapple Leaf Discs with Potassium Silicate Reduce Feeding Damage by Adult Japanese Beetle

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The role of silicon for protecting plants from insect damage is not well known. Previously, we reported that root applications of potassium silicate (PS) at 100 ppm reduced feeding damage to *Malus sargentii*, (Sargent crabapple) leaf tissues by adult Japanese beetles. This study reports on leaf disc pre-treatment with PS. Sargent crabapple leaves were harvested in August 2001 and 3 leaf discs (12 mm) were taken from each leaf and samples placed in polyethylene bottles with 200 ml of 0, 100, 200 or 400 ppm, PS solutions with pH adjusted to 7 ± 0.2 . Samples were placed in an orbital shaker at 100 rpm for 24 hours at room temperature. After PS treatment, samples were blotted dry and five leaf discs were placed per petri dish. One adult female Japanese beetle was placed into each petri dish and placed in an incubator held at 23°C and a 16:8 photoperiod for 48 hours. The percent leaf tissue removed was visually estimated using defoliation template and frass weight was measured to the nearest 0.1 mg. Potassium Silicate at 200 ppm significantly reduced the feeding damage to the leaf discs by adult Japanese beetle. The frass weight of beetles were also significantly lower for 200 ppm treatment. There was no difference on feeding damage and frass weight for 0, 100 and 400 ppm treatments. The differences between root application and leaf disc PS pre-treatment and effective concentration of PS to reduce feeding damage in vivo and in vitro will be discussed.

Plant-Soluble Silicon in Foodstuffs

The Food Factor

Approximate values for soluble silicon in foodstuffs are given in Table 1.

There is a wide range in the amount of soluble silicon in foodstuffs. The highest values are found in cereals, particularly in rice, which contains about 100 mg of soluble silicon per 100 g of dry weight. Other foodstuffs with high values are wheat, barley, and oats. The lowest values are found in fruits and vegetables, which contain less than 10 mg of soluble silicon per 100 g of dry weight. The amount of soluble silicon in foodstuffs is also affected by the growing conditions, particularly the amount of silicon in the soil.

Session 2

Silicon in Soils

Plant-Available Silicon in Paddy Soils

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In the northern part of Japan, five years in 1980's had cool summer and rice production was damaged. In 1993, a nationwide extraordinary cool summer made a very poor harvest of rice. Rice blast easily spreads and damages rice plant under such a cool temperature. Silicon (Si) plays an important role in reducing the incidence of several rice diseases including rice blast. Suitable Si status of rice plant may reduce the use of agricultural chemicals, thus contributing to environmental conservation. Si likewise improves photosynthetic activity and lodging tolerance, which in turn may lower grain protein content in rice, thus producing rice with good eating quality. This is why the role of Si is being watched once again.

Si is a beneficial nutrient for healthy and productive development of rice plant, and is required about ten times as much as nitrogen (N). It is therefore important to study the Si supplying capacity of paddy soils to make it highly productive and environmentally sound which fits well the call for sustainable and productive environment as well as the need to produce "healthy food" in the 21st century.

(1) Si balance in paddy fields

A balance sheet of "demand and supply" of Si between rice plant and paddy field was shown by Imaizumi *et al.* (1958). According to them, while the amount of Si taken up by rice plant is $950 \text{ kg ha}^{-1} \text{ y}^{-1}$, over 70% of Si taken up by rice plant comes from paddy soil and the remaining 30% originates from irrigation water, assuming that all of Si supplied by irrigation water is utilized by rice plant. In lysimeter experiment, Kurashima *et al.* (1973) observed that the amount of Si contained in percolating water varied with soil types, mainly soil texture. Considering that the Si derived from irrigation water was the difference between Si supplied from irrigation water and Si leached by percolating water, they concluded that 10 to 20% of Si taken up by rice plant came from irrigation water, and the remaining 80 to 90% originated from paddy soil. The Si concentration in irrigation water in Yamagata prefecture decreased by almost 50% compared with the level observed forty years ago (Kumagai *et al.*, 1998). Similar observation was reported in Iwate prefecture where the Si concentration in irrigation water was 25% less than the level obtained ten years ago (Iwate prefecture, 1994). In the region where the Si concentration in irrigation water tends to decrease, the Si supply from paddy soil should be given prior attention. Hanzawa *et al.* (2002) designed a lysimeter experiment where in irrigation water of two different Si concentrations were supplied to lysimeters filled with three soil types (granite soil, gray lowland soil and ando soil). The Si supply from paddy soil was estimated by deducting the Si supplied by irrigation water from the sum of the Si taken up by rice plant and the Si leached by percolating water. According to them, when the Si taken up by rice plant was made to be 100%, 8 to 37% of Si was supplied from irrigation water, 11 to 15% of Si was leached by percolating water, and 78 to 104% of Si was supplied from paddy soil.

Sumida (1992) calculated the Si balance in each growth stage of rice plant on a gray lowland soil at NARCT (National Agricultural Research Center for Tohoku Region, Omagari, Akita prefecture) (Table 1). The Si concentration in irrigation water was $15 \text{ mg SiO}_2 \text{ L}^{-1}$. It was assumed that the daily amount of irrigation water and percolating water were 12 mm day^{-1} and 10 mm day^{-1} , respectively, and the Si concentration in percolating water was equal to that in soil solution. From transplanting to maximum tiller number stage, the daily Si uptake by rice plant, the daily Si leaching by percolating water and the daily Si supply from paddy

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Table 1. Daily Si balance sheet in each growth stage of rice plant.^a

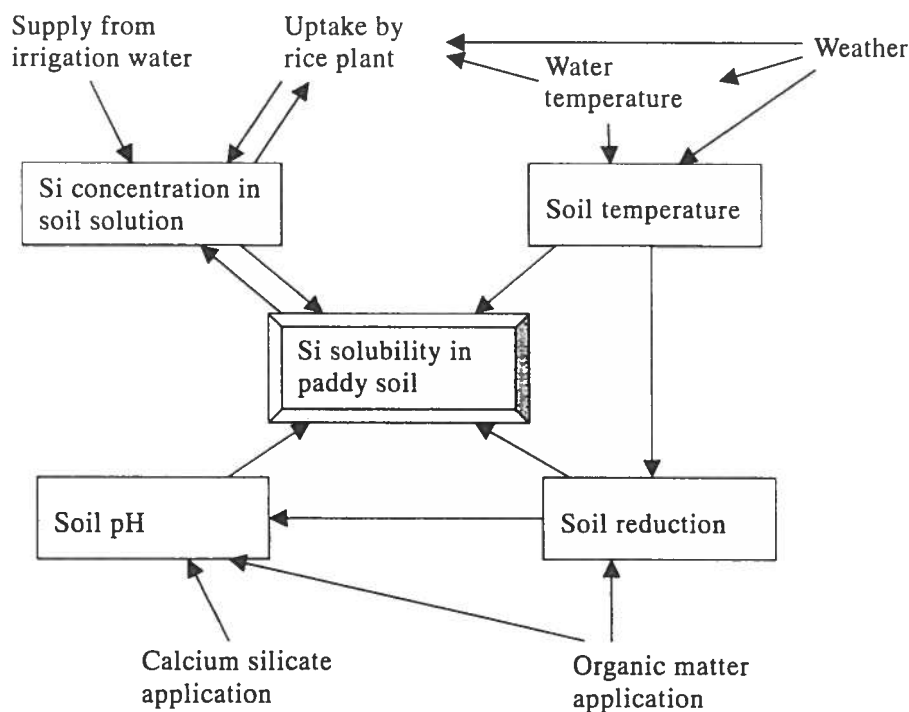
Growth stage	Si (mg SiO ₂ /m ² /day) balance			
	Irrigation	Leaching	Uptake by rice plant	Supply from paddy soil
Taransplanting Tillering	180	– 120	– 100	40
Panicle initiation	180	– 90	– 570	480
Meiosis	180	– 40	– 840	700
Full heading	180	– 20	– 1,260	1,100

^aSumida (1992).

soil were 100 mg SiO₂ m⁻² day⁻¹, 120 mg SiO₂ m⁻² day⁻¹ and 40 mg SiO₂ m⁻² day⁻¹, respectively. On the other hand, from meiosis stage to full heading stage when Si uptake by rice plant was the most active, the daily Si uptake was 1,260 mg SiO₂ m⁻² day⁻¹, the Si leaching by percolating water, 20 mg SiO₂ m⁻² day⁻¹, and the Si supply from paddy soils, 100 mg SiO₂ m⁻² day⁻¹. On this paddy field, the daily Si supply of only 40 mg SiO₂ m⁻² day⁻¹ from paddy soil did not decrease the Si concentration in soil solution. Besides, even when the Si concentration in soil solution fell considerably due to active Si uptake by rice plant, the daily Si supply of 1,100 mg SiO₂ m⁻² day⁻¹ is still sufficient. During this period, 86% of the Si uptake was derived from paddy soil. Thus, a large amount of Si uptake by rice plant depends on the Si supply from the paddy soil, the degree of dependence of which is higher when Si uptake by rice plant is active. Therefore, it is imperative to evaluate the Si supplying capacity of paddy soils.

(2) Factors controlling availability of Si in paddy soils

The potential factors controlling solubility of Si in paddy soils are summarized in Fig. 1. The dissolution of Si in paddy soils is influenced by soil temperature, soil redox potential (Eh), soil pH and Si concentration in soil solution. The amount of Si dissolved from paddy soil increases with increasing soil temperature and also

**Figure 1.** Factors controlling Si solubility in paddy soils (Sumida,1992).

increases with decreasing soil Eh. This increase would be attributed to Si release from ferrisilica complexes under reductive soil condition. The amount of Si dissolved from paddy soil decreases with increasing soil pH when pH is between 4 to 9. This pH dependency is largely due to pH dependent adsorption of Si by sesquioxides. The Si uptake by rice plant may decrease the Si concentration in soil solution, while paddy soil has the ability of maintaining the Si concentration at certain value. Therefore, it is necessary to consider all these factors for evaluating Si supplying capacity of paddy soils.

(3) Evaluation of Si supplying capacity of paddy soils

An acetate buffer (pH 4) extraction has been widely used in Japan as a method for evaluating plant available Si of paddy soils in soil testing. However, Si extracted by the acetate buffer solution evidently increases in paddy soils that were previously amended with calcium silicate fertilizers (slags). It seems that the "too strong" acetate buffer dissolves some non-available Si from the residual calcium silicate fertilizers. That is why the use of the acetate buffer method is likely to underestimate the need for Si fertilization of paddy soils previously amended with calcium silicate fertilizers. On the other hand, decreased soil Eh of a submerged soil, in which rice plant grows and absorbs Si, increases water-soluble Si. From these points of view, several new methods for evaluation of available Si of paddy soils have been proposed in Japan.

Nonaka *et al.* (1988) developed a method for measuring water-soluble Si in paddy soils that involved submerged soil incubation. In this method, a 10 g air-dried soil sample is submerged in a 100 mL cylindrical bottle (about 45 mm in diameter) with 10 mL water and incubated at 40°C for a week, after which time the supernatant is analyzed for Si content. Sumida *et al.* (1988) developed a soil incubation method (supernatant method) that required even longer submergence time than the method of Nonaka *et al.*. Although Si extracted by either method generally correlates better with Si in rice straw than Si extracted by the acetate buffer method, both methods have some disadvantages such as requiring longer time to obtain soil testing results and neglecting Si adsorbed on soil solid phase. Actually, the amount of Si extracted by either method ranges approximately from 50 to 250 mg SiO₂ kg⁻¹ soil. This means the amount of Si in plow layer ranges approximately from 5 to 25 g SiO₂ m⁻², assuming that the weight of plow layer per m² is 100 kg. Considering that Si is taken up by rice plant in large amounts that are about ten-fold greater than those of N and are supplied mainly from paddy soils, both methods measure merely the intensity factor. Sumida *et al.* (1991) mentioned that the supernatant method is not appropriate in the comparison among soils largely differing on soil texture or clay mineral.

Kitada *et al.* (1992a) designed a modified surface water dissolution (SWD) method in which the surface water obtained by submerged soil incubation was exchanged for distilled water every week. The amount of Si supplied from soil was estimated by summing up Si dissolved in every surface water during the period corresponding to rice cultivation duration. Because the SWD method may be able to evaluate Si adsorbed on soil solid phase and dissolved gradually from soil, it seems that this method measures Si nearly under the field condition in which rice plant absorbs Si. They showed that the sum of Si estimated by the SWD method and Si supplied from irrigation water was close to the amount of Si taken up by rice plant. However, the SWD method is not suitable for use in a routine soil testing, because of the considerably long time requirement (twelve weeks) and its complexity.

Sumida (1991) designed another method in which the dissolution and adsorption of Si in a submerged soil were examined by incubating the soil with silicate solutions containing from 0 to 100 ppm SiO₂ at 30°C using a soil:solution ratio by 1:10. After the incubation (five days), the amounts of dissolution or adsorption of Si unit soil (v mg SiO₂ kg⁻¹ soil) were proportional to the Si concentration in supernatant solutions (u mg SiO₂ L⁻¹) like the following equation: $u/a + v/c = 1$, where constant a (mg SiO₂ L⁻¹) is the Si concentration of solution at which neither dissolution nor adsorption occurs. Constant c (mg SiO₂ kg⁻¹ soil) indicates the amount of Si dissolved from soil with vast amounts of water, that is, evaluates plant available Si including Si adsorbed on soil solid phase which can easily dissolved when the Si concentration in a soil solution decreases due to Si

uptake by rice plant. Therefore, constant c was denominated “potentially soluble Si”. Sumida concluded that the dissolution and adsorption method provided the most suitable indices of the Si supplying capacity of paddy soils amended with calcium silicate fertilizer and/or organic matter and paddy soils with varying clay minerals and soil texture. The potentially soluble Si concept may be good, but the dissolution and adsorption method is also not suitable for a routine soil testing because of its long incubation period (five days) and its complexity.

Recently, two phosphate buffer methods for measuring plant available Si of paddy soils were proposed independently. While Shigezumi *et al.* (2002) have used 20 mM phosphate buffer (pH 6.95) solution, Kato (1998) has done 40 mM phosphate buffer (pH 6.2) solution (made by titrating 40 mM Na_2HPO_4 with 40 mM NaH_2PO_4 to pH 6.2). In the method of Kato, a soil with the phosphate buffer (pH 6.2) solution using a soil:solution ratio by 1:10 is shaken for five minutes, and is incubated for 24 hours at 40°C , and then is shaken for five minutes. After centrifugation and/or filtration, the Si concentration in the supernatant is measured colorimetrically. Kato has made the evaluation accuracy improved due to the increase of Si extracted by optimizing the extraction condition such as the concentration and pH of the phosphate buffer solution and incubation period. On the other hand, Shigezumi *et al.* has shortened the extraction time (for 5 hours at 40°C), considering its rapidity. Assuming that the origin of Si taken up by rice plant from paddy soils is mainly Si adsorbed on soil solid phase, both methods extract it by exchanging for phosphorus (P) which is adsorbed for competition with Si. Although for some soils such as ando soil neither method produced a good correlation, Si extracted by the phosphate buffer solution generally correlated better with Si uptake by rice plant than Si extracted by the acetate buffer solution. The phosphate buffer methods are superior to the other methods on rapidity, but the disadvantage that Si dissolved slowly in reduced condition has not been evaluated has remained on the methods.

As early as 1958, Imaizumi *et al.* proposed a criterion for Si fertilizer application. Si concentration of rice straw, with promising beneficial effect as a silicate fertilizer application was less than $110 \text{ mg SiO}_2 \text{ g}^{-1}$. In the northern part of Japan, rice blast is a serious problem, but it may be considerably suppressed if the Si concentration of rice plant at full heading stage can be higher than $70 \text{ mg SiO}_2 \text{ g}^{-1}$ (Sumida, 1992). Its value of $70 \text{ mg SiO}_2 \text{ g}^{-1}$ corresponds approximately to the value of $110 \text{ mg SiO}_2 \text{ g}^{-1}$ in rice straw at harvest. Based on the relationship between the potentially soluble Si of soil with the dissolution and adsorption method and the Si concentration of rice plant at full heading stage, Sumida (1992) proposed a criterion for Si fertilizer application to paddy fields in the northern part of Japan. The criterion states that if the potentially soluble Si is less than $300 \text{ mg SiO}_2 \text{ kg}^{-1}$ soil, beneficial effects of a Si fertilizer application would be observed. On the other hand, Yamagata prefecture (2002) also put forward another new criterion for Si fertilizer application, based on the relationship between the Si extracted with the phosphate buffer (pH 6.2) method and the Si concentration of rice straw at harvest. According to the Yamagata's criterion, the measured value of Si expecting beneficial effects of the Si fertilization is less than $300 \text{ mg SiO}_2 \text{ kg}^{-1}$ soil. Though the relationship between the dissolution and adsorption method and the phosphate buffer (pH 6.2) method has not been investigated, the range of the measured values with the dissolution and adsorption method corresponds nearly to that with the phosphate buffer (pH 6.2) method.

(4) Fluctuation Si supplying capacity of paddy soils with soil management

Since the middle in 1970's, some paddy fields in Japan have been utilized with irrigated paddy rice and upland crop rotation. In this system, the N supplying capacity of paddy soils greatly changed. Kitada *et al.* (1992b) reported that the Si supplying capacity of paddy soils tended to be higher, as “soil uplandization index” (Naganoma *et al.*, 1983) was bigger. The soil uplandization index of an upland-paddy rotational soil is calculated from the relative relation among the sedimentation volumes of the upland-paddy rotational soil, a continuous paddy soil and a permanent upland soil. This index ranges from 0 to 1, where 0 would indicate nearer to the continuous paddy soil and 1 nearer to permanent upland soil. Kitada *et al.* (1992b) mentioned

that the Si uptake by rice plant was 750 kg SiO₂ ha⁻¹ when the soil uplandization index was less than 0.3, on the other hand, the Si uptake by rice plant was 900 kg SiO₂ ha⁻¹ when the soil uplandization index was more than 0.3. Sumida *et al.* (2001) demonstrated that the Si extracted with the phosphate buffer (pH 6.2) method increased when the upland field returned to paddy field. The Si uptake of rice plant in the first year remarkably increased but the Si uptake in the second year slightly increased in comparison with the Si uptake of rice plant in continuous paddy field. The proper management of Si fertilizer based on soil testing is required, because Si supplying capacity of paddy soils greatly fluctuates when paddy-upland rotation is employed.

Recently, rice cultivation with non-tillage or non-puddling which aimed at labor saving and avoiding excess moisture injury of an upland crop in paddy-upland rotation is noticed. Kato *et al.* (2001) investigated the influence of the soil management of non-puddling or non-tillage on distribution of Si in a soil profile. According to them, in the non-tillage paddy field, plant available Si with either the submerged soil incubation method or the phosphate buffer (pH 6.2) method is accumulated in the surface layer (0 to 3 cm), partially because of rice straw spread over the paddy field as Si source. In the next soil layer (3 to 18 cm), while the water-soluble Si of the non-tillage paddy soil with the submerged soil incubation method has been less than that of either the non-puddling paddy soil or the puddling paddy soil, the exchangeable Si of the non-tillage paddy soil with the phosphate buffer (pH 6.2) method has been slightly more than that of the non-puddling paddy soil and considerably more than that of the puddling paddy soil. Therefore, they suggested that the soil management of non-puddling or non-tillage increases Si adsorption capacity of paddy soils.

(5) Attempts to establish a new criterion for Si fertilizer application in paddy soils

Yamagata agricultural experiment station, examined both the phosphate buffer (pH 6.95) method proposed by Shigezumi *et al.* and the phosphate buffer (pH 6.2) method proposed by Kato. In 2002, this station set a new criterion for Si fertilizer application based on the relationship between the Si extracted with the later method and the Si concentration of rice straw at harvest (above mentioned). Recently, the diagnosis for available Si in paddy soils which would be used in a soil survey project conducted by Ministry of Agriculture, Forestry and Fisheries of Japan was revised from the conventional acetate buffer method to the submerged soil incubation method and the phosphate buffer (pH 6.2) method. In main rice-producing districts, a new criterion that can be applied in each region will soon be determined.

Table 2. Characteristics of methods for evaluating plant available Si of paddy soils.

Method	Reagent and condition for extraction	Disadvantage and advantage	Reference
Acetate buffer (pH 4) method	N acetate buffer, 30°C/5 h, 1 : 10	Dissolving non-available Si	Imaizumi <i>et al.</i> , 1958
Submerged soil incubation method	Water, 30°C/4 W or 40°C/1 W, 1 : 6	Long time requirement and measuring only intensity factor High accuracy (capacity factor)	Nonaka <i>et al.</i> , 1988
Dissolution and adsorption method	Si solution, 30°C/5 D, 1 : 10	Long time requirement and complexity of the procedure High accuracy (capacity factor)	Sumida, 1991
Surface water dissolution method	Water, 30°C/12 W, 1 : 6	Long time requirement and complexity of the procedure High accuracy (capacity factor)	Kitada <i>et al.</i> , 1992
Phosphate buffer (pH 6.2) method	40 mM phosphate buffer, 40°C/24 h, 1 : 10	Cannot be adopted for Ando soil High accuracy (capacity factor) and rapidity	Kato, 1998
Phosphate buffer (pH 6.95) method	20 mM phosphate buffer, 40°C/5 h, 1 : 10	Cannot be adopted for Ando soil Rapidity	Shigezumi <i>et al.</i> , 2002

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Silicon Status of Selected Louisiana Rice and Sugarcane Soils

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The addition of silicon (Si) as calcium silicate has been shown to increase yields in sugarcane and rice, especially in Florida, USA. Si additions to rice grown in Florida have also improved disease resistance. Preliminary research with rice grown under field and greenhouse conditions in Louisiana has indicated potential for improving grain yield and reducing the incidence of disease. The response of sugarcane to Si in Louisiana is currently being studied. Louisiana produces rice and sugarcane on a wide range of soils, but little is known about the Si status of these soils. Fifteen soils were collected throughout Louisiana from areas where rice and sugarcane have been grown. The contents of monosilicic acids, polysilicic acids, acid-extractable, and acetic extraction Si were determined in surface (0-15 cm) soils. Actual Si in the form of monosilicic acid was found to be low in seven soils, ranging from 20.78 to 33.96 mg Si kg⁻¹ soil. Silicon determined on an acid-extractable basis indicated that nine of the soils were low in Si content, ranging from 307.29 to 556.20 mg Si kg⁻¹ soil, and this group included the seven soils found to be low in monosilicic acid. Five soils were considered to be either deficient or critically deficient as determined by the relationship between monosilicic acid and acid-extractable Si. This study suggests that most Louisiana soils from areas where rice and sugarcane have been grown contain marginal levels of Si and, therefore, may respond to calcium silicate amendments, especially those soils found to be low in monosilicic acid.

INTRODUCTION

Silicon (Si) is not currently recognized as an essential element for plant growth, but numerous beneficial effects have been reported in rice and sugarcane (Osuna-Canizalez *et al.*, 1991; Snyder *et al.*, 1986; Anderson *et al.*, 1987; Datnoff *et al.*, 1991). In Florida, most of the rice production occurs on the Everglades Histosols, and significant grain yield increases have been shown with the addition of calcium silicate slag (Snyder *et al.*, 1986). Rice yield increases and reduced grain discoloration have also been reported on Alfisols and Ultisols (Yamauchi and Winslow, 1989). Preliminary research in Louisiana indicated that modest increases in grain yield and slight improvement in disease resistance could occur, but these results were quite variable even with soils possessing very similar properties (Bollich *et al.*, 2001). The objective of this study was to determine the Si content of 15 Louisiana soils previously cropped to rice and/or sugarcane.

METHODS

Soils were collected from 15 field sites in 10 Louisiana parishes for this experiment. Fresh soil samples were collected from a 0- to 15-cm depth at each site. Soil moisture was variable across samples collected, but earlier research determined that changes in soil moisture from 5 to 50% had no effect on the sensitivity of laboratory methods to determine the content of soluble silicic acids, which are mobile and weakly adsorbed (Matichenkov *et al.*, 1997; Matichenkov and Snyder, 1996). Soluble monosilicic acid was determined on water extracted from fresh soil samples using the Mallen and Raily method (Iler, 1979). Six g of fresh soil were

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placed into beakers with 30 ml of water. The samples were shaken for 1 hour, filtered through No. 40 filters, and the filtered extract was analyzed for soluble monosilicic acids. An aliquot of extract (20-25 ml) was subjected to ultrasound for 1 minute under medium power for depolymerization. All of the polysilicic acid was transformed into monosilicic acid using the molybdate method (Matichenkov *et al.*, 1997). Biogeochemically active amorphous silica was determined from 2-g air-dried samples after a 1-day extraction with 20 ml 0.1 M HCL (Barsykova and Rochev, 1979). After 24 h, the extract was filtered through No. 40 filters and analyzed for Si using the colorimetric method (Iler, 1979). The concentration of Si was measured with a photometer at 660 nm.

RESULTS

To characterize Si deficiency, actual Si (mobile monosilicic acid) and potential Si (source for monosilicic acid) need to be described (Matichenkov, personal communication). Monosilicic acid content ranged from 6.68 to 33.96 mg Si kg⁻¹ soil (Table 1). Seven of the 15 soils were determined to be low in Si based on monosilicic acid content. Acid-extractable Si content ranged from 90.14 to 546.98 mg Si kg⁻¹ soil. Potential Si contents of 500-600 mg kg⁻¹ as determined by acid extraction are considered low. Nine of the 15 soils were determined to be low in Si based on potential Si content. Since monosilicic acid Si is derived from the pool of biogeochemically active amorphous silica, these low contents indicate concern regarding the Si status of most soils evaluated in this study. Matichenkov *et al.* are currently developing a scale to categorize soil Si status. According to their estimations, all of the soils evaluated in this study were considered at best to be low in Si based on the summarizing parameter (SP = acid-extractable Si + 10*monosilicic acid). Three soils were deficient and two soils were critically deficient when Si content was based on SP (Table 2).

Table 1. Content of monosilicic acid, polysilicic acid, and acid-extractable Si in selected Louisiana soils.

Order/Series	Monosilicic	Polysilicic	Acetic extraction	Acid-extractable
(mg Si kg ⁻¹ soil)				
Alfisols				
Crowley sil	20.78	29.93	180.3	276.47
Midland sil	11.22	13.57	102.91	163.89
Crowley sil	23.44	23.77	201.50	307.29
Crowley sil	7.26	6.26	46.03	104.47
Hebert sil	31.71	14.49	252.65	514.20
Gallion sil	6.68	10.22	94.21	143.40
Grenada sil	12.99	90.67	227.55	419.97
Entisols				
Norwood sil	14.70	27.95	136.89	373.87
Vacherie sil	18.01	14.89	193.97	457.87
Rita m	8.27	9.19	61.73	160.82
Rita m	9.51	6.15	37.12	90.14
Inceptisols				
Alligator c	33.96	13.14	298.72	546.98
Sharkey c	29.53	12.16	281.72	556.20
Perry c	24.80	13.62	183.67	430.21
Mollisols				
Moreland c	29.64	10.13	259.61	546.98
LSD (0.05)	2.3	5.9	15.0	25.0

Table 2. Levels of actual and potential deficiency of activated Si in Louisiana soils.

Order/Series	Si deficiency level ¹		
	Actual	Potential	Summarizing parameter
Alfisols			
Crowley sil	L	D	L
Midland sicl	D	D	D
Crowley sil	L	L	L
Crowley sil	C	D	C
Hebert sicl	L	L	L
Gallion sil	C	D	L
Grenada sil	D	L	L
Entisols			
Norwood sil	D	L	L
Vacherie sil	D	L	L
Rita m	C	D	D
Rita m	C	C	C
Inceptisols			
Alligator c	L	L	L
Sharkey c	L	L	L
Perry c	L	L	L
Mollisols			
Moreland c	L	L	L

¹C = critically deficient; D = deficient; L = low; actual = monosilicic acid; potential = acid-extractable Si; summarizing parameter = Acid-extractable + 10*monosilicic acid.

DISCUSSION

The 15 Louisiana soil samples evaluated for Si content represent a very diverse selection of soils that are commonly cropped to rice and/or sugarcane, and in some instances, other rotation crops. This group represented four soil orders and included clays, silty clay loams, silt loams, and mucks. In previous field research (Bollich *et al.*, 2001), yield responses to calcium silicate slag applications were measured on two Crowley silt loam soils and the Rita muck. There was no rice yield response to slag applications on a Sharkey clay, Moreland clay, Hebert silty clay loam, or a Crowley silt loam from a different area. But according to the categorization of Si soil content in this current study, all would be considered either deficient or low in Si. Since calcium silicate slag was incorporated very minimally and at planting in the field experiment, accumulation of Si by the rice plants may have been hindered. There was also no evaluation during the following cropping season to determine whether a successive rice crop would respond to the calcium silicate previously applied. Future research should address these issues when attempting to evaluate the response of Si amendments on rice.

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Persistence and Availability of Rice Plant Si in Soils

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Recycling of plant silica (Si) seems to be important for sustainable rice cultivation. Rice plant requires very high amount of Si, but majority of Si is accumulated in straw and hull. Even though Si that comes from irrigation water is becoming lesser (Kogawa *et al.*, 1982; Kumagai *et al.*, 1998), we can minimize additional input of Si if we utilize Si-phytoliths of rice plant effectively. But there is a big discrepancy about stability and/or availability of plant opaline Si.

Most of geologist and archaeobotanical researchers consider Si-phytoliths as fairly stable and very useful tool to interpret past vegetation and paleoclimate conditions even ten thousands years ago. They determine plant species and its production rate from type and amount of Si-phytoliths detected in soil profile (Kondo, 1988; Sase & Kondo, 1974). This method was used also to estimate the amount of organic materials applied into paddy field since ancient times (Hashikawa *et al.*, 1980; Sasaki & Fujiwara, 1975).

On the other hand, there are many reports that show Si-phytoliths may provide available Si to plants. Ma and Takahashi (1991) reported that the short-term availability of Si in the rice straw is as low as about 9% per two years of cultivation. Takahashi (1987) estimated that about 70% of Si brought in as rice straw manure was utilized by rice crop, based on 40 years long-term experiment. This corresponds to 8% of Si utilized yearly if first order reaction is applied. Only 1% of Si-phytoliths will survive 60 years later, if this decomposition rate is assumed.

Ishibashi (1954) reported that rice hull Si was highly effective to young seedling, but the data is not necessarily applicable to actual field. There are several reports that indicate availability of Si-phytoliths. Nishikawa, *et al.* (1981) investigated the effects of rice hull and straw to rice plants and found increases of Si content comparable to calcium Si slag. Seki *et al.* (1989) and Sistani *et al.* (1997) reported rice hull ash provides available Si. But the quantitative availabilities of Si-phytoliths are not shown clearly.

It must be important to study about availability of Si-phytoliths and clarify the mechanism that ensures stability of Si-phytoliths.

METHOD

Pot experiment

A pot culture experiment was conducted during summer 2001. Si sand (<210 μm , "Yanagisawa Co., No.7") was washed by decantation and used as main potting medium to minimize supply of soluble Si. Plastic pot with 15 cm diameter and 25 cm high was filled with 5.5 kg of the sand. Polyolefine coated urea, superphosphate, potassium chloride (1 g / pot each as N, P_2O_5 and K_2O), dolomite (1 g / pot) and surface soil of Kan-non-dai experimental field (25 g / pot) were added to supply essential nutrients. Si containing materials like rice hull (26 g), rice hull ash (11.4 g), rice straw manure (40 g), and calcium silicate slag (13.3 g) were added or not added (control) to each pots with three replications. Three rice plants (cultivar "Nihonbare") were transplanted per pot and irrigated with distilled water to maintain flooded condition. Dry-matter production, grain yield, and uptake of Si were measured.

Si-phytoliths in long-term experiment field soil

Surface soil samples were obtained from long-term experimental fields of National Agricultural Research

Center and analyzed for amount of Si-phytoliths. Experimental fields are; Plot A (rice straw applied since 1987), plot B (rice straw manure and/or chemical fertilizer applied since 1931), and plot C (rice straw and/or chemical fertilizer applied since 1987).

One g of soil is pretreated with 30% hot H_2O_2 and shaken for 1 h in 0.02% hexametaphosphate solution. Particles those smaller than 20 μm were removed by gravity sedimentation method. The sediment was transferred into heavy liquid (1.92 g / mL) and mixed. Mixture was centrifuged for 60 min at 10,000 rpm and then floating Si-phytoliths was separated. The separation by floatation-centrifugation was repeated five times. Whole heavy liquid samples with Si-phytoliths were mixed into one, filtered with Millipore and washed with distilled water. Si-phytoliths on the filter are subjected to quantitative measurement.

RESULTS

Growth of rice plant in pot with Si sand as main potting medium seemed healthy and no apparent deficiency symptom was observed. But lower half of root showed black color suggesting occurrence of sulfide.

Dry-matter production between treatments was found to be significant but rather small. Effect of different Si sources on grain yield was rather large. Rice hull or rice straw manure treatment recorded 50% higher yield than control (Fig. 1). The Si content of rice plant in control treatment was very low (Fig. 2), and it can be attributed to the least amount of available Si in control pots. Si content of straw and hull increased considerably by the application of different Si materials when compared with control. The extent of increase was different from each material and rice hull treatment recorded highest increase. Rice hull ash and rice straw manure gave comparable increase with chemical calcium Si slag (Fig. 2). Availabilities of Si in each material based on difference from control treatment were 12% for rice straw manure, about 25% for rice hull ash and Si slag, and 67% for rice hull (Table 1). These values show high solubility of Si-phytoliths.

Larger amount of Si-phytoliths were found from long-term field treated with rice straw and/or rice straw manure. The differences were considerably large (Table 2). The larger amounts of Si-phytoliths noticed in plots treated with rice straw and or rice straw manure indicated that there is accumulation of Si-phytoliths with the continuous recycling of plant residues in rice fields. Additional data like amount of recycling of plant residues and recovery rate are needed to estimate stability and availability of Si-phytoliths applied into these paddy field condition.

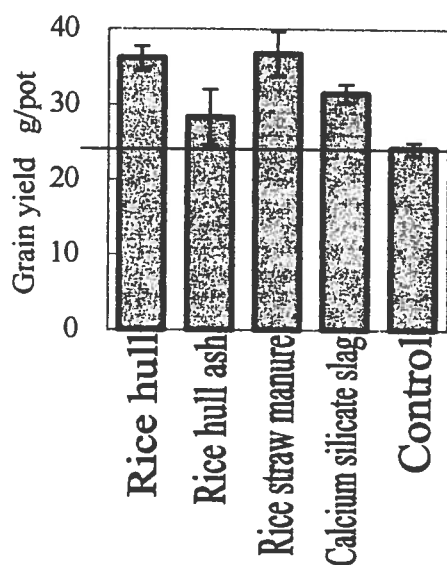


Fig. 1. Effect of silicate material on grain yield

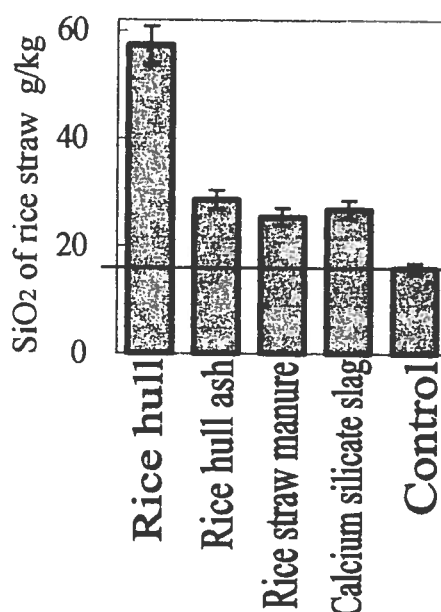


Fig. 2. Effect of silicate material on silicate concentration of rice straw

Table 1. Availability of silicate material by rice plant

Treatment	Rice hull	Rice hull ash	Rice straw manure	Calcium silicate slag	Control
SiO ₂ in rice hull (g/pot)	0.5	0.3	0.3	0.2	0.1
SiO ₂ in straw (g/pot)	4.4	2.3	2.1	2.3	1.3
Total SiO ₂ (g/pot)	4.9	2.6	2.4	2.5	1.4
Increase (g/pot)	3.5	1.2	1.0	1.1	—
SiO ₂ % in material	20	41	20	30	—
SiO ₂ applied (g/pot)	5.2	4.7	7.9	4.0	0.0
Availability (%)	67	25	12	26	—

Table 2. Silica-phytoliths in long-term experiment field soil

Plot	Soil	Treatment	SiO ₂ mg/g soil (Difference)
A	Kohnosu	With rice straw	7.9
		Without rice straw	5.1 (2.8)
B	Kohnosu	With rice straw manure	5.1
		Without rice straw manure	2.8 (2.3)
C	Yawara	With rice straw	14.6
		Without rice straw	8.4 (6.1)

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Effect of Ca-Silicate Amendments on Soil Chemical Properties under a Sugarcane Cropping System

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Under high leaching environments, common to tropical regions, soils undergo significant weathering that results in base stripping and declining charge capacity. The consequences of this natural weathering are acidification and de-silication of soils. The application of a basic Ca-silicate slag (0, 4.5, 9.0 and 12.0 t / ha) to these degraded soils under a sugarcane monoculture, was investigated in two field trials established on contrasting soil types in north Queensland Australia. Changes in soil chemical properties were monitored over a two year period. Surface charge fingerprinting was used to quantify changes in the charge capacity of the soil associated with amendment applications. Significant increases in CEC were observed in all treatments receiving Ca-silicate additions one year after application. In this respect the CEC increased from 1.51 to 4.53 cmol(+)/kg with the application of 12 t / ha of Ca-silicate. In order to differentiate CEC generation associated with an increase in pH, surface charge fingerprints were undertaken on composite samples from each treatment. With increasing additions of Ca-silicate there was a vertical shift in the charge curve over a pH range, suggesting the increase in charge is not associated with an increase in pH *per se*. At a nominal pH of 5.5, the CEC increased from 2.69 cmol(+)/kg in the control treatment to 4.42 cmol(+)/kg with the application of 12 t / ha Ca-silicate. There was no increase in the pH buffering capacity of the soil associated with Ca-silicate additions. By plotting CEC values of the charge fingerprint at pH 5.5 against the rate of Ca-silicate application, it was estimated that for every tonne of Ca-silicate applied, CEC increased by 0.15 cmol(+)/kg. These trends were still present two years after the implementation of treatments. These results would suggested that the application of Ca-silicate slag significantly improves the CEC of degraded soils and may contribute to the observed yield increases of sugarcane on these Si deficient soils.

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Comparison of Three Methods for Evaluation of Available Silicon in Paddy Soils

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INTRODUCTION

In formulating adequate recommendations for silicate fertilizer application, it is really necessary to estimate precisely the amount of plant-available Si in soils. The chemical extraction method using an acetate buffer solution (1 M, pH 4), which had been developed in 1958 (Imaizumi and Yoshida), was most widely used in Japan to measure available silicon (Si) in paddy soils. However, when the silicate fertilizers were previously applied to soils, the Si availability of soils is very likely overestimated by this method. Because Si in the silicate fertilizers residue remaining in soils which shows a low availability for plant is dissolved by the acidity of this extractant. Therefore many studies have been conducted to develop a new method for the estimation of the amount of available Si in soils.

In a soil incubation method proposed by Takahashi (1981), the amount of Si which dissolved from the soil during the incubation under flooded conditions (40°C, 1 week) has been used as a laboratory index of Si availability of paddy soils. It was shown in his report that the amount of Si dissolved from the submerged soil was positively correlated with the Si concentration in the shoot of rice plant. However, it was reported that the soil incubation method was not suitable for comparing the Si availability of soils differing in clay minerals and texture (Sumida 1991). This result could be attributed to the fact that the Si adsorbed on the surface of soil solid phase that can be desorbed when the Si concentration in soil solution decreases is not taken into consideration in this method.

Recently, a simple extraction method using a phosphate buffer solution (20 mM, pH 6.9, 5 h) was proposed (Shigezumi *et al.* 2002). One of our authors (Kato 1998) also examined extraction conditions in detail and proposed another phosphate buffer solution method (40 mM, pH 6.2, 24 h, hereafter referred to as P-buffer method). In both the methods, it was assumed that rice plant takes up the Si present in the soil solution and the Si adsorbed by the soil solid phase that can be desorbed easily. Since the Si adsorption by soils is a competitive reaction with the phosphorus adsorption, the adsorbed Si could be efficiently extracted by the phosphorus solution. It has been reported that the concentration of Si increased with time of submergence. This fact suggests that there is the Si in soils that can slowly dissolve under reducing condition. However, the slow release of Si from submerged soil is not taken into account in the P-buffer method. A pre-incubation before extraction by the P-buffer method could provide more precise estimation of available Si in soils.

In this study, the available Si in paddy soils collected in Yamagata prefecture, Japan was measured by both the soil incubation method and the P-buffer method. As another evaluation method, the P-buffer method with a pre-incubation of submerged soil (hereafter referred to as combination method) was also tested. The correlation studies were carried out between the amount of Si extracted by the three evaluation methods and the Si concentration in the shoot of rice plant (*Oryza sativa*).

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MATERIALS AND METHODS

The 15L soil samples were collected from paddy fields in Yamagata prefecture, Japan. The 72 soil samples were Gley Lowland soils, and 31 samples were Gray Lowland soils. The 11 Wet Andosols samples and 9 Brown Lowland soils samples were also included. All the soil samples were air-dried and sieved (<2 mm) before soil analysis. The amount of available Si in the soils was measured by the soil incubation method, the P-buffer method and the combination method. The experimental procedures of these extraction methods were as follows:

1) Soil incubation method

The soil (10 g) was put into a 120 mL volume plastic bottle and 60 mL of deionized water was added. The bottle was shaken lightly to remove air bubbles and allowed to stand in an incubator without shaking (40°C). After 1 week, the supernatant was taken and filtered through a 0.2 µm-pore size filter to obtain a clear sample solution.

2) P-buffer method

A sodium phosphate buffer solution (40 mM, pH 6.2, 50 mL) was added to 5 g of the soil and shaken for 5 min. The soil suspension was put into an incubator without shaking (40°C, 24 h). After the incubation, the soil was shaken again during 5 min and then filtered using a filter paper and a 0.2 µm filter.

3) Combination method

The soil (10 g) was incubated under flooded condition during 1 week as described in the soil incubation method. After incubation 40 mL of a phosphate buffer solution (100 mM, pH 6.2) was added and mixed. Therefore the P concentration in the solution can be 40 mM just after mixing. The soil was incubated again during 24 h at 40°C to extract Si from the soil. The shaking and filtration were performed in the same manner as those in the P-buffer method.

There were two replications for all the extraction methods. The amount of Si extracted was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Rice plant tops were sampled in a maturing stage from the same paddy fields where the soil samples were collected. The Si concentration in the shoot was analyzed and compared with the amount of Si extracted by three methods mentioned above.

RESULTS AND DISCUSSION

The amount of Si extracted by the P-buffer method was large as compared with that extracted by the soil incubation method in all the soil samples. The combination method extracted more Si than the P-buffer method in almost all the soils. However in a large number of Wet Andosols samples the pre-incubation of soils decreased the amount of Si extracted by the phosphate solution. This decrease could be attributed to the increase in the solution pH during the pre-incubation. Because it was confirmed that the amount of Si extracted by the phosphate solution decreased with increase in the solution pH and this tendency was large in Wet Andosols (Kato 1998). In fact, the solution pH after the extraction of Si was high in the combination method as compared with that in the P-buffer method (data was not shown). The decrease in the Si extracted from Wet Andosols samples caused by the pre-incubation also suggested that the amount of Si dissolved slowly under reducing condition was small in these soils.

The relationship between the Si concentration in the shoot of rice plant and the amount of Si extracted from the soils by the soil incubation method was shown in Fig. 1. Although a positive correlation was obtained for each soil types, the correlation coefficient (0.641***) for all the soils tested here was not high. The values of available Si in Wet Andosols measured by the soil incubation method tended to be low in comparison with those in other soil types. A plausible reason of these results is a high silicon adsorption in Wet Andosols. It has been reported that the soil ability to adsorb Si is different among soils and positively correlated with a

phosphate adsorption capacity. Therefore in the Wet Andosols samples that showed a high phosphorus adsorption capacity the amount of Si adsorbed by the soils could be large relatively. Since the adsorbed Si in soils was not taken into account in the soil incubation method, the available Si in Wet Andosols might be underestimated.

As shown in Fig.2, a better result was obtained in the P-buffer method. The correlation coefficient (0.678***) obtained by the P-buffer method was higher than that obtained by the soil incubation method. However there was no correlation in Wet Andosols. The amount of Si extracted from Wet Andosols tended to be large as compared with that in the other soils which showed the same level of Si concentration in the shoot.

In the combination method, a highly positive correlation was obtained (Fig. 3). The correlation coefficient

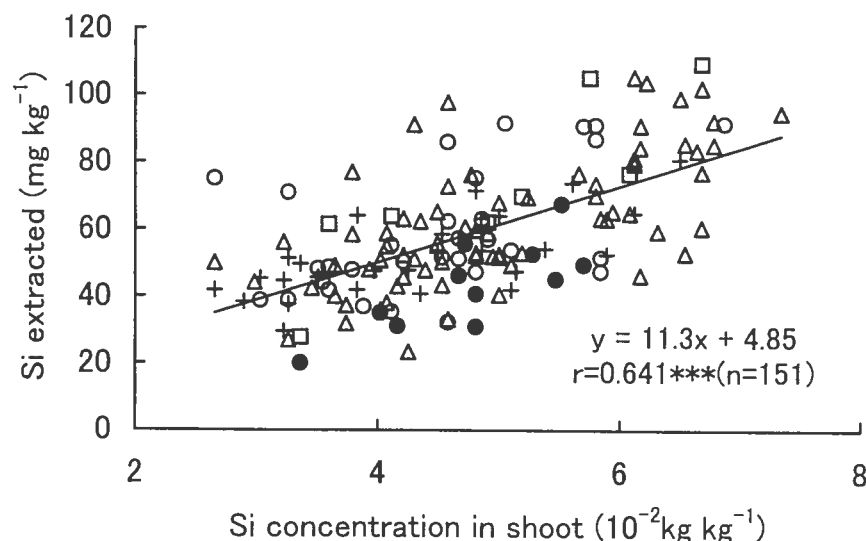


Fig. 1. The relationship between the Si concentration in shoot of rice plant and the amount of Si extracted by the soil incubation method.

○ ; Gray Lowland soils, △ ; Gley Lowland soils, ● ; Wet Andosols, □ ; Brown Lowland soils, + ; other soils

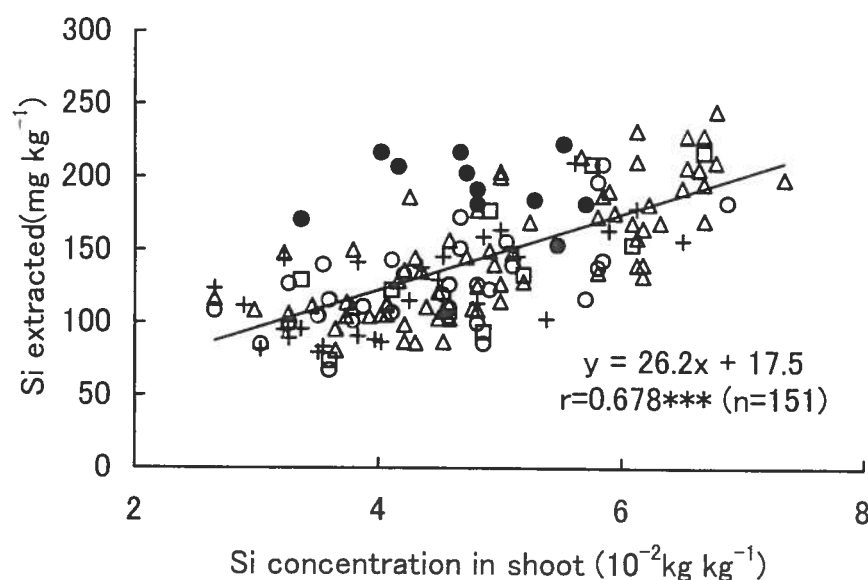


Fig. 2. The relationship between the Si concentration in shoot of rice plant and the amount of Si extracted by the P-buffer method.

○ ; Gray Lowland soils, △ ; Gley Lowland soils, ● ; Wet Andosols, □ ; Brown Lowland soils, + ; other soils

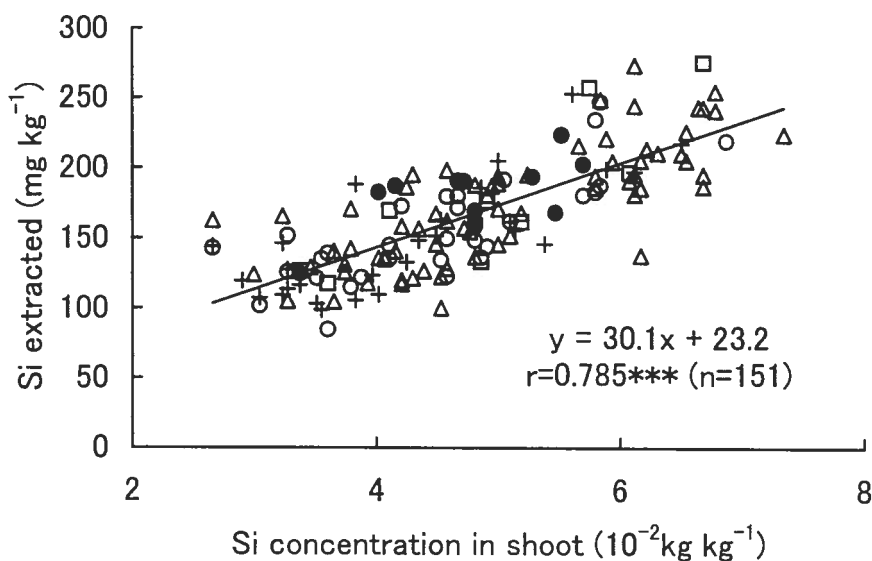


Fig. 3. The relationship between the Si concentration in shoot of rice plant and the amount of Si extracted by the combination method.

○ ; Gray Lowland soils, △ ; Gley Lowland soils, ● ; Wet Andosols, □ ; Brown Lowland soils, + ; other soils

(0.785***) for all the soils was the highest among the evaluation methods tested in this study. Even in Wet Andosols, the values of available Si were correlated ($r=0.657^*$) with the Si concentration in the shoot and did not deviated from the regression curve calculated by using the values of all the soil samples.

It was concluded that the combination method in which the Si in soils was extracted by the phosphate buffer solution after the incubation of submerged soils was found to be the best evaluation method to estimate available Si in paddy soils.

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Enhancement of Available Silicate in a Soil by Potassium Uptake of some Crop Species

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INTRODUCTION

Long-term field experiments suggest that some plants can grow well even on a potassium (K) deficient soil, whereas other plants poor. This implies that plants may have an ability to absorb insoluble K forms in a soil. K has three types of existing sites, namely, 1) planar sites, 2) edge sites and 3) inner sites. If a crop takes up K from 2) and/or 3), silicate (SiO_2) is expected to release from a soil and/or to be absorbed by crops. We have tested this hypothesis from a long term field experiment that has been in operation since 1976.

MATERIALS AND METHODS

A long-term field experiment has been conducted to evaluate of N, P and K of Memuro soil since 1976 (Brown Andosol, SL. Total K; 7236, exchangeable K; 99 mg K kg^{-1}). Sugar beet, maize, potato, soybean, kidney bean, winter wheat and spring wheat were grown in crop rotation system. We analyzed soil K in 0 plot, no-K plot (i.e. without K fertilizer but with application N and P), NPK plot (i.e. the plot applied complete fertilizer). We plowed the control plot (i.e. unplanted and unfertilizer plot) to evaluate change of K and SiO_2 . Soils of these plots were collected before fertilization in 2000. We measured 1M- NH_4OAc (pH 7) extracted K as exchangeable soil K and total K. K and SiO_2 uptake by crops were estimated from their yields and % K and SiO_2 in dry matter.

RESULTS AND DISCUSSION

Potato showed severe K deficiency under no-K treatment and yielded poorly compared to NPK treatment plot (data not shown). The total K uptake by potato was about 18 kg ha^{-1} in no-K plot and 20% of that in NPK plot (Table 1). Potassium deficiency didn't have serious effect on other crops. Yield and K uptake of winter wheat in no-K plot were almost same as NPK plot (Table 1). These results showed that there were the most difference in K uptake among crop species and potato appears to be least tolerant to low available K among 6 crops studied.

The total amounts of K uptake by crops during 23 years were 475 kg K ha^{-1} in 0 plot, 1321 kg K ha^{-1} in no-K plot and 2855 kg K ha^{-1} in NPK plot (Table 1). NPK plot had received 2450 kg ha^{-1} fertilizer during long-term experiment. We estimated that the decrease in the amount of K in each plot within 30 cm depth compared to control plot as exploitation during 23 years. The supply of exchangeable K to these crops was evaluated from the decreasing in exchangeable K of these three plots compared to the control plot. The exploitation of exchangeable soil K were 100 kg ha^{-1} in 0 plot and 230 kg ha^{-1} in no-K plot and weren't found in NPK plot because of K fertilizer (Table 2). The exploitation of exchangeable K couldn't explain K uptake by crops, especially in no-K plot. The highest amount of this exploitation of total K was 1750 kg K ha^{-1} in no-K plot, second highest was 1450 kg K ha^{-1} in NPK plot and the lowest was 560 kg K ha^{-1} in 0 plot (Table 2). The results indicated that crops in 0 plot and especially no-K plot utilized insoluble forms of K from the soil.

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Table 1. K uptakes by crops.

Year	Crop	0 plot	No-K plot (kg K ha ⁻¹)	NPK plot
1976	Kidney bean	7 (22) ^{a)}	26 (79)	32 (100)
1977	Winter wheat	13 (29)	52 (113)	46 (100)
1978	Sugar beet	49 (21)	159 (69)	231 (100)
1979	Kidney bean	3 (10)	19 (59)	33 (100)
1980	Winter wheat	10 (23)	49 (114)	43 (100)
1981	Sugar beet	8 (3)	116 (50)	231 (100)
1982	Soy bean	19 (14)	53 (39)	135 (100)
1983	Potato	17 (13)	24 (19)	126 (100)
1984	Winter wheat	8 (11)	60 (89)	68 (100)
1985	Sugar beet	29 (12)	97 (42)	232 (100)
1986	Maize	38 (18)	45 (21)	210 (100)
1987	Soy bean	19 (16)	37 (31)	117 (100)
1988	Potato	17 (15)	19 (17)	113 (100)
1989 ^{b)}	Winter wheat	8 (11)	60 (89)	68 (100)
1990	Sugar beet	17 (7)	108 (48)	226 (100)
1991	Maize	42 (18)	100 (42)	237 (100)
1992	Soy bean	23 (21)	38 (34)	112 (100)
1993	Potato	14 (20)	16 (22)	72 (100)
1994	Spring wheat	18 (45)	18 (43)	41 (100)
1995	Sugar beet	37 (28)	92 (70)	131 (100)
1996	Maize	26 (16)	86 (54)	160 (100)
1997	Soy bean	37 (32)	33 (28)	115 (100)
1998	Potato	16 (21)	14 (18)	76 (100)
The Total amount of K uptake (1976-1998) (kg K ha ⁻¹)		475	1321	2855 ^{c)}

a) Relative % of that in NPK plot in the same year

b) No data in this year, substitution data in 1984

c) 2450 kg K ha⁻¹ fertilizer applied (1976-1998)**Table 2.** Estimates of exploitation^{a)} of soil K and SiO₂^{b)} since 1976 and uptake by crops^{c)}.

			control plot	0 plot	No-K plot	NPK plot ^{d)}
Soil K	Exchangeable K	(mg K kg ⁻¹)	99	70	34	116
	Total K	(mg K kg ⁻¹)	7236	7073	6740	6827
Soil SiO ₂	Extractable SiO ₂ with acetic acid	(mg SiO ₂ kg ⁻¹)	1083	1090	900	967
Exploitation	Exchangeable K	(kg K ha ⁻¹)	-	100	230	-60
	Total K	(kg K ha ⁻¹)	-	560	1750	1450
	Extractable SiO ₂ with acetic acid	(kg SiO ₂ ha ⁻¹)	-	-20	650	410
K uptake		(kg K ha ⁻¹)	-	475	1321	2855
SiO ₂ uptake		(kg SiO ₂ ha ⁻¹)	-	1036	3855	3441

a) The decrease in the amount of K in each plot within 30 cm depth compared to control plot

b) Topsoil 0-30cm before fertilization in 2000

c) Total amounts of uptake by crops (1976-1998)

d) 2450 kg K ha⁻¹ fertilizer applied (1976-1998)

If a crops take up insoluble K (2) edge sites and/or 3) inner sites), SiO₂ may be release into a soil and/or absorbed by crops. To confirm this hypothesis we estimated cumulative amount of SiO₂ uptake by crops during 23 years. Neither silicate fertilizer nor improvement materials with silicate applied to long-term field. SiO₂ taken up by crops came only from soil. The highest of SiO₂ was 3855 kg SiO₂ ha⁻¹ in no-K plot, second highest was 3441 kg SiO₂ ha⁻¹ in NPK plot and the lowest was 1036 kg SiO₂ ha⁻¹ in 0 plot (Table 2). In no-K plot the decrease in the amount of total K was the highest. These results showed that crops in no-K plot utilized insoluble forms of K from soil and released SiO₂, which is then taken up by the crops. In NPK plot

both SiO_2 uptake and the exploitation of total K were the second highest. Amount of K uptake by crops from soil in NPK plot was unknown because efficiency of K fertilizer wasn't clear, but these results support that crops in NPK plot utilized insoluble forms of K from soil. In long-term field experiment we observed that uptake of K from insoluble forms of K in the soil is related to solubilization of SiO_2 . These results suggested the relationship between decrease of total K and SiO_2 uptake.

In order to confirm the relationship between K uptake by crop species and solubilization of SiO_2 we conducted a pot experiment. We used Edosaki soil (Andosol, LiC, Total K; 7400, exchangeable K; 31 mg K kg^{-1}) which has poor K supplying power. We applied no K source and then examined K and SiO_2 uptake among three dicotyledon crops (groundnut, soybean and sunflower). Three dicotyledon crops showed slight SiO_2 uptake (Fig. 1). Extractable SiO_2 with acetic acid in the soil after harvesting only groundnut is smaller than that in the soil of the control treatment pot (i.e. unplanted pot) and groundnut was inferior to other crops and mainly absorbed exchangeable K (Fig. 1). Though the reason why SiO_2 in groundnut pot was less than the control treatment pot still was unclear, these results suggest groundnut has neither an ability to take up insoluble K forms from a soil nor an ability to release SiO_2 from a soil. Extractable SiO_2 with 2.5% acetic acid in the soil after harvesting soybean and sunflower were more than that of control treatment pot (Fig. 1). Soybean and sunflower also took up more K than exchangeable K (Fig. 1). It means they could take up K which fixed to soil more hardly than exchangeable K. These results suggested that soybean and sunflower had an ability to absorb insoluble K form from a soil and they could release SiO_2 . The results of this pot experiment supports our hypothesis that insoluble K uptake by crop species accelerates release of SiO_2 from a soil.

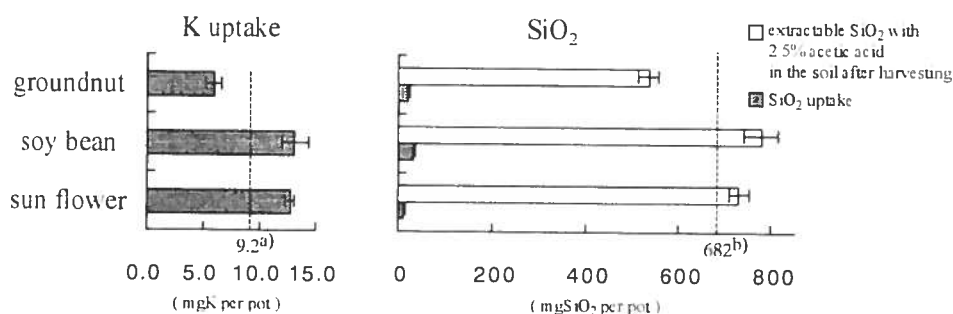


Fig. 1. K and SiO_2 uptake by crops and extractable SiO_2 with 2.5% acetic acid from the soil after harvesting
a) Exchangeable K in control treatment pot (unplanted pot)
b) Extractable SiO_2 with 2.5% acetic acid in the soil of control treatment pot after experiment term

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Silicon Behavior in Terrace Paddy Fields Located in Hilly and Mountainous Regions

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INTRODUCTION

The growth and grain yield of rice grown in a terrace paddy field located in hilly and mountainous regions of North Japan is often influenced by cool summer damage. To maintain high productivity, to decrease rice blast and insect damage, and to reduce the lodging of rice plants in these areas, silicon fertilizers have been applied for a long time. However, the application rate of silicon fertilizer utilized by farmers tends to be decreasing because of saving production cost of rice due to the productive adjustment and the low price of rice. On the other hand, irrigation water in the terrace paddy field is usually supplied successively from the upper to the lower fields (plot-to-plot irrigation). This irrigation system can save both irrigation water and labor. Irrigation water supplies silicon to rice plants, however the silicon concentration of irrigation water decreased significantly in last 40 years (Kumagai *et al.* 1998). Therefore, we investigated in detail the behaviors of silicon both in flooded water and soil solution, and the silicon absorption by rice plants in the terrace paddy field where plot-to-plot irrigation was carried out.

MATERIALS AND METHODS

Experiments were conducted on Andosol in terrace paddy fields located in hilly and mountainous regions in 2001. This rice terrace had a slope of 17‰ and an area of 120a (Fig.1). Water was irrigated from a neighbor stream, and has been managed by plot-to-plot irrigation maintaining the depth of the flooded water of 4 to 5

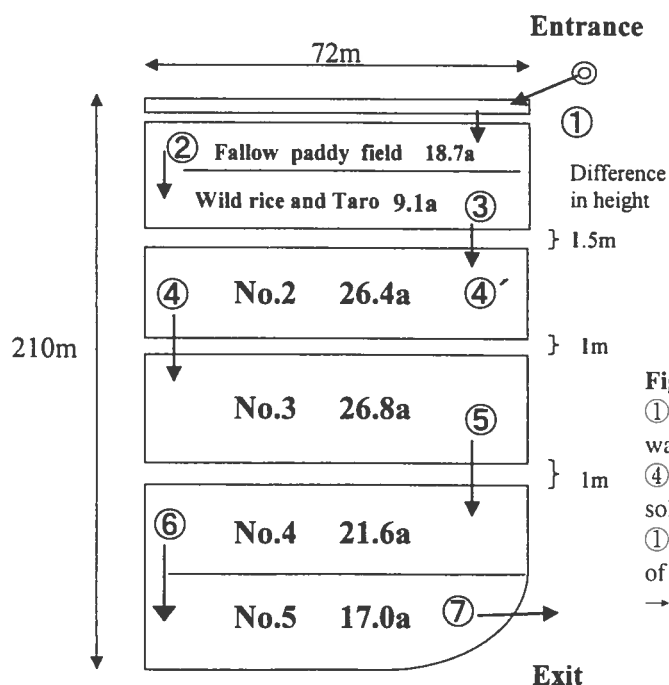


Fig.1. Outline of experimental terrace paddy field
①~⑦ ; Numbers show the places taken the flooded water
④'~⑦ ; Numbers show the places taken the soil solution
①~⑦ ; Numbers show the places where temperatures of flooded water and surface soil were measured
→ ; flooded water flow

cm since 1999. The No.2, No.3, No.4, and No.5 fields were used for rice (*Oryza sativa* L., cv. Manamusume) cultivation and were managed following conventional way. Flooded water was taken into a polyethylene bottle. Soil solution was taken by a suction method using ceramic cups every two weeks at 6 cm in the soils of the water outlet points of the paddy fields. Silicon concentrations of flooded water and soil solution were analyzed by a colorimetric molybdenum blue method. The rice plants of 4 hills were taken from each paddy field at the booting stage (24-July, 2001) and at harvest time (25-Sept, 2001). The silicon contents of the leaf blades and stems were analyzed by a colorimetric method using 1.5 mol L^{-1} hydrofluoric acid extraction (Saito *et al.* 1986), while those of the panicles were analyzed by a gravimetical method with dry ashing digestion.

RESULTS AND DISCUSSION

The silicon concentrations in irrigation water at the entrance of the first field ranged from 29.4 to 38.6 $\text{mg SiO}_2 \text{ L}^{-1}$ throughout the experimental period (Fig.2). However, they decreased gradually at the lower paddy fields and were between 0.41 and 14.5 $\text{mg SiO}_2 \text{ L}^{-1}$ at the lowest field. Silicon in flooded water of the No.2 field could not be detected from the middle to the end of May, while from the middle of June, when flooded water was covered with rice plants, it increased slowly. This phenomenon seems to result from silicon consumption by diatoms or rice plants. Ohtsuka *et al.* (2001) identified 92 diatom taxa, belonging to 28 genera, in a paddy field in Central Japan. Kikuchi *et al.* (1975) reported the numbers of diatoms in both water and soil were the highest in early June, and thereafter declined rapidly. Thus, diatoms absorbed silicon from flooded water at the earlier growth stage of rice plants. Thereafter, the numbers of diatoms decreased because the canopy of rice plants shaded the flooded water. A decrease of silicon concentration in flooded water at the latter growth stage was mainly attributed to silicon absorption by rice plants.

The silicon concentrations in soil solution were not significantly changed among the fields (Fig.3). They increased from the middle of May to the end of June, and then decreased. These results suggested that the

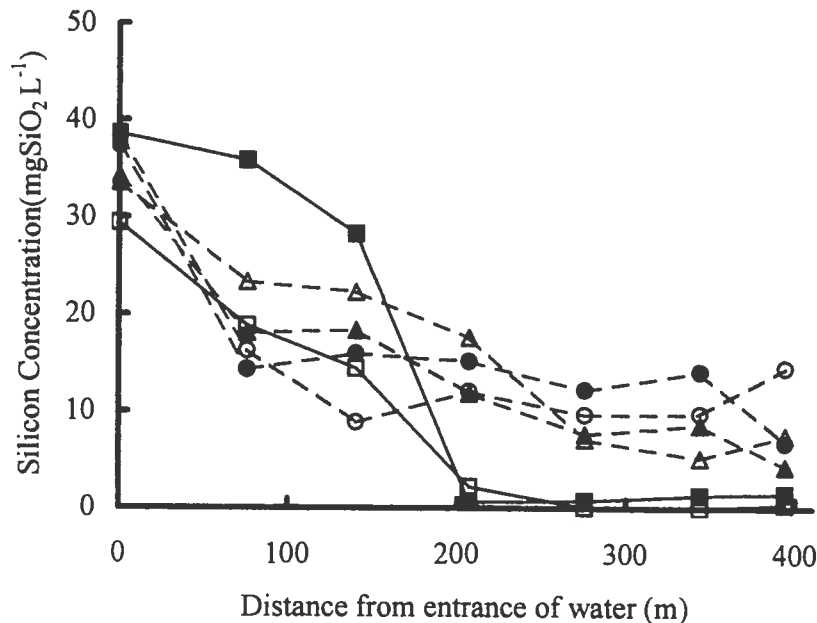


Fig.2. Changes of silicon concentration in flooded water of terrace paddy fields in 2001

—■— 14-May —□— 30-May -▲- 8-Jun
 -△- 22-Jun -●- 14-Jul -○- 20-Aug

Outlet of fallow paddy field : 76 m, Outlet of Wild rice and Taro field : 140 m, Outlet of No.2 field : 207 m, Outlet of No.3 field : 275 m, Outlet of No.4 field : 344 m, Exist : 394 m

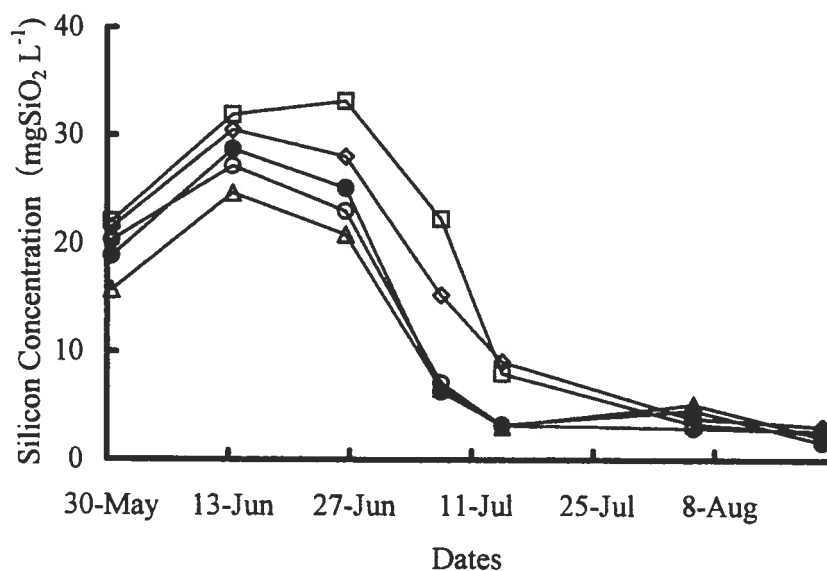


Fig.3. Changes of silicon concentration in soil solution of terrace paddy fields

●—④' ○—④ □—⑤ △—⑥ ◇—⑦

④~⑦ : Number shows field number taken soil solution from upper most

increase of silicon concentrations in the soil solution was caused by a rising of soil temperature from the middle of May to the end of June, and then a decrease was caused along with the growth of rice plants.

The silicon contents of rice leaf blades in each field were not significantly different at the booting stage, but they were different between the No.3 field and No.4 field at harvest time.

The silicon concentration in flooded water of terrace paddy fields decreased from the upper field to the lower field, but might not necessarily reflect the silicon concentration of soil solution and rice plant, because the plot-to-plot irrigation system was managed for only three years. In the future, the difference of silicon concentration of rice plants between the upper field and the lower field will be expanded. The silicon concentration of flooded water in the fields lower than the No.2 field increased along with the growing stage of rice plants, while that of the soil solution decreased. Therefore, we may conclude that the growth of diatoms influenced the silicon concentration of flooded water and soil temperature, while the growth of rice plants influenced that of the soil solution.

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Soil Classification on Deficiency of Activated Si

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The absent of simple, universal and highly informative methods for classification of the soil on the deficiency of plant-available and active forms of silicon (Si) in the soil had a great negative influence on the practical use of Si fertilizers in the world. The soil fertility and crop responses to silicon (Si) fertilization depend on the following soil factors: (1) actual (soluble and weakly adsorbed) forms of Si and (2) potential forms of Si which can be the sources for the actual forms of Si. The unit deficiency parameter of activated Si and the soil classification of deficiency of activated Si were discussed using both factors. The content of plant-available Si (water extraction from fresh soil) and amount of biogeochemically active Si (0.2 N HCl extraction from dry soil) are parts of the parameter under discussion. This parameter was tested on various soils from Russia, Ukraine, UK, US and other countries and was found to have a good relationship with the Si content of plants ($r^2=0.96-0.98$). Four gradations for Si deficiency in soils were determined: (1) soil without deficiency of activated Si, (2) soils with low level of deficiency, (3) soils with deficiency and (4) soil with critical deficiency of activated Si. Soil samples taken from various regions of Florida were analyzed for their active Si content and the value were used to create a map showing the Si deficient areas of Florida. Determination of Si deficiency in the soil and the mapping should help to solve practical problems involving the need for Si fertilizers and soil amendments.

Estimation of Critical Level of Available Silicic Acid and Application Rate of Silicate Fertilizer for Paddy Fields

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INTRODUCTION

The decrease of application rate of silicate fertilizer would reduce the yield and degrade the eating quality of rice. On the other hand, it is hard to estimate the amount of silicic acid (Si) in rice plant from the amount of available Si extracted by acetate buffer solution (pH 4) or hydrochloride solution (0.5 M). Kato (1998) proposed a new method for determination of available Si in paddy soil by phosphate buffer reagent and that in fertilizer by water-weakly acidic cation-exchange resin.

The objectives of this study are to estimate the critical level of available Si in paddy soil and to establish a standard rate of silicate fertilizer in paddy fields.

MATERIALS AND METHODS

1) Determination of amount of available Si in silicate fertilizer

We used 8 type of silicate fertilizers were used. Two procedures employed were as follows:

- 1 Resin method: Ground silicate fertilizer (0.2 g) and 0.5 g of the cation exchange resin (AmberliteIRC-50, pK6.1, type H) were poured into a plastic bottle. Four hundred mL of distilled water was added and then the bottle was shaken by a reciprocal shaker at 25°C for 96 hours. The amount of Si in extracted solution was determined by the molybdenum blue method.
- 2 Extraction Method: Silicic fertilizer diluted in hydrochloride (0.5 M) was shaken for 1 hour under 30°C. The amount of Si in extracted solution was estimated by the potassium fluoride method.

2) Determination of the amount of Si in rice plant

Application rate of silicate fertilizer was 0 - 8 t ha⁻¹. The rice plant was collected at harvest stage and the concentration of Si in rice plant was measured by wet digestion post-gravimetric method.

3) Relationship between the amount of available Si in paddy soil and concentration of Si in rice plant

The amount of available Si in soil of 4 fields was estimated by phosphate buffer method (40 mM, pH 6.2). The total treatments were 19. The concentration of Si in rice plant was determined using the same method as previously described.

RESULTS AND DISCUSSION

- 1) The amount of Si in silicate fertilizer (calcium silicate (slag) and potassium silicate) extracted by water (water soluble) was less than that extracted by HCl (HCl soluble).

The ratio of water soluble-Si to HCl-soluble Si ranged from 0.2 - 0.75. This ratio depends on the type of silicate fertilizer. The order of this ratio was as follows: fused magnesium>phosphate(high silicate)>fused silicate phosphate>ground aerated light-weight concrete (ALC)>fused magnesium phosphate>potassium silicate>calcium silicate.

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The concentration of Si in rice plant increased with the increase of the application rate of silicate fertilizer. There is a significant relationship between the increase of concentration of Si in rice plant because of Si application, and the amount of available Si in soil. The relationship between the concentration of Si in rice plant and the amount of available Si estimated by resin method was more significant than that estimated by extraction method. This result agrees with the previous report (Kato 1998) that the amount of Si in rice plant was regulated by the amount of available Si in soil estimated by resin method.

The relationship between the amount of Si in rice plant at harvest (Y) and the amount of available Si estimated by resin method (X) was presented by linear equation ($Y=0.658X$ ($r=0.747$)) (Fig. 1). Table 1 showed the estimated rate of silicate fertilizer for increasing the concentration of Si in rice plant around 10 mg g^{-1} using this equation.

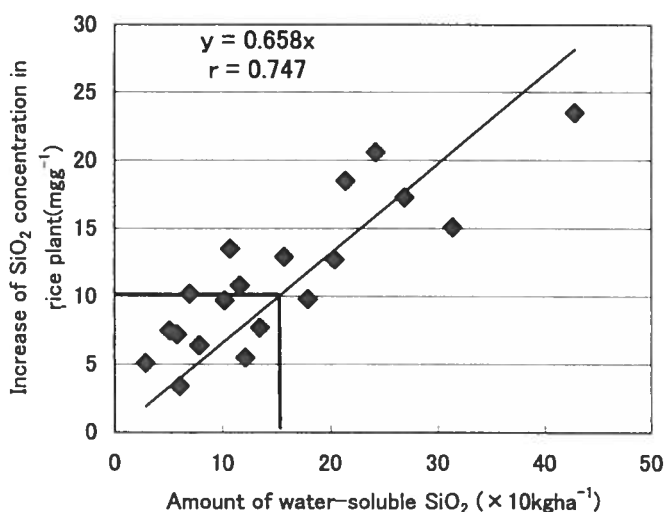


Fig.1. The relationship between the increase of SiO₂ concentration in rice plant and amount of water-soluble SiO₂ in silicate fertilizer

Table 1. Amount of water-soluble SiO₂ in silicate fertilizer and estimated rate of fertilizer for 10 mg kg^{-1} SiO₂ g⁻¹ increasing of rice plant

Name	Water-soluble SiO ₂ (mg g ⁻¹)	Amount of fertilizer application (kg ha ⁻¹)
Fused magnesium phosphate	150	1,000
Calcium silicate (slag)	60	2,500
Ground aerated light-weight concrete (ALC)	150	1,000
Fused magnesium phosphate (high silicate)	200	750
Fused silicate phosphate	200	750
Fused magnesium phosphate+slag	100	1,500
Fused magnesium phosphate+ALC	100	1,500
Potassium silicate	80	2,000

2) A significant relationship between the amount of available Si in paddy soil prior to cropping season estimated by phosphate buffer extraction method and the concentration of Si in rice plant at harvest was observed (Fig. 2). When the concentration of Si in rice plant at harvest is less than 110 mg g^{-1} , it is recommended to apply the silicate fertilizer (Sumida 1992). According to Fig.2, this critical level of the amount of available Si in soil was around 300 mg kg^{-1} . The estimated rate of silicate fertilizer with the reference of the concentration of Si in rice plant was shown in Table 2.

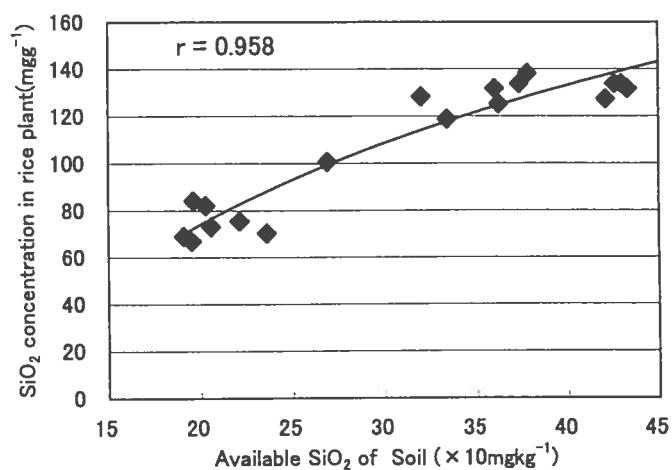


Fig.2. The relationship between the SiO₂ concentration in rice plant and available SiO₂ extracted by the P-buffer method(pH 6.2)

Table 2. The relationship between the available SiO₂ in soil and SiO₂ concentration in rice plant, amount of silicate fertilizer application

Available SiO ₂ of soil by P-buffer method (mg kg ⁻¹)	SiO ₂ concentration in rice plant (mg g ⁻¹)	Silicate fertilizer (water-soluble SiO ₂ kg ha ⁻¹)
200	70	600
	80	450
250	90	300
	100	150
300	110	—
350	120	—
400	130	—
450	140	—

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The Concentration of Silica in Rice Plant with Reference to the Silica Status in Paddy Field and River Water in Yamagata Prefecture

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INTRODUCTION

Dry weight and yield of rice plant, and disease resistance of rice decrease with the decrease of silicic acid (Si) content of rice. Si in rice plant derives from soil, irrigation water, organic matter, and fertilizer. The amount of available Si in paddy soils and the amount of Si in rice plant during the ripening stage of the rice plant are lower in present condition of Yamagata Prefecture compare to 1980's.

The objective of this study was to estimate the amount of Si in rice plant with special reference to the concentration of Si in irrigation water and amount of available Si in paddy soil in Yamagata Prefecture.

MATERIALS AND METHODS

1) Irrigation water. Hundred water samples were collected in August, 1996 from the mean rivers of Yamagata Prefecture used as irrigation. Water samples collected was filtered and amount of Si was measured by the molybdenum yellow method.

2) Soil. Three hundred and forty eight soil samples were gathered from paddy fields after harvest in 1997. The soil samples were air-dried and sieved (<2 mm). The amount of Si in soil was measured by molybdenum blue method using ascorbic acid (Yanai *et al.* 1996).

3) Plant. Two hundred and fifty seven rice plants were sampled at maturing stage at which 152 of the plant samples were collected from where the soil samples were collected. The concentration of Si in above ground of the rice plant was estimated

RESULTS AND DISCUSSION

1) The concentration of Si in irrigation water. Figure 1 shows the concentration of Si in irrigation water. The Si concentration ranges from 5.5 to 21.3 mg L⁻¹ and the average of Si concentration in the irrigation water was 10.2 mg L⁻¹. Yamagata Prefecture consists of 4 districts, i.e. Murayama, Saihoku, Okitama and Shonai. The average of Si content in the irrigation water in Shonai, Murayama and Saihoku district are 9.8, 10.6 and 11.7 mg L⁻¹ respectively. The lowest value of Si concentration of irrigation water was found in Okitama district (8.9 mg L⁻¹) among 4 districts.

In 1956 Kobayasi had reported the Si concentration of irrigation water, analyzed using the molybdenum blue method. He collected the irrigation water from 89 points in July and August. We showed their data in Fig. 1 and Table1. The average of Si concentration is 13.7 mg L⁻¹ in 1996. This amount was smaller than that in 1956. The biggest difference in the concentration of Si in irrigation water between 1956 and 1996 was found in Murayama district (19.8 mg L⁻¹), while the difference in that ranged from 9.9 - 10.9 mg L⁻¹ in other districts. In addition, the standard deviation of concentration of Si in irrigation water in 1956 is bigger than in 1996. Therefore, a large difference in concentration of Si and a high average of that in irrigation water were

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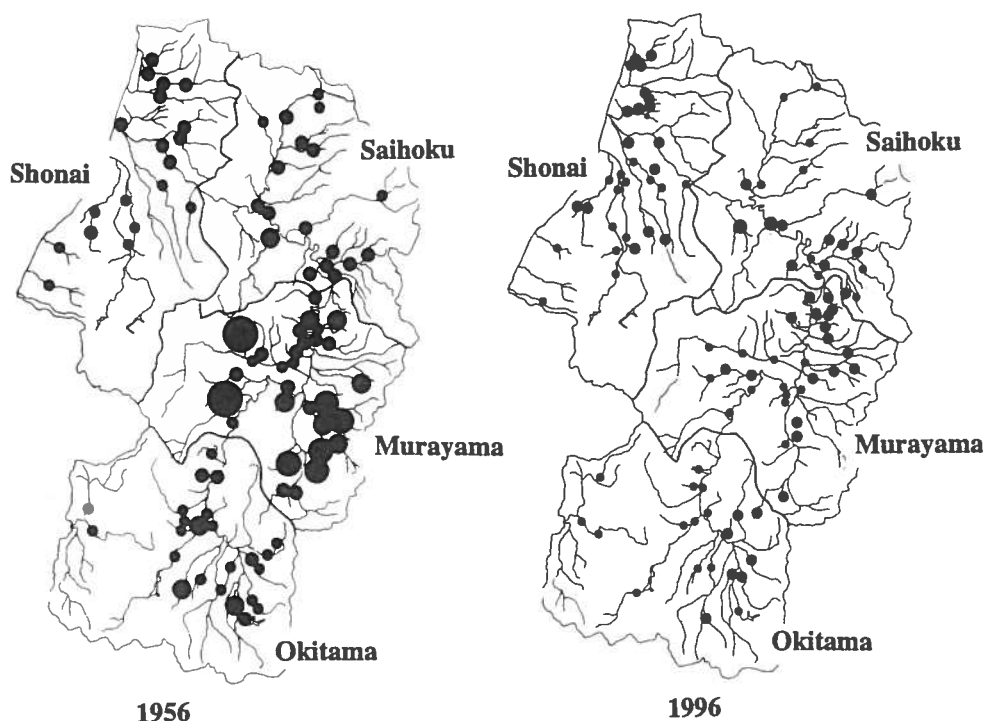


Fig. 1. SiO_2 concentration of irrigation water in 1956 and 1996.



Table 1. Concentration of Si in irrigation water.

district	$\text{SiO}_2 \text{ (mg L}^{-1}\text{)}$	
	1956	1996
Murayama	30.4 ± 4.2	10.6 ± 2.6
Saihoku	22.6 ± 5.5	11.7 ± 4.5
Okitama	19.4 ± 11.9	8.9 ± 1.7
Shonai	19.7 ± 4.2	9.8 ± 3.0
avg.	23.9 ± 9.7	10.2 ± 3.2

Table 2. The amount of Si extracted by the P-buffer method.

district	$\text{SiO}_2 \text{ (mg g}^{-1}\text{)}$				
	avg.	max.	min.	S.D.	n.
Murayama	105	358	41	5.0	101
Saihoku	88	384	35	5.7	61
Okitama	63	132	27	2.2	67
Shonai	86	190	43	2.8	119
avg.	87	384	27	2.2	348

observed in 1956 compare to in 1996.

Matsumura reported that pH value of irrigation water increased 0.3 - 0.8 unit from 1954 to 1979 in Gunma Prefecture. Our result showed that pH of irrigation water increased around 0.8 unit from 1956 to 1996. The amount of soluble Si in soil related to soil pH (Kato 1996). Consequently, the increase of pH of irrigation water might reduce the concentration of Si in irrigation water in last four decades in Yamagata Prefecture.

2) The amount of available Si in soil ranged from 27 - 384 mg kg^{-1} with average of 87 mg kg^{-1} (Table 2). The average amount of available Si in Murayama and in Okitama districts was 105 and 63 mg kg^{-1} , respectively. These differences of available Si among districts are related to the concentration of Si in irrigation water. The parent material of paddy soil in Okitama district is granite. The amount of available Si in granite is low (Imaizumi and Yoshida 1958).

3) The amount of Si in rice plant at harvest ranged from 51 to 164 mg g^{-1} with average of 101 mg kg^{-1} . Imaizumi reported that the average concentration of Si in rice plant was 107.9 mg g^{-1} , indicating that the concentration of Si in rice plant reduced during past 40 years. The critical concentration of Si in rice plant to meet

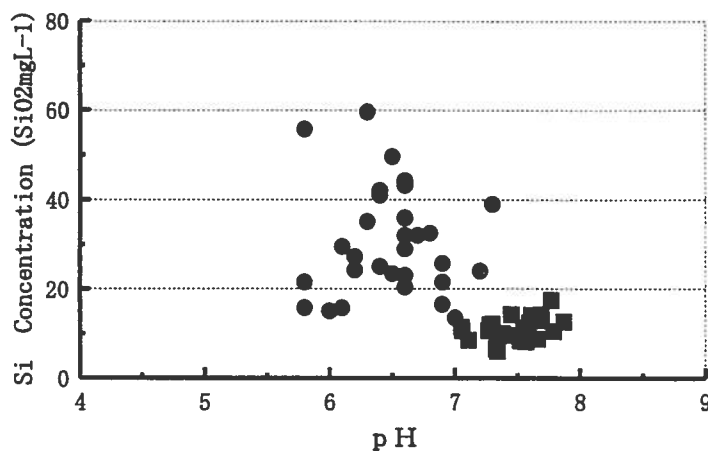


Fig. 2. The relationship between the pH and Si concentration of Irrigation water (Murayama district).

● : 1956 ■ : 1996

Table 3. The Si concentration in rice plant.

variety	SiO ₂ (mg g ⁻¹)			S.D.	n.
	avg.	max.	min.		
Haenuki	102	157	57	1.9	152
domannaka	104	164	56	4.5	32
Sasanishiki	115	155	70	3.7	30
Hananomai	91	122	65	5.8	11
Satonouta	81	109	55	6.6	9
Akitakomachi	83	122	51	6.6	14
Koshihikari	90	123	58	11.4	5
avg.	101	164	51	1.5	257
district (variety : Haenuki)					
Murayama	106	147	69	2.4	63
Saihoku	89	109	70	4.5	8
Okitama	87	132	57	4.7	23
Shonai	106	157	57	3.4	58
avg.	102	157	57	1.9	152

the efficiency of the Si fertilizer is 110 mg kg⁻¹ and 67.7% of plant samples showed the concentration of Si in rice plant was less than 110 mg kg⁻¹. In addition, 3.5% of rice samples show that the concentration of Si in rice plant was less than 5 mg g⁻¹, indicating that there was no silicified cell (Ishizuka 1949).

The concentration of Si in rice plant (cv. Haenuki) was 106 mg g⁻¹ in Murayama and Shonai districts, and those were 89 and 87 mg g⁻¹ in Saihoku and Okitama, respectively. When the parent materials of paddy fields are granite or volcanic ash, the amount of Si in rice plant is low. The concentration of Si in rice plant was related to the amount of available Si in soil and concentration of Si in irrigation water in Okitama district. However, the concentration of Si in irrigation water was higher in Saihoku district than the average of it in Yamagata Prefecture, while the amount of available Si in soil in Saihoku district was comparable. The parent material of paddy soil is volcanic ash. Further study should be conducted about the availability of Si in Andsol.

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Evaluation of Silicon Sources by Biological and Incubation Test

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The objective of this study was to evaluate different materials as silicon (Si) sources applied granulated and powder form using biological and soil incubation test. The Si sources (treatments) were: Wollastonite, Albright & Wilson, granulated Siligran and powder Siligran. The experiment was carried out in the greenhouse and the soil was a clay red-dark Latossolo. The biological test (flooded rice crop) was a factorial design 4×4 (4 Si rates and 4 Si sources) with four replications. All pots received calcium carbonate to balance calcium and pH in the soil. The rates used in the incubation test were: 0, 100, 200, 400 and 800 kg ha⁻¹ of Si while in the biological test was: 0, 100, 200 and 400 kg ha⁻¹ of Si. The effect of Si rates in the soil and plant variables was analyzed by regression analysis while average among sources by the test of Tukey. The Siligran powder and granulated were less efficiency compared to the others (Wollastonite and Albright & Wilson). Siligran applied in powder form was more reactive than the granulated when pH, Ca, Mg and Si in the soil were determined.

Dynamics of Silicon in Paddy Field with the Reference of the Amount of Silicon in Irrigation Water

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INTRODUCTION

It is well known that silicon (Si) is one of the beneficial elements for rice plant, having an important role in increasing the plant resistance to diseases and insect, preventing lodging, promoting photosynthetic activities and improving canopy structure. Rice blast disease has been one of the major diseases of rice plant in Fukushima Prefecture. However, few rice blast disease was found in rice plant cultivated at along the river bank containing a considerable high amount of Si in river water. This fact indicates that the rice plant grown under Si-applied condition is more tolerant to rice blast disease than that grown in the field without Si application. In rice plant, Si is originated from irrigated water, soil and applied fertilizer. It is thought that the Si in irrigation water is necessary for rice plant, but is not clear that when and how Si is supplied from irrigation water. In paddy soil, Si is derived from two groups, i.e.: available Si under oxidized and available Si under reduced condition. Si Fertilizer are useful, however, farmers do not know how to apply a variety of the Si fertilizers. Consequently, the fate and dynamics of Si of different groups of Si in paddy soil should be known when Si is applied as fertilizer. The objective of this experiment is to estimate the dynamics of Si in paddy field using lysimeter from the view point of Si in soil water and irrigated water.

MATERIAL AND METHOD

Cultural practices:

The experiments were conducted at Koriyama, Fukushima Prefecture for 3 years using the lysimeter. The experimental area was 1 m² and the effective soil depth was 0.7 m. The soils used were Brown Forest Soil, Gray Lowland Soil and Andosolsl. Four 23-d-old seedlings of rice (*Oryza sativa* L., cv. Koshihikari) per hill were transplanted on May. Planting density was 24 hills m⁻². Rate of water percolation was about 6 L day⁻¹, and water requirement in depth was about 1.2 cm during growing season of rice plant. Planting density was 24 hills m⁻².

Treatment:

In Fukushima prefecture, the amount of Si in irrigated water ranged from five to 33 mg L⁻¹ with the average of 16 mg L⁻¹. Therefore, two treatments were established, i.e. high concentration of Si (HS) and low concentration of Si (LS). The amount of Si in irrigated water was 15 mg L⁻¹ in LS, while it was about 42 mg L⁻¹ in HS.

MEASUREMENTS

The soil temperature at the depth of 10 cm, the concentration of Si in soil solution, the amount of Si in percolated water at the depth of 70 cm, the amount of Si absorbed by rice plant and amount of available Si in paddy soil were measured. The six of the ceramic filter, 8 cm in length and 0.3 cm in diameter placed in the

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depth of 2 – 10 cm were used the sampling of soil solution. The extraction method using N acetic acid buffer solution (pH 4) was employed to evaluate the amount of available Si in paddy soil.

RESULTS

Figure 1 showed the amount of Si in percolation water and soil solution and soil temperature. Since there were same patterns among 3 soils, we only showed the figure of Gray Lowland soil (Fig. 1). Fig.1. showed that the amount of Si in soil solution of LS treatment in Gray Lowland Soil was 16.4 mg L^{-1} (as SiO_2 , in this paper Si was presented as SiO_2) in May 29 and slightly decreased around 15 mg L^{-1} during June. Then, it was decreased rapidly to $3 - 5 \text{ mg L}^{-1}$ in the first of July.

Thereafter, the concentration of Si in soil solution was constant at a low level ($3 - 5 \text{ mg L}^{-1}$) for 2 months (from first of July to middle of Sept.) and was about 2 times higher than the level of Aug. at harvest time of rice plant. While, the concentration of Si in HS treatment were 20.2 mg L^{-1} in May 29 and 20 mg L^{-1} during June. After July, the concentration of Si in soil solution in HS treatment was comparable to that of LS treatment. The concentration of Si in percolated of LS treatment water was 17.3 mg L^{-1} on May 29. It increased with the increase of soil temperature in one month after transplanting. The maximum concentration of Si in percolated water (28.4 mg L^{-1}) was observed in Aug. 3 and then it decreased. On the other hand, it was 27.0 mg L^{-1} in HS treatment on May 29. Thereafter, a similar trend of concentration of Si in percolated water was observed in HS and LS treatment. There was a positive correlation between the amount of Si in rice plant at harvest and the total amount of Si in percolated water ($R^2=0.720^{***}$). The rapid decrease of the concentration of Si in soil solution at 10 cm below the soil surface in both treatments might be caused by the absorption of Si by rice plant. It was supposed that dissolution rate of Si from paddy soil was regulated by the absorption rate of Si by rice plant in after maximum number of tiller stage of rice plant. Therefore, it could be said that there was no difference in concentration of Si in soil solution after maximum number of tiller stage of rice plant irrespective of the concentration of Si in irrigated water. Increasing the concentration of Si in percolated water during early and middle growth stage of rice plant indicated that all of the Si dissolved from paddy

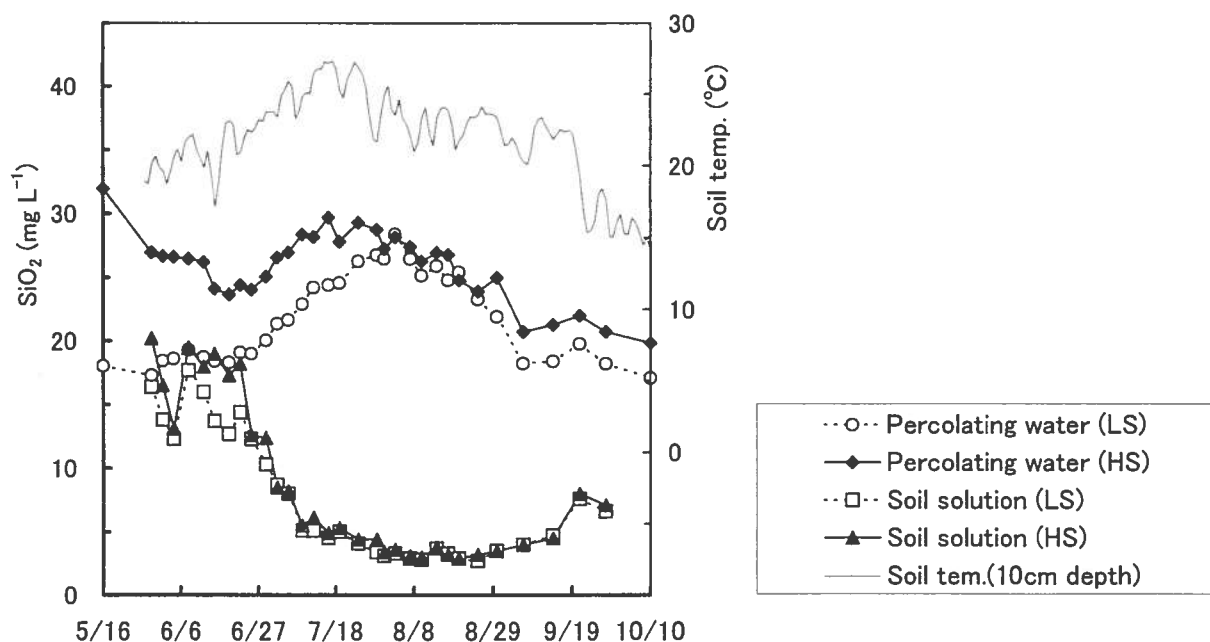


Fig. 1. SiO_2 concentration in the percolating water, and soil solution in paddy soil planted with rice (2001).

soil could not be absorbed by rice plant because of an insufficient root density at all soil layers. Therefore, the concentration of Si in percolated water depends on the concentration of Si in irrigated water. On the other hand, elongated roots of rice plant might absorb Si in soil water located at all soil layers during the late growth stage of rice plant, resulting the decrease of the concentration of Si in percolated water. The Si balance in paddy soil was shown in Table 1. The amount of Si supplied by irrigated water was 11 – 37 g m⁻² and the amount of Si dissolved from paddy soil was 71 – 150 g m⁻². While, rice plant absorbed 80 – 162 g m⁻² Si from paddy field and as much as 12 – 20 g m⁻² of Si was loss through percolation. Seventy-eight to 104% of Si in rice plant was originated from paddy soil and 8 – 37% of Si in rice plant was derived from irrigated water (Fig. 2). Irrigated water could not be said as an important source of Si for rice cultivation, but the higher amount of Si in HS treatment than LS treatment (Table 1).

Table 1. SiO₂ balance in paddy filed planted with rice. (Mean value for 3 y from 1999 to 2001 in lysimeter)

Soil Group	Concentration		Irrigation water supply (a)	Rice absorbs (b)	Percolation water (c)	Paddy field Lost (d=b+c)	Dissolved from Soil (e=d-a)	Available SiO ₂ in top soil	
	irrigation water (mg L ⁻¹)	soil solution (mg L ⁻¹)						Before tillage (g m ⁻²)	After harvest (g m ⁻²)
Brown Forest Soils	15	3.8	11	80	12	92	81	34	32
	42	4.4	33	90	14	104	71	37	33
Gray Lowland Soils	15	7.6	12	137	17	154	142	73	68
	42	8.6	37	162	20	182	145	76	85
Andsol Soils	15	11.5	12	146	16	163	150	97	98
	42	12.3	37	161	18	179	142	92	89

Parent material of Brown Forest Soils is granite. Flooding level was 6 cm. Water requirement in depth is about 1.2 cm. The rate of water percolation was 6 L m⁻² day⁻¹. Topsoil depth was 15 cm. Concentration of Si in irrigated water and in soil solution is mean value of growing season of rice plant. Available SiO₂ was extracted with N acetic acid buffer solution (pH=4).

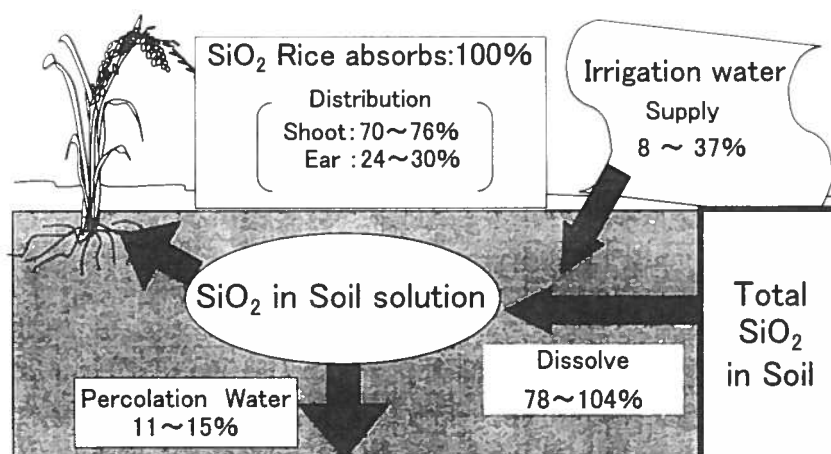


Fig. 2. Percentage of SiO₂ balance in paddy field if the amount of SiO₂ rice absorbs is 100.

Effects of Cultivated Conditions on Si Uptake from Subsoil by Rice Plant

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Two field experiments aimed at investigating the effects of cultivated conditions on Si uptake from subsoil by rice plants were carried out. The study was made with three levels of N supply (experiment 1) and two kind of rice seedlings with different leaf stages (experiment 2).

In experiment 1, Si uptake from plow layer by rice plants increased with increasing N supply. On the other hand, Si uptake from subsoil by rice plants in plot applied 80 kg N ha⁻¹ was lower than that in plot applied 40 kg N ha⁻¹ (ordinary N level for *Oryza sativa* L c.v. Sasanishiki). Then root depth index decreased with increasing N supply. In experiment 2, especially at heading stage, Si uptake from subsoil by rice plants in mature seedling plot was much more than that in young seedling plot. And at heading stage, the Si content in rice plants growing in mature seedling plot was higher than that in young seedling plot. Then root depth index of rice plants in mature seedling plot was larger than that in young seedling plot. These results suggest that less N supply as basal fertilizer and/or planting of seedlings with elder leaf stage contribute to growth into more healthy plants with large rhizosphere and high Si content.

INTRODUCTION

Silicon (Si) is essential and important element for rice plant. The rice plants with low Si content become weak in rice blast epidemics. In Fukushima prefecture, northern Japan, the rice plants grow with low Si content at some districts such as Abukuma district. In these districts, to lead to more Si content in rice plants, two ways almost exist. One way is silicate application on paddy field which is ordinary method. And second way is to cultivate healthy rice plant which absorb more Si derived from soil. Since the uptake of Si derived from soil is much more than that of Si derived from fertilizer, the second way should be considered. But until now the suitable method for second way couldn't be clarified. In this study, I assumed that the increase of Si uptake from subsoil contribute to growth into more healthy rice plants with large rhizosphere and high Si content. Therefor I investigated the effects of cultivated conditions on Si uptake from subsoil by rice plants.

MATERIALS AND METHODS

In 1993, two field experiments were carried out at Fukushima prefectural agricultural experiment station, Japan. I prepared frames for investigation of Si uptake from subsoil. The frames were made from thin wooden board to the size of 60 × 30 cm and 15 cm high. And half of frames were spread polyester-fiber-made sheets (Toyobo Ltd) to block off roots except water. One pair of frames consisted of bottomless frame (Si1-frame) and Si2-frame spread sheet was used to distinguish between Si derived from plow layer and Si derived from subsoil. After four rice seedlings per hill were transplanted at the spacing of 30 × 15 cm, frames were placed on soil surface at the interval of 90 × 45 cm. Then seedlings of four hills in frame were pulled out. And frames were installed at the bottom of plow layer, 12 cm in depth. After installing of frames, rice seedlings were re-planted in the frames to be micro plots. The rice plants in frames were harvested, dried, weighed and ground at heading and harvesting stages. Si contents of the samples were analyzed by conventional method. In this study, total Si uptake (tSi) was regarded as the Si uptake by rice plants growing in Si1-frames. And Si uptake

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from plow layer (pSi) was also regarded as the Si uptake by rice plants growing in Si2-frames. Therefore Si uptake from subsoil was calculated by the balance, $tSi - pSi$. Monolith samples of the top 30 cm soil layer were taken at harvesting stage. After the roots were washed out, roots length of rice plants was measured by root length scanner (Comair Ltd). On these data, root depth index was calculated with the equation of Oyanagi *et al* (1993). This study consists of two experiments. The different methods between both experiments were as follows.

Experiment 1

Before puddling, P_2O_5 and K_2O were applied at the rates of 80 kg ha^{-1} . Nitrogen were applied at the rates of 0 (N-0), 40 (N-40) and 80 kg ha^{-1} in each frames. Each treatment was replicated 2 times at heading stage, and 3 times at harvesting stage. Sasanishiki (*Oryza sativa* L) was used as an experimental cultivar.

Experiment 2

Before puddling, nitrogen, P_2O_5 and K_2O were applied at the rates of 80 kg , 80 kg and 60 kg ha^{-1} respectively. After installing of frames, rice seedlings (*Oryza sativa* L. c.v. Koshihikari) with two different leaf stages (young seedling with 3.0-3.3 leaf stage and mature seedling with 5.0-5.5 leaf stage) were re-planted at the spacing of $30 \times 15 \text{ cm}$ in each frame. Each treatment was replicated 3 times.

RESULTS AND DISCUSSION

Experiment 1

As shown in Fig.1, Si uptake from plow layer increased with increasing N supply at both stages. On the contrary, Si uptake from subsoil at heading stage slightly decreased with increasing N supply. And furthermore, at harvesting stage, Si uptake from subsoil by rice plants in N-8 plot was clearly lower than that in N-0 plot or N-4 (ordinary N level for c.v. Sasanishiki) plot. This result indicates that surplus N supply lead to less Si uptake from subsoil. The roots distributed in the top 10 cm soil layer increased with increasing N supply. In contrast, the roots distributed in 10-30 cm soil layer were smallest in N-8 plot. Consequently, root depth index decreased with increasing N supply (Table 1). In these results, the percentage of Si uptake from subsoil to total Si uptake increased with rising of the root depth index (data not shown). It has been reported by Sumida (1992) that the main limiting factor of Si uptake by rice plant under abundant N cultivation is Si supply ability of paddy field at later life stage. Experiment 1 showed that restraint of root elongation under abundant N cultivation was another limiting factor of Si uptake by rice plants.

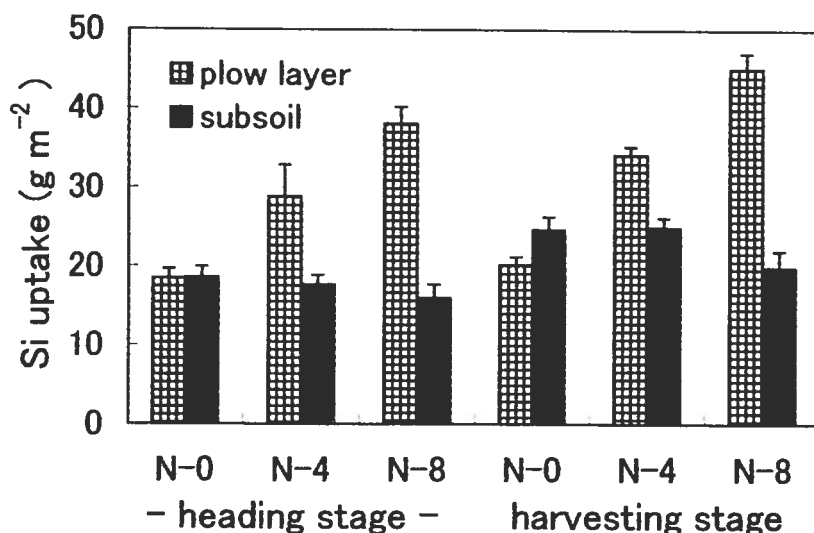


Fig. 1. Effect of three N levels (N-0; 0 kg ha^{-1} , N-4; 40 kg ha^{-1} N-8; 80 kg ha^{-1}) supplied as basal fertilizer on Si uptake from plow layer and subsoil by rice plants. Bars indicate SE.

Table 1. Effects of the amount of N supply and plant age of rice seedlings on the distribution of rice roots and root depth index

cultiver	kinds of seedlings	N supply (kg ha ⁻¹)	root length (m hill ⁻¹)			RDI (cm)*
			0-10**	10-20**	20-30**	
Sasanishiki	young seedling	0	332	159	64	10.2
Sasanishiki	young seedling	40	411	169	56	9.4
Sasanishiki	young seedling	80	552	100	32	7.4
Koshihikari	young seedling	60	584	163	105	9.3
Koshihikari	mature seedling	60	511	378	110	11.0

*RDI, root depth index. **soil depth (cm)

Experiment 2

At heading stage, Si uptake from subsoil by rice plants in mature seedling plot was much more than that in young seedling plot (Fig. 2). At harvesting stage, Si uptake from subsoil by rice plants in mature seedling plot was more than that in young seedling plot (Fig. 2). And at heading stage, Si content in rice plants growing in mature seedling plot was higher than that in young seedling plot (data not shown). Then root depth index of rice plants in mature seedling plot was larger than that in young seedling plot (Table 1). These results imply that mature seedlings easily grow into healthy plants with large rizhosphere and high Si content. As rice plants become sensitive to rice panicle blast epidemics at just before and after heading stage (Tokunaga *et al.*, 1966), it is implied that the rice plants growing from seedlings with elder leaf stage are advantage to tolerance of rice blast epidemics.

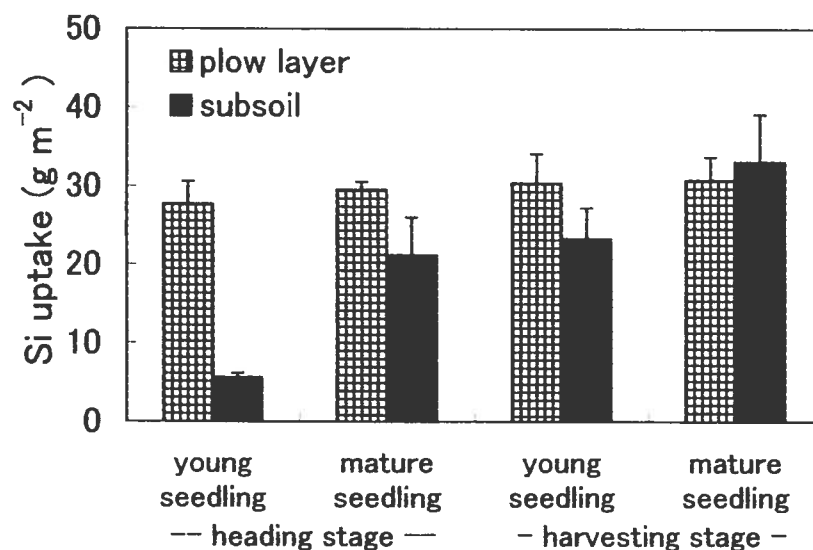


Fig. 2. Effect of two different rice seedlings (young seedlings with 3.0-3.3 leaf stage and mature seedlings with 5.0-5.5 leaf stage) on Si uptake from plow layer and subsoil by rice plants. Bars indicate SE.

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Enhancement of Phosphorus Leaching by Soybean Cultivation Especially in Brazilian Oxisol

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Introducing soybeans to the pastures of the Cerrado (Brazilian savanna) region accompanied with fertilizer application, especially phosphorus (P), is expected to improve soil fertility. The effect of P fertilizer application on soil fertility was examined in the soybean fields. We found that the available P, determined by the Bray 2 and Mehlich 1 methods, increased. However, we could not detect any increase in the total P in these soils applied with P fertilizer at 200 kg P ha⁻¹. Result of column cultivation experiment suggests the relationship between P leaching and Si, especially in leachate passed through 0.2 µm filter. Furthermore, effect of increase in Si concentration on the displacement of phosphate was observed. These results imply that phosphorus leaching may be enhanced due to displacement for available Si increased by soybean cultivation when P fertilizer was applied.

INTRODUCTION

The central plateau of Brazil, which used to be covered by savanna vegetation, is characterized by highly weathered acid soils (Oxisol), which are rich in iron and aluminum oxides and low in phosphorus and bases. Pastures where Gramineae *Brachiaria species* have been introduced and beef cattle production are important in this region. For the persistence of the pasture, minimum amount of fertilizer and lime is necessary. Also, introducing soybeans to these pastures, called an agropastoral system, accompanied with fertilizer application that is expected to improve soil fertility. After soybean cultivation accompanied with P fertilizer, we can detect increasing of available P in soil but not total P. This result implies that soybean cultivation stimulated P leaching. In order to confirm the hypothesis, we will discuss the enhancement of phosphorus leaching by soybean cultivation when P fertilizer was applied.

MATERIAL AND METHODS

Experimental design and cumulative P fertilizer

An experimental design for an agropastoral system that started in 1993 at EMBRAPA-CNPQC was described from the viewpoint of phosphorus (P) in Figure 1. A cropping system is based on continuous 4-year cultivation of soybeans and millet and 4 years of grazing on *Brachiaria* pasture. In the first year, P fertilizer was applied at 140 kg P₂O₅ ha⁻¹ and then 80 kg P₂O₅ ha⁻¹ was applied either every year or on alternative years. Harvested soybean seeds were taken away from the field and all residues, including the shell and stalks were incorporated back to the soil. The first sample soils were collected in 1997 from the field for evaluating P availability and total P. Cumulative application of P fertilizer on experimental plots up to 1997 was follows:

- 1) Cerrado vegetation; no fertilizer
- 2) *Brachiaria* pasture; P fertilization every 2 years, 300 kg P₂O₅ ha⁻¹ (130 kg P ha⁻¹)
- 3) Continuous no-tillage soybeans; 460 kg P₂O₅ ha⁻¹ (200 kg P ha⁻¹)

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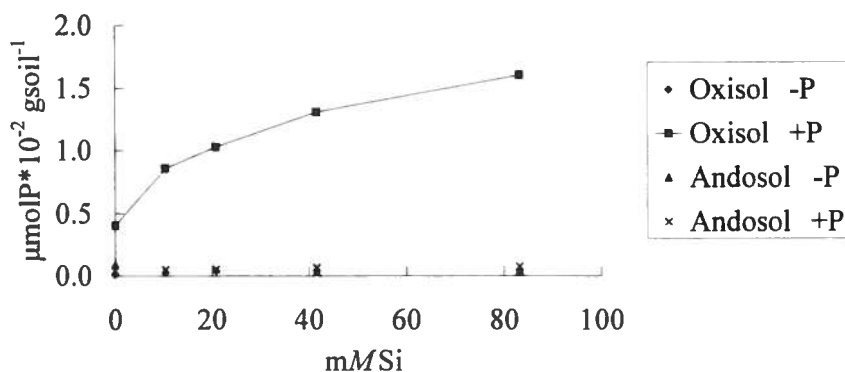


Figure 1. Effect of Si concentration on P displacement in soil samples which had previously 0 (-P) or 0.25 mg P soil⁻¹ (+P).

Column cultivation

We cultivated soybean and upland rice on the column filled with soils in 3 replicates. Two soils are Oxisol of EMBRAPA-CNPQC and Andosol of NIAES. 500 mL of each soil was filled. Plants were cultivated for two months, 5 weeks after seeding water was applied carefully not to prevent leaching from column, then later 300 mL of water was applied and leachate from column was collected. P and Si in soils and leachate was determined using the colorimetric molybdenum blue method and ICP-AES.

Phosphorus desorption experiment

To test P desorption, 10 g of soil samples which containing previously received 0 or 0.25 mg P g soil⁻¹ as superphosphate was incubated for 5 days at 20°C with 25 mL of 0.01 M CaCl₂ containing 0, 0.73, 1.46, 2.92, 5.84 mg Si as silicic acid prepared by the Okuda and Takahashi method (1961). Phosphorus in the supernatant was determined using the colorimetric molybdenum blue method.

RESULT AND DISCUSSION

Table 1 shows P availability in these 3 plot soils, natural vegetation (No. 1) and *Brachiaria* pasture plot (No. 2) and Continius soybean plot (No. 3). The P availability, expressed by Bray 2, is 1.2 mg P kg⁻¹ in No. 1. However, Bray 2 of the No. 2 and No.3 plots showed higher values of 5.9 mg P kg⁻¹ and 5.8 mg P kg⁻¹, respectively, compared to the No.1 plot. Mehlich 1 method also shows that P availability increased with fertilization in plots No. 2 and No.3.

The total P of these plots is also shown in Table 1. We could not observe any effect of P fertilization on the total P of the soil layers from 0 to 60 cm in depth in plots No. 2 and No.3, compared to plot No. 1. These

Table 1. Total P and available P in the experimental plots.

Plot	Sampling site	depth (cm)	Available P (mg P kg ⁻¹)		Total P (mg P kg ⁻¹)
			Bray 2	Mehlich 1	
1	Cerrado Vegetation	0-20	1.2	0.8	332
		20-40	0.5	0.3	319
		40-60	0.3	0.2	286
4	<i>Brachiaria</i> pasture	0-20	5.9	3.1	376
		20-40	1.3	0.6	312
		40-60	0.8	0.4	310
8	Cont. soybeans No-tillage	0-20	5.8	3.1	293
		20-40	1.4	0.7	235
		40-60	0.8	0.5	219

Table 2. Mineral contents including P in leachate from column soils cultivated.

		P		Al		K	
		0.2 μ filtered ($\mu\text{mol} \cdot 10^{-2}$ 500 mL soil $^{-1}$)	Total	0.2 μ filtered ($\mu\text{mol} \cdot 10^{-2}$ 500 mL soil $^{-1}$)	Total	0.2 μ filtered (μmol 500 mL soil $^{-1}$)	Total
Oxisol	Soybean	4.6	45.7	203.2	295.9	103.7	1579.3
(Brasil)	Upland rice	1.1	36.7	197.5	281.8	64.5	1266.0
Andosol	Soybean	1.3	315.2	16.6	949.0	38.1	10518.1
(NIAES)	Upland rice	1.1	329.3	12.3	3670.7	28.0	10092.0
		Si		Mg		Mn	
		0.2 μ filtered (μmol 500 mL soil $^{-1}$)	Total	0.2 μ filtered (μmol 500 mL soil $^{-1}$)	Total	0.2 μ filtered (μmol 500 mL soil $^{-1}$)	Total
Oxisol	Soybean	946.0	1798.3	1363.3	8048.4	64.9	1059.0
(Brasil)	Upland rice	330.4	1757.4	1636.9	9726.7	37.3	585.0
Andosol	Soybean	607.8	808.8	894.9	262180.4	6.1	2831.7
(NIAES)	Upland rice	488.0	1566.9	1278.9	367858.0	1.3	620.9

results may imply the possibility of P leaching, even though it is a soil low in P.

Table 2 shows mineral contents including P in leachate from column soils cultivated. Phosphorus in leachate passed by 0.2 μm filter from column soils (Oxisol) of soybean cultivation is about 4 times higher value of $4.6 \mu\text{mol} \cdot 10^{-2}$ 500 mL soil $^{-1}$ compared to that of upland rice cultivation. However, in case of Andosol, phosphorus in leachate passed through 0.2 μm filter in soybean cultivation is almost the same value as that of upland rice cultivation. Si in leachate passed through 0.2 μm filter from column soils (Oxisol) of soybean cultivation is about 3 times higher value of $946 \mu\text{mol}$ 500 mL soil $^{-1}$ compared to that of upland rice cultivation. Relationship between total P and Si is almost the same as that of passed by 0.2 μm filter, but is not so clear. We could not detect same relationship between P and Al, K, Mg, Mn.

Figure 1 shows the effect of increase in Si concentration on the displacement of phosphorus was observed on Oxisol, but not Andosol.

CONCLUSION

Four years of P fertilizer application caused an increase of Bray 2 and Mehlich 1 values in Oxisol. However, we could not detect any increase in total P content in these soils even though P fertilizer was applied at 200 kg P ha^{-1} . Result of column cultivation experiment suggests the relationship between P leaching and Si, especially in leachate passed through 0.2 μm filter. Furthermore, effect of increase in Si concentration on the displacement of phosphate was observed on Oxisol, but not Andosol. These results imply that phosphorus leaching in Oxisol may be enhanced due to displacement for available Si increased by soybean cultivation when P fertilizer was applied.

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Prospectives of Si Fertilization for Reduction of P and N Leaching from Cultivated Areas

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Eutrophication of natural waters has been accelerated by nutrient-bearing runoff water from cultivated areas and today is one of the important problems in the USA as well as in other parts of world. Liming of agricultural lands has been found to be responsible for transformation of plant-available P into plant-unavailable forms. Another approach to reduce P leaching is to decrease its application rate in mineral fertilizers. Both approaches can dramatically reduce agricultural benefits. We hypothesized that using Si-rich substances would reduce P leaching and keep the P in plant available forms. This hypothesis was examined in column experiments utilizing Alfisols, Spodosols, Entisols, and pure quartz sand. Chemically pure SiO_2 , CaSiO_3 , agricultural grade limestone, and two commercial Si fertilizers were incorporated into the soils. Columns were irrigated with deionized water, P-bearing and NO_3^- -bearing solutions. A greenhouse experiment was conducted on the Entisol treated with limestone and Si-rich fertilizers with or without poultry manure as a source of P and N. Bahiagrass (*Paspalum notatum* Fluegge) was used as the test plant. Limestone reduced P leaching in the column and greenhouse experiments, but reduced root and shoot growth in the latter. The use of Si fertilizers and chemically pure Si-rich substances decreased P leaching 50 to 90% and maintained P in plant-available forms in the column experiments. The leaching of N (NO_3^-) was reduced from 5 to 40%. In the greenhouse experiment, both commercial Si fertilizers accelerated root and shoot growth by 30 to 200%. Thus, the study showed that Si fertilization both reduced P and N (NO_3^-) leaching and increased plant growth. This probably is due to increased P adsorption by the soil, improved P availability, acceleration of N mineralization and the growth response due to Si.

Effect of Recycling of Plant Silicon on Phosphorus Utilization in Paddy Soils of Karnataka, South India

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INTRODUCTION

Although Silicon is not considered as essential element for growth and development, addition of this element can increase growth and yield of rice (Savant *et al.*, 1997a; Takahashi, 1995; Takahashi *et al.*, 1990; Yoshida, 1981). Depletion of plant-available Si in soils where rice is grown could be a possible limiting factor contributing to declining or stagnating yields (Savant *et al.*, 1997a; 1997b). Research studies revealed that an adequate supply of silicon to rice plants can decrease the incidence of important rice diseases and inhibit the toxicities of iron, aluminum and manganese besides improving the availability and utilization of P by rice plants (Savant *et al.*, 1997b). However, silicon fertilizers are too expensive to afford for most of the Indian farmers and many of them are not aware of its importance.

A huge amount of rice hull is generated every year in all the rice growing countries. On an average 50% of rice hull obtained is being used as a source of fuel in rice mills, hotels and brick making industries in South India. The Rice Hull Ash (RHA) thus obtained contains silicon as major constituent is being used in the rice nursery and main fields in different parts of South India. Application of black to gray rice hull ash at 0.5–1.0 kg m⁻² to seedbed produced healthy and strong rice seedlings (Kumbhar *et al.*, 1995; Sawant *et al.*, 1994). Although, it is not possible to replenish all the silicon removed by rice crop, proper method of recycling of plant silicon will help in solving the problem of soil silicon depletion. Hence, there is need to evaluate the effect of continuous application of RHA on growth and yield of paddy which otherwise would go as waste.

Research on the interaction of Si and other plant nutrients has been conducted, but results are sometimes contradictory (Deren, 1997). Silicon is said to improve the P status of rice, some times by chemical reactions in the soil that make more P plant available (Silva, 1971), whereas others emphasize that P balance within the plant is improved with Si (Deren, 1997; Epstein, 1994; Lin and Hung, 1980). The positive effects of applied Si on the availability of P by rice plants are also reported by Blair *et al.*, 1990. Phosphate fertilizer efficiency has increased when applied along with Si (IARI, 1988). Though phosphorus management is not a problem under submerged condition, studies on the application of RHA as a source of silicon and its effect on phosphorus availability in rice farming is limited. Hence, the present investigation was undertaken to know the effect of continuous recycling of RHA with different sources of phosphorus on growth and yield of paddy.

MATERIALS AND METHODS

Field experiments were conducted to know the effect of RHA as a source of silicon along with two sources of phosphorus viz. Diammonium Phosphate (DAP) and Rock Phosphate (RP) on growth and yield of rice (*Oryza sativa* L.). The experiment was conducted at Regional Research Station, Southern Transition Zone, Shimoga (*Isohyperthermic typic haplustalfs*), and Agricultural Research Station, Coastal Zone, Mangalore

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(*Typic kandiustults*) of Karnataka, South India for two seasons (summer and kharif) of 2001. The texture of the experimental sites was loamy sand and sandy loam with a pH of 5.11 and 5.06 at Shimoga and Mangalore respectively.

The study was conducted in a randomized complete block design with nine treatments and three replications. Three week old seedlings were planted at 20×10 cm spacing in a treatment plot size of 18 m^2 and 16.25 m^2 at Shimoga and Mangalore respectively. RHA was applied at 2 and 4 Mg ha^{-1} along with and without phosphatic fertilizers. The recommended fertilizers for paddy ($100:50:50 \text{ NPK kg ha}^{-1}$ at Shimoga and $60:30:45 \text{ kg ha}^{-1}$ at Mangalore) were applied as per the package of practice adopted in the Zones for both seasons. Required amount of N and K were applied as DAP, Urea and Muriate of Potash during planting and Urea as split application to all the plots. Recommended amount of P was applied as DAP during planting whereas RHA and RP were applied one week before planting. The field experiment was conducted in the same layout for two seasons without disturbing the bunds and by operating all necessary practices. The details of the treatments used in the study are given below.

Treatments

- | | |
|--|--|
| T ₁ – Control (Recommended N & K without P) | T ₇ – Recommended NPK (P as RP) |
| T ₂ – T ₁ +RHA at 2 Mg ha^{-1} | T ₈ – T ₇ +RHA at 2 Mg ha^{-1} |
| T ₃ – T ₁ +RHA at 4 Mg ha^{-1} | T ₉ – T ₇ +RHA at 4 Mg ha^{-1} |
| T ₄ – Recommended NPK (P as DAP) | |
| T ₅ – T ₄ +RHA at 2 Mg ha^{-1} | |
| T ₆ – T ₄ +RHA at 4 Mg ha^{-1} | |

The data were analyzed statistically by analysis of variance and the mean separation was done by using the least significant difference (LSD)

RESULTS AND DISCUSSION

The effect of RHA alone and with two phosphorus sources on grain yield of paddy recorded during summer and kharif seasons of 2001 is presented in Table 1. Application of RHA with and without P sources was found to be significant at both the locations except during kharif season at Shimoga.

The application of RHA at 2 and 4 Mg ha^{-1} without P sources increased the grain yield of paddy over control (T₁). The results are in agreement with other reports stating that the application of silicate materials

Table 1. Effect of RHA with different P sources on grain yield (kg/ha) of paddy

Treatments	SHIMOGA				MANGALORE			
	Summer	Kharif	Average	% Increase over control	Summer	Kharif	Average	% Increase over control
T ₁	3856	2800	3328	–	4300	1930	3115	–
T ₂	4560	3170	3865	16.14	5193	2750	3971.5	27.51
T ₃	4910	3190	4050	21.69	5193	3100	4146.5	33.13
T ₄	5185	3170	4177.5	25.54	5307	3350	4328.5	38.97
T ₅	5412	3250	4331	30.14	5380	3510	4445	42.7
T ₆	5409	3270	4339.5	30.4	5527	3280	4403.5	41.38
T ₇	3970	2710	3340	0.36	4853	3240	4046.5	29.92
T ₈	3883	2860	3371.5	1.3	5520	3740	4630	48.63
T ₉	4674	3110	3892	16.95	5467	3480	4473.5	43.63
F Test	*	NS			*	*		

increased the yield and yield components of rice (Hossain *et al.*,1999; Jakhro,1984; Savanth *et al.*, 1997b; Snyder *et al.*,1986). Results of field experiment conducted in Sri Lanka have also shown that the application of 740 kg ha⁻¹ of RHA yielded an additional 1.0-1.4 t ha⁻¹ of rice (Amarasiri,1978). Hossain *et al.*, (2001), observed that application of powdered rice chaff with a biodecomposer was more effective in increasing grain yield than the inorganic silicate materials. Sistani *et al.* (1997) noticed more dry matter in RHA treated seedlings over the untreated seedlings and emphasized the application of RHA to rice nurseries as an efficient way of recycling plant Si especially in developing countries.

Addition of phosphorus as DAP or RP without RHA also increased the grain yield over control (Table 1). However, addition of RHA along with either DAP or RP as P sources has further increased the paddy grain yield at both the locations and seasons. Application of DAP as P source at recommended level without RHA was found to be more effective than RP at both the locations in achieving higher yields. However, at Shimoga, the highest grain yield average (4339.5 kg ha⁻¹) of two seasons was obtained by the application of RHA at 4 Mg ha⁻¹ along with DAP fertilizer (T₆). Whereas, in Mangalore, the highest grain yield average of 4630 kg ha⁻¹ was noticed by the application of RP as P source with RHA at 2 Mg ha⁻¹. Higher yield in the treatments receiving RHA without phosphorus than control may be attributed to increased availability of phosphorus by the RHA silicon in soil.

The average grain yield of two seasons shown that addition of RHA at 2 and 4 Mg ha⁻¹ increased the yield by 16.14 and 21.69% at Shimoga and by 27.51 and 33.13% at Mangalore respectively over control. Addition of RHA at 2 and 4 Mg ha⁻¹ with DAP as P source increased the yield to an extent of 30.14 and 30.40% at Shimoga and with RP as P source to an extent of 48.63 to 43.63% at Mangalore respectively. An additional yield increase of 5% achieved by application of RHA with DAP at Shimoga and 14-19% with RP at Mangalore indicated that application of RHA along with phosphatic fertilizer was also beneficial in rice farming. Though higher yields were noticed by the application of RHA along with P sources, the application of RP along with RHA at 2 Mg ha⁻¹ was more beneficial at Mangalore.

The effect of RHA alone and with P sources on straw yield during both seasons was found to be significant at both locations (Table 2). In general, the average straw yield was higher at Mangalore than at Shimoga during both the seasons. The percent increase in the straw yield was higher with DAP application at Shimoga and with either DAP or RP at Mangalore.

The positive effects of applied Si on the availability and utilization of P by rice plants are reported in literature. On certain mineral soils, Si may decrease P sorption on clays, making the P available to plants (Silva,1971; Okuda and Takahashi,1964). Silicon fertilization with no additional P fertilizers increased the P content of rice straw and grain during 1964 wet and 1965 dry seasons (IRRI,1965,1966). This increased P

Table 2. Effect of RHA with different P sources on straw yield (kg/ha) of paddy

Treatments	SHIMOGA				MANGALORE			
	Summer	Kharif	Average	% Increase over control	Summer	Kharif	Average	% Increase over control
T1	4016	2570	3293	—	5480	5520	5500	—
T2	4954	3040	3997	21.38	5740	5530	5635	2.45
T3	5267	3240	4253.5	29.18	5780	6330	6055	10.09
T4	5607	3560	4583.5	39.2	5913	6570	6241.5	13.49
T5	5720	3530	4625	40.45	5980	6260	6120	11.27
T6	5681	3750	4715.5	43.18	6167	6620	6393.5	16.25
T7	4283	2520	3401.5	3.31	5573	4920	5246.5	-4.62
T8	4120	2690	3405	3.4	6027	6440	6233.5	13.34
T9	4974	3130	4052	23.04	6040	6460	6250	13.64
F Test	*	*			*	*		

content has been attributed to better availability of soil P and/or enhanced mobility of P from the roots to the stems. Under low P adsorbing conditions, application of Si has been found to reduce the P requirement (Blair et al. 1990).

The application of RHA at 2 and 4 Mg ha⁻¹ without P recorded an additional grain yield of 537 and 722 kg ha⁻¹ at Shimoga and 856 and 1031 kg ha⁻¹ at Mangalore over control respectively. The straw and grain yield obtained by the application of RHA at 4 Mg ha⁻¹ without P source was on par with DAP and superior over RP fertilizer in the present investigation indicated that recycling of plant silicon material like RHA helps in achieving sustainable rice yield besides mobilizing soil phosphorus.

CONCLUSION

The study conducted at different agro climatic zones of South India for two consecutive seasons concluded that continuous recycling of RHA with and without P fertilizers in the main fields of paddy increased the grain and straw yield of rice. Recycling of relatively inexpensive and efficient Si material like RHA would ensure additional grain yield without harming the environment in rice farming of developing countries.

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Reaction of Phosphate and Silica-alumina Solutions

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Phosphate (P) is generally reactive with active forms of Al in soil-water systems. The active forms of Al in soils are $=\text{Al}-\text{OH}$ and $=\text{Al}-\text{OH}_2^+$ groups that exist in allophane, imogolite, Al complexed with humus, edge of layersilicate minerals, polymeric hydroxyaluminum in the interlayer of chloritized 2:1 clay minerals, KCl-extractable Al and so on (Wada, 1980). The active forms of Al in water or in soil solution are monomeric and partially neutralized polymeric hydroxyaluminum ions and possibly sub-colloidal hydroxyaluminosilicate, although concentration of these materials appears highly dependent on conditions of water and soils. Several papers showed that added Si increased water-soluble and easily extractable P to some extent and previously-added Si decreased the amount of P sorption by soils (Adams, 1980). However, in P-deficient soil, silicic acid does not increase P availability by rice plants (Savant *et al.*, 1997, and cited literatures). Complexation of hydroxyaluminosilicate with vermiculite (Saha and Inoue, 1997) and with montmorillonite (Saha *et al.*, 1998) increased P sorption by these minerals at the initial P concentration range from 0.25-0.5 to 50 mM and at pH value around 4.5.

In a water system, solubility of proto-imogolite sol which is hydroxyaluminosilicate was reported and the proto-imogolite is more stable than microcrystalline-gibbsite in 0.1 mM H_4SiO_4 (Lumsdon and Farmer, 1995). Chemical Si-O-Al linkage of freeze-dried silica-alumina (Si-Al) solutions was well-demonstrated using infrared absorption spectrometry (Wada and Wada, 1980). Effect of Si on the amelioration of Al toxicity for plants was also discussed using the Si-Al solutions (Cocker *et al.*, 1998; Gu *et al.*, 1998). In the present study, reactions of P and Si-Al solutions were compared with those of P and partially neutralized AlCl_3 (PN-Al) solutions to examine how Si affects the reaction of P and Al in the water system. Although the PN-Al solutions were highly reactive with P (Veith, 1977), reaction of P and the Si-Al solutions is not fully understood.

MATERIALS AND METHODS

The PN-Al solutions and Si-Al solutions were prepared after Hiradate *et al.* (1998). To mixtures of 0 or 1.85 mM $\text{Si}(\text{OH})_4$ solution (775 mL) and 0.1 M AlCl_3 solution (25.6 mL), 0.1 M NaOH solution was added dropwise at a rate of 0.3 mL min^{-1} and then distilled water was added to make volume of 1 L. The amount of the 0.1 M NaOH solution added was 51.2, 61.4 and 71.7 mL to prepare the solutions of different OH/Al molar ratios. The OH/Al molar ratio was 2.0 and 2.4 for PN-Al solutions and 2.0, 2.4 and 2.8 for Si-Al solutions. Al concentration of these solutions were 2.56 mM and Si/Al molar ratio was 0.56 for the Si-Al solutions.

To 25 mL of the PN-Al or Si-Al solutions, 5 mL of distilled water, 1.5 mM Na_3PO_4 or 1.5 - 20 mM NaH_2PO_4 was added and shaken for various periods up to 2 weeks at 25 degrees C in a screw-capped centrifuge tubes. After measuring final pH with glass electrode, the mixtures were centrifuged at 10,000 rpm for 10 min and the supernatant solution was filtered through 0.45 μm pore-size membrane filter. P concentration in the supernatant solutions was determined by molybdenum blue method using ascorbic acid and Al and Si concentration by atomic absorption spectrophotometry using $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame. When a precipitate was formed, after washing it thrice with distilled water, the precipitate was dried on a slide glass and used for X-

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ray diffraction, scanning electron microscope (SEM) observation and energy dispersive X-ray (EDX) analysis. Electric conductivity (EC) was also determined for the mixtures of PN-Al or Si-Al solutions (25 mL) and distilled water, 1.5 or 4 mM NaH_2PO_4 solutions (5 mL).

To examine the effect of coagulating sub-colloidal materials in the Si-Al solutions on the reaction with P, 2 mL of 50 mM Na_2SO_4 was added to 25 mL of Si-Al solutions and shaken for 4 hr before the addition of NaH_2PO_4 in the procedure described above.

RESULTS AND DISCUSSION

When the initial P concentration was lower than 0.6 mM, the amount of P precipitated in the Si-Al solutions ($\text{OH}/\text{Al}=2.0$ and 2.4) was much smaller than those in the PN-Al solutions (Fig. 1). The high P concentration in solution was obtained in the Si-Al solutions in 24 hr after preparation although P concentration was slightly lower in 1 hr after preparation of the Si-Al solutions. This result suggests that formation of Si-O-Al linkage was in an almost stable stage in 24 hr after preparation. However, 10 to 20 percent of initially added P was removed from the solution even when the initial P concentration was about 0.6 mM or lower. This result suggests that the solute in the Si-Al solutions was not homogeneous and that a minor portion of the sub-colloidal hydroxylaluminosilicate precipitated with P.

To examine the form of P in the PN-Al and Si-Al solutions, EC of the reaction mixture was determined in 24 hr after mixing (Fig. 2). When 5 mL of 1.5 mM NaH_2PO_4 was mixed with 25 mL of PN-Al solutions, EC values were only slightly greater than those for 5 mL of distilled water and 25 mL of PN-Al solutions. In contrast, greater increase in EC values were obtained for the mixtures of 1.5 mM NaH_2PO_4 (5 mL) and 25 mL of Si-Al solutions than those for the water-added solutions instead of NaH_2PO_4 (Fig. 2). The increase in EC for the Si-Al solution having $\text{OH}/\text{Al} = 2.8$ was smaller than those having $\text{OH}/\text{Al} = 2.0$ and 2.4 possibly due to partial precipitation of P with hydroxylaluminosilicate in the Si-Al solution ($\text{OH}/\text{Al}=2.8$). The EC values for

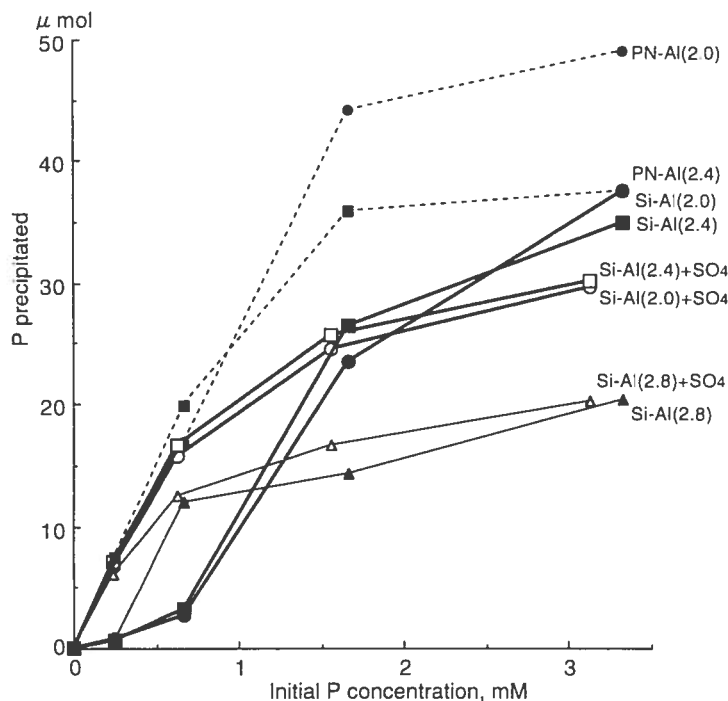


Fig. 1. Changes in the amount of P precipitated in silica-alumina (Si-Al) solutions or in partially neutralized AlCl_3 (PN-Al) solutions with the addition of NaH_2PO_4 or $\text{Na}_2\text{SO}_4/\text{NaH}_2\text{PO}_4$. Numerals in parentheses show OH/Al molar ratios of the solutions.

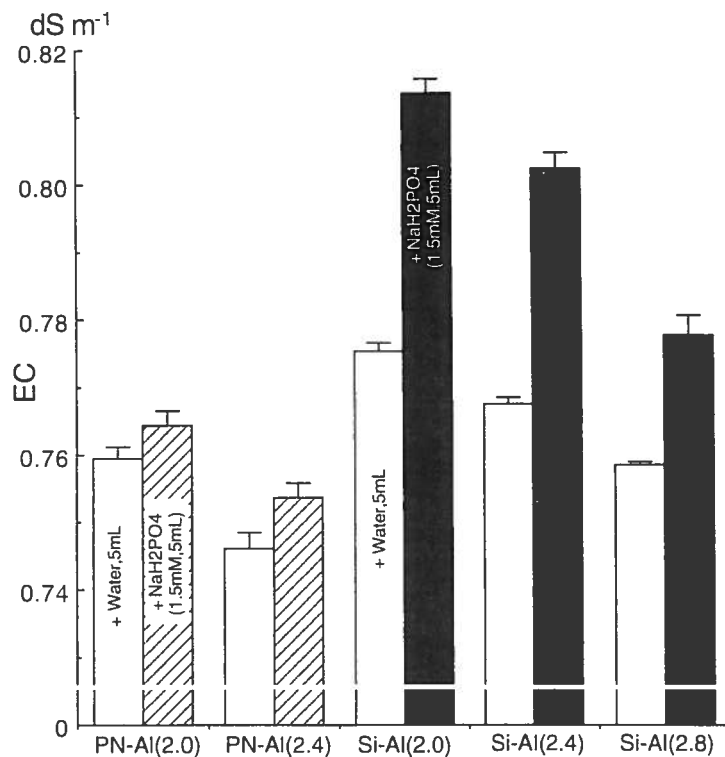


Fig.2. Electric conductivity of mixtures containing 25 mL of partially neutralized AlCl_3 (PN-Al) or silica-alumina (Si-Al) solutions and 5 mL of water or 1.5 mM NaH_2PO_4 in 24 hr after preparation. Numerals in parentheses show OH/Al molar ratios of the solutions.

the P-added Si-Al solutions slightly decreased with time in 2 weeks and it may suggest some chemical reaction were partly taking place but the EC values were still greater than those for water added Si-Al solutions by more than 0.02 dS m^{-1} that approximately corresponds to the amount of the added NaH_2PO_4 . This result suggests that the added P to the Si-Al solutions having OH/Al molar ratio of 2.0 and 2.4 was largely in the form of free ion in the mixtures.

In the higher initial P concentration range more than about 1.5 mM, the amount of P precipitated from the solution gradually came closer to those for PN-Al solutions (Fig. 1). Thus, in the higher P concentration range, the effect of Si to interfere the reaction of P and Al was small.

When Na_2SO_4 was added to the Si-Al solutions (OH/Al=2.0 and 2.4), P precipitated from the solution phase when the initial P concentration was 0.6 mM or lower (Fig. 1). The sub-colloidal hydroxyaluminosilicate precipitated even when the 5 mL of 1.5 mM Na_2SO_4 was added to the Si-Al solutions. This result suggests that Na_2SO_4 is more reactive with the Si-Al solutions than NaH_2PO_4 . This is possibly because SO_4^{2-} is divalent anion and it can coagulate hydroxyaluminosilicate more effectively than monovalent H_2PO_4^- . Further, SO_4^{2-} may alter the conformation of the hydroxyaluminosilicate to react with H_2PO_4^- .

The Si-Al solution (OH/Al=2.8) is more reactive with low concentration of P than those having OH/Al molar ratio of 2.0 or 2.4 (Fig. 1). However, in the P concentration range higher than 0.6 mM, reactivity with P was lower than those for PN-Al solutions and Na_2SO_4 -added Si-Al solutions having OH/Al molar ratio of 2.0 and 2.4. The difference in the amount of P precipitated between with and without Na_2SO_4 addition was small for the Si-Al solution (OH/Al=2.8) (Fig. 1). Thus, reactivity of the Si-Al solution (OH/Al=2.8) and P is different from those having OH/Al molar ratio of 2.0 and 2.4 and the former was considered to be analogous to silica-alumina gel of which OH/Al molar ratio is close to 3.

The Na_2SO_4 treated hydroxyaluminosilicate in the Si-Al solutions reacted with P releasing $\text{Si}(\text{OH})_4$, SO_4^{2-} and OH^- , and the precipitate was noncrystalline by X-ray diffraction. Using SEM, the precipitates formed

from the Si-Al solutions appeared noncrystalline. The S/Al and Si/Al molar ratio of the precipitate decreased with an increase in P/Al molar ratio according to EDX analysis. However, the decrease in Si/Al molar ratio is less than the increase in P/Al value. P was probably reacted mainly displacing OH or OH₂⁺ groups and bridging more than two hydroxyaluminosilicate clusters. Thus, P and Al might react without releasing much Si and Si did not interfere the reaction of P and Al strongly in the Si-Al solutions under the P concentration of 0.6 mM or higher under the present experimental conditions.

ACKNOWLEDGEMENTS

The author thank Mr. T. Sato, Graduate School of Agricultural Science, Tohoku University for operating SEM and assistance for EDX analysis. This work was partly supported by a Grant-in-Aid for Scientific Research (No. 12660055) from the Ministry of Education, Science, Sports and Culture of Japan.

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Adsorption of Phosphate onto Silica Gel under Ferric Ion Coexisting Condition

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INTRODUCTION

Phosphate is an essential substance for growth of plants, and its behavior in soils and sediments have been studied in detail. Iron hydroxide is one of important constituents which control the behavior of phosphate. The adsorption of phosphate onto this hydroxide has been investigated under different pH¹ and pE². On the other hands, silicate is one of major constituents of soils, but its effect on adsorption of phosphate is not known well.

In this study, we investigated adsorption percent vs. pH curves (adsorption curves) of phosphate onto silica gel under coexisting ferric ion. As a result, we found that the form of adsorption curve varied by the order in which phosphate, ferric ion and silicate reacted, and that the maximum of adsorption percent was at pH 5.5–6 for adsorption of iron phosphate onto silica gel; pH 3 for phosphate onto iron silicate.

EXPERIMENTAL

The dispersed silica gel solution was prepared in the following manner. A 7.10 g-dose of $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ was dissolved in 40 mL of distilled water to which 5 mL of conc. HCl was added at once and mixed thoroughly. When necessary, a 1.35 g-dose of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was added. The solution was continuously mixed while NaOH solution was added by drops to gradually increase the pH to 8. This gel was recovered with a centrifuge, washed 5 times with distilled water, and dispersed in distilled water and diluted to 50 mL. The silica gel prepared from Na_2SiO_3 with this method will be written as [Si], and iron silicate from Na_2SiO_3 and FeCl_3 as [SiFe]. Phosphate was determined according to Tabushi and Ishibashi³ and Hori *et al.*⁴

RESULTS AND DISCUSSIONS

The adsorption curves of phosphate

Curve 1 in Fig. 1a is the adsorption curve of phosphate onto [Si] under ferric ion coexisting condition. This adsorption curve had two local maxima. The curve began to rise around pH 3 and reached the first maximum with a constant value at pH 5.4–6.2. It then dropped off to 80% of the peak value at pH 6.5, and then rose again above pH 6.5 to reach the second maximum at pH 8.

For comparison, the adsorption curve of phosphate onto [Si] without coexisting ferric ion was investigated. It was found that phosphate was scarcely adsorbed onto [Si] alone. Next, the adsorption of phosphate to iron hydroxide (curve 2) was studied and it was found that the adsorption was around 20% in the pH range of 3–6.2, but increased above pH 7 and reached a maximum at pH 8. This adsorption curve resembled curve 1 in the pH range of 7–10. This indicates that the adsorption of phosphate at pH 7–10 can be attributed to the adsorption solely onto iron hydroxide, but that the presence of both ferric ion and [Si] is necessary for the adsorption at pH 5.4–6.2.

Curve 3 in Fig. 1a is the adsorption curve of phosphate onto [SiFe]. This adsorption curve had a maximum at pH 3.

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Figure 1b shows the distribution of chemical forms of phosphate and ferric ion as functions of pH. Among the possible combinations of chemical forms of phosphate ion (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}) and ferric ion (Fe^{3+} , FeOH^{2+} , $\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})_3(\text{aq})$, $\text{Fe}(\text{OH})_4^-$), the combinations of the dominant species are (i) H_3PO_4 and Fe^{3+} at $\text{pH} < 2.2$ (curves 1 & 5), (ii) H_2PO_4^- and FeOH^{2+} (curves 2 & 6) at $\text{pH} = 2.2 - 3.5$, (iii) H_2PO_4^- and $\text{Fe}(\text{OH})_2^+$ (curves 2 & 7) at $\text{pH} = 3.5 - 6.3$, (iv) H_2PO_4^- and $\text{Fe}(\text{OH})_3(\text{aq})$ (curves 2 & 8) at $\text{pH} = 6.3 - 7.2$, (v) HPO_4^{2-} and $\text{Fe}(\text{OH})_3(\text{aq})$ (curves 3 & 8) at $\text{pH} = 7.2 - 9.6$, and (vi) HPO_4^{2-} and $\text{Fe}(\text{OH})_4^-$ (curves 3 & 9) at $\text{pH} > 9.6$.

As aforementioned, the adsorption of phosphate onto [Si] occurs under ferric ion coexisting conditions, especially with adsorption rate of over 50%, in the pH range of 4–6.5 (curve 1 in Fig. 1a). The combination of the chemical forms of phosphate and ferric ion which may interact together is (iii), *i. e.*, the combination of H_2PO_4^- and $\text{Fe}(\text{OH})_2^+$. The reaction product, therefore, is considered to be $\text{H}_2\text{PO}_4 \cdot \text{Fe}(\text{OH})_2$ or FePO_4 . These are neutral species, and when phosphate and ferric ion are present in high concentrations, the solubility product is easily exceeded and precipitation occurs. However, when the two are present only in extremely low concentrations and the solubility product is not exceeded, there is almost no precipitation present. When [Si] coexists as a third component in such a condition, FePO_4 is collected onto the [Si]. This is in good agreement

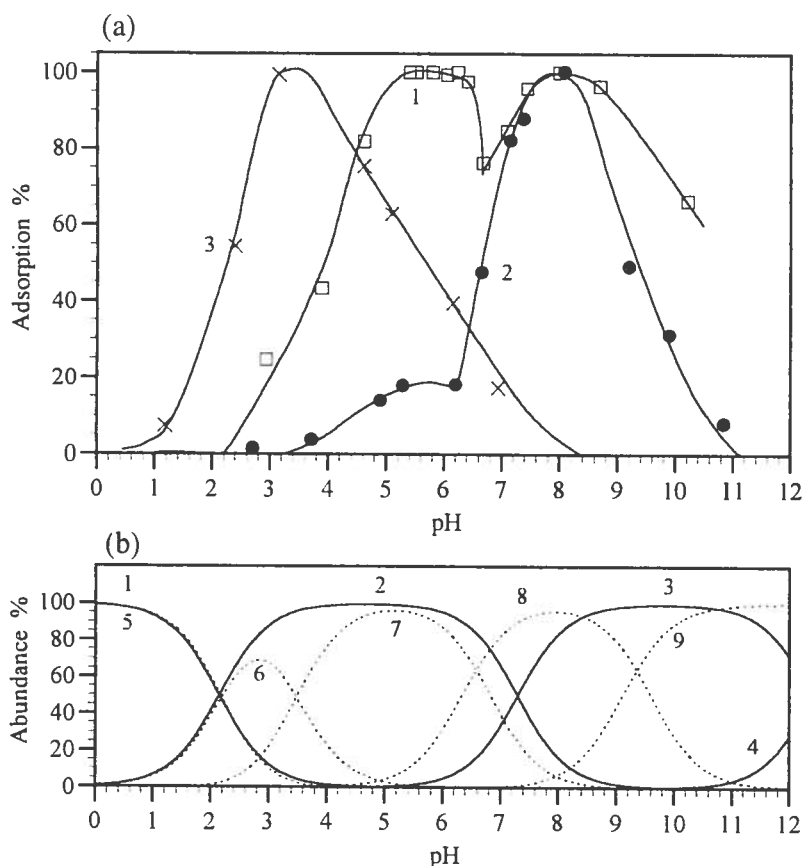


Fig. 1. (a) Adsorption curves of phosphate onto [Si] under ferric ion coexisting conditions: the adsorption curves of phosphate ($0.02 \mu\text{mol l}^{-1}$) in FeCl_3 solution ($10 \mu\text{mol l}^{-1}$) with addition of [Si] (curve 1) and without addition of [Si] (curve 2); the adsorption curve of phosphate ($100 \mu\text{mol l}^{-1}$) onto [SiFe] (curve 3).

(b) The distribution curves for the chemical species of phosphate and ferric ion: the abundance of the respective forms the phosphate ion 1: H_3PO_4 ; 2: H_2PO_4^- ; 3: HPO_4^{2-} ; 4: PO_4^{3-} (solid lines) and the ferric ion 5: Fe^{3+} ; 6: FeOH^{2+} ; 7: $\text{Fe}(\text{OH})_2^+$; 8: $\text{Fe}(\text{OH})_3(\text{aq})$; 9: $\text{Fe}(\text{OH})_4^-$ (dashed lines) are given as a function of pH. The equilibrium constants ⁵ used in the calculations were, for phosphate ion, $\log K_{a1} = -2.2$, $\log K_{a2} = -7.2$, and $\log K_{a3} = -12.3$, and for ferric ion, $\log K_{a1} = -2.19$, $\log K_{a2} = -3.48$, $\log K_{a3} = -6.33$, and $\log K_{a4} = -9.6$.

with the observation of the remarkable phosphate adsorption in the pH range of 5.4-6.2 (Curve 1 in Fig. 1a) in a mixed system of phosphate, ferric ion, and [Si].

The combinations of dominant phosphate and ferric ion species at pH = 6.5-9 are (iv) H_2PO_4^- and $\text{Fe}(\text{OH})_3(\text{aq})$ or (v) HPO_4^{2-} and $\text{Fe}(\text{OH})_3(\text{aq})$. This also implies that the phosphate form adsorbed onto iron hydroxide are either H_2PO_4^- or HPO_4^{2-} .

The combinations of dominant phosphate and ferric ion species at pH = 2.5-5 are (ii) H_2PO_4^- and FeOH^{2+} . It is difficult to estimate the chemical form of ferric ion in iron silicate. When H_2PO_4^- is adsorbed onto [SiFe], the phosphate ion might react to ferric ion which bond with one hydroxide ion and some silicates.

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Session 3

Silicon in Plants

Silicon and Abiotic Stress

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INTRODUCTION

The very fact that we are meeting for the "Second Silicon in Agriculture Conference" suggests that the importance of silicon in agricultural systems is becoming more recognised. However, this is not the case in plant physiology, where the element is still rarely considered [1,2]. The problem of interesting plant physiologists in an element that is often regarded as "non-essential" is quite a major one, and this is certainly the case when we come to consider the abiotic stresses. Relatively few researchers in this area have even dabbled with silicon, and even fewer have made a concerted attempt to understand its potential roles in stress physiology.

What are the Abiotic Stresses?

Before we go any further we should attempt to define the words "abiotic" and "stress". The first of these is relatively simple to define and "abiotic" can be taken to mean "the nonliving components of the environment" [3]. So the remit of this paper will not include stresses caused by plant pathogens or herbivores. The word "stress" is decidedly more difficult to define, and has been the cause of some controversy, but seems to be associated with strain [4]. In the context of this conference we could define a stress as anything that causes a decrease in plant growth or yield. Plant stress physiology is a vast area of research, and one that now even has it's own web site, www.plantstress.com. That site covers almost all of the major stresses, but if we want a complete listing, then we are best to consult the classic book on the topic [4].

Silicon- plus or minus?

In the natural or agricultural environment most plants will obtain enough silicon for their needs from the soil. In some cases, however, silicon fertilizers are added to the soil to improve crop performance, and we will hear more about these at this conference. Moving into the laboratory many plant scientists conduct experiments in hydroponic culture as it is easier to control what the plants obtain from solution culture than in soil. They often leave Si out of their formulations altogether, and the plants must then survive with what they can get from Si contamination of water and chemicals. Usually these plants seem to grow reasonably well, but there may be all sorts of hidden effects of growing plants with low levels of silicon, and Epstein [1,2] has gone so far as to call such plants "experimental artefacts". Even those scientists who do acknowledge silicon to be an important element in plant nutrition often have a problem in deciding what is their control. In the context of stress physiology, the most common experimental set-up will involve plants grown at a range of silicon concentrations including "zero" (true zero is almost impossible to attain, and the term "silica minimal" is often used). All these groups of plants then have the same stress applied, and the aim is to see whether added Si increases the growth of the plants under stress. Most scientists would tend to regard the "silica minimal" plants as their control, as they are "treating" plants with Si in the other groups. But would it be better to regard the Si plus plants as the control, and the Si minus plants as the "treatment", as the former are closer to "natural"? Maybe.

Aims

We will now reflect on the effects of silicon on each of the stresses in turn. The aims of this paper will be two-fold: to assess what is known of the effects of silicon across the range of abiotic stresses; and then to concentrate on one particular stress, aluminium toxicity, where there has been much progress in recent years.

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THE STRESSES

Table 1 shows our rough guide to the progress so far on the effects of Si on abiotic stresses. The table is intended to show how much work has been carried out on a particular stress, and how much is known about the mechanisms of any Si effects. It is apparent that there is much more known in some areas than others, and some stresses have hardly been considered. Even where considerable work has been carried out (e.g. on aluminium), we are still far from a complete understanding of the mechanisms.

Temperature stress- heat, chilling, freezing

Relatively little seems to be known about the effects of silicon on the various temperature stresses. Recently, Wang Lijun (pers. comm.) has found that creeping bent grass plants that were grown with Si had lower leaf temperatures than those grown without Si, and that this increased heat tolerance. We are not aware of any work showing effects of Si on relieving stress in chilling sensitive plants. However, it has been suggested that silica deposition in the walls of palm leaves may favour supercooling, and thus increase tolerance to freezing stress [5].

Wind and other physical stresses

It has long been known that silica deposition in the shoots of higher plants can have considerable strengthening effects [6]. A lodging resistant wheat cultivar has been shown to have a higher Si content in the culm epidermis than a sensitive one [7]. Deposition of silica in rice increases the thickness of the culm wall and the size of the vascular bundle, preventing lodging during typhoons in Japan [8]. Deprivation of Si may result in plants with physical abnormalities, such as a decrease in roughness [9].

Light- too little, too much

One can imagine situations where Si could be beneficial to plants growing where there is either too little or too much light, but there is certainly a paucity of work in this area. Kaufman and his co-workers speculated that leaf phytoliths might act as "windows" or "light pipes" bringing light to the leaf mesophyll cells and increasing photosynthesis. However, when this hypothesis was tested the data did not support the idea [10]. Silicon-fertilized rice often has more erect leaves, and this has often led to suggestions that these plants would

Table 1. Summary of work carried out on the effects of Si on abiotic stresses.

Abiotic Stress	Work on ameliorative effects of Si	Understanding of mechanism(s) of Si effect
Temperature		
(a) High	+	+
(b) Chilling	-	-
(c) Freezing	+	+
Physical stress		
(a) Wind	++	++
Light intensity		
(a) High light	-	-
(b) Low light	+	+
Radiation	-	-
Drought	-	-
Waterlogging (anaerobiosis)	-	-
Mineral deficiency		
(a) Of Si itself	+++	++
(b) Of other elements	+	+
Mineral toxicity		
(a) Salinity	++	+
(b) Heavy metals	++	+
(c) Manganese	++	++
(d) Aluminium	+++	+++

Key: +++, considerable; ++ moderate; +, some; -, little/none.

have greater canopy photosynthesis. However, individual leaves of Si-fertilized rice do not show increased photosynthesis, despite better growth [11]. White hairs are well known to have reflective properties in groups such as the cacti, and undoubtedly light reflection is a mechanism for keeping xerophytic plants cool. Siliceous hairs would also probably reflect light, but any reflection would probably be due to hair colour rather than to Si *per se*.

Radiation

As far as we know there are no papers suggesting that Si can have an effect on tolerance to radioactivity. However, it does seem that rare earth elements are often associated with Si [12] and it may be that radioactive elements, many of which are trivalent or quadrivalent, may become co-deposited with Si in the plant in a similar way to Al (see below).

Water- drought and waterlogging (anaerobiosis)

Although the relationship between water uptake, transpiration rate and silica deposition in the plant is well established [13], there seems to be a distinct lack of work on the effects of Si on water stress. Similarly, any role(s) for silicon in alleviating anaerobiosis due to waterlogging are obscure, despite the fact that rice routinely grows in such environments, is a heavy silicon accumulator, and even has substantial Si deposits in the root endodermis [14].

Minerals- deficiency

There is considerable evidence that silicon is beneficial for crop plants [1,2,8,11], and it is now routinely added as a fertilizer for several silicon accumulating crops. Growth and yield reductions have frequently been reported when it is supplied in sub-optimal amounts. Deficiency of silicon may also have quite complex effects on other nutrients. For example, in cucumber growth enhancement by silicon depended on an imbalance in phosphorus and zinc supply [15].

Minerals- toxicity

There has been a considerable amount of work on the effects of silicon on mineral toxicity. This can be broken up as follows:

- a) *Salinity*. There is a reasonable body of literature that suggests that Si can have beneficial effects for plants growing under saline conditions. It seems that Si restricts sodium uptake to the shoot of sensitive plants [16,17], and the mechanism is by partial blockage of the transpirational bypass flow [18].
- b) *Manganese*. Our understanding of manganese toxicity in general, and specifically the ameliorative effects of Si on toxicity, owe much to the Germans, W.J. Horst and the late H. Marschner. In cowpea Si nutrition reduced leaf apoplastic manganese content suggesting that Si modified the cation exchange properties of cell walls [19]. Electron paramagnetic resonance has shown that Si decreased leaf manganese content, and decreased the accumulation of oxidation products in the leaves [20].
- c) *Other heavy metals*. There have also been relatively infrequent reports that Si can ameliorate the toxicity of various heavy metals. Until recently, the mechanism(s) behind these effects have been obscure, but it now seems likely that some type of co-precipitation, often in the cell walls, is involved [21,22].

ALUMINIUM TOXICITY

In the last ten years it is true to say that there has been more progress on the effects of Si on aluminium (Al) toxicity than any other abiotic stress. Why this has been the case is difficult to ascertain, but it may partly be due to the parallel interest in Al/Si interactions shown by chemists and animal scientists, stimulated by the pioneering work of J.D. Birchall and his colleagues in the late 1980's [23]. There is no doubt that Al toxicity in plants is a major problem, both for agriculture on naturally acidic soils and for forest areas affected by acidic rain. Many publications have now shown that under some conditions added Si can ameliorate Al toxicity in hydroponic culture. Two reviews have considered Al/Si interactions [24,25], and we will not reiterate all of this material here, but rather will concentrate on recent developments in this topic.

Solution Chemistry

One of the key problems in researching Al and Si in plants over the last ten years has been that the basic chemistry of how Al and Si interact in solution has been a fairly controversial topic. It is known that at neutral pH, Al and Si form hydroxyaluminosilicates (HAS), and that the formation of HAS reduces Al toxicity. What happens at acidic pH has been unclear, and this is the range of most interest to plant scientists. It now seems, however, that improvements in both speciation modelling and experimental procedures are throwing some light on this topic [26,27]. The formation of HAS at pHs of 4.0 and below has been shown to be negligible, and formation gradually increases as pH increases to pH 5.0 and beyond. These findings have considerable importance for agriculture on acidic soils.

Plant Growth Effects

It is now apparent that amelioration of Al toxicity by Si in hydroponic culture is a rule, and not an exception. The few cases in the literature where amelioration has not been found are probably due to workers using a low background pH. The other exception may be Al accumulating plants like the Old World shrub, *Melastoma malabathricum*, [28], but this needs confirmation. Three recent examples showing amelioration, at least under some conditions, concern rice [29], barley [30] and *Holcus lanatus* [31].

Mechanisms

The mechanisms behind the amelioration effect are still somewhat obscure even in experiments conducted in hydroponic culture. It now seems that bulk solution effects due to the formation of HAS can account for almost all amelioration at pH 5.0 and above, and almost none below pH 4.0. Between these two values HAS formation will be increasingly important as pH is increased. However, there is growing evidence that *in planta* effects are also involved:

- a) When experiments have been conducted near pH 4.0 or below, and amelioration has still been observed, then we can only conclude that that bulk solution phenomena are not involved. For example amelioration was observed at pH 4.2 in *Holcus lanatus*, and the authors went to great lengths to show that no HAS formed in their hydroponic solutions [31].
- b) In very Al-sensitive plants amelioration can be observed at very low Al and Si concentrations, well below levels where solution chemistry effects are likely. For example in the wheat cultivar, Scout 66, the toxicity caused by only 1.5 μM M Al could be partially overcome by 5.0 μM M Si [32].
- c) There has recently been a lot of interest in organic acids in relation to Al toxicity. Two papers have concerned Al/Si interactions. In the first it was shown that even under conditions where Si ameliorated Al toxicity, the addition of Si did not reduce Al-induced malate efflux [32]. In the second paper it was found that Si could actually stimulate greater organic acid exudation in some plants [33].
- d) The one attempt so far to investigate Al/Si interactions in cell suspension culture showed that in rice Si caused no amelioration in cell culture, but a significant amelioration in intact plants [34]. This suggests that organized plant structures may be necessary for amelioration effects to occur.
- e) Finally, the number of cases of codeposition of Al with Si in plant tissues is increasing- this merits a section of its own.

Codeposition of Al with Si

At the same time that plant physiologists have been discovering the conditions under which Si can ameliorate Al toxicity, electron microscopists, using x-ray microanalysis, have been finding more and more cases where Al and Si are codeposited in plant tissues. It has even been suggested that these Al/Si deposits may represent a new type of biomineral [25]. There are two main locations where Al/Si deposits have been found: in the roots, particularly of grasses and cereals that have been grown in hydroponic culture; and in the needles of conifers. Recent examples include: (a) our own work [35-39] on Al/Si deposits in the needles of the conifers (Table 2). Silicon and Al levels vary with the species, but codeposition is commonest in the epidermis and transfusion tissue. (b) Al/Si deposits in *Lotus pedunculatus* roots [40]. (c) an investigation of Al in phytoliths in the above ground tissues of 20 species (Gramineae, Cyperaceae, Ericaceae and Coniferae) [41]. Only

Table 2. Silicon and aluminium content of the cell walls of five tissues of mature needles of six conifer taxa as determined by EDX microanalysis and expressed as mean values in mmol/kg (n= 5).

Conifer species	Soil pH	Leaf age (yr.)	Element	Epid-ermis (a)	Hypo-dermis (b)	Meso-phyll (b)	Endoder-mis (a)	Transfusion tracheid (b)
Pinus strobus L., White pine [35,36]	4.2	2	Si	200	200	1500	500	5800
			Al	15	5	25	15	45
Tsuga canadensis (L.) Carr., Eastern hemlock *	4.2	3	Si	60	1200	330	300	1000
			Al	60	65	10	20	40
Picea glauca (Moench.) Voss, White spruce [37]	4.2	5	Si	280	630 ^m	nd	1100 ^m	nd
			Al	70	35 ^m	nd	25 ^m	nd
Abies balsamea (L.) Mill., Balsam fir [38]	4.2	4	Si	75	25	15	25	nd
			Al	70	15	30	10	nd
Larix laricina (Du Roi) K. Koch, American larch [39]	6.7	1	Si	1900	250	60	25	25
			Al	60	20	10	15	18
Larix decidua (L.) Mill., European larch [39]	6.7	1	Si	2300	700	25	25	15
			Al	30	15	12	15	10

Unless otherwise stated all determinations were conducted using frozen hydrated sections from the needle tip.

(a), outer tangential wall; (b), radial wall; m, mid-point of needle; nd, not determined.

*, approx. values derived from unpublished data; all other data derived from our previous publications [35-39].

the woody species produced phytoliths containing much Al.

Ten years ago there were very few such examples of Al/Si codeposition, and now they have become almost commonplace. The key questions now concern the chemistry of the deposits (are they aluminosilicates?), and their role. Intuitively, we feel that sequestering toxic Al in an Al/Si deposit should be beneficial for plant growth, but there is still no proof!

In the Field??

Although there has been some work using silicate slag to decrease Al toxicity [e.g. 42], and the results have been positive, there is not a lot known in this area. One of the problems will undoubtedly be distinguishing Si effects from those of raised pH. According to the latest data, even liming a suitable mineral soil at pH 4.0 with standard calcitic or dolomitic limestone will radically affect the speciation of Al, Si and HAS, leading to decreased Al toxicity. Adding additional soluble Si to an acidic soil (above pH 4.0) would also be expected to decrease Al availability through bulk solution effects. Even below pH 4.0 it is possible that *in planta* effects will decrease Al toxicity. Hopefully in the next few years we will be able to take Al/Si interactions into the field. If we can, then the benefits for agriculture on acid soils may be considerable.

CONCLUSIONS

We conclude that there is still a lot to do! In many topic areas almost nothing is known, and even in areas where most progress has been made (e.g. aluminium), we are still far from understanding the mechanisms of any effects. As resources are not great we need to prioritise research goals, and concentrate our attention on a few topics, preferably those that will stand the best chance of increasing agricultural production under stress conditions.

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The Role of Silicon in Turfgrass Disease Management

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Silicon is the second most abundant element in the earth's crust (Epstein, 1999). Although not generally considered an essential element, when silicon is amended to soils or nutrient solutions low in soluble silicon, plants show improved growth and development. Other benefits include increased insect and disease resistance, reduced mineral toxicities, and enhanced drought and frost tolerance. These relationships have been demonstrated in many plant species including cucumbers, rice, sugarcane, and turfgrass (Datnoff *et al.*, 2001a).

In turfgrass, silicon has been associated with improvement in host resistance to abiotic and biotic stresses in Canada, China, Japan and the United States (Hamel and Heckman, 1999; Linjuan *et al.*, 1999; Rondeau, 2001; Schmidt *et al.*, 1999; Saigusa *et al.*, 2000). For example, silicon amendment has led in several turfgrass species to better blade rigidity, elasticity, traffic or wear resistance, and heat tolerance (Linjuan *et al.*, 1999). Silicon also has improved resistance to insect feeding by *Rusidra depravata* and disease resistance to *Rhizoctonia solani* in zoysiagrass (Saigusa *et al.*, 2000). Creeping bentgrass growth and establishment were better with silicon amendment as well as resistance to *Pythium aphanidermatum*, *Sclerotinia homoeocarpa* and *R. solani* (Gussack *et al.*, 1998; NC State, 1997; Rondeau, 2001; Schmidt *et al.*, 1999). In Kentucky bluegrass, powdery mildew (*Sphaerotheca fuliginea*) was suppressed by the addition of silicon (Hamel and Heckman, 1999). More recently, a leaf spot on bermudagrass caused by *Bipolaris sorokiniana* was reduced by silicon depending on inoculum concentration (Datnoff and Rutherford, 2002).

In Florida, gray leaf spot caused by *Magnaporthe grisea* (*Pyricularia grisea*) has been demonstrated to reduce prostrate growth (bare ground coverage) of St. Augustinegrass in new sod fields by 36%. It can also reduce growth in newly sprigged lawns and regenerating sod fields. Recently, gray leaf spot development was reduced by silicon amendment over a range of 19 to 78% on several cultivars of St. Augustinegrass in the greenhouse (Figure 1) (Datnoff and Nagata, 1999). In addition, this study showed that levels of silicon were increased in these cultivars more than two fold in comparison to the non-amended control (Figure 2). In the field, silicon alone was compared to foliar sprays of chlorothalonil, and silicon plus chlorothalonil for managing gray leaf spot development (Brecht *et al.*, 2001). This disease was reduced by 17-27, 31-63 and 56-64% for silicon alone, chlorothalonil alone and silicon plus chlorothalonil, respectively, compared to a non-treated control (Figure 3). Final percent bare ground coverage also was significantly increased between 17 to 34% using silicon (Figure 4). At harvest, the treatments that had been amended with silicon had sod mat weights 11 to 13% higher than the non-treated grass (Figure 5) (Datnoff *et al.*, 2001b). In addition, these sod pieces when transplanted to the field showed greater root lengths, 0.8 to 1 cm, (Figure 6) and improved turf quality ratings, 7.1 to 7.6 vs. 6.6 to 7.1 (Figure 7).

In the case of St. Augustinegrass, silicon appears to be a viable method for increased growth and reduced disease. Silicon may help in the management of fungicide use, thus promoting good environmental stewardship. Therefore, the use of silicon amendments for turfgrasses may be a viable option for reducing biotic and abiotic stresses for commercial sod growers, golf course superintendents, and homeowners.

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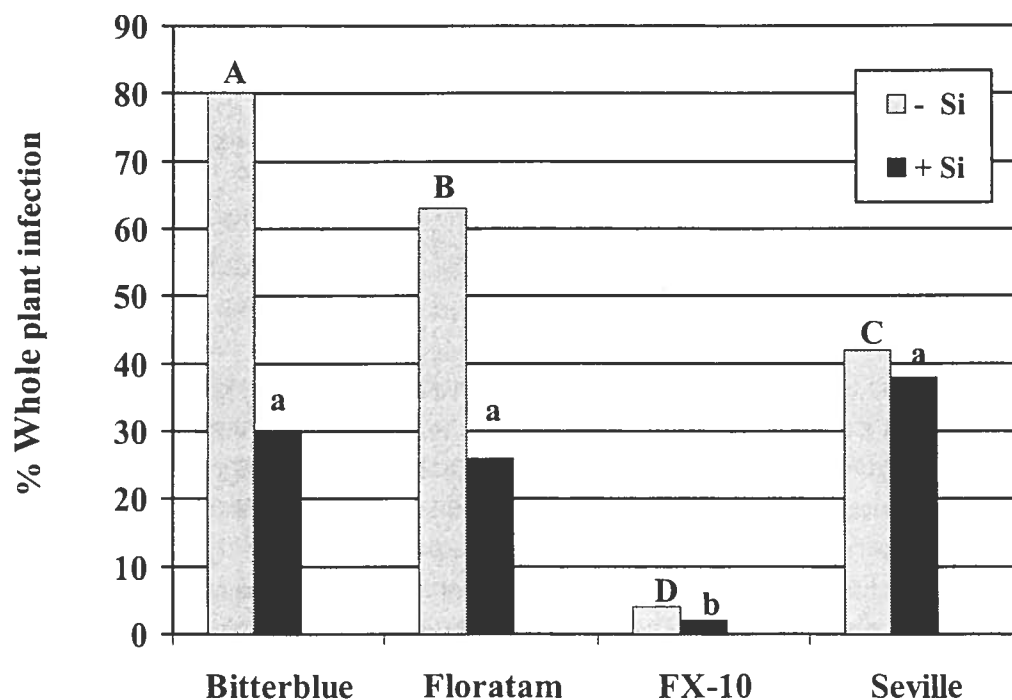


Figure 1. Percent infection by *P. grisea* on St. Augustinegrass cultivars grown in soil amended without and with silicon.

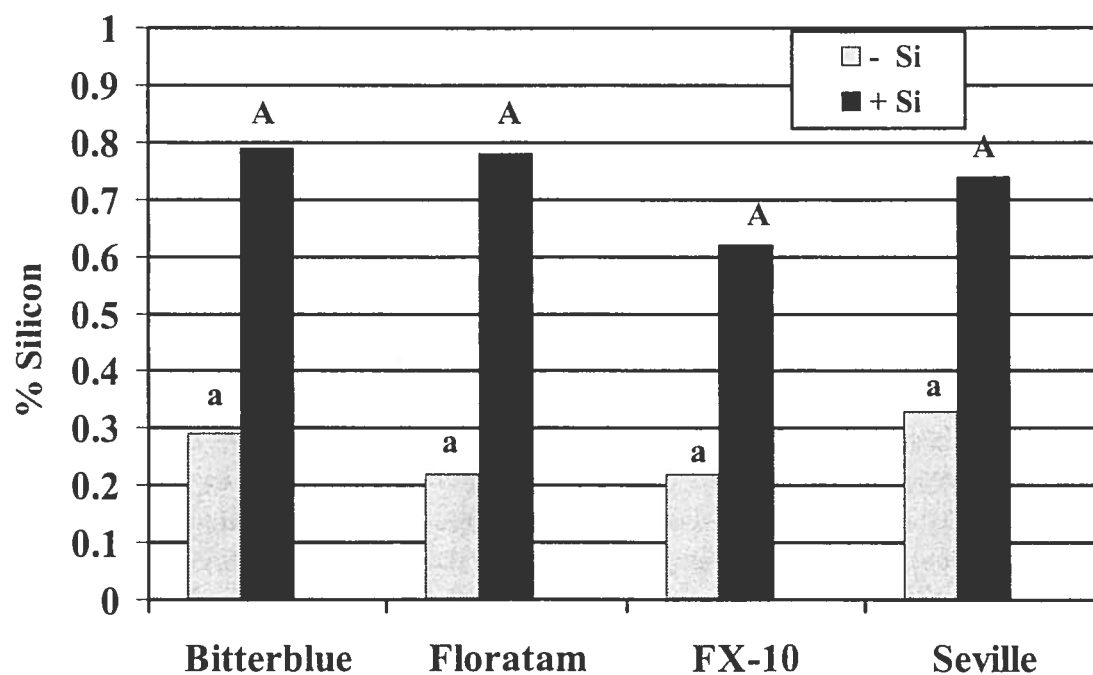


Figure 2. Percent silicon content in leaves of St. Augustinegrass cultivars grown in soil amended without and with silicon.

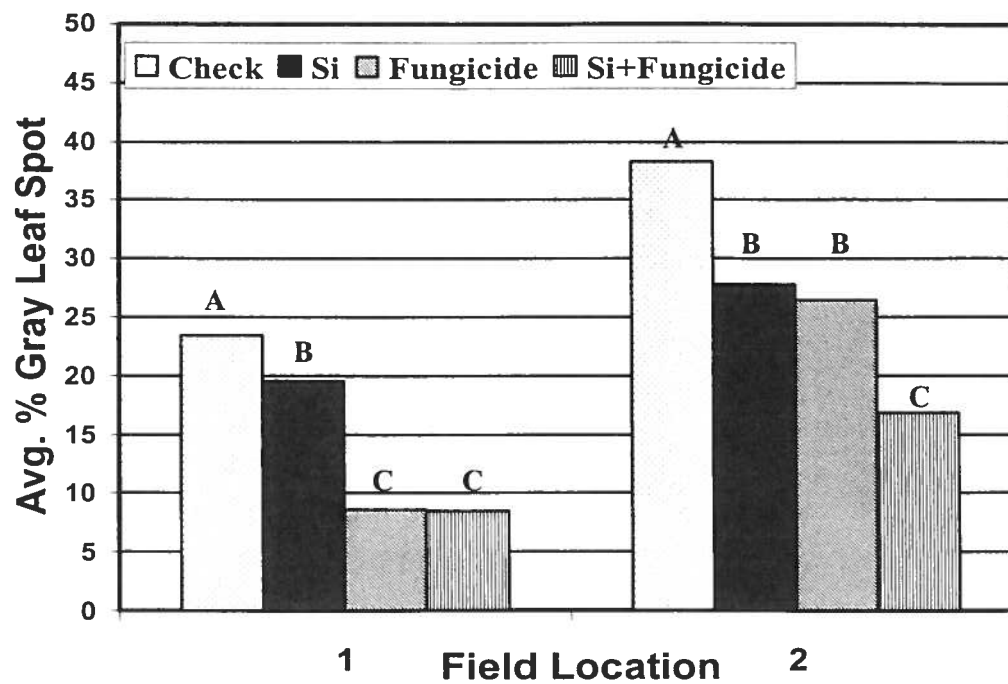


Figure 3. Percent whole plant infection by *P. grisea* at two field locations.

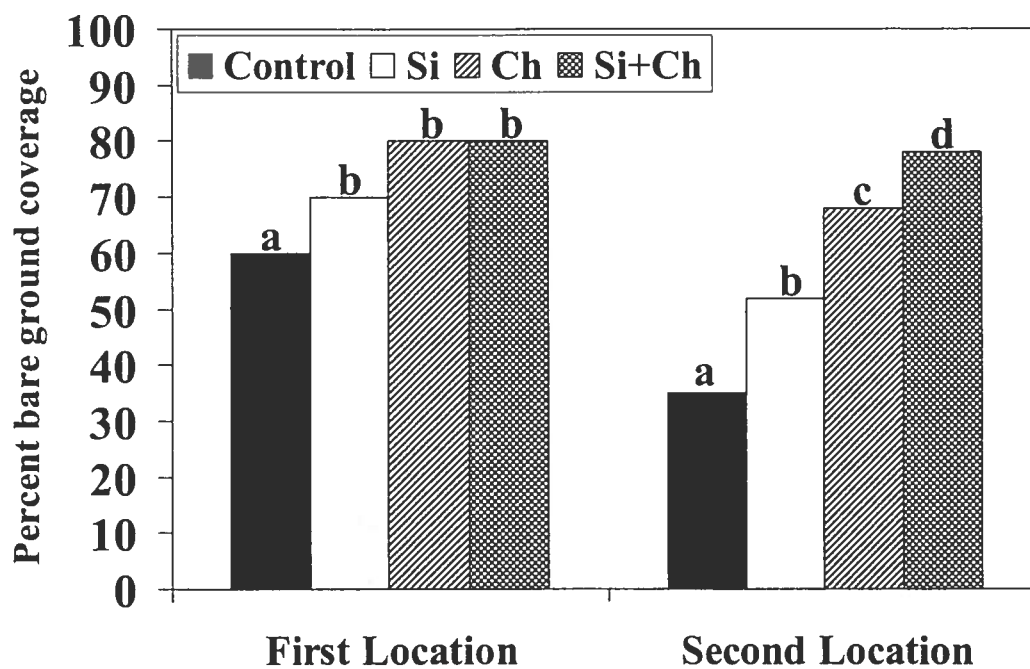


Figure 4. Influence of silicon on prostrate growth of St. Augustinegrass at two field locations.

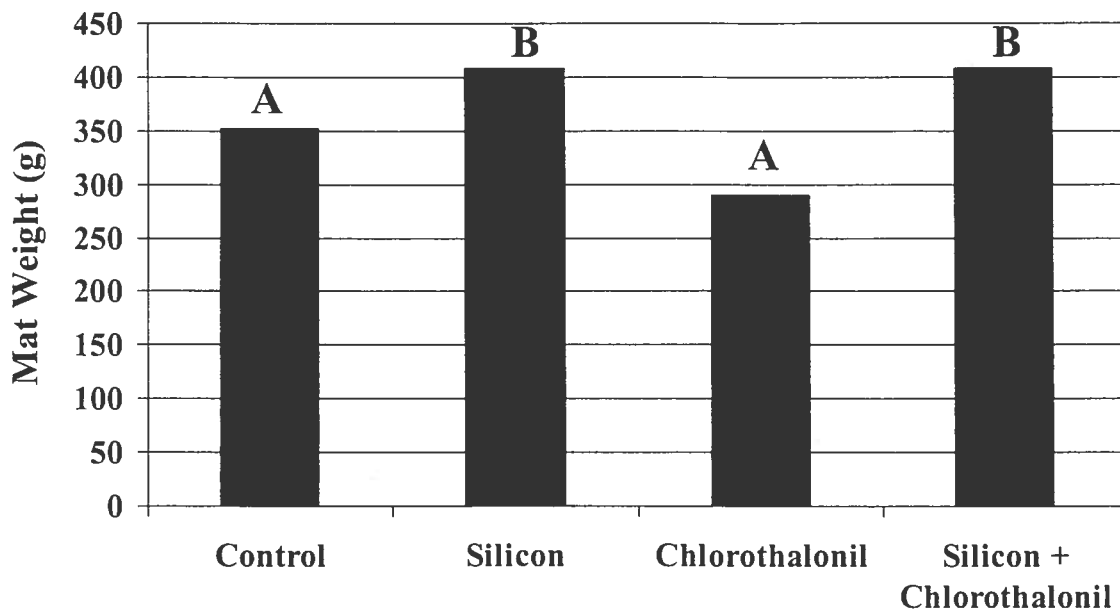


Figure 5. Influence of silicon and chlorothalonil alone and in combination on sod dry mat weights.

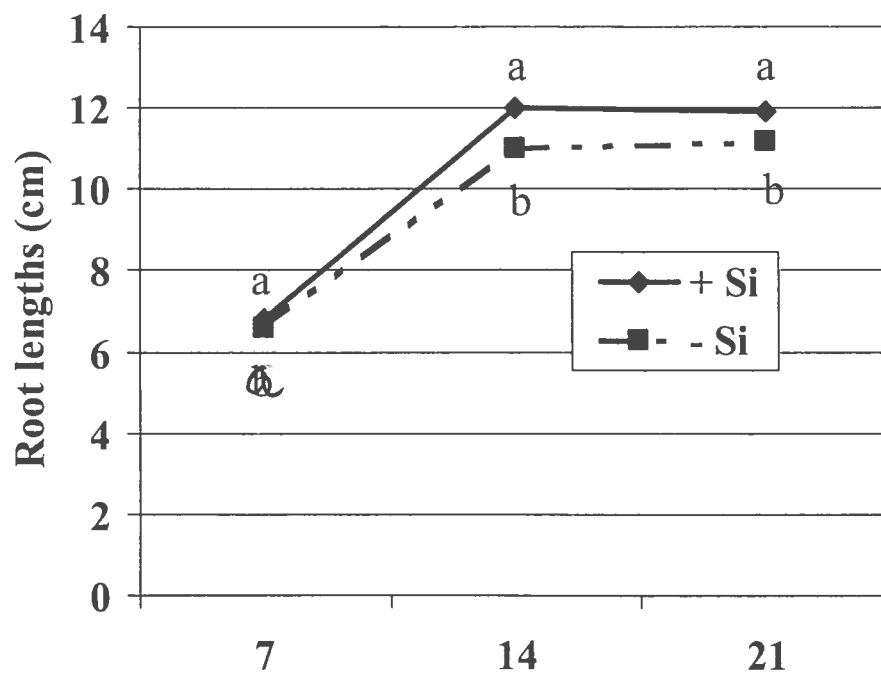


Figure 6. Root lengths of St. Augustinegrass sod mats 7, 14 and 21 days after transplanting.

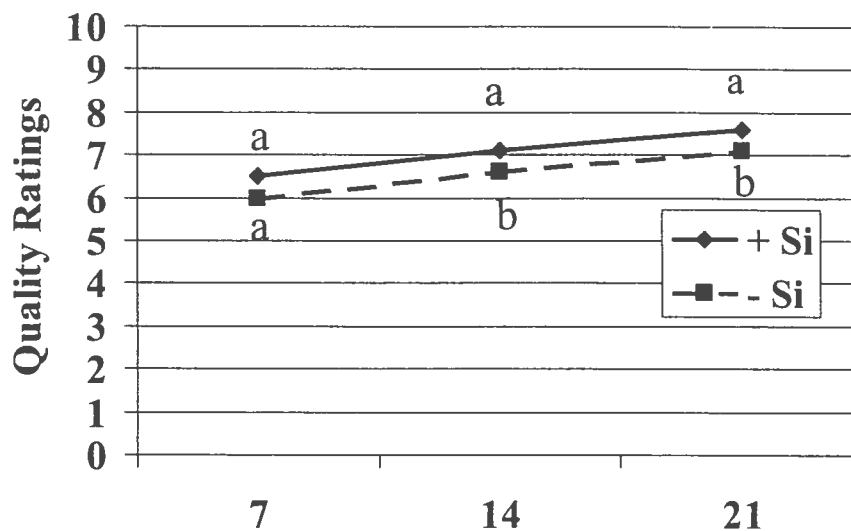


Figure 7. Quality ratings of St. Augustinegrass sod mats 7, 14 and 21 days after transplanting.

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Characterization of Silicon Uptake by Rice

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Silicon is the second most abundant element in the earth's crust, therefore, all plants rooting in the soil contain Si. However, the Si content of the top greatly varies with the plant species, ranging from 0.1 to 10% Si in dry weight. These differences have been ascribed to the uptake capacity of roots.

Rice is a typical Si-accumulating plant, which accumulates Si in the shoot up to 10% Si. Many studies have shown that rice roots have a specific transport system for silicic acid, which is the form of Si to be taken up. However, the mechanisms for Si uptake by rice roots have not been well understood. In the present study, we characterized the Si uptake by rice roots from following aspects.

1. Comparison of Si uptake ability among various gramineous plants

Silicon uptake was compared among various gramineous plants. Young seedlings (17-d-old) were allowed to take up Si in a 0.5 mM CaCl_2 solution containing 0.6 mM Si as silicic acid for 24 h. Obviously, rice showed the highest uptake ability of Si, followed by wheat>sorghum>maize • rye • barley (Fig. 1). This result further confirms that a specific uptake system for silicic acid exists in the rice roots.

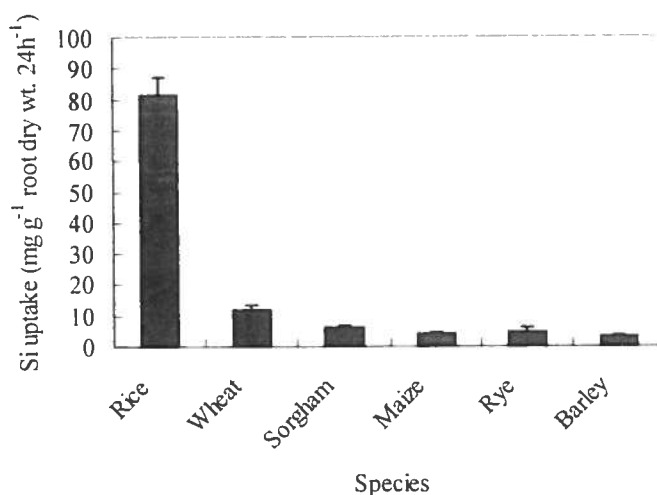


Figure 1. Si uptake by different gramineous plants from a solution containing 0.6 mM Si as silicic acid during a 24-h period

2. Genotypical difference in Si uptake

The genotypical difference in Si content has been reported to be smaller than that of other nutrients, suggesting that all cultivars of rice have a high ability to take up Si. The Si content of the shoot is related to both Si-uptake ability of each individual root and development of whole root system. A comparative study on Si uptake by the individual root and root system was conducted between a *japonica* variety, Nipponbare, and an *indica* variety, Kasalath. When both varieties were grown in a nutrient solution containing 0.15 mM silicic acid, the content of Si in the shoot was higher in Nipponbare than in Kasalath (Table 1). When grown in a solution

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containing 1.5 mM silicic acid, it was nearly the same in Nipponbare and Kasalath. The amount of Si taken up per plant was larger in Kasalath than in Nipponbare, but the amount per g dry weight of root was higher in Nipponbare than in Kasalath. Kasalath has a larger root system than Nipponbare. These results suggest that although the Si content of the shoot is nearly the same in Nipponbare and Kasalath, different mechanisms are involved in accumulation of Si in the two varieties. The high Si content in Kasalath relies on larger root system, while that in Nipponbare on higher uptake ability per root. This speculation was supported by the results of a multi-compartment transport box experiment. Si uptake per root in Nipponbare was 30% higher than that in Kasalath.

Table 1 Comparison of Si uptake between a *japonica* variety, Nipponbare and an *indica* variety, Kasalath*

	0.15 mM Si		1.5 mM Si	
	Nipponbare	Kasalath	Nipponbare	Kasalath
Si content (Si %)				
Shoot	1.72	1.30	4.31	4.54
Root	0.15	0.20	0.35	0.24
Dry weight (g)				
Shoot	3.36	4.10	3.08	4.86
Root	0.71	1.12	0.66	1.27
Uptake				
mg Si/plant	58.66	55.47	134.43	223.86
mg/g root dry wt.	83.59	49.82	205.40	176.89

*Two cultivars were grown in a nutrient solution containing 0.15 mM or 1.5 mM Si as silicic acid for 1 month.

3. Kinetics of Si uptake

Kinetics of Si uptake by rice was examined. To investigate whether the uptake of Si is inducible, rice seedlings were pre-cultured in a solution with or without Si (1.5 mM Si as silicic acid). The uptake of Si increased linearly with time, but, there was no difference in the Si uptake between the plants previously exposed to Si and not (Fig. 2), suggesting that the uptake of Si by rice roots is not inducible.

The uptake of Si by rice roots from a solution with various concentrations of Si was investigated. The uptake was saturated at 1.28 mM Si (Fig.3). From this uptake curve, the K_m was estimated to be 0.32 mM. This result suggests that Si uptake is mediated by a kind of transporter in rice roots. From K_m value, this transporter has a low affinity for silicic acid.

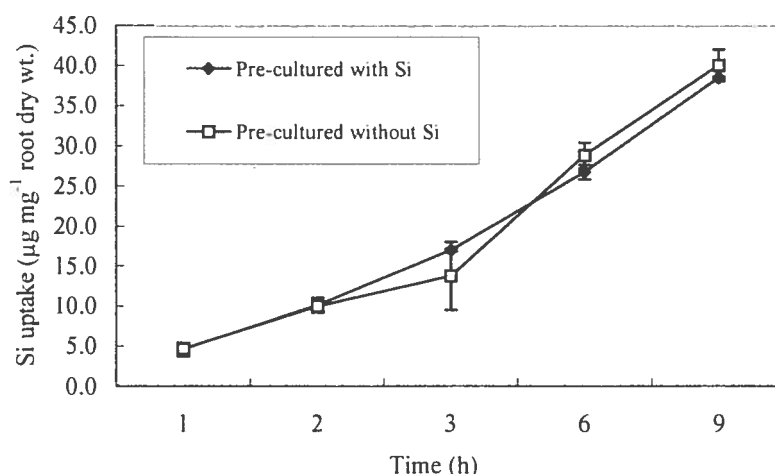


Figure 2. Change in the Si uptake by rice roots precultured in the solution with Si (1.5 mM Si) or without Si for 1 day. The uptake experiment was conducted in a nutrient solution containing 1.5 mM Si as silicic acid.

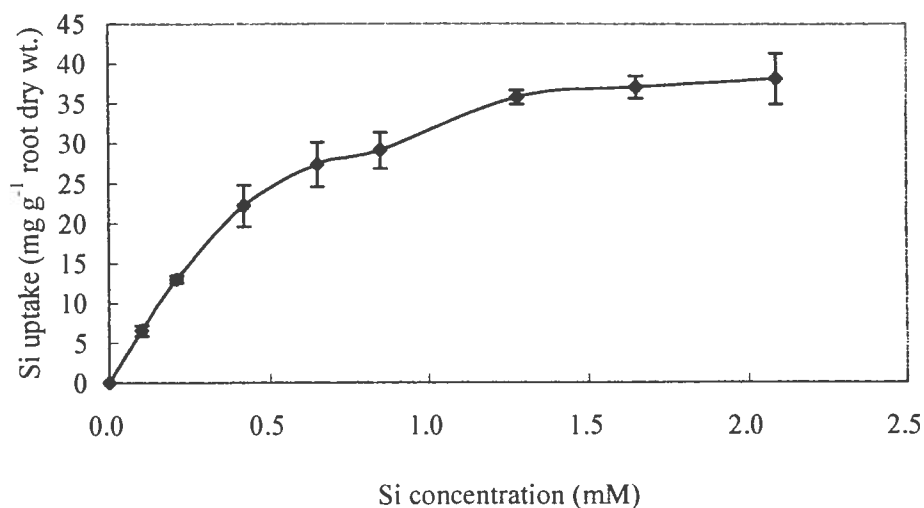


Figure 3. Si uptake by rice roots from a solution with various Si concentrations. The seedlings were cultured for 6 hours.

4. Effect of boric acid on Si uptake

Boron is taken up by the roots also in the form of an undissociated molecule, boric acid. There is a possibility that B is taken up by the same transporter as Si. To examine this possibility, the Si uptake was examined in the presence of various B concentrations. As shown in Fig. 4, the Si uptake was not affected by the presence of B. It is unlikely that the same transporter works for B and Si.

Recently, the B uptake has been reported to be transported across the plasma membrane through aquaporins from a series of inhibitor experiment in squash (Dordas and Brown, 2001). The effect of channel inhibitors such as HgCl₂, phloretin, and DIDS on the Si uptake by rice roots is under investigation.

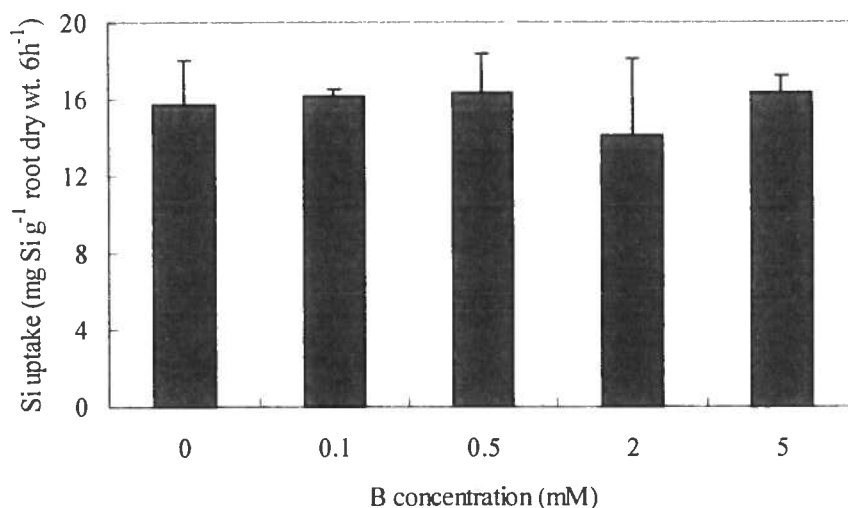


Figure 4. Effect of B as boric acid on the uptake of Si by rice roots. Seedlings (16-d old) were allowed to take up Si for 6 hours from a solution containing 0.75 mM Si as silicic acid in the presence of various B concentrations.

Reference

Dordas, C. and Brown, P. H. 2001. Evidence for channel mediated transport of boric acid in aquash (*Cucurbita pepo*)

Comparison of Silicon Uptake Characteristics between Two Cultivars of Pumpkin (*Curcubita moschata* Duch)

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INTRODUCTION

Cucumber in Japan is usually produced by grafting onto pumpkin stocks. It has been reported that the occurrence of a white powder of silica (blooms) on the cucumber fruit is mainly affected by the type of stock used, and the shoot Si concentrations of cucumber grafted onto the bloom-type stocks are higher than those grafted onto the bloomless-type stocks (Yamanaka and Sakata 1993, Iwasaki and Matsumura 1999). However, the reason for this difference in Si uptake by the roots of these pumpkin cultivars is still unknown. In this study, to clarify the physiological reasons for these differences, Si uptake from solutions of different Si concentrations by both cultivars and Si concentrations in the apoplastic and symplastic sap extracted from the root tips were examined.

MATERIALS AND METHODS

Two cultivars of pumpkin (*Curcubita moschata* Duch) plants for stocks, Shintosa (ST) and Super unryu (SU), were used for the following experiments. The ST cultivar has been used as a bloom-type stock while SU is used as a bloomless-type stock. All the experiments were conducted under greenhouse conditions.

Exp. 1: Silicon uptake from solutions of different Si concentrations. The seeds of both cultivars were soaked in tap water overnight and then sown on trays containing vermiculite. The seedlings were transferred to plastic pots (1 seedling per pot with a volume of 1.0 L) with continuously aerated half-strength Hoagland nutrient solution (pH 5.5) without addition of Si. The solution was replaced with fresh solution once a week. After 14 days of pre-culture, plants were treated with the nutrient solution containing 0.1, 0.2, 0.5, or 1.0 mM Si for 3 days. During treatment, the decrease in the volume of the nutrient solution in each pot was measured every day, which was regarded as the volume of water used by plant transpiration. A small portion of the nutrient solution was subjected to determination of Si after filling up to 1.0 L with distilled water. Based on this Si concentration and the decrease in volume of the nutrient solution until the next day, the amount of Si supplied to the roots by transpiration (mass flow) over 24 hours was estimated. The Si levels in the solution and plant samples were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, Shimadzu ICPS 1000IV).

Exp. 2: Apoplastic and symplastic Si concentrations within the root tips. Both cultivars were pre-cultured for 7 days without Si by the same method as described above, except that plastic pots with a volume of 3.5 L were used for each seedling. The plants were treated with nutrient solution containing 0.5, 1.0 or 1.5 mM Si for 25 days. The nutrient solution was exchanged once a week during the treatment. After treatment, the roots were harvested and 2 cm tips of the tap roots were excised. The apoplastic and symplastic saps were collected by centrifugation, according to the method described by Yu *et al.* (1999) with some modifications. Briefly, about 25 root tips from one plant were arranged in a filter unit (Millipore Ultrafree-MC, 0.45 μ m) with the cut end facing down, and the apoplastic sap was collected by centrifugation at 3,000 g at 4°C for 15 min. The symplastic sap was prepared from the frozen-thawed root tissues after collection of apoplastic sap by

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centrifugation at 3,000 g at 4°C for 15 min. Subsequently, Si remaining in the root residue was extracted with a mixture of 1.0 M HCl and 2.3 M HF (1:2). For determination of the apoplastic and symplastic Si concentrations, polymerized Si was digested with the addition of 5 M HCl and 11.5 M HF (1:2). Silicon concentration in each sample was determined by a colorimetric method (van der Vorm 1987).

RESULTS AND DISCUSSION

1. Silicon uptake from solutions of different Si concentrations.

The concentrations of Si in shoots of the ST cultivar were significantly higher than those of the SU cultivar, and the difference between the two cultivars was more pronounced with increases in Si level. On the other hand, Si concentrations in the roots of the SU cultivar were slightly lower than those of the ST cultivar (Fig. 1A).

Based on the estimated amounts of Si supplied by transpirational water flow (mass flow) during the 3 days of the experiment and the Si contents in the roots and shoots of both cultivars, a schematic representation of Si flow from the root medium to the roots and shoots at 0.5 mM Si level is shown in Fig. 1B. The estimated amounts of Si supplied around the roots by mass flow were higher in the ST cultivar than in the SU cultivar. In the case of the ST cultivar, about 73% of Si supplied around the roots by mass flow was absorbed by the plants and more than 95% of the absorbed Si was distributed in the shoots. On the other hand, only 7% of the estimated amount of Si supplied around the roots by mass flow was absorbed by the SU cultivar and about 60% of the absorbed Si was translocated to the shoots.

These results clearly showed that in the case of the SU cultivar, the large amounts of Si which delivered by

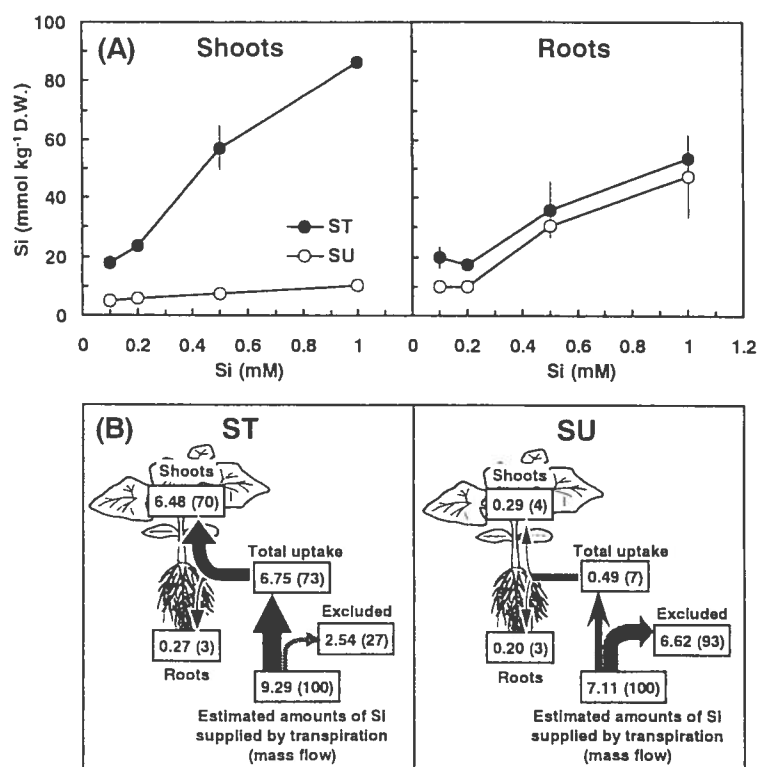


Figure 1. (A) Si contents in the shoots and roots of two cultivars of pumpkin after treatment with solutions containing different concentrations of Si for 3 days. Vertical bars indicate standard deviations of the means of 3 replicates. (B) Schematic representation of Si flow from the root medium to the roots and shoots (mg Si). The data on the plants treated at 0.5 mM Si for 3 days were used (See Materials and methods). Values in parentheses indicate the relative distribution of Si for each cultivar when the estimated amounts of Si supplied by mass flow were taken as 100%.

mass flow were excluded before entering into the flow from the roots to shoots. Therefore, it was considered that the low ability of Si uptake by the roots of the SU cultivar was the major factor responsible for the low Si contents in the shoots, in addition to the smaller amounts of Si supplied by transpirational water flow and the lower translocation of Si from the roots to the shoots compared with the ST cultivar.

2. Apoplastic and symplastic Si concentrations within the root tips.

In the case of the ST cultivar, the apoplastic Si concentration was not markedly different from the initial external solution, and the symplastic Si concentration was significantly higher than the apoplastic Si concentration at all Si levels examined (Fig. 2A). On the other hand, the apoplastic Si concentration of the SU cultivar at 1.5 mM Si was markedly higher than the initial external solution. With the exception of the 0.5 mM Si solution, the apoplastic Si concentration was significantly higher than the symplastic Si concentration at the same Si level. The symplastic Si concentration of the SU cultivar was lower than the initial external solution at all Si levels examined. Consequently, the symplastic Si concentration of the SU cultivar was significantly lower than that of the ST cultivar at all Si levels examined, whereas the apoplastic Si concentration was significantly higher than that of the ST cultivar at 1.5 mM Si. These results indicated that the higher capacity to retain Si in the root symplast of the ST cultivar seemed to induce translocation of larger amounts of Si from the roots to shoots compared with the SU cultivar.

The amounts of Si retained by the root residue of the SU cultivar were significantly greater than those of the ST cultivar at all Si levels examined, and this tendency was more marked at higher Si levels (Fig. 2B). At all Si levels examined, about 50% of the total Si within the root tip of the ST cultivar was found in the symplast, while more than 80% was associated with the root residue in the case of the SU cultivar. The amounts of residual Si in the SU cultivar increased significantly at Si levels of 1.0 and 1.5 mM compared with 0.5 mM Si. As a result, the total content of Si within the root tips of the SU cultivar was higher than that of the

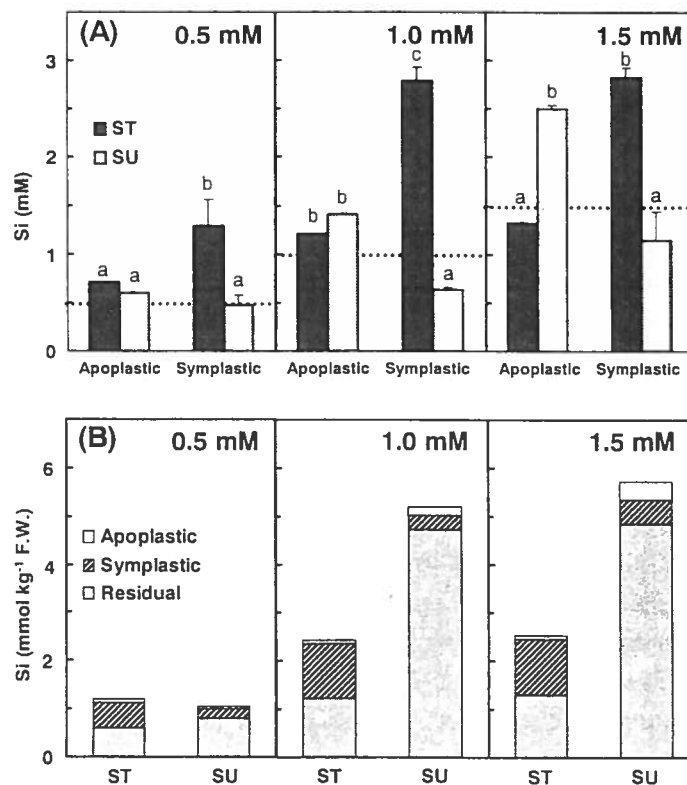


Figure 2. (A) Apoplastic and symplastic Si concentrations within the root tips of two cultivars of pumpkin. Dotted lines indicate the Si concentration in the fresh nutrient solution of each treatment. (B) The contents of Si within the root tips of two cultivars of pumpkin.

ST cultivar at 1.0 and 1.5 mM Si. However, in Exp. 1 the Si concentrations in the roots of the SU cultivar were slightly lower than those of the ST cultivar at 1.0 mM Si (Fig. 1A). This discrepancy was probably due to the long uptake period of 25 days in Exp. 2. As the observed apoplastic Si concentrations of the SU cultivar at 1.0 and 1.5 mM Si were almost equivalent to or higher than the saturated concentration of Si, some of the apoplastic Si would precipitate on the root surface during treatment.

In summary, the present study suggested that a higher capacity to retain Si in the root symplast of the ST cultivar was responsible for the higher Si contents in the shoots as compared with the SU cultivar. The transmembrane flux of Si into the symplast was thought to be restricted in the case of the SU cultivar. This might be one reason why the Si delivered by mass flow was largely excluded before entering into the flow from the roots to shoots (Exp. 1). On the other hand, the accumulation of Si was evident in the root apoplast of the SU cultivar, especially at high Si levels (Exp. 2). These results could be explained by the precipitation of the excluded Si from the symplast on the surface of the cell walls. However, it is possible that suberized cell walls may act as an apoplastic barrier to Si movement from the root medium to the xylem. Further studies will be needed to clarify the reasons for the low capacity to retain Si in the root symplast of the SU cultivar and for the poor Si transfer from the root apoplast into the xylem.

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Silicate Accumulation in Aging Leaves of Dicotyledonous Canopy Trees: Effects of Phylogeny, Climate and Soil Silicate Availability

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BACKGROUND

Woody dicotyledonous species are known to accumulate silicon (Si) to various degrees. In temperate dicot tree species, SiO_2 concentrations in old leaves are typically less than 10 mg/g dry mass and never more than 38 mg/g. Here, I report that some tropical dicot trees accumulate silicate to much higher concentrations up to 230 mg/g, and summarize some findings from my recent comparative studies that explored phylogenetic, ecological and physiological correlates of species differences in silicate accumulation in leaves of tropical and temperate dicot trees.

Study 1. Ash and silicate accumulation in seven dicot tree species in a neotropical dry forest.

In seven canopy tree species in a seasonal dry forest in Panama, Central America, silicate and mineral ash accumulated linearly with leaf aging. Species differed widely in ash accumulation rates; mineral ash contents in mature senesced leaves ranged from 100 to 300 mg/g leaf dry mass among species. In high ash accumulating species in Urticaceae and Moraceae, which are known as producers of opal phytolith, 30-60% of ash in old leaves was accounted by acid-insoluble SiO_2 that was extractable with alkaline solution (Table 1). *Urera caracasana* was the only species that also contained detectable concentrations of non-structured silicate that was spontaneously soluble. Silicate contents (mg SiO_2 per g leaf dry mass) in these species were much higher than the published values for live and dead leaves of any temperate dicot trees (Table 1). Calcium (CaO) also accumulated with leaf age (12-16% of mineral ash in old leaves), but species differences in Ca concentrations were independent of Si concentrations (Table 1). Potassium (K_2O) was the third major component of mineral ash, but it did not accumulate with leaf age. Proportional abundance of silicate in ash increased with leaf age in silicate accumulating taxa. Ash and silicate concentrations in old leaves were positively correlated with ash and silicate concentrations in young leaves. Thus, silicate accumulated during leaf expansion and continued throughout the leaf lifespan at species-specific rates.

Table 1. Seven canopy tree species studied in a tropical dry forest at the Parque Natural Metropolitano, Republic of Panama. Also shown are abundance of phytolith (Doloris Piperno, personal communication) and concentrations (mg per gram dry mass) of ash, silicate (SiO_2), and calcium oxide (CaO) in mature old leaves.

Species	Family	Phytolith	Ash	SiO_2	CaO
<i>Urera caracasana</i>	Urticaceae	abundant	260.0	90.7	59.6
<i>Castilla elastica</i>	Moraceae	common	186.3	111.8	23.5
<i>Cecropia longipes</i>	Moraceae	common	160.2	53.8	39.0
<i>Anacardium excelsum</i>	Anacardiaceae	none	106.1	43.0	17.5
<i>Annona spraguei</i>	Annonaceae	rare	80.3	10.3	29.0
<i>Luehea seemannii</i>	Tiliaceae	none	75.5	8.0	24.3
<i>Antirrhoea trichantha</i>	Rubiaceae	none	64.9	2.7	21.4

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Study 2. Comparison of silicate accumulation in common tree species at two temperate and two tropical sites.

The main objective of this study was to examine whether leaves of tropical canopy trees accumulate more silicate than temperate trees *in situ*. Common tree species were chosen in two temperate and two tropical sites that also differed in soil types and silicate availability (Table 2). Because phylogeny strongly affects uptake and accumulation of silicate, care was taken to allow pair-wise comparisons of related species between tropical and temperate sites (e.g., temperate vs. tropical Moraceae species). At each site, 9-17 common woody species were chosen. Most of the study species were canopy trees but a few woody vines and shrubs were also included at each site. Three exposed branches were marked in each of 1-5 individuals of each species, and young fully expanded 1-3 leaves were sampled during the early growing season, (May-June, 2000). Canopy was accessed with 40-50 meter tall construction cranes in Panama, while they were accessible from the ground at the temperate sites. About 4-6 months later, I sampled old mature leaves closest to the position of the initially-sampled leaves in each branch. This sampling scheme of young and old leaves with a known difference in leaf ages allowed calculation of the Si accumulation rate per unit leaf area per unit time for each branch, as well as comparisons of maximum ash and Si values in old mature leaves. When leaves in the marked branches had been lost before the second sampling, senesced leaves that were ready to abscise in a nearby branches were sampled. Here I report only the results of the analysis of maximum ash and silicate concentration for each species, although Si accumulation per unit time may be a better correlate of the species differences in physiology (e.g., transpiration rates, regulation or silicate entry at the root endodermis, etc.).

Table 2. Site characteristics and mean (s.d.) soil silicate availability determined by acetic acid extraction (mg SiO₂/g air dry soil).

Site	Forest type	Soil type	Silicate availability
Parque Natural Metropolitano, Panama (PNM, 9° N)	50-100 year old seasonally dry secondary forest	Moderately weathered alfisol	0.466 (0.080)
Fort Sherman, Panama (FTS, 9° N)	Mature evergreen tropical rainforest	Highly weathered oxisol from sandstone bedrock	0.076 (0.027)
Archbold Biological Station, Florida, USA (ABS, 27° N)	Scrub dominated by evergreen shrubs and open pine flats.	Subtropical spodosols with high quartz (white sand) content	0.011 (0.010)
Institute of Ecosystem Studies, New York, USA (IES, 41° N)	Secondary mature temperate deciduous forests.	Slaty silt loam from glacial till derived from shale and slate	0.061 (0.05)

Overall, species mean SiO₂ content was positively correlated with ash content in old leaves. However, species differed greatly within and among sites. Across the biomes, species in certain families, such as Moraceae, Celtidaceae, Ulmaceae, were high silicate accumulators, Anacardiaceae, Annonaceae Malvaceae ssl. (including Tiliaceae, Bombacaceae) were moderate accumulators, and Lauraceae and Rubiaceae hardly accumulated Si. Within each phylogenetic branch, tropical taxa accumulated more Si. Difference among sites in ash and Si concentrations were significant ($P < 0.01$ and $P < 0.003$, respectively, with ANOVA after log-transformation to achieve normality). However, there was a large difference between the two tropical sites; mean leaf SiO₂ concentration was significantly higher at PNM with a higher soil Si availability than at FTS (Table 3). However, because these two tropical sites completely differed in species composition, it is not clear whether environment or species was largely responsible for the observed differences in leaf Si concentration between PNM and FTS.

Between the two temperate site, species at ABS (latitude 27° N) accumulated more Si in leaves than species

Table 3. Range of maximum ash and silicate content in old or senesced leaves for woody species sampled at each study site.

Site	Climate	Soil Si	No. spp.	Ash (mg/g)	SiO ₂ (mg/g)
PNM	Tropical	high	16	129 (65-276)	59 (3-229)
FTS	Tropical	low	16	94 (26-188)	31 (1-112)
ABS	Temperate	very low	9	68 (24-130)	25 (3-107)
IES	Temperate	low	17	91 (39-169)	12 (2-52)

at IES (latitude 41°N), even though soil Si availability was much lower at ABS than at IES. However, ash content was higher at IES because of higher Ca and K contents in many species at IES than at ABS.

QUESTIONS AND HYPOTHESES FOR FUTURE RESEARCH

Why do some tropical woody dicots accumulate more Si than their temperate relatives? Why do many primarily tropical taxa, such as Moraceae and Chrysobalanaceae, contain many species with extremely high Si accumulation? The results of the studies reported here suggest several hypotheses to be tested in future studies. 1) High silicate accumulation is a byproduct of high transpiration rates. This is likely because many tropical species that were found to be high Si accumulators are pioneer trees with high transpiration rates. 2) High silicate accumulation may reflect an adaptive mechanism to ameliorate aluminum toxicity, which is a more likely problem in highly weathered tropical soils. 3) Silicate is more important as defense against insect herbivory and fungal pathogens in tropical communities. 4) At the ecosystem level, rates of Si recycle between soil and biomass may be affected strongly by species composition, as high Si accumulators uptake soil solution Si and return it in less soluble form (= opal phytolith) in leaf litter. Conversely, distribution and abundance of high Si accumulating taxa may be constrained by the low soil Si availability in highly weathered soil. A global survey of Si availability in tropical soils may be useful for better understanding of tropical ecosystems, including both natural and agroforestry systems.

Silicon Stimulates Oat Leaf Growth by Modifying Cell Wall Properties

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INTRODUCTION

The beneficial effects of silicon on plant growth have been reported in a wide variety of crops including rice, oat, barley, wheat, cucumber, and annual brome (Epstein 1999). Especially, silicon improves plant resistance to abiotic and biotic stresses, thereby promoting plant growth (Epstein 1999). Recently, we reported that silicon at 5-10 mM stimulated growth of some Poaceae seedlings under conditions without stress by promoting cell expansion (Hossain *et al.* 2002). The rate of cell expansion is directly determined by the cellular osmotic potential and the mechanical properties of the cell wall (Cosgrove 1986). The present study was carried out to clarify the mechanism by which silicon promotes growth of Poaceae seedlings. For this purpose, we analyzed the osmotic concentration of the cell sap and the mechanical and chemical properties of the cell wall in oat second leaves.

MATERIALS AND METHODS

Caryopses of oat (*Avena sativa* L. cv. Victory) were soaked in distilled water for 1 h. They were germinated and grown in 10 mM silicic acid (H_4SiO_4) solution under dim red light for 42 h, and then in the dark at 25°C. Silicic acid solution was prepared by the method of Hossain *et al.* (2002) and the pH of the solution was adjusted to 5.5 with 5 mM MES-KOH buffer solution.

Second leaves of oat seedlings grown for 10 days were harvested and then boiled for 10 min in 80% ethanol. Segments of 10 mm were cut from the subapical, middle, and basal regions of the leaves. After the ethanol-fixed leaf segments were rehydrated, the mechanical extensibility of the cell wall of the segments was measured with a tensile tester as previously described (Hossain *et al.* 2002).

Second leaves grown for 10 days were harvested and then 30 mm-long segments were cut from the subapical, middle, and basal regions of the leaves. The cell sap was collected from frozen-thawed segments by the centrifugation at 1000 g for 10 min at 4°C (Parvez *et al.* 1996). The osmotic concentration of the collected cell sap was measured with a vapor pressure osmometer (Model 5500, Wescor Inc.).

After measuring the mechanical properties of the cell wall, the leaf segments were homogenized and the cell wall materials were prepared. The cell wall polysaccharides were fractionated into two fractions (hemicellulose and cellulose) according to the method of Wakabayashi *et al.* (1997). Sugar content in each fraction was determined by the phenol-sulfuric acid method and expressed as glucose equivalents. The molecular mass of hemicellulosic polysaccharides was determined using an HPLC-gel permeation chromatography system, as reported by Soga *et al.* (1999).

RESULTS AND DISCUSSION

Figure 1 shows the effect of silicon at 10 mM on growth of oat second leaves. Growth promotion by silicon

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was first observed 8 days after imbibition, and the difference in length between control and silicon-treated leaves increased gradually with seedling growth. At the 12th day, promotion of growth reached about 50% over control. This result indicates that silicon clearly promotes growth of oat second leaves under conditions without stress.

To clarify the mechanism of silicon-induced expansion growth in oat second leaves, we measured the cell wall extensibility and the osmotic concentration of the cell sap in the various regions of oat second leaves after 10 days of culture (Fig. 2). The basal regions showed a high cell wall extensibility and silicon treatment increased it about 50% over control. In the middle and subapical regions, the cell wall extensibility was fairly lower than that in the basal regions, and silicon treatment did not affect the extensibility in these regions. These results suggest that silicon stimulates growth of oat second leaves by increasing the cell wall extensibility, particularly in the basal regions, which were shown to be the major site of cell expansion and also of silicon action (Hossain *et al.* 2002). On the other hand, the osmotic concentration of the cell sap slightly decreased

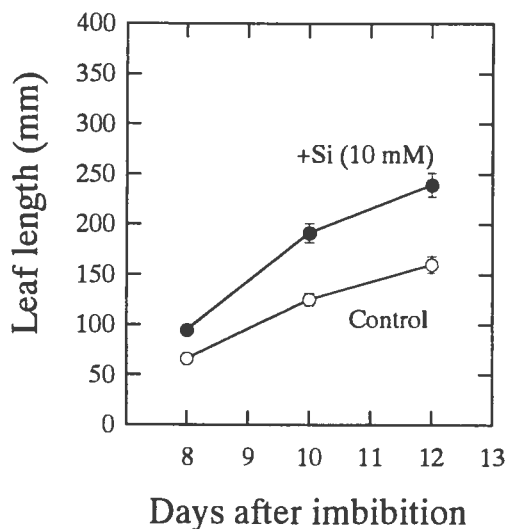


Fig. 1. Time course of growth of oat second leaves in the presence or absence of silicon. Imbibed oat caryopses were incubated in 10 mM silicon (H_4SiO_4) solution. The pH of the solution was adjusted to 5.5 by using 5 mM MES-KOH buffer solution. Data are means \pm SE ($n=20$).

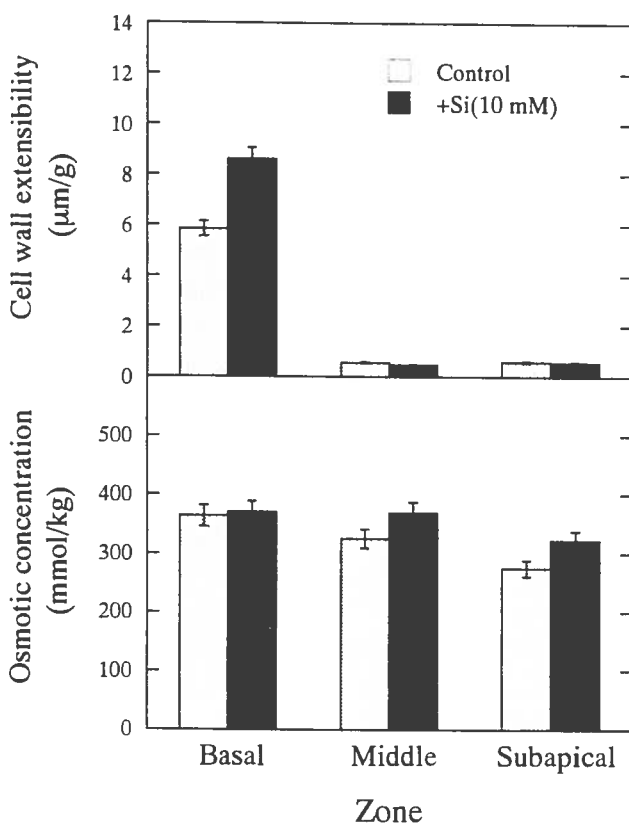


Fig. 2. Effects of silicon on the cell wall extensibility and the osmotic concentration in the subapical, middle, and basal regions of oat second leaves. Oat seedlings were grown under conditions shown in Fig. 1 for 10 days. Data are means \pm SE ($n=30$ and 3 for the determination of the cell wall extensibility and the osmotic concentration, respectively).

from the basal to the subapical regions (Fig. 2). Silicon treatment did not significantly affect the osmotic concentration in any region, suggesting that the osmotic concentration in the cell sap is not directly involved in the silicon-induced cell elongation process.

Figure 3 shows the amounts of hemicellulose and cellulose and the molecular mass of hemicellulosic polysaccharides in the basal regions of oat second leaves after 10 days of culture. Physico-chemical properties of cell wall polysaccharides, such as levels and molecular mass of polysaccharides, are shown to determine the mechanical properties of the cell wall. Silicon treatment significantly reduced the amounts of hemicellulose and cellulose per unit length, suggesting that silicon decreased the thickness of the cell wall in the basal regions of second leaves. However, silicon did not affect the molecular mass of hemicellulosic polysaccharides. The thickness of the cell wall is considered to be one of factors determining the mechanical extensibility of the cell wall in seedlings grown under hypergravity conditions (Soga *et al.* 1999). Also, Tsurumi *et al.* (1996) showed that chromosaponin increased the mechanical extensibility of the cell wall in the elongating regions of lettuce roots by decreasing the thickness of the cell wall in the regions, when it stimulated root growth. The results suggest that the thinning of the cell wall is involved in the increase in the cell wall extensibility in the basal regions of oat second leaves by silicon treatment.

In summary, silicon decreases the thickness of the cell wall in the basal regions of oat second leaves, which, in turn, may cause an increase in the cell wall extensibility, thereby promoting whole-leaf growth under conditions without stress.

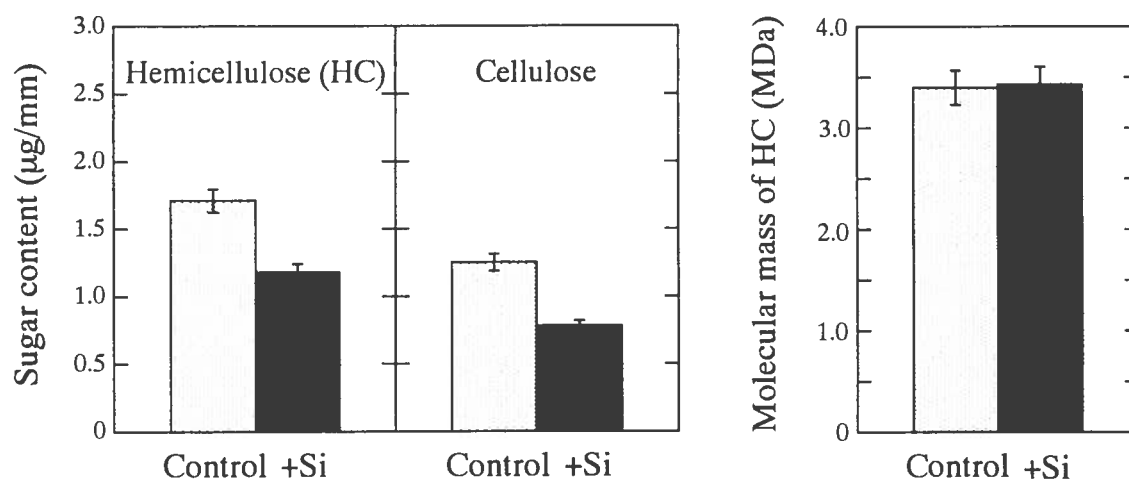


Fig. 3. Effects of silicon on the amounts of hemicellulose and cellulose and on the molecular mass of hemicellulosic polysaccharides in basal regions of oat second leaves. Oat seedlings were grown under conditions shown in Fig. 1 for 10 days. Data are means \pm SE (n=3).

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The Aqueous Chemistry of Organosilicate Complexes

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In aqueous solution, silicon exists primarily in four fold, tetrahedral coordination with oxygen, often as monomeric silicic acid, SiO_2H_4 , or higher polymers thereof. How such a simple molecule interacts with living systems is unclear, since organosilicates have been conspicuous in nature by their almost total absence. Using ^{29}Si NMR spectroscopy we show that aqueous silicates are indeed capable of interacting with carbon compounds, giving rise to a variety of organosilicates in which silicon can be in either four, five or six fold coordination with oxygen (1). For example, many types of **aliphatic mono-** and **polyhydroxy alcohols** can combine with silicate anions to form alkoxy substituted silicon (IV) complexes. The complexing ability is greater for smaller alcohols, and increases with the number of attached hydroxy groups. Thus simple polyols containing a pair of hydroxy groups in *threo* configuration, such as **threitol**, **xylitol** and **sorbitol**, bind so tightly to silicon that they yield organosilicates in which silicon is either **five** or **six coordinate**. Aliphatic polyol chains which also contain a terminal carboxylic acid group, like **gluconic**, **saccharic** or **glucoheptonic acid**, give even more stable penta- or hexavalent organosilicates. Many cyclic sugars, both **pyranosic** and **furanosic**, also readily yield penta- and hexavalent organosilicates. Since all these organic molecules are common in nature, it is tempting to speculate that any naturally occurring organosilicates are likely to be based on species containing either penta- or hexavalent silicon sites.

Nature is rich in organosilicates.

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Selective Uptake of Silicate Minerals by Plants

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INTRODUCTION

Rare earth elements are a series of lanthanide elements whose atomic numbers are from 57 to 71. Their oxides are stable and REEs occur most abundantly in the form of oxide in silicate rocks. REEs have very similar chemical behaviours. They systematically increase in the number of f-electrons with atomic number within the lanthanide suite that causes chemical differences from La through Lu. REEs tend to be present as a group and slight variation of REE abundance can record subtle process in natural systems. By benefit of these properties, geologists and geochemists have been using REE abundance as a useful tracer.

For easier comparison of REE abundance, the concentrations of REEs in a sample were normalized to chondrite and the relative concentrations are plotted in a logarithmic scale against atomic numbers. The plot is called REE patterns. Most of naturally-occurring samples show simple characteristic patterns. Silicate minerals in soil are originated from rocks. Rocks normally show atypical monotonously-decreasing abundance pattern.

In this study, REE abundance in plants was studied to understand the source of REEs, and indirectly silicon in plants, based on the tight linkage between REEs and silicon.

MATERIALS AND METHODS

Soil-grown plants and soils

Six species of plant including three dicotyledons (*Populus sieboldii*, *Thea sinensis* and *Vicia villosa*), one monocotyledon (*Sasa nipponica*), one gymnosperm (*Taxodium japonicum*) and one pteridophyte (*Matteuccia*) were sampled in the campus and accessory experimental fields of Tokyo University of Agriculture and Technology. They range from an active Si uptaker, moderate accumulator, to an excluder. Each sample was divided into organs such as leaves, stem (or trunk) and root.

Soil on which each sample grew was sampled on the same day of each plant sampling. All soils are andosol. The soil was dried at 105°C.

Seaweed and seawater

Five species of seaweed (*Ecklonia cava*, *Delisea fimbriata*, *Ptilonia okadai*, *Ulva fasciata* and *Codium fragile*) were sampled along the coast of Aburatsubo Bay, Japan. Each sample was washed separately in a plastic beaker with Milli-Q water. Each of the samples was dried under 105°C.

Surface seawaters were sampled around the sites of seaweed sampling. Each seawater sample was filtered through a 0.4 µm Nuclepore filter immediately after sampling. pH of filtered seawater was adjusted to about 2 with an HNO₃ solution and was stored in a clean plastic tank. Several pieces of filter on which suspended particles were collected were soaked in a HNO₃ solution for 2 days and the residue was dried and stored.

Other filters were dried without acid soaking.

Analysis of REEs and Si

Each sample was ashed at 700°C and remaining ash was dissolved with an HNO₃ acid. The REEs in the solution were preconcentrated with solvent extraction using HDEHP and H₂MEHP as chelating agents. REEs in seawater were preconcentrated by a C₁₈ cartridge loaded with a mixture of HDEHP and H₂MEHP. The REEs collected in the cartridge was stripped by an HCl solution. REEs were determined by ICP-MS.

Ashed sample was fused with sodium carbonate in a platinum crucible. The fused cake was dissolved by acids and Si was determined with ICP-AES.

RESULTS AND DISCUSSION

Soil-grown plants

The REE patterns of each plant sample and each soil fraction are shown in Fig. 1 and 2. From the REE patterns some features are evident. Firstly, two upward concave curves (Gd-Tb-Dy-Ho, Er-Tm-Yb-Lu) in REE patterns of plant samples were observed. This is called the 'tetrad effect'. Secondary aberration of Ce from lines can be observed in the REE patterns of most plant samples. Thirdly, REE patterns of plant species resemble each other, but the slope of pattern became steeper generally in order of secondary roots, main root and leaf or trunk (stem). From Figure 2, the silicate fraction from each soil showed similar REE patterns independent of location, but the soluble fraction of soil showed different concentrations and REE patterns depending on location. The soil samples for *Sasa nipponica* and *Taxodium japonicum* were collected from the same area and soluble fraction of soil for the two soils gave almost identical patterns. REE patterns of the soil-grown plant samples are shown in Fig. 1. Ce has a different valency (+4) under oxidizing condition from that of the other REEs (+3) and the anomalous behavior of Ce is commonly observed in natural samples.

Unlike the concentrations, the REE patterns did not vary and seemed to be independent of species and location. The REE patterns of secondary root were very similar to each other in slope and overall variation of curve except for Ce. This observation seems to imply that REEs in plants are originated from silicate minerals in soil, which also indicates the silicon in plants must also be originated from silicate minerals.

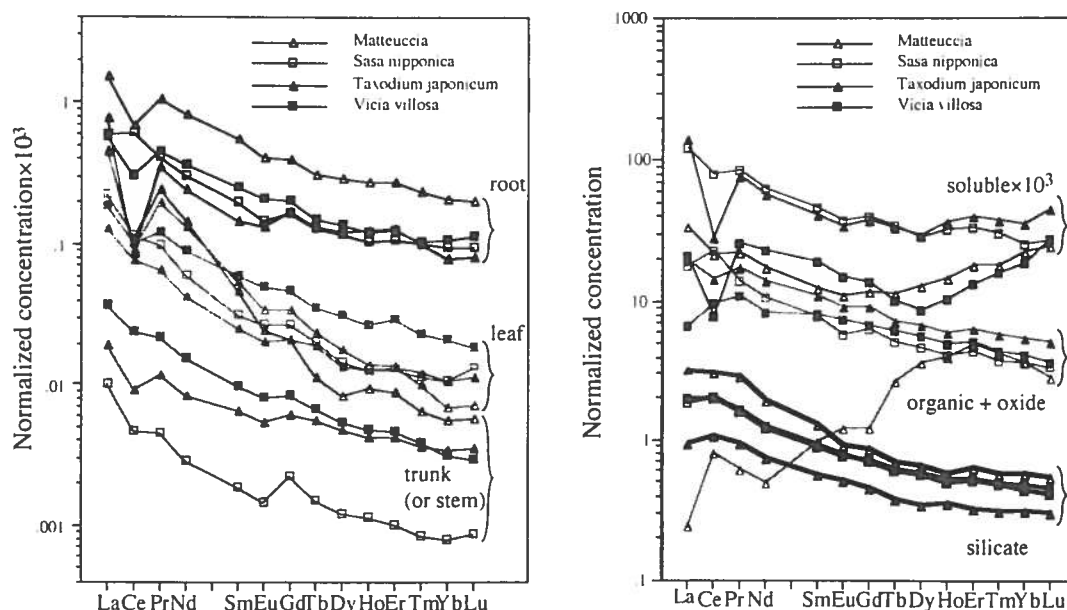


Figure 1. REE patterns of four species of plants and different fraction of soils sampled from the grounds where the plants grew.

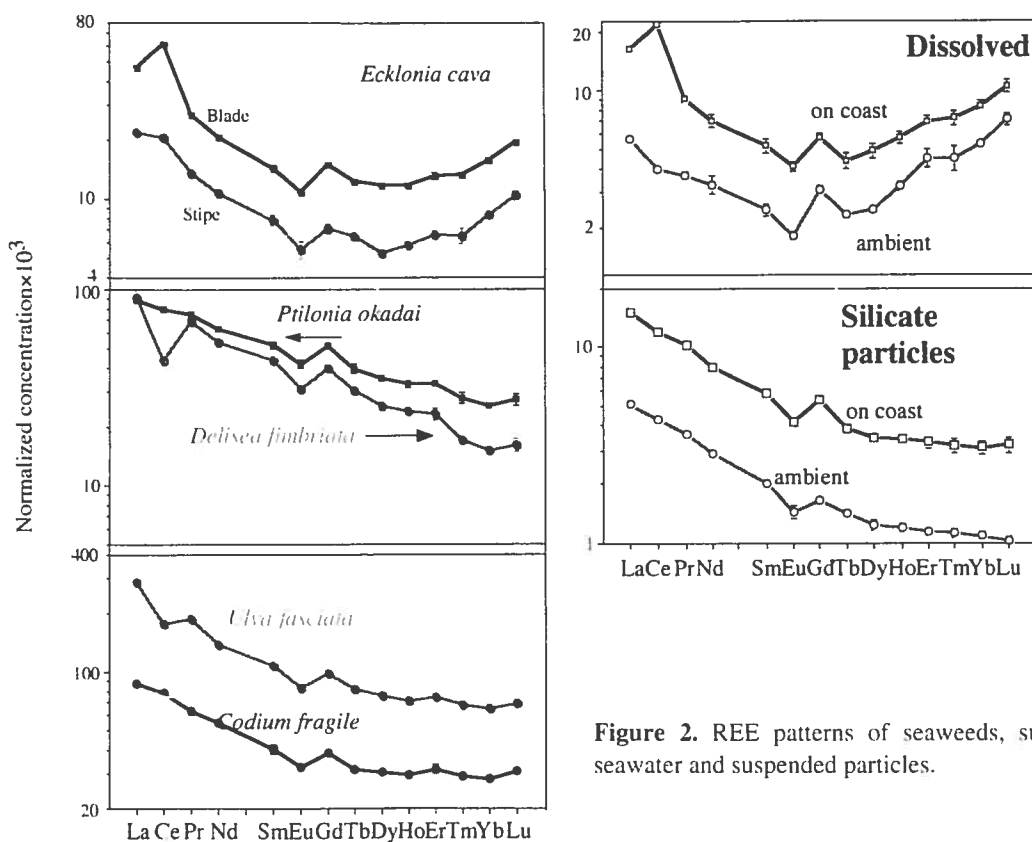


Figure 2. REE patterns of seaweeds, surface seawater and suspended particles.

Seaweed

The REE patterns of seaweeds are evidently different from those of the soil-grown plants, while the aberration of Ce from lines and two upward concave curves (Gd-Tb-Dy-Ho, Er-Tm-Yb-Lu) are seen in common with the soil-grown plants. Two types of different REE patterns are observed in seaweeds: one is similar to that of seawater (*E. cava*), and another is rather similar to that of suspended particles in seawater (*D. fimbriata*, *P. okadai*, *U. fasciata*, *C. fragile*). A clear relationship between REE patterns and Si concentration in seaweeds can be found. *Ecklonia cava*, whose REE patterns was the most similar to that of seawater, had very low concentration of Si, while *D. fimbriata*, whose REE pattern was the most similar to that of suspended particles, showed the highest concentration of Si.

The relationship between REE pattern and Si concentration in seaweeds could be explained if solid silicate particles serve as one of the source of Si. We assumed that seaweeds have two sources of REEs, specifically seawater and silicate particles. The contribution proportion of these two sources was estimated by comparing the overall variation of REE pattern of seaweed with that composed of the patterns of the two sources at a different weight. The relationship between the proportion and abundance of Si is shown in F.-F. Fu et al. (2000). Silicon concentration becomes greater with the increase of the contribution proportion of silicate particles. The linear relationship between the contribution proportion value and Si concentration implies that seaweeds obtain REEs not only from seawater solution but also from solid silicate particles. The proportion is higher for green and red algae and the lowest for brown algae.

Generally, seaweed samples may be contaminated with silicate particles on their surface. Silicate particles are enriched in REEs. This contamination would confound our results, because it also could explain our experimental results. Several lines of evidence itemized below suggest that the contamination was negligible.

1. Length of sonification time in the washing procedure did not influence either the overall variation of REE pattern or the Si concentration.
2. The REE patterns of seaweeds showed two upward concave sections (Gd-Tb-Dy-Ho and Er-Tm-Yb-Lu), which has been attributed to the tetrad effect. The contamination from silicate particles would yield only a

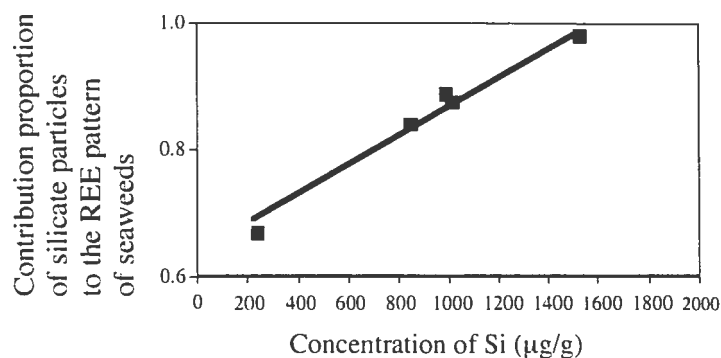


Figure 3. The relationship between the proportion of REEs originating from silicate particles and abundance of Si in seaweeds.

flat pattern with respect to the tetrad effect.

3. The use of HF in the digestion procedure made no difference in the analytical results of REEs.

CONCLUSION

The relationship between the shape of REE patterns and Si concentration of seaweeds indicated that silicate minerals could be absorbed rather directly from their blade. Soil-grown plants shows common REE patterns especially for the roots, independent of places and soils. This suggests that the soil-grown plants might absorb silicon from silicate minerals from the roots, the same as seaweeds do from blades.

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Difference in Silicon Concentration in Leaves among Tree Species : Implication for Supplement of Mechanical Strength

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INTRODUCTION

It has not been accepted yet that silicon is essential to plants, although the benefit of Si to plants has been acknowledged widely. Some plants, such as rice, absorb large amounts, and Si has been considered as an essential element for these plants.¹⁾ The biochemical role of Si in plant tissues has not been elucidated. Si is rather inactive physiologically and the beneficial effect seems to appear through the physical and mechanical properties of SiO_2 . It is reported that if a plant is short of Si, the weakness of its structure causes the deterioration of plant growth and the plant may suffer from disease and/or to be attacked by insects.²⁾ It has been also reported that Si mitigates the toxicity of aluminum.³⁾ Studies of Si benefits have focused on agricultural productivity of plants such as rice. Because experiments controlling Si availability are impossible in mature trees, the benefits of Si to trees is not known. From recent studies using rare earth elements as a proxy of the origin of Si, many plants, including trees absorb Si not in soil water but in silicate particles.⁴⁾ It was also reported that the weathering rate of rock was enhanced by existence of trees.⁵⁾ These studies imply that trees might benefit by absorbing Si. In the case of trees Si is most accumulated in leaves without exception. In this study the relationship between the concentration of Si in leaves and the mechanical strength of leaves has been explored.

MATERIALS

The leaves of 29 tree species (dicotyledons), which were growing on the campus of Tokyo University of Agriculture and Technology, agricultural department (Fuchu, Tokyo, Japan) were sampled in September 2001. Leaves of similar size were sampled from a tree. The number of sampled leaves from a tree varied depending on the leaf weight. Family and species of trees sampled in this study are listed Table 1. Leaves were classified into four types (see Table 1, A or standard-shape, B or spade-shape, C or hart-shape and D or crown-shape) by their shapes as shown in Figure 1.

Surface area of leaves, thickness of central venation, thickness of blades and dry weight were measured. Approximate tree height was also measured (Table 1). The thickness of central venation was measured at the one third length from the bottom of a leaf. The thickness of blade was measured at the both sides of the position of central venation where the thickness had been measured. Leaves were washed with water by using a plastic sponge and were washed with distilled water. They were dried in aluminum wraps for three days at 80°C and dry weight was measured. The dried leaves were cut into size of 1cm by 1cm carefully with scissors and tweezers. After being

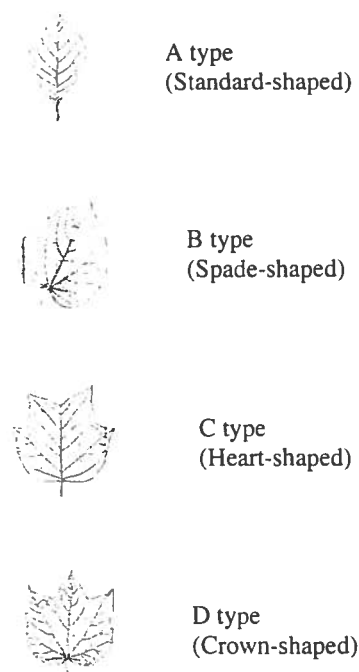


Figure 1.

Table 1. Measurement data

Family	Species	Number of leaves	Leaf area (cm ²) ¹⁾	Thickness of central venation (mm) ¹⁾	Thickness of blade (mm) ¹⁾	Dry weight (g) ¹⁾	Height (m)	Leaf type ²⁾
Magnoliales	<i>Liriodendron tulipifera</i>	10	319	2.4	0.2	1.84	10	C
	<i>Magnolia obovata</i>	10	305	3.2	0.3	1.94	10	A
	<i>Magnolia Kobus</i>	10	112	1.7	0.2	0.43	8	A
Bignoniaceae	<i>Catalpa ovata</i>	10	276	2.7	0.2	1.28	11	B
	<i>Paulownia tomentosa</i>	10	289	2.8	0.2	1.67	12	B
Flacourtiaceae	<i>Idesia polycarpa</i>	10	154	1.3	0.2	0.54	10	B
Alangiaceae	<i>Alangium platanifolium</i>	10	191	1.7	0.2	0.49	10	D
Saxifragaceae	<i>Hydrangea macrophylla</i>	10	128	1.6	0.2	0.34	1	A
Cercidiphyllaceae	<i>Cercidiphyllum japonicum</i>	15	51	0.6	0.2	0.23	12	B
Hamamelidaceae	<i>Corylopsis pauciflora</i>	25	15	0.5	0.2	0.06	2	B
Styracaceae	<i>Styrax japonica</i>	20	27	0.7	0.1	0.12	8	A
Celastraceae	<i>Euonymus japonica</i>	20	15	1.2	0.4	0.17	1	A
Pittosporaceae	<i>Pittosporum Tobira</i>	10	34	1.2	0.3	0.27	1.5	A
Cornaceae	<i>Aucuba japonica</i>	10	65	1.6	0.3	0.41	1.5	A
Rubiaceae	<i>Gardenia jasminoides</i>	10	42	0.9	0.2	0.21	1.5	A
Aquifoliaceae	<i>Ilex latifolia</i>	10	77	2	0.4	1.06	8	A
	<i>Ilex integra</i>	10	21	0.8	0.3	0.2	8	A
Oleaceae	<i>Osmanthus fragrans</i>	10	27	1.1	0.3	0.32	5	A
	<i>Ligustrum japonicum</i>	10	19	0.9	0.4	0.21	5	A
	<i>Ligustrum lucidum</i>	20	19	0.7	0.2	0.1	2	A
Thymelaeaceae	<i>Daphne odra</i>	20	11	1.1	0.5	0.11	0.5	A
Berberidaceae	<i>Nandina domestica</i>	30	7	0.6	0.2	0.05	1.5	A
Ericaceae	<i>Rhododendron pulchrum</i>	30	10	0.8	0.2	0.06	1.5	A
	<i>Pieris japonica</i>	30	8	0.5	0.3	0.07	2	A
Theaceae	<i>Camellia japonica</i>	10	10	0.6	0.3	0.1	2	A
	<i>Cleyera ochracea</i>	10	27	0.9	0.3	0.27	2	A
	<i>Ternstroemia japonica</i>	15	25	1	0.4	0.25	3	A
	<i>Eurya japonica</i>	25	17	1	0.5	0.15	2	A
	<i>Camellia Sasanqua</i>	25	10	0.6	0.2	0.08	3	A

1) Average date for the leaves sampled.

2) A, B, C, D are referred to in Fig. 1.

homogenized a part of the sample was analysed.

DETERMINATION OF SI IN PLANT SAMPLES

About 0.4 g of each plant sample was placed in a platinum crucible and was ashed at 700°C for 1h. The remaining ash was blended with 1.0 g of anhydrous sodium carbonate. Then the mixture was heated at 850°C for 30 min in order to fuse the ash with sodium carbonate. After cooling to room temperature, the fused matter was dissolved with 5 ml of 1 N HCl. The solution was then transferred to a Teflon beaker and was evaporated to dryness by heating it slowly. The residue was dissolved again with 15 ml of 0.2 N HCl. Si in the solution was determined with ICP-AES after ten times dilution.

RESULTS AND DISCUSSION

The reason for the accumulation of Si in tree leaves

It was clearly seen that the concentration of Si in larger leaves was higher (Table 1). For the greater concentration of Si in larger leaves, three reasons can be considered. (1) Si was passively absorbed from roots in the transpiration stream. (2) Si might have been physiologically incorporated in order to mitigate the toxicity of the aluminum. (3) On the analogy of rice, Si in trees was incorporated to supplement the mechanical strength of a leaf. The validities of the three reasons were examined here each by each. (1) It could be assumed that the total amount of absorbed Si in the leaf was "the concentration of Si in liquid in xylem sap"

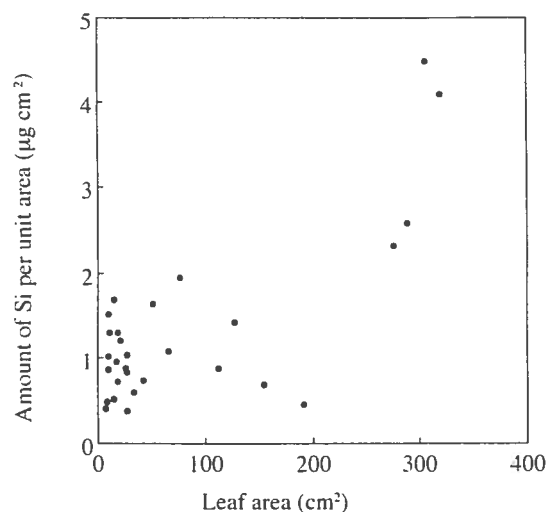


Figure 2. Correlation between leaf area and the amount of Si per unit area.

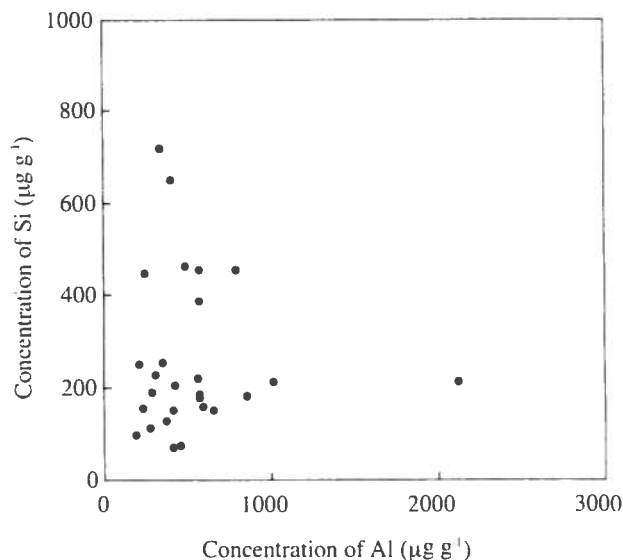


Figure 3. Concentrations of Si v.s. Al in leaves.

× “transpiration”. Transpiration generally increases as leaf area becomes larger. Transpiration rate per unit area, however, was reported to decrease with increasing size of leaves.⁶⁾ Results showed that the amount of Si per unit area increased with the increasing size of leaves (Fig. 2). Therefore the reason (1) is incompatible with the observation. (2) Si could be absorbed actively to mitigate the toxicity of aluminum. However the correlation was not found between the concentration of Si and that of Al in leaves (Fig. 3). Therefore the reason (2) is not compelling to explain the observational results. (3) If Si is incorporated to supplement the mechanical strength of leaves, the relationship between the concentration of Si in leaves and the measure of mechanical weakness of leaves would be found. A central venation in a leaf of a dicotyledonous plant plays a role of the frame to support a blade. A leaf with larger surface area and with smaller thickness of central venation would be difficult to support flat. This is the reason why we applied “the ratio of leaf area to thickness of central venation” as the index of mechanical weakness of leaves. In Figure 4 is shown the relationship between the measure and the concentration of Si in leaves. Results showed that the concentration of Si in leaves was corre-

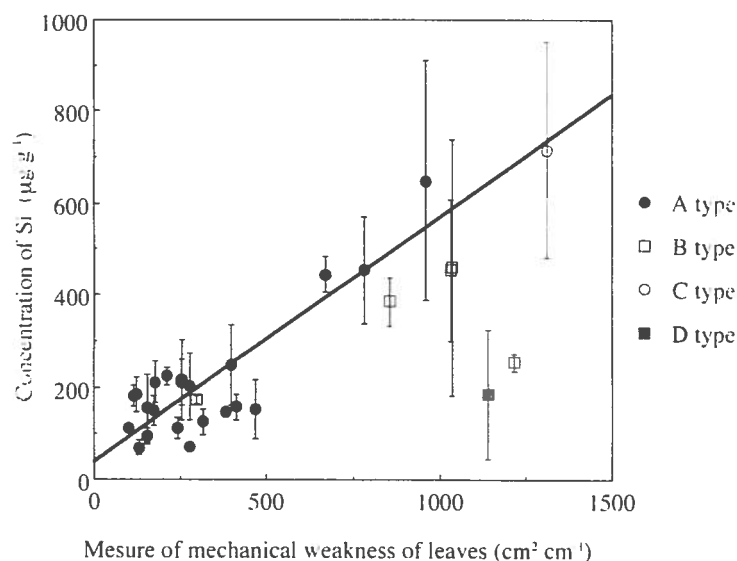


Figure 4. Correlation between concentration of Si and “measure of mechanical weakness of leaves”. The straight line was drawn by the least square method using data of type A leaves.

lated with this measure. (Fig. 4) A leaf with greater mechanical weakness had a higher concentration of Si. Therefore it can be considered that Si can be actively accumulated in leaves of trees to strengthen the leaves. This consideration seems to be better supported by taking account of individual shapes of leaves.

The leaf shape difference and the concentration of Si

Most of plants belonged to A type (standard shape, see Figure 1) and data points for the A type are generally distributed along the straight line in Figure 4. The data for B type (spade-shaped) were plotted somewhat lower or near the straight line except for *Indesia poylcarpa*. The leaves of this type could be easier to support than the those of type A and therefore lesser amount of Si is required. Only *Liriodendron tulipifera* is of C type (hart-shaped). Because the area of a leaf is located near the top along the central venation, this leaf could be hardest to support only with the central venation. The leaf of this type gave data plotted above the line, indicating that more Si is needed to support the leaf. Only *Alangium platanifolium* is of D type (crown-shaped). In the case of this type several venations run from the bottom to the top of a leaf. Therefore, compared with other leaves, it is easier to support the leaf. With the burden being shared with the other venations, leaves of this type gave data below the line, implying that Si is less required by this species to support the leaf.

CONCLUSION

In the trees of dicotyledonous plants, Si may play an auxiliary role to supplement the mechanical strength of a leaf. The study of the chemical speciation of Si in leaves would lead us to the better understanding of the role of Si in leaves of trees.

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Silicon Forms in Cell Wall of Rice and Tomato Plants

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INTRODUCTION

Silicon is one of the most abundant elements in the earth's crust, and most soils contain considerable quantities of this element; however, repeated cropping can reduce the levels of plant-available silicon to the point that supplemental silicon fertilization is required for maximum production.

Deficiency in silicon at the panicle forming stage resulted in slowing down the growth in a straw before heading (Inanaga *et al.*, 2002). What is more, silicon might exist in associated with lignin-carbohydrate complexes (Inanaga and Okasaka, 1995), implying that silicon may participate into a formation of cell wall in a straw of the rice plant. Thus, we attempted to fractionate silicon in the cell wall of rice straw and tomato stem.

MATERIALS AND METHODS

Rice plant: Seventy-two seedlings, which were sown on 22 May 1998, were transferred on 19 June to a container of 20 L filled with Kimura culture solution containing 50 mg L⁻¹ silicon. Since 29 July, the rice seedlings were transferred to the nutrient solution containing silicon either 0 or 50 mg L⁻¹. The main straw was harvested at 5 days before heading, at the heading day, and 5, 10, 20 and 40 days after heading.

Tomato plant: Ten-cm-high tomato plant seedlings were grown in a 4 L pot filled with a culture solution without or with 50 mg L⁻¹ silicon for 50 days since 20 April. The harvested plants were cut into the upper stem (stem to 3 node below a node with developing leaf), the lower stem, and root.

Fractionation of cell wall: The prepared cell wall sample was successively extracted with 80°C DI water (F1), 0.25% ammonium oxalate and 0.25% oxalate solution at 80°C (F2), acetate and NaClO solution for 4 h at 80°C (F3), and 24% KOH (F4). The extracted residue was F5. The F2 and F3 extracts were dialyzed against water before they were freeze-dried as F1. After acidified with acetate, the F4 was precipitated with the addition of ethanol. The F1, F2, F3, F4 and F5 are corresponding to soluble pectin material in hot water, soluble pectin material in oxalate, oxidized lignin, hemicellulose and cellulose, respectively.

RESULTS

Fig.1 shows changes in the silicon content of each fraction in cell wall of rice plants. At 5 days before heading, cell wall treated with 0 mg L⁻¹ Si contained more silicon than that treated with 50 mg L⁻¹ silicon. After heading, however, the later increased remarkably while the former decreased abruptly. About 56% of silicon was in soluble form at the 40 days after heading in +Si treatment.

Although silicon content in tomato plants had the order of root > lower stem > upper stem in -Si treatment, while in +Si treatment, it was mainly deposited in roots and then lower stem. The upper stem contained only 3% of total silicon (Fig 2). Over ninety percent of silicon existed in soluble form in roots (95%), and stems (92%), while in upper stem, soluble form took 52% of the total silicon amounts in +Si treatment. Each of other forms of silicon in roots and lower stems of +Si treatment was lower than 5%. In upper stem, silicon in soluble pectin took 24% and hemicellulose and cellulose took 11% and 10% respectively.

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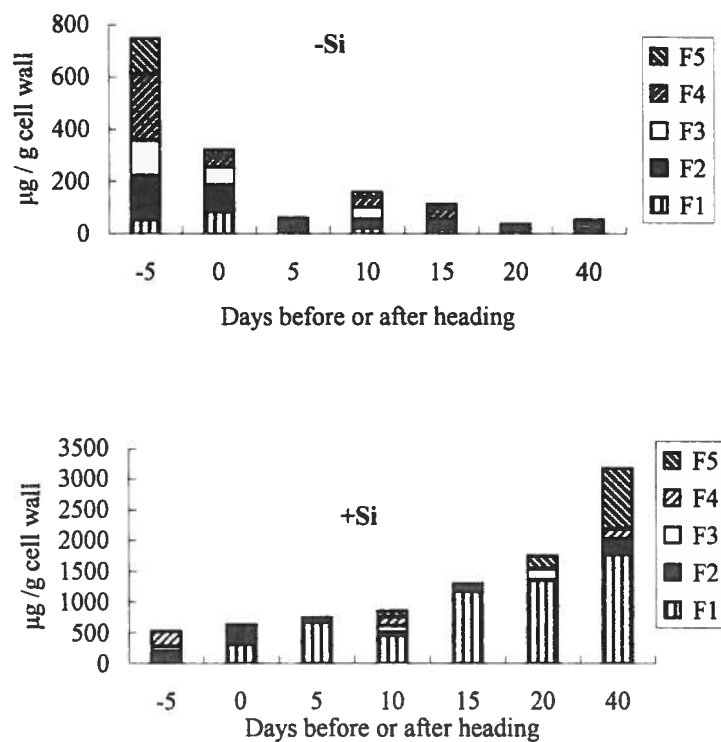


Fig 1. Si content of each fraction in cell wall of rice straw

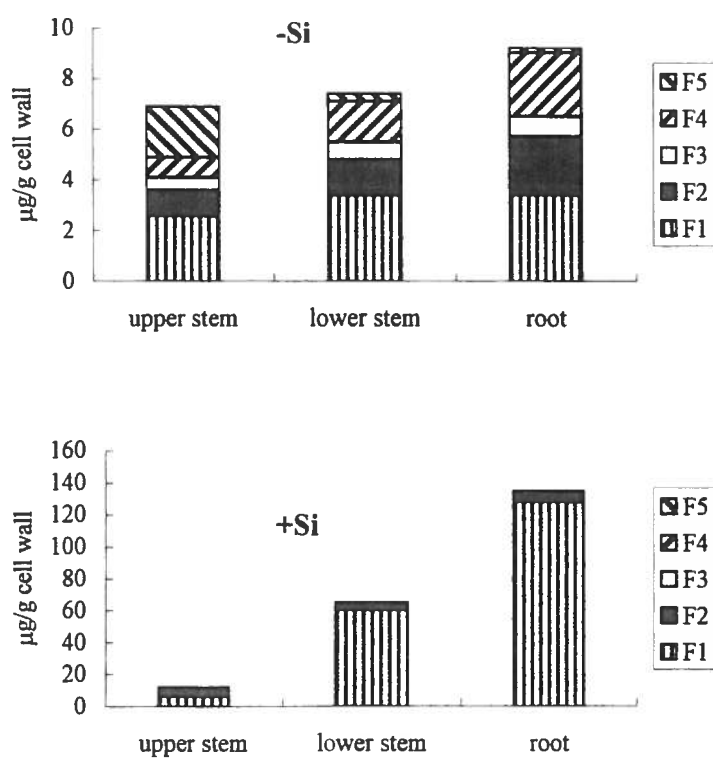


Fig 2. Silicon content of each fraction in cell wall of tomato plant

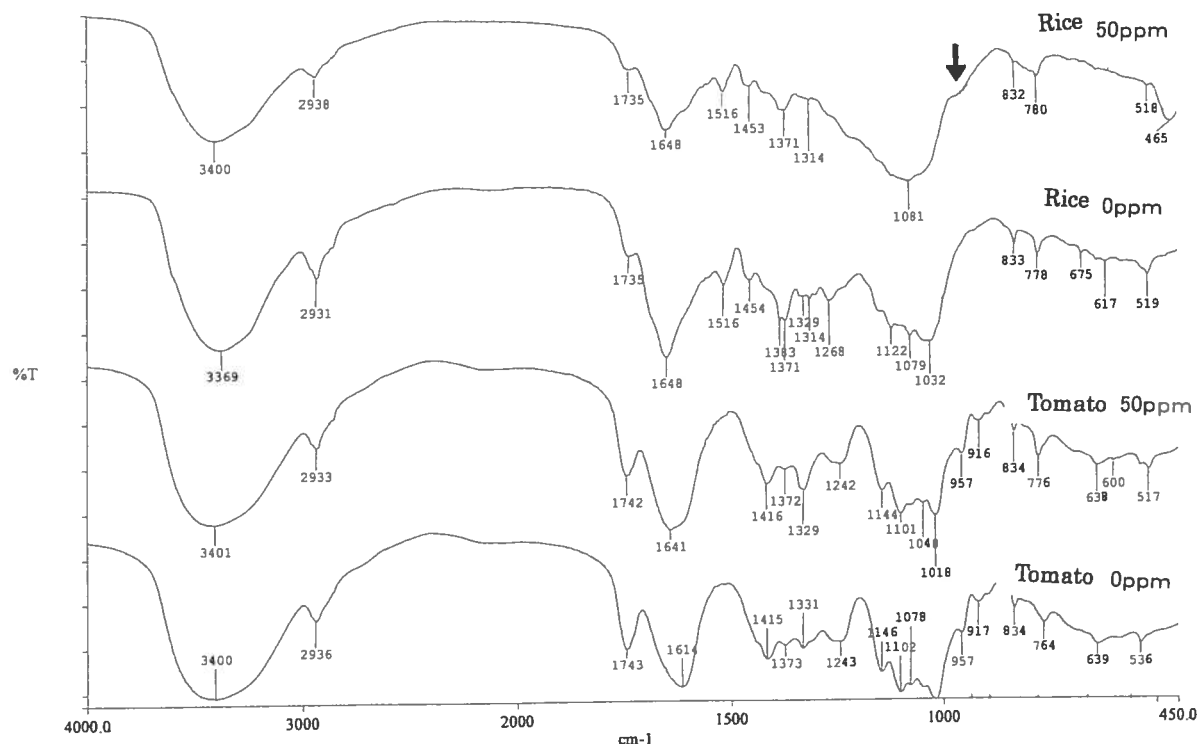


Fig 3. Infrared absorption spectra of soluble pectin material in hot water in cell wall of rice or tomato

Fig.3 shows the infrared absorption spectra of soluble pectin material in hot water in the cell wall of rice plant on 40 days after heading and tomato plant. The IR spectra of soluble pectin material in hot water were similar in all treatments. However, in the case of rice plant, the spectra were shifted to longer cm^{-1} at 3369 cm^{-1} and had stronger signal at 465 cm^{-1} in +Si treatment. While in the case of tomato plant, only shift to longer cm^{-1} at 1614 cm^{-1} was observed.

DISCUSSION

It is well known that the heading in rice plant is delayed when silicon is deficient in the culture solution. We reported that this was due to shorter straw length during panicle stage (Inanaga *et al.*, 2002, in press). At 5 days before heading in -Si treatment, the dry weights of pectin fraction and lignin fraction were less when compared with those in the +Si treatment (data not shown). The amount of lignin-carbohydrate complexes in vegetative parts of rice plant decreased in the case of silicon deficiency (Inanaga *et al.* 1995). These results suggest that the formation of cell wall maybe slow down when silicon is deficient.

Miyake and Takahashi (1983) reported that at the flowering stage of tomato plant, the malformation of newly developed leaf occurred when silicon was deficient. Furthermore, the fiber length in the cotton became shorter in poor silicon level than in rich silicon level (Boylston, 1988). The dry weights of F2 and F3 were lower in -Si treatment than in +Si treatment of tomato plants, indicating that the formation in pectin and lignin material of cell wall was depressed in tomato plant under poor silicon condition.

Silicon concentration in -Si treatment was higher at 5 days before heading than in +Si treatment, but it decreased remarkably after heading. This result is in accordance with our previous report that silicon content of spikelets grown in silicon-deficient solution was remarkably higher at 10 and 5 days before heading than that in silicon-rich solution (Inanaga *et al.*, 2002), indicating that silicon may be necessary for the formation of cell wall of rice straw.

In the cell wall of rice plant treated with 50 mg L^{-1} , there exist more silicon in soluble pectin material in

oxalate (F2) and hemicellulose (F4) forms at 5 days before heading than the other fractions, and then only Si in soluble and cellulose forms remarkably increased at 40 days after heading. While in the cell wall of tomato plant, the silicon content in soluble form was the largest in the upper stem (52%), lower stem (92%) and root (95%) among other fractions. Inanaga *et al* (1995) described that when the cell wall was extracted with acetate buffer (pH 4.5), silicon was eluted into fractions of lower molecular weight. These results indicate that silicon incorporated into the cell wall of plant is accumulated in soluble pectin material in hot water, in which silicon may be easily utilized for plants.

Calculated from Fig 1, the silicon concentration in soluble form was 964 and 70 mg kg⁻¹ in -Si treatment of rice and tomato respectively, and 276875 and 1121 mg kg⁻¹ respectively in +Si treatment on 40 days after heading. The IR spectra didn't show the absorption at 1090 cm⁻¹ and 800 cm⁻¹ characteristic of silica gel. However, a signal at 465 cm⁻¹ became stronger. It had a shoulder near 910 cm⁻¹ and shifted to longer cm⁻¹ at 3360 cm⁻¹ in +Si treatment. While in tomato plant, only shift to longer cm⁻¹ at 1612 cm⁻¹ was observed in +Si treatment. In spite of existence in higher silicon concentration in soluble pectin material in hot water, the fact that the IR absorption spectra characteristic of silica gel didn't observe in rice plant but did only at 465 cm⁻¹ suggests that part of silicon in the cell wall may exist in associated with pectin materials.

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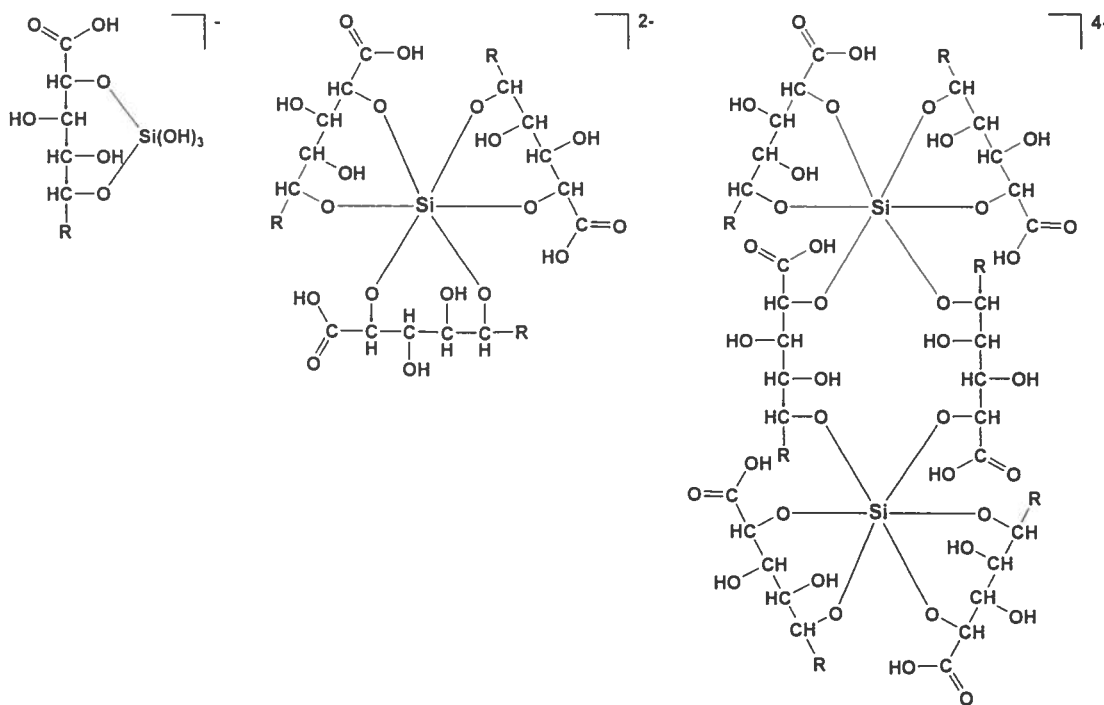
Organosilicates in Nature

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Although the beneficial effects of silicon in plants and animals are now established there is almost no understanding of how, on a molecular level, these are bestowed. Our approach to investigating the biochemistry of silicon has been to attempt to create and identify model organosilicates in solutions approximating physiological conditions (1) in order to establish the type of chemistry that is likely to be found *in vivo*. Using ²⁹Si NMR spectroscopy we have shown that common carbohydrates – including many aliphatic, pyranosic and furanosic sugars (such as gluconic acid, lyxose and ribose, respectively) – form stable organosilicates in aqueous solution. Surprisingly, the silicon in these complexes is not tetravalent, but is instead either five or six coordinated. Some examples of organosilicates formed with aliphatic sugar acids are:



For aliphatic sugars, the criterion for complexation is that there are at least four adjacent hydroxy groups, with two being in *threo* configuration. For pyranose sugars, there must be hydroxy groups in the 1,3 axial positions, while furanose rings require hydroxy groups in either the 2,3- or 3,4-*cis* positions. Extracellular matter, cell membranes, biofluids and soil solutions are of course rich in such molecules. Silicon-29 NMR spectra of a dense, *living* colony of the fresh water diatom, *Naviculla pelliculosa*, reveal two signals, that of monosilicic acid at -71 ppm and a weak but reproducible signal at -131.5 ppm, as shown below (2). Because of its chemical shift, we tentatively assign the latter signal to a pentaoxo-azo-silicon complex. This observation provides the very first evidence that hypercoordinated silicon species containing Si-O-C linkages exist in living systems.

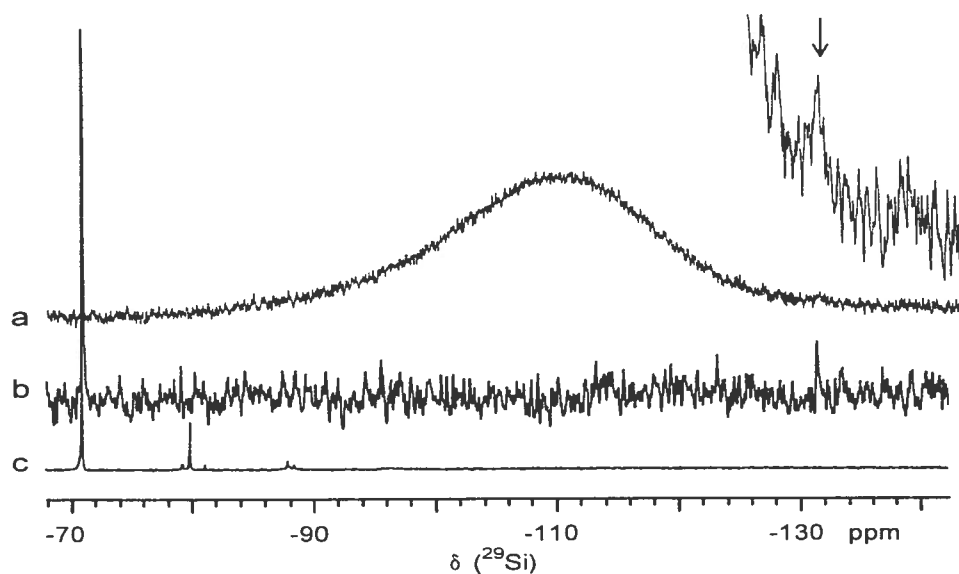


Figure a) ^{29}Si NMR spectrum (149 MHz) of a synchronized culture of *Navicula pelliculosa* following 6 h feeding on ^{29}Si -enriched silicon at 298 K. b) Spectrum of a second culture (now also enriched in ^{15}N) recorded on a second spectrometer (99.4 MHz). c) Spectrum of the nutrient medium for the culture in b).

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Exogenous Silicon (Si) Increases Antioxidant Enzyme Activities and Reduces Lipid Peroxidation in Roots of Salt-Stressed Barley (*Hordeum vulgare* L.)

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INTRODUCTION

Salinity toxicity is a worldwide agricultural and eco-environmental problem. Approximately one-third of the world land surface is arid and semi-arid, of which one half is affected by salinity (Liang *et al.*, 1996). There are two major limiting factors to affect crop production in the agricultural soils of China, one being aluminium toxicity in highly weathered acidic soils distributed mainly in the tropics and subtropics, and the other salinity stress in salinized and/or sodic soils in the coastal and inland areas (Liu *et al.*, 1987; Liang, *et al.*, 1996; Liang *et al.*, 2001). It is estimated that there are approximately 27 million ha of salinized soils in China's coastal and inland areas (Liang *et al.*, 1996). Therefore, how to improve the plant growth and development in salinized soils is crucial to sustain agriculture in China where there is little opportunity for opening up new land. Studies have shown that plasma membrane injury induced by excess Na and Cl ions accumulated in plant tissues is one of the most important toxic mechanisms under salt stress (Liu *et al.*, 1987; Hernandez *et al.* 1993; Singha and Choudhuri, 1990). Increasing evidence exists that plasma membrane injury induced by salt stress is related to an increased production of highly toxic oxygen free radicals (Liu *et al.*, 1987; Hernandez *et al.* 1993; Singha and Choudhuri, 1990). Under salt stress, both superoxide dismutase (SOD) and catalase (CAT) activities decline in plants (Hernandez *et al.* 1993; Singha and Choudhuri, 1990) and malondialdehyde (MDA), the end product of peroxidation of membrane lipids, accumulates rapidly (Fadzilla *et al.*, 1997, Hernandez *et al.* 1993; Lutts *et al.* 1996), which results in an increase in permeability of plasma membranes.

Silicon (Si) has not yet been considered a generally essential element for higher plants. The main difficulties in establishing its essentiality lie in that Si roles in plant biology remain poorly understood and direct evidence is still lacking that Si is a part of plant constituents or enzymes (Okuda and Takahashi, 1965; Lewin and Reimann, 1969; Epstein, 1994). However, numerous studies have demonstrated that Si is a beneficial or "agronomically essential" element for higher plants, particularly for gramineous plants (Okuda and Takahashi, 1965; Lewin and Reimann, 1969; Epstein, 1994; Liang *et al.*, 1996; Epstein, 1999; Liang, 1999; Liang and Ding, 2002). More recently, Si roles in alleviating both abiotic (aluminium, heavy metals and salt toxicity) and biotic (plant diseases and pests) stresses in plants have received much attention (Epstein, 1994; Liang *et al.*, 1996; Epstein, 1999; Liang, 1999; Liang and Ding, 2002).

Previous studies showed that salt tolerance of wheat (*Triticum aestivum*) (Ahmad *et al.* 1992), mesquite (*Prosopis juliflora*) (Bradbury and Ahmad, 1990), and barley (Liang *et al.*, 1996; Liang, 1999) could be markedly enhanced by the addition of small amount of soluble Si. We demonstrated that added Si decreased the permeability of plasma membrane of leaf cells (Liang *et al.* 1996), and significantly improved the ultra-structure of chloroplasts which were badly damaged by the added NaCl with the double membranes disappear-

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Abbreviations: AOS, active oxygen species; DBI, double bonding index; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; IUFA, index of unsaturated fatty acids; MDA, malondialdehyde; NBT, (p-nitro blue tetrazolium chloride; POD, peroxidase; PVP, polyvinylpyrrolidone; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid;

ing and the granae being disintegrated in the absence of Si (Liang, 1998). Our previous investigations also showed that added Si increased leaf SOD activity and suppressed lipid peroxidation induced by salt-stress and stimulated root H^+ -ATPase in the membranes, suggesting that Si may affect the structure, integrity and functions of plasma membranes by influencing the stress-dependent peroxidation of membrane lipids (Liang *et al.*, 1996; Liang 1999). The stimulation of root plasma membrane H^+ -ATPase by added Si under salt stress was responsible for the increased uptake and transport of K^+ and decreased uptake and transport of Na^+ and Cl^- from roots to shoots by plants (Liang, 1999; Liang and Ding, 2002). However, information is still scant regarding the influences of exogenous Si on root antioxidant enzyme activities and non-enzymatic antioxidants responsible for scavenging the active oxygen species and oxidative damage induced by salt stress in plants.

Based on our previous works (Liang *et al.* 1996; Liang 1998, Liang 1999, Liang and Ding, 2002), this paper reports the results of Si effects on time-dependent changes of antioxidant enzyme activities and lipid peroxidation in roots of salt-stressed barley using two contrasting barley cultivars differing in salt tolerance. The objectives of this study are to clarify the involvement of exogenous Si in antioxidative enzymes and lipid peroxidation in barley under salt stress and elucidate the possible mechanisms of silicon-enhancement of salt tolerance in barley.

MATERIALS AND METHODS

Growth conditions and treatment

Two barley (*Hordeum vulgare* L.) cultivars were selected: Jian 4, a relatively salt tolerant cultivar, and Kepin No.7, a relatively salt susceptible cultivar (Liang *et al.* 1996, Liang, 1999). Seeds of barley were surface sterilized with $HgCl_2$ (1.0 g/L) for 10 minutes, rinsed thoroughly with distilled water, and germinated on moist filter paper for three days in an incubator at $25^\circ C$. Then the germinated seeds of uniformity were selected to sow in plastic containers filled with quartz sand in the greenhouse at room temperature and watered with 1/3 strength Hoagland nutrient solution. When the second leaf emerged, seedlings of uniform size were planted into hydroponics plastic pots fitted with insulated covers. Each pot was covered with a polythene lid through which plants were supported over the nutrient solution. The pot contained 4000 ml of full-strength Hoagland nutrient solution, which was aerated for 20 min daily and renewed every other day. Daily photoperiod was 12 hours and maximum temperature was $25^\circ C$ while the daily minimum temperature at night was adjusted to $15^\circ C$. The pH of the nutrient solution was adjusted to 6.0 daily using 0.01 mol L^{-1} KOH and/or H_2SO_4 .

Salinity and silicon treatments were initiated by adding sodium chloride (NaCl) and silicic acid to the nutrient solution immediately after the seedlings were transplanted to the nutrient solution. Silicic acid was prepared following the method described elsewhere (Liang, 1998). The initial pH of the nutrient solution was adjusted to 6.0 using dilute H_2SO_4 before transplanting. Six treatments with three replicates were established including CK (no added NaCl or Si), 1.0 mmol L^{-1} Si (added as silicic acid), 60 mmol L^{-1} NaCl, 60 mmol L^{-1} NaCl+ 1.0 mmol L^{-1} Si, 120 mmol L^{-1} NaCl and 120 mmol L^{-1} NaCl + 1.0 mmol L^{-1} Si. Four separate experiments were performed, three to study root antioxidant enzyme activities and the other GSH and MDA concentrations.

Assays of antioxidant enzyme activities in roots

Root tips were harvested 0, 2, 4 and 6 days after treatments commenced, and used for assays of SOD, POD and GR activities, and GSH and MDA concentrations. Three separate extractions were made of the activities of each antioxidant enzyme and the concentrations of GSH and MDA.

SOD (EC 1.15.1.1) was assayed by the photochemical method described by Giannopolitis and Ries (1977). One g of fresh root segments (< 2 mm) was homogenized in an ice bath in 5 mL HEPES-KOH buffer (pH 7.8) containing 0.1 mmol L^{-1} EDTA. The homogenate was centrifuged at 10,000 g for 15 min at $4^\circ C$. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT

(p-nitro blue tetrazolium chloride) reduction measured at 560 nm.

POD (EC 1.11.1.7) activity was assayed following the method of Xu and Ye (1989). For this, 0.5 g of fresh root segments (< 2 mm) was homogenized in an ice bath in 5 mL 50 mmol L⁻¹ borate buffer (pH 8.7) containing 5.0 mmol L⁻¹ sodium hydrogen sulphite and 0.1 g PVP. The homogenate was centrifuged at 10,000 g for 25 min at 4°C. The supernatant was used as enzyme extract. To 0.1 mL of the enzyme extract, a substrate mixture containing acetate buffer (0.1 mol L⁻¹, pH 5.4), ortho-dianside (0.25% in ethyl alcohol) and 0.1 mL 0.75% H₂O₂ was added. Absorbance change of the brown guaiacol at 460 nm was recorded for calculating POD activity.

GR (EC 1.6.4.2) activity was extracted from 0.5 g fresh root segments in an ice bath in 2.5 mL of 0.05 mol L⁻¹ Tri-HCl buffer (pH 7.6) containing 0.1 mmol L⁻¹ EDTA and 1% (W/W) PVP (polyvinylpyrrolidone). The homogenate was centrifuged at 18,000 g for 30 min at 4°C. GR activity was assayed by following the oxidation of NADPH at 340 nm as described by Schaedle (1977). The reaction mixture contained 100 mmol L⁻¹ potassium phosphate buffer (pH 7.8), 0.2 mmol L⁻¹ NADPH, 0.5 mmol L⁻¹ oxidized glutathione (GSSG) and the root extract.

For the measurement of lipid peroxidation in roots, the TBA (thiobarbituric acid) test was adopted which determines MDA (malondialdehyde) as an end product of lipid peroxidation. For this, root tissues (500 mg) were homogenized in 3 mL 0.1% TCA (trichloroacetic acid) solution. The homogenate was centrifuged at 2,500 g for 10 min and the supernatant was assayed for MDA concentration using the method given by Heath and Packer (1968).

Glutathione (GSH) concentration was determined by reading the absorbance at 412 nm following the method by Guri (1983). For this, 0.5 g of fresh root segments was homogenized in an ice bath in 5 mL of 5% (w/v) TCA, and the homogenate was centrifuged at 16,000 g for 20 min. The supernatant was used for GSH assay by using 5,5'-dithio-2, 2'-dinitrobenzoic acid as reagent.

Statistical analysis

Variance analysis was performed on all experimental data in the figures and statistical significance ($P < 0.05$) of means of 3 replicates was judged by the least significant difference (LSD) values.

RESULTS

For the salt-sensitive cultivar Keping No.7, salt (NaCl only) addition at two levels significantly increased SOD activity of barley roots 2 days after treatment compared with control (with neither salt nor Si added) or Si treatment alone ($p < 0.05$) (Figure 1). The incorporation of 1.0 mmol L⁻¹ Si into the salt treatments significantly increased this enzyme activity compared to the corresponding treatments with no Si added. At lower

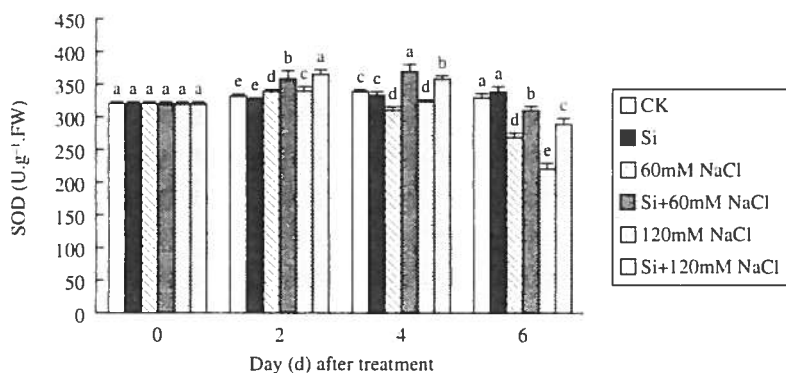


Figure 1. Changes of superoxide dismutase (SOD) activity of the roots of barley (salt-sensitive cultivar, Keping No.7) exposed to varied concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p < 0.05$) different by Duncan's New Multiple Range Test.

(60 mmol L⁻¹ NaCl) and higher (120 mmol L⁻¹ NaCl) level of salt, added Si increased SOD activity of the plants by 5.94% and 7.7%, respectively compared with the corresponding NaCl treatments (Figure 1). This enzyme activity was significantly inhibited 4 days after the plants were exposed to salt stress, while Si treatment considerably stimulated SOD activity of salt-stressed plants regardless of salt level (Figure 1). SOD activities of the plants treated with salt were much higher than those with no salt treated on Day 2, however, this trend changed with progression of experiment. At the sixth day of treatment, SOD activity of the salt-treated plant declined sharply (Figure 1). For example, SOD activity of the plants treated with 60 mmol L⁻¹ NaCl and 120 mmol L⁻¹ NaCl was only 81.4% and 66.8% that of control, respectively. In contrast, added Si counteracted this decline significantly. At the lower level of salt, incorporation of 1 mmol L⁻¹ Si increased this enzyme activity by 15.4% and at the higher level of salt, by 31.4% (Figure 1). For the salt-tolerant cultivar Jian 4, very similar time-dependent change was observed with respect to SOD activity for the treatments with salt and those with salt with Si (Figure 2). However, much higher SOD activity was found for salt-tolerant cultivar Jian 4 than for salt-sensitive cultivar Kepin No.7, irrespective of salt or Si level.

Peroxidase (POD) activity of salt-stressed plants greatly and significantly increased compared to the control (no salt or Si added) for both cultivars used at the 2nd day of exposure to NaCl salt, independent of Si, (Figs 3 and 4). However, no significant difference was noted in this enzyme activity between the salt treatments and those with salt with Si (Figs 3 and 4). But at the fourth day of treatment, POD activity of salt stressed plants declined sharply for both cultivars and both salt levels compared with the other treatments. For examples, POD activity in the salt-sensitive plants exposed to 120 mmol L⁻¹ NaCl was only 87.4% that of the control, and in the salt-tolerant plants exposed to 60 and 120 mmol L⁻¹ NaCl was 69.6% and 62.7% that of the corresponding control, respectively (Figs 3 and 4). The salt-tolerant cultivar exhibited much higher POD activity than the salt-sensitive cultivar though slower decline of this enzyme activity was observed for the salt-sensitive cultivar than for the salt-tolerant cultivar when exposed to salt stress for 4 days. It is interesting to note that addition of Si significantly enhanced POD activity of the salt-stressed plants irrespective of salt level or barley cultivar (Figs 3 and 4). As expected, POD activity for the salt stressed plants declined with the prolonged duration of exposure to salt stressed environments, while the salt-stressed plants had significantly higher POD activity in the presence of Si than in the absence of Si (Figs 3 and 4).

The effects of salt and Si on GR activity are shown in Figs. 5 and 6. GR activity of the plants under salt stress was stimulated significantly at the 2nd day of salt treatment excluding the salt-sensitive barley treated with 120 mmol L⁻¹ NaCl compared with the control. The inclusion of Si significantly increased GR activity of the salt-sensitive barley grown under 120 mmol L⁻¹ NaCl and that of salt-tolerant barley grown under 60 mmol

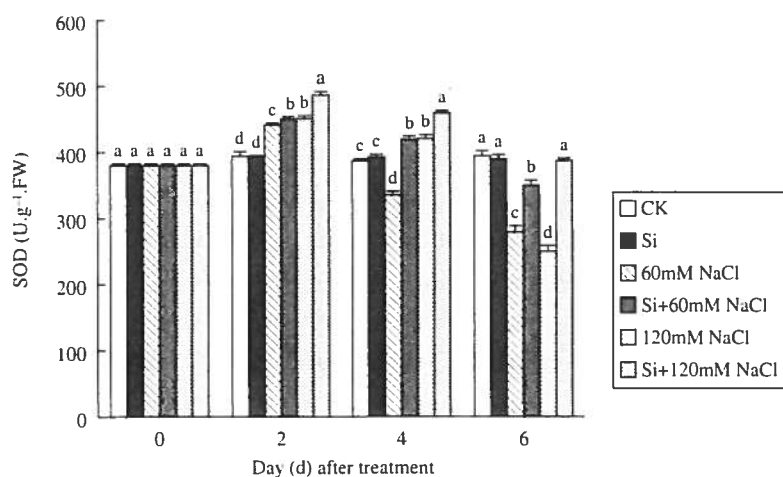


Figure 2. Changes of superoxide dismutase (SOD) activity of the roots of barley (salt-tolerant cultivar, Jian No.7) exposed to varied concentrations of NaCl and Si. Data are expressed as means±SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p < 0.05$) different by Duncan's New Multiple Range Test.

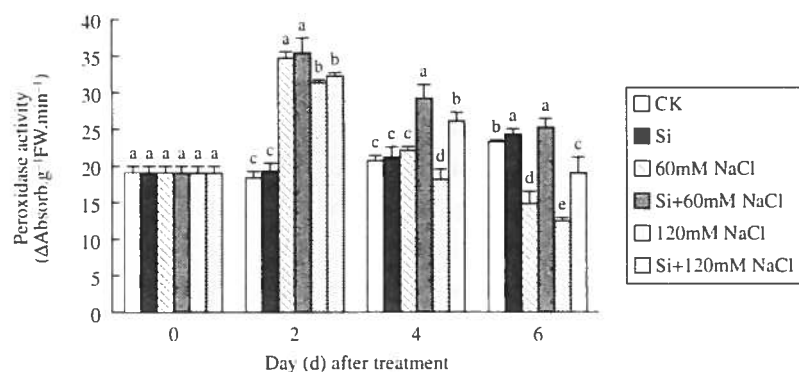


Figure 3. Changes of peroxidase (POD) activity of the roots of barley (salt-sensitive cultivar, Keping No.7) exposed to varied concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly (p<0.05) different by Duncan's New Multiple Range Test.

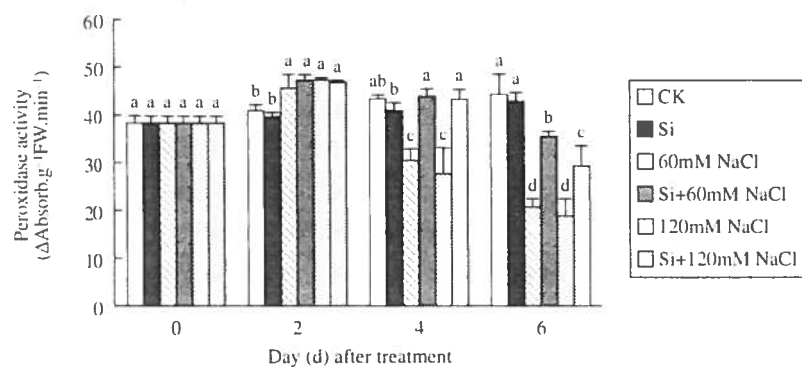


Figure 4. Changes of peroxidase (POD) activity of the roots of barley (salt-tolerant cultivar, Jian No.7) exposed to varied concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly (p<0.05) different by Duncan's New Multiple Range Test.

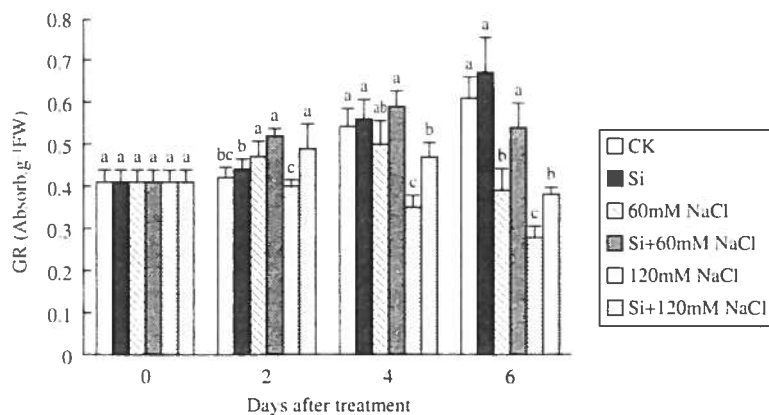


Figure 5. Changes of glutathione reductase (GR) activity of the roots of barley (salt-sensitive cultivar, Keping No.7) exposed to varied concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly (p<0.05) different by Duncan's New Multiple Range Test.

L^{-1} NaCl from Day 2 through to Day 4 (Figs 5 and 6). For salt-sensitive cultivar, a considerable decline of GR activity of salt stressed plants was observed and this was more pronounced for the plants treated with higher salt concentration (Fig.5). However, the salt-tolerant barley exhibited much higher GR activity compared to the control and no significant difference existed between the Si-deprived and Si-fed plants grown under 60 $mmol L^{-1}$ NaCl. At 120 $mmol L^{-1}$ NaCl, GR activity was significantly and greatly depressed, which was significantly stimulated by added Si.

As illustrated in Figs. 7 and 8, for the salt-sensitive cultivar, GSH concentration in plants was significantly enhanced at 60 $mmol L^{-1}$ NaCl compared to the control but was significantly depressed at 120 $mmol L^{-1}$ NaCl on Day 2 (Fig.7). Added Si increased GSH concentration at both salt levels but this increase is not statistically significant. For the salt-tolerant cultivar, the plants had significantly higher GSH concentration when exposed to salt environments compared to the control on Day 2, and added Si significantly enhanced GSH concentration of the plants grown under both lower (60 $mmol L^{-1}$ NaCl) and higher (120 $mmol L^{-1}$ NaCl) salt levels (Fig.8). However, on Day 4, GSH concentration was greatly reduced for the salt-sensitive barley under salt stress, but was significantly enhanced by inclusion of Si (Fig.7). In case of salt-tolerant cultivar, GSH concentrations in salt-stressed barley greatly decreased on Day 4 compared to those on Day 2, but enhanced significantly

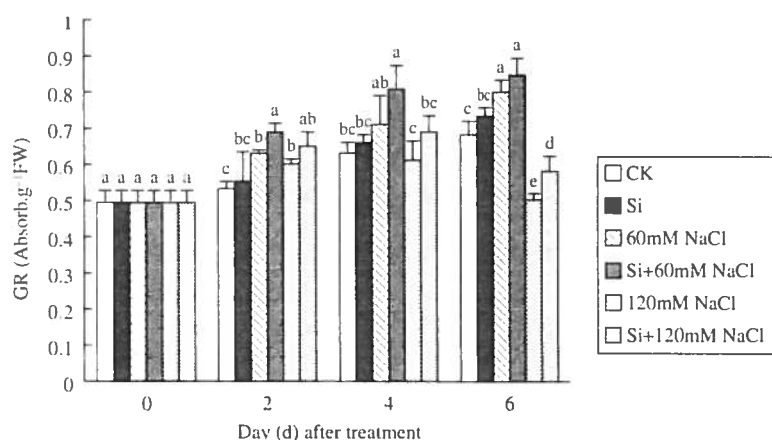


Figure 6. Changes of glutathione reductase (GR) activity of the roots of barley (salt-tolerant cultivar, Jian No.7) exposed to varied concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test.

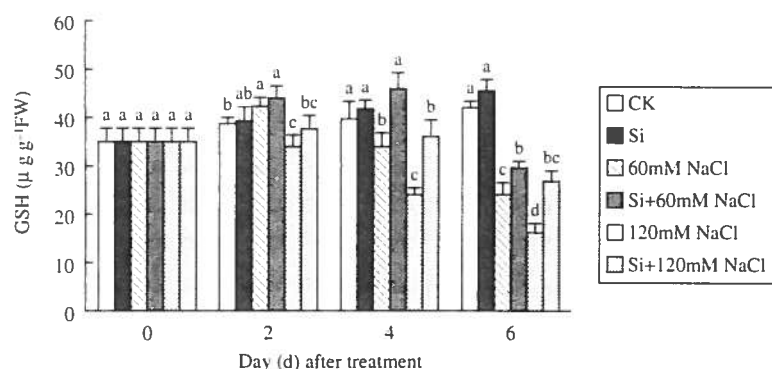


Figure 7. Changes of glutathione (GSH) concentration of the roots of barley (salt-sensitive cultivar, Keping No.7) exposed to varying concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test.

cantly by the added Si (Fig. 8). On Day 6, GSH concentration of the salt-stressed plants was sharply declined regardless of cultivars or salt level, but was significantly increased by the addition of Si (Figs. 7 and 8).

The data for malondialdehyde (MDA) concentration was presented in Figs. 9 and 10. The MDA levels increased on all days showing a dose-response for both salt levels added and for both cultivars used. For the salt-sensitive cultivar, inclusion of Si reduced MDA concentration of salt-stressed plants. A significant reduction for MDA was noted for the treatment with salt with Si compared to that with salt alone on all days except for the treatment with lower level of salt on Day 6 where the reduction of MDA induced by Si added was not statistically significant (Fig. 9). However, for the salt-tolerant cultivar, less lipid peroxidation indicated by MDA concentration induced by salt was observed compared to the salt-sensitive cultivar (Figs. 9 and 10). A significant difference was found with respect to MDA concentration between the plants treated with 60 mmol L⁻¹ NaCl and those treated with 60 mmol L⁻¹ NaCl with Si on all days except on Day 2 (Fig.10). Very similar results were obtained in case of higher level of NaCl.

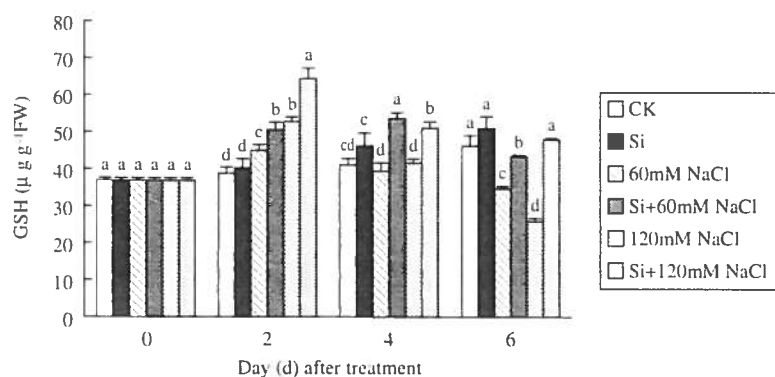


Figure 8. Changes of glutathione (GSH) concentration of the roots of barley (salt-tolerant cultivar, Jian No.7) exposed to varying concentrations of NaCl and Si. Data are expressed as means±SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p < 0.05$) different by Duncan's New Multiple Range Test.

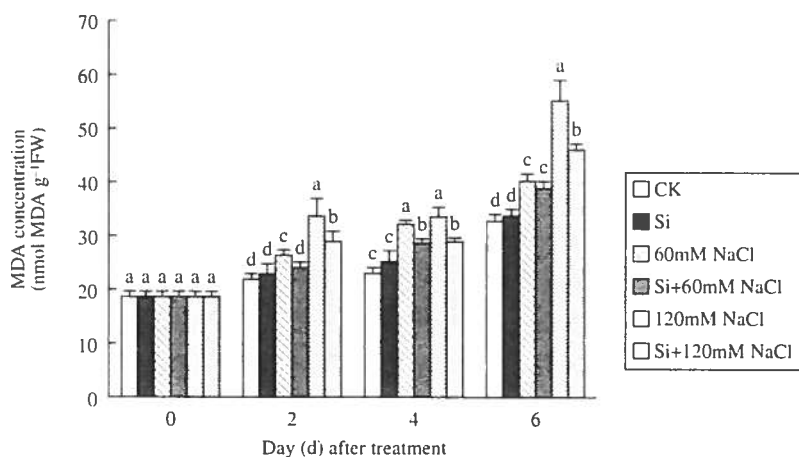


Figure 9. Changes of malondialdehyde (MDA) concentration of the roots of barley (salt-sensitive cultivar, Keping No.7) exposed to varying concentrations of NaCl and Si. Data are expressed as means±SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p < 0.05$) different by Duncan's New Multiple Range Test.

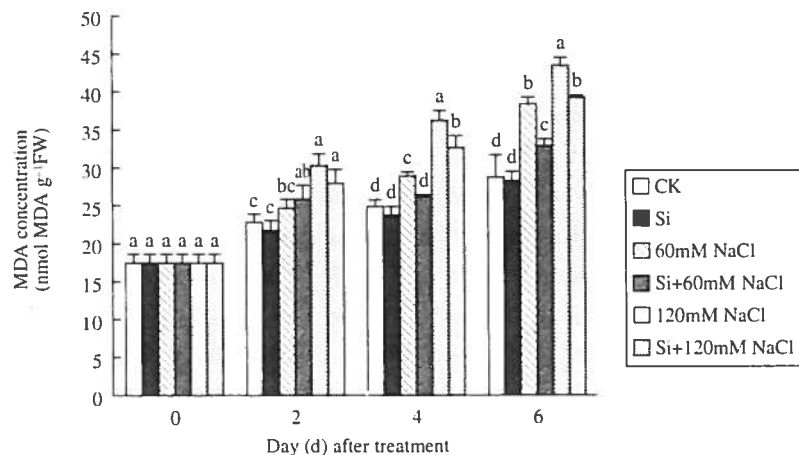


Figure 10. Changes of malondialdehyde (MDA) concentration of the roots of barley (salt-tolerant cultivar, Keping No.7) exposed to varying concentrations of NaCl and Si. Data are expressed as means \pm SD (n=3). Means marked with the same letter on the same day after treatment are not significantly ($p < 0.05$) different by Duncan's New Multiple Range Test.

DISCUSSION

Free oxygen radicals accumulated in plant cells under stressed conditions result in the lipid peroxidation via oxidation of unsaturated fatty acids leading to membrane damage and electrolyte leakage (Liu *et al.*, 1987; Marschner, 1995). Membrane lipid peroxidation, a typical symptom indicating membrane injury mediated by the elevated free oxygen radicals is proved to be one of the most important mechanisms of salt toxicity in higher plants (Fadzilla *et al.*, 1997; Hernandez *et al.*, 1993). SOD, CAT and POD are the major antioxidant enzymes associated with scavenging the active oxygen species and SOD is likely to be central in the defense against toxic active oxygen species (Marschner, 1995). However, it detoxifies superoxide anion free radicals accompanying the formation of H_2O_2 , which is very toxic to the chloroplasts, nucleic acids and proteins (Fridovich 1986, Rabinowitch and Fridovich 1983) and can be decomposed by catalase and peroxidase (Marschner, 1995; Scandalios, 1990; Elstner and Osswald, 1994). Glutathione reductase also plays a key role in oxidative stress by converting the oxidized glutathione, GSSG, to GSH and maintaining a high GSH/GSSG ratio (Alscher, 1989, Fadzilla *et al.*, 1997). GSH is a major antioxidant that is known to protect cells from oxidative stress (Smith *et al.*, 1990). Changes in processes that regulate GSH concentration and/or redox status are considered to be one of the important adaptive mechanisms of plants exposed to the stressed conditions (Alscher, 1989, Fadzilla *et al.*, 1997, Smith *et al.*, 1990). It was reported that severe oxidative damage occurred with elevated concentration of H_2O_2 and reduced concentration of GSH in rice plants at early stage when exposed to salt stress condition (Fadzilla *et al.*, 1997).

In the present study, on Day 2, all antioxidant enzymes were remarkably stimulated in the plants exposed to salt. This may be a general adaptive defense response of plants to toxic salt environments at early stages. The significant lipid peroxidation occurred progressively in barley plants induced by the added salt as the exposure time prolonged and this apparently followed a dose-response (Figs 9 and 10). The induction of lipid oxidative damage expressed as MDA by salt was closely related to the declined activities of antioxidative enzymes and concentration of non-enzymatic antioxidants in plant roots (Figs 1-8). However, exogenous Si significantly increased the activities of SOD, POD and GR, and the concentration of GSH in the roots of barley (Figs. 1-8). The induction of these enzymes coincided with a decrease in concentration of MDA, an end product of membrane lipid peroxidation (Figs. 9 and 10), suggesting that oxidative damage induced by salt

was alleviated by the addition of Si. It is noted that the induction of SOD activity coincided with an increase in the activity of the enzyme (POD) scavenging H_2O_2 . This is considered to be of particular importance since the increase in H_2O_2 resulting from higher SOD activity induced an increased capacity of enzymatic H_2O_2 decomposition (Wu *et al.*, 2002). Bowler (1992) agrees that the cooperation between H_2O_2 scavenging enzymes and SOD plays an important role in resistance of plants to environmental stresses. Similar adaptation responses were reported for barley exposed to ozone (Wu *et al.* 2002). We believe that such coordination also plays a crucial role in preventing plants from salt injury. The results of this study was in line with our previous findings which showed that added Si decreased the permeability of the plasma membrane of leaf cells, enhanced leaf SOD activity and decreased MDA concentration under salt stress (Liang *et al.*, 1996; Liang, 1999). Our results (unpublished data) showed that the index of unsaturated fatty acids (IUFA) and double bonding index (DBI) of root plasma membrane for salt-treated barley increased by 18.5 and 16.5%, respectively compared to salt-untreated barley, but inclusion of Si significantly decreased IUFA and DBI. Furthermore, the fluidity of leaf and root plasma membrane and tonoplast decreased under salt stress conditions, but the addition of Si was observed to stimulate the fluidity of leaf plasma membrane and tonoplast, and root plasma membrane (unpublished data). Similar results were also reported (Agarie *et al.*, 1998) that Si enhanced the stability of lipids in cell membranes of rice plants exposed to drought and heat stresses, suggesting that Si prevented the structural and functional deterioration of cell membranes when rice plants were exposed to environmental stress. Wang and Galletta (1998) found that foliar application of silicate enhanced the ratios of fatty acid unsaturation ((18:2+18:3)/18:1) in glycolipids and phospholipid and elevated amounts of membrane lipids in strawberry. It thus seems to strongly suggest that Si decreases the permeability of plasma membranes and membrane lipid peroxidation and maintains the membrane integrity and functions of salt-stressed barley, thus mitigating against salt toxicity and improving the growth of plants (Liang *et al.*, 1996; Liang, 1999).

In our previous experiments with barley, root dehydrogenase activity (Liang *et al.*, 1999) and plasma membrane H^+ -ATPase activity (Liang, 1999) were significantly enhanced by the inclusion of Si under salt stress, thus improving the uptake and translocation of K^+ ion and diminishing the uptake of Na^+ and Cl^- ions (Liang and Ding, 2002). Under salt stress, added Si significantly improved the growth of roots which showed to be brittle, stunted and stubby with less lateral roots and hairs in the absence of Si (Liang *et al.*, 1996; Liang and Ding, 1999). Higher root activity for the Si-fed plants under salt stress facilitates the uptake of nutrients and their transport to shoots from roots. This plays an important role in the tolerance of plants to salt toxicity.

Genotypical difference exists in salt tolerance between different plants and plant species (Marshner, 1995). As shown in our previous publications (Liang *et al.*, 1996; Liang, 1999), cultivar Jian 4 used in the present study is a relatively salt-tolerant cultivar, while cultivar Kepin No.7 is a salt-sensitive one. This is strongly supported by the data showed in this study regarding the antioxidant enzyme activity, and MDA and GSH concentrations. The higher antioxidant levels in salt-tolerant barley (Figs. 1-10) coincided with our previous results which showed that salt-tolerant barley suffered much less severely from damage by salt and produced significantly higher dry matter yield than salt-sensitive barley (Liang *et al.*, 1996; Liang, 1998; Liang and Ding, 1999). However, inclusion of Si significantly alleviated the salt toxicity of both barley cultivars and improved the growth of plants compared to the corresponding plants treated with salt alone (Liang *et al.*, 1996).

Si has still not been listed among the generally essential elements in plants due to the fact that direct evidence is still lacking that Si is part of the molecule of an essential plant constituent or metabolite (Epstein, 1994). One of the most difficulties in establishing the necessity of Si in higher plants is the difficulty to purge solution cultures thoroughly of Si. Numerous previous studies showed that Si was inert in plants and not involved in the physiological metabolism (Epstein, 1994). However, Si roles in plant metabolism have received ever-increasing attention since last decade. Si has been proposed to be included routinely in the formulation of nutrient solutions (Epstein, 1994; Epstein, 1999; Rafi *et al.*, 1997). It was reported that foliar

application of silicate stimulated antioxidant superoxide dismutase (SOD) activity in the drought-stressed bentgrass (Schmidt *et al.*, 1999) and induced metabolic changes in strawberry plants by influencing the ratios of fatty acid unsaturation ((18:2+18:3)/18:1) in glycolipids and phospholipid (Wang and Galletta 1998). Cherif *et al.*, (1994) reported that amendment of nutrient solutions with soluble Si resulted in a marked increase in stimulation of chitinase activity and in a more intense and rapid activation of peroxidases and polyphenoloxidases in cucumber after infection with *Pythium* spp. The results in the present study coupled with the reports in the literature strongly suggest that Si be involved in the metabolic or physiological activity in higher plants exposed to abiotic and biotic stresses, though these effects of Si may be indirect or secondary. Therefore, further studies in our laboratory will be strengthened aiming at better understanding of the physiological or biochemical roles Si plays in higher plants.

ACKNOWLEDGMENTS

This research is jointly supported by the grants from National Natural Science Foundation of China (Approved Nos. 39470424 & 39770441) and by the grant from International Foundation For Science (Agreement No. C/2528-2). The author is grateful to Dr. B. Forster of the Cell and Molecular Genetics Department, Scottish Crop Research Institute, Dundee, UK, for his encouragement in this study.

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Influence of Silicon on the Tolerance of the Upland Rice to the Soil Water Stress

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Silicon in the rice plant is taken up passively from the root with the transpiration stream to the evaporating surfaces and accumulated in plant tissue. This work was designed to evaluate the effect of the silicon application using calcium silicate in two soils of the Brazilian savanna Typic Acrustox (red-yellow latosol - LVa) and Ustoxic Quartzipsammentic (sandy quartz - AQa) on the Si accumulation of an upland rice and the its tolerance to water stress. The hypothesis was that a plant that accumulates Si in the epidermis of the leaves can be more tolerant to the water stress because it gets to be more efficient in regulating respiration. The experiment was set up in a complete factorial design ($2 \times 3 \times 4$) with 4 replications: 2 soils classes, 3 water level in the soil (60, 70 and 80% of moisture holding capacity) and 4 Si rates (0, 200, 400 and 600 kg ha⁻¹). The variables evaluated in the experiment were: grain yield, plant height, soil soluble Si and Si uptake by the plant at harvest. The "available" Si in the soil and the Si content in the rice straw increased with calcium silicate application. The LVa type of soil showed higher "available" Si compared to the AQa because of the higher clay content. Silicon promoted increase on rice yield and enhanced water stress tolerance. Silicon effects on yield were larger when the soils were submitted to a larger water stress.

**Accumulated Silicon in Tropical Forage Species
(*Brachiaria decumbens* and *Brachiaria brizantha*)
and Tolerance to Hydric Deficit**

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The beneficial effects of silicon (Si) fertilization have been observed in several vegetable species especially when these are submitted to stress, be it biotic or abiotic. One of possible reasons for larger adaptability and resistance of brachiaria grass in areas of low fertility of soil Brazilian savannas regions, can be associated with its capacity in to absorb and to accumulate Si in aerial part of the plant. This work had as objective to evaluate the effect of Si on dry matter yield of two grass species (*Brachiaria decumbens* and *Brachiaria brizantha*), cultivated under two moisture regimes in soil. It was used the completely randomized factorial design (5x2x2) with five Si rates: 0, 242, 484, 968 and 1452 kg ha⁻¹, two water tensions in soil (60% and 80% of field capacity), two brachiaria species and five replication. The experiment was installed in greenhouse, using one of the most representative soils of area under savannas, Typic Acrustox (Latosol). Both brachiaria species can be considered Si accumulator plants. The application of Si in the soil significantly increased the contents of this element in the aerial part of the two species tested. The application of Si didn't changed the tolerance of two grasses species to the water stress, and didn't affect the dry matter yield.

Isolation and Characterization of a Rice Mutant Defective in Si Uptake

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Silicon (Si) is a beneficial element for plant growth. Silicon increases photosynthesis by improving light interception, enhances strength of tissues, reduces transpiration of plants, resulting in increased resistance of plants to multiple stresses including physical, chemical, and biological stresses such as water-deficiency, radiation damage, nutrient imbalance, metal toxicity, diseases and pests (Ma *et al.*, 2001). Most of these functions of Si are expressed mainly through silica deposited on the tissue surface. The more Si accumulates in the shoot, the larger is the beneficial effect that is gained. Therefore high accumulation of Si on the top is a prerequisite for benefiting the plant growth.

Rice can accumulate Si in the top up to 10% Si in dry weight and this high accumulation has been ascribed to the high uptake ability by the roots. An abundance of evidence shows that the rice root has a transport system specific to silicic acid. However, neither the gene encoding this transporter nor that encoding the transporter protein has been isolated. A gene family encoding a Si transporter has been cloned from marine diatom (*Cylindrotheca fusiformis*), which requires Si as an essential element (Hildebrand *et al.*, 1993, 1997). However, similar genes were not found in rice from homology search. In order to clone the Si transporter in rice roots, we isolated a rice mutant, which is defective in active Si uptake and further characterized this mutant.

A rice mutant defective in active Si uptake was isolated by screening M_2 seeds (64000) of rice (cv. Oochikara) that were treated with 10^{-3} M of sodium azide for 6 h at 25°C. Mutants were screened in half strength Kimura B solution containing 50 μ M GeO_2 . As Ge taken up in a manner similar to Si, is toxic to the plants, which appears as brown spots in the leaf blades, plants without brown spots in the leaves were selected. After performing progeny test for M_3 and M_4 seeds, a mutant (GR1, Germanium Resistant), which showed resistance to Ge, was obtained. The dry weight of both shoot and root of WT was reduced by 40% after exposure to 20 μ M Ge for 12 days, while the growth of GR1 was hardly affected by the same treatment.

There were no differences in the phenotype between the wild type (WT) and GR1 in terms of root morphology and top performance. The short-term and relatively long-term uptake experiments showed that Si uptake by the roots in GR1 was significantly lower than that in WT at either a low or high Si concentration (Figure 1). However, there was no difference in the uptake of other nutrients such as P and K (Table 1). When two lines were grown in a soil amended with or without Si (2 g Na_2SiO_3 /kg soil), the Si content of the top was much lower in GR1 than that in WT. However, there were no differences in the P and K content. The Si uptake per root was further compared between GR1 and WT by using multi-compartment transport box. At 12 h, the Si uptake per root was three times more in the WT than in the GR1. All these results consistently indicate that a mutation in the Si uptake occurred in GR1.

Silicon uptake by rice roots is known to be an active process (Okuda and Takahashi, 1962). The Si uptake is not affected by transpiration, but suppressed by metabolic inhibitors. We further examined GR1 in terms of active process. The concentration of Si in the xylem sap and external solution was compared between GR1 and WT. When the external solution contained 0.15 mM Si, the Si concentration in the xylem sap of WT after 1 h was 33-times as high as that in the external solution, whereas that of GR1 was only 3-times as high. When the external solution contained 1.5 mM Si, the Si concentration in the external solution and in the xylem sap was similar in GR1, but it was 4 times higher in the xylem sap than in the external solution in the WT. The

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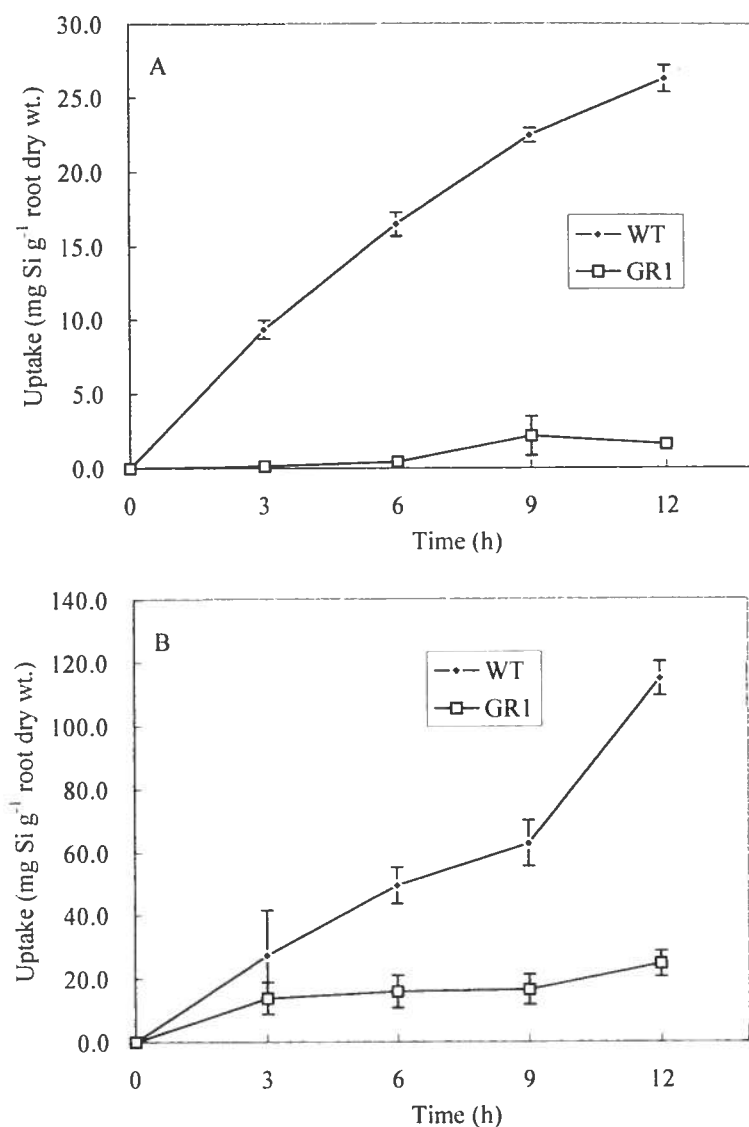


Figure 1. Uptake of Si by WT rice, cv Oochikara, and a mutant (GR1) defective in Si uptake. Twenty-day-old seedlings were placed in a nutrient solution containing 0.15 (A) and 1.5 mM (B) Si as silicic acid.

Table 1. Contents of P, K, and Si in the shoot and root of a wild type rice (cv Oochikara) and a mutant (GR1) defective in Si uptake. Two lines were grown in a nutrient solution for 4 weeks.

	Shoot		Root	
	0.15 mM Si	1.5 mM Si	0.15 mM Si	1.5 mM Si
P content (%)				
WT	0.57	0.54	0.23	0.21
GR1	0.51	0.57	0.20	0.25
K content (%)				
WT	3.08	3.18	1.02	1.14
GR1	3.12	3.23	1.01	1.17
Si content (%)				
WT	1.46	4.62	0.03	0.12
GR1	0.26	1.43	0.04	0.08

uptake of Si by WT was inhibited by metabolic inhibitors such as NaCN, 2,4-dinitrophenol and low temperatures, while the Si uptake by GR1 was not inhibited (Figure 2). These results suggest that the active transport system for Si uptake is defective in GR1. In the population of F2 between GR1 and WT, the ratio of the plants

with a high ability to take up Si to those with a low ability was 3:1 (Table 2), suggesting that the low ability to take up Si in GR1 is controlled by a recessive gene. This mutant is expected to be a powerful tool for isolation and identification of the Si transporter in rice roots.

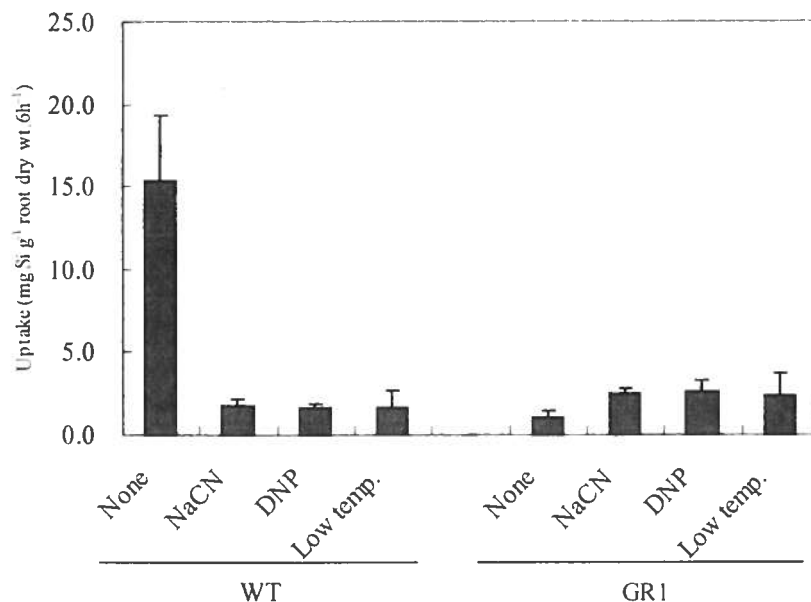


Figure 2. Effect of metabolic inhibitors and a low temperature on the Si uptake by a wild type rice (cv Oochikara) and a mutant (GR1) defective in Si uptake. The uptake experiment was conducted in a nutrient solution containing 0.75 mM Si in the presence or absence of inhibitors (1 mM for DNP and 10 mM for NaCN) for 6 hours. For low temperature treatment the plants were exposed to 4°C.

Table 2. Segregation ratio of progeny resulting from genetic crosses between a wild type (WT, cv Oochikara) of rice and a mutant (GR1). Si uptake by each seedling in a nutrient solution containing 0.68 mM Si was determined during 24 h

	Phenotype of progeny		Ratio tested	X ²	P
	High Si uptake	Low Si uptake			
WTxGR1 F2.	71	18	3:1	1.08	0.3-0.5
Si uptake (mg Si g ⁻¹ root dry wt. 24 h ⁻¹)	42.6±10.9	8.07±1.70			

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RFLP Mapping of a Gene for Si Uptake in Rice (*Oryza sativa* L.)

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Nutrients for higher plants can be classified into two categories, namely essential elements and beneficial elements. Effective use of both types of nutrients is important for high crop production.

Silicon (Si) is one of essential elements for rice, and rice actively absorbs Si and accumulates a high level of Si (Takahashi *et al.* 1990). Si gives a variety of beneficial effects on the rice plants, such as disease resistance (Seebold *et al.* 2000), insect resistance (Salim *et al.* 1992) and heavy metal resistance (Wang *et al.* 2000). Although beneficial effects of Si are well documented, little is known about the mechanism of active Si uptake in rice.

To investigate the mechanism for Si absorption, we isolated a rice mutant that shows a reduced level of Si uptake and attempted the mapping of the mutant gene responsible for low Si uptake using restriction fragment length polymorphism (RFLP) markers.

Seeds of a japonica rice cultivar Oochikara were mutagenized by 1 mM sodium azide (pH 3.0) for 6 hours. To select mutants of low Si uptake, about 64,000 M₂ seedlings were tested for germanium resistance. Usually rice absorbs germanium (Ge) as an analog of Si, and absorbed Ge induces brown speck on the leaves of rice plant due to its toxicity. Seedlings were water cultured in 1/2 Kimura's B solution for initial 10 days, and then cultured in the solution containing 50 μ M of GeO₂ for 5 days. After the GeO₂ treatment, brown speck on the leaves of M₂ seedlings were observed, and seedlings without or with less brown speck were selected.

In this screening, a plant showing a high level of Ge resistance was obtained. M₃ progenies derived from this resistant plant were tested for Ge resistance, and all plants showed Ge resistance (Fig.1). We named this

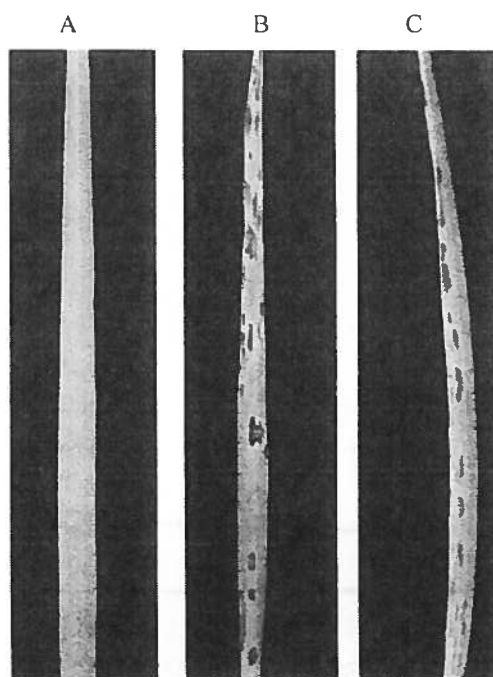


Fig. 1. Leaves of GR1, Oochikara and their F₁ plant grown in the solution containing 50 μ M GeO₂.

A : GR1, B : (GR1 \times Oochikara)F₁, C : Oochikara

mutant line GR (Germanium Resistant) 1.

To determine whether GR1 is a mutant of Si absorption, Si uptake of GR1 and Oochikara was measured. Seedlings of the GR1 and Oochikara were water cultured in 0.5 mM CaCl_2 for initial 10 days and then cultured in 1/2C Kimura's B solution for 10 days. Seedlings were further cultured in 1/2C Kimura's B solution containing 1.5 mM of silicic acid for 12 hours. Concentration of silicic acid in the solution was measured at 4, 8 and 12 hours after the start of Si treatment by the molybdenum blue method. In this experiment, GR1 showed a lower level of Si uptake than Oochikara at any time (Fig. 2).

F_1 hybrid plants derived from a cross between GR1 and Oochikara were tested for Ge resistance by the same method as used for mutant screening. The mode of inheritance of the low Si uptake in GR1 was examined using two F_2 populations derived from crosses of GR1 with the original cultivar Oochikara and an indica cultivar Kasalath. Si uptake were individually measured by the same method as used for mutant characterization. In these experiments, F_1 hybrid plants were sensitive to Ge (Fig. 1), and the both F_2 populations segregated in Si uptake. Of 98 F_2 plants of the GR1 \times Oochikara cross, 77 plants showed high Si uptake and 21 showed low uptake. This segregation ratio matched a 3:1 ratio ($\chi^2 = 0.69$, $0.3 < P < 0.5$). Also of 100 F_2 plants of the GR1 \times Kasalath cross, 83 plants showed high Si uptake and 17 showed low uptake. This segregation matched a 3:1 ratio ($\chi^2 = 3.41$, $0.05 < P < 0.10$). These results indicated that the low absorption of Si uptake in GR1 is controlled by a single recessive gene.

To map the mutant gene for low Si uptake in GR1, RFLP analyses were performed. Genomic DNA were isolated by the CTAB method from the (GR1 \times Kasalath) F_2 population and its parental lines, and 96 RFLP markers evenly distributed on rice chromosome 1-12 were used for RFLP linkage analysis. The genotype data were analyzed by the computer software MAPMAKER/EXP ver. 3.0 (Lander *et al.* 1987).

The mutant gene for low Si uptake was mapped between RFLP markers, C1408 and C560 located on the long arm of chromosome 2. The genetic distances of the mutant gene from the flanking markers were 6.5 cM for both C1408 and C560 (Fig. 3). We tentatively designated this mutant gene *lsi* (low Si uptake). Further mapping efforts are under the way toward map-based cloning of this gene.

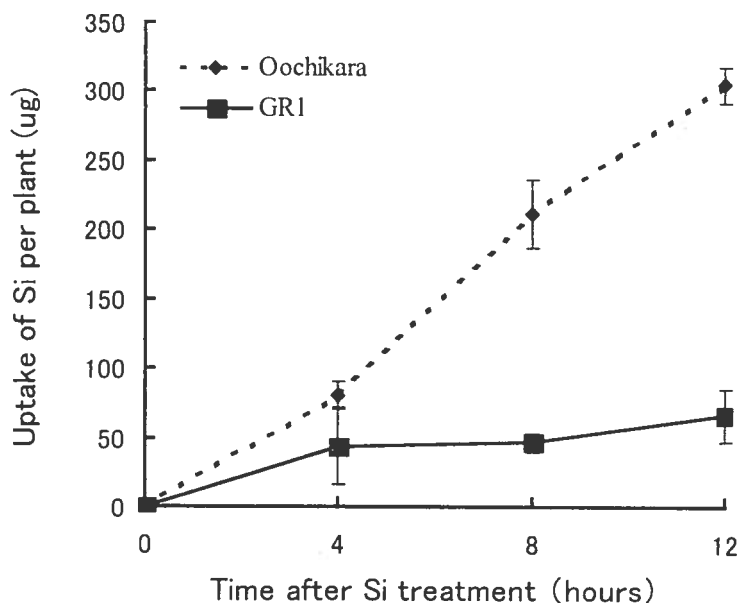


Fig. 2. Changes in Si uptake of GR1 and Oochikara.

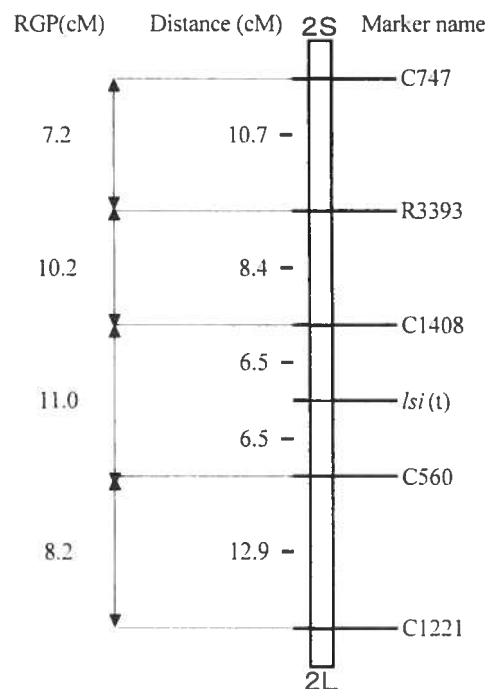


Fig. 3. RFLP mapping result of the mutant gene for low Si uptake in GR1. The mutant gene was localized to the long arm of chromosome 2. Genetic distances on the left under RGP were cited from the High-Density rice genetic map constructed by Rice Genome Research Program.

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Microarray Analysis of Transcript Profiles in Response to Si Nutrition

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Si has a number of beneficial effects on growth of rice plants. However, to our knowledge, genes regulated by Si nutrition have not been described. Here we carried out microarray analysis of gene expression profiling in response to Si nutrition in rice. Rice plants were grown for four weeks with nutrient solution (Kimura B solution) containing 0, 0.015, 0.15, or 1.5 mM silicate. Leaves were harvested and Si contents were determined to ensure proper Si treatment. Total RNA was extracted from the leaf samples and fluorescently labeled. Microarray analyses were carried out with slide glass containing 8987 cDNA clones. Statistical analysis of hybridization data revealed a number of clones regulated by Si nutritional status. Those upregulated by application of Si include xyloglucan endotransglycosylase, calmodulin binding protein, calcium dependent protein kinase, translation and elongation factors. Those downregulated by Si include chlorophyll a/b binding proteins and several stress related proteins. These data represent first genome scale analysis of transcript profiling in response to Si nutrition.

INTRODUCTION

Si has a number of beneficial effects on higher plants. Si application enhances disease resistance, increases carbon fixation, yield and quality of grains, especially in rice plants. Si application is also known to affect activities of several enzymes including H⁺-ATPase, superoxide dismutase (Liang 1999), peroxidase, β -glucosidase (Cherif *et al.*, 1994), and phenylalanine ammonia-lyase (Carver *et al.* 1999). However, genes regulated by Si nutrition has not been reported.

Microarray analysis is a powerful tool for genome scale analysis of transcript profiling. This technique has been applied to describe transcriptome analysis of yeast cells in response to sugar supply (DeRisi *et al.* 1997). As a part of the rice genome project by the Ministry of Agriculture, Forestry and Fisheries of Japan, microarrays were generated for genome scale analysis of transcript accumulation. We used microarrays containing 8987 cDNA clones for transcript profiling in response to Si nutrition in rice plants.

MATERIALS AND METHODS

Rice plants (*Oryza sativa* L., cv. Nipponbare) were grown hydroponically in a greenhouse with natural light at 30°C in the day and 25°C in the night. Growth media used were Modified Kimura-B solution (pH5.5) with varying concentrations (0, 0.015, 0.15 and 1.5 mM) of silicate. After five weeks from sowing, leaves were harvested and subjected for Si determination and RNA extraction.

For Si determination, rice leaves were digested with alkali and Si concentration were determined using the molibdenum blue method.

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Total RNA was extracted with Sepasol-CTAB method and forty microgram of total RNA was subjected to fluorescence labeling with Cy5 using Atlas Glass Fluorescent Labling Kit (Clontech). Hybridization was carried out using ExpressHyb Hybrideization Buffer (Clontech) according to the manufactures' procedure. The image was scanned using Gene Pix 4000 (Axon instruments) and analyzed with Array Gauge (Fuji Film). Local background was subtracted and data was normalized using median. Each slide glass contains duplicate spots for all the clones and two independent hybridizations were performed, resulting in four data sets for each spot for each sample. These data were subjected to Student's t-test and $p < 0.05$ was used for the judgment of significance.

RESULTS AND DISCUSSION

Rice plants grown with four different concentrations of silicate were indistinguishable from their appearance (data not shown). Si contents of leaves were determined (Fig. 1). Si concentration in leaves was increased as the Si concentration in nutrient solution higher, suggesting proper Si treatment in our experiments.

Microarray analysis of the four RNA samples were carried out and list of clones that gave significant difference between the treatments were listed. Eighty clones were identified whose signal intensities were significantly upregulated between 0 mM Si and 0.015 mM Si. The difference in signal intensities was mostly around 2 fold, though some clones exhibited more than 3 fold differences. There was no clone identified that exhibited more than 10 fold differences. These results suggest that effect of Si nutrition is moderate compared with hormonal treatments or other stress treatments such as temperature, salt or inhibitors. The clones identified include genes related to signal transduction, transcriptional regulation.

Among the clones that were upregulated by 0.015 mM Si, some exhibited further upregulation as the concentration of Si in nutrient solution, others were not further increased.

Similarly, 80 clones were identified whose transcript accumulation were reduced by application of 1.5 mM Si. The extents of reduction were in most cases less than two fold. Some clones, however, exhibited nearly four-fold reduction. Again, similar to the case of the analysis of upregulated clones, impact of Si nutrition is less intense compared with other treatments such as hormone treatments. The list of clones include chloro-

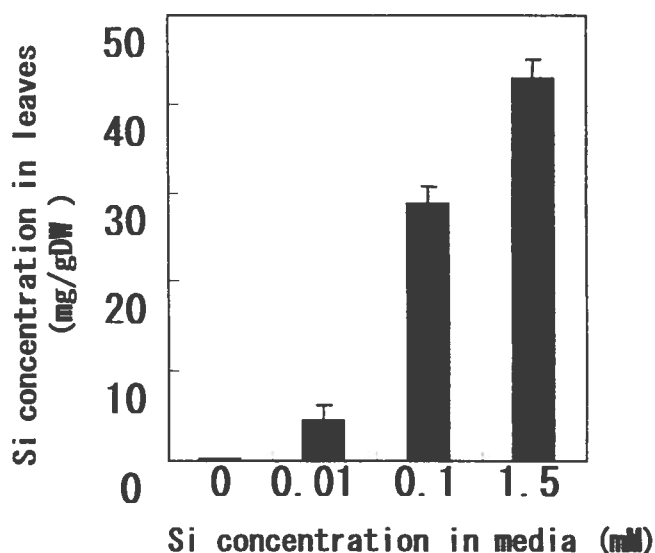


Fig. 1 Si contents in leaves of rice plants grown with various concentration of silicate in media. Rice plants were grown hydroponically with media containing 0, 0.015, 0.15 and 1.5 mM silicate for five weeks and concentration of silicate in leaves were determined, $n=3$.

phyll a/b binding proteins, disease resistant genes and stress related gene such as heat shock proteins and metallothioneins.

The patterns of changes in transcript accumulation were confirmed with the RT-PCR analysis. Among the seven clones picked up to confirm the results of microarray analysis, four clones exhibited similar patterns of regulation with the results of array analysis.

The present results represent first genome scale analysis of transcript profiling. Clones identified that are up and down regulated support physiological knowledge and revealed novel aspects of Si nutrition.

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Fine Structure and Development of Rice Husk Accompanied with Silica Shell

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Rice plant constructs peculiar and elaborate husks covering their grain. Those husks are worthy of notice from two points of views that they are covered with thick silica layer and also they are biomaterial resources being produced enormously every year. Some industrial utilizations based on these two peculiarities will be reported in the following paper, so cell structure of husks and fine elaborated structure of silica layers are shown here.

General structures of rice husks had been examined for a long time and shown in the classical textbooks, being observed by light microscopy(LM), and recently their convex surface structures of epidermis have been observed by scanning electron microscopy(SEM). However, the precise fine morphology of cell wall and silica layer remained uncertain. Here, the combination technique between LM and SEM was designed as shown in Figs. 1-3. Another combination technique is designed between some removal treatments such as hydrofluoric acid and SEM observation as shown in Figs. 5,6. The clue of formation mechanism of silica layer seems to be get by these ways.

MATERIALS AND METHODS

Young(just after flowering on Aug 6th) and mature husks(Aug 26th) of japonica rice (Akihikari) and commercially supplied mature ones of japonica rice were offered for the SEM and LM.

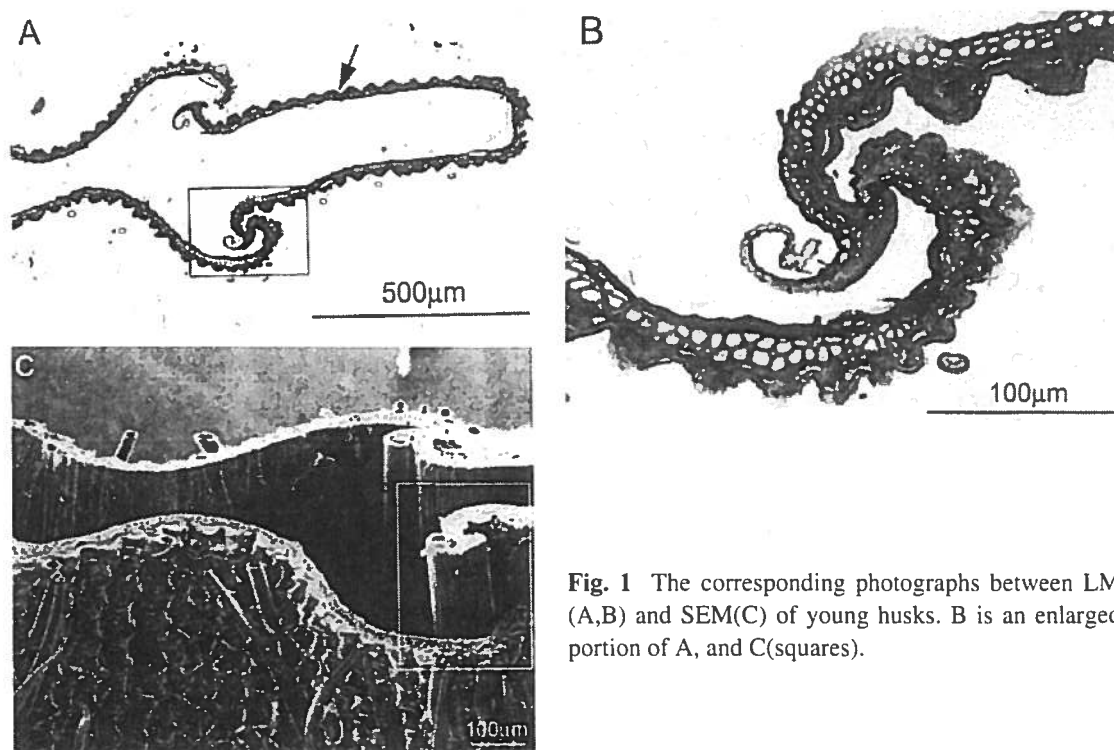


Fig. 1 The corresponding photographs between LM (A,B) and SEM(C) of young husks. B is an enlarged portion of A, and C(squares).

In relation to LM, husks were macerated by Jeffrey reagent and sonications as the traditional method. Outline of tissues and cells could be observed. Next, these husks were embedded in Aron-Alfa(cyano-acrylate adhesive resin) and offered to the glass knife microtomy. 3 μm sections were sliced off from the transverse or longitudinal directions and then observed by LM(for instance Fig.1A,B), while the remained husk after the glass-knife microtomy were washed in acetone to remove the resin. The husk having a sharply sliced surface were observed by SEM(Fig.1C).

For SEM observations, some kinds of component-removal treatments to reveal the interior fine structure as follows. ① hydrofluoric acid treatment to remove silica layers. ② Repeated washings of 72% H_2SO_4 and NaClO solutions to remove all cell wall components. ③ Chloroform washing to remove cuticle layers. ④ thermal treatments to remove only cuticle layer under about 200-300 $^{\circ}\text{C}$, ⑤ carbonization to get sharp fracture surface, and ⑥ ashing treatment to remove all organic elements except of silica.

RESULTS AND DISCUSSION

The convex overall views of a husk shows long periodical ridges of with deep cavities ca. 50 μm intervals (Fig. 1C). Surface of ridges are very smooth and periodically(ca. 80 μm) protruded with two breasts making bust-like appearance(Fig. 2A,B). The husk development can be observed by the combination between LM and SEM(Figs. 1-3). The husk tissues are already formed at the just after the flowering stage, although secondary cell walls are a little thin and less lignified(Fig.3A), being compared with the mature ones(Fig.3B). The outermost cuticle layer is also thickened. Thin silica layer covering the tissues has already initiated to secrete on the epidermal cells underneath the cuticle layer at the early stage(Fig.3A), although its non-stainable property is difficult to discern the layer. At the end of August general development of husks were already completed judging from the sectional view of husks under LM, so the major constructing period of the silica layer is judged on mid August. Concave-side silica layer facing the grain is also constructed during the period,

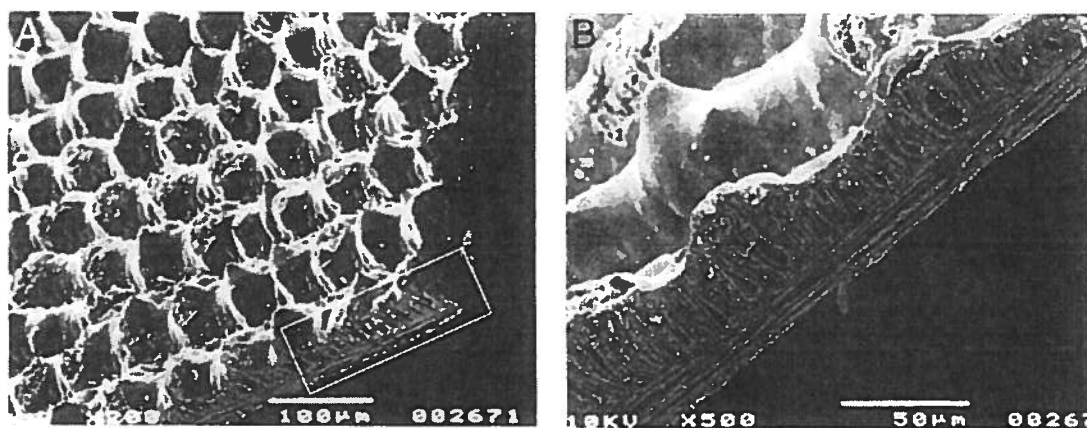


Fig. 2 Outside view of a rice husk and transverse(T) and longitudinal(L) sectional views of a mature rice husk.

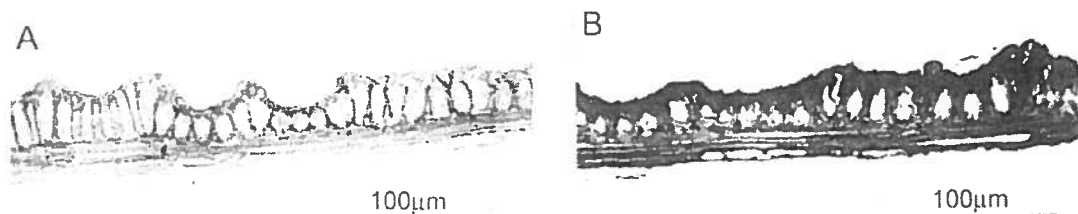


Fig. 3 Comparison between young(A) and mature(B) husks sliced longitudinally. Fig. A is corresponding to a portion of Fig.1A(arrow), and Fig. B to Fig. 2A(arrow). The silica layers are contrasted by the bright defocussing line (arrow heads), while the thicker layers of mature husk having no contrast at the just-focussing.

although it is very thin.

Judging from the various observations, the most peculiar morphological shapes are the outer(convex-side) epidermal cells(Fig. 4) and the silica layer(Figs. 3,6). Each cells showed just like a spider shape(Fig. 4). Their long legs bulge out from the body, being interlocked with those of the neighboring cells with one another. These epidermal cells construct a series of firm shells composed of highly lignified walls and then cover with silica. We call the epidermis-silica layer compounds the macro-shell(Fig. 9A). The four corner legs of each spider are especially protruded making a bust and two breasts joined with neighboring ones. We call these swelling of the bodies and busts the micro-shell(Fig. 9B).

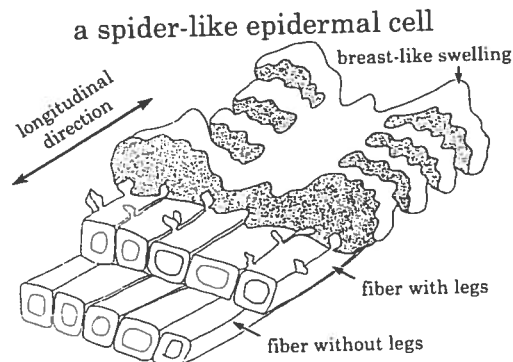


Fig. 4 Illustration of a spider-like epidermal cell covering the convex husk surface.

Outer surface of convex-side epidermal tissue exposed after the removal of silica are very interesting(Fig. 5), although cuticle layers still often cover them. Periodical micro-shell structure having ridges and breast on them are clear. It is worthy to note that long legs of a spider-like cell are inserted with neighboring ones and each legs also teeth like(Fig. 5B). Sides of each tooth are uneven and interlocked with those of neighboring teeth. Their uneven structure are result in the strong anchoring effect to join neighboring ones. More, at edges of teeth many small mounds could be observed(Figs. 5B,9C).

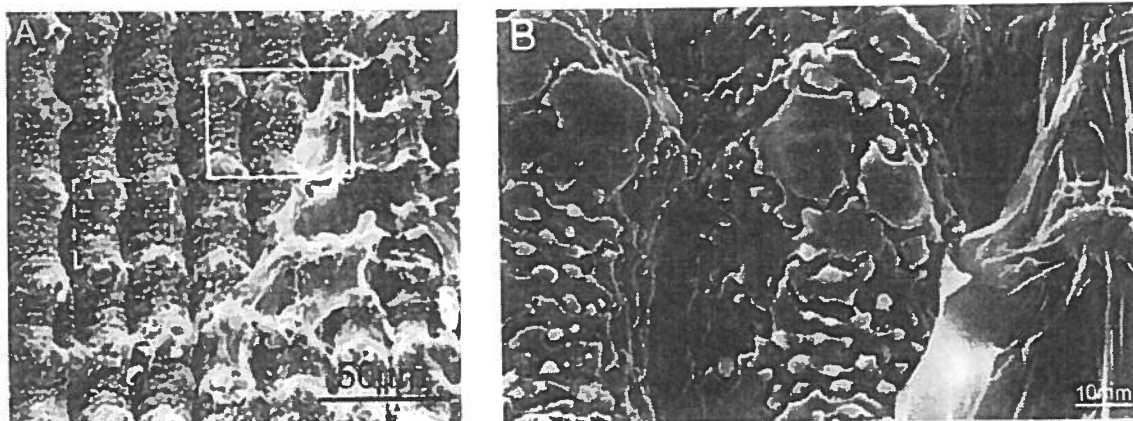


Fig. 5 Outside view of a rice husk after the removal of silica layer by the hydrofluoric acid treatment. Thin membrane at the right-side is residue of the cuticle layer. B is an enlarged portion of A(arrow head). A dotted square shows the area of an epidermal cell(A). Legs are inserted with those of neighboring cells and more interlocked with wavy teeth(B). Small protrusions with a hole(arrow heads) are abundant along their edges(arrows).

In focusing the silica layer, the outer surface is smooth judging from thermal removal of cuticle layer(see the following paper Figs. 1B,3B). However, the inner surface was really replica of the outer structure of cell wall(Fig.6). Many hollows on the inner surface of silica layer are considered to be corresponding to the mounds. The interlocked structure between cell wall and silica layer can be detected on the fractured face of

the carbonized husks(see the following paper Fig. 3B). We call these smaller unevenness the ultra-shell structure(Fig. 9D).The interesting structures are fine sticks located at the small hollows(Fig. 6B). One stick on one hollow suggests a residue of eruption of silica from cell lumen of epidermal cells to the intermediate space between wall and cuticle layer.

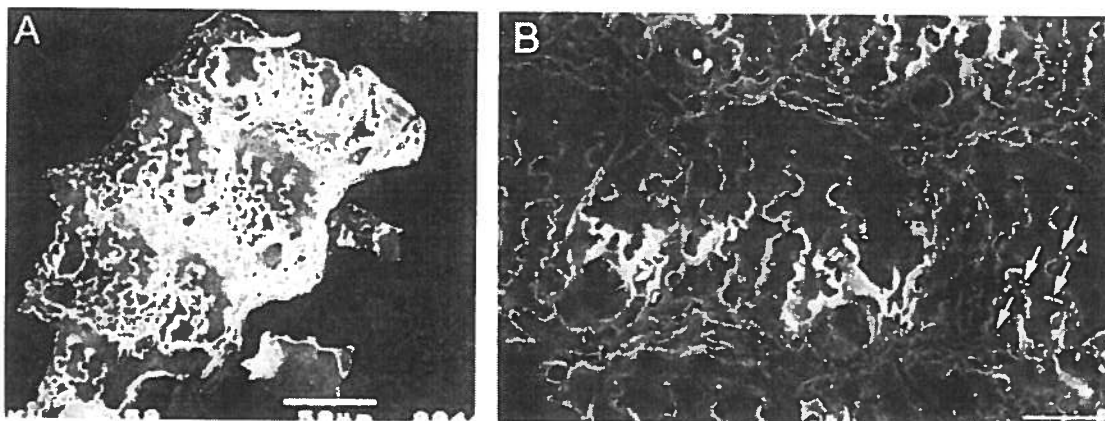


Fig. 6 Inner surface of fragment of silica layer after the removal of cell wall components(A) and the enlarged view(B). Note the replicating picture of Fig. 5 and a small sticks accompanied with hollows(arrows).

More impressive structures are obtained in a white ash which observed from the inner-side(Figs. 7, 8). Transverse bars and central axis are coincided with the legs and the body of a spider, respectively. These frameworks are connected to outer silica shell with the stick, although their connections seem to be very feeble. The sticks in Fig. 6B are residue of the connection. Judging from these photographs, cell lumens of the epidermal cells are filled with the silica. Therefore, the liquefied silica is stocked in cell lumen of epidermal cells and then erupt to the outside, being solidified in both regions. If so, new question will occur. From which route the silica filling cell lumens can be supplied passing through? At any rate the inner-side of micro-shells are reinforced by the peculiar sandwich structure composed of the lumen silica and the leg walls(Fig. 9D). Rice husks are result in the graded shell structures(Fig. 9).

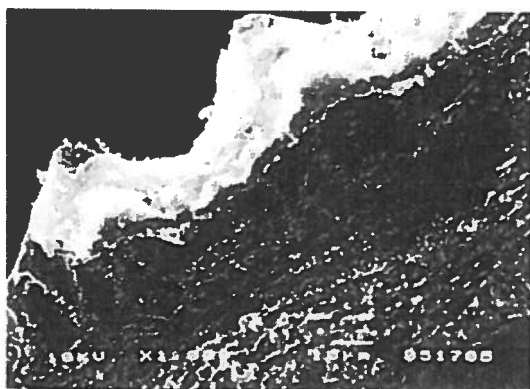


Fig. 7 Side view of a white ash. Inner side of the micro-shell are reinforced with the lumen silica.

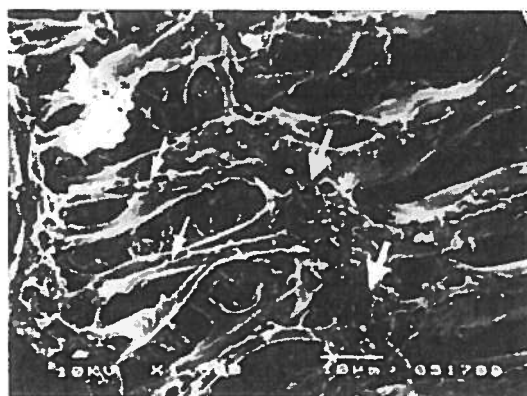


Fig. 8 Adaxial view of a white ash. Cell lumen of a spider body(double arrow) and legs(arrows) are also fulfilled with silica. The frameworks of lumen silica link the outer shell through the sticks(arrow heads).

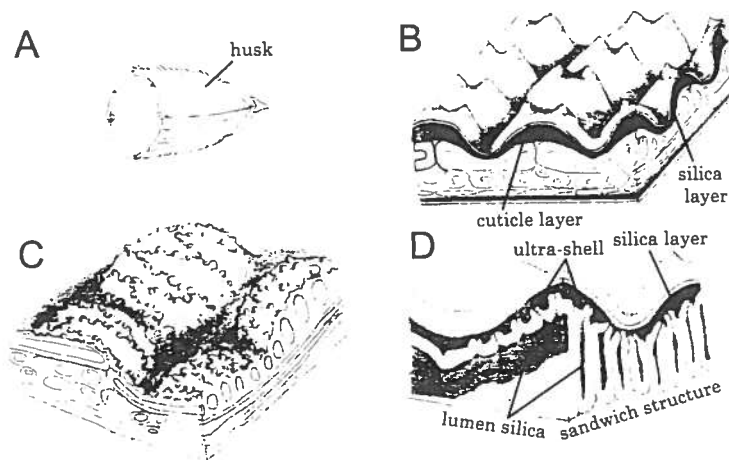


Fig. 9 Illustration of graded shell structures of the rice husk. A:the macro-shell having a big concave space, B:the micro-shells composed of outer protrusion of epidermis-silica complex, C:the outer surface of spider-like epidermal cells. D:the ultra-shells(small arrows) at the interface between silica layer and outermost cell wall, and the sandwich-structure(big arrows) of leg walls and lumen silica reinforcing the micro-shells.

Some Industrial Utilizations of Rice Husks and Silica-Carbon Shells

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Rice husks have very complicated structures being composed of cell walls, layered and lumen silica, and cuticle as shown in the previous report. Organic elements are about 80% composed of lignocellulose, while inorganic ones, mainly of silica, amount 20%. The outer convex-side epidermis has stiff and highly-lignified thick wall. Outer silica layer on them are also thick (1-5 μm). The most interesting structure seems to be the closely-combined cooperation between organic cell walls and inorganic silica layer. Husks are bulky having a large concave space. They are forming the macro-shell (2-5 mm level) of husks themselves having a concave space, the micro-shell (50-80 μm) constructed by the periodical protrusion, and the sandwich structure (ca. 5 μm intervals) transversely reinforcing the micro-shells, and ultra-shell (under 1 μm) distributed on the boundary phase between silica layer and cell wall (see Fig. 8 in the previous paper). That is, the interface between organic and inorganic phases are folded in the four steps and result in very wide. The key of rice husk utilizations must be such graded shell structures.

Even if a husk itself is very small, total ones after the threshing amount to 90 million tons every year. Although they were dispersed on local areas in old time, threshing plants are constructed now around big cities. Especially, husks as the by-products of the rice are discharged enormously at export ports in some south-Asian country. They are valuable and sustainable bio-resources accumulated without any cost by the rice distribution, if their proper usage will be developed. They have high-potential as bio-energy and silicon ingredient. We tried developments of the husk- utilization and will propose some effective grading systems from original husks to ashes.

PROPOSED UTILIZATIONS FROM NATIVE RICE HUSKS TO ASHES

As powder materials

Rice husks are uniform size and may be used as powder materials. However, they are difficult to be processed at the original state because of their poor chemical processing property. The cuticle layers covering husks were shown to protect the permeation of chemicals into husk tissue and to reject the adhesive property (see Fig. 8B in the previous paper). The layers could be removed easily by the short-time thermal treatment of 200-300°C (Fig. 1B). The operation is used to condense the bulky husks temporary for the storage or the transportation, because lignocellulosic walls can be mollified and the macro-shells are compressed. The compressed husks are possible to be fragmented for finer powder materials. In such fine powders, their micro- and ultra-shell structures are still maintained without the cuticle layers (Fig. 1B).

As termite evasion

Wood tips and rice husks were solidified under high pressure respectively and then fed to the termites (*Coptotermes formosanus*). The termites evaded apparently the husk (Fig. 2). Silica layers lining lignocellulosic epidermis are supposed to damage the termite teeth. As termites can not swallow the micro-shells to say nothing of macro-shell, the native shell and ash are expected an effective termite barrier, being different

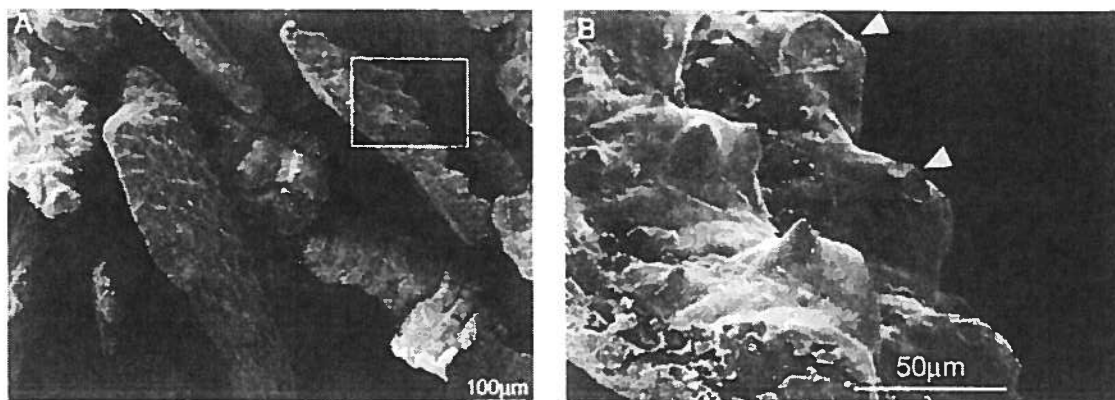


Fig. 1 Husk fragments(A) and the enlarged view(B). The cuticle layer has been breakdown judging from the sharp fractured edges at the breast(arrow heads).

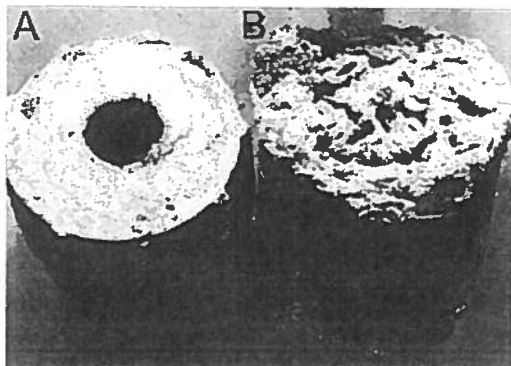


Fig. 2 Difference of termite evasion effect between two lumps of condensed husks (A) and wood chips (B). The lump of husks is uninjured(A), whereas wood-chip one has been attacked(B), severely.

from anti-termite chemicals which are inevitably harmful to us.

As carbonized materials

Organic elements of husks are not flamed up but carbonized under the ordinary burning condition of bio-materials such as wood chips and agricultural wastes. The main reason of nonflammable property must be the non-organic silica layer and the lumen silica cooperated on the organic elements, preventing the oxygen supply. Especially, cell wall layers of each leg are covered with silica layer and impregnated with the lumen silica are difficult to be oxidized in the sandwich-shells and carbonized. This condition results in the carbon-silica complex. The complex also play an important role to support their original morphology of husks(Fig. 3). The strong points of carbonized husks are good liquid- and gas-permeability preserving their bulky macro-shell structure. They will be expected as environmental recovery substance, to say nothing of fuels.

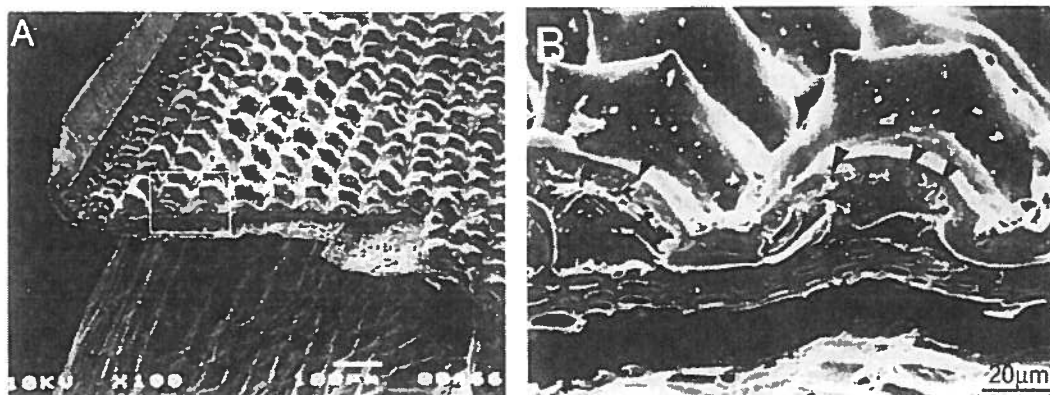


Fig. 3 Fractured surfaces of a carbonized husk(A) and an enlarged portion(B). Tissues reveal their sharp interior structures. Note the interface between silica layer and carbonized walls shows the ultra-shell structure(arrow heads).

As bio-energy resources and silica ash resources

Although rice husks are generally poor flammable as described above, they have high potential of bio-energy resource because of their enormous production ever year. Excreted husks from the big threshing factories are result in very large. In such factory, burning plants are equipped with gas supply system and collect the thermo-energy. Such plants can cover all energy of the factory by the recovered one.

On the other hand, a large amount of ash is also excreted again from the burning plant, although they are smaller than those of husks. Such ashes are also valuable resources as a silicon material. In the actual energy recovery plant, combinations between carbon and silica are changeable under the burning schedules. Ashes are pure white under the aired condition, resulting in silica without carbon, namely, white ash(Fig. 4A, Fig. 7 in the previous paper). On the contrary, grayish carbon-rich ash will be obtained under the deficiency of oxygen(Fig. 4B). Their proper combination is case by case as shown in the following examples. The key of effective utilizations of ashes will be the control of their balance.

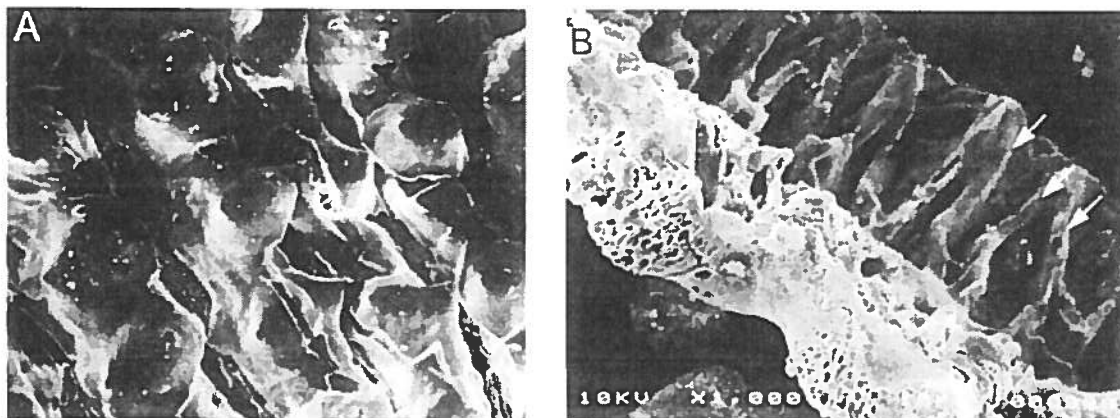


Fig. 4 Outer surface of white ash(A) and inner surface of gray ash under the high-temperature(ca.1000°C)(B). Although the silica layers are cracked sometimes along the cavities, the micro-shells are preserved(A), while the lumen silicas are covered with carbonized cell wall of the sandwich-structures(B).

As silicon resources

Silicon is a very important material for the high-technical industry. The silicon tetrachloride is an intermediate chemicals to produce various silicon goods. Rice husk ashes are inexhaustible silicon resource, because the silicon tetrachloride can be transformed from by the ash. In the transformation from silica, namely silicon dioxide, to the silicon tetrachloride, reduction of oxygen is very important. Carbon elements remained in the gray ash are effective for the remover of oxygen. It is very interesting to note that the close-combination between carbon and silica became possible such transformation.

As inorganic powder materials such as cement or plastic filler

White and gray ashes are still bulky having large concave space of the macro-shell and very light. All organic elements are removed or changed to carbon, so ashes are resistance to flame and not rotten of course. They have very wide surface area ranging from the macro-shell to the ultra-shell, and their surfaces are activated by the burning treatment. For instance, cuticle layers which are trouble in the compound formation have been burnt out. These characters can be used for the building materials. When they are used as cement fillers, we can manufacture the cement board(Fig. 5) and artificial wood which come up the Building Standard.

As major component of new porous ceramics

Melting property of silica layers is favorable to the ceramic materials(Fig. 5). The property is changeable

under the thermal condition and the combination with other components. When ash-based ceramics are manufactured by way of trial(gray ash 40%: siliceous sand 40%: aluminous cement 20%, and during 2 hours under 1300°C), excellent new porous ceramics (bulk density 1.2 g/cm^3 , bending strength 14 N/mm^2 , specific strength 11 N/mm^2 , dimensional stability during the heating 2.5% , water permeation 31%). The ceramics conducts liquids and gases. The interior structures are rich in conductive channels(Fig. 6). These peculiar structures are concluded to be derived from the ashes which have the graded shell structures of the macro-, micro-, narrow-, and ultra-shells. As the porous ceramics is still gray, so the carbon beams of the narrow-shell reinforcing the micro-shells are concluded to be surviving after the thermal treatment.

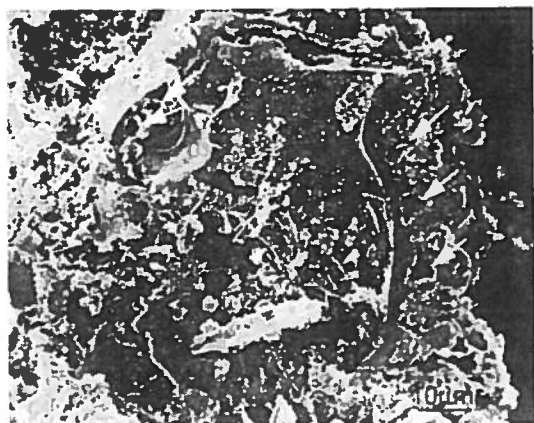


Fig. 5 Inner structure of a cement board fractured and washed in 1N HCl. The micro- and ultra-shell structures(big and small arrows) are remained and fine fibrils are occurred(arrow heads).

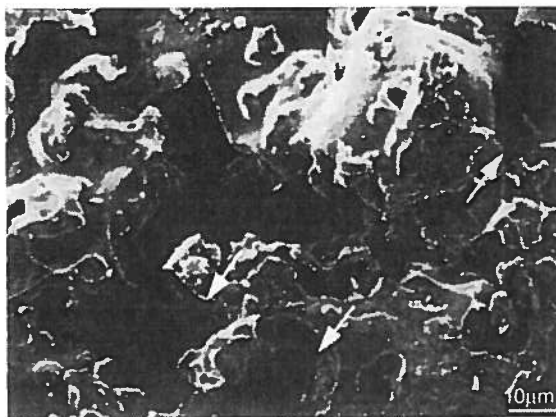


Fig. 6 Fractured structure of ash-base ceramics. Many channels of various sizes are making network(arrow heads).

CONCLUSION

Rice husks themselves are complete natural product, being different from ordinary agricultural wastes such as oil palm or bark. They are designed elaborately to protect rice grain from insects and bacteria. Especially, the graded shell complex constructed with silica layers and cell walls are very beautiful, and the key of effective utilization of bio-materials are shown to apply the original morphological structures.

Increased Co-accumulation of Iron and Silicon may be Responsible for Greener Leaves in Sugarcane Treated with Silicated Amendments

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A silicon research program was established in the Australian sugar industry in 1999 to quantify response to rates of imported calcium silicate slag and local silicated materials (Berthelsen *et al.*, 2001). The rates of calcium silicate experiment at Bundaberg became infected with Orange rust disease (*Puccinia kuehnii* E. J. Butler) in 2000. Plots treated with more than 3 t calcium silicate / ha appeared to producing fewer diseased new leaves during the autumn recovery phase in April 2000. Measurements of "leaf greenness" in SPAD units (Minolta leaf chlorophyll meter) confirmed first fully expanded leaves in treatments receiving 4.5, 6.0, 9.0 or 12.0 t calcium silicate / ha were significantly greener ($p < 0.05$) than treatments receiving 0, 1.5 or 3.0 t / ha applications. This response was maintained through first and second ratoon crops in 2001 and 2002.

The increase in SPAD values at higher rates of calcium silicate was not associated with elevated nitrogen levels in leaf tissue, but was correlated with Si% in the leaf lamina. There was no functional explanation for the impact of silicon on leaf greenness, other than qualitative observations about enhanced recovery from Orange rust. However interpretation of leaf nutrient analysis revealed a significant asymptotic relationship between levels of silicon and iron in leaf tissue. A similar, but stronger, relationship existed between concentration of iron in the leaf lamina and SPAD values. The significant association between silicon and iron in leaf tissue has since been quantified in rates of silicated products experiments at another two sites and as trends in data in an additional three experiments.

Inclusion of iron in the chlorophyll molecule and enhancement of iron accumulation by application of silicates may provide a functional explanation for the apparent association between silicon and greener leaves.

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In Vitro Effects of Potassium Silicate Application and Nitrogen Fertilization on Shoot and Root Growth in *Tagetes erecta* 'Lady First'

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Role of silicon nutrition in growth and stress (biotic and abiotic) resistance in plants has been under investigation in recent years. The interaction of silicon and nitrogen fertilization (NF) on plant growth are not well known. In vitro study is a fast method to understand the role of silicon on plant growth and physiology. Phytatrays (Sigma Co. St. Louis, MO, USA) were lined with Anchor seed germination and steel blotter papers and ml of potassium silicate (PS) solutions at 0, 100, 200 and 400 ppm concentrations were added weekly to each phytatray. Twelve *Tagetes erecta* seeds ('Lady First') were planted per phytatray and kept under 16 hours of light (49.9 μmol) at room temperature. Seeds uniformly germinated within 24-36 hours, and measurements were taken after 28 days. Stem height, and total fresh weight were significantly higher in PS treated seedlings than the controls. Root length, average diameter, surface area and volume were significantly lower than the controls. In a companion study, PS solution supplemented with 0, 200 and 400 ppm Peters fertilizer (N-P-K, 20-10-20) were used in seed germination in phytotrays. Nitrogen fertilizer with PS solutions at 200 ppm significantly increased stem height compare to the controls, but no difference were observed on total fresh weight. Root length, average diameter, and surface area increased with 400 / 200 ppm, (PS/NF), but no difference were observed for root volume. Nitrogen fertilizer with PS solutions at 400 ppm reduced and/or naturalized the effect of silicon. The differences in vitro and vivo application of PS and NF on seed germination and growth of *Tagetes Spp.* will be discussed.

Effect of Silicon on Photosynthetic Rate, Chlorophyll Fluorescence and Chlorophyll Content of Tomato (*Lycopersicon esculentum* Mill.) Plants under Salt Stress

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Physiological response of salt-stressed tomato plants (*Lycopersicon esculentum* Mill.) in the presence and absence of silicon was studied in water-cultured plants under controlled conditions. The treatments in experiment I were; control, 2.5 mM Si as Na_2SiO_3 , 2.5 mM Si with 147.5 mM NaCl and 150 mM NaCl. Experiment II, control, 2.5 mM Si, 2.5 mM Si with 97.5 mM NaCl and 100 mM NaCl. Response to treatments was determined by analysis of growth parameters (leaf, stem and root dry weight), measurements of physiological parameters such as, photosynthesis activity, leaf chlorophyll content, and chlorophyll fluorescence. Exposure of plants to salt stress led to noticeable decrease in photosynthesis rate, stomatal conductance, and chlorophyll content and chlorophyll fluorescence. Plants treated with Si and 97.5 mM salt increased photosynthesis parameters and chlorophyll content, but had little effect in salt treated plants at 147.5 mM in the presence of Si. Chlorophyll fluorescence was measured on dark-adapted leaves, and results indicated that Si added to salt treated plants enhances photochemical efficiency. Leaf, stem and root dry matter content were depressed as salinity increased in plants lacking additional Si, which was alleviated by the addition of 2.5 mM Si to salt treated plants. These results suggest that Si is beneficial in improving the growth of plants in areas of high soil salinities.

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Session 4

Silicate Fertilizers and Crop Production

Silicate Fertilizers in Japan

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INTRODUCTION

The official standard of silicate fertilizer was designated in conformity with the Fertilizer Control Law in 1955 in Japan on the basis of the experimental results on the slag application in rice field, and acid-soluble silicic acid was recognized as one of the main nutrients of fertilizer for the first time in the world. Since then, the slags discharged from various steel industries, which had been handled before then as byproduct calcium fertilizer, have been utilized as slag silicate fertilizer for rice plant. At present, four standardized silicate fertilizers and several standardized phosphate and potassium fertilizers, which are guaranteed with acid-soluble silicic acid, are commercially available in Japan.

OFFICIAL STANDARD OF SILICATE FERTILIZER IN JAPAN

In the official standard of silicate fertilizer, raw material, the minimum content of acid-soluble (0.5 M HCl-soluble) silicic acid and alkalinity, the maximum permissible content of toxic substances and a particle size are set down to maintain the quality of the fertilizer.

1 Slag silicate fertilizer

Slag silicate fertilizer is the major silicate fertilizer in Japan and is made from large variety of slags. Its raw material is restricted to blast furnace slag, ordinary steel slag, stainless steel slag, ferromanganese slag, silicomanganese slag, ferronickel slag, nickel slag, ferrochrome slag, magnesium slag, phosphorus slag and convertor slag. For a fertilizer with guaranteed acid-soluble silicic acid, alkalinity and citric acid soluble magnesium, the minimum content of these main nutrients are 10.0% SiO_2 , 20% CaO or CaO+MgO and 1% MgO respectively. However, the guaranteed content of main nutrients in commercially available silicate fertilizers are to a great extent about 30% for acid-soluble (0.5 M HCl-soluble) silicic acid, 35~45% for alkalinity and 2~5% for citric acid soluble magnesium. As to contaminated toxic substances, for instance, for a fertilizer with guaranteed not less than 20% SiO_2 of acid-soluble silicic acid, maximum permissible content is 0.01% Ni, 0.1% Cr and 0.04% Ti per 1.0% acid-soluble silicic acid and/or maximum limited content is 0.4% Ni, 4.0% Cr and 1.5% Ti and particle size of the fertilizer shall be 100% through a wire sieve with 2.00 mm openings and also the fertilizers other than quenched slag must be 60% more through a wire sieve with 600 μm openings.

2 Porous lightweight concrete powder fertilizer

Porous lightweight concrete powder fertilizer is made from the industrial waste of autoclaved lightweight concrete or autoclaved aerated concrete. Its main component is 11Å Tobermorite, which is a calcium silicate hydrate and prepared synthetically from lime, silica and Portland cement at relatively low temperature by hydrothermal technique. The minimum content of main nutrients of this fertilizer is 15% SiO_2 for acid-soluble silicic acid and 15% CaO or CaO+MgO for alkalinity. For toxic substances, maximum permissible content of Titanium is 1.0% Ti. Particle size shall be 100% through a wire sieve with 4.00 mm openings.

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3 Calcium Silicate Fertilizer

It is natural mineral powder, of which component is $\text{SiO}_2 \cdot \text{CaO}$. The minimum content of nutrients is 20.0% for acid-soluble silicic acid and 25.0% for alkalinity. The particle size of fertilizer must be 100% through a wire sieve 2.00 mm and more than 60% through a wire sieve with 600 μm openings.

4 Silica Gel Fertilizer

The silica gel desiccant for packing is directly used as silica gel fertilizer. It contains 99.7% SiO_2 and little amount of CaO and other components. Particle size must be less than 30% through a wire sieve with 75 μm . It has been proved in some experiments that it is suitable silicate fertilizer for a nursery bed of rice, because soil pH shall not rise by its application.

TRENDS IN SUPPLY AND DEMAND OF SILICATE FERTILIZERS IN JAPAN

Though a demand of the silicic acid fertilizer was over the megaton in 1960's, it decreased continuously from the latter half in 1970's to less than 300,000 ton in 2000, because of a decrease of the paddy field area and tendency of labor saving of farm work. In the meantime, other fertilizers guaranteed with acid-soluble silicic acid in addition to silicate fertilizer were developed, including the phosphate fertilizers such as fused magnesium phosphate, mixed phosphate fertilizer and fused phosphate silicate fertilizer, and the potassium fertilizers such as potassium silicate fertilizer, liquid potassium silicate fertilizer and fused potassium silicate fertilizer. These fertilizers are guaranteed with phosphate or potassium and silicate but also calcium and magnesium and/or manganese. The total production of these fertilizers is almost the same as that of the silicate fertilizer in recent years. About 80 companies have registered about 350 varieties of these fertilizers. On the other hand, about 70 companies have registered about 200 varieties of silicate fertilizers

DISSOLUTION MECHANISM OF SI IN SLAG FERTILIZER IN PADDY SOIL

Based on the results obtained in soil incubation and pot experiment, the reaction in a paddy soil in which the slags were applied are summarized by the scheme shown in Fig.1. The application of the slags brings about increases in the pH and Ca concentration in the soil solution, which may depress the continuous dissolution of the slags, since the solubility of the slags in an aqueous solution decrease with increasing solution pH and Ca concentration in the solution. The supply of CO_2 gas to a soil that received the slags decreased the soil solution pH and increase the Si concentration in the soil solution. These facts suggest the soil pH decreased by the neutralization effect of CO_2 gas, hence, the Si dissolution from both the slags and soil is enhanced. It is assumed that in paddy fields, CO_2 gas which originated from organic materials such as rice straw and root

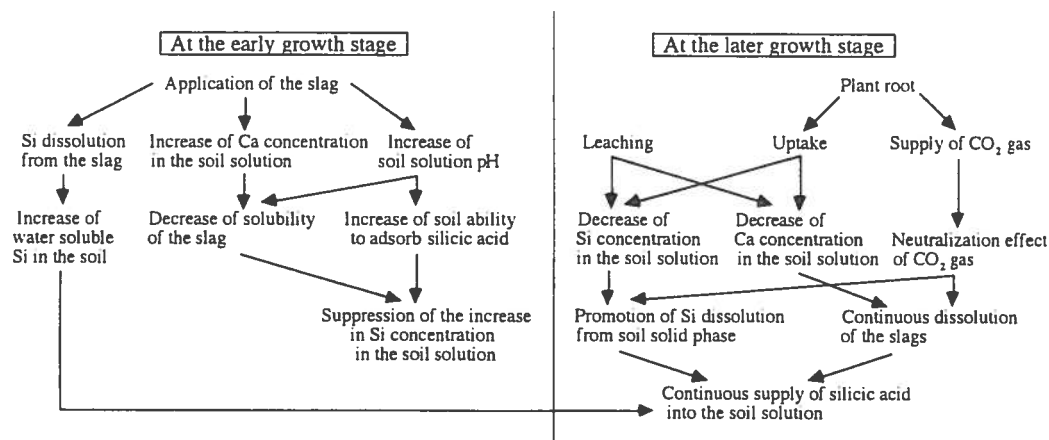


Fig.1. Scheme describing the possible reactions in a paddy soil when a slag is applied.

residue of rice plant, promote the dissolution of the slags. Plant roots influence the environment of the rhizosphere. Nutrient uptake by plant roots is likely to reduce the concentration of the nutrients in the soil solution. This decrease in the concentration of, especially of Ca and Si, may influence the solubility of the slags and/or Si dissolution from soil.

ADSORPTION AND AVAILABILITY OF SI IN SOIL AMENDED WITH SLAG FERTILIZER

It has been reported that the Si concentration in the soil solution decreases when the application rate of the slag is large or when alkalinity of the applied slags is high. In order to clarify the above-mentioned phenomenon, the Si adsorption by a soil where slags were applied was examined by ^{30}Si tracer method. The amount of Si in the solid and liquid phases of soil, which was involved in the isotope dilution during 1 h after the addition of labeled Si (D_{60} -value), was calculated from the ^{30}Si content in the solution. The percentage of the amount of labeled Si that was adsorbed by the soil to that of labeled Si added, %Ar is shown in Fig 2. The %Ar ranged widely from 40% to 87% and was positively correlated with the pH of the soil solution. The solubility of slags in aqueous solution is closely related to the A/Si ratio which is the ratio of alkalinity to 0.5 M HCl-soluble SiO_2 content in the slags and consequently the D_{60} -value tended to increase with the A/Si ratio as shown in Fig.3. The buffering capacity (D_{60}/C , C:Si concentration in the solution measured in slag only) of the soil for Si was

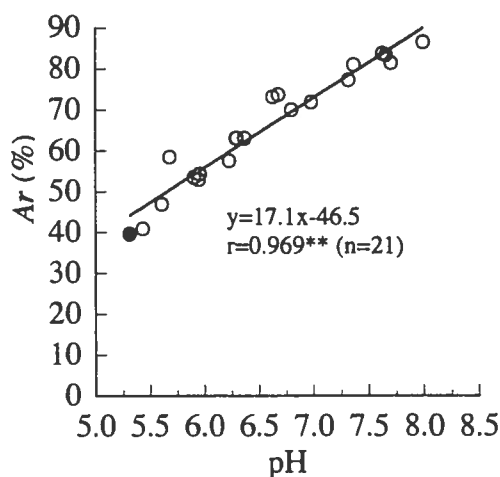


Fig.2. Percentage of the amount of labeled Si which was adsorbed by the soil to that labeled Si which was added to the soil(%Ar). ○, with slag; ●, without slag.

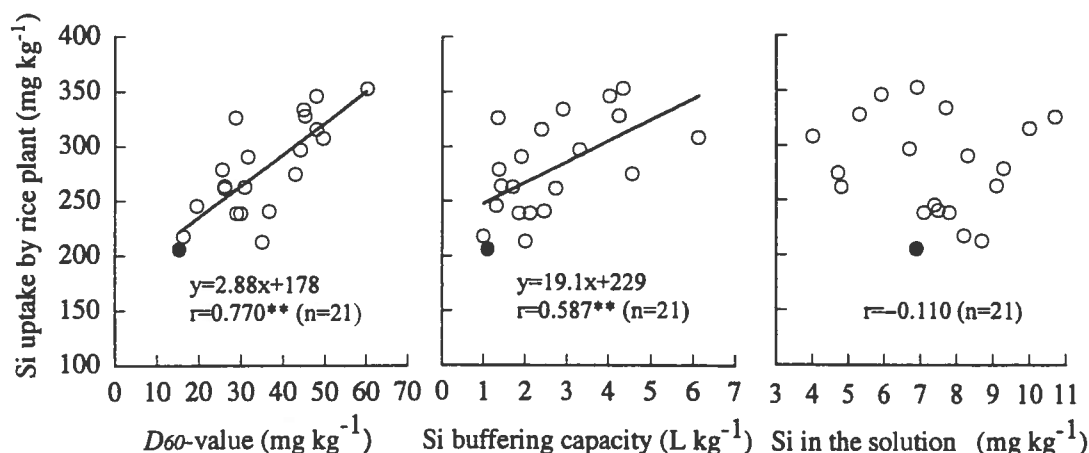


Fig.3. Relationships between the Si uptake by rice plant and the D_{60} -value, the Si buffering capacity, or the amount of Si in the solution obtained in $^{30}\text{Si}(I_0)$. ○, with slag; ●, without slag.

also determined to characterize the soil ability to supply Si continuously into the soil solution. A correlation study was carried out between the amount of Si taken up by rice plant and the D_{60} -value. As shown in Fig.3 the amount of Si taken up by rice plant was positively correlated with the D_{60} -value. A positive correlation was also obtained between the Si uptake and the Si buffering capacity, although the correlation coefficient was lower than that obtained in the D_{60} -value. On the other hand, there was no correlation between the Si uptake by rice plant and the amount of Si in the solution.

THE CONTRIBUTION OF FERTILIZER SILICON TO SILICON NUTRITION OF RICE PLANT

Uptake of Si in silicate fertilizers by rice plant grown on a paddy soil amended with blast furnace slag or calcium silicate hydrate including the main component of porous lightweight concrete powder fertilizer was studied by the ^{28}Si or ^{30}Si tracer method.

1 Blast furnace slag

Two ^{28}Si -labeled blast slag samples with same mineral component, air-cooling and water-cooling one were prepared from ^{28}Si concentrated SiO_2 (34%, ^{28}Si :99.0%), CaO (44%), MgO (75%) and Al_2O_3 (15%). Two soils with different available Si, Fujimi sandy soil(43.0 mg Si kg^{-1}) and Arakawa alluvial soil (117.8 mg kg^{-1}) were selected for the experiment. At one application of ^{28}Si -slags, the pot experiments in the same soil were repeated three times for the straight year. Some leaves were sampled in the growing period, in addition to straw and rough rice at harvest time. The percentage of Si derived from the slag(Sidfsg) was estimated from the isotopic composition of Si in the samples, which was determined by mass spectrometry. The Sidfsg and Si content from slag of the first crop experiment are shown in table 1. The value of Sidfsg was higher for Fujimi sandy soil than for Arakawa alluvial soil in almost all the experimental treatments. It was around 60% at the panicle formation stage(7/28) in the both soil and gradually decreased with progression of growth stage to 20 ~40% in the Fujimi soil and 10~15% in the Konosu soil at harvest time. It shows that the soil Si is uptaken by rice plant further than the fertilizer Si at the late growth stage. No appreciable difference was observed in the amount and recovery rate of slag Si(Rsgsi) between two slags and also two soils and Rsgsi was 23~30% at 2.0 g S- SiO_2 /pot rate and 32~33% at 4.0 g S- SiO_2 /pot rate. Si uptake from slag at 4.0 g S- SiO_2 /pot was 1.7 times for air-cooling slag and 1.5 times for water-cooling slag at 2.0 g S- SiO_2 /pot rate. %Rsgsi at the second harvest decreased to 50~75% for Fujimi soil and 6th for Kounosu soil compared to that of first harvest. The sum of the %Rsgsi for first and second harvest was 40.0~48.6% at 2.0 g S- SiO_2 /pot rate and 29.5~43.9% at

Table 1. The percentage, content and recovery rate of Si derived from slag at the first crop experiment

Soil type	Slag type	Rate of slag application (g S- SiO_2 /pot)	% Sidfsg				Sidfsg (mg/pot)					Recovery rate of slag Si (%)
			7/28	8/25	Straw	Rough rice	7/28	8/25	Straw	Rough rice	Total	
Fujimi Sandy soil	Air-cooling	2.0	56.3	58.9	37.1	25.2	75	158	331	73	637	31.9
		4.0	68.8	64.9	50.9	40.8	107	275	592	135	1109	27.7
	Water-cooling	2.0	56.2	56.7	37.3	21.8	89	177	347	56	669	33.5
		4.0	64.9	62.9	50.3	32.3	92	301	595	100	1088	27.2
Arakawa Alluvial soil	Air-cooling	2.0	56.0	30.6	16.1	8.5	136	129	344	47	656	32.8
		4.0	70.9	42.7	24.7	16.1	161	312	631	90	1194	29.9
	Water-cooling	2.0	38.7	32.1	17.2	9.6	104	226	291	48	669	33.5
		4.0	44.2	38.8	23.6	13.8	122	191	544	65	922	23.1

0.1M-HCl soluble SiO_2 content of ^{28}Si -slag : Air-cooling sample : 33.24%, Water-cooling : 33.15%

^{28}Si atom % excess of slag sample : Air-cooling : 5.67, Water-cooling : 5.50

4.0 g S-SiO₂/pot rate. At the third harvest it was impossible to estimate the Sidfsg of rice plant samples for Konosu soil, because those ²⁸Si content were near to natural abundance. The sum of the %Rsgsi of three harvest for Fujimi soil was 46.5~56.6%.

2 Calcium silicate hydrate

The five kinds of ³⁰Si-labelled calcium silicate hydrate(CSH) were prepared synthetically from ³⁰Si concentrated SiO₂, CaO and concrete by hydrothermal method. The rational formula and SiO₂ content of CSH are shown in table 3. The content of Si in CSH is 42.7~50.5% and appreciably higher than that of slag silicate fertilizer. Among CSH, Tobamorite is the main component of porous lightweight concrete powder fertilizer. As described in the blast furnace slag experiment, %Sidfsg was estimated from the isotopic composition of Si in the some parts of rice plant, which were sampled from ³⁰Si-treated pot with Fujimi sandy soil at tillering stage, heading stage and harvest time(Table 3). Table 3 suggests that calcium silicate hydrate would be classified into 3 types by the pattern of change of Sidfsg with the growth of paddy rice. ①Tobamorite and Pseudo-wallastonite: Though the %Sidfsg was very high at early growth stage, it appreciably decreased at harvest stage. ②C-H-S and Xonotolite: The %Sidfsg was kept fairly constant throughout the cropping period. ③ Gyrolite: The %Sidfsg was lower than other calcium silicate hydrate to a large degree at early growth stage, whereas it increased gradually and was highest in rough rice. The %Rsgsi showed great difference among the compounds and was 73% for Tobamorites, around 60% for C-S-H and Xonotolite, 53% for Pseudo-wallastonite and 38% for Gyrolite.

Table 2. Rational formula and SiO₂ content of Calcium Silicate Hydrate (CSH)

Name of CSH	Rational formula	SiO ₂ content (%)
Tobamorite (A)	Ca ₅ [(SiAl) ^(a) ₆ O ₁₈ H ₂]4H ₂ O	50.42
Tobamorite (A)	Ca ₅ [(SiAl) ^(b) ₆ O ₁₈ H ₂]4H ₂ O	42.74
C-S-H	5CaO · 6SiO ₂ · 5H ₂ O	44.24
Gyrolite ^(c)	Ca ₆ (Si ₁₂ O ₃₀)(OH) ₄ · 7H ₂ O	50.49
Xonotlite	Ca(Si ₆ O ₁₇)(OH) ₂	54.13
Pseudo-wallastonite	CaO · SiO ₂	51.72

(a) Al/(Al+Si)=0.13, high degree of crystallization

(b) Al/(Al+Si)=0.10, medium degree of crystallization

(c) ill-crystallized calcium silicate hydrate

Table 3. Percentage of Si derived from CSH and recovery rate of Si in CSH

Calcium silicate hydrate	Tillering stage			Heading stage			Harvest time		Recovery rate of Si in CSH (%)
	Lower leaves	Upperr leaves	Tillers	Lower leaves	Upper leaves	Tillers	Straw	Roough rice	
Tobamorite A	76.0	76.0	75.7	69.0	61.7	32.3	62.5	25.0	73.1
Tobamorite B	74.6	76.3	75.3	70.8	68.4	39.9	66.4	31.2	73.4
C-S-H	61.8	61.8	60.2	58.7	62.5	62.2	59.7	52.8	58.4
Gyrolite	28.8	23.7	26.3	31.4	56.4	68.6	50.6	61.9	38.7
Xonotlite	60.2	65.9	62.8	57.9	64.4	59.4	59.9	55.1	60.9
Pseudo-wallastonite	71.2	73.1	69.7	64.4	54.9	41.3	53.5	45.8	53.2

EVALUATION OF SI AVAILABILITY IN SLAG FERTILIZERS

In Japan, Si availability in the slag has been evaluated by a chemical extraction method using 0.5 M HCl (Official Method of Fertilizers (Ministry of Agriculture, Forestry and Fisheries). However, this extraction method often overestimates the amount of Si actually available in slags. Although another extraction method

by an acetate buffer solution (1 M, pH 4.0) has been devised as a better evaluation method, it is still not suitable for the evaluation of Si availability in slags. These facts show that unavailable Si is concurrently dissolved by the acidity of the extractants. For the compensation of these discrepancies, it is considered that the amount of available Si in the slags should be estimated under conditions similar to those in paddy soils. When the slags are applied to soils, the increase in the soil pH is relatively small because of the pH buffering capacity of soils. The soil solution pH increase by the dissolution of Ca and Mg from the slags and the development of soil reduction and is depressed by the neutralization effect of CO₂ gas in the rhizosphere. These neutralization effects should be taken into account for the estimation of Si availability in the slags. Based on these findings on the dissolution process of the slags in paddy fields, a new extraction method for the evaluation of Si availability in slag fertilizers was developed. In the method, the slags were dissolved in water with the addition of a weakly acidic cation exchange resin (H form). The Si dissolution from the slags was enhanced by the addition of the resin. The pH of the extractant was well controlled between 6 and 7 during extraction. The percentage of Si extracted by the new method was in the same range as that of the Si recovery rate by rice plant and a positive correlation was obtained as shown in Fig.4. As a result, Si availability in the slags could be evaluated more precisely by the method proposed here than by using the traditional methods.

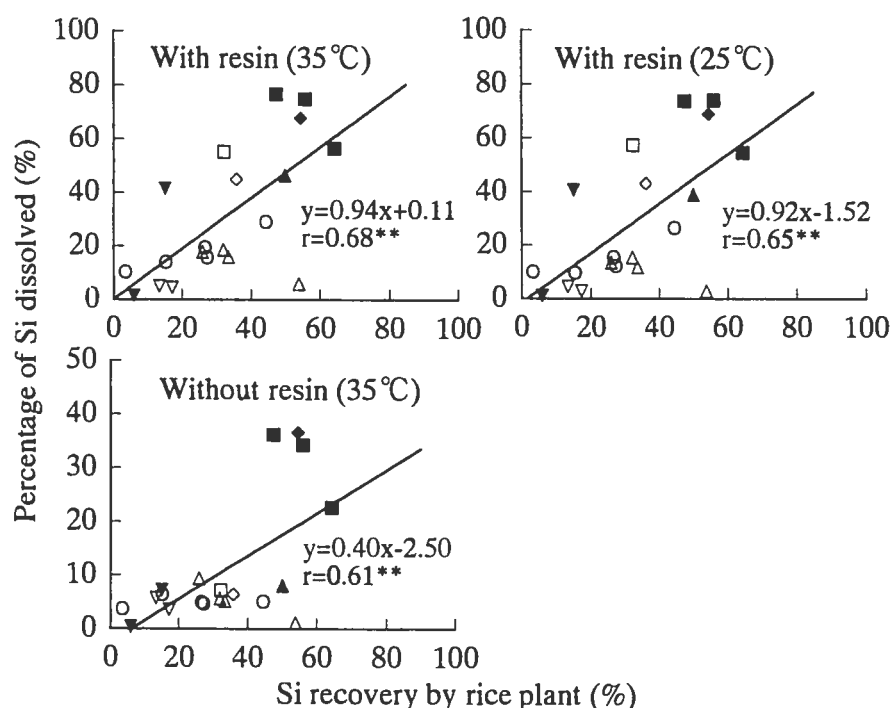


Fig.4. Comparison between the Si recovery rate by rice plant and the solubility of the slags in water with or without the addition of resin. ○,blast furnace slag; △,silico-manganese slag; ▽,ferro-nickel slag; ▲,phosphorus slag; ▼,ordinary steel slag; ◇,stainless steel slag; □,ferrochrome slag; ◆,magnesium slag; ■,convertor slag. $^{**}p < 0.01$

Silicon Application in Nutrient Solutions for Horticultural Crops

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INTRODUCTION

The significant role of silicon in the plant nutrition for specific horticultural crops has been demonstrated by several researchers (Adata and Besford, 1986; Horst and Marschner, 1978; Vaughan and O'Neal, 1989) and has been worked out in detail in Voogt and Sonneveld (2001). Silicon supply is most relevant for cucumbers (and other cucurbitaceae like courgette and melon) and roses in protected cultivation since in northwestern Europe these crops are practically all grown in substrates. These are high value crops, grown in high-tech production systems with complete control over nutrient supply, adapted to the crop requirements in time (Voogt and Sonneveld, 1997). This requires sophisticated methods to supply the crop with sufficient silicon during the growing period. Important in this connection is to weigh up the qualitative and quantitative aspects of Si supply at one hand and the total costs of the Si fertilisers and the dosing system on the other. In this contribution the emphasis is laid on the search for substrates with sufficient silicon for plant growth and possibilities of Si supply in the nutrient solution and on estimations of the profitability of Si supply.

SILICON IN GROWING MEDIA

In order to find out the potential in supply of silicon to plant roots of some growing media, a survey was made on a number of materials, which are used as growing medium, or could be used as component in mixtures. The total silicon content was looked up or determined and the potential release of soluble silicon was determined (Kipp *et al.*, 2000). The substrates from mineral origin contain high quantities of total Si, however the effective concentrations in the root environment are eventually poor in all of them (Table 1). In the organic substrates, the quantities are low, except for rice-hull, but they do not differ much in concentration in the root environment from the mineral substrates. Rice-hull is an exception among the organic substrates, not surprisingly because of its origin (Gascho, 2001). The total Si quantity is as high as in some mineral substrates, but most important is the high concentration in the root environment, which is highest of all substrates tested.

Tests were carried out with some of the substrates listed in Table 1, in order to monitor the Si release in time. With rockwool, the substrate commonly used for both cucumbers and roses, the release is rather poor (Figure 1). Only in the case of used rockwool in the first weeks, a relevant quantity is released. The crop uptake, expressed on the water uptake, is estimated at $0.5 - 0.7 \text{ mmol Si l}^{-1}$. With an average water consumption of $3 - 4 \text{ l m}^{-2} \text{ day}^{-1}$, the total demand is roughly $1 - 3 \text{ mmol Si m}^{-2} \text{ day}^{-1}$.

The release of silicon from growing media was also studied in combination with plant growth. Cucumber was grown in four types of growing media, the release was monitored throughout the growing season. The treatments were: Rice Hull mixed with white peat in a 1:1 volume based ratio and laid out in grow-bags; New standard commercial high density rockwool slabs; The same type of slabs, but used for two years; Pumice stone substrate. In the rockwool slabs, both new and used, and in the rice hull/peat mixture, the concentrations were much higher in the beginning of the growing period than later on (Figure 2). With rice hull substrate, significantly higher Si concentrations in the root environment were found than with the other treatments. Pumice

Table 1. Silicon contents in several materials, used as growing medium in horticultural crops, either in pure form or in mixtures with other materials, the range of total silicon in mmol Si kg^{-1} dry matter and the average, minimum and maximum concentration found in the root environment in mmol Si l^{-1} (after Kipp et al. (2000)).

Medium	Total Si		average	Si concentration	
	mmol kg ⁻¹			mmol l ⁻¹	
	min	max		min	max
<u>Mineral</u>					
Rockwool new slab	7200	8000	0.2	0.1	0.4
Rockwool old slab	7200	8000	0.3	0.1	0.7
Glasswool	13300	15000	0.1	0.0	0.2
Perlite	11600	13500	0.1	0	0.2
Pumice stone	7500	8400	0.1	0.1	0.3
Expanded clay	8500	9500	0	0	0
<u>Organic</u>					
Wood fibre	100	1800	0.1	–	–
Peat	15	220	0.1	0.0	0.2
Bark	76	850	0.1	–	–
Rice Hull	2200	2600	0.8	1.1	2.5
Compost	2000	4500	0.2	0.1	0.7
Coir chips	1200	1250	0.0	–	–
Coir dust	1200	1250	0.1	–	–
<u>Artificial</u>					
Polyurethane	0	0			

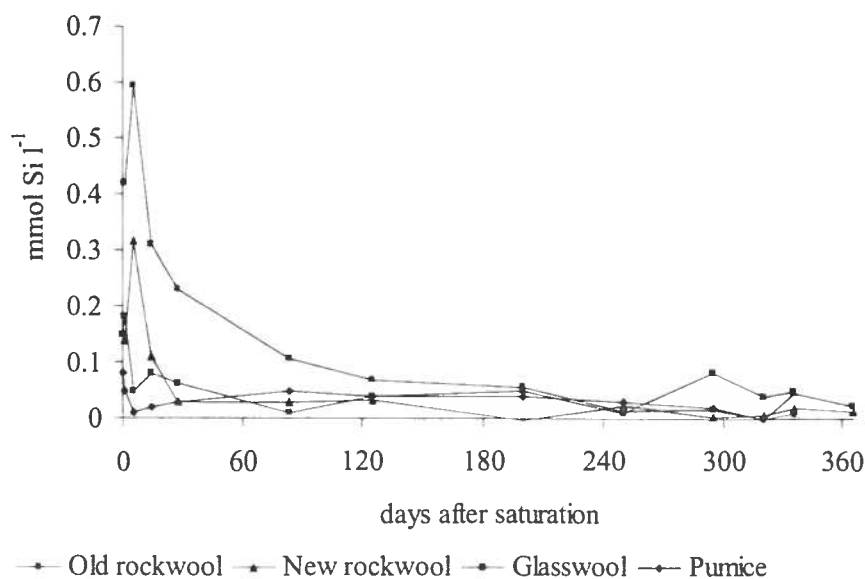


Figure 1. Release of Si from four growing media used for growing cucumbers, expressed in $\text{mmol Si l}^{-1} \text{ day}^{-1}$. The media were saturated with nutrient solution and monitored throughout one year

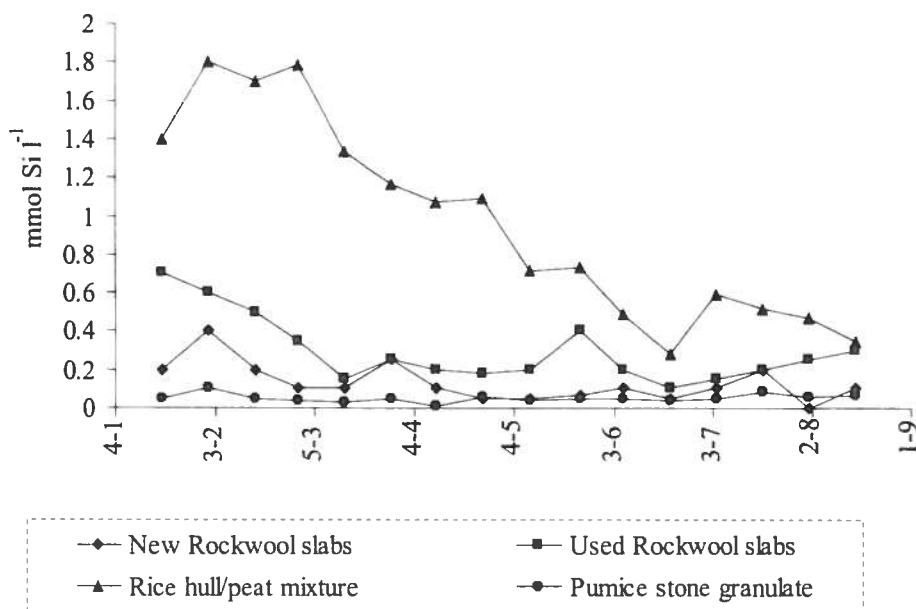


Figure 2. The course of the Si concentration in mmol l^{-1} in the root environment of four growing media, without additional Si supply, during a long term cucumber crop

substrate was almost devoid of soluble Si, also at the beginning. The strong depletion of Si from the root environment indicates an active uptake of this element. This phenomenon has been described before (Voogt and Sonneveld, 2001). It also indicates that the demand of the crop is higher than the quantity of Si released from the substrate. So it can be concluded that the supply of Si from rockwool and pumice substrate is greatly insufficient. Rice Hull did perform better, but the release in time does likely not match with the crop requirements. In addition, Rice hull, contains a high quantity of Mn, which is released rapidly. The concentrations of Mn in the root environment found with this substrate are as such that Mn toxicity could easily occur in susceptible crops (Sonneveld and de Bes, 1984).

SILICON SUPPLY

To overcome the lack of control over the Si release from the growing medium itself, intensive search have been carried out looking for possible soluble silicon sources. From the chemical properties of silicon it follows that, within the pH range required for optimum plant growth, silicon will be present either in form of mono silicic acid ($\text{Si}(\text{OH})_4$), or in stable polysilicates, however, the monosilicic acid form only in limited concentrations (Iler, 1979; Lindsay, 1979). From experimental work it was found that Si concentrations of $0.75 - 1 \text{ mmol l}^{-1}$ are sufficient to meet the Si requirement for most crops (Voogt and Bloemhard, 1992).

A number of experiments have been conducted to find an applicable way to distribute silicon in the nutrient solution (Voogt, 1989¹; Voogt, 1989²; Voogt and Kreuzer, 1989). Polysilicates with large particle size are very stable in nutrient solutions and could even be mixed with the concentrated fertiliser stock solutions. Application in this form has a lot of advantages, however the uptake appeared to be very limited. A close relationship was found between the particle size and the Si quantity in the plant tissue (Figure 3). The best results were obtained with mono silicic acid.

In an experiment with substrate grown cucumber, a comparison was made between the different methods of making Si available for the plant. In comparison with a control treatment without Si supply, Si was supplied continuously in the nutrient solution as monomere $\text{Si}(\text{OH})_4$ and was compared with Si supply by the growing medium itself; a rice hull-peat mixture substrate, a self prepared rockwool slab with silica gel and a factory prepared silica containing rockwool slab. The yield response was clearly affected by the treatments, showing

significant negative results for the rice hull-peat substrate (Table 2) . The other treatments with Si supply tend to give better yields than the zero treatment (although not significant). The Si concentrations were definitely high in the Si containing substrates in the first two months of the experiment, but were much lower (rice hull) to even devoid of Si (silica gel slab) in the last couple of months.

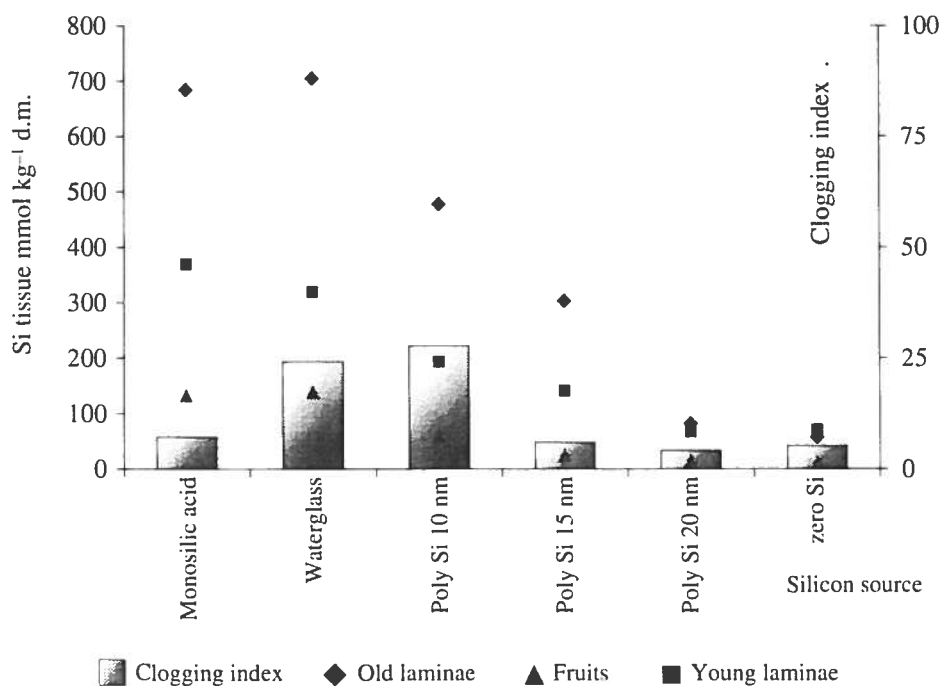


Figure 3. The performance of some Si forms: monosilic acid, waterglass and three polysilicates with particle size ranging from 10 – 20 nm, with regard to the stability in nutrient solutions, expressed as index for clogging of the drip irrigation, and its effectiveness for plant uptake, expressed as the Si content in some tissues of cucumber, grown in waterculture with these Si forms.

Table 2. The effect of different methods of Si application to the root environment on the yield of cucumber (kg m^{-2}), the Si concentration in the root environment in mmol Si l^{-1} , averaged over two periods and the Si content in young full grown leaves of the plant at two moments, in the growing period, in mmol Si kg^{-1} dry matter (Voogt 1989; Voogt 1990)

Treatment		Yield		Si Root environment		Si tissue	
		2 months	6 months	3 months	6 months	3 months	6 months
Control, zero Si	38.2 ^a	0.12	0.15	68	125		
Si supplied in nutrient solution	40.1 ^b	0.41	0.68	425	625		
Rice hull-peat mixture	36.1 ^c	0.75	0.48	459	602		
Slab with silica gel	38.9 ^{ab}	0.63	0.25	380	420		
Prepared slab	39.2 ^{ab}	0.38	0.32	289	302		

PREPARATION OF NUTRIENT SOLUTIONS

Monosilic acid in a nutrient solution could be derived from several sources. However, not all of them are applicable because of practical reasons. In soilless culture systems, plant nutrients are supplied by the irrigation water continuously. Solutions are composed from soluble fertilisers, mixed in stock tanks and diluted by a fertiliser supply unit. None of the sources of mono silicic acid, however, could be supplied in this way, since they could not be mixed with other fertilisers, without getting problems with precipitation (Iler, 1979). As an

alternative, the fertiliser could be supplied separately by injection in the water stream, provided that the Si source is in liquid form. However, if mono silicic acid is concentrated, it becomes unstable and polymerises rapidly. A settlement for this problem is the use of mixtures of KOH and Si(OH)_4 . Essential for the stability is a KOH : Si(OH)_4 mole ratio of at least 1.8, to avoid polymerisation. At high pH, the equilibrium of monomere Si(OH)_4 and polymeric silica is extremely shifted towards Si(OH)_4 (Iler, 1979). For use as fertiliser in horticulture a special compound is developed, called potassium-metasilicate. It has a mole ratio of K : Si of 2 and is obtainable as a concentrated solution with 13% SiO_2 . Corrections must be made for the quantity of potassium and hydroxid in this fertiliser naturally, and could be easily achieved by the calculation methods of fertiliser formulas from standard nutrient solutions (de Kreij et al., 1999). In this way, introduction of acids in the formulas is unavoidable in equilibrium with the OH^- input from the potassium-metasilicate. An example of the result of such calculation is made in Table 3.

With the use of potassium-metasilicate as Si source some practical problems are involved.

1. A complication is the necessity of separated supply of the potassium-metasilicate from the other fertilisers. The supply of fertiliser solution is tuned and controlled on the basis of the EC setpoint. This setpoint is not fixed, but will fluctuate throughout the growing season, or even per day or per hour, and is determined by the grower and a number of parameters. Since the required acid – tuned to the Si concentration to be supplied in the solution – is inevitably included in the fertiliser solution, the acid supply is fluctuating consequently. It is therefore strongly recommended to synchronise the Si supply with the fertiliser supply in a fixed rate. A disadvantage however is the changing Si concentration in the nutrient solution supplied to the plants.
2. The necessary acid supply could easily disturb the normal pH control and subsequent supply of acid or base after the fertilisers supply. One possible solution is to move the final pH control to a position further downstream the nutrient solution.
3. For some water sources the bicarbonate present must be neutralised by including acid in the fertiliser formula as well. The acid required for the silicon in addition can result in such high quantities of acid in the fertiliser formula, that a stable pH control is strongly hampered. In such a situation it is recommended to supply the potassium-metasilicate beforehand in the water, for instance in a buffer tank. This will work out also for the problem outlined in 2. The disadvantage of this is that because of the changing supply of fertiliser solution and subsequent correction with acid or base from the pH control, the concentration of NO_3 and K (in case of HNO_3 and KOH as pH correction, respectively) can fluctuate.

OPTIMISATION OF SI SUPPLY

The most significant effects of Si supply on cucumber and rose found, are on the reduction of the susceptibility for powdery mildew (Menzies *et al.*, 2001). Other reports also mentioned yield increase (Voogt and Sonneveld, 2001). However, with cucumber specifically the bloom on the fruit does negatively affect the fruit quality (Voogt and Bloemhard, 1992). So the benefits of Si and the costs of the supply must be considered against each other. A calculation was made to assess the break-even point for the supply of the Si compound, estimated yield increase, reduction in fungicide treatment, etc. The calculations were based on trials with a range in Si concentrations.

The costs of Si supply is linearly connected with the Si consumption, the current price is €1.65 kg^{-1} . The Si consumption is strongly correlated with the transpiration (Voogt, 2001). Depending on the cropping system and climatical situation (transpiration), the total Si consumption ranges from 0.2 to 0.3 $\text{kg m}^{-2} \text{ year}^{-1}$ for cucumbers and from 0.18 to 0.25 $\text{kg}^{-1} \text{ year}^{-1}$ for roses. The changing in the fertiliser formula, involve also an increase in costs (€0.05 per mmol supplied Si $\text{m}^{-2} \text{ year}^{-1}$). The costs of the supply equipment (€0.045 $\text{m}^{-2} \text{ year}^{-1}$) are taken into account as well. Opposites that are savings, made on the necessary fungicide treatments (powdery mildew). The savings could be assessed from the reduction in labour and costs of the fungicide, which was maximum €0.22 at the highest Si treatment. These costs must be weighed up to the yield increase.

Table 3. Scheme for the calculation of a fertiliser formula from the basic composition of a nutrient solution (in mmol L⁻¹) to a 100 times concentrated stock solution (in kg m⁻³). Scheme A in case of a standard nutrient solution for cucumber, without Si. Scheme B with silicon. To make things easy, nutrient solutions and fertiliser formulas are presented without micro elements

Scheme A (without Si)

Standard composition

		NH ₄	K	Ca	Mg	NO ₃	SO ₄	H ₂ PO ₄
	mmol L ⁻¹	1.25	8	4	1.375	16	1.375	1.25
Fertiliser								
KH ₂ PO ₄	1.25		1.25					1.25
Ca(NO ₃) ₂	4			4		8		
NH ₄ NO ₃	1.25	1.25				1.25		
MgSO ₄	1.375				1.375		1.375	
KNO ₃	6.75		6.75			6.75		

Fertiliser formula for concentrated stock tanks

	1 m ³	100 times concentrated
A tank		
Calcium nitrate		72 kg
Ammonium nitrate		19.5 kg or 15.7 L
B tank		
Monopotassium phosphate		17 kg
Magnesium sulphate		33.4 kg
Potassium nitrate		68 kg

Schema B (with Si)

Standard composition

		NH ₄	K	Ca	Mg	NO ₃	SO ₄	H ₂ PO ₄	Si	OH ⁻ *	H ⁺ *
fertiliser	mmol l ⁻¹	1.25	8	4	1.375	16	1.375	1.25	0.75		
KOH + Si(OH) ₄	0.75		1.5						0.75	1.5	
HNO ₃	1.5					1.5					1.5
KH ₂ PO ₄	1.25		1.25					1.25			
Ca(NO ₃) ₂	4			4		8					
NH ₄ NO ₃	1.25	1.25				1.25					
MgSO ₄	1.375				1.375		1.375				
KNO ₃	5.25		5.25			5.25					

* OH⁻ and H⁺: not as part of the basic composition but for settlement purposes.

Fertiliser formula for concentrated stock tanks

	1 m ³	100 times concentrated
A tank		
Calcium nitrate		72 kg
Ammonium nitrate		19.5 kg or 15.7 L
B tank		
Monopotassium phosphate		17 kg
Magnesium sulphate		33.4 kg
Potassium nitrate		53 kg
Nitric acid (38%)		25.1 kg or 18.5 L
Silicium solution		
Potassium metasilicate		0.23 g/L**

** Supply of pure commercial product (solution), in g/L nutrient solution

The yield response was derived from several experiments with a range in Si concentrations (Voogt and Sonneveld, 2001) The yield increase of Si supply was estimated on the safe side, at 2%, 3% and 4% for Si levels of 0.25, 0.5 and 0.75 mmol l⁻¹ and up respectively. The net profit of the yield increase is obtained by corrections for the direct costs.

With the data specified above, the results of the calculation show that the break-even point for Si supply is a concentration of 1.5 mmol l⁻¹ (Figure 4 top). However, in addition, at Si supply above 1 mmol Si l⁻¹, the blooming on the fruit is as such, that it probably reduces the price in the market and should be taken into account as well. The effect of the quality downgrade on the turnover was estimated, based on the observations on the incidence of blooming in relation to the Si supply and the assumption that the price reduction is at least

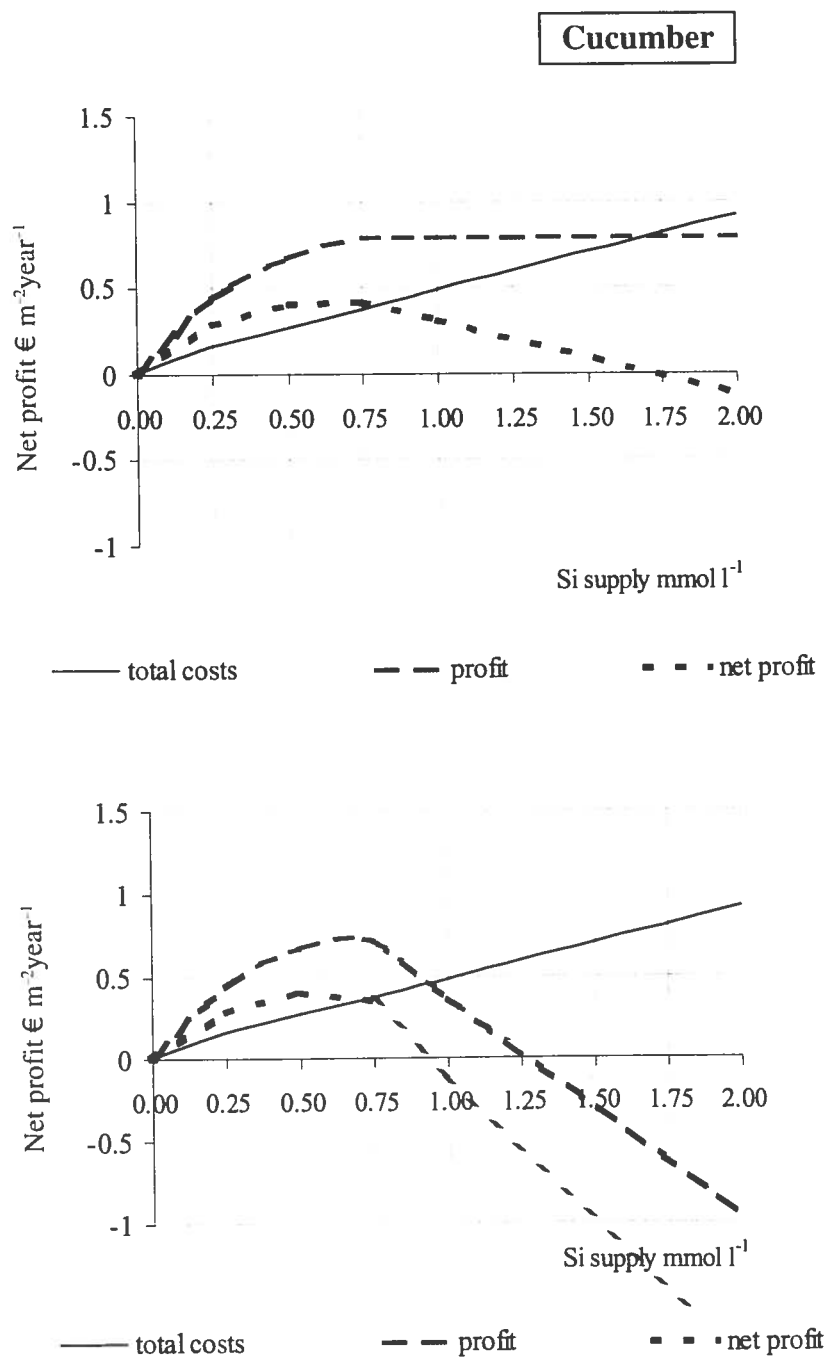


Figure 4. The results of calculations of the break-even point of Si supply in form of potassium-metasilicate, for cucumber, taking into account the effect on yield and powdery mildew (top graph) and in addition the effect on fruit quality (bottom graph).

20%. The result of the calculation in that case is a break-even point about 0.85 mmol/l with a dramatic decrease in the net profit of Si supply above 1 mmol l⁻¹ (Figure 4 bottom). For rose, the break even point is much higher, since the total turnover of this crop is much higher (Figure 5 top). No negative aspects on Si supply on product quality of rose have yet been observed (Voogt and v. Elderen, 1991). The calculation model also show that Si supply for just powdery mildew control is only profitable if the number of additional fungicide applications is reduced to zero (Figure 5 bottom). However, this won't be the case, since silicon cannot prevent mildew infection completely (Belanger *et al.*,1998). From practical experience it was estimated

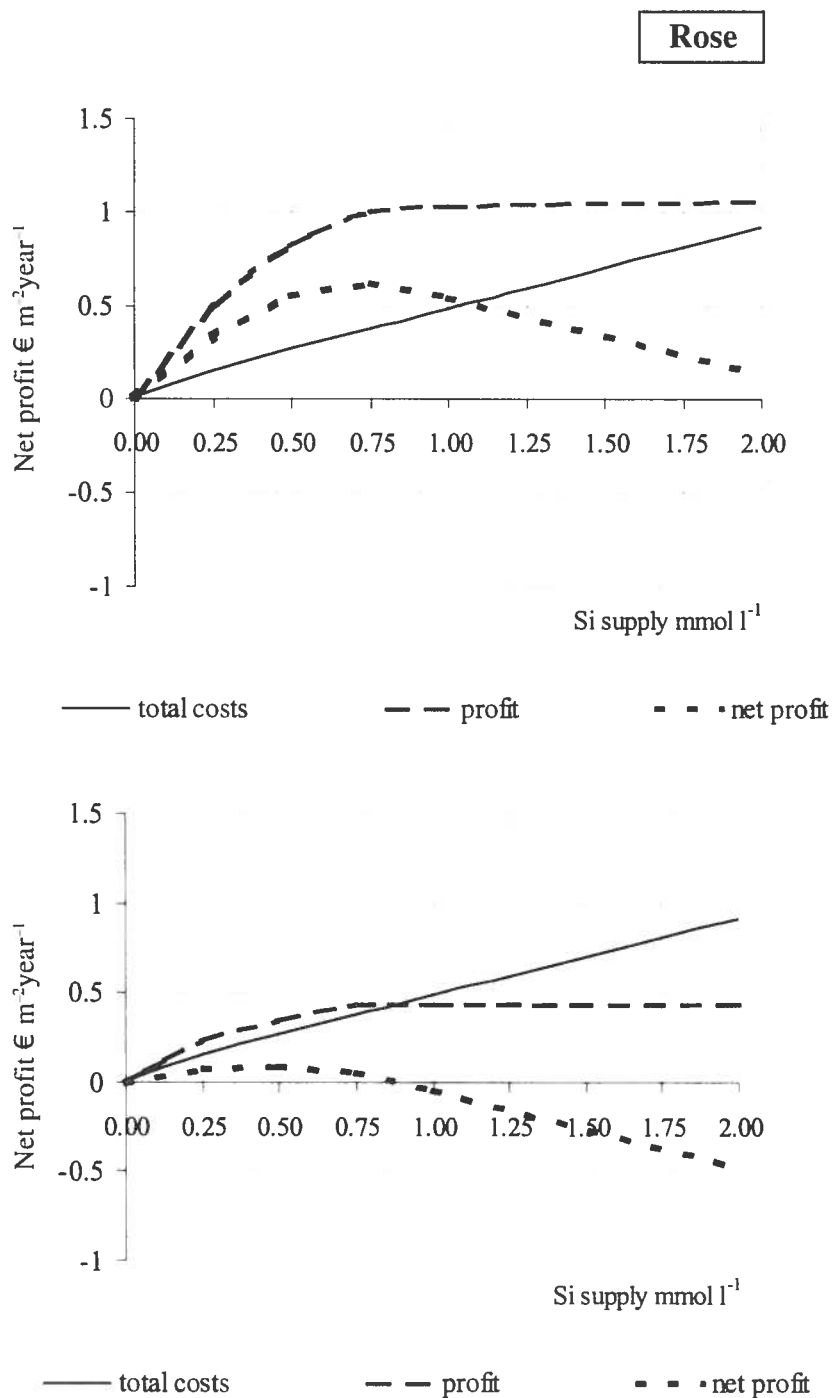


Figure 5. The results of calculations of the break-even point of Si supply in form of potassium-metasilicate, for rose in rockwool, taking into account the effect on yield and powdery mildew (top graph). and in case the Si supply could substitute the chemical mildew treatment completely, calculated without effect of Si on yield (bottom graph).

that the number of treatments is reduced from 15 times without Si, to 5 times with high Si supply (Voogt, 1990; Voogt and Elderen, 1991).

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Role of Silicon in "Potassium Silicate Fertilizer"

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INTRODUCTION

In Japan, about 4 million tons of fly ash are discharged annually from coal-fired power plants. "Potassium silicate fertilizer" (SiK) is produced by calcinations of a granulated mixture of fine fly ash, potassium hydroxide, magnesium hydroxide and dolomite. Since 1978, SiK has been on the market and its annual consumption increased during 1980s and reached a plateau of about 50,000 tons in 1990s. The guaranteed composition of SiK is: 2% citric acid soluble $K_2O \geq 20\%$, $MgO \geq 4\%$, $B_2O_3 \geq 0.1\%$ and 0.5 M HCl soluble $SiO_2 \geq 30\%$. SiK was registered and officially classified as a potassium fertilizer. However, about 90% of SiK has been used by rice farmers who expect benefits from silicon. Kubota (1984) and Tokunaga (1991) reviewed the unique properties and effectiveness of SiK as a slow-release potassium fertilizer.

This paper briefly reviews recent research and field observations in relation to the role of silicon in SiK fertilization.

COMPOSITION AND SOLUBILITY OF SiK

Esaki (2002) examined the conditions of calcinations and mixing ratio of raw materials of SiK in detail, and identified the major component species of the reaction products, e.g., K_2CaSiO_4 , K_2MgSiO_4 , $K_2Al_2O_4$, $K_2(AlSi)_4O_{12}$, $K_2MgSi_3O_9$, $Ca_2MgSi_2O_7$, and amorphous silicates. Amorphous forms of silicates occupy nearly 54% of weight in SiK samples at stores. For evaluation of available potassium and silica of SiK, Esaki recommended adopting 2% ammonium citrate buffer (CA, pH 4.5) instead of 2% citric acid solution (pH 2.0) of the official standard method, because the solubility in CA of individual components of SiK corresponded much better with the efficiency of absorption by test plants than in case of the official standard method.

A close relationships was found between dissolution percentage of SiK in CA, at 30°C in 60 minutes, and molar ratio of M/Si, where $M=K+Ca+Mg$ (Fig.1). Amorphous forms of silicates appear to dissolve faster than

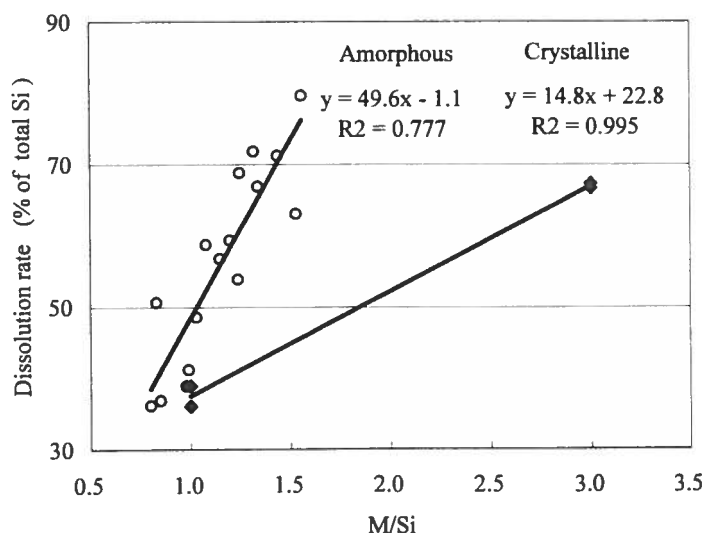


Fig. 1. Effect of metal/silicon ratio (M/Si) and form of SiK components on solubility of silicon in 2% ammonium citrate solution (Original data in Esaki, 2002).

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crystalline forms in relation to the M/Si ratio. Dissolution percentage of Si (Y) parallels to that of K (X): $Y=0.845X+4.4$ ($R^2=0.955$). These facts suggest that Si plays a role of controller for release of K from SiK while release of K induces dissolution of Si.

ENHANCEMENT OF NUTRIENT ABSORPTION

Hayase (1998,1999) compiled and analyzed the data of SiK application trials on paddy rice conducted by 215 public organizations from 1989 to 1996. SiK application increased shoot dry matter by 1%, accumulation of Si 8%, K 7%, N 1%, P 4%, and Mg 14% on average at harvest. Even in case of equal K application, SiK promoted K absorption from maximum tillering stage to ear emergence (Fig. 2).

A marked enhancement of K absorption was reported also in case of Ca-silicate application to rice plants in peat soil (Dep. Soils Fert., Hokkaido Nat. Agr. Ex. Sta. 1971). It is interesting that Si stimulates root exudation of phenolics (Kidd *et al.* 2001) and some phenolics enhance K absorption by plant roots (Tomita *et al.* 2001).

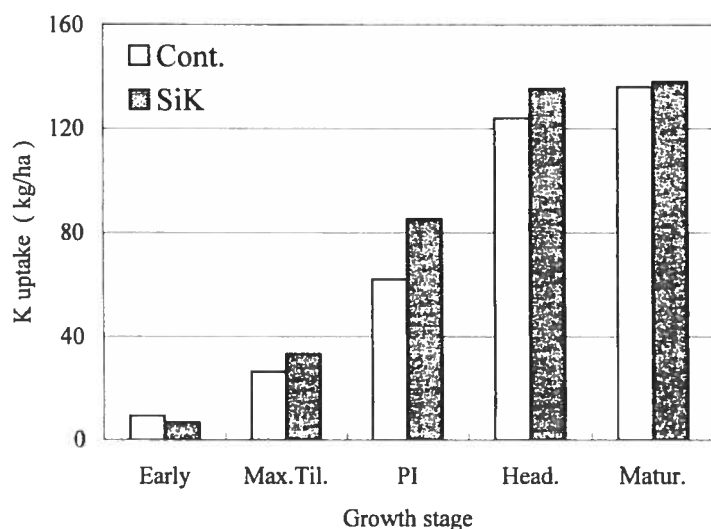


Fig. 2. Effect of SiK application on K uptake by rice crop at equal rates of K fertilization. Average amount of basal K: total K=74 kg/ha; SiK K=56 kg/ha, SiO_2 =102 kg/ha. Relative amount of K uptake at panicle initiation stage was significantly different between SiK and control at 1% level (Original data in Hayase (I), 1998).

HIGH ABSORPTION EFFICIENCY OF Si

On average of 50 trials in rice crops, Si absorption efficiency of basally applied SiK (0.5 M HCl soluble SiO_2 158 kg/ha) was 46% (Fig.3). If the data of a cool injury year and Andosols (with high available Si) were

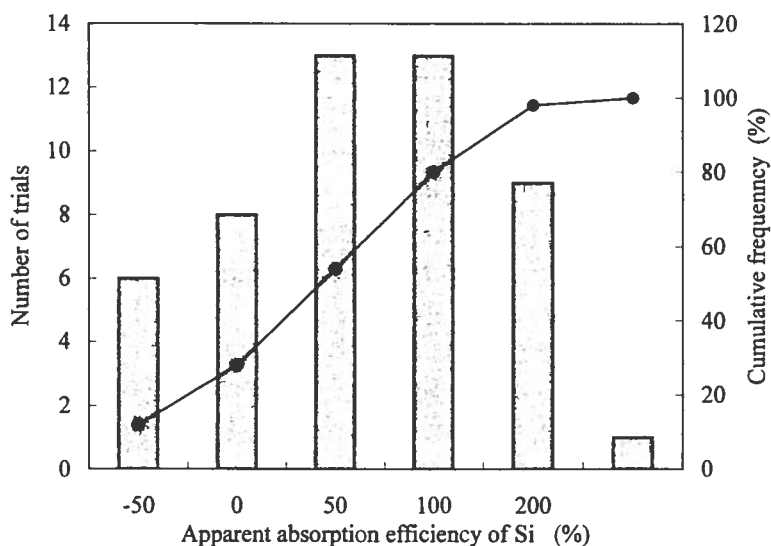


Fig. 3. Frequency distribution of apparent absorption efficiency of silicon in SiK basally applied to rice crop. Rates of SiK application as 0.5 M HCl soluble SiO_2 : average=158 kg/ha (n=50), range= 45-360 kg/ha. Apparent absorption efficiency = 100 (amount of Si uptake by SiK treated crop - amount of Si uptake by control crop) / amount of Si applied (Original data in Hayase (IV), 1999).

deleted, then the efficiency rose to 73%. Significant correlations ($r=0.646-0.749$, depending on variety and region) were found between Si and K accumulation at ear emergence stage (Hayase IV, 1999). Coupled release of K and Si from SiK might be a factor of the high efficiency.

IMPROVEMENT OF RICE YIELD AND QUALITY

The average increases in rice straw and grain yields due to basal application of SiK in 91 trials were by 1% and 2%, respectively. Corresponding figures for top dressing (around panicle initiation stage) of SiK in 63 trials were 1% and 4%, respectively. Increase in grain yield was due to both increases in number of grains and in percentage of ripened grains (Hayase II, 1998).

SiK application was found effective in improving "eating quality" of rice mainly by lowering protein content of polished grain (Hayase III, 1999). Silicon application generally tends to enhance N absorption of rice during the period of reproductive growth stage and, as a result, to reduce the amount of available N in soil of the ripening period. Such modification of N accumulation pattern in the rice plant might bring higher productive efficiency of the absorbed N and lower protein concentration of the grain.

SILICIFICATION

According to our calculation, the hull or glume of a single rice grain would accumulate 8-25 $\mu\text{g SiO}_2$ per day even before ear emergence (Dep. Soils Fert. Hokkaido Nat. Agr. Ex. Sta. 1971, Mizuno 1987). If we assume a cross section of 25 mm^2 per grain and a transpiration of 1mm from grain per day, then the concentration of SiO_2 in transpiration stream will be 320-1000 ppm. This unrealistic SiO_2 concentration indicates a possible existence of an active excretion mechanism in the rice glume cells. Topdressing of SiK was effective for silicification of young glume growing inside the sheath and also for fulfillment of the grain after anthesis (Mizuno 1987). Adequate silicification is a prerequisite for healthy ripening of rice in some stressed environments.

MISCELLANEOUS BENEFICIAL EFFECTS

There are many case reports on beneficial effects (high yield and quality, healthier roots, disease resistance etc.) of SiK application to various kinds of crops including non-silicon-accumulators such as *Alliaceae* and *Solanaceae*. While K, Mg, B and Ca of SiK might play important parts in these cases of beneficial effects, further researches on role of silicon in plant life are needed.

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Si Fertilizers: Past, Present and Future

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In agriculture of ancient Roman and China Empires some types of silicon (Si) fertilizers including plant ash were used for optimization of plant Si nutrition and for restoration of degraded soils. In the XIX century, the first investigation of the role of Si in plant growth and the use of Si fertilizers were just beginning. Sir Humphrey Day, Alexander von Humboldt, Justius von Liebig, J.B. Lawes, D. Mendeleev, Isenoke Onodera, V. Vernadsky were pioneers in this area that involved Si chemistry. Laboratory, greenhouse and field experiments on all continents and all climatic zones have shown substantial benefits for silicon fertilization of rice, corn, wheat, barley, sugar cane as well as several other crops. There are three main reasons for silicon fertilization: (1) improved silicon nutrition in the plant reinforces the plant natural protective properties against diseases, insect attack and unfavorable climatic conditions; (2) the soil treatment with biogeochemically active substances containing silicon optimizes soil fertility through improved water, physical, and chemical soil properties and aids the maintenance of other nutrients in plant-available form; and (3) various ecologically safe industrial by-products may be used as silicon fertilizers, many of which help solve the problem of proper utilization of numerous industrial wastes. Because of these reasons it is evident that silicon fertilizers should be used more extensively than they are presently. Frankly, silicon fertilizers are not used more extensively worldwide because there is a critical lack of specialists working to show the importance of silicon as a fertilizer material.

Solubilities of New Silicon Source, Fused Potassium Silicate Fertilizer, Produced from Steelmaking Slag

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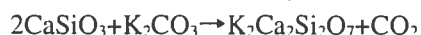
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Steelmaking slag is commonly used to produce silicate fertilizers, such as the blast furnace slag which is used as calcium silicate fertilizer. Besides this slag, steelmaking slag, especially the slag from the desiliconization process of hot metal in steel mills, also contains a large amount of silicon dioxide (SiO_2). The main component of the desiliconization slag is wollastonite (CaSiO_3), whose silicon dioxide is hard to dissolve in water and weak acidic solutions.

Fused potassium silicate fertilizer has been made to enable the desiliconization slag to be used as a silicate fertilizer (Yao *et al.*, 2001). Potassium carbonate (K_2CO_3) pellets were added to the melted slag in the hot-metal ladle and melted uniformly at a high temperature. The melted mixture was then collected from the ladle, cooled, pulverized, and granulated.

In this fusion process, polymeric silicate CaSiO_3 , was reacted with K_2CO_3 to form dimeric silicate $\text{K}_2\text{Ca}_2\text{Si}_2\text{O}_7$, by the following reaction:



The resulting compound, $\text{K}_2\text{Ca}_2\text{Si}_2\text{O}_7$, is a major chemical compound in fused potassium silicate fertilizer (Figure 1) (Akiyama *et al.*, 2001). It is categorized as a slow-release form: only 10.1% of the K_2O is water-soluble while 88.5% of K_2O is soluble in only 0.2 g L^{-1} citric acid, and 49.3% of SiO_2 is soluble in 0.5 mol L^{-1} hydrochloric acid (Table 1).

Actual fused potassium silicate fertilizer contains minor components such as magnesium oxide (MgO), manganese oxide (MnO) and iron oxide (FeO), which are derived from the slag (Table 1). In the present study, the effects of MgO and MnO on the solubilities of the potassium silicate fertilizers were examined using

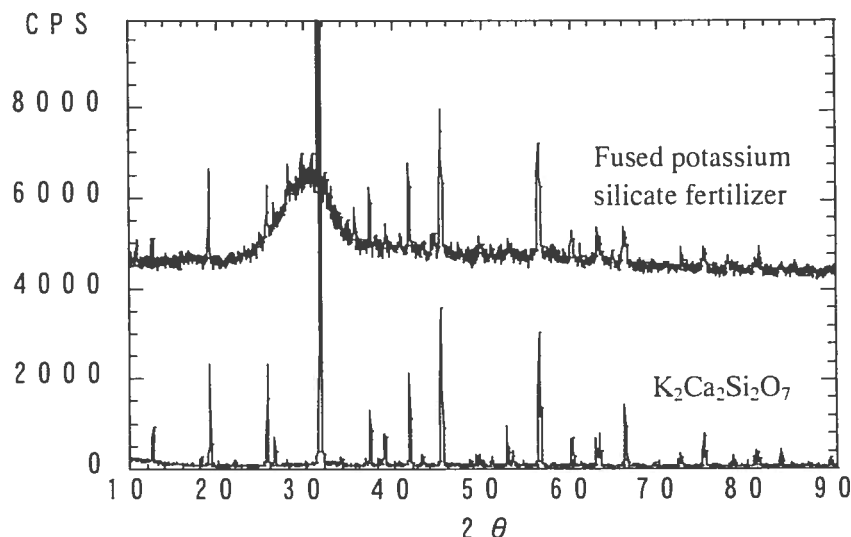


Figure 1. X-ray diffraction of the fused potassium silicate fertilizer and pure $\text{K}_2\text{Ca}_2\text{Si}_2\text{O}_7$.

Table 1. Chemical compositions of fused potassium silicate fertilizer and $K_2Ca_2Si_2O_7$

Sample	contents (mole kg ⁻¹)							solubilities (%)		
	SiO ₂	CaO	MgO	MnO	FeO	Al ₂ O ₃	K ₂ O	S-SiO ₂ /SiO ₂	C-K ₂ O/K ₂ O	w-K ₂ O/K ₂ O
potassium silicate fertilizer	6.86	3.71	0.20	0.35	0.51	0.38	2.55	64.1	97.5	12.9
$K_2Ca_2Si_2O_7$	6.14	6.13	0.00	0.00	0.00	0.00	3.05	49.3	98.6	10.1

S-SiO₂ was soluble SiO₂ in 0.5 mol L⁻¹ hydrochloric acid solution.

C-K₂O and W-K₂O were soluble K₂O in 0.2 g L⁻¹ citric acid solution and water, respectively.

water- and citric acid-soluble K₂O as an index of the fertilizer solubilities.

$K_2Ca_2Si_2O_7$ compounds, in which CaO was substituted by MgO or MnO, were prepared by fusing a stoichiometric mixture of K₂CO₃, CaCO₃, SiO₂, Mg(OH)₂ and MnCO₃ at 1150 °C. The slag from the desilicization process was melted with K₂CO₃, CaCO₃ and MnCO₃ at different molar ratios at 1400 °C to produce fertilizer containing different amounts of MnO. The melted mixtures were then cooled on a steel plate and crushed to pass a 1-mm sieve. Solubilities of these compounds and fertilizers were determined using water, 0.2 g L⁻¹ citric acid solution or 0.5 mol L⁻¹ hydrochloric acid solution.

The solubilities of $K_2(Ca,Mg)_2Si_2O_7$ and $K_2(Ca,Mn)_2Si_2O_7$ are shown in Figure 2. On substituting MgO for a part of CaO in $K_2Ca_2Si_2O_7$, the water-solubility increased because of the formation of K_2MgSiO_4 . In the case of adding MnO instead of MgO, the solubilities did not change until replacing 9.2 mole % of CaO by MnO, but increased above 10 mole %. This results suggested that a solid solution with MnO substituted for at most 10% of CaO in the silicate was formed, and so the solubility hardly increased. Furthermore, MnO substitution at less than 10 mole % of CaO did not increase the solubilities of the fused potassium silicate fertilizer. These results indicated that the amounts of MgO and MnO included in the fused potassium silicate fertilizer were sufficiently small to control the solubilities of this fertilizer.

The results of the present study showed that fused potassium silicate fertilizers, produced from steelmaking slag, contained $K_2Ca_2Si_2O_7$ as a major compound, resulting in a slow-release of SiO₂ and K₂O. The amounts of minor compounds, MgO and MnO, were adequate to maintain low solubilities in water. The SiO₂ and K₂O contents in acidic solutions were sufficiently high to suggest this product could be used as a slow-release fertilizer.

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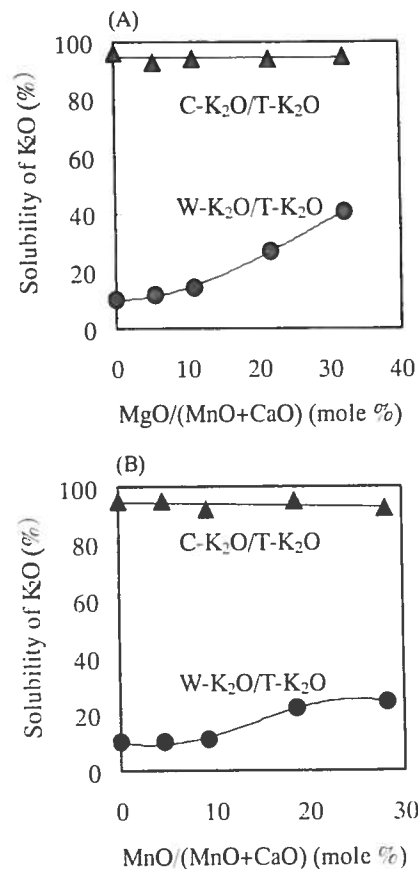


Figure 2. The effects of replacing CaO by MgO (A) or MnO (B) on K₂O solubility in $K_2Ca_2Si_2O_7$.

Effect of Silica Gel Application on Growth and Silicon Contents of Rice Seedlings in Nursery Beds with Different Available Silicon Contents

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The silica gel application to a nursery bed of rice is effective on early growth of rice plants (Fujii *et al.*, 1999) and on the control of seedling blast (Hayasaka *et al.*, 2000). In Japan various soils are commonly used as commercial nursery bed soils. Therefore it is important to evaluate Silicon(Si) in soils for nursery beds during raising of seedlings. We measured available Si in 15 commercial nursery bed soils by using the soil incubation method (Takahashi *et al.*, 1981). The method is as follows: 10 g of soil is placed into 100 mL plastic bottle, submerged with 60 mL of water and incubated at 40°C for a week. Si in supernatant is measured spectrophotometrically by the ammonium molybdate method. We also studied the effect of silica gel application to three bed soils that had different Si availabilities.

The range of available Si of 15 nursery bed soils was wide, from 33 to 206 mg Si/kg dry soil. The rice seedlings raised on these bed soils contained from 0.43 to 1.94% Si DW. The available Si contents in soils were significantly correlated with the Si contents in rice seedlings. Thus, the soil incubation method was good to evaluate the Si availability of bed soil.

We applied various amounts of silica gel to three bed soils (available Si; 33, 87 and 206 mg Si/kg dry soil respectively) and raised rice seedlings. Silica gel was mixed into commercial bed soils in rice nursery boxes (30 × 60 × 3 cm) at the rates of 0, 50, 100, 200, 400 g and then rice seeds were sown. At 21 days after sowing seedlings were sampled to investigate the growth and Si contents. Dry weight of rice seedlings grown on bed soils amended with silica gel tended to be higher than that without silica gel (Table 1). Si contents in

Table 1. Effect of Silica gel application to nursery beds with different Si availabilities Si (Low-Si, 33; Middle-Si, 87; High-Si, 206 mg/kg dry soil) on the growth of rice seedlings.

Bed soil	Available Si mg/kg	Silica gel g/box	Shoot FW (g/100 plants)	Shoot DW (g/100 plants)	Plant Height (cm)
Low-Si	33	0	5.64	1.23	9.8
		50	5.64	1.17	10.6
		100	6.20	1.25	10.8
		200	6.20	1.31	10.3
		400	6.34	1.35	11.0
Middle-Si	87	0	6.58	1.28	11.7
		50	7.42	1.37	11.5
		100	7.24	1.37	12.1
		200	7.30	1.40	12.4
		400	7.55	1.42	12.7
High-Si	206	0	7.66	1.38	12.2
		50	7.23	1.44	11.9
		100	6.50	1.37	11.6
		200	8.76	1.56	12.6
		400	7.92	1.53	12.8

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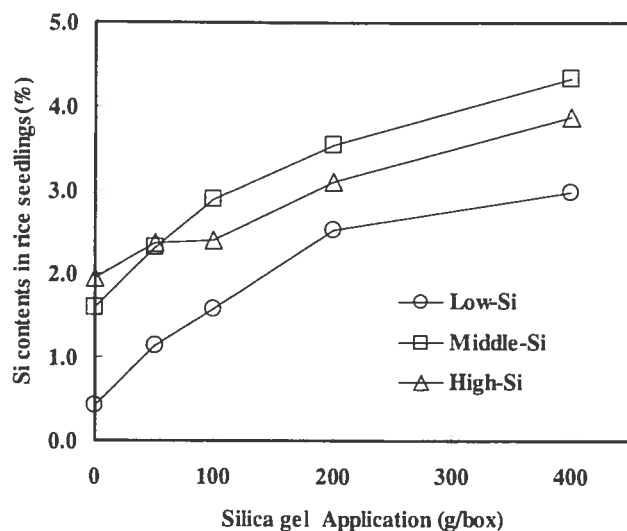


Fig. 1 Effect of Silica gel application to nursery beds with different Si availabilities (Low-Si, 33; Middle-Si, 87; High-Si, 206 mg/kg dry soil) on Si contents of rice seedlings.

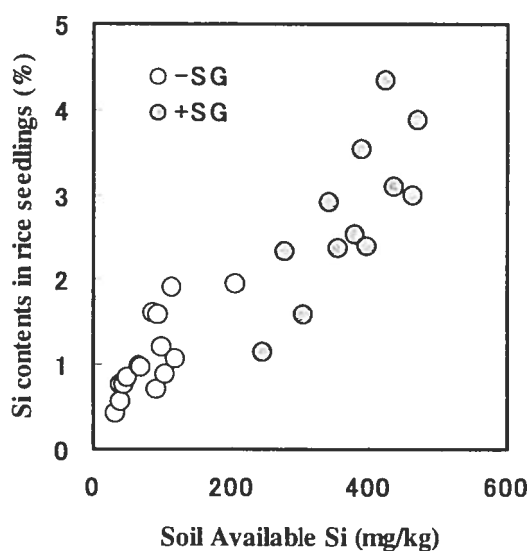


Fig. 2 Relationship between soil available Si and Si contents in rice seedlings with and without silica gel. -SG= without silica gel; +SG= with silica gel.

the seedlings increased in proportion to the rates of silica gel application. The silica gel application to the bed soils increased Si contents of plant regardless of the levels of available Si (Fig. 1). The soil incubation method was also applied to evaluate the Si availability of bed soils previously amended with silica gel (Fig. 2).

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Effect of Acidified Porous Hydrate Calcium Silicate Applied in a Nursery Bed Soil on Growth and Nutrient Uptake of Rice Seedling

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INTRODUCTION

The production of healthy seedlings is an important factor to improve the yield and quality of rice in Japan. A high ratio of dry weight to plant length is one of prerequisites to obtain healthy rice seedlings.

It is well known that silicon fertilization increases the photosynthesis rate of rice plants and, consequently, the dry matter production is increased too (Takahashi, 1987). Another effect of silicon on rice is the increase of resistance to diseases and insects. Thus, the silicon application in a nursery bed could result in healthy seedlings.

In Japan, silica gel (SG) is the most used source of silicon applied in a nursery bed of rice because it has high soluble silicon content (>80%). Fujii *et al.* (1999) reported that the silicon content, dry weight and the ratio of dry weight to plant length of seedlings were increased by SG application. However, despite the higher content of soluble silicon, this fertilizer is expensive and its use is limited in large scale. Many researchers have been proposed alternative sources of silicon to rice cultivation. For example, Saigusa *et al.* (2000) proposed the use of Porous Hydrate Calcium Silicate (PS), which has been produced as industrial waste in the manufacturing process of the light autoclaved concrete, as a cheap and effective material to supply silicon to the rice. However, the PS is an alkaline material (pH 10) and its application in the nursery bed can increase the soil pH more than 5.5 increasing the susceptibility of rice seedling to diseases.

Thus, to avoid this problem the acidified porous hydrated calcium silicate (APS) was developed by the addition of sulfuric acid to the PS material. With the acidification of PS material the tobermorite [$\text{Ca}_5(\text{Si}_6\text{O}_{18}\text{H}_2) \cdot 4\text{H}_2\text{O}$], which is the principal component of PS, is transformed to silica gel and gypsum as the follow reaction:



The APS can probably be applied in a nursery bed without a problem and an increase of soil pH will be found. Consequently, a positive effect of silica gel and gypsum can be expected with the application of APS in a nursery bed. Thus, the objectives of this study were to: (i) evaluate the effect of APS applied on the growth and nutrient uptake of rice seedling, and (ii) determine the aimed application rate of APS, and (iii) evaluate the efficiency of APS material on the supply silicon to rice seedlings in the soils with different levels of available silicon content.

MATERIALS AND METHODS

Two experiments were conducted at the Experimental Farm, Graduate School of Agricultural Science, Tohoku University, Japan. 125 g of pre-germinated rice seeds (*Oryza sativa* L. cv. Hitomebore) were sown in a

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nursery tray (60 × 30 × 3 cm). The nursery bed soil was chosen among thirty-three commercial nursery bed soils (numbered from 1 to 33, see Figure 1) according to their level of available silicon content.

For the first experiment, nursery bed No. 25 that was used because it is the most used nursery bed in the Kawatabi region and it has a steady medium level of available silicon content. The treatments of this experiment were described as follows: APS treatments = acidified porous hydrate calcium silicate (APS) was mixed with each nursery bed soil at the 4 ratios of 0:1, 1:5, 1:3, and 1:1 (APS 0, APS I, APS II, and APS III, respectively); Silica gel treatment (SG): 200 g of SG per nursery tray was applied.

For the second experiment three nursery bed soils were used: with low (bed No. 1), medium (bed No. 25) and high (bed No. 33) available silicon content. The second experiment was carried out to evaluate the efficiency of APS material on the supply silicon to rice seedlings in soils with different levels of available silicon content. The treatments were the almost same as the first experiment except for the SG treatment.

For both experiments plant length, leaf age and leaf color (with chlorophyll meter) were measured. The angle between the leaf blade and the leaf sheath of rice seedlings was measured only in the second experiment.

The available silicon of nursery bed soils (No. 1-33) was extracted with a 40 mmol L⁻¹ phosphate buffer solution (Kato, 2000) in the soil: solution ratio of 1:10, and measured by a colorimetric method (Weaver *et al.* 1968). 100 seedlings were sampled and separated into shoot and root for dry matter measurement and chemical analysis. The plant material was dried in an oven at 70°C for 48 h. Nitrogen content was analyzed with a NC analyzer. Samples were digested with sulfuric acid and hydrogen peroxide for analysis of phosphorus, potassium, calcium, and magnesium content. Phosphorus was analyzed with a colorimetric method, and other elements were analyzed with atomic absorption spectrometry (Mizuno *et al.* 1980). For sulfur analysis, the samples were ashed in an oven, and sulfur in the ash was extracted with hydrochloric acid / water at a 1:1 ratio solution, and analyzed by ion chromatography (Tsuji, 2000). Silicon in the samples was extracted with 1.5 mol L⁻¹ hydrofluoric acid, and analyzed by a colorimetric method (Saito *et al.* 1986).

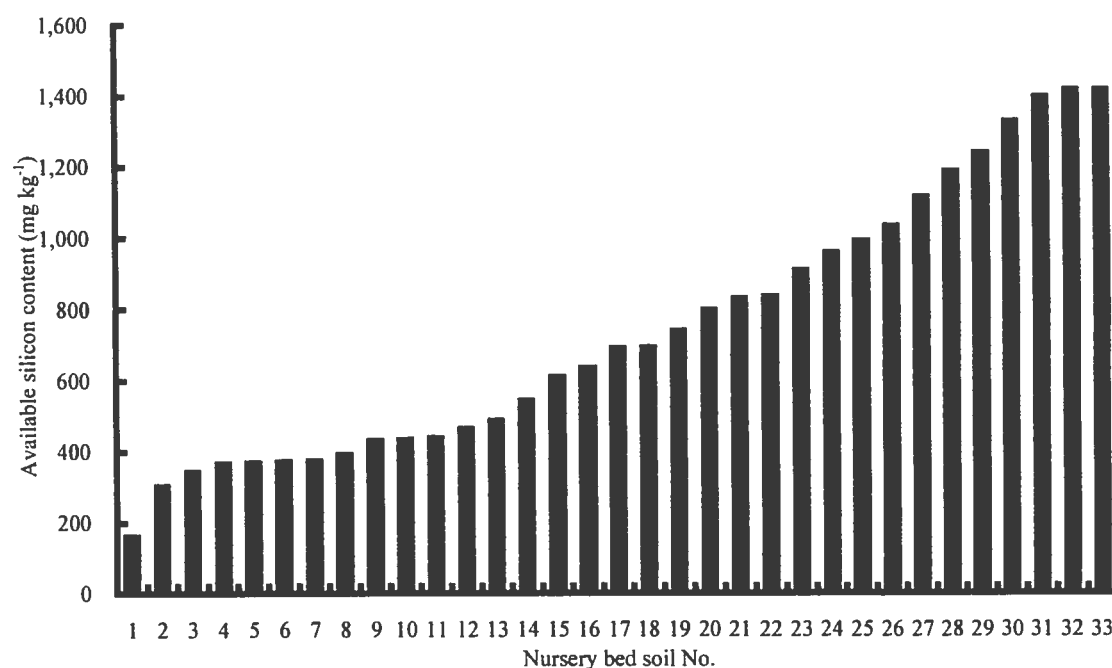


Fig. 1. Available silicon content of 33 commercial nursery bed soils.

RESULTS AND DISCUSSION

Effects of APS on growth and nutrient uptake of rice seedling

Table 1 shows the effect of silicon treatments on some of the rice seedling characters. No significant differences ($p < 0.05$) were found between the treatments for plant length, leaf age and the leaf color of seedlings. On the other hand, the shoot dry weight of 100 seedlings and the ratio of that to the plant length in APS treatment plots were 13–15% higher than those of the APS 0 plot, those in the APS and SG plot were not different statistically.

The dry weight of roots in the APS II and SG plots were higher than those of the APS 0 plot, and the angle between the third leaf blade and the leaf sheath in the APS treatment and SG plots were 37–49% smaller than that of the APS 0 plot. On the other hand, the angle between the fourth leaf blade and the leaf sheath in all the plots were statistically the same ($p < 0.05$).

Table 2 shows nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur contents in the shoot of rice seedlings. There were no significant differences ($p < 0.05$) for nitrogen, phosphorus, and potassium contents between all the plots. However, the calcium and sulfur contents of plants in the APS plots were higher than those in the APS 0 and SG plots, which was clear that the calcium and sulfur supply were increased by the APS application. The magnesium content in plants of the APS treatment plots decreased after APS application. It was probably due to the increased calcium from the APS fertilizer. It is well documented in the literature that the increased Ca application, decreased the Mg absorption by plants because these cations compete for the same site of absorption. Despite the decrease in Mg absorption by the rice seedlings, no problems occurred because the calcium to magnesium ration was less than 2, which is considered to be within the limit that causes magnesium deficiency in rice.

Fig. 2 indicates silicon content in the shoot of rice seedlings. The silicon content in these shoots increased with the APS application. Higher silicon contents were found in the APSII, APSIII and SG which were not different statistically for Tukey ($p < 0.05$). Silicon content in APS 0, APS I, APS II, APS III and SG plots were 29, 37, 41, 44 and 41 mg kg^{-1} respectively.

Table 1. Effect of silicon treatments on some character of rice seedlings.

Plot	Length of plant (cm)	Leaf age	Leaf color (SPAD)	Dry weight (g per seedling)		Dry weight/Length of plant	Angle of between leaf and sheath	
				Shoot	Root		Third leaf	Fourth leaf
APS 0	19.5 a ^(a)	4.9 a	27.6 a	23.9 a	5.5 a	1.2 a	45 b	11 a
APS I	18.9 a	4.8 a	24.8 a	27.0 b	6.0 ab	1.4 b	24 a	12 a
APS II	19.0 a	4.8 a	28.5 a	27.4 b	6.7 b	1.4 b	23 a	11 a
APS III	18.8 a	4.8 a	28.9 a	26.8 b	5.3 a	1.4 b	25 a	10 a
SG	19.1 a	4.8 a	27.7 a	26.9 b	6.4 b	1.4 b	28 a	11 a

^(a) within the columns, means follow of different letters differ statistically (Tukey, $p < 0.05$)

Table 2. Effect of silicon treatments on nutrient contents of rice seedling.

Plot	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur
	g kg ⁻¹					
APS 0	29.7 a (a)	8.2 a	25.5 a	4.3 a	4.0 b	5.2 ab
APS I	29.7 a	9.3 a	28.3 a	5.0 b	2.9 a	5.4b
APS II	28.8 a	8.8 a	26.7 a	5.1 b	2.9 a	7.3 c
APS III	29.5 a	9.2 a	30.3 a	5.3 b	2.5 a	5.8 b
SG	28.2 a	9.1 a	23.1 a	4.2 a	3.7 b	4.2 a

^(a) within the columns, means follow of different letters differ statistically (Tukey, $P < 0.05$)

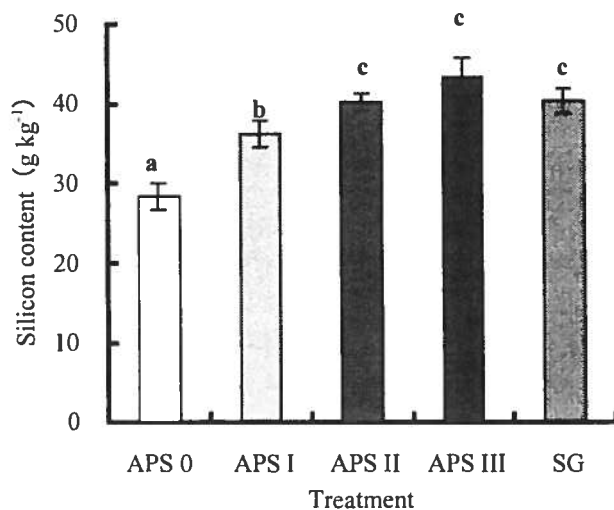


Fig. 2. Effect of silicon treatments on silicon content of rice seedlings. Vertical lines indicate the standard error. Bars followed of different letter differ statistically (Tukey, $p < 0.05$)

Evaluation of APS material on silicon supply to rice seedlings in soils with different levels of available silicon content.

Fig. 3 shows the silicon content in the shoot of rice seedlings shoot grown in nursery bed soils No. 1, No. 25 and No. 33, respectively. The silicon content in the shoot of rice seedlings grown in the plots were APS was not applied reflected the available silicon content of each bed soil. When silicon was not applied, the highest values of silicon content were found in the No. 33 bed soil. The degree of response to silicon fertilization was high when a nursery bed with low available silicon content was used. The silicon content of the shoot in the APS 0, APS I, APS II, and APS III plots of the No. 1 bed soil were 11, 24, 35, and 39 mg kg⁻¹, respectively. Those of the No. 25 soil were 29, 38, 44, and 44 mg kg⁻¹, and those of No. 33 were 42, 41, 45, and 45 mg kg⁻¹, respectively.

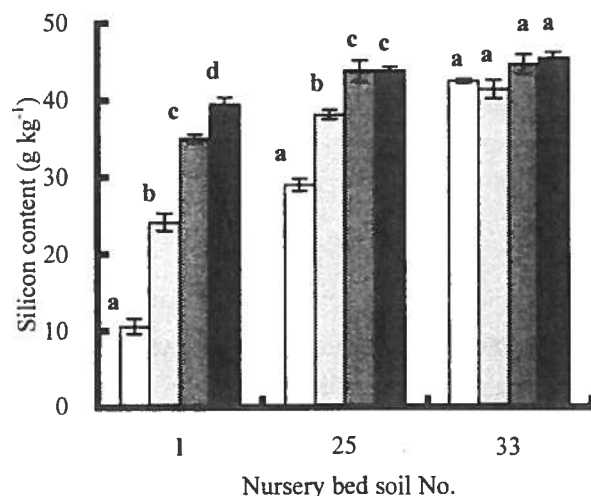


Fig. 3. Effect of silicon treatments on silicon content of the shoot of rice seedling. Vertical lines indicate the standard error at same Nursery bed soil. Bars followed of different letter differ statistically (Tukey, $P < 0.05$)

□ APS 0 □ APS I ■ APS II ■ APS III

From the above results we conclude that the rice seedlings response to silicon fertilization depends on the available silicon content of the nursery bed soil. Therefore, the amount of APS necessary for rice seedlings can be determined according to the available silicon content of the nursery bed soil. The APS can be applied successfully in a nursery bed to raise healthy rice seedlings.

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Effects of Porous Hydrated Calcium Silicate on Silicon and Nitrogen Concentration of a Young Leaf Blade in a Rice Plant (*Oryza sativa* L.) as Nutrients Influencing its Resistance to Rice Blast (*Magnaporthe grisea*)

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INTRODUCTION

Tobermorite, which is a kind of porous hydrated calcium silicate (PS), is used as a light concrete material. We have reported that the application of PS for rice plants is effective for increasing rice blast resistance through the mechanical strength of leaf epidermis (Saigusa *et al.* 2000). However, there are no reports on the effect of PS on those silicon and nitrogen concentrations of a young leaf blade, which are important nutrients related to blast resistance.

In this study, the change of silicon and nitrogen concentrations of young leaf blades were investigated to understand the reinforcement mechanism of rice blast resistance by PS application.

MATERIALS AND METHODS

Experiments were conducted on Andisol at the experiment farm of Tohoku University, Miyagi, Japan in 1998. PS waste (256 mg Si g⁻¹) was applied at the rate of 0 - 1000 g m⁻² before transplanting. Rice plants (*Oryza sativa* L. cv. Hitomebore) were transplanted in mid-May. A bulk blend compound fertilizer at the rate of 1 g N pot⁻¹ was applied for the pot experiment, and 7 g N m⁻² for the field experiment.

Rice blast (*Magnaporthe grisea*) was inoculated into the plants grown in the pots at last in June, while the time blast fungus starts infection to rice plants at the Tohoku region in Japan. The infection rate of the rice blast was shown by the ratio of the number of infected stems to the number of total stems at 10 days after inoculation. The leaf blade, which was the most upper leaf developed at the inoculation, was collected for silicon and nitrogen determination at 10 days after inoculation.

Leaf blades of the plants grown in the field were collected at the maximum tiller number stage. Young leaf blades, that were emerging and newly developed leaf blades, were separated from the aged leaf blades. The 12th leaf blade was collected from the field at 0, 3, 12, 19, 26, 41, 53 days after emergence.

RESULTS

Rice blast inoculation in the pot experiment

Plant height, stem number, and leaf age of rice were not significantly different among the treatments at the time of inoculation. Silicon concentration increased with an increasing PS application rate. The difference of nitrogen concentration among plots was not significant (Table 1).

The infection rate in PS300 and PS1000 plots was significantly lower than that in the PS0 plot. The infection rate in the PS150 plot was unstable and not significantly different from the PS0 plot (Figure 1). The infection rate correlates with the silicon concentration of the leaf blade ($r=-0.64$), but does not correlate with the nitrogen concentration.

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Change of silicon and nitrogen concentration in leaf blades with elongation.

Silicon concentration of the young leaf blades was lower than that of the aged leaves. An increase of silicon concentration by PS application on the young leaves was also smaller than that on the aged leaves (Figure 2).

Silicon concentration of the 12th leaf increases rapidly up to 10 days after emergence, above which it increases gradually. On the other hand, nitrogen concentration decreases abruptly up to 10 days after emergence, above which it decreases insensibly. The difference of silicon concentrations among PS treatments was significant during elongation (Figure 3).

DISCUSSION

It has been reported that PS application is useful to improve blast infection resistance of rice plants by the field experiments (Saigusa *et al.* 2000). The effect was confirmed by the blast inoculation in this study (Figure 1). Silicon concentration of young leaves correlated with the blast infection, but did not correlate with the nitrogen concentration.

The susceptibility to blast on the aged leaves is normally less than that on the young leaves. One reason is that silicon accumulation of the aged leaves was more than that of young leaves. It is also known that silicon nutrition of young leaves is influenced by silicon absorption at the leaf emergence (Volk *et al.* 1958). PS is a fertilizer to supply silicon for rice plants from transplanting to harvesting time (Saigusa *et al.* 1998). The results illustrated by Figure 2 shows the continuous supply of silicon by PS contributions, increasing the silicon concentration of young leaf blades.

Table 1. Plant length, leaf age, stem number, dry weight, and silicon and nitrogen concentration of rice at inoculation.

PS application rate (g m ⁻²)	Plant length (cm)	Leaf age	Stem num. (pot ⁻¹)	Dry weight* (g pot ⁻¹)	Concentration in LB	
					Silicon (mg g ⁻¹)	Nitrogen (mg g ⁻¹)
0	45.0	10.5	37.0	22.1	20.2	39.6
150	45.0	10.4	37.5	22.0	24.6	35.6
300	45.7	10.3	33.7	22.6	28.5	39.8
1000	44.8	10.4	33.5	22.6	33.0	50.3

*dry weight, silicon and nitrogen concentration in leaf blade were measured at 16 days after inoculation.

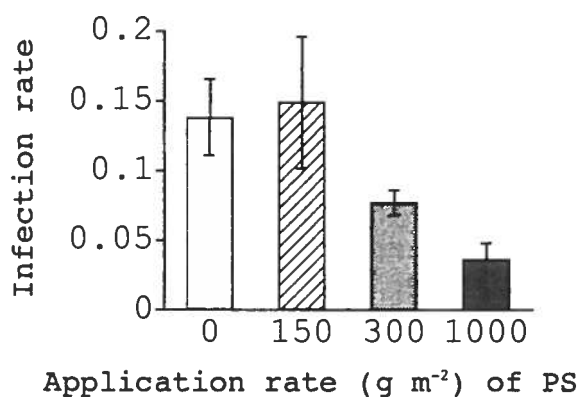


Figure 1. Effect of PS application on infection rate of stem after inoculation.

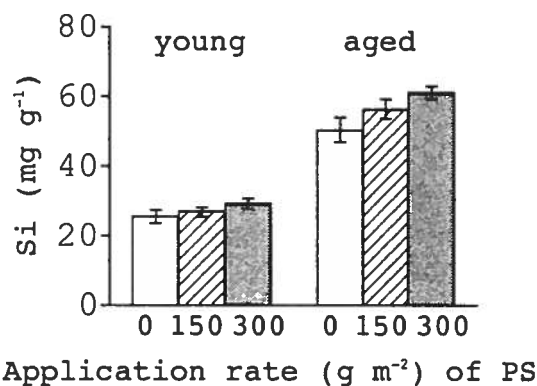


Figure 2. Silicon concentration of the young and aged leaf blades at the maximum tiller number stage.

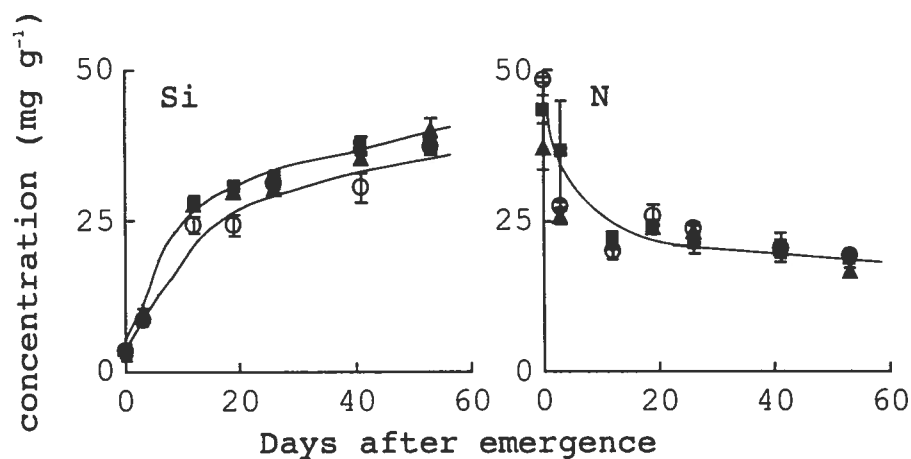


Figure 3. Change of silicon and nitrogen concentration after leaf emergence.

○, PS0 ; ▲, PS150 ; ■, PS300

It is concluded that the PS application does not influence nitrogen concentration, but increases silicon concentration of the new leaf blades at the time blast fungus infects to rice plant. The increase of silicon concentration of the new leaves contributes to improving blast resistance of rice plants.

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Neutralized Autoclaved Aerated Concrete as Silicate Fertilizer for Rice Seedlings

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INTRODUCTION

Autoclaved Aerated Concrete (AAC) is widely used as a lightweight construction material with a bulk density of 500 kg/m³ and volume porosity of 80%. AAC is mainly composed of porous hydrated calcium silicate such as tobermorite ($5\text{CaO} \cdot 6\text{SiO}_2 \cdot 5\text{H}_2\text{O}$). The availability of AAC as silicate fertilizer in paddy fields was proved to improve the silica nutrition effectively for rice plants [1]. A Silica gel was applied to the seedlings of rice plants and a high rooting ability was reported [2]. AAC, as a silicate fertilizer, although, had not been applied for the seedlings of rice plants owing to its relatively high pH. In this study, neutralized autoclaved aerated concrete (NAAC) was prepared, and its effects as a silicate fertilizer on seedlings of the rice plants was investigated

EXPERIMENTAL PROCEDURE

The raw material of NAAC was prepared by acidifying the alkaline AAC with diluted sulfur to pH 5.3. The NAAC was granulated to a particle size of 2 - 4.75 mm in diameter to use it as a silicate fertilizer. The raising of seedlings was carried out under the conditions shown in Table 1. The experiments were conducted in a green house at the Laboratory of Asahi Kasei Corporation from March 17 to April 24, 2001. The pHs of the external solutions for the seedling bed were between 4.8 and 5.3 through the experiments.

Table 1. Conditions for raising seedlings¹⁾ of rice plants

Treatment	Nursery bed			Cover soil
	NAAC fertilizer (g/vessel)	Soil ²⁾ for the nursery bed (g/vessel)	Agricultural medicine ³⁾ (g/vessel)	Cover soil ²⁾ (g/vessel)
Control	none	2700	5.94	800
NAAC400	400	2300	5.94	800
NAAC800	800	1900	5.94	800
NAAC1600	1600	1500	5.94	800
NAAC2700	2700	0	5.94	800

¹⁾ Conditions for raising seedlings:

Seedling rice: *Oryza sativa* L., cv. Hitomebore, 160g seed/vessel

Seedling vessel : 300 mm W × 600 mm L × 35 mm H

N-P₂O₅-K₂O content in a seedling vessel: N-P₂O₅-K₂O =1.2 g-1.2 g-1.2 g/vessel.

²⁾ Soil: Granular Parumatto Ichigo (Katakura Chikkarin Co., Ltd.) was used. The weight of the soil is indicated as dried soil.

³⁾ Agricultural medicine: Tachigare Ace (Sankyo Co., Ltd.) was supplied in the nursery bed.

RESULTS AND DISCUSSION

The NAAC fertilizer contains 26% (w/w) of easily soluble silicate (S-SiO₂). The S-SiO₂ content in NAAC fertilizer was analyzed from the silicate concentration which was treated with 0.5 N HCl to eliminate Ca, and then it was solved with 0.5 N NaOH at 20°C for 8 hours. The water-soluble ions which elute from the NAAC fertilizer were measured from the external solution of 15 g of NAAC and 75 g of pure water after leaving to stand at 20°C for 10 days. The pH and electric conductivity of the external solution were 5.3 and 2.9 mS/cm, respectively. The concentrations of SiO₃²⁻, Ca²⁺, SO₄²⁻, Al³⁺, Fe³⁺, and Zn²⁺ ions in the external solution were 140, 550, 1800, 1.0, 1.2, and 0.8 mg/L, respectively. The concentrations of other elements such as Cd, Pb, Cu, Ni, Cr, Hg, Se, and As were below 0.01 mg/L. The eluting materials of the external solution after 20 days were very close to those after 10 days.

The feasibility of the NAAC as a silicate fertilizer was examined for use in raising the rice seedlings. The influence of the NAAC supply on the growth of rice seedlings was indicated in Table 2. As can be seen from Table 2, the NAAC supply promoted the growth of rice seedlings in every case compared with the control. The proper quantity of NAAC supply was considered to be 800-1600 g/vessel, which corresponds 200-400 g of S-SiO₂ supply per vessel. When 800 g/vessel of NAAC fertilizer was supplied (NAAC800), the rice seedlings showed the most robust growth. The fullness factor, which is represented by shoot dry weight per shoot length, indicated the highest value in the case of NAAC800. When the nursery bed was composed of only the NAAC fertilizer shown as NAAC2700 in Table 2, both shoots and roots represented slow growth compared with the cases in which the soils were included in the bed.

The influence of the NAAC fertilizer supply on the contents of nutrients in dried rice shoots is shown in Table 3. Silicate contents of the dried rice shoot were gradually increased according to the amount of NAAC fertilizer supply. The relationship between the shoot silicate content and the amount of S-SiO₂ supply is indicated in Figure 1. As can be seen from Figure 1, the increase of silicate concentration in the shoots has a deep relationship with the quantity of S-SiO₂ supply from the NAAC fertilizer. Table 3 shows that the NAAC

Table 2. Influence of the NAAC fertilizer supply on rice seedlings

Treatment	S-SiO ₂ Supply from NAAC ¹⁾ (g/vessel)	Growth of rice seedling			
		Foliar age	Shoot length (mm)	Shoot dry weight (g/100 seedlings)	Root dry weight (g/100 seedlings)
Control	none	4.3 ± 0.1	138 ± 8	1.50	0.58
NAAC400	104	4.4 ± 0.1	140 ± 5	1.89	0.63
NAAC800	208	4.3 ± 0.1	144 ± 7	2.06	0.70
NAAC1600	416	4.3 ± 0.1	157 ± 11	2.03	0.69
NAAC2700	702	4.3 ± 0.1	141 ± 10	1.76	0.59

¹⁾ S-SiO₂ supply from NAAC: amount of easily soluble silicate (S-SiO₂) supply from NAAC fertilizer in a seedling vessel.

Table 3. Influence of the NAAC fertilizer supply on the contents of nutrients in dried rice shoots

Treatment	Contents of nutrients in dried rice shoots (%)					
	SiO ₂	N	P ₂ O ₅	K ₂ O	CaO	MgO
Control	1.55	3.05	1.10	2.62	0.41	0.60
NAAC400	5.76	3.12	1.13	2.85	0.63	0.49
NAAC800	8.12	3.08	1.25	3.40	0.58	0.46
NAAC1600	9.89	2.98	1.33	4.53	0.52	0.34
NAAC2700	11.23	3.08	1.17	3.64	0.43	0.30

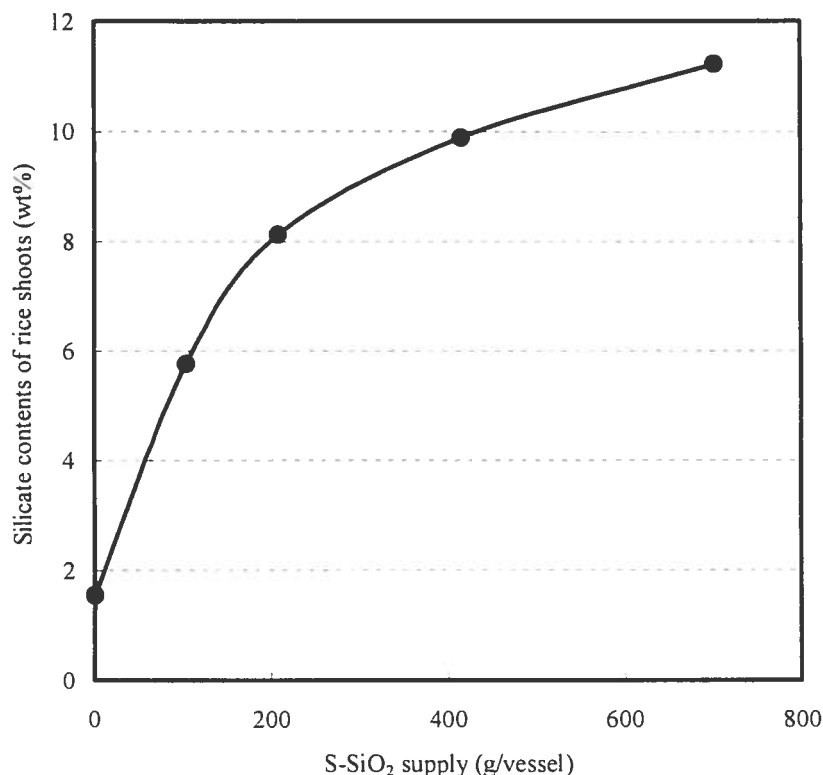


Figure 1. Influence of S-SiO₂ supply on the silicate content of dried rice shoots

fertilizer supply did not affect shoot concentrations of main nutrients such as N and P₂O₅. The distinctive feature of this NAAC fertilizer to act on the rice seedlings was found to be an increase in the shoot concentration of K₂O and CaO nutrients.

CONCLUSIONS

- (1) The feasibility of NAAC as a silicate fertilizer was found to promote growth of rice seedlings.
- (2) The proper quantity of NAAC supply for rice seedlings was considered to be 800-1600 g/vessel, which corresponds to 200-400 g of S-SiO₂ supply per vessel.
- (3) The increase of silicate concentration in the rice shoots has a deep relationship with the quantity of S-SiO₂ supply from the NAAC fertilizer.
- (4) The distinctive feature of this NAAC fertilizer to act on the rice seedlings was found to be increase in the shoot concentration of K₂O and CaO nutrients.

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A New Determination Method for the Solubility of Silicate Fertilizers: Examination of Silicate Dissolution using Citrate Buffer Solutions

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INTRODUCTION

Silicate fertilizers are widely used in rice plantations to increase resistance to pests and prevent lodging. In the development of silicate fertilizers, it is important to correctly evaluate their solubility. It has been pointed out that the dissolved Si determined by commonly-used extraction does not correlate with the quantity of Si taken up by rice plants^{1, 2)}. For example, silicate absorption by rice plants from blast furnace slags including calcium silicate fertilizers, is observed to be half that from fused phosphate fertilizers. This order of difference, however, is not observed in the percentage of dissolved silicate determined by the various extraction methods. Since the extraction procedure is carried out under strongly acidic conditions, greatly different from those in paddy fields, almost 100% Si is dissolved from slags. Therefore, these methods are unsuitable for studying the dissolution properties of the fertilizers.

In this work, we have investigated extraction methods in which the pH is close to that of paddy soils (pH 5.5~7) and a high buffer capacity is maintained, on the basis of the official citric acid method. When a 4% citrate buffer solution was used, a trial silicate fertilizer fabricated in our laboratory, a calcium silicate fertilizer, and a fused phosphate fertilizer, clear differences in the solubility of silicate throughout the acidic to neutral region, whereas such differences were not observed by the official method. Since our best results were obtained at pH 5.5, we have employed a citrate buffer solution (4%; pH 5.5) for determination of the solubilities of a variety of silicate fertilizers developed in our laboratory. The results obtained were well correlated with the results of pot cultivation experiments, which determined the quantity of Si taken up by rice plants.

Our new extraction method may be useful for examination of the solubility of silicate fertilizers, because the experimental procedure conforms to the official method.

EXPERIMENTAL

Samples and quantitative analyses

Materials studied were trial silicate fertilizers fabricated in our laboratory, and commercially available fertilizers including calcium silicate, potassium silicate and fused phosphate fertilizers. Each sample was pulverized in a vibrational mill, and sieved to a particle size < 150 μm . Chemical analyses were determined using X-ray fluorescence analysis (Rigaku Denki SYSTEM 3370E). Samples were prepared with a glass bead sampler (Tokyo Kagaku NT-2000). Standard samples were prepared by adding appropriate concentrations of reagents (e.g. SiO_2 and CaCO_3), to the standard matrix for X-ray fluorescence analysis. This was prepared with a composition adjustment that of the materials studied. The dissolved Si in aqueous solution was determined using ICP (Rigaku SPECTRO CIROS and Nippon Jarrell-Ash ICAP-575 spectrometers).

Extraction of silicate with citrate buffer solutions

The official method of extraction with a 2% citric acid solution was modified as follows: To sample material (1.0 g) was added citrate buffer solution (4%; 150 ml; pH 5.5) preheated at 30°C. The resulting

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mixture was shaken at 30°C for 60 min., and then cooled rapidly to room temperature. The total volume was adjusted to 250 ml with purified water. The solid was removed by filtration with dry filter paper. The diluted filtrate (400×) was used for Si analysis, carried out by means of ICP-OES at a wavelength of 256.1 nm. From the total Si content obtained for the same sample material by X-ray fluorescence spectroscopy, the percentage of dissolved Si was determined.

RESULTS AND DISCUSSION

Examination of extraction methods

Three methods have been widely used for determination of soluble silicate in fertilizers: (1) the official method using a 2% citric acid solution (pH 2) or 0.5 M hydrochloric acid; (2) the semiofficial method with a 1M acetate buffer solution (pH 4); (3) the industry standard method using an ammonium citrate solution (2%; pH 4.5), for the analysis of potassium silicate fertilizer.

From point of view of the lack of correlation between measured dissolved silicate and its uptake by rice plants, we have investigated the effect of citrate buffer solutions on silicate dissolution, bearing in mind the requirements of the official method, and the observed pH's of paddy soils. Studies of the influence of electrolyte concentration and pH showed that, at low buffering capacity, dissolution of silicate fertilizer caused the solutions to become strongly alkaline. At high electrolyte concentration however, the silicate materials were completely dissolved and no difference was observed in solubility. When a 4% citrate buffer solution was used, a clear difference in solubility was observed throughout the acidic to neutral pH range (Figure 1). Since the difference was clearest at pH 5.5, a 4% citrate buffer solution (pH 5.5) was employed for further examinations of silicate fertilizers.

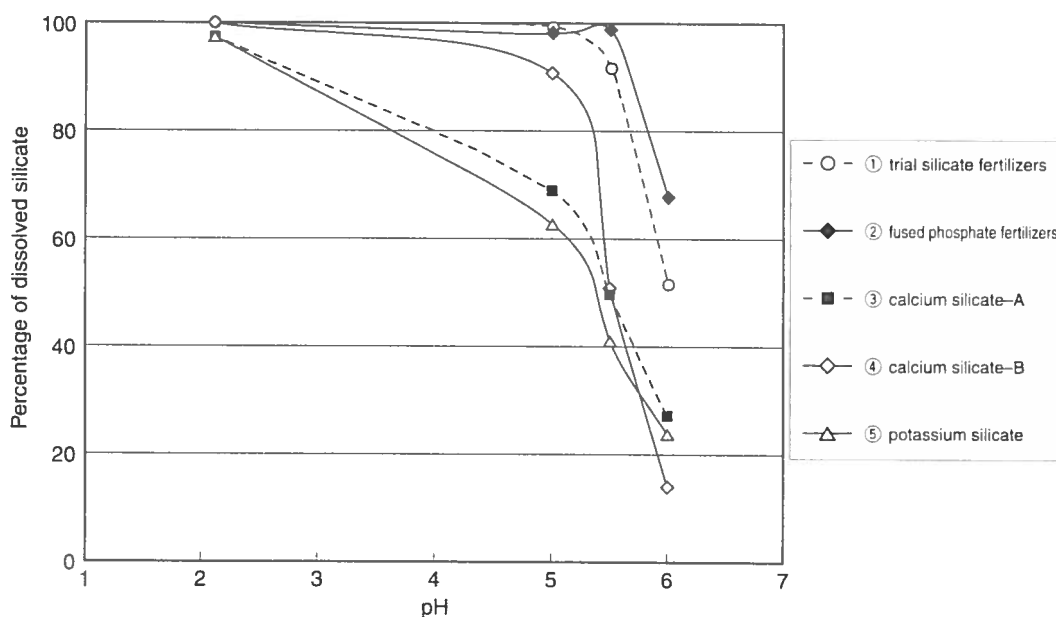


Figure 1. Percentage of dissolved silicate as a function of pH.

Comparison with the official method

Common silicate fertilizers and a trial fertilizer were examined by the three most widely used methods, i.e., the official, semi-official and industry standard methods, and the results were compared with those obtained by our method. When the official and semi-official methods were employed, the percentage of dissolved silicate was determined to be almost 100% for the trial, the fused phosphate and the calcium silicate fertilizers. No difference in silicate dissolution could be detected by the three methods. In contrast, our method clearly showed the differences (Figure 2).

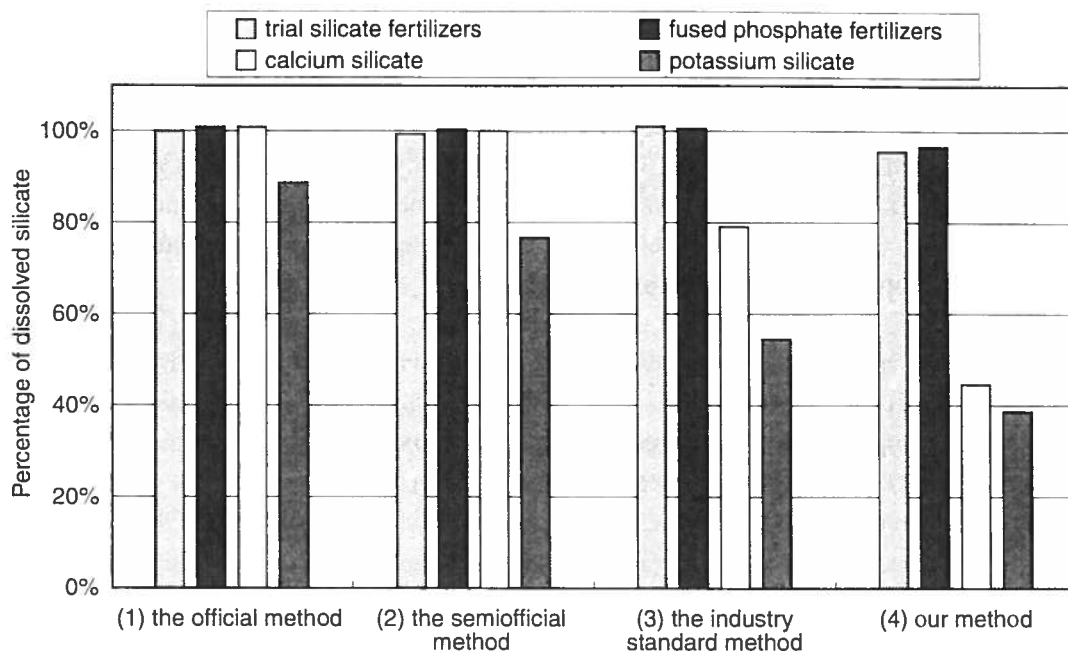


Figure 2. Comparison of the percentages of dissolved Si determined by different methods for each silicate fertilizer.

Correlation with pot experiment

The uptake of Si by rice plants for the new fertilizers developed was studied in pot cultivation experiments. In each pot experiment, single rice plants were cultivated in different pots containing soil fertilized by each sample fertilizer. Other cultivation conditions were kept identical. The quantity of Si taken up in the harvested plant was determined. The value was referred to the quantity of Si taken up by rice plant from a calcium silicate fertilizer, and the relative value (assuming 100 for the reference fertilizer) was defined as the coefficient of Si uptake. Three sample materials, A, B and C, in which the alkali (Ca + Mg) : acid (Si + P) ratio was gradually increased showed a correlation between the chemical composition and the coefficient of Si uptake. The coefficients of the trial fertilizers were around fourfold higher than the value for the calcium silicate fertilizer (Figure 3). In addition, the coefficient indicated a correlation with the percentage of soluble Si determined by our method in which a 4% citrate buffer solution was used for extraction.

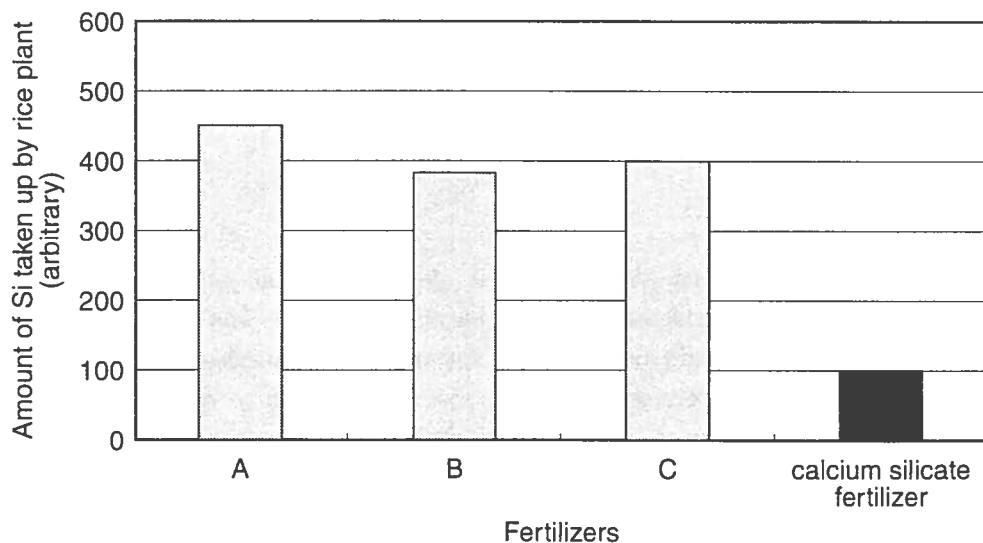


Figure 3. Coefficients of Si uptake from fertilizers. A, B and C : new fused silicate-phosphate fertilizers with different (Ca + Mg) : (P + S) ratios

CONCLUSION

In this study of silicate dissolution, a 4% citrate buffer solution was used instead of the 2% citric acid solution employed in the official method. The buffer solution pH was also adjusted to 5.5, which is close to that of normal paddy soils. As a result, a clear difference in solubility was observed for a variety of silicate fertilizers for which only minimal differences were observable by other widely used methods. This new method may be generally useful for the investigation of other fertilizers, since the experimental procedure is conducted in accordance with the official testing methodology.

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Evaluation of the Solubility of Silicate Fertilizers using an Ion Exchange Resin

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INTRODUCTION

Silicate fertilizers have been used mainly in rice planting where they improve the resistance of plants to disease and insect pests, and prevent it from lodging. As useful silicate fertilizers for rice plant growing, both calcium and potassium silicates have been used. However, under the acidic environment of paddy fields, the dissolution of silicate ion from potassium silicate and calcium silicate fertilizers is not sufficient, and they are often applied in combination with fused phosphate fertilizers. Therefore, it is vital to develop silicate fertilizers which can efficiently provide silicate ion components. Equally important as the development of fertilizers is the establishment of a method which allows evaluation of the dissolution behavior of the silicate ion from trial silicate fertilizer products. It has been pointed out^{1, 2)} that the silicate dissolution tests which have been used officially in fertilizer analysis do not correlate well with the amount of silicate ion taken up by rice plants.

The dissolution of silicate ion components from a fertilizer has been determined by the use of a 2% aqueous solution of citric acid (pH *ca.* 2) or a sodium acetate-buffered solution (initial pH 4). However, both dissolution tests are performed in a low pH range, and are inappropriate in evaluating the dissolution behavior of silicate ion within the pH range of soils (pH 5-7). Therefore, the present official testing methods are not precise enough to obtain a correlation between the amount of dissolved eluted silicate ion components and that absorbed by rice plants. For example, it is reported that the availability of silicate ion to rice plants from blast furnace slag such as calcium silicate fertilizer is low, and is less than half that from fused phosphate fertilizers. From the dissolution tests described above however, approximately 100% of the silicate ion can be dissolved because the tests are performed under strongly acidic conditions which are quite different from actual fields.

Kato, *et al.*³⁾ have reported a method which, by the use of an ion exchange resin instead of soil, enables an evaluation of the behavior of silicate ion dissolution under conditions similar to those of paddy fields. This method is characterized by the use of a weakly acidic cation exchange resin possessing a buffering activity very similar to that of paddy field soils. As an application of this new ion exchange method, we have investigated procedures to measure the dissolution of the silicate ion from several trial silicate fertilizer samples. Further studies have shown that a high fertilizer effect is observed for fertilizers from which more than a specified amount of silicate ion is dissolved over one-month period.s

EXPERIMENTAL

The samples examined for the present study are trial silicate fertilizers, calcium silicate fertilizers, potassium silicate fertilizers, and commercial silicate-containing products. Each sample was crushed with a vibration mill and sieved. Samples of particle diameter <150 μm were used.

The chemical composition of the fertilizers was determined by a X-ray fluorescence analyzer (Rigaku Denki SYSTEM 3370 E). Samples for X-ray fluorescence analysis were prepared by a glass bead sampler (Tokyo Kagaku NT-2000). The reference sample was prepared from a cement reference sample for X-ray fluorescence analysis as the matrix because of its similarity in elemental composition, and to this sample were

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added reagents such as SiO_2 and CaCO_3 in order to adjust the concentrations to a desired range. The amount of silicate ion components which dissolved in water was determined by ICP-OES (Rigaku SPECTRO CIROS; Nippon Jarrell-Ash ICAP-575).

A weakly acidic ion exchange resin (Fig.1, Amberlite IRC-50, carboxylic acid type) was used as the buffer. The silicate ion dissolution tests were performed as follows : sample (0.2 g), ion exchange resin (2 g), and pure water (1 L) were added to a 1 L polyethylene bottle, and the mixture allowed to stand for periods of up to 5 months. At the end of the chosen period, the mixture was stirred gently (ca. 5 min.) with a magnetic stirrer and its pH measured. The supernatant solution was decanted, hydrochloric acid added, diluted, and analyzed for Si using ICP-OES (256.1 nm).

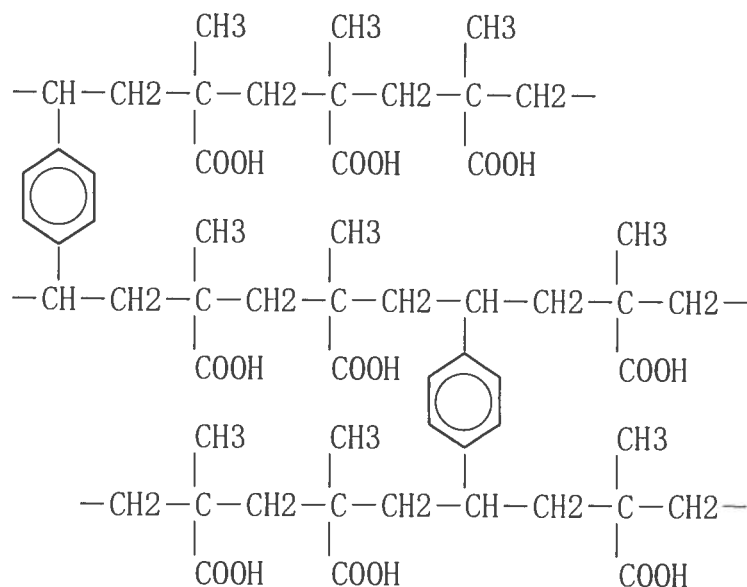


Figure 1. Chemical structure of weakly acidic ion exchange resin (Amberlite IRC-50).

RESULTS AND DISCUSSION

The goal of investigating the dissolution behavior of silicate ion in the presence of ion exchange resin was to confirm its behavior in the silicate fertilizers applied to paddy fields. This method allows, within a given pH range, an observation of dissolution behavior of silicate ion, which depends only on the hydrogen ion concentration, and is independent of the kind and amount of dissolution reagents such as citric acid and acetic acid.

It is generally considered that, on impregnation of a silicate fertilizer by water, alkali metals (Ca, Mg) initially dissolve out from the solid fertilizer yielding a honeycombed structure. Subsequently, silicate ion also dissolves out. As a consequence, the pH of the solution is increased at the initial stage of dissolution. The ion exchange resin acts as a buffer and can maintain the solution pH at a certain constant value. The reaction equation is shown below:



We carried out tests to determine the conditions under which [i] the initial pH increase is suppressed and [ii] it is maintained to 6-7 during the dissolution process. We found that using sample (0.2 g), ion exchange resin (2 g), and pure water (1 L) was most appropriate.

Measurements of pH during the dissolution showed that while the initial pH values of fused phosphate and

calcium silicate fertilizers returned rapidly to neutrality, about one day was required for the trial silicate fertilizers to return to the neutral state. On and after the second day, the pH of every sample was within 6-6.5, and stayed almost constant thereafter (Fig. 2).

The amounts of dissolved silicate ion were determined next. Over the whole period of the test, the largest amount of silicate ion was dissolved from the trial silicate fertilizer. It also showed a high solubility particularly during the initial stage of dissolution. On the other hand, the fused phosphate fertilizer dissolved readily and almost all silicate ion dissolved within two months. The calcium silicate fertilizer was most difficultly soluble, and the amount of dissolved silicate ion over two months was about 1/2 of that of the trial silicate fertilizer. Although the difference between the two decreased gradually (since then) over time, the amounts of dissolved silicate ion over the five months test were 36% for the trial silicate fertilizer and 24% for the calcium silicate fertilizer. The rates of dissolution of silicate ion from the two samples were 92% and 70% respectively (Fig. 3).

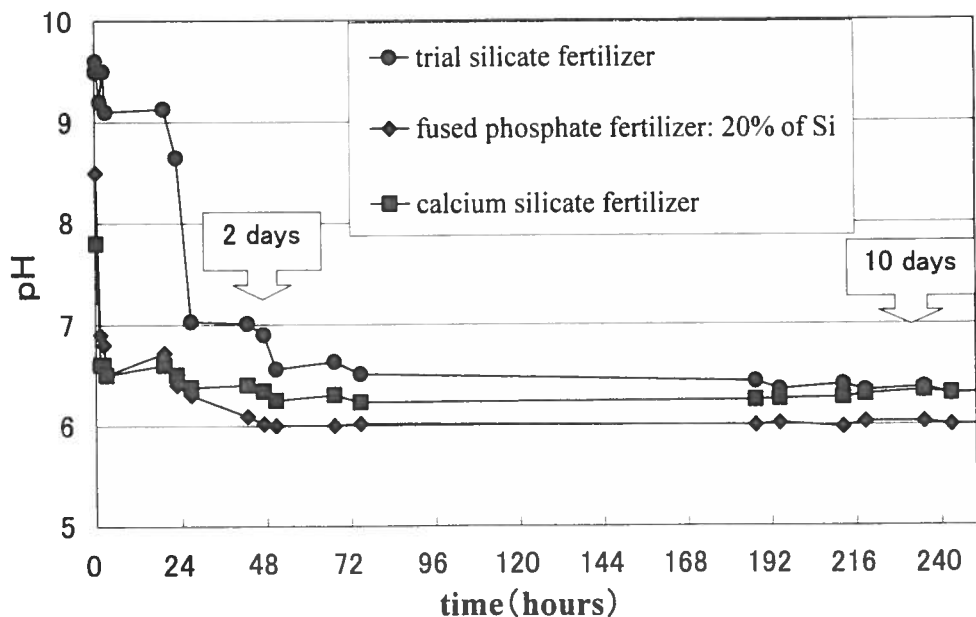


Figure 2. Change of pH with the time passed after start of dissolution.

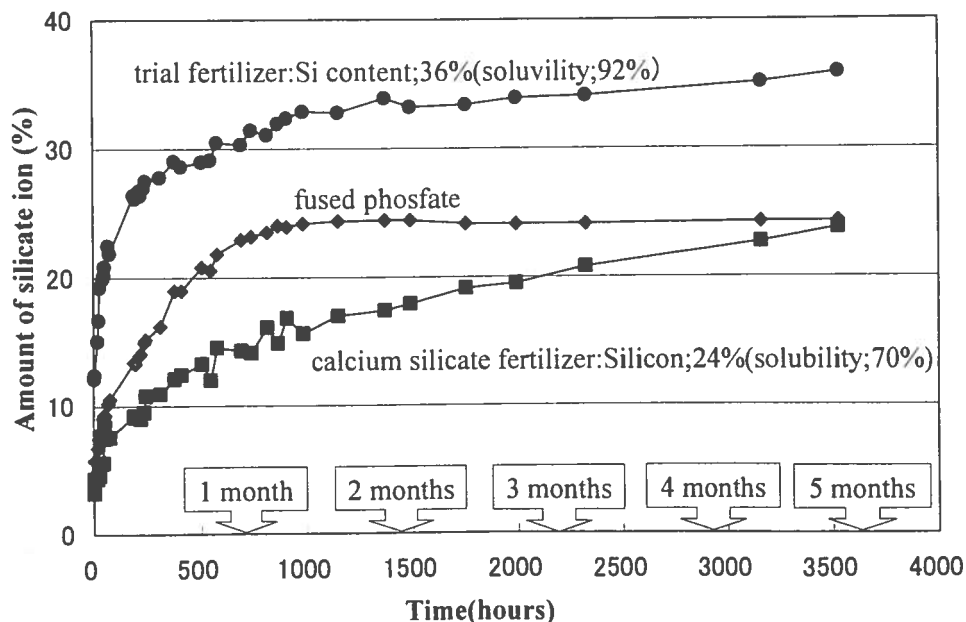


Figure 3. Dissolution behavior of silicate ion in the presence of an ion exchange resin.

In order to evaluate the rate of silicate ion absorption during an actual fertilization to a rice plant, a pot cultivation test was performed next, and the absorbability of silicate ion by a plant under development was evaluated. The pot cultivation tests consisted of simultaneous cultivation of rice plants on soils treated with the trial fertilizers followed by determination of the silicate ion content in the harvested rice plant. The absorption of silicate ion from the trial silicate fertilizers was measured relative to that of a rice plant cultivated with a calcium silicate fertilizer selected as control (taken as 100). The trial silicate fertilizers A, B, C are those in which the ratio of alkali (Ca + Mg) to acid (Si + P) is increased gradually. A correlation was observed between the composition ratio and amount of silicate ion taken up (Fig. 4).

The trial silicate fertilizers showed high rates of silicate ion absorption and were more than 4 times as effective as calcium silicate fertilizers.

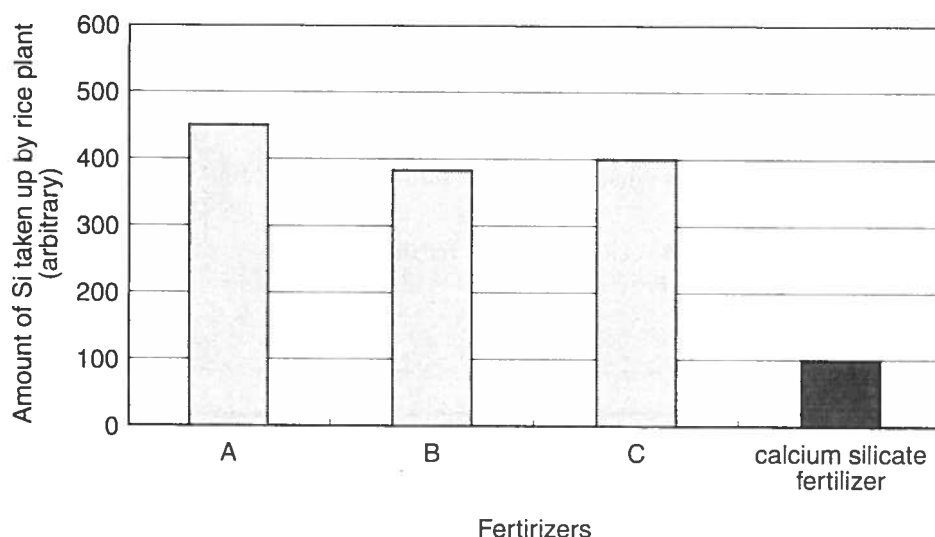


Figure 4. Coefficients of Si uptake from fertilizers. A, B and C : new fused silicate-phosphate fertilizers with different (Ca + Mg) : (P + S) ratios.

CONCLUSION

The ion exchange resin method is considered as an effective evaluation method of estimating the solubility of silicate ion under an environment similar to that of paddy fields. However, the test does require a long period.

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Silicon in Fertilizers Evaluated by Sodium Carbonate Plus Ammonium Nitrate

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An extraction method of silicon in slag and fertilizers was developed to quantify the potentially available Si to the plants. The method consist on breakup the Si content materials in an alkaline extractor of $\text{Na}_2\text{CO}_3 + \text{NH}_4\text{NO}_3$. For this study were used different shaking times (1, 3, 6 and 12 hours), different extractor concentrations (1+1.6%, 3+4.8%, 5+8% and 10+16% of $\text{Na}_2\text{CO}_3 + \text{NH}_4\text{NO}_3$, respectively) and different times of contact with the fertilizer (1, 2, 5, 10, 14 and 21 days). They were examined in order to determine the appropriate extraction condition. Simultaneously, an experiment was carried out in the greenhouse using flooded rice crop with an application of 125 kg ha^{-1} of Si from 12 different Si sources. As a conclusion, shaking time did not show to be significant although 3 hours shaking looks to be more feasible than the others. The concentrations 1.0+1.6% and 3.0+4.8% of $\text{Na}_2\text{CO}_3 + \text{NH}_4\text{NO}_3$ were the most promising extraction. It was also observed that all the Si sources increased solubility along the rest time period, and that 5 and 10 hours of rest period get better correlation with Si uptake by the rice plants. According to the results, the $\text{Na}_2\text{CO}_3 + \text{NH}_4\text{NO}_3$ extractor for Si in the fertilizer looks to have high potential to be used as an index of the available Si to the plants.

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Comparison of Silicon Methods for Fertilizers and Slag

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There are no official methods in Brazil to available silicon in the fertilizer and slag. A study was carried out to evaluate different extractors to predict the available Si to the plants. They were: water, HCl 0.5 M, 5% Na_2CO_3 , Na_2CO_3 1% + NH_4NO_3 1.6%, citric acid 5%, acetic acid 0.5 M, cation exchange resin (Ambilite IR-50, pK 6.1) and leaching column method. Simultaneously, a greenhouse experiment was conducted were 125 kg ha^{-1} of Si was applied from 12 different Si sources on flooded rice crop. The results showed that the most efficient Si sources were Rhodia followed by Wollastonite and the less effective were MB-4 and furnace slag. The acid extractors seem to be adequate to the furnace slag but not to the Wollastonite. The water extraction was the lowest Si recovery. The methods that showed higher correlations between Si extracted from the fertilizers and Si uptake by the plants (flood rice) were resin and Na_2CO_3 1% + NH_4NO_3 1.6% followed by leaching column.

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Role of Silicon on the Production of Rice

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There exists more than 60 elements in higher plants. However, only 17 elements are considered to be "essential" for higher plants: (C, H, O, N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B, Mo, Cl, Ni). Adding to these essential elements, other elements such as Na, Si, Co are called as "beneficial elements" because of their promoting effects on the growth of some limited plants.

On the other hand, silicon is also called an "agronomically essential element" for increasing and/or sustaining rice production (Savant *et al.* 1997). Silicon has important multifunctions such as improving the resistance to lodging, pest and disease, depressing excessive transpiration and keeping the leaves erect, and thus it promotes the use of water efficiency and the photosynthesis rate etc through their deposition in leaf, culm and chaff (Savant *et al.*, 1997; Takahashi, 1995, 1999; Takahashi *et al.*, 1990).

The silicon content in soils is 33% on average, but the amounts of available silicon in rice plants in the paddy soil is not sufficient enough. Furthermore, in intensive rice cultivation under relatively high humid conditions in Japan, a large amount of silicon contained in both rice straw and hull is removed from the paddy field during harvesting time. Therefore, the application of silicate fertilizer has been one of the important common practices for Japanese farmers in order to maintain the silicon supply in paddy fields. An excellent review on silicon management and sustainable rice production until 1995 was done by Savant *et al.* (1997). Therefore, in this paper, I will focus on reviewing the recent progresses of silicon researches on rice production mainly in Japan.

WHY DOES SILICON NOW DRAW ATTENTION TO RICE CULTIVATION IN JAPAN?

Recently many scientists have been paying close attention to silicon application for rice production in Japan because of the following reasons (Saigusa, 2000).

- 1) **A remarkable decrease in the natural supply of silicon by irrigation water:** Rice plants absorb silicon nearly 1t/ha during one cropping seasons and about 1/4 of silicon was originated from irrigation water. However, according to Kumagai *et al* (1999), the average silicon content of irrigation water in Yamagata Prefecture measured in 1996 (10.2 ppm) was less than half of that measured in 1956 (23.9 ppm). The reasons for the decrease in the silicon content of irrigation water were attributed to the construction of an artificial dam in the upper stream region and also the concretion of the canal facility. Adding to these problems, the amount of irrigation water itself applied in one season remarkably decreased, because of subsoil compaction by the use of heavy machinery.
- 2) **A decrease in the amount of rice straw recycling:** Rice straw contains more than 12% silicon and has been recycled in the form of a matured rice straw compost even in soil with poor drainage. However, recently, Japanese rice farmer have been having other interests or labor problems such as side business or old age, which have forced them to stop recycling the rice strew as a compost.
- 3) **A decrease in the application rate of silicon fertilizer and its availability:** International liberalization of the rice market in Japan, forced Japanese farmers to lower the rice production cost. Adding to this, it was clarified that some of the calcium silicate slag fertilizers used for a long time did not work as much as the

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guaranteed availability by 0.5 N HCl extracted Si (Saigusa *et al.* 1998a,b).

- 4) **The consumer's demand for chemical free rice and good tasting rice:** After 1970, Japanese government has forced to conduct a rice production adjustment (about 30%) because of an over production of rice. Farmers wanted to produce valuable rice such as chemical free rice, organic farming rice and good tasting rice. Therefore, silicon was beneficial because it suppressed pests and diseases (Savant *et al.* 1997) and also decreased protein content in rice grain (Fujii, 2002; Miyamori *et al.*, 1996).
- 5) **The development of a new silicon fertilizer:** Based on the reasons mentioned above, sophisticated farmers and companies were interested in developing a new type of silicon fertilizer which is highly efficient, for example, autoclaved light concrete (ALC) fertilizer, silica gel, fused phosphorus-silicate fertilizer etc (Kato, 2002)
- 6) **Soil silicon availability in cooler regions:** Rice is originally a tropical crop and Japanese scientists have contributed to a great extent in the development of both rice variety tolerance to cool damage and rice cultivation methods effective in cool temperate zones. Consequently, main rice-producing districts have shifted to northern Japan (cooler). However, both the soil silicon availability and rice absorption in cooler regions are inferior to those in warmer regions, thus silicon nutrition in rice production is extremely important in northern Japan rather than southern Japan. Adding to these phenomena, in northern Japan some paddy soil are classified into Spodosols and Andisol, which require a high silicon application for rice cultivation.
- 7) **An increase in world population and food security.:** Remarkable increases in the world's population is still continuing and it is expected to 100 billion by 2050, while the land area for agricultural use is decreasing year by year. Therefore the yield of crops in unit land areas has to be increased in a great extent in order to maintain the same food supply. On the other hand, the global environment is getting worse and worse and food security is one of the greatest concerns. Consequently, we should try to improve the crop yield without increasing environmental loading attributed to crop production. Silicon may contribute greatly to environmental friendly high-yielding cultivation of rice by the reasons mentioned above.

PHYSIOLOGICAL ASPECTS OF SILICON IN RICE PLANTS

Rice plants are known to be silicon accumulators and actively absorb silicon from soil solution in the form of monosilicic acid. The active absorption of silicon in the lateral roots rather than root hairs were clarified using two mutants of rice :one defective in the formation of roots hairs and another in the formation of lateral roots (Ma *et al.*, 2001).

The silicic acid absorption of rice has been believed to occur in the uncharged and monomolecular state $[\text{Si}(\text{OH})_4^0]$. However, a new hypothesis, the absorption of ionic state of silicic acid $[(\text{OH})_3\text{SiO}^-]$, was proposed using nourishment composition and a cation-anion balance of paddy rice (Ito, 2001).

Absorbed monosilicic acid are polymerized as Si gel or plant opal ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) when silicon concentration increases by losing absorbed water through transpiration. Association of polymerized silicon with cellulose and hemicellulose in the cell wall have been suggested, and recently, a significant role in the formation of cross-links between lignin and carbohydrate via association with phenolic acids or aromatic rings in the cell wall of rice shoot were proposed as a hypothesis (Inanaga *et al.*, 1995).

Polymerized silica also exists in the epidermal cells of the leaf sheath, leaf blade and hull mainly as plant opal, a very slightly soluble form. In the leaf, it is deposited as a 2.5 μ thick layer (cuticle-Si double layer) in the space immediately beneath the cuticle layer in the epidermal cells. The location and mechanical strength of this cuticle-Si double layer contribute to maintain erect leaves, minimize transpiration, and suppress fungal disease and insect pest (Savant *et al.*, 1997).

ROLE OF SILICON IN RICE PRODUCTION

1) The nursing of healthy rice seedlings

In Japan, the transplanting of rice seedlings is a common practice as opposed to direct seeding cultivation because of a cool weather and a high demand from consumers on good tasting rice. Therefore, raising healthy seedlings is considered to be one of the most important practices for growing good tasting rice and also the application of silicate fertilizer in nursery bed soil contribute very much in raising healthy seedlings (Fujii, 2000; Fujii *et al.*, 1999; Maekawa *et al.*, 2002a,b).

Application of silica gel enhanced both the rooting ability and the root activity of rice plants and was more effective on dry matter production than tiller production. (Fujii *et al.*, 1999; Fujii, 2002). These results attributed to an increased photosynthetic rate caused by a high stomatal aperture and a high light extinction coefficient with silica gel application. Silica gel application in the nursery box resulted in a grain yield increase and a protein content decrease. This means high-yielding and high-quality rice production is highly feasible by silica gel application in the nursery box.

The application of potassium silicate solution into nursery bed soil improved the silicon nutrition of rice seedlings and suppressed rice seedling blast (Maekawa *et al.*, 2001 and 2002a). After inoculating rice blast fungus to the seedlings treated by potassium silicate, accumulation of silicon at the penetration sites (near appressoria) and remarkable generation of active oxygen was observed. Therefore, the possibility of amplifying the recognition signal by silicon right after rice blast fungus infection was postulated. (Maekawa *et al.*, 2002a; 2002b).

The rice hull ash-treated rice seedlings showed more dry matter, P, K and Si, and less N, Al and Mn than the untreated ones (Sistani *et al.*, 1997).

2) Suppression of diseases and pests in rice cultivation

The reinforcement of the mechanical strength of leaves protects the rice plants from fungal diseases and insect pests (Savant *et al.*, 1997). Among the rice diseases, rice leaf and neck blast are the most serious diseases of rice cultivation in high humid temperate or tropical regions and a large number of researches on the relationships between silicon nutrition and rice blast infection have been done in many countries. As mentioned above, Si application on nursery bed soil is very effective in repressing the rice blast incidence of rice seedlings, which are generally raised in warm and high humid glasses or vinyl houses (Fujii 2000, Maekawa *et al.*, 2001, 2002a).

Advanced environmental friendly rice cultivation does not require chemical pesticide application for controlling fungal diseases without any decreases in the rice grain yield. Effects of silicon application on the managing leaf and neck blast were demonstrated on Histosols in the same degree as a fungicide (Deren *et al.*, 1994; Datnoff *et al.*, 1997).

The blast infection in the porous hydrate calcium silicate plot was much less than that in the control plot, and the number of silicified bulliform cells (main invasion site of rice blast) increased exponentially with increasing silicon content in the leaf blade, while the number of silicified short cells had no relation to the silicon content (Saigusa *et al.*, 2000).

3) Promotion of rice grain production improving their photosynthetic activity.

Increases in dry matter and grain yield production in rice with silicon fertilization have been well documented in many countries (Savant *et al.*, 1997; Fujii 2002; Ando *et al.*, 2002) and are mainly attributed to improving or maintaining photosynthetic activities (Agarie *et al.*, 1992; Fujii, 2002; Ando *et al.*, 2002). Effects of silicon on photosynthetic activity in rice cultivation may be divided into two parts: promoting the individual leaf photosynthesis rate and improving the canopy structure. Effects of silicon application were often found in the rice grown under a cool climate (weak sunshine) or shade conditions. Furthermore, the depression of photosynthetic activity in the afternoon improved by silicon application (Ando *et al.*, 1997). The reason for this was because silicon application prevented over transpiration and increased water use efficiency

in leaves (Agarie *et al.*, 1991, 1998; Matoh *et al.*, 1991). On the other hand, the distance between the desirable and actual leaf position of the top leaf was shorter in the rice treated by silicon than that in the rice grown without silicon application. This meant that silicon application enhanced the erectness of the leaf blades and thus the canopy structure of rice plants for the photosynthesis much improved (Ando *et al.*, 2002).

4) Production of a high quality rice under silicon fertilization.

The quality of good taste rice is closely related to the protein content of brown rice that is also in close correlation with the efficiency of brown rice production per unit nitrogen (Miyamori, 1996). Silicon application improves photosynthetic activity through both structural changes and physiological changes. Thus the promotion of the carbohydrate translocation from the leaf and stem to the panicle takes place (Fujii, 2002). Consequently, both the efficiency of brown rice production per unit nitrogen and the ripening ability (percentage ripened grain and thousand grain weight) are much improved by the silicon application (Miyamori, 1996). The possibility of both high yielding and low protein rice cultivation was pointed out (Fujii, 2002)

SILICON AVAILABILITY AND NEW SILICATE FERTILIZER

1) Silicon availability in paddy soil

For evaluating the available silicon in paddy soils, the acetate buffer method has been widely used in Japan. However, this method seems to be too strong to extract the available silicon in the soil as previously amended with calcium silicate slag, and it may dissolve some non-available silicon from the slag remained (Savant *et al.*, 1997). Some incubation methods such as one week at 40°C, 4 weeks at 30°C, 4 weeks at 30°C with silicate solution and the successive extraction method were developed and generally showed a much better correlation between the available soil silicon and the silicon absorbed by rice plants than the acetate buffer method in the soils that applied silicate fertilizer.

2) Silicon availability in slag fertilizer.

Calcium silicate slags have been practically applied to paddy soils as silicon source in Japan. However, their official guaranteed silicon availability by 0.5 N HCl was not necessarily reflected to plant-available silicon (Saigusa *et al.*, 1998). Thus, a new water extraction method with a cation exchange resin was developed in order to evaluate the silicon availability in slag fertilizers (Kato and Owa, 1997a, b)

3) New silicate fertilizer

The industrial waste of autoclaved light concrete (PS:porous hydrate calcium silicate) were able to supply a much higher amount of silicon to rice plants throughout the growing season than the commercial slag silicate (SS) fertilizers (Saigusa *et al.* 1998). The crystalline structure of tobermorite ($\text{Ca}_5[(\text{SiAl})_6\text{O}_{18}\text{H}_2] \cdot 4\text{H}_2\text{O}$), main component of PS, altered to calcite and silica skeletons by 53 days after transplanting, and the silica skeleton remained as a silicon source for rice plants even after disappearance of tobermorite (Saigusa *et al.*, 2000; Yamato *et al.*, 2000). The use efficiency of PS-Si by rice plants was more than 70% at harvest, while that of SS was only 30-40% (Kato, 2002). Silica gel containing $\text{SiO}_2 > 99\%$ with pH 4.5-5.5 was registered as a silicate fertilizer in 1999 in Japan and showed significant effects on the growth, grain yield and silicon content of rice as mentioned above (Fujii *et al.*, 1999; Fujii, 2002)

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The Role of Silicon on Tropical Crops

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For tropical crops, silicon fertilization (slag) has been used to reduce soil acidity and increase insect, fungal and water stress resistance. Sugarcane, rice and pasture are some of the tropical crops that benefit from silicon especially when the soils are low or limiting in this element. Rice yields increased by 84% in comparison to the control while a 15% increase for sugarcane was observed when there was a Si deficiency in the soil. Silicon fertilizer effects on plant yield were much greater when the soils were submitted to water stress. Sometimes the expected positive agronomic results cannot be observed in the field because of the low solubility of the Si source used. This has caused much confusion and lack of credibility in the use of silicon among growers. Currently, there are no standard procedures to determine available silicon in the fertilizer or slag sources being used in Brazil. A study was conducted to evaluate different extractors to predict Si availability in fertilizers and slags. These methods demonstrated that the best correlations between Si extracted and Si accumulation in the plants were resin and Na_2CO_3 (1%) + NH_4NO_3 (1.6%) followed by a leaching column.

Effect of Root and Foliar Applications of Silicon on Growth and Quality of Five-Selected Vegetables in Deep Flow Technique

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At present, continuing evidence suggests that silicon (Si) does enhance the growth of a wide range of crops, from rice, sugarcane and wheat, to citrus, strawberry, cucumber, tomato and rose. Expressly, Si supplements have been widely used in China, Japan and Korea in rice and sugarcane production and in Europe for the production of greenhouse crops. Regarding the above-mentioned beneficial effect of Si on crops mostly grown under field condition, the Faculty of Agricultural Technology, KMITL, Thailand has conducted the research to determine such beneficial effects of Si (supplemented as root and foliar application) on DFT-grown selected vegetables widely consumed in Thailand in order to increase and/or sustain crop productivity. Besides, silicon source was put into consideration for economical crop production in this regard. Three \times five \times two factorials in Completely Randomized Design with 9 replications was employed. Factor A and B were sources (Sodium silicate : $\text{Na}_2\text{Si}_3\text{O}_7$, Potassium silicate : $\text{K}_2\text{Si}_3\text{O}_7$ and Zeolite) and concentrations (0, 50, 100, 150 and 200 ppm) of Si applied as root application while factor C was two concentrations (500 and 0 ppm) of $\text{Na}_2\text{Si}_3\text{O}_7$ applied as foliar application. Five vegetables, namely celery (*Apium graveolens*, Umbelliferae), Chinese cabbage (*Brassica campestris* var. *chinensis*, Cruciferae), lettuce (*Lactuca sativa*, Compositae), phakkat kung (*Brassica* sp., Cruciferae) and watercress (*Nasturtium officinale*, Brassicaceae) were tested for this regard. For overall, the results showed that Si application has significantly improved the growth of all tested vegetables (except celery) compared to its corresponding control. Besides, an additive effect of foliar application on root application was found on two vegetables (watercress and Chinese cabbage). No additive effect of foliar application was observed on phakkat kung, meanwhile its adverse effect was detected on lettuce. In terms of root application, zeolite was shown to be the best Si source for all four Si-responsive vegetables (Chinese cabbage, lettuce, phakkat kung and watercress) and its most effective concentration was ranged from 50-200 ppm. Crop quality was evaluated on Chinese cabbage by sensory evaluation using multiple comparison test. It showed that Si significantly improved some qualities such as color and crispness, while bitterness, texture and fiber were not different from the un-supplemented one. In terms of disease occurrences, natural contamination of Pythiaceae was not found throughout the experiment.

Table 1. Effect of root and foliar applications of silicon on growth of Celery (*Apium graveolens*; Umbelliferae) in Deep Flow Technique.

Root application		Foliar application (Na ₂ Si ₃ O ₇)	Fresh weight (g)		Dry weight (g)		
Source	Conc ^a		Top	Root	Top	Root	Top/root
Na ₂ Si ₃ O ₇	0	500 ppm	16.89	3.44	1.839	0.179	10.58 a-c ^{1/}
	50		20.50	4.56	2.235	0.225	10.10 a-c
	100		18.43	3.81	1.931	0.190	10.09 a-c
	150		18.06	4.00	1.912	0.204	9.76 a-c
	200		17.68	4.00	1.936	0.221	8.87 bc
	0	0 ppm	17.57	3.72	1.936	0.206	10.41 a-c
	50		20.12	4.09	2.070	0.186	11.37 a
	100		17.49	3.93	1.930	0.189	10.23 a-c
	150		18.06	3.56	1.940	0.179	11.17 ab
	200		18.74	4.77	2.140	0.252	8.57 c
K ₂ Si ₃ O ₇	0	500 ppm	16.89	3.44	1.839	0.179	10.58 a-c
	50		18.23	4.03	1.971	0.209	9.75 a-c
	100		17.86	3.47	1.830	0.211	9.15 a-c
	150		16.16	3.23	1.642	0.166	10.80 a-c
	200		17.17	3.86	2.157	0.215	10.16 a-c
	0	0 ppm	17.57	3.72	1.936	0.206	10.41 a-c
	50		18.83	4.13	2.008	0.211	9.61 a-c
	100		16.02	3.10	1.636	0.178	10.02 a-c
	150		16.93	3.18	1.777	0.170	11.26 a
	200		17.80	3.90	2.153	0.291	9.85 a-c
Zeolite	0	500 ppm	16.89	3.44	1.839	0.179	10.58 a-c
	50		20.49	4.07	2.200	0.220	10.01 a-c
	100		19.63	3.88	2.115	0.217	10.18 a-c
	150		19.09	3.83	2.125	0.216	10.08 a-c
	200		17.88	3.46	1.869	0.180	10.63 a-c
	0	0 ppm	17.57	3.72	1.936	0.206	10.41 a-c
	50		19.18	3.81	2.065	0.217	9.74 a-c
	100		19.38	3.94	2.062	0.215	9.78 a-c
	150		17.82	3.38	1.923	0.194	9.80 a-c
	200		18.39	3.43	1.995	0.184	11.16 ab
C.V. =			29.07%	35.46%	31.74%	31.51%	19.84%
Factor A (source of silicon)			Ns	ns	ns	ns	ns
Factor B (silicon conc ^a in nutrient)			Ns	ns	ns	ns	ns
Factor C (foliar application)			Ns	ns	ns	ns	ns
Factor A*B			Ns	ns	ns	ns	ns
Factor A*C			Ns	ns	ns	ns	*
Factor B*C			Ns	ns	ns	ns	ns
Factor A*B*C			Ns	ns	ns	ns	ns

1/ = Values within column followed by the same letter are not significantly different (P < 0.05) by Duncan's Multiple Range Test.

Table 2. Effect of root and foliar applications of silicon on growth of Chinese cabbage (*Brassica campestris* var. *chinensis*; Cruciferae) in Deep Flow Technique.

Root application		Foliar application (Na ₂ Si ₃ O ₇)	Fresh weight (g)		Dry weight (g)		
source	conc [□]		Top	Root	Top	Root	Top/root
Na ₂ Si ₃ O ₇	0	500 ppm	41.43 f ^{1/}	3.13 d-f ^{1/}	1.606 ef ^{1/}	0.329 a-f ^{1/}	5.14 cd ^{1/}
	50		76.64 a-d	2.62 f	3.269 a-c	0.458 a	7.32 a-d
	100		65.30 b-f	4.69 a-f	1.846 d-f	0.246 d-f	7.92 a-c
	150		70.70 b-e	4.48 a-f	1.752 ef	0.297 c-f	6.87 a-d
	200		62.21 b-f	3.10 d-f	2.095 d-f	0.275 c-f	7.76 a-d
	0	0 ppm	41.66 f	3.04 ef	1.444 f	0.220 ef	6.88 a-d
	50		67.19 b-e	4.68 a-f	3.373 ab	0.366 a-e	9.33 a
	100		46.86 ef	3.92 b-f	1.884 d-f	0.334 a-f	6.42 b-d
	150		70.33 b-e	5.30 a-e	2.256 d-f	0.425 a-c	5.01 d
	200		65.41 b-f	4.98 a-f	2.012 b-e	0.275 b-f	8.92 ab
K ₂ Si ₃ O ₇	0	500 ppm	41.43 f	3.13 d-f	1.606 ef	0.329 a-f	5.14 cd
	50		59.09 c-f	6.40 a	1.949 d-f	0.291 c-f	7.58 a-d
	100		62.59 b-f	3.37 c-f	1.974 d-f	0.271 d-f	7.50 a-d
	150		75.39 a-d	3.51 c-f	2.131 d-f	0.279 c-f	7.46 a-d
	200		84.73 a-c	5.46 a-d	2.521 b-e	0.391 a-d	6.44 b-d
	0	0 ppm	41.66 f	3.04 ef	1.444 f	0.220 ef	6.88 a-d
	50		62.33 b-f	5.58 a-c	1.70 ef	0.230 ef	7.65 a-d
	100		54.89 d-f	4.06 b-f	1.897 d-f	0.306 b-f	6.48 a-d
	150		78.13 a-d	4.43 a-f	2.238 d-f	0.342 a-f	6.82 a-d
	200		68.87 b-e	6.21 ab	2.222 d-f	0.364 a-e	6.55 a-d
Zeolite	0	500 ppm	41.43 f	3.13 d-f	1.606 ef	0.329 a-f	5.14 cd
	50		95.43 a	3.68 c-f	3.74 a	0.447 ab	8.58 ab
	100		70.33 b-e	4.00 c-f	2.044 d-f	0.279 c-f	8.03 ab
	150		78.56 a-d	5.43 a-d	2.553 b-e	0.329 a-f	8.46 ab
	200		67.97 b-e	5.09 a-e	1.595 ef	0.212 f	7.59 a-d
	0	0 ppm	41.66 f	3.04 ef	1.444 f	0.220 ef	6.88 a-d
	50		66.21 b-f	4.39 a-f	2.389 c-f	0.278 c-f	9.08 ab
	100		65.47 b-f	3.84 c-f	1.883 d-f	0.268 d-f	6.97 a-d
	150		85.51 ab	4.33 a-f	2.799 b-d	0.330 a-f	8.42 a-d
	200		69.83 b-e	5.59 a-c	2.402 c-f	0.382 a-d	6.28 b-d
C.V. =			30.70%	48.00%	29.70%	31.51%	25.88%
Factor A (source of silicon)			Ns	ns	ns	ns	ns
Factor B (silicon conc [□] in nutrient)			**	**	**	*	**
Factor C (foliar application)			Ns	ns	ns	ns	ns
Factor A*B			Ns	*	**	*	ns
Factor A*C			Ns	ns	ns	ns	ns
Factor B*C			Ns	ns	ns	**	**
Factor A*B*C			Ns	ns	ns	ns	ns

1/ = Values within column followed by the same letter are not significantly different (P < 0.05) by Duncan's Multiple Range Test.

Table 3. Effect of root and foliar applications of silicon on growth of lettuce (*Lactuca sativa*; Compositae) in Deep Flow Technique.

Root application		Foliar application (Na ₂ Si ₃ O ₇)	Fresh weight (g)		Dry weight (g)		
Source	conc ^a		Top	Root	Top	Root	Top/root
Na ₂ Si ₃ O ₇	0	500 ppm	35.91 f ^{1/}	2.26 h ^{1/}	1.536 g ^{1/}	0.085 d ^{1/}	19.28 ab ^{1/}
	50		66.73 a	4.28 a	2.511 ab	0.175 ab	14.61 a-c
	100		60.81 a-c	4.32 a	2.660 a	0.187 a	14.59 a-c
	150		51.57 a-f	3.96 a-c	2.008 a-g	0.160 a-c	12.74 c
	200		44.56 c-f	2.72 f-h	1.573 fg	0.125 b-d	13.87 bc
	0	0 ppm	45.22 c-f	2.94 d-e	1.715 d-g	0.108 cd	17.09 a-c
	50		65.77 a	4.09 ab	2.482 a-c	0.176 ab	14.28 a-c
	100		58.01 a-d	3.70 a-d	2.421 a-d	0.153 a-c	15.85 a-c
	150		49.01 b-f	3.76 a-d	1.818 b-g	0.140 a-d	12.95 c
	200		52.22 a-f	3.14 c-g	1.908 a-g	0.137 a-d	15.58 a-c
K ₂ Si ₃ O ₇	0	500 ppm	35.91 f	2.26 h	1.536 g	0.085 d	19.28 ab
	50		47.77 c-f	3.1 d-g	1.830 b-g	0.129 b-d	14.44 a-c
	100		53.44 a-e	3.80 a-d	2.139 a-g	0.161 a-c	13.18 c
	150		40.73 ef	2.81 e-h	1.633 e-g	0.124 b-d	13.65 c
	200		43.20 d-f	2.65 gh	1.699 e-g	0.134 a-d	12.97 c
	0	0 ppm	45.22 c-f	2.94 d-e	1.715 d-g	0.108 cd	17.09 a-c
	50		57.68 a-d	3.64 a-e	2.169 a-g	0.151 a-c	14.86 a-c
	100		47.96 b-f	3.01 d-h	1.799 c-g	0.130 b-d	13.95 a-c
	150		48.43 b-f	3.27 b-g	1.837 a-g	0.130 b-d	14.37 a-c
	200		45.97 c-f	3.17 c-g	1.845 b-g	0.190 a-c	12.09 c
Zeolite	0	500 ppm	35.91 f	2.26 h	1.536 g	0.085 d	19.28 ab
	50		51.94 a-f	3.12 c-g	1.967 a-g	0.114 cd	17.26 a-c
	100		45.26 c-f	3.16 c-g	1.737 d-g	0.143 a-c	12.47 c
	150		50.42 a-f	2.97 d-h	1.812 b-g	0.123 b-d	19.30 a
	200		54.26 a-e	3.59 a-e	2.059 a-g	0.131 b-d	16.67 a-c
	0	0 ppm	45.22 c-f	2.94 d-e	1.715 d-g	0.108 cd	17.09 a-c
	50		60.94 a-c	3.69 a-d	2.318 a-e	0.137 a-d	17.29 a-c
	100		55.53 a-e	3.06 d-h	1.992 a-g	0.142 a-c	14.24 a-c
	150		64.48 ab	3.88 a-d	2.287 a-f	0.150 a-c	16.18 a-c
	200		56.73 a-e	3.52 a-f	2.099 a-g	0.131 b-d	16.29 a-c
C.V. =			28.86%	23.06%	31.74%	36.41%	30.32%
Factor A (source of silicon)			**	**	ns	*	*
Factor B (silicon conc ^b in nutrient)			**	**	**	**	**
Factor C (foliar application)			**	*	ns	ns	ns
Factor A*B			Ns	**	ns	ns	ns
Factor A*C			Ns	ns	ns	ns	ns
Factor B*C			Ns	**	ns	ns	ns
Factor A*B*C			Ns	ns	ns	ns	ns

1/ = Values within column followed by the same letter are not significantly different (P < 0.05) by Duncan's Multiple Range Test.

Table 4. Effect of root and foliar applications of silicon on growth of Phakkat kung (*Brassica* sp.; Cruciferae) in Deep Flow Technique.

Root application		Foliar application (Na ₂ Si ₃ O ₇)	Fresh weight (g)		Dry weight (g)		
Source	conc ^a		Top	Root	Top	Root	Top/root
Na ₂ Si ₃ O ₇	0	500 ppm	38.4 b-d ^{1/}	3.133 d-f ^{1/}	1.986 cd ^{1/}	0.219 b ^{1/}	9.24 cd ^{1/}
	50		37.40 cd	2.62 f	2.20 b-d	0.313 a	11.62 a-d
	100		43.03 a-d	4.69 a-f	2.549 a-d	0.264 ab	9.85 a-d
	150		50.38 a-d	4.48 a-f	2.831 a-d	0.300 a	10.05 a-d
	200		31.49 d	3.10 d-f	1.835 d	0.197 b	9.52 b-d
	0	0 ppm	38.94 b-d	3.04 ef	2.203 b-d	0.197 b	10.99 a-d
	50		51.92 a-c	4.68 a-f	2.816 a-d	0.290 ab	10.23 a-d
	100		49.39 a-d	3.92 b-f	3.000 a-c	0.237 ab	13.00 a-c
	150		58.64 a	5.30 a-e	3.238 ab	0.300 a	13.30 ab
	200		49.69 a-d	4.98 a-f	2.822 a-d	0.301 a	9.72 b-d
Ksi	0	500 ppm	38.40 b-d	3.133 d-f	1.986 cd	0.219 b	9.24 cd
	50		61.24 a	6.40 a	3.506 a	0.332 a	11.60 a-d
	100		45.59 a-d	3.37 c-f	2.523 a-d	0.240 ab	10.96 a-d
	150		42.72 a-d	3.51 c-f	2.421 a-d	0.257 ab	9.85 a-d
	200		48.51 a-d	5.46 a-d	2.853 a-d	0.316 a	9.36 cd
	0	0 ppm	38.94 b-d	3.04 ef	2.203 b-d	0.197 b	10.99 a-d
	50		59.58 a	5.58 a-c	3.111 a-c	0.304 a	11.10 a-d
	100		49.18 a-d	4.06 b-f	2.756 a-d	0.253 ab	11.75 a-d
	150		45.10 a-d	4.43 a-f	2.52 a-d	0.29 ab	9.58 b-d
	200		54.94 a-c	6.21 ab	3.517 a	0.347 a	10.85 a-d
Zeolite	0	500 ppm	38.40 b-d	3.133 d-f	1.986 cd	0.219 b	9.24 cd
	50		51.43 a-c	3.68 c-f	2.957 a-d	0.270 a	11.44 a-d
	100		51.61 a-c	4.00 b-f	2.476 a-d	0.302 a	8.53 d
	150		57.00 ab	5.43 a-d	3.113 a-c	0.312 a	10.41 a-d
	200		53.10 a-c	5.09 a-e	2.902 a-d	0.313 a	9.51 b-d
	0	0 ppm	38.94 b-d	3.04 ef	2.203 b-d	0.197 b	10.99 a-d
	50		56.24 a-c	4.39 a-f	3.142 ab	0.287 a	13.74 a
	100		54.13 a-c	3.84 c-f	2.918 a-d	0.277 a	10.74 a-d
	150		55.39 a-c	4.33 a-f	2.947 a-d	0.247 ab	13.04 a-c
	200		50.93 a-c	5.59 a-c	2.647 a-d	0.315 a	9.02 d
C.V. =			34.67%	48.00%	36.92%	48.26%	31.98%
Factor A (source of silicon)			Ns	ns	ns	ns	ns
Factor B (silicon conc ^a in nutrient)			**	**	**	**	*
Factor C (foliar application)			*	ns	*	ns	**
Factor A*B			Ns	ns	ns	ns	ns
Factor A*C			Ns	ns	ns	ns	ns
Factor B*C			Ns	ns	ns	ns	ns
Factor A*B*C			Ns	ns	ns	ns	ns

1/ = Values within column followed by the same letter are not significantly different (P < 0.05) by Duncan's Multiple Range Test.

Table 5. Effect of root and foliar applications of silicon on growth of watercress (*Nasturtium officinale*; Brassicaceae) in Deep Flow Technique.

Root application		Foliar application (Na ₂ Si ₃ O ₇)	Fresh weight (g)		Dry weight (g)		
source	conc ^a		Top	Root	Top	Root	Top/root
Na ₂ Si ₃ O ₇	0	500 ppm	14.10 ef ^{1/}	0.26 c-f ^{1/}	0.752 d-f ^{1/}	0.065 c-g ^{1/}	11.67 c-e ^{1/}
	50		24.74 a	0.27 c-f	1.305 a	0.067 c-g	22.22 a
	100		23.53 a-d	0.39 a	1.222 a-c	0.097 a-d	14.60 a-e
	150		24.48 ab	0.40 a	1.237 ab	0.100 a-c	12.77 c-e
	200		24.03 a-c	0.43 a	1.205 a-d	0.108 a	11.46 d-e
	0	0 ppm	13.56 ef	0.20 f-h	0.717 ef	0.050 fg	15.52 a-e
	50		13.44 ef	0.20 f-h	0.689 ef	0.049 fg	14.72 a-e
	100		17.08 a-f	0.28 c-e	0.923 a-f	0.070 b-g	13.76 b-e
	150		16.56 b-f	0.25 c-g	0.878 a-f	0.063 d-g	17.00 a-e
	200		16.48 b-f	0.23 d-h	0.757 c-f	0.057 fg	15.28 a-e
K ₂ Si ₃ O ₇	0	500 ppm	14.10 ef	0.26 c-f	0.752 d-f	0.065 c-g	11.67 c-e
	50		17.30 a-f	0.25 c-g	0.891 a-f	0.063 d-g	15.70 a-e
	100		23.21 a-d	0.41 a	1.22 a-d	0.102 ab	12.24 c-e
	150		18.66 a-e	0.38 ab	0.952 a-e	0.094 a-e	10.65 e
	200		15.79 d-f	0.28 c-e	0.853 a-f	0.070 b-g	14.75 a-e
	0	0 ppm	13.56 ef	0.20 f-h	0.717 ef	0.050 fg	15.52 a-e
	50		9.07 f	0.16 h	0.470 f	0.04 g	12.40 c-e
	100		19.6 a-e	0.32 bc	1.029 a-e	0.08 a-f	13.13 c-e
	150		14.64 ef	0.27 c-f	0.782 b-f	0.066 c-g	11.88 c-e
	200		14.14 ef	0.30 cd	0.748 d-f	0.102 b-g	11.61 c-e
Zeolite	0	500 ppm	14.10 ef	0.26 c-f	0.752 d-f	0.065 c-g	11.67 c-e
	50		22.86 a-d	0.26 c-f	1.255 a	0.065 c-g	20.30 a-c
	100		19.72 a-e	0.21 e-h	1.045 a-e	0.053 fg	21.65 ab
	150		23.63 a-d	0.29 cd	1.210 a-d	0.072 b-g	17.10 a-e
	200		20.50 a-e	0.32 bc	1.107 a-e	0.079 a-f	13.77 b-e
	0	0 ppm	13.56 ef	0.20 f-h	0.717 ef	0.050 fg	15.52 a-e
	50		18.63 a-e	0.23 d-h	1.000 a-e	0.057 fg	22.46 a
	100		16.31 c-f	0.18 gh	0.850 a-f	0.045 fg	19.56 ad
	150		16.81 a-f	0.21 e-h	0.885 a-f	0.052 fg	18.71 a-e
	200		16.31 c-f	0.24 c-g	0.851 a-f	0.062 e-g	15.60 a-e
C.V. =			40.32%	35.46%	31.74%	31.51%	19.84%
Factor A (source of silicon)			*	ns	ns	ns	ns
Factor B (silicon conc ^a in nutrient)			**	ns	**	*	ns
Factor C (foliar application)			**	ns	ns	ns	ns
Factor A*B			Ns	ns	ns	*	ns
Factor A*C			Ns	ns	ns	ns	*
Factor B*C			Ns	ns	ns	**	ns
Factor A*B*C			Ns	ns	ns	ns	ns

1/ = Values within column followed by the same letter are not significantly different ($P < 0.05$) by Duncan's Multiple Range Test.

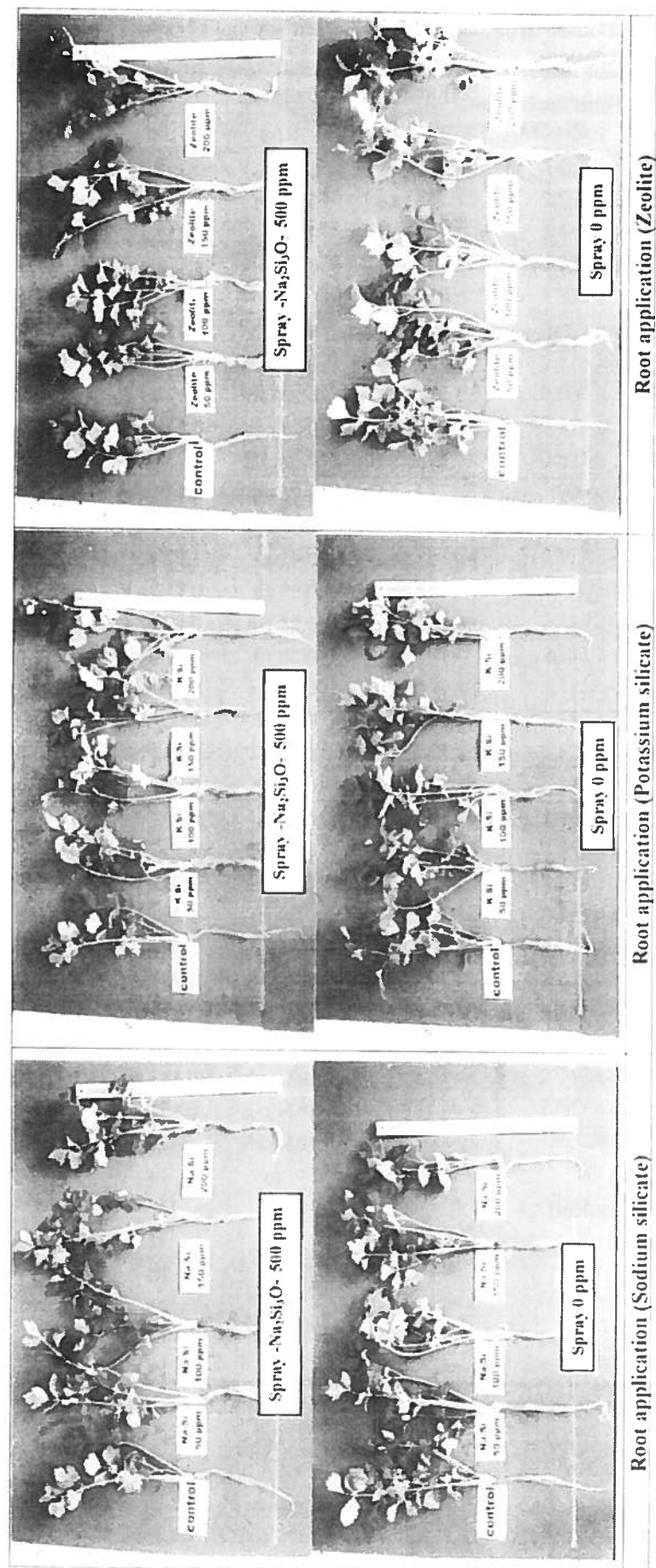


Fig. 1. Effect of root and foliar applications of silicon on growth of Celery (*Apium graveolens*; Umbelliferae) in Deep Flow Technique.

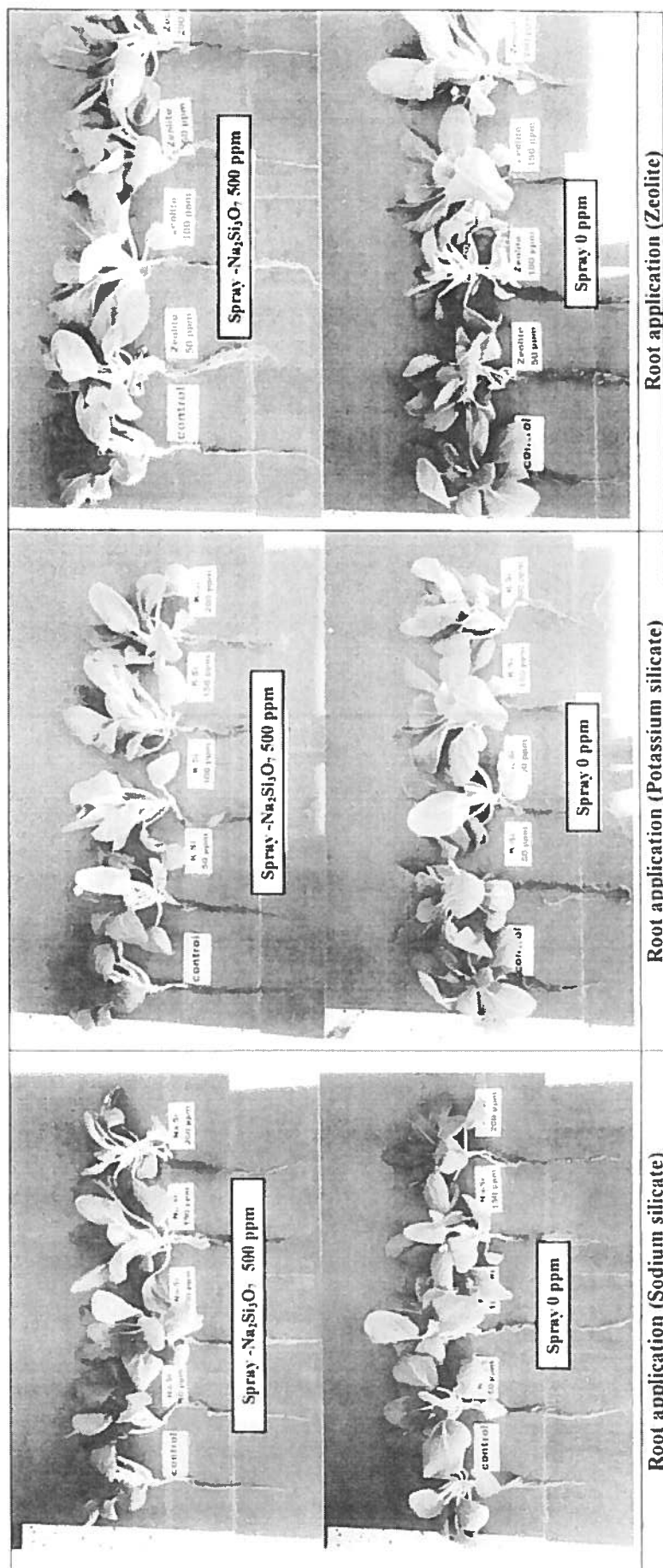


Fig. 2. Effect of root and foliar applications of silicon on growth of Chinese cabbage (*Brassica campestris* var. *chinensis*, Cruciferae) in Deep Flow Technique.

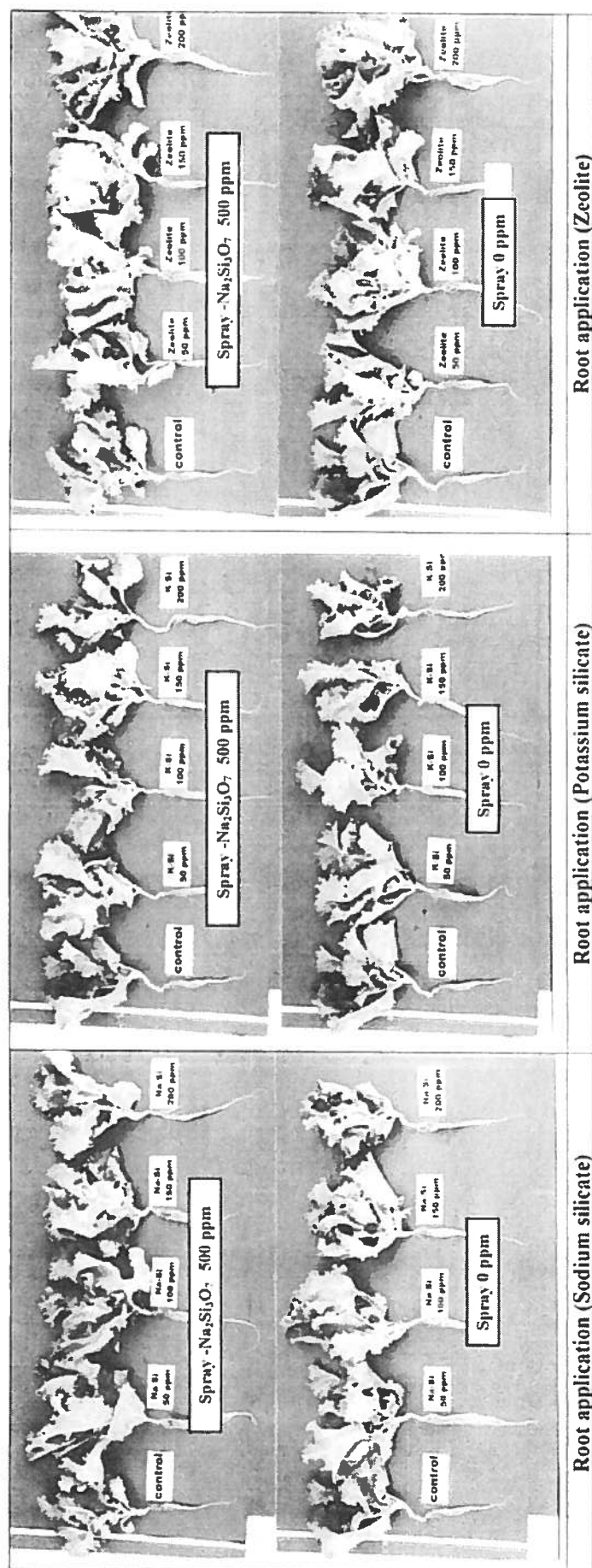


Fig. 3. Effect of root and foliar applications of silicon on growth of lettuce (*Lactuca sativa*; Compositae) in Deep Flow Technique.

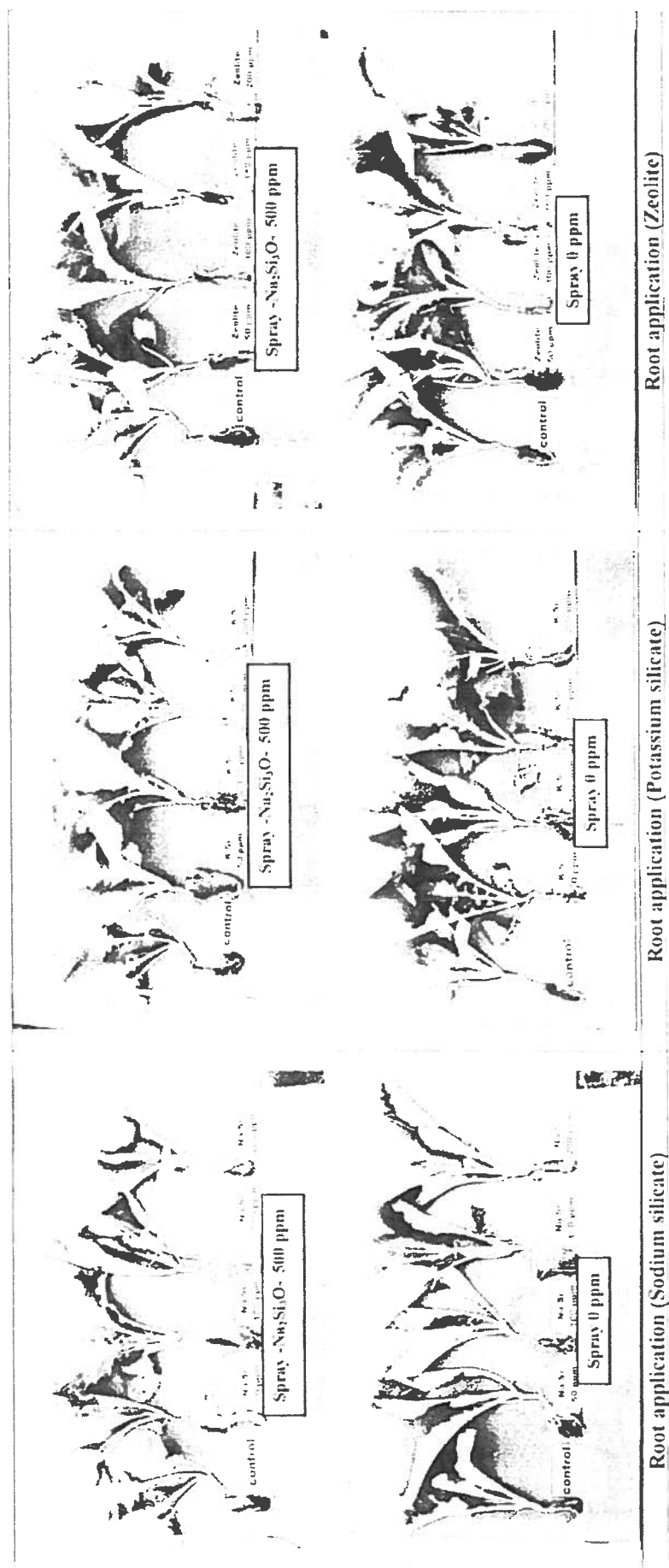


Fig. 4. Effect of root and foliar applications of silicon on growth of Phakkat kung (*Brassica* sp.; Cruciferae) in Deep Flow Technique.

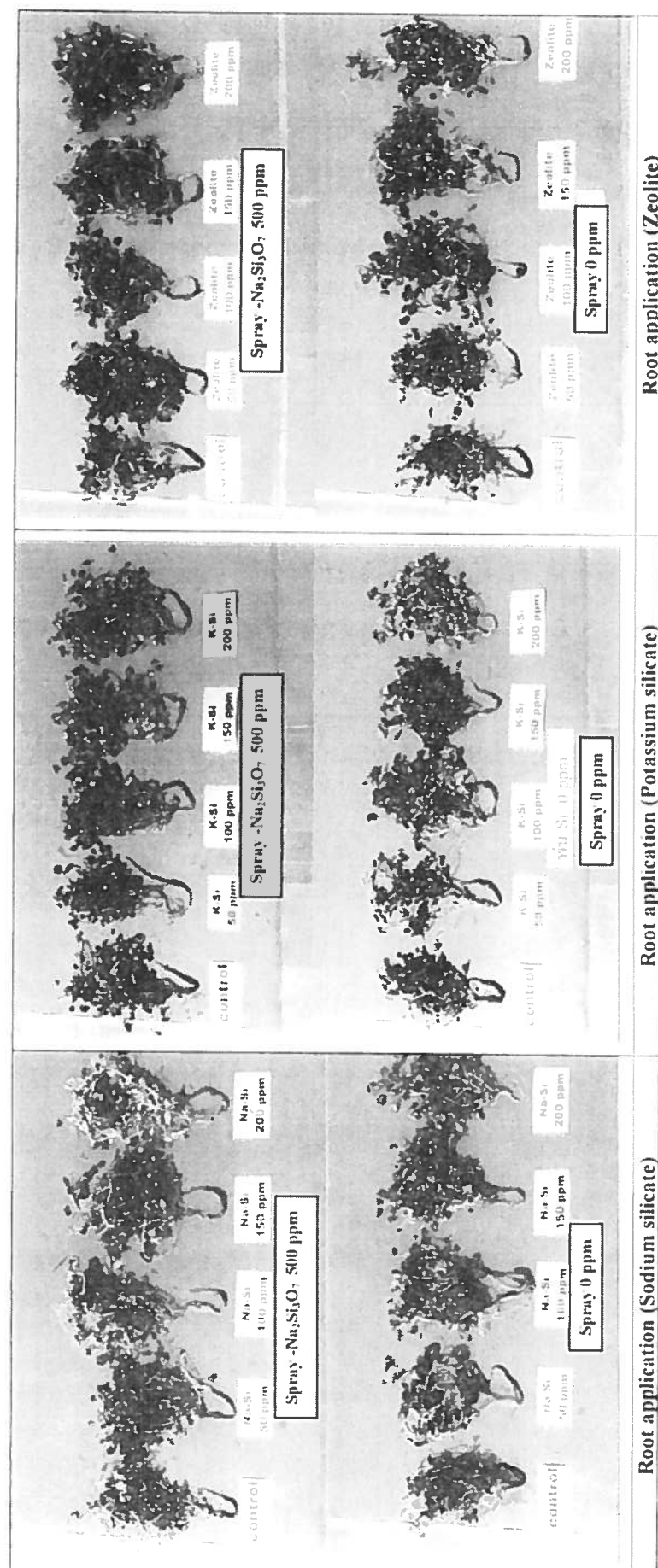


Fig. 5. Effect of root and foliar applications of silicon on growth of watercress (*Nasturtium officinale*; Brassicaceae) in Deep Flow Technique.

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The Application Method of New Silicon Sources

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INTRODUCTION

It is known that a rice plant adsorbs a lot of silicon (Si), and the amount of Si in rice plant is 1 – 1.2 t ha⁻¹ at harvest. This Si amount is around 10 times as much as Nitrogen in rice plant. Lack of Si causes poor root elongation, growth and yield of rice plants. In the meantime, a rice plant does not photosynthesize as regularly because its leaves hang down and shade each other. As a result, the leaves of rice plant easily fall down and yield poor grain filling. However, the usage of silicon fertilizer has decreased due to several changes in the construction of farming family and agricultural administration, such as aging farmers, a decrease of full-time farmers, and so on. Furthermore, the concentration of Si in irrigation water reduced during the past 4 decades as reported by Kumagai *et al.* (2002), Nitrogen and potassium compound fertilizer (NK fertilizer) has been used for ear. NK fertilizer is commonly also applied as topdressing when paddy rice requires silicon at most, so that NK fertilizer and silicon should be applied at the same time.

Consequently, the objectives of this study are to establish a new Si application technology from the view points of reducing the labor and of increasing the yield and quality of rice.

MATERIALS AND METHODS

Experiment 1: Establishment of new application method

1. Si dissolved from fertilizer

Si fertilizer used were Hydrogel, Inergy (Silica Gel), Aerated light-weight concrete (ALC) and calcium silicate (CSi). Ten gram of each Si fertilizer was placed in a beaker and mixed with 50 mL of distilled water. The mixture was incubated under 25°C for 24 hours, then the mixture was filtrated and the amount of Si in solution was determined. The residue was mixed with 50 mL of distilled water and incubated and filtrated as previously described. This procedure was conducted 10 times.

2. Application of Si for water inlet

According to the above result, Hydrogel was used in this experiment. The size of paddy field was 40 a (40 by 100 m), and cultivar variety used was Koshihikari (*Oryza sativa* L.). Ten kg of Hydrogel in mesh bag was put in a punched container. The container was set at water inlet and introduced the irrigated with a regular irrigation method. Irrigation was done from 25 days before heading to yellow ripe stage of rice plant. The concentration of Si in irrigation and surface water and the amount of Si in rice plant at harvest were determined.

Experiment 2: Effect of Si fertilizer on quality and yield of rice

1. Effect of Si fertilizer to nursery box on growth of rice plant

Hundred gram of Inergy (with) or 0 g Inergy (without) was applied to a nursery box (30 × 60 × 3 cm).

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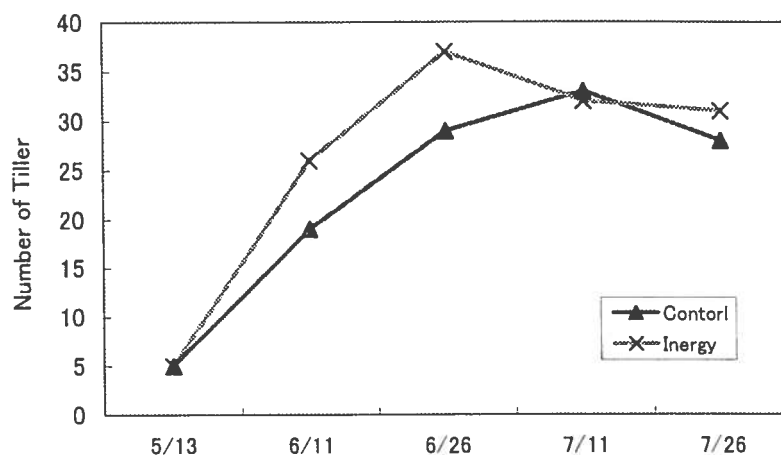


Figure 1. The number of tiller as effected by Inergy application

The seedling was cultivated using conventional cultural practices. The seedlings were transplanted in the paddy field (20a) and number of tillers was counted periodically.

2. Effect of topdressed Si fertilizer mixed with NK fertilizer on quality and yield of rice

The seedling was grown with Inergy and the seedlings were transplanted in the paddy field. The conventional cultural practices were employed except for topdressing. Two kind of fertilizers as topdressed fertilizer were used, i.e.: first, NK fertilizer (NK) and second, NK fertilizer mixed with Inergy (NK-Si). These fertilizers were applied on July 17 (panicle formation stage of rice plant). The application rate of fertilizer was as follows: N:K:Si= 30:30:93 kg ha⁻¹. The rice plant was harvested on Sept. 13 to determine the yield and yield components.

RESULTS AND DISCUSSION

Experiment 1

1. Selection of high dissolve Si fertilizer

The concentration of Si in solution originated from Hydrogel was highest among Si fertilizer. The concentration of Si in solution from Hydrogel was constant at 50 mg L⁻¹ during the experimental period. The concentration of Si from Inergy was 30 mg L⁻¹ and that from ALC and CSi ranged from 8 to 10 mg L⁻¹. To evaluate the amount of Si in seedling, solution culture including with or without Hydrogel was employed. The concentration of Si in solution and the amount of Si in seedling ranged from 40 to 65 mg L⁻¹ and 66 to 100 g kg⁻¹ with Hydrogel, respectively, while without Hydrogel those ranged from 2 to 4 mg L⁻¹ and 10 g kg⁻¹, respectively. Therefore, Hydrogel showed an excellent performance as Si fertilizer.

2. Application of Si at water inlet

The average of Si concentration was 8.5 mg L⁻¹ in irrigation water and 3.7 mg L⁻¹ in surface water before application of Si. It increased to 7 mg L⁻¹ 24 hours after Si application at water inlet. The concentration of Si in rice plant at harvest was 10 g kg⁻¹ higher in Si application than without Si application. Therefore, the new Si application technique from the view points of reducing the labor benefits the farmers.

Experiment 2

1. Effect of Si fertilizer to nursery box on growth of rice plant

There was some obvious difference in growth of rice plant between with and without Inergy application (Table 1). The maximum number stage of rice plant with Inergy was on June 26, which was 2 weeks earlier than without Inergy. In addition, tiller number was around 40 per hill with Inergy, while that was 35 per hill

Table 1. Yiled and Yield Componets of Rice as Affected by Si topdressed

Type of Fertilizer*	Weight of Rice Plant g hill ⁻¹	Number of Panilce per hill	Brown Rice Yield t ha ⁻¹	Grain Weight mg garin ⁻¹	Amount of Si in Rice Plant kg ha ⁻¹
NK fertilizer	79.5	20.0	7.58	22.0	790
NK +Si fertilizer	98.7	24.5	8.13	22.9	918

* : Application rate of fertilizer : N ; K : Si = 30 : 30 : 92 kg ha⁻¹

Fertilizer was applied at panicle initiation stage of rice plant

without Inergy.

2. Effect of Si fertilizer mixed with NK fertilizer topdressed on quality and yield of rice

The amount of Si in rice plant was 918 kg ha⁻¹ with NK-Si, as contrasted with 790 kg ha⁻¹ with NK. The yield of rice with NK-Si was 550 kg ha⁻¹ higher than with NK, which was attributed to the heavier grain weight with NK-Si. The heavier grain weight resulted the high eating quality of rice (Matsuda *et al.*, 2000).

CONCLUSION

According to the results, Hydrogel is presently recognized as an effective silicon fertilizer with high solubility in water and should be applied to water inlet from the panicle formation stage through yellow ripe stage to achieve high yield. The application technique is simple and easy. The topdressed application of Si is remarkably effective for gaining high yield and maintaining high quality of rice.

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How Dose Silicon Influence on Resistance of Rice Blast Disease?

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INTRODUCTION

It is well known that silicon is one of the most beneficial elements for rice plant. One of the important roles of silicon for rice plant is to encourage the resistance against blast disease. Although the resistance mechanisms of silicon against blast disease from the physical, chemical and physiological view points has been proposed, the mechanism is still unknown. Our objective is to verify the resistance mechanism against blast disease from the physical viewpoint.

MATERIALS AND METHODS

1. Treatment: The application rates of silica gel were 0, 1, 2, and 4 g / seedling case.
2. Rice cultivation: Seedlings of Sasanishiki (*Oryza sativa* L.) were planted into seedling case with 0.6 g N / seedling case. Seedling case was placed in green house at 25-30°C. Sasanishiki is susceptible variety.
3. Pathogen: *Magnaporth grisa* (race 003) were sprayed as spore suspension at 7th leaves stage of rice plant.
4. Incubation and invasion time: Inoculated rice plants were placed in a mist chamber (25°C) during 9 to 20 hours (incubation time). After incubation, dewes on leaves were dried by electric fan and seedling case was replaced in the greenhouse for 7 days.
5. Measurements of hyphae: The invaded hyphae of blast fungus and host responses in leaves were evaluated at 96 hours after inoculation. To remove chlorophyll, leaves were immersed in alcoholic lactophenol solution about 1 week. Crystal violet in metyle salicylate (0.2%) was used for staining the hyphae.
6. Silicon in middle parts of uppermost leaf of rice plant was observed by SEM (JEOL JED) and Energy Dispersive X-ray Spectrometer (EDS)

RESULTS

1. The amount of Si in leaf increased and the number of sporulating lesion reduced with increase of application rate of silica gel. The positive relationship between number of lesions and invasion time (incubation duration after inoculation) was observed and the higher number of lesion was recognized in lower amount of silicon in leaf (Fig. 1). These results indicated that the invasion of rice blast fungus into leaf took a longer time when the amount of silica in the leaf was high.
2. There were no differences in the percentage of spore germination and appressorial formation among the treatments. The reactions in leaf cell at the early stage of infection were grouped into 3 types, i.e. no-reaction, granulation of host cellular contents, and browning of both epidermal and parenchyma cells. The rate of no-reaction at penetration site reduced with decrease of silica gel application (Fig. 2).
3. Silica mainly accumulated at the surface of leaves. No silicon was observed in cytoplasm, in contrast to carbon and oxygen (Fig.4). It is clear that the bright image in Fig.4 (Si) and Fig. 5(E) was due to a large amount of silicon deposition on leaf surface with high silicon content.

4. In conclusion, the accumulated of silicon in leaf surface resulted the hardness of fungal penetration into leaf inside and encouraged the physical resistant against rice blast.

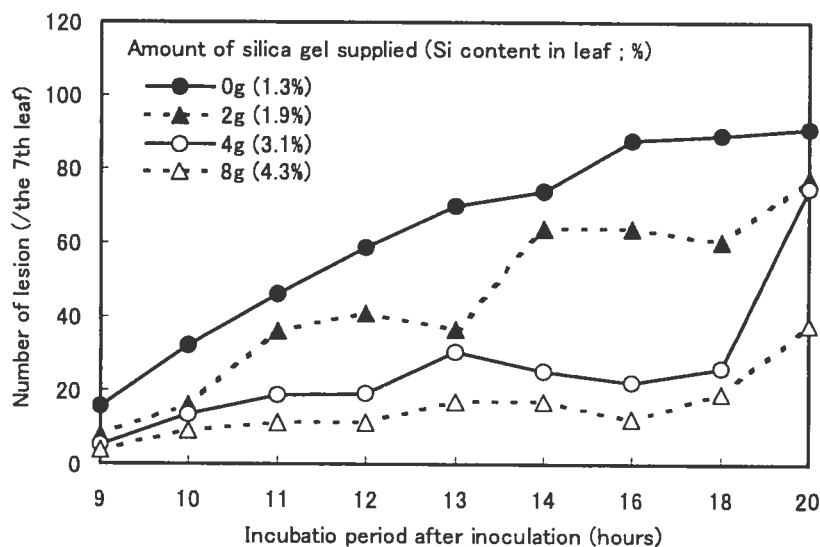


Fig. 1. Influence of silicon concentration of the leaf on invasion time of rice blast fungus. Inoculated plants were transferred to a mist chamber (25°C) for different incubation periods between 9-20 hours. Dew droplets formed on inoculated leaves were dried up immediately by the electric fan after taking out of plant from the chamber. The number of susceptible blast lesion was counted at 7 days after inoculation.

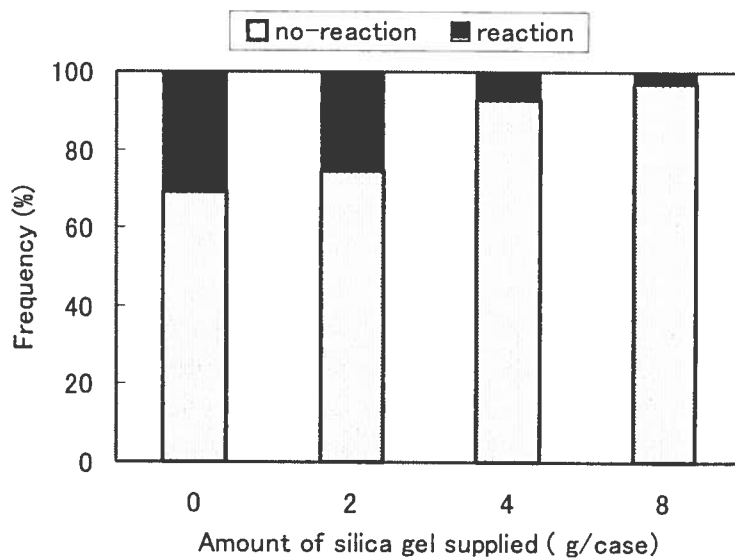


Fig. 2. The rate of reaction at appressorial formation sites in leaf cells 96 hours after inoculation. The reaction cells are granulation of host cellular contents and browning of both epidermal and parenchyma cells.

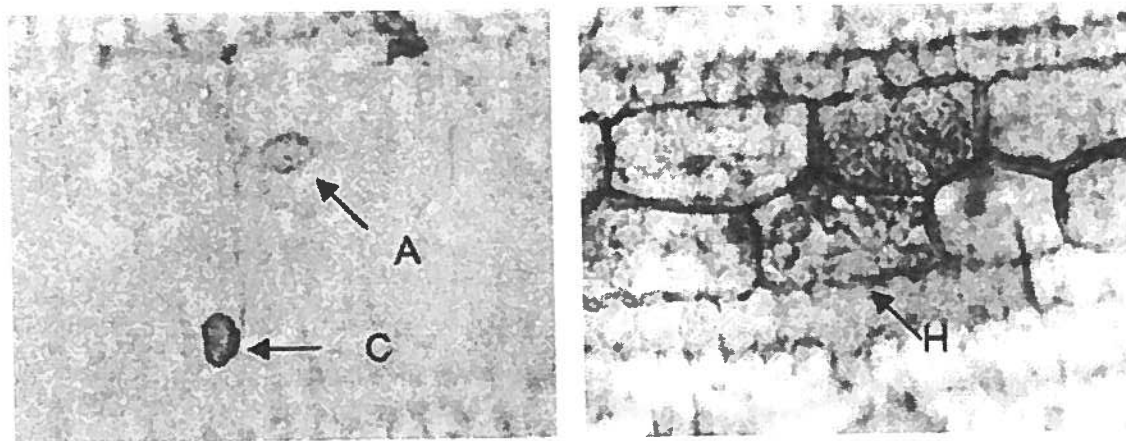


Fig. 3. A microscopic photograph of blast infection in cleared and stained susceptible rice leaf 114 hr after inoculation. Abbreviations; A:appressorium, C:conidium, H:hyphae

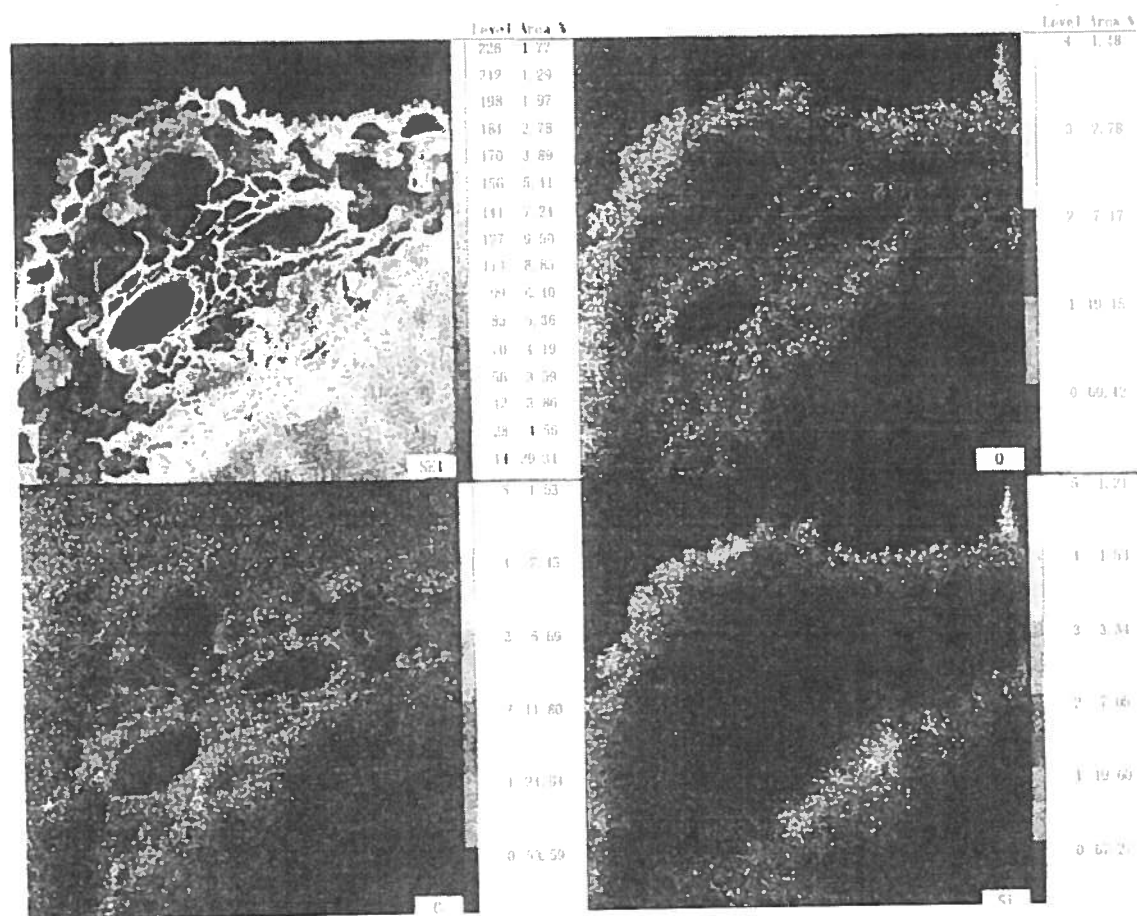


Fig. 4. Scanning electron micrographs and silicon X-ray maps of the section of a leaf at the 7-leaf stage. SEM is scanning electron micrograph of the section of a leaf. C, O and Si are X-ray maps of Carbon, Oxygen and Silicon, respectively.

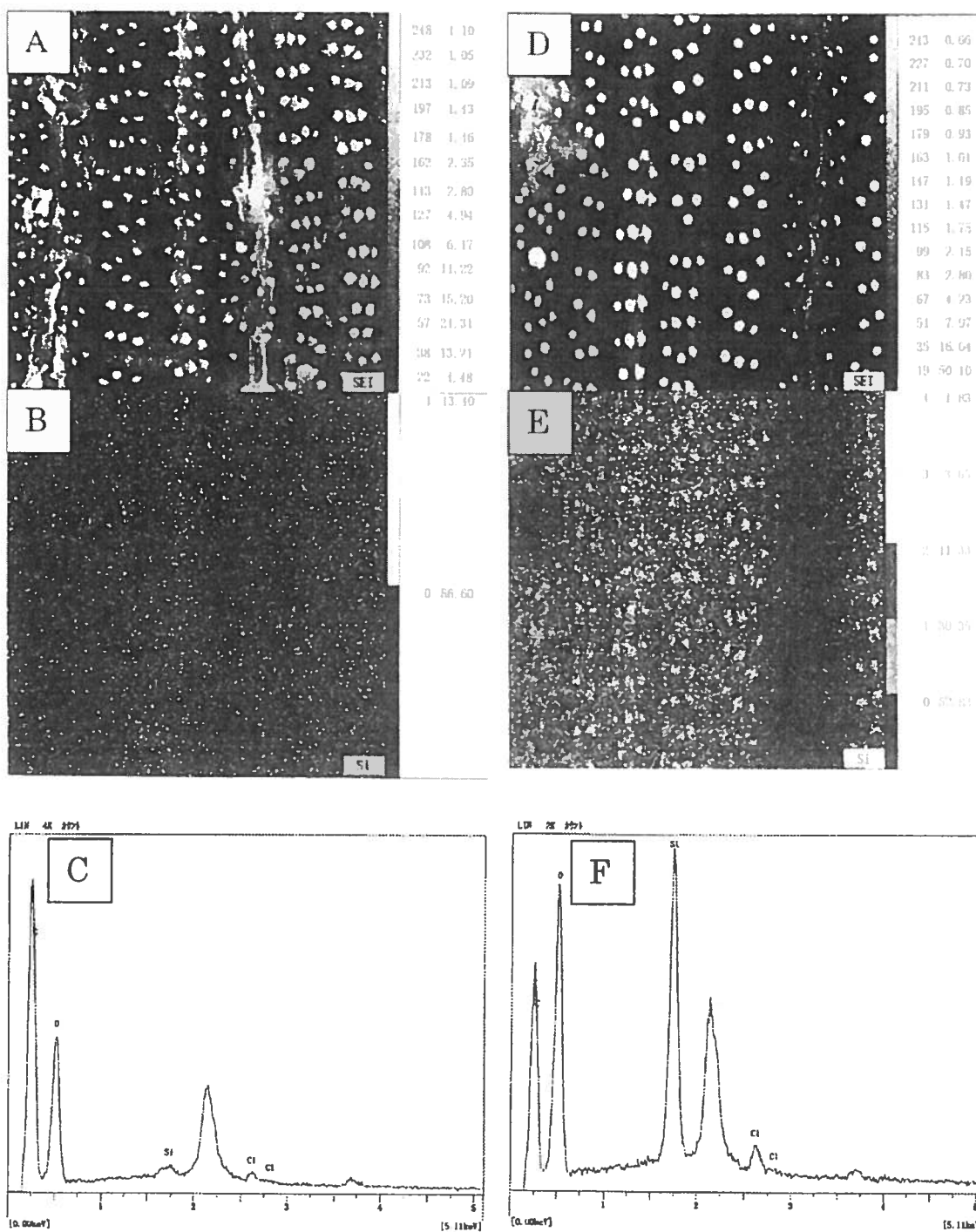


Fig. 5. Scanning electron micrographs and silicon X-ray maps and X-ray spectra of leaf surfaces. A (silica gel ; 0 g/case) and D (8 g/case) are SEM images of leaf surfaces without and with silicon treatment, respectively. B and E are X-ray maps of A and D, respectively. C and F are X-ray silicon spectra of A and D, respectively.

Categorization of Soil Type with Reference to Behavior of Silicon in Soil

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INTRODUCTION

Silicon (Si) is beneficial elements to rice plant and it is commonly applied to cultivated soil in Japan. However, even though there was a considerable amount of available Si in soil evaluated by conventional method or a high application rate of Si, the amount of Si in plant grown in volcanic ash soils was relatively low compare to grown in the alluvial soils. This phenomenon was related to the Si dissolution and adsorption processes in soil. This fact indicated that effective Si application method has not been established yet.

The objectives of this study were to evaluate the behavior of Si in soil from the viewpoint of dissolution and adsorption and to categorize the soil type.

MATERIALS AND METHODS

Soil sample: Fourteen alluvial soils and 10 allophanic volcanic ash soils in Yamagata Prefecture and 8 allophanic volcanic ash soils in Kumamoto Prefecture were used.

Analysis:

Characteristics of silicon dissolution and adsorption: The soil was placed in the test tube. Zero to 100 mg of $\text{SiO}_2 \text{ L}^{-1}$ was applied under soil: solution (1:10). The test tubes were kept at 30°C , 5 days.

Amount of available Si in soil is evaluated by incubation method. Phosphate retention capacity and soil texture were estimated by conventional methods.

RESULTS AND DISCUSSION

The relationship between Si absorption and dissolution was shown in Fig. 1. Relative intensity of Si index of Si adsorption (a) and Si availability (b) were evaluated using following equation; $Y=aX+b$, where X was Si concentration added and Y was the value of concentration of Si in solution added subtracted from of the concentration of Si in supernatant. Negative correlation was observed between X and Y. Phosphate retention capacity increased with the decrease of "a" (Fig.2). When phosphate retention capacity was more than 1,500, "a" value was less than -0.6, indicating that Si adsorption was regulated by active aluminum and more than

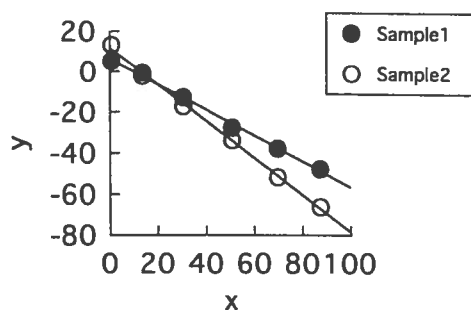


Fig. 1. Exemplification of the characteristics of silicon dissolution and adsorption. "x" value is silicate concentration of the slution added to soil sample, and "y" value is concentration changes of supernatant compared with "x".

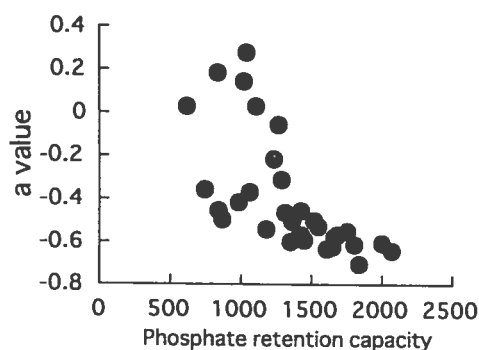


Fig. 2. The relationship between phosphate retention capacity and relative intensity index of silicon adsorption represented by "a".

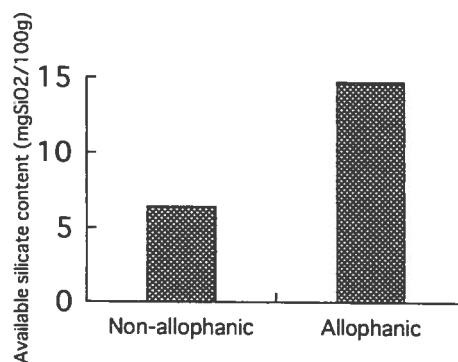


Fig. 3. Mean silicate availability of Allophanic and Non-allophanic volcanic ash soils.

60% of dissolve Si could be adsorbed by soil.

Figure 3 showed Si availability in volcanic ash soils. The amount of Si availability in allophanic soils was higher than in non-allophanic soil. Therefore, it could be said that Si availability in volcanic ash soil was influenced by weathering processes of volcanic ash soils.

Soil texture affected the availability of Si in alluvial soils (Fig.4). Negative correlation between the amount of sand in alluvial soil and Si availability and positive correlation between the amount of silt in alluvial soil and Si availability were observed (Table 1). The origin of available Si was still unclear, further study is needed.

The categorized soil type according to these results was shown in fig. 5. Si adsorption affected the availability of Si in soil and effectiveness of Si fertilizer. Index of Si adsorption in alluvial soils with low phosphate retention capacity was lower than in volcanic ash soils. Volcanic ash soils might be divided into 2 groups according to Si availability. A relatively higher amount of Si was dissolved in allophanic volcanic ash soil than in non-allophanic volcanic ash soil. On the other hand, availability of Si in alluvial soil depended on particle size.

Table. 1 Relationship between each fraction content of particle size and silicate availability

	regression equation	r
sand	$Y = -0.35X + 13.4$	-0.463**
silt	$Y = 0.18X + 3.8$	0.348*
clay	$Y = 0.07X + 8.3$	0.169

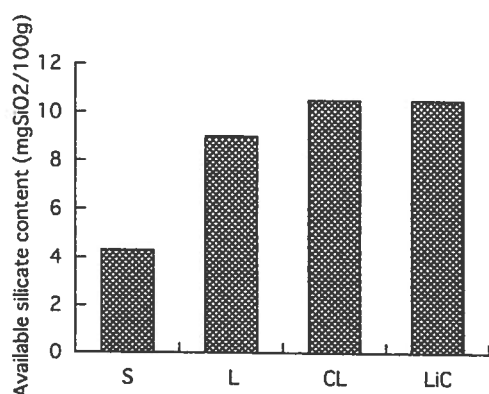


Fig. 4. Mean silicate availability of alluvial soils with different texture.

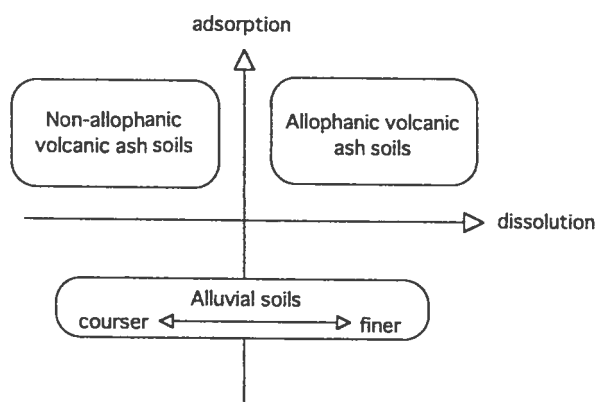


Fig. 5. Categorization of soil type in the light of silicate supply

Early Growth of Rice Plants as Affected by Silica Gel Application to the Nursery Bed in Different Condition of Wind

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INTRODUCTION

It is well known that the early growth of rice plant in temperate area is affected by viability of rice seedling. There is strong wind blowing during nursery and early growth stages of rice plant in Shonai area. When the wind blows hardly, seedling house should be closed but air temperature in the seedling house increases, resulting viability of rice seedling becomes low. Furthermore, the wind affects the surface water temperature. Hence, the dry weight of rice seedling during nursery season and early growth stage of rice is inhibited.

One of the factors, which relates to the viability of rice seedling, is Si content of rice seedling. However, the application of silicon to nursery bed of rice is difficult because high pH status of silicon fertilizer. New silicon fertilizer, silica gel (Si), which the pH is around 5, has been released.

One of the objectives of this study was to estimate the rooting ability, as one of the indexes of viability of rice seedling, and growth of rice plant during early growth stage of rice plant under Si application. Another objective of this study was to estimate the effects of Si on the rice seedling under shading and on the early growth of rice under hard wind conditions.

MATERIALS AND METHODS

Experiment 1 (Researcher's Experiment)

Cultural Practices:

The application rate of rice seeds (*Oryza sativa* L.), N, P₂O₅ and K₂O were 150 g, 2 g, 2 g and 2 g per nursery box (0.3 × 0.6 × 0.03 m), respectively, were applied. One gram of N per nursery box was applied as topdressed at 1.5 leaf age of rice seedling. The nursery box was placed in a vinyl house.

Sixty, 90 and 70 kg ha⁻¹ of N, P₂O₅ and K₂O, respectively, were applied as basal fertilizer. Around five of 25-days-old seedlings of rice per hill were transplanted on May 10. The plant spacing was 0.3 × 0.5 m, which was at 0.3 m distant in E-W rows. Other cultural practices followed the conventional practices.

Treatment:

1. Si application rate to the nursery box: 0, 250 and 500 g per nursery box.
2. Shading during nursery stage: with black cheese cloth (50% shading) and without.
3. Wind-blow: with (WBN) and without (OBN) wind-break net (1.8 m height).

Measurement:

1. Dry weight of above ground and root of seedling, amount of Si in the seedling, and number of leaves of seedling were evaluated.
2. Number of tillers was counted in the field.

Experiment 2 (Farmers' experiment)

Cultural practices done in the nursery box followed the conventional practices except for Si application.

One treatment, i.e. with (300 g Si to nursery box) and without, was employed to evaluate the viability of seedling as affected by Si content in seedling. Shoot dry weight, root dry weight number of root and the amount Si in the seedling, which were cultivated by 19 farmers in Shonai district were evaluated.

RESULTS AND DISCUSSION

The amount of Si in the seedling increased with the increase of Si application regardless of the researcher's and farmers' experiment (Table 1). No significant differences in shoot dry weight, number of root and root dry weight of seedling between 250 and 500 g Si treatments were observed, while there were significant differences in those measurements parameters between 0 and 250 g Si treatments. Therefore, we concluded that the application of Si at the rate of 500 g to the nursery box was the luxury application rate.

Table 1. Dry weight of above ground and root, and amount of Si in plant as affected by silica gel application

	Above ground		Root	
	Dry weight (g/100 seedlings)	Si content (g/kg)	Number (/seedling)	Dry weight (g/100 seedlings)
Researchers experiment				
Without Si application	1.35	18.8	7.1	0.22
250 g Si/nursery box application	1.69	47.1	8.7	0.49
500 g Si/nursery box application	1.71	57.1	8.8	0.51
Average of 19 farmers				
Without Si application	1.34	20.7	8.7	0.43
300 g Si/nursery box application	1.51	61.7	9.7	0.57

There was no difference in number of tillers between with and without Si application in WBN treatment. While, a higher number of tillers was observed in with Si application than without Si application in OBN treatment (Fig.1). The same trend was observed under shading treatment (Table 2). Significantly a higher dry weight of seedling in with Si than without Si application under shading treatment was obtained.

Consequently, Si might have a more beneficial effect on the rice plant under stress conditions and therefore, application of Si to the nursery box was the beneficial practices to get a favorable condition for seedling.

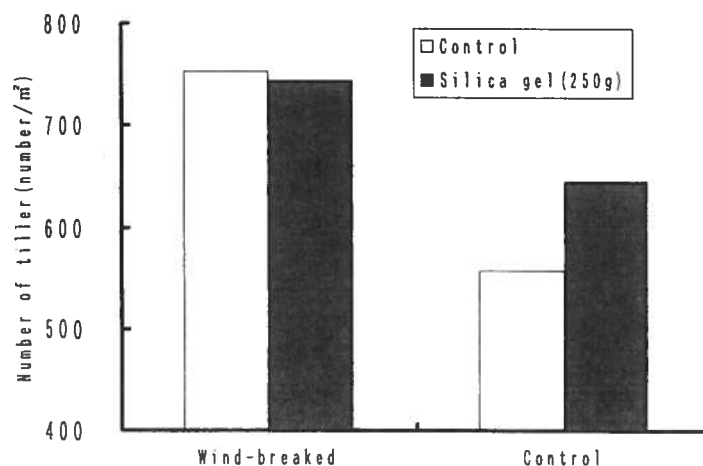


Fig. 1. Number of tiller of rice plant at 40 days after transplanting with reference to wind-break and silica gel application to nursery box

Table 2. Dry weight of above ground and number of leaves of seedling (shaded or not), and number of tiller at early growth stage as affected by silica gel application

	Above ground of seedling		At early growth stage
	Dry weight (g/100 seedlings)	Number of leaf (number)	Number of tiller (number/m ²)
Control (not shading)			
Without Si application	1.17	2.6	581
250 g Si/nursery box application	1.57	3.0	627
Shading by black cheese cloth			
Without Si application	0.95	2.3	450
250 g Si/nursery box application	1.39	2.7	490

- Transplanting date : May 13
- Sampling date : June 21 (early growth stage)

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Effect of Silicon on Growth of Hydroponically Grown Cotton Genotypes

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Silicon has many beneficial effects on plant growth largely due to its unique physiological role in metabolism of many plant species. A solution culture experiment was conducted to study the beneficial effects of Si on growth of eleven different cotton genotypes infected with root rot. One-week-old seedlings of cotton genotypes were transplanted in iron tubs containing half strength of Johnson's nutrient solution, with and without addition of silicon. The plants were harvested month after transplanting. The symptoms of root-rot were obvious in both the treatments, however the addition of Si significantly increased shoot dry weight (SDM) and root dry weight (RDM). There were significant differences among genotypes for SDM and RDM in both the treatments. The SDM of the BH-118 and BH-124 was higher compared to other genotypes in both the treatments. The RDM of BH-118 and CIM-443 were higher in both treatments while there is significant interaction among treatments and genotypes for SDM and RDM. Addition of Si significantly increased silicon concentration in leaves of cotton plants. There was also genotypic variability for silicon concentration in leaves of cotton plants. The genotypes FDH-228, FH-634 and CIM-448 had significantly higher silicon concentration in both the treatments. Results of the present study showed that Si addition partially controlled the root-rot in cotton genotypes and improved overall growth of the plant.

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Effect of Si Fertilizers on Citrus

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Most citrus in Florida is grown on sandy soils that could be deficiency or critical deficiency in activated silicon (Si). The field testing of soils and plants, as well as greenhouse and field experiments on experimental and commercial groves with Si fertilizers are being conducted on just germinated, as well as young and mature citrus trees. A relationship was determined between soil silicon status, content of Si in leaves and health of citrus trees. The obtained data has demonstrated that citrus trees may actively transport monosilicic acid from the soil thereby increasing the trees resistance against outside biotic and abiotic stresses. Optimization of Si nutrition was responsible for an increase in the mass of roots and green mass of germinated grapefruit and oranges seedlings from 20% to 60%. The Si-rich slag application increased citrus tree height from 10 to 40% and accelerated the growth of the tree branches from 30 to 45% over a 6-month period. The application of Si fertilizer on a Temple orange (*C. reticulata* × *C. sinensis*) and Valencia orange (*Citrus sinensis* L. Osbeck) grove had a significant effect on the quality of the fruit. The total Brix increased for Si treated plots. The content of juice per box increased and thus the content of solids per box increased as well. All obtained data was statistically significant at the $P < 0.05$ levels. Si fertilizers or soil amendments may be useful in (1) improving soil properties, (2) providing Si nutrition for citrus, and (3) plant protection by providing increasing resistance against outside diseases and insect stresses. Although more work needs to be done, it appears that citrus could benefit from improved Si nutrition.

Yield Response of Sugarcane from Uptake of Applied Silicon in Australia

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A program of research was commenced in Australia in 1999 to define environments in which sugarcane was responsive to application of siliceous amendments and to identify suitable materials for commercial use by the industry (Berthelsen *et al.*, 2001a; 2001b). Two series of field experiments were established; one included seven rates of imported crushed calcium silicate slag (0 to 12 t/ha), while the second evaluated performance of local materials such as cement and sugar mill by-products against the calcium silicate.

Yield data for plant and first ratoon crops demonstrated significant residual value of calcium silicate, with cumulative average yield responses at two sites of approximately 20 tonnes cane / ha to 4.5 t product / ha and 35 tonnes cane / ha to 9.0 t product / ha. Similar yield responses to 3 or 6 t calcium silicate / ha were recorded in the second series of experiments, and yield from application of 50 or 60 t sugar mill ash / ha were similar to those from 3 t calcium silicate / ha. Largest yield response (40 t / ha) was achieved from use of a filter mud / sugar mill ash mixture at 50 t / ha.

Increasing rates of calcium silicate resulted in higher concentration of silicon in leaf dry matter, with 95% of maximum yield occurring between 0.4 and 0.7% silicon across sites. Calcium silicate was the most effective product for increasing silicon levels in leaf tissue, with only 80% of latter values being achieved from application of the high rates of sugar mill ash. Levels of silicon in soil (extracted with 1 M CaCl_2 and 0.1 M H_2SO_4) increased in response to applied calcium silicate. There was no significant yield response above 20 mg Si / kg (CaCl_2) or 165 mg Si / kg (H_2SO_4) on a red ferrasol soil in the wet tropics.

Silicon acquisition by biomass generally increased in response to rate of applied calcium silicate and higher yield. Uptake by plant cane in un-amended soil was ranked in accordance with CaCl_2 -Si and ranged from 49 to 108 kg Si / ha, whereas silicon uptake ranged from 128 to 177 kg Si / ha when 4.5 t calcium silicate / ha was applied. The silicon concentration in plant components was ranked dead leaf > green leaf + tops > stalk. The average increment of silicon in crop biomass associated with 4.5 t calcium silicate / ha was equivalent to 5.2% of applied silicon in plant cane at Innisfail and Bundaberg sites and equivalent to 5.7% of residual silicate in the first ratoon at Innisfail.

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Silicon Content in the Native Brazilian Savanna's Fruits

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The Brazilian Savanna region, which covers 2.1 million km², generally has a small amount of silicon in its soils. However, most of its native vegetation showed to be silicon accumulators probably as consequence of burn and silicon cycle throughout the litter. Locals use to eat native savanna's fruit fresh as whole fruit or choosing only its pulp or nut. There is a great need to establish the nutritive value of those fruits and to use them as supplements, helping local population to have access to better nutrition and avoid hunger. Some research have shown that native savanna's fruits are rich in energy, vitamins and minerals, but there is no records on their silicon content. The presence of this element was determined in 17 fruit species using an average pool of 25 fruits for each specie and considering the part usually eaten. Seven species showed considerable amount of silicon in its eatable parts. Nut silicon content in g/kg of dry matter was 12 and 5.4 respectively for *Dipteryx alata* Vog. and *Syagrus flexnosa*, and between 1.2 and 1.7 for pulp in the following fruits: *Ananas anassoides*; *Brosimum gaudichaudii*; *Campomanesia cambedessiano* and *Syagrus romanzoffiana*.

Silicon Uptake by *Brachiaria decumbens* and its Influence on Rumen Dry Matter Degradability

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Grasses are usually classified as silicon accumulators. Silicon deposits may occur in cell walls, cell lumens or in extra cellular locations, which are generally associated with lowered cell wall digestibility by ruminants, although it has been hard to demonstrate a consistent relationship. This field experiment involved the effect of calcium silicate application at 0, 2 and 6 t ha⁻¹ rates on *Brachiaria decumbens* pasture, a tropical grass with great resistance to drought. Silicon uptake by the plants was correlated with the rumen dry matter degradability on the third cut, 18 months after calcium silicate application. Silicon content in the leave increased from 18.6 (control) to 25.5 g kg⁻¹. Grass was cut 15 cm from the ground and *in situ* degradability assay was conducted with the samples at 3, 8, 16, 24, 48, 72 and 96 h of incubation times. Besides the significant differences on silicon concentration on leaves, it did not affect the rumen degradability of the tested materials. There is not concordance at animal nutrition specialists in how silicon affects forage digestibility, if it has influence on activity of the enzymes involved in forage digestion or if it is due to protection of the cell wall. We believe that silicon was not a limit to microbial degradation.

The Silicon Role on *Brachiaria decumbens* Degraded Pasture

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The relationship between forage and the role of silicon on tropical grass production are practically unknown. Silicates, besides supplying silicon to the soil and plants, also have the capability to decrease soil acidity. In this study, the application of 0, 1, 2, 4 and 6 t ha⁻¹ of calcium silicate on soil surface of a degraded pasture of *Brachiaria decumbens* was evaluated under field conditions. Soil pH, silicon uptake and dry matter production of *Brachiaria decumbens* were determined. The results showed that calcium silicate raised pH, Ca and Si content in soil sampled 0-10, 10-20 and 20-40 cm and was positively related to forage dry matter production. Silicon content on *Brachiaria decumbens* leaves increased with calcium silicate rates and ranged from 8.0 through 15.2 g kg⁻¹. It was also observed that calcium silicate could increased pH in deeper soil layers and not only in the surface soil.

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Foliar Silicon Content, Extrafloral Nectaries, Ants and Herbivory at Brazilian Tropical Savannah

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Plant chemistry has been receiving more attention in ecological studies, however few importance has been giving to silicon as a defensive agent in plants. In the present study we investigated biotic (extrafloral nectaries, EFNs) and chemical defenses (foliar silicon content) against herbivory at Cerrado (Brazilian Tropical Savannah). It was sampled 15 tree species (five species bearing EFNs and 10 without these glands) according to its foliar herbivory, arthropods and ants diversity. The results indicated that an increase on silicon accumulation was directly correlated with reduction on herbivory by chewing insects. In general EFNs bearing plants presented a low level of silicon, what can justify the plant-ant relationship observed. The herbivory due to phytophagous was higher in plants bearing coriaceous leaves. The results also suggested that the plant choice by a biotic or chemical defense is dependant of specific ecological constraints and so one cannot say that one choice is better than the other.

Evaluation of Candidate Silicon Fertilizers

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Silicon fertilization for rice and sugarcane production on organic and sand soils in south Florida has become routine, giving rise to an interest among growers in finding suitable Si fertilizers in addition to the currently-used calcium silicate. A laboratory determination of Si-release from candidate Si sources has been found useful for identifying materials that have sufficient potential as Si fertilizers to warrant field testing.

Session 5

Silicon Studies in Asian Countries

30 70% of crop area
damaged by pathogens
& insects

Recent Research of Si-alleviated Stresses in Plants in China

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1 million tons Si
used
20 million ha of
paddy soil
Si-def

Silicon (Si) has not yet been considered a generally essential element for higher plants, partly because roles Si plays are poorly understood and direct evidence is still lacking that Si is a part of plant constituents or enzymes. However, numerous studies have demonstrated that Si is a beneficial or "agronomically essential" element for higher plants, particularly for grasses. More recently, roles for Si in alleviating both abiotic (e.g. aluminium, heavy metals and salt toxicity) and biotic (plant diseases and pests) stresses in plants have received worldwide attention over the last two decades. This paper briefly overviews the recent research work regarding Si-enhanced resistance and/or tolerance to salt toxicity and fungal attack in plants in China.

Exogenous Si significantly alleviated salt toxicity in barley and improved the growth of barley under salt stress. Added Si reduced Na but enhanced K uptake and transport by plants and altered microdistribution of ions in plant roots with both Na and Cl being more evenly distributed in the epidermal, cortical and stelar cells. Si decreased the permeability of plasma membrane by increasing antioxidative enzyme activity and reducing lipid peroxidation induced by salt stress and improved ultrastructure of chloroplasts, which were badly damaged by the added NaCl with the double membranes disappearing and the granae being disintegrated in the absence of Si. The addition of Si significantly increased H^+ -ATPase and H^+ -PPase activity in plasma membranes and tonoplast, and significantly increased the plasma membrane fluidity under salt stress. However, tonoplast fluidity of salt stressed barley was decreased significantly by the addition of Si, suggesting that Si may help to decrease ionic damage by the compartmentation of Na^+ into the vacuole through tonoplast Na^+/H^+ antiporter and to decrease electrolytic leakage to cytoplasm from vacuole. It can be concluded that Si may regulate plasma membrane and tonoplast fluidity and maintain membrane stability, structure and functions to enhance salt tolerance of barley.

Exogenous Si significantly increased leaf POD, PPO, PAL and chitinase activities in cucumber infected by *Sphaerotheca fuliginea* and significantly decreased the disease index, but did not in cucumber infected by *Colletotrichum lagenarium*. However, Si failed to significantly enhance the leaf POD, PPO and chitinase activities in plants un-inoculated with *Sphaerotheca fuliginea*. EDX analysis showed that silicon was concentrated on the base of the trichomes on the surface of the epidermis. There wasn't any relationship between distribution of silicon and penetration sites of fungal hyphae. In the regions where Si was heavily deposited, fungal hyphae penetrated the epidermis of leaf through gaps of cell, while some penetration sites were almost free from any specific Si accumulation.

It appears that enhanced plant defence to *Sphaerotheca fuliginea* arose from the ability of signal materials produced by pathogene and host to activate the natural host defence mechanisms, and Si enhanced the resistance of cucumber plants against powdery mildew. It seems that Si accumulation was unlikely induced by the fungus. Si accumulated played no roles in enhancement of resistance against fungal attack as a physical barrier and did not directly hinder the fungal growth and the penetration of fungal hyphae into plant tissues.

antioxidant enzyme activity \uparrow w/ Si
super-oxide dismutase

Research on Agricultural Utilization of Silicon in Korea: Progress and Prospects

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INTRODUCTION

The Korean peninsula has mountainous topography principally consisting of acid rocks such as granite and granite-gneiss. Paddy soils were usually derived from medium to coarse-textured local alluvium types from valleys and fan deposits. Rice crop is always affected by precipitation, particularly by typhoons in the summer season, resulting to lodging and yield loss.

The effect of the ground wollastonite (β -calcium silicate) in increasing the yield of rice crop was evaluated in late 1950s. It was demonstrated that silicate fertilizer could improve soil fertility as well as rice yields. Subsequently, research on silicate was actively carried out up to 1980s. Silicate has been used in rice cropping system to control excessive inputs such as fertilizer and chemicals.

There is a need to utilize silicate as source for low input-sustainable culture toward production of high quality rice. The government of Korea has been supplying silicate fertilizers derived from furnace slag for all of paddy fields since 1965 and for phosphorus-deficient upland fields in the island of Jeju since 1998.

This paper summarized the results of current researches on the development and use of silicon fertilizer for improvement of paddy soils, crop response, and increased resistance to environmental stresses. Finally, future research strategies are discussed.

Silicon resources used in Korea

Silicon resources used in Korea as soil amendment or fertilizer are rice straw, ground wollastonite, blast furnace slag, lightweight concrete silicate, and silicate complex fertilizers. Rice straw is returned to paddy soils as compost; ashes, cattle manure and fresh straw are also returned to the soil. Ground wollastonite was found to be the best source of Si, and contained about 8~10% of SiO_2 , 35-40% of CaO and about 2% of MgO. It was applied as silicate fertilizer in powder form up to the end of 1970s. Recently, wollastonite was replaced by slag, the by-product of furnace in steel industry, because of higher production cost and poor quality. Slag having pH around 9.7-10.7 contained 36.0-37.1% of SiO_2 , 13.1-15.7% of Al_2O_3 , and 37.9-40.4% of CaO and 5.2% of MgO.

Lightweight concrete silicate was registered in 1997 as fertilizer, and the chemical composition contained 15% of SiO_2 and 15% of alkalinity, with less than 1.0% of titan as regulated through the fertilizer management law. There are several types of complex fertilizer with Si such as silicate-phosphate, 15-18% with 30% alkalinity, silicate-phosphate-potash, 15-10-10% with 30% alkalinity and silicate mixed with micro-nutrients used as foliar spray of horticultural crops and composed of water soluble SiO_2 (24%), boron (0.052%) and iron (0.11%). The availability in Si contents from these sources is in the order of rice hull> ground wollastonite> bulked rice hull> slag> rice ash (NIAST, 1983).

Improvement of soils

Si contents in paddy soils

Paddy soils of Korea as studied by NIAST (1999) showed distribution patterns of SiO_2 , with 31.5% of paddy soil having lower than 50mg kg^{-1} , and 42.1% having $50\text{-}100\text{ mg kg}^{-1}$. The Si content of paddy soils

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classified by soil types was in order of saline paddy (115 mg kg^{-1}) > acid sulfate paddy > highly productive paddy > sandy textured paddy > less-paddified paddy > poorly drained paddy (79 mg kg^{-1}). The contents of SiO_2 in the major paddy types “highly productive” and “less-paddified” were 89 to 81 mg kg^{-1} respectively. Therefore, most of the paddy fields needed more application of silicate.

Evaluation of silicate in paddy soils

The highest yield of rice was attained with 130 mg kg^{-1} of soluble silicate. The amount of silicate fertilizer to be applied was calculated by Park (1970). He developed a “multi-nutrient factor balance concept” using SiO_2/OM as key factor in determining demand for nitrogen and potash. He proposed equation models based on SiO_2 content of paddy soils for the highest yield of rice. Lee (1986) revised Park’s model of nitrogen recommendation to “ $12.74 - 1.52(\text{OM}) + 0.028(\text{SiO}_2 \text{ gained by analysis})$ ”, and it is now being tested for recommendation as fertilizer.

Effects of soil improvement

The residue effects based on rice yield using silicate after rice cropping were evaluated for 3 years (NIAST 1984), and the results were used as basis in the government-subsidized supply of once in every four years. In the long term, yearly application of 2.5 t / ha of silicate for 28 years in paddy fields resulted to increased percentage of water soluble aggregate and degree of aggregate stability (Park *et al.*, 2000). In upland fields, applying 2.5 t / ha of silicate every years for 18 years increased SiO_2 content in soil up to 512ppm and increased barley yield by 22% (Kim *et al.*, 1994).

Crop response

Deposits of silicon and its role

Korean scientists are studying the deposit patterns of silicon in plant tissues as it applies to the classification of plant species, protecting mechanism from diseases, water-saving culture through reduction of transpiration of the plant. Si within the rice plant could provide beneficial conditions for photosynthesis and translation of substrates through maintenance of erect leaves, reduction of leaf senescence and inhibition of the evolution of ethylene that destroys the chlorophyll, and reduction of volatilization loss of nitrogen from leaves (Kang 1980; Kang *et al.*, 1984). Lee *et al.* (2000a) found that silicon was more accumulated in the damaged parts such as a cut and spots of diseases in the leaves of cucumber. Kang (unpublished) found by experience that it was mainly deposited in root parts than in shoots of rice seedling grown with high concentration of Si. Based on two patterns of deposits and mobilization of silicon in the protection of plants against diseases by foliar spraying of silicon, it is now possible to make use of “micro-spaces where it is accumulated”.

Resistance to stress conditions

Most of the silicon researches were concerned with the endurance to unfavorable environments due to biotic and abiotic stresses. In Korea, it was shown that Si had significant effects on the control of crop diseases such as rice blast, helminthosporium spot of rice, rice black streaked dwarf virus, powdery mildew of cucumber and stem rot of gymnocalycium. Lee *et al.* (2000a) found that the elongating hyphae of fungal colony of powdery mildew in the cucumber leaves were inhibited by the silica deposited surrounding the invaded pathogen and in the lesions of diseases. Cho *et al.* (1998), also found out that the foliar spray of Si could reduce the population of fungal colony of powdery mildew of cucumber as well as in the surfactants “tween 20”. It would be expected that research on environment-friendly protection of crops, particularly on improvement of the effects of Si foliar spray, would be conducted for control of powdery mildew in many plants.

As rice crop production in Korea is governed mainly by temperature and precipitation, Kang and Park (2001) evaluated the response of temperature of the rice plant with given Si supply. Compared with the rice plant without Si, the temperature of the rice plant with Si was highly maintained in the range of $23\text{--}38^\circ\text{C}$ of air temperature, and lower in the range of $43\text{--}48^\circ\text{C}$ of higher temperature. The function of Si to maintain the temperature in plants seems defined, as well as the role of Si in the Si-containing construction materials in the

maintenance of temperature. Kang *et al.* (1991) reported that stomatal population relating to the large stoma and low frequency distributed on the surface of leaf as a means of reducing water loss was lower in rice plants with Si. Results of the field trials (Kang *et al.*, 2000) showed that, 17% of the irrigation water applied with Si was saved in non-flooding and 27.9% in flooding conditions.

The nutrient disorders of soils in Korea are due to excess of N resulting in lodging, salinity, soil toxicity and other agro-chemicals. Jung *et al.* (1997) reported that severe damage to rooting and withering leading to death of rice plants after it is transplanted in the paddy field irrigated with highly acid water originated from the acid sulfate soil. However, rice plants were found to grow well with application of Si at the 3t/ha. The residual effect of the herbicide "Butachlor" in paddy water could be reduced by the application of Si. Damage of rice plants due to the application of non-selective herbicide "Glufosinate Ammortium" in the early vegetative stage of rice at the farmer's field in 2001 was reduced by application of Si (Kang unpublished). Kang and Park (2001) reported the participating possibility of Si by measuring the electric voltage induced to the rice plant with and without Si. The change in electronic voltage in Si-treated plants was stable, while it was unstable in silicon-free plants.

Increase of crop yields and improvement of quality

Application of silicate as fertilizer resulted in increase in yield by 2-3 t / ha and improved the quality of staple food crops such as rice, barley, wheat, rye, and sesame; horticultural crops such as garlic, onion, cucumber, Chinese cabbage, strawberry, potatoes, tomatoes, gymnocalycium, and grape fruits; and foliage crops such as orchard grass. Recently, concerns with food quality, food safety and environmental preservation, particularly the pollution of the soil, water, and plants by heavy metals were reported. With silicate application, there was an increase in the perfect grains of rice, and low protein content, higher ratio of Mg/K.N, Mg/K.N. Higher analytical eating values related to eating quality of rice grains were also reported (Nam *et al.*, 1995; Jung *et al.*, 1996; Kang *et al.*, 1997). Immobilization of heavy metals in the soils by the application of Si caused to reduce the heavy metal contents in brown rice (Jung *et al.*, 1996).

PROSPECTS

In Korea, crop production is governed by year-round weather conditions and unfavorable topography. Heavy dosage of agro-chemicals such as herbicides, fungicides and fertilizers to crop production are unavoidable. Therefore, utilization of Si as the means of environment-friendly agriculture is urgently needed to keep not only environment safe but to improve the quality of the products and to ensure food safety. Recently the UN has classified Korea as a potentially water-short country; we hope that silicate could lessen save water demand in agriculture. In the future, strengthen intensive research toward development of silica materials for improvement of upland soils and productivity of upland and horticultural crops will be strengthened.

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Status and Utilization of Silicon in Indian Rice Farming

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INTRODUCTION

Rice occupies a pivotal place in India's national food and livelihood security system. Under conditions of expanding urbanization and per capita income, a minimum growth of 2.5% will be needed annually in food grain production to balance the food budget. This will involve producing 220 million tonnes of food grains by 2002-3 and 240 million tonnes by 2006-7. Rice occupies an area of over 44 million hectares and has the largest untapped yield potential even at currently available levels of technology.

In India, though research on Si has been initiated long back, the available data is not adequate to make any recommendations for Si fertilization in rice farming. The necessity of Si fertilization to the rice crop in India has not been widely evaluated as in other countries. The rice which contributes more than 40% of the total food production expectedly gets lion's share of important inputs such as fertilisers (about 40%) and water (65%) in India. Even though at national level the progress *per se* in agricultural sector is commendable, if one examines partial factor productivity it looks rather that all is not well especially with respect to rice response to fertilizers. Intensive rice cropping with modern rice varieties and with adequate NPK inputs, it is possible that application of silicate materials may help further increasing grain yields, especially through efficient use of applied N, increased availability of P and imparting resistance of the plant to lodging, pests and diseases. This however, needs confirmation from large scale field trials at different agro-climatic conditions.

There is no comprehensive report on research work related to silicon in Indian soils and this study attempts to compile the information regarding the status and availability of silicon in rice soils of India.

CONSTRAINTS PERTAINING TO SOIL FERTILITY IN PADDY SOILS

Rice yields in intensely cultivated irrigated areas are either decelerating/stagnating/declining in the post-green revolution era depending on the degree of imbalance in fertilizer use, soil degradation and type of cropping system practiced. Recalcitrant organic matter formed due to continuous submergence is considered the smoking gun of rice yield decline in rice-rice system while loss of soil organic matter is regarded as the major factor for decline in rice-wheat system. Depletion of soil nutrient reserves (negative nutrient balances), emerging nutrient deficiencies/toxicities, degraded soil structure and reduced microbial processes are some of the major constraints in rice soils.

Intensive agriculture involving growing of two to three crops a year and producing 10 to 14 Mg ha⁻¹ of grain (or grain equivalents) results in heavy depletion of plant nutrients. Adequate balanced NPK fertilization needs to be practised to check the decline in soil fertility.

The most of Indian soils analyse low for N, low to medium for P and medium to high for K. Intensive cropping can lead to deficiencies of secondary and micronutrients in soil. Deficiency of Zn is wide-spread in India and recommendations for adequate Zn and S fertilization are made for most soils and crops. Imbalanced use of NPK fertilizers (9.5:2.7:1 instead of 4:2:1) is common.

The Indian soils, in general, and those of rice-wheat system in particular, are poor in organic matter, and under intensive cropping, with imbalanced fertilizer use, soil organic matter content declines further as evidenced from the long term fertility experiments (Swarup *et al.*, 1998).

There is decline in the productivity of the rice-wheat rotation in the Indo-Gangetic alluvial plains, despite the application of optimum NPK fertilizer inputs (Nambiar & Abrol, 1989). Deficiencies of secondary nutrients and micronutrients are also affecting the performance of this region and in newer areas (Hegde & Dwivedi, 1992).

STATUS OF SILICON IN RICE SOILS

Rice requires large amounts of Si for healthy plant growth and development. Under the warm subhumid tropical conditions of India, Si removed by 12 rice cultivars (90-140 days duration) grown on an Inceptisol during the dry season varied from 205 to 611 kg Si ha⁻¹ when grain yields ranged from 4.6 to 8.4 t ha⁻¹ (Nayar *et al.*, 1982b).

The average available silica status of eight different soil types of Kerala (South India) as adjudged by four different extractants revealed that Silica extracted by 0.025 M citric acid ranged between 200 to 1500 kg ha⁻¹ with an average of 700 kg ha⁻¹ (Nair & Aiyer, 1968b). River waters have a higher silica content (12 ppm) than well waters (5 to 7 ppm). On an average irrigation water contributes about 30 kg of silica per hectare per rice crop. An average rice crop removes about 250 kg of silica per hectare under Kerala conditions. In 5 out of 9 soils (mostly belonging to red and laterite groups) studied, silicon content ranged from 8 to 83 ppm and considered to be highly deficient (Nayar *et al.*, 1982a).

However, there is no national database on silicon availability in Indian soils, and it is apparent from the reviewed literature (Table 1) that most of the paddy soils studied are deficient in silicon. Based on the removal of silica by an average rice crop and the available silica status of the rice soils a strong case exists for application of silicate fertilizers especially for achieving sustainable rice yields in India.

Table 1. Status of available silicon in different rice soils of India

Location	Available Si (mg kg ⁻¹)	Extractant used	Reference
Kerala (Average of Eight soils)	350	0.025 M citric acid	Nair & Aiyer (1968)
Laterite sandy soil, Kerala	89	NaOAC (pH4.0)	Anilkumar <i>et al.</i> (1990)
Laterite, Vellayani, Kerala	435	Acid soluble	Sadanandan & Verghese (1968)
Red loam, Vellayani, Kerala	201	NaOAC (pH4.0)	Padmaja & Verghese (1972b)
Sandy clay loam, Kanyakumari, TN	29	NaOAC (pH4.0)	Subramanian & Gopalaswamy (1990)
Madukkur Series, Madurai, TN	55	NaOAC (pH4.0)	Subramanian & Gopalaswamy (1991a)
Madukkur Series, Madurai, TN	70	NaOAC (pH4.0)	Subramanian & Gopalaswamy (1991b)
Anaiyur Series, Sholavandan, TN	80	NaOAC (pH4.0)	Subramanian & Gopalaswamy (1991b)
Vylogam Series, Chinnamanur, TN	40	NaOAC (pH4.0)	Subramanian & Gopalaswamy (1991b)
Thuckalay, Padmanathapuram, TN	29	NaOAC (pH4.0)	Subramanian & Gopalaswamy (1991b)
Alluvial clay loam, CRRI, Orissa	139	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Alluvial, coastal sand, Kerala	8	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Laterite, sandy clay loam, Bastar	49	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Laterite, sandy clay loam, Sukinda	43	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Laterite, sandy clay loam, Pattambi	179	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Laterite, sandy loam, Burdwan	43	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Laterite, loam, Alwaye	83	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Red soil, sandy clay loam, Berhampur	248	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)
Black soil, clay, Hyderabad, AP	278	NaOAC (pH4.0)	Nayar <i>et al.</i> (1982a)

INTERACTION OF AVAILABLE SILICON WITH OTHER NUTRIENTS IN SOIL AND PLANT UPTAKE

The effect of sodium silicate on the yield of dry matter and uptake of P by wheat, rice and berseem was investigated by Datta *et al.* (1961). Dry matter and uptake of P by wheat increased irrespective of whether the silicate was applied alone or in combination with phosphate, but in rice the former treatment alone was effective.

Silicon removal from lateritic soil by rice cultivars is reported to be as high as 1000 kg ha⁻¹ (Sahu, 1990). The response of silicon to P uptake by individual cultivars of rice was positive as inferred from the significant interaction effect between silica and cultivars. Application of silica as sodium silicate (Goswami & Kamath, 1984) and other silicate materials (Subramanian & Gopalaswamy, 1991a) had increased the available phosphorus content and decreased fixation of phosphorus in soil. Addition of Poha industry waste ash (PIWA) as a source of silicon increased the availability of phosphorus in soil (Khatik & Vishwakarma, 1994).

A low content of silica in the rice plant is associated with high values for phosphorus. A higher percentage of silica, however, does not seem to influence the phosphorus content. A high value for silica is invariably associated with a low value for the metallic ions iron, aluminum and manganese (Nair & Aiyer, 1968b). Chinnaswamy & Chandrashekharan (1976) noticed synergistic interaction between silica and phosphate in soil, which causes release of adsorbed phosphate ions due to replacement by silicate ions from the exchangeable site.

Sodium silicate treatments proved better at the initial stage in increasing the number of tillers, but calcium magnesium silicate treatment took the lead at the later stages especially at 60 lb nitrogen level (Sadanandan & Vergheese, 1968). The influence of silicon on straw and grain yield was better pronounced at higher levels of nitrogen. They revealed that silicon nutrition of rice at the rate of 100 lb SiO₂ per acre brings about various beneficial effects.

Silicon either alone or in combination with calcium or magnesium or both considerably increased the available phosphorus status of soils (Padmaja & Varghese, 1972a). The contents of phosphorus and potassium in grain and straw are also increased by the application of silicon either alone or in combination with calcium or magnesium (Padmaja & Varghese, 1972b). However, the availability of Ca decreased when it was combined with silicon.

A field experiment was conducted on an Inceptisol in the dry season of 1974, to study the changes in the content and uptake of silica (SiO₂) in relation to growth and yield of 12 rice varieties in the duration range of 90-140 days (Nayar *et al.*, 1982). The grain yield of the varieties was in the range of 4.6 to 8.4 t ha⁻¹, which resulted in the total removal of 439 to 1308 kg silica/ha by the crop. The silica content in the harvest straw of all the varieties except Vijaya was less than 11%, the reported critical limit for optimum growth and yield. The silica content of the leaf blade, culm and whole plant increased with progress of growth and was low during the vegetative period and high after flowering.

Majumder *et al.* (1985) studied the silica uptake in diallel cross involving seven genotypes. Maximum profile of this element was found in leaf followed by stem and root. Regarding its uptake both additive and non-additive genes were involved while the former was higher in magnitude indicating the scope of individual selection in segregating generations. Heterotic effect was also evident. The assessment for efficiency of uptake can be made at an early stage (60 days of growth). They noticed IR 28 as the best combiner for exploitation of heterotic Si-content under P-stress conditions, though the parental line itself had poor silicified stem.

A field experiment was conducted to know the release and uptake of silicon in relation to different levels of major nutrients in the lateritic flooded rice soils (Anilkumar *et al.*, 1990). The uptake of silicon followed a progressive increase from 13 to 75 days after transplanting. The highest rate of uptake and content of silicon was noticed in the treatment receiving higher dose of fertilizers (80:40:40).

It has been reported that various sources of silicate materials (sodium meta silicate, furnace slag and rice

husk) at 500 ppm SiO_2 level and phosphate materials (superphosphate and rock phosphate) at 25 ppm P_2O_5 level in acid soils increased the available silicon and phosphorus content in soils (Subramanian & Gopalswamy, 1990). The yield, contents of these nutrients and their uptake by rice also increased by the application of silicate and phosphate materials. Rice husk ranked first in increasing the availability of Si and P in soil and their nutrient content in plant as well as uptake by rice.

FACTORS AFFECTING SILICON AVAILABILITY

The solubility of Si minerals in soil is variable and is influenced by temperature, pH, particle size, chemical composition, and the presence of disruptive layers. Their dissolution kinetics is also affected by soil factors such as organic matter, moisture content, redox potential and sesquioxides (Drees *et al.*, 1989).

The silica content of the rice plant is independent of the variety but is dependent on the available silica status of the soil. The ratio of silica content of straw and grain is maintained at 2:1 irrespective of varietal differences and soil variations (Nair & Aiyer, 1968b).

An incubation study was conducted for a period of 45 days to know the influence of moisture, organic matter, phosphate and silicate on the availability of Si and P in four rice soils of Tamil Nadu (Subramanian & Gopalswamy, 1991b). Continuous submergence of soil and addition of FYM @ 5000 and 12500 ppm resulted in an increase in the availability of Si and P. The addition of Si significantly increased the available Si in all soils and P in acid soils. The available Si and P increased initially and subsequently decreased with passage of time.

A series of investigations were made by Japanese researchers to know the relationship between Si in soils and plants and Si fertilization. In India Nayar *et al.* (1977a; 1982a) compared N sodium acetate buffer (pH 4.0) with three other chemical extractants (distilled water, 0.2 N HCl and 0.025 M citric acid) and reported the extracting power of the reagents for Si as 0.2 N HCl > 0.25 M citric acid > N acetate buffer > water with some exceptions in some soils. In a green house experiment, they found better correlation of 0.025 M citric acid extractable Si in soils with Si uptake by rice plants. The atomic ratios of Si/Al, Si/Fe+Al could also serve as indices of silica supplying power of soils for growing rice (Nayar *et al.*, 1982a).

Since solubility of silica is pH dependent, the reclamation of alkali soil, which essentially means lowering of pH and ESP, is likely to bring changes in silica and phosphorus content of the soil profile. Mongia and Chhabra (2000) studied the effect of reclamation of alkali soil on water soluble silica content of soil profiles and its relation with different forms of P and extractable Fe and Mn. The concentrations of both water soluble Si and phosphate were higher in all the unreclaimed soils as compared to the reclaimed ones at all depths. A positive and significant correlation was found between pH of the soil and water soluble silica. Reclamation of alkali soils resulted in loss of silica from the surface and its deposition in the lower layers of the profile in all the three soils studied. Water soluble P was found to bear a positive and significant correlation with water soluble Si, whereas Olsen's P had a weak and non-significant but positive correlation.

RECYCLING OF PLANT SILICON IN INDIAN RICE FARMING

Silicate slags are expensive Si sources, and therefore most rice farmers of tropical and subtropical regions probably will be unable to use them at the rates of 1 or 2 t/ha/year (Savant *et al.*, 1997). Agricultural wastes comprising of rice bran, rice husk and rice straw accounts to 106 Mt whereas, wheat and other cereal straw accounts to 140 Mt in India. Nearly 100 to 115 Mt of crop residues are either wasted or burnt. The organic wastes available in India are estimated to supply about 7.1, 3.0 and 7.6 mt of N, P_2O_5 and K_2O respectively. The crop residue alone can supply about 1.13, 1.41 and 3.54 Mt of N, P_2O_5 and K_2O , respectively (Ramaswami, 1999). However, plant residues are used as Si sources both intentionally and incidentally everywhere in the world.

Assuming grain: straw ratio to be 2:3 in HYVs, the amount of straw produced in India is about 300 Mt. Half of these (150 Mt) are used as cattle feed and the rest 50% can be recycled for their beneficial effects on soils and plants. Rice straw contains 40% C, 0.6% N, 0.1% P and S, 1.5% K and 5% Si (Ponnamperuma, 1982). Thus one ton of rice straw thus contains 400 kg C, 6 kg N, 1kg P and S, 15 kg K and 50 kg Si and thus a good source of K (water soluble) and Si. Assuming an average of 5 % Si in rice straw, 150 million tons of rice straw containing roughly 7.5 million tons of Si is available for recycling. Similarly, assuming an average of about 20% rice hull in unmilled rice and nearly 8% Si in rice hull, probably 40 million tons of rice hull containing 3.2 million tons of Si is available for recycling.

However, proper recycling of rice straw and rice hull is not common among most rice farmers of India. Some of the probable reasons for this could be bulkiness of the material, additional labor requirement for recycling (mainly for transport and spreading), difficulty in incorporating uncut straw, insect, disease and rat problems associated with rice straw, low benefit: cost ratio and demand for nonagricultural uses.

RECYCLING OF RICE STRAW

In the mechanised rice culture, the straw is spread on the land and can be incorporated into the soil by disking/ploughing provided it does not delay the turn around period due to unfavourable low temperature. In Indo-Gangetic belt, wheat planting will be delayed if incorporation of rice straw is attempted due to prevalence of cold weather. Thus burning is a major convenient method of straw disposal in Punjab and Haryana where combines are commonly used for harvest of rice. At present about 14 million tonne of rice straw, left in the field after machine harvesting of the rice crop in northern India (Punjab, Haryana and Western Uttar Pradesh), is burnt in situ to clear the fields, because this is a cheap and rapid method of straw disposal (Mishra *et al.*, 2001).

Sharma and Mishra (2001) inferred that burning of rice-wheat crop residues would result in total loss of organic matter and nitrogen, and three-fourth loss of sulphur, even if the ashes were incorporated into the soil. This should be a cause of concern to the farmers because they invest substantial amount on fertilizers and need to apply organic manures to maintain soil organic matter status and productivity. However, this practice recycles some amount of P and K and most of micronutrient cations such as Fe, Mn, Zn and Cu to the soil if the ash is incorporated into the soil before it is blown away by winds.

Repeated straw application improves physical properties of soil that benefit both rice (improving internal drainage) as well as non-rice crops like wheat in rice -wheat system (soil structure improvement). However, major objection to the use of straw as a manure is that it immobilises available soil N which of course is temporary and is much less in flooded soils than in dryland soils obviously due to lower N requirement coupled with slow rate of decomposition under flooded condition.

RECYCLING OF RICE HULL AND RICE HULL ASH

On an average 50% of rice hull obtained in India is being used as a source of fuel in rice mills, hotels and brick making industries. The Rice Hull Ash (RHA) thus obtained contains silicon as major constituent is being used in the rice nursery and main fields of rice farming. Application of black to gray rice hull ash at 0.5 – 1.0 kg m⁻² to seedbed produced healthy and strong rice seedlings (Kumbhar *et al.*, 1995; Sawant *et al.*, 1994). Savant & Sawant (1995) observed increased P content in rice seedling when black to gray RHA at 0.5 to 2.0 kg m⁻² was applied to rice seedbed.

Rice hull has some value as a soil amendment and nutrient source, but its use as a silicon source has been very limited. Incorporation of RHA into soil normally occurs near rice mills and/or their disposal sites. Sharma *et al.* (1988) and Sahu (1989) demonstrated that increased flooded rice yields have been attributed to recycling of silicon in rice hulls and straw and/or their ash.

Greenhouse experiments conducted by Sawant & Patil (1994), showed that SiO₂ content of rice seedlings increased when black or gray RHA was applied to the seedbed. They reported that the RHA treatment improved the plant vigor and at times increased the number of tillers/hill and increased the grain yield. Further, Kumbhar & Nevase (1995) reported that application of black to gray RHA at 1 kg m⁻² to the soil in the seedbed markedly reduced the incidence of leaf blast in the rice seedlings.

The integrated use of RHA at 2.0 kg m⁻² and rice straw (RS) at 2 t ha⁻¹ significantly reduced the severity of leaf blast (24.9%) and incidence of neck blast (29.7%) incited by *Pyricularia oryzae* in comparison to the non-treated control during 1996-97 wet season (Kumbhar & Savant, 2001a) and reduced the incidence and severity of leaf scald disease caused by *Monographella albescens* to an extent of 29.5 and 25.6%, respectively, over the control during 1995, 1996 and 1997 wet seasons in the tropical region of Maharashtra (Kumbhar & Savant, 2001b). They also recorded higher grain yield over nontreated control and concluded that use of RHA at 1 kg m⁻² of seedbed combined with RS at 2 t ha⁻¹ may be helpful to farmers in increasing the rice yields without the use of fungicides.

Silicon content of many of the cultivars released by Konkan Krishi Vidyapeeth (KKV), Dapoli are reported to be low (8-10%) in silicon. Silicon uptake by all the varieties under study increased with the aging of the crop irrespective of addition of rice hull ash (Talashilkar and Chavan, 1996). Addition of rice hull ash significantly increased silicon content of rice at all the three stages of growth. However, interaction effect of silicon and cultivars was observed to be non-significant at maximum tillering and panicle stages. Sahu (1990) used rice hulls as a source of silicon to rice and also noticed significant increase in silicon uptake by rice.

Sawarkar *et al.* (1995) noticed significant increase in grain, straw and total dry matter yields of paddy by the application of Poha industry waste-ash (PIWA). The highest grain yield was recorded with 120 kg P₂O₅ ha⁻¹ added through SSP along with 3 t ha⁻¹ of PIWA.

CONCLUSION AND FUTURE RESEARCH

The comprehensive report on silicon research in Indian rice soils suggests that the majority of the soils studied are deficient in silicon and its fertilization has beneficial effects in rice soils besides increasing the yield. However there is need to study the distribution and availability of silicon in different rice growing soils of India. Researchers need to give greater attention towards the research on different ways of utilization of silicon and its importance in integrated nutrient and pest management programs in large scale field trials for its practical feasibilities by the farmers.

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Fertilizer Application and Integrated Crop Nutrition Management in Vietnam

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In the recent years, Vietnam Agriculture has obtained far-reaching achievements. From having been a regular rice importer, Vietnam has become the second biggest rice exporter in the world. The cultivation of other crops such as coffee, rubber trees and cashew... have also been increasing quickly.

So the position of Vietnam agriculture has been increasingly heightened in the world. In the above-mentioned achievements, there has been no less important contribution of fertilizers. According to the estimations, fertilizer application has contributed to 30% of the total productivity increasing in the whole country. For over a long period of time, the amount of fertilizers applied has been increased with the average of 9% annual increase and it tends to increase with the average of 10% in the future (next period). But there are many shortages in the real situation of fertilizer application in Vietnam:

- The application of fertilizers has not been equal to all kinds of crops as well as to different ecoregions.
- The fertilizer application has been unbalanced, which tendency to apply more of nitrogen.
- The amount of fertilizers application has been still low as compared with developed countries.
- Due attention has not been paid to the complete supply of nutrition. Micro and secondary - elements rarely have been applied to production.
- Misunderstanding in fertilizer application leads to severe consequence to environment.

Thus, the application of fertilizers in Vietnam should be based on the main concepts on integrated crop nutrition management such as;

- The integrated crop nutrition management should be based on the knowledge of soil fertility, geographical distribution, the present status of and trend in soil fertility changes.
- The integrated crop nutrition management should be carried out with full knowledge understanding of strong points and weak points of order to take full advantage of the climatic factors for production.
- The integrated crop nutrition management must assure the nutrition balance at necessary ration, between the input and output in order to improve crop fields but also to avoid soil degradation and over-exploitation soil fertility.
- Because of humid tropical condition, we should take full advantage of organic matter cycles in order to give back the maximum amount of organic matter to soil.
- Concentrating on the study of using secondary and micro element for each crop (at present less attention has been paid to this)

In short: Inadequate understanding of the role of fertilizers leads to unreasonable application of fertilizers which degrades soils, and causes environmental pollution, breaking the ecological balance, and especially leads to low effect. From the above-mentioned reality, soil nutritionists, under the coordination of FAO, have established the concept on Integrated crop nutrition management. With this knowledge, in the recent time and in the future, in Vietnam the supply of nutrition to crops has and will become more balanced and sufficient that enables higher and higher effects on agricultural production, contributing to assure the national food safety and build up a good sustainable agriculture.

Soil Sciences Research in Thailand

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INTRODUCTION

Thailand is located in the tropic monsoon zone of southeast Asia between 5° and 21° north latitude and between 97° and 106° of east longitude. The main part of the country is bordered on the west and the north by Myanmar, on the east by Laos and Cambodia. Peninsular Thailand extends to Malaysia, with Myanmar and the Andaman Sea on the west, and Gulf of Thailand on the east. Thailand is divided into four regions, namely the North, the Central Plain, the Northeastern and the Southern regions. The whole country covers approximately 51.31 million hectares (Mha), of which 28.90 Mha or 56.3% is agriculture area, 37.3% for forest and water area including lakes, swamps and rivers, housing area (3.4%) and non-agriculture area (3.0%) (Table 1).

Table 1. Land utilization in Thailand 2000.

	Mha	%
Total land area	51.31	100
Housing area	1.76	3.43
Agriculture area	28.90	56.27
Forest land	18.38	35.82
Water sources	0.75	1.46
Non agriculture land	1.53	3.00

AGRICULTURAL LAND

Rice is a major crop in Thailand. About 48.9% of agriculture area or 14 Mha is planted rice. For upland field crops, cassava and corn are the main crops, planted in about 2.28 and 2.01 Mha, respectively. The fruit trees are planted in about 12 Mha or 5.45%. Coconut, longan, citrus and durian are popular fruit trees in Thailand (Table 2).

Northern region: Mountain ranges with abundant forest resources surround the upper north, while the inner area is a high plain. In the lower north area, the lowland is mainly occupied by paddy rice, and the upland by maize, bean and cotton.

Northeastern region: Poor soils and erratic rainfall with a long drought period limit farming potential. Only 10% of the farmland is irrigated. Paddy occupies most of the lowland, while cassava and sugar cane are the main crops in the upland.

Central region: The lowland is mainly occupied by paddy, vegetables and flowers, while the upland is mainly occupied by cassava, maize and sugarcane. Fruit and rubber trees are mainly grown in the coastal area.

Southern region: Mainly rubber and oil palm trees are planted on the hilly and mountainous area; about 10.8 and 0.82% of agriculture area, respectively. The lowland is very limited.

Table 2. The Major crops in Thailand (2000).

crop	Mha	% of agriculture area
Rice	14.13	48.91
Corn	2.01	6.96
Cassava	2.28	7.90
Sugarcane	0.21	0.73
Pine apple	0.20	0.70
Soy bean	0.02	0.06
Rubber	3.25	10.89
Oil plam	0.24	0.82
Eucalyptus	0.16	0.55
Cafe	0.06	0.22
Mix fruits tree	1.16	4.02
Citrus	0.05	0.17
Durian	0.01	0.05
Coconut	0.27	0.94
Lychee	0.01	0.03
Longan	0.07	0.24
Total	28.90	100

SOIL CLASSIFICATION IN THAILAND

Currently Thailand soils have been identified about 300 series, and they are differentiated on the basis of USDA soil Taxonomy into 8 orders, namely Spodosols (0.19%), Oxisols (0.02%), Vertisols (0.78%), Ultisols (45.90%), Mollisols (1.35%), Alfisols (8.90%), Inceptisols (8.81%), Entisols (3.14%) and Slope complex and others (30.8%) (Table 3).

Table 3. Distribution of soil order in Thailand.

Soil Orders	Area (ha)	%
Entisols	1.61	3.14
Inceptisols	4.53	8.81
Mollisols	0.7	1.35
Alfisols	4.56	8.9
Ultisols	23.58	45.9
Vertisols	0.40	0.78
Spodosols	0.07	0.19
Oxisols	0.02	0.02
Slope complex	15.49	30.8

SOIL RESOURCES IN THAILAND

There are four major problems in soil resources.

1. Misuse of land
2. Land mismanagement such as
 - Soil erosion loss of nutrients, minerals and organic matter (33% of the whole country).
 - Low organic matter (more than 50% of the whole country).
3. Topology and environment, as coastal land area, peat swamps, old-mine soil, etc.
4. Others

SOIL FERTILITY STATUS IN THAILAND

During 1992-2000, about 63,311 samples were analysed by Department of Land Development (DLD). The soils are very low in CEC, organic matter and available plant nutrients. Upland soils, which cover a substantial part of the country, are red yellow podzolics, grey podzolics, and red yellow latosols are usually strongly acidic, low in organic matter, relatively low in available phosphate and low to medium in available potassium.

Table 4. Plant nutrition status of Thai soils.

	Very low to low (%)*	Very high to toxic (%)**
P	66.0-92.4	2.5-10.1
K	62.5-86.9	3.1-9.5
Ca	6.0-25.8	3.1-21.6
O.M.	54.3-87.0	2.2-23.7
pH	acidic 4.6-80.3	basic 2.3-19.2

*soil can produce 50-75% of maximum yield.

** enough nutritional level in 2-3 years.

PROBLEM SOILS

Table 5 shows that problem soils occupy about 60% of the whole area of Thailand. In the northern region, about 50% of area is sloping complex soils. Low and medium fertility soils are found. Low fertility and saline soils are mostly found in the northeastern region due to sandy texture of weathered sandstone. The Central plain area has a medium to high fertility soil, but acid sulphate soil was found in this region. In the southern region sloping complex soil and organic soil (peat and muck soil) are found and this area has a high rainfall. Rapid weathering of parent materials together with leaching and erosion converts primary materials into Kaolinite.

Table 5. Distribution of soil types in Thailand.

	Mha	%
A. Problem soils	30.8	60.09
organic soils	0.04	0.09
acid sulphate soils	0.67	1.30
saline or/and sodic soils	0.73	1.42
sandy soils	7.46	14.52
shallow soils	6.56	12.78
sloping complex soils	15.40	29.98
B. Normal soils	19.20	37.34
C. Other	1.32	2.57
Total	51.3	100

SOIL SCIENCE RESEARCH IN THAILAND

From the published papers, research on soil sciences in Thailand are summarized in Table 6.

Researches on the application of chemical and organic fertilizers (compost, green manure and mulching)

Table 6. Numbers of research papers in Thailand (~ 2001).

	DLD (229)*	DOA (92)*	Universities (81)*
Soil erosion	medium	few	few
Soil and water conservation	many	few	medium
Soil improvement	many	many	many
Soil fertility	medium	many	many

() * number of research papers.

DLD: Department of Land Development, DOA: Department of Agriculture.

have long been carried out in Thailand for increasing crop production and soil qualities. Bio-fertilizers are also a significant factor to increase crop production, which include many soil microorganisms such as Rhizobium, mycorrhiza, azolla and blue green algae.

POTENTIAL OF SOIL AND PLANT ANALYSIS IN PLANT NUTRIENT MANAGEMENT

Fertilizer application recommendation for crops had been given empirically, however, soil analysis is done before fertilizer recommendation to farmers recently, according to the government policy. There are many soil analysis laboratories in DOA and DLD, and they give their services free of charge to farmers. Some laboratories, universities or academic institute or private companies, also can do on soil analysis.

For the soil and plant analyses, soil and plant laboratories are connecting network to make standard qualities of analysis and collection of data to improve the fertilizer recommendations in the future.

Researches on soil testing and plant responses in the field were reported for upland field crops such as corn, sorghum, mungbean, and soybean. For oil palms, mango, durian, longan, lychee and some other prominent crops, researches are now in progress.

CONCLUSION

Soil problems in Thailand are common among the other tropical countries. Major problems are soil erosion, low in organic matter, and limited plant nutrition. Research on application of Biofertilizers, chemical fertilizers, green manure, compost and organic matter were carried out to improve fertility and quality of soil. Soil or/and plant testing is needed to increasing fertilizers use efficiency and increasing crop production.

Field Excursion

Outline of the Yamagata Prefectural Agricultural Experiment Station Shonai Branch

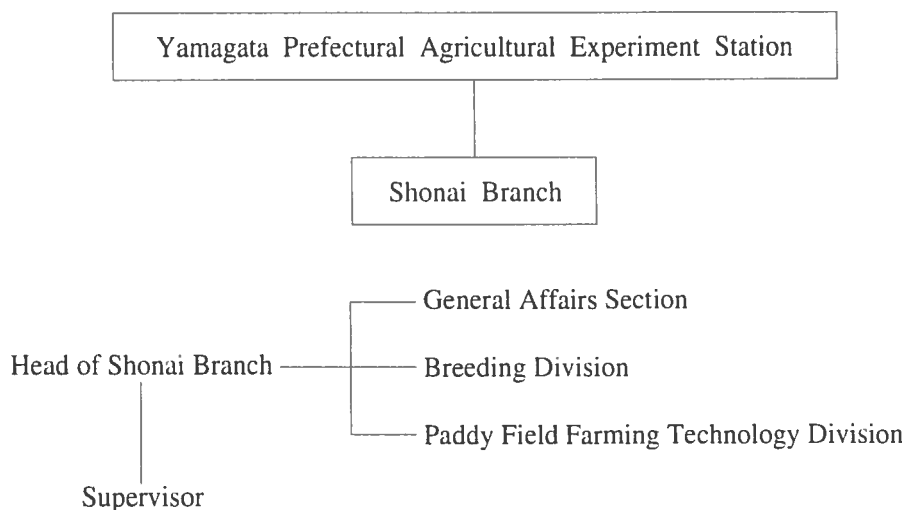
Address

Yamagata Prefectural Agricultural Experiment Station Shonai Branch
 25 Yamanomae, Fujishima-machi, Higashitagawa-gun Yamagata Prefecture, Japan
 TEL : (+81-235)64-2100 FAX : (+81-235)64-2382
<http://www.agri.pref.yamagata.jp/shounai/>
 E-mail : shounai@aff.pref.yamagata.jp

History

1920: Yamagata Pref. Agr. Exp. Sta. Shonai Branch was established at the present location
 2000: The 80th Anniversary of Establishment.

Organization



Structures and Fields(ha)

Paddy	5.10 ha
Upland	0.40 ha
Land of Buildings	1.41 ha
Roads and Waterways	0.01 ha
Total	7.01 ha

Introduction of Our Research

Objectives

- ① Development of the original brand of paddy rice variety in Yamagata Prefecture
- ② Development of Technology for Improving Eating-quality and Sustainable Yield-Production

Breeding Division

Current Research Project

- ①Development of the original brand of paddy rice variety which has high quality, palatability cold resistance and disease resistance
- ②Development of brew's rice and glutinous rice corresponding to processor and consumer needs
- ③Development of the efficient breeding technique for strengthening characteristic grasp of a paddy rice variety and line
- ④Development of an new breeding method by using a DNA marker etc

Results

Since 1964, a total of 10 varieties, consisting of 7 staple varieties, 2 high yielding varieties and 1 brewer's variety has been bred.

Especially "Haenuki", which had been registered variety since 1993, performance outstanding cultivation traits, such as plant type and cold resistance. "Haenuki" is also a staple cultivar of super-high quality of brown rice and has very good eating quality.

By for 2001, a total cultivation area of "Haenuki" reached to 43,000 ha which successfully the 7th hit position of cultivation area ranking in the national level, and it is adopted as a recommended variety of Yamagata Prefecture, Oita Prefecture and Akita Prefecture, and greatly contributes profits to producers, etc.

Technology of Paddy Field Farming Division

Current Research Project

- ①Establishment of cultivation techniques to produce a high-quality and a delicious rice.
- ②Establishment of the cultivation methods to get a best eating quality of rice related to leaf color
- ③To development cultural practices to get high-yield and good quality of soybean
- ④Establishment of Integrated pest management
- ⑤Development of the main-pest control technology

Research accomplishment on Silicon in Sustainable Rice-Production

I Characteristic of Silica gel as a New Silicon Source

Silicon is known as one of the most important element for rice growth. Many Researchers have been studying the effect of Silicon on plant for many years. It is hard to investigate the effect of silicon on growth and yield of plant grown under field condition exclusively, because silicon fertilizer contains some alkali indicating a high pH. Furthermore, plant disease is often observed when silicon fertilizer is applied. These facts arise efforts to discover a new silicon source for crop production. We have developed a new silicon source, namely silica gel, that substituting the existing silicon fertilizer commonly used by farmers. This fertilizer has following the characters:

1. Silica gel are characterized by an amorphous and a large surface area, and which means easily dissolved in water.
2. The particle size can be flexibly controlled by milling and sieving process, and the pH ranged from 3 to 9 and the pH could be adjusted between 3 and 9.
3. No significant difference in the amount of mineralized $\text{NH}_4\text{-N}$ was found between the treatment with and

without silica gel incorporation into the soil.

II Growth and yield of wetland rice under the influence of silica gel application

It is a fact that dry matter production and yield of rice grown under solution culture are affected by its silicon content. Measuring the effect of silicon on growth and yield of rice grown under field condition is difficult, because the amount of mineralized N increases when Si source is applied to the field. The objective of this study was to evaluate the dry matter production and yield of rice plant grown under field condition with reference of canopy structure and plant type.

The results obtained were as follows:

1. No significant difference in amount of mineralized $\text{NH}_4\text{-N}$ was observed between with and without silica gel application.
2. A higher photosynthetic rate of leaf blade was recognized in Si treated plot than control in the afternoon. Light transmission ratio of Si treated plot as affected by the plant canopy structure was higher than the plots no Si -treated as.
3. Increasing rate of Si application enhanced dry weight, amount of N/leaf area(LA), and chlorophyll/LA of rice.
4. A higher number of grains per unit area, ripening grain percentage and yield of rice were obtained in Si-treated plot than the plots without any Si.

III Rooting ability and early growth of wetland rice as affected by silica gel application to the nursery bed

The early growth of rice plant is affected by variability of rice seedling in temperate area. One of the factors, which relates to the variability of rice seedling is Si content of rice seedling. The application of silicon to nursery bed of rice is difficult because of its high pH status. The object of this study was to estimate the rooting ability and early growth of rice plant using silica gel(Si). The results obtained were as follows:

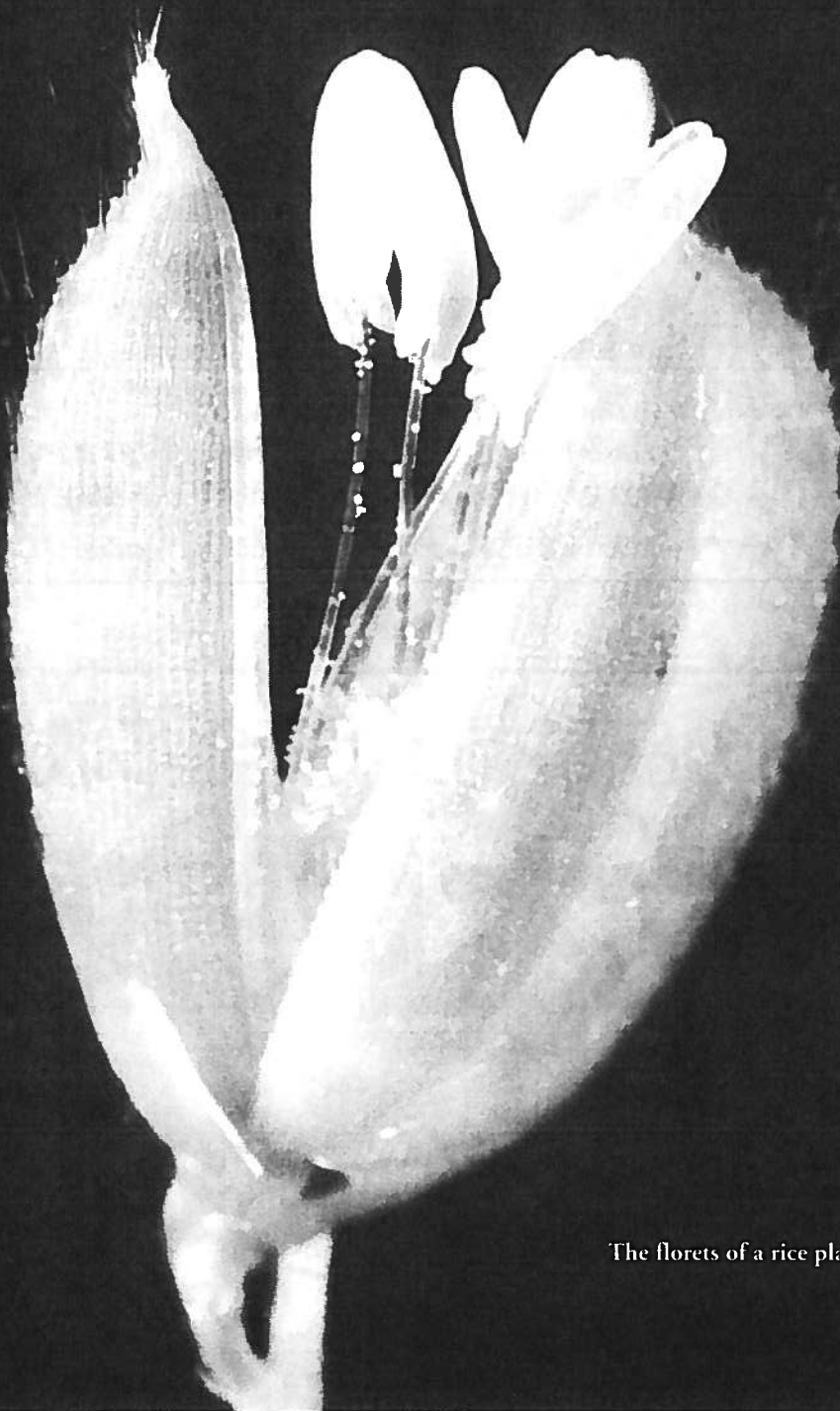
1. Seedling treated with Si showed bigger dry weight, higher dry weight to plant height ratio and increased the amount of silica content compared to control.
2. The photosynthetic rates of individual leaf and of plant canopy of rice seedling were higher in the Si treated seedling. A higher amount of TAC was observed in the seedling treated with Si compared to the untreated one.
3. A bigger number of roots and a heavier dry weight were found in the Si treated plots than control.

IV How does silicon influence host resistance against rice blast disease

While many researchers have demonstrated that silicon in rice plants plays an important role in the resistance against blast disease, the mechanism is not clearly defined. Based on the work of earlier researchers, we put forward several hypotheses to explain how silicon confers this resistance. One hypothesis was that the absorption of silicon in rice reduces nitrogen uptake, which consequently reduces the susceptibility to rice blast. However, our experiments did not support this hypothesis, since applying silica gel to rice did not reduce nitrogen uptake. Another hypothesis was that at least one the process of pathogenesis was inhibited in rice plants of higher silicon content. However, our results showed no significant differences between high and low silicon concentration on spore germination rates, appressorium formation rates, the size of lesions, and sporulation capacity. Therefore, these mechanisms are unlikely the primary factors of the resistance. The possible explanations remained are that silicon acts as a physical barrier against fungal penetration on the surface of leaves or that silicon promotes some physiological resistance mechanisms within the plant. Consequently, we reified these hypotheses during the early infection phase.

Calcium Silicate Fertilizer

For creating an excellent soil suitable
for growing strong rice plants
and producing high-quality rice.



The florets of a rice plant

Calcium Silicate Fertilizer Association

Daiich-inoue Bldg., 2-14-1 Nihonbashi Kayaba-cho, Chuo-ku, Tokyo 103-0025 Japan

Zen-Noh-Type Soil Analyzer ZA-II



**A Compact Set of ZA-II and
Related Equipment (Optional)**

ZEN-NOH

(National Federation of Agricultural Cooperative Association)