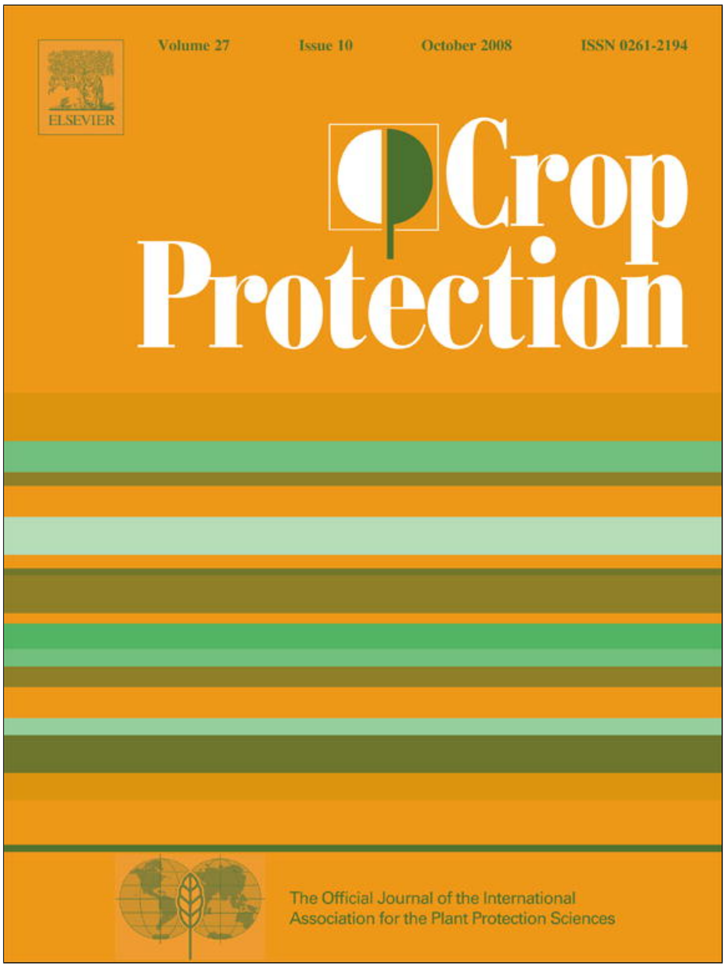
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Short communication

Effect of silicon solubilizers on silica transportation, induced pest and disease resistance in rice (*Oryza sativa* L.)

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ABSTRACT

Promoter or carrier-induced silicon transportation into rice (*Oryza sativa* L.) in relation to yellow stem borer, *Scirpophaga incertulas* (Walker) and blast (*Pyricularia grisea*) disease resistance has been investigated. Simple amino acids, such as histidine, imidazole, glutamic acid, glycine and glutamine significantly enhanced the levels of $Si(OH)_4$ in the stem and 14–18% silicon transport into the leaf surface, as evidenced by scanning electron micrograph (SEM) and silicon mapping studies. Studies on plants grown with these molecules in greenhouse (500 μmoles of the carrier X twice) or 1500 μmoles of the carrier X four times in field were conducted. The work has led to the identification of a novel class of bio-compatible molecules, which exhibit remarkable resistance to damage by yellow stem borer and blast infections and generate higher dry matter and increased yields.

Rice (*Oryza sativa* L.) depends on the availability of silicic acid at all phases of its growth as well as protection from abiotic stresses such as water, salinity, and metal toxicity and also biotic stresses such as rice yellow stem borer, *Scirpophaga incertulas* (Walker), and blast (*Pyricularia grisea*) (Kim et al., 2002; Rodrigues et al., 2003; Ranganathan et al., 2006). Thus, the evolution of the rice plant has ensured mechanisms for uptake of silicic acid, which is normally available at concentration 10^{-4} mole l^{-1}, within a narrow pH range 6–9. Also, mechanisms for controlled transport of silicic acid exist (Mengel et al., 2006) and its deposition occurs in all parts of plant (Yoshida et al., 1962). Recent studies suggest that the yields from rice may be seriously affected if adequate silica is not available (Savant et al., 1997; Esser, 2002). Recently, we demonstrated that molecules like pyridine-*N*-oxides can enhance solubility of silica in water and its physiological effects on rice blast disease and yellow stem borer damage (Ranganathan et al., 2004, 2006). Because pyridine-*N*-oxides are organic molecules, they might lead to soil residual effects. Therefore silicon solubilizers which do not leave deleterious influences on soil microbial organisms was explored under ambient conditions, leading to identification of natural compounds. In this search some of the simple amino acids were found to have the desirable characteristics; among these, histidine, imidazole, glutamic acid, glycine and glutamine were found to be promising models as promoters or carriers. The influence of these solubilizers in relation to yellow stem borer damage and blast disease was investigated during 2004–2005.

The effect of the application of selected enhancers namely histidine, lysine, glutamic acid, glutamine, glycine and imidazole were studied using five varieties of rice, including BPT 5204, IR 64, Rasi, Kasturi and Krishnahamsa during the wet season of 2004 and dry season of 2005. Experiments were conducted both in the field (4.8 m^2 per replication) and also in pots in the greenhouse. Based on the solubility factors, the amino acids were dissolved in water to a concentration of 1500 μmoles of the carrier, and applied four times in the field experiment starting from transplanting, active tillering, primordial initiation and grain filling stages. For pots, 500 ml of either the blank solution or mixed with 500 μmoles of the carrier was applied twice. The results were analysed as previously reported (Ranganathan et al., 2006).

Greenhouse experiments were performed in a total of 35 pots (45 cm diameter), 5 for each carrier and the blank to study the effect of silicon on incidence of yellow stem borer. In each pot 3–4 plants (1 month old) were planted and irrigated. The effect of silicon on stem borer damage was assessed by releasing two neonate larvae per tiller. This study was carried out with rice variety BPT 5204 during the wet season of 2004. Plant damage, in vegetative phase was assessed 2

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weeks after infestation as percentage of dead hearts and as percentage white ears at reproductive phase. The results are presented in Table 1.

As could be seen from Table 1, histidine and glutamic acid notably reduced the dead hearts by 11.5% and 20.9%, respectively, in comparison to the control value of 48.6%. In a similar manner, imidazole remarkably reduced the percentage of white ear damage to 8.4% from a control value of 54.4%.

In the field studies both in wet and dry seasons there was a reduction in stem borer damage and on application of solubilizers the incidence was further reduced (Tables 2 and 3).

The beneficial results resulting from the application of carriers on natural field incidence of stem borer at the vegetative stage in variety BPT 5204 is shown in Table 2. Since the field results were encouraging with BPT 5204, variation in stem borer damage, under natural conditions was evaluated by including several varieties for testing during the 2005 dry season. Results indicated that stem borer damage was significantly reduced (Table 3). The carriers cited in Table 3 enhanced the solubilization of silica by 3–5-fold as indicated by the augmented silicic acid present in the stem of the rice plant and that of silica in the leaves (Figs. 1 and 2). Earlier Savant et al. (1994), Chandramani (2003) and Usharani et al. (2006) reported a reduction in stem borer damage due to increased silicon content by application of soil lignite fly ash and various soil amendments. Also, in sugarcane, silicon was observed to reduce damage by the sugarcane borer, *Eldana saccharina* (Keeping and Meyer, 2006; Kverdaras et al., 2007).

Leaves collected from the treated greenhouse plants of HR 12 (susceptible cultivar) were cut into small segments of upper, middle and lower regions, and were artificially inoculated with the blast pathogen and left for 5 days at room temperature and then observed for infected area.

Table 1
Effect of carriers on yellow stem borer damage in rice (variety BPT 5204) under greenhouse conditions (wet season 2004)

Crop growth phase	Treatments	Dead heart (%) Mean±SEM	*t*-Value, d.f
A. Vegetative phase	Control (no carrier)	48.6±26.0	
	Histidine	11.5±2.3	14.99*, 14 d.f
	Glutamic acid	20.9±2.0	6.25*, 13 d.f
		White ear (%) Mean±SEM	
B. Reproductive phase	Control (no carrier)	54.4±18.0	
	Imidazole	8.4±3.4	8.179*, 15 d.f

Treatment means (*) significant ($p \leq 0.05$).

Table 2
Effect of carriers on yellow stem borer damage in the rice at vegetative phase in the field (wet season 2004)

Carrier	Dead hearts (%) Mean±SEM[a]	*t*-Value (*p*-value)
Control (no carrier)	10.27±0.4	–
Lysine	7.4±0.6	2.1081* (≤0.05)
Imidazole	8.1±0.6	2.7458* (≤0.05)
Glutamic acid	8.4±1.9	2.3210* (≤0.05)
Glutamine	8.5±1.1	1.698, NS

NS—not significant.
[a] Each value is a mean of observations from 40 plants.

Table 3
Beneficial effect of carriers on yellow stem borer damage in the field at reproductive phase (dry season 2005)

Rice variety	White ear (%) No carrier Mean±SEM	With carrier Mean±SEM[a]		*t*-value, *p*-value (≤0.05)
Kasturi	9.3±0.8	Imidazole	2.5±0.9	5.7696*
		Glycine	3.3±0.1	7.9803*
		Lysine	3.4±0.2	7.3103*
		Histidine	3.5±0.7	5.5430*
IR 64	3.0±0.9	Glycine	0.5±0.3	NS
		Lysine	1.4±0.2	NS
		Imidazole	1.4±0.1	NS
Rasi	4.4±0.8	Glycine	0.9±0.3	NS
		Imidazole	1.2±0.3	NS
		Lysine	1.9±0.4	NS
		Glutamine	2.9±0.6	NS
		Histidine	3.0±0.9	NS
Krishnahamsa	8.3±1.1	Imidazole	2.1±0.5	5.51482*
		Lysine	2.5±0.3	5.2427*
		Glycine	2.7±0.7	4.3358*
		Histidine	3.1±0.6	4.1570*

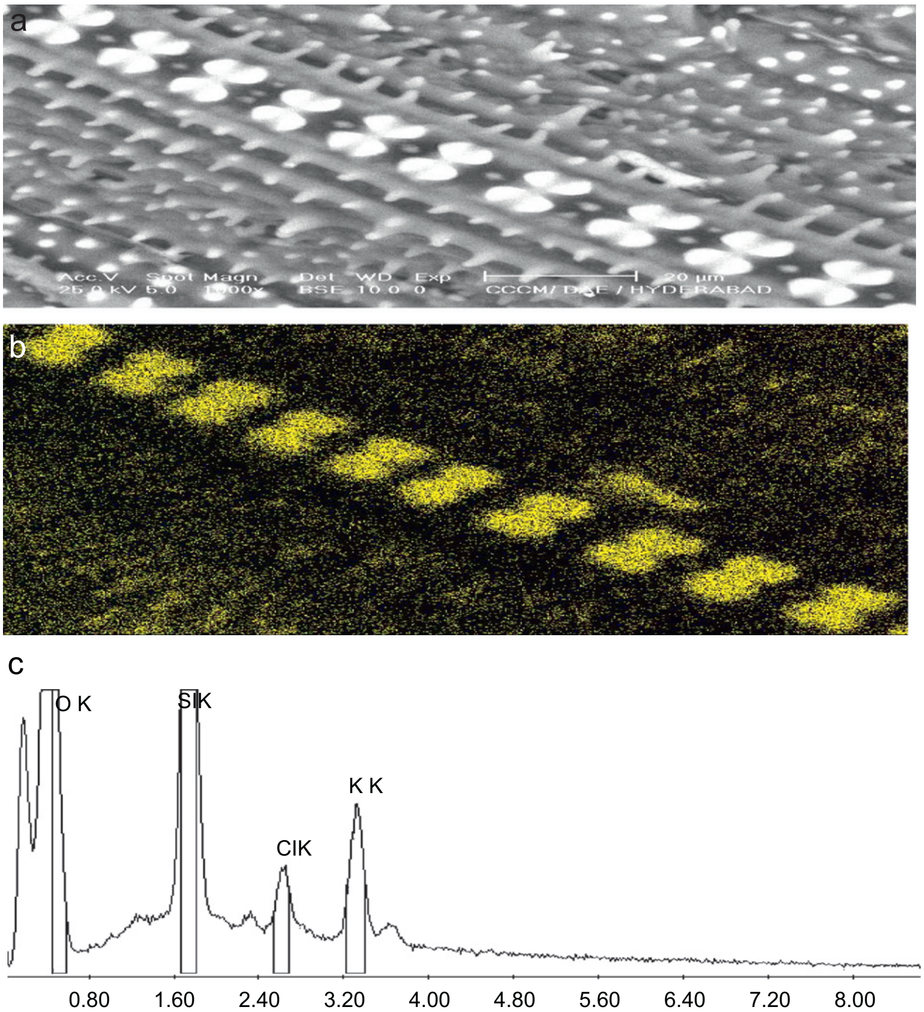
[a] Each value is a mean of two replications with observations from 20 plants per replicate.
* Significant at 5% L.

Infestation of the leaf blast disease on rice exhibited notable differences in infected area. For instance, in the leaf segments from the lower region or the area nearer to leaf sheath, infection was less pronounced than that of leaf segments from upper or leaf tip region (Table 4).

Leaf segments of treated plants from lower region to apical or upper regions showed an increase in per cent infection, particularly with lysine, glycine and imidazole; leaf segments from histidine and glutamine treatments were found to be more resistant. Interestingly, leaf segments from the lower region, irrespective of treatments (except glycine and sodium silicate) have shown resistance which might be due to the vicinity to the intercalary meristematic region, where cell size is small and compact while the opposite is true at tip regions. Indirect evidence supports this hypothesis, in the form of infected area and per cent infection in the middle and upper regions.

In the field, whole leaves were inoculated with blast pathogen. The infection was manifested throughout the leaves as blast spots. These were counted 5 days after inoculation and the results are presented in Table 4. From Table 4 it can be seen that histidine and glutamine provided exceptional protection while the other carriers also provided some resistance.

At the outset it was envisaged that the enhanced presence of $Si(OH)_4/SiO_2$ would be beneficial for the rice plant. To complement the experiments cited above (Tables 1–4), we also obtained direct evidence for the ability of the carriers to enhance silicic acid in the stem of the rice plant and silica in the leaf (Figs. 1 and 2). To study the influence of carriers on the $Si(OH)_4$ content in the stem, extract of the mature rice plant after harvesting, the green stem was cut into pieces, transferred into polycarbonate syringe and squeezed to collect the fluid and the amount of silicic acid assessed by the



Element	Wt%	At%
SiK	63.33	69.89
ClK	12.68	11.08
K K	24	19.02
Total	100	100

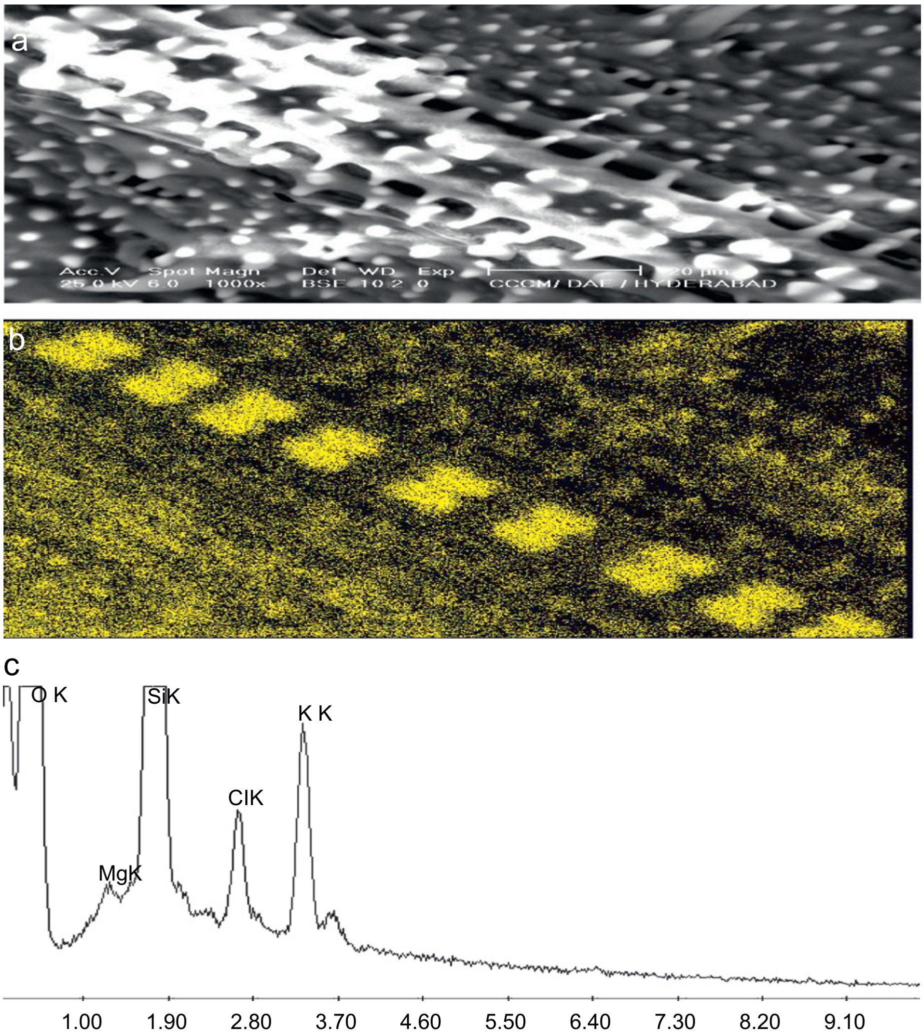
a. SEM profile at the leaf.
b. Silicon contrast map.
c. Energy Dispersive X-ray (EDX) analysis
d. Silica enhancement % : 1.96 %

Fig. 1. Scanning electron micrograph (SEM) profile: *histidine*-treated rice leaf.

ammonium molybdate method (Hartley and Jones, 1972). The results indicated an increase in silicic acid content in the stem extract (data not shown).

In the field, the influence of carriers on the deposition of silica on rice leaf was determined. Rice was grown in natural soil (control), and with added carriers lysine, imidazole, inositol, 1-*N*-hydroxyl imidazole 3-*N*-oxide, glutamic acid and glutamine. Two-month-old leaves in each case were cut into 2 cm sections, wiped with tissue paper to remove moisture, taped to aluminium stubs, sputter coated with gold and loaded on to the scanning electron microscope equipped with Energy dispersive X-ray micro analysis (EDX), with an accelerating voltage of 15 kV. Surface scan was performed on an integrated analytical SEM (Philips, The Netherlands). The SEM results are presented in Figs. 1 and 2 to provide an integral view. Table 5 provides clear evidence for the per cent enhancement of deposition of silica by the carrier molecules. As could be seen from Table 5 most carriers showed enhancement of silica deposition on the leaf notably imidazole, lysine and glutamine.

In the present study, we established that promoters and carriers can enhance the availability of silica in rice. These findings show that silicon contributes to reduction in the incidence of yellow stem borer and blast infection. The carriers studied have been subjected to scrutiny in terms of their efficiency and found to be beneficial with little detriment to rice. Based on the results reported in this paper, large-scale field experiments are being planned with glycine, imidazole and glutamine.



Element	Wt %	At %
SiK	76.68	76.38
ClK	12.36	9.74
K K	10.98	13.88
Total	100	100

a. SEM profile of the leaf.
b. Silicon contrast map.
c. Energy Dispersive X-ray (EDX) analysis
d. Silica enchancement %: 13.86%

Fig. 2. Scanning electron microscope (SEM) profile: *glutamic acid*-treated rice leaf.

Table 4
In vitro screening of rice plants (variety HR 12) for blast disease resistance (treated with carriers in field)

Carrier	Leaf area portion (%)			Number of spots (5 days after inoculation)
	Lower portion	Middle portion	Upper portion	
Histidine	0	0	0.05	1
Lysine	0	25	100	7
Glutamine	0.05	12	0	3
Glycine	62	0.01	100	4
Imidazole	0	25	100	6
Sodium silicate	100	62	63	12
Control	Leaf tissue became dry			18
LSD (treatment)			0.035	0.015
Position			0.024	NS
Interaction			0.066	NS
d.f			53	17

Table 5
Effect of carriers on percentage (%) silica deposition

Carrier	Si (%)[a]	Increment (%)
Control	62.11	–
Histidine	63.3	1.96
Lysine	78.7	16.56
Imidazole	80.56	18.45
Myo-Inositol	76.61	14.36
IMNO[b]	74.21	12.10
Glutamic acid	76.68	13.86
Glutamine	77.18	15.97

[a] Remaining elements were Cl and K (see Figs. 1 and 2).
[b] IMNO: 1-*N* hydroxyimidazole-3-*N*-oxide.

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