## STATUS OF NUTRIENT ELEMENTS IN RICE GRAIN IN RELATION TO SILICON ACCUMULATION PATTERN DURING GRAIN FILLING

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## Abstract

Rice varieties Tadukan (indica), Nanjing 11(indica), Nipponbare (japonica) and Arborio (javanica) were tested to find out the content and accumulation pattern of Si in the hull and brown rice during the grain filling periodand the changes in the weight or the contents of N, P, K, Ca and Mg both in the hull and brown rice. The largest increment in the hull weight during heading to maturity was observed for Arborio. The content of Si in the hull and brown rice increased gradually until 30 days after heading. At maturity, the amount of Si in the brown rice was one twentieth of the hull, irrespective of the cultivars. During the GFP, the increment of Si content was highest in Arborio among the cultivars, and the increase of Si content in the hull of was more remarkable than that of Si concentration. Furthermore, Si concentration in the hull of Tadukan was comparable to Arborio, despite the low Si content. These results suggested that the high amount of Si in the hull of Arborio was accomplished by the high number of silica cells on the large grain surface rather than the increased deposition of Si in each silica cell. Both the content and concentration of N and P in the hull decreased until maturity and twas negatively related to the accumulation of Si in the hull. In brown rice, the content of these elements increased with time although the changes in concentrations were slight andmost pronounced in Arborio. Therefore, it was supposed that the heavy accumulation of Si in the hull was the cause of enhanced accumulation of other elements in the brown rice. In addition, observation of Si distribution on the abaxial surface of lemma by electron-probe X-ray microanalysis revealed heavy deposition of Si on the dentate protuberances of the hull, which was supposed to be one of the reasons for different hull weight among the cultivars.

#### Introduction

Silicon (Si) has been recognized as an agronomical essential element for increasing and/or sustaining the rice production (Takahashi *et al.*, 1990; Epstein, 1994; Savant *et al.*, 1997; Hassanuzzaman *et al.*, 2014). Adequate supply of Si has been reported to induce resistance/tolerance in the rice plant to biotic and abiotic stress (Okuda and Takahashi, 1965; Yamauchi and Winslaw, 1987; Hossain *et al.*, 1999; Ma, 2004; Ma and Yamaji, 2006; Hassanuzzaman *et al.*, 2014). Also, several researchers have shown the improvement of physiological characteristics and increase of dry matter production by Si application in rice (Ma *et al.*, 1989; Liang *et al.*, 1994).

At the reproductive growth stage, Si can increase the number of spikelets on the secondary branch, the ripening percentage, and the weight of grains (Seo and Ota, 1983; Nhan *et al.*, 2012). Mizuno (1987) reported that the hulls of poor quality milky-white grains are generally low in Si content, which is directly proportional to the Si content in the straw. According to Inanaga *et al.* (2002), Si may positively

affect the cell division and formation of cell walls in the spikelet before heading. These studies indicate that Si plays an important role in hull formation which in turn influences grain quality.

Besides the above mentioned physiological importance, Si in the rice hull has been attracting the attention as an environmentally friendly soil amendment. Since large amounts of Si absorbed by the rice plants are deposited as amorphous  $SiO_2$  mainly in the leaves and hulls, their utilization as soil amendments is effective for plant Si recycling (Hashim *et al.*, 1996; Savant *et al.*, 1997). Therefore, the determination of Si concentrations in the hulls of various rice cultivars would give valuable information for the utilization of rice hulls.

Silicon regulation on other mineral nutrient uptake indicated that Si fertilization made soil phophorus (P) more available, potassium (K) uptake improved and a better absorption of nitrogen (N) and calcium (Ca) to plants (Eneji *et al.*, 2008; Mali and Aiery, 2008a, 2008b). In this study, the content and accumulation pattern of Si in the hulls and brown rice during the grain filling period of several cultivars, and compared their relations to the changes in the weight and in the contents of other mineral elements in the hulls and brown rice. Furthermore, the differences in morphological structure and Si distribution on the abaxial surface of the rice hull of different cultivars were examined by electron-probe X-ray microanalysis.

## Materials and Methods

#### Plant material and sampling

A field experiment was conducted in 2001 at a paddy field of grey lowland soil (Typic Endoaquepts) in Faculty of Agriculture, Kochi University, Japan. The surface soil (0-15 cm) with characteristics of pH (H<sub>2</sub>O, 1:2.5 w/v), 5.40; EC, 0.072 dS m<sup>-1</sup>; total N, 1.17 g kg<sup>-1</sup>; total C, 12.7 g kg<sup>-1</sup>; exchangeable cations Ca, 2.87 cmol<sub>c</sub> kg<sup>-1</sup>; Mg, 0.38 cmol<sub>c</sub> kg<sup>-1</sup>; K, 0.26 cmol<sub>c</sub> kg<sup>-1</sup>; and CEC, 7.67 cmol<sub>c</sub> kg<sup>-1</sup>. Basal fertilizer was supplied at the rate of 100 kg N, 100 kg P<sub>2</sub>O<sub>5</sub>, and 100 kg K<sub>2</sub>O ha<sup>-1</sup> by using a coated slow release fertilizer (E-80, 140 d-type, Kumiai, Co. Ltd.). Using NH<sub>4</sub>Cl, additional 20 kg N ha<sup>-1</sup> was also applied with the slow release fertilizer. Silicate fertilizers were not incorporated in the field throughout the cultivation.

Four rice (*Oryza sativa* L.) cultivars Tadukan (indica), Nanjing 11 (indica), Nipponbare (japonica), and Arborio (javanica) were selected. Grain characteristics of the above mentioned cultivars are shown in Table 1. Two seedlings of 19 days old of each cultivar per hill were transplanted with spacing was 30 cm 4 15 cm. Other cultural conditions were conventional ones. Plots of each cultivar were arranged following the randomized block design with two replications.

Cultivar	Ecospecies/	Grain size	Length	Width	Thickness	Volume	
	type			(mm)			
Tadukan	Indica	Small	11.79 с 10.43	2.64 a 3.87	1.87 a	24.73 a	
Nanjing 11	Indica	Medium	b	b	2.39 b	30.46 c	
Nipponbare	Japonica	Medium	9.05 a 12.28	4.13 с 4.87	2.47 b	28.20 b	
Arborio	Javanica	Large	d	d	2.58 с	47.08 d	

Table I. Characteristics of grains of different cultivars.

All the means for each parameter are average of 50 seeds.

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Means in a column do not differ significantly for the same letter by DMRT at 5%. Measurement of seeds was done by vedio micrometer (VM-30, Olympus Co. Ltd.).

Ten panicles of equal size were collected from different spots in each plot at heading, 10 days after heading (DAH), 20 DAH, 30 DAH and at maturity. All of the grains from panicles in each plot were mixed and composite samples were obtained. All of the spotted, distorted or abnormal seeds were discarded from the samples. In every composite sample, each grain was separated into a hull and a brown rice, and their dry weights were recorded.

The contents of Si and other mineral elements in the samples were determined by using an inductively coupled plasma atomic spectrometry (ICP-AES, Shimadzu ICPS 1000IV) after fusion with lithium metaborate (Goto and Ninaki, 1991), unless otherwise stated. Potassium and Mg were determined by using atomic absorption spectrometer (Shimadzu AA-610S), and N was determined according to the Kjeldhal method.

#### Electron probe X-ray microanalysis (EPMA) of lemma

Inflorescence bract lemma and palea was dissected from the mature seeds at maturity. Lemma was then cut along the length. Prepared lemma was fixed to aluminum disks with adhesive and was coated with a carbon layer approximately 30nm thick using a vacuum evaporator (JEOL, JEE-400). Analysis was carried out using a wavelength dispersive type electron probe X-ray microanalyzer (JEOL, JXA-8600MX). The analysis of Si on the abaxial surface around the tip area of the hull were conducted at an accelerating voltage of 15 kV, a sample current of  $5 \sim 10^{-8}$ A, and with a beam diameter of 5  $\Box$ Km. The X-ray spectrometer was peaked for the first order silicon K $\Box$  line (0.7126 nm), using a thallium acid phthalate (TAP) diffracting crystal. The detection of Si was performed by X-ray distribution mapping with stage scanning mode. Morphological observation was performed using the secondary electron scanning microscope (SEM) image at a sample current of  $2 \sim 10^{-9}$ A.

Least significant difference (LSD) for the comparison of any pair of the data were analysed.

## Results and Discussion

#### Dry weights of hull and brown rice

Among four cultivars, the largest increase in the hull weight was observed for Arborio and the increment was 2.5 mg, while the increases in other three cultivars were less than 1.5 mg (Fig. 1). At the same sampling time, the hull weight was maximum in Arborio, followed by Nanjing 11, Nipponbare and Tadukan. The difference of the hull weight between Arborio and Nanjing 11 was larger than the other three cultivars.

From 10 DAH to the maturity, the weight of the brown rice of Arborio increased from 7.5 to 35 mg, which was the largest increase among four cultivars, while the increment in the weight of the brown rice of other three cultivars was less than 15 mg (Fig. 1B). At every sampling time, the maximum dry weight among the cultivars was observed for Arborio, followed by Nipponbare, Nanjing 11, and Tadukan, except for the sampling at 10 DAH but no significant difference was observed among four cultivars. Due to the significant weight increase in the brown rice of Arborio during the late stage of the grain filling period, the difference in the weight of the brown rice between Arborio and the other three cultivars was remarkable especially at 30 128

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DAH and at maturity.

## Contents and concentrations of Si and other elements in hull

The lowest content of Si per hull was found at heading in all the cultivars (Fig. 2). The content increased gradually up to 30 DAH and then remained almost no change. This tendency was most remarkable for Arborio which accumulated 0.35 mg of Si in the hull during the period from heading to 30 DAH, while other three cultivars accumulated less than 0.20 mg Si. As a result, Si content per hull at every sampling time was highest in Arborio and no significant difference was found among the other three cultivars. On the other hand, Si concentrations in the hulls of Nanjing 11 and Nipponbare increased significantly from heading to 20 DAH and those of Tadukan and Arborio continued significant increasing until 30 DAH (Table 2). Different from the Si content per hull, the remarkable difference in Si concentration between Arborio and the other three cultivars was not observed, although the Si concentrations of Tadukan and Arborio were higher than those of Nanjing 11 and Nipponbare at every sampling time.

Table 2. Concentrations of Si and other elements in the hulls during the grain filling period.

Element	Cultivar	Heading	10 DAH	20 DAH	30 DAH	Maturity	
			g/kg dry weight				
Si	Tadukan	29.94	47.91	62.04	73.74	72.71	
	Nanjing 11	23.81	44.27	51.98	54.48	62.58	
	Nipponbare	25.33	37.73	52.38	59.55	62.68	
	Arborio	33.88	53.66	65.69	77.64	71 .57	
LSD(0.05)				7.65			
Ν	Tadukan	11.3	6.99	5.02	4.18	3.99	
	Nanjing 11	9.93	5.21	4.64	3.5	3.26	
	Nipponbare	9.74	5.94	4.32	3.76	3.49	
	Arborio	11.31	7.29	5.471	4.4	4.04	
LSD(0.05)				0.96			
Р	Tadukan	1.65	1.00	0.68	0.43	0.25	
	Nanjing 11	1.66	0.85	0.6	0.36	0.23	
	Nipponbare	1.34	0.87	0.45	0.26	0.14	
	Arborio	1.8	1.08	0.78	0.63	0.28	
LSD(0.0s)				0.11			
K	Tadukan	6.85	6.97	5.04	2.7	1.14	
	Nanjing 11	6.64	5.29	5.22	5.0	2.4	
	Nipponbare	5.3	5.67	9.77	4.7	3.72	
	Arborio	8.95	7.03	7.48	6.19	4.72	
LSD(0.0s)				1.42			
Ca	Tadukan	1.37	1.4	1.48	1.6	1.01	
	Nanjing 11	1.19	1.39	1.41	1.37	1.45	
	Nipponbare	1.11	0.88	1.08	1.43	1.16	
	Arborio	1.95	2.04	2.16	2.2	1.72	
LSD(0.0s)				1.42			
Mg	Tadukan	1.02	0.88	0.79	0.65	0.62	
-	Nanjing 11	1.09	0.75	0.62	0.45	0.46	
	Nipponbare	0.84	0.59	0.29	0.22	0.23	

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Arborio	1.2	0.91	0.71	0.59	0.52
LSD(0.0s)			0.11		

Contrary to the accumulation pattern of Si, the contents and the concentrations of N and P in the hull were highest at heading and gradually decreased until maturity in all of the cultivars (Fig. 2 and Table 2). The drastic decreases in the contents of these elements were observed for Arborio and Nanjin 11 within 10 DAH. From heading to maturity, the largest decrease in the content of N and P was recorded for Arborio, followed by Nanjin 11. Although the contents and the concentrations of Mg and K decreased in the same tendency with N and P, those of Mg showed almost no change after 30 DAH. In the case of K, the decrease was found clearly only during the late stage of grain filling period. The content and the concentration of Ca increased until 30 DAH and then decreased, except for Nanjin 11 where they continued increasing gradually until maturity. The increase in the Ca content until 30 DAH was more pronounced in Arborio than in the other three cultivars. On the other hand, the contents of the examined elements were always highest in Arborio at the same sampling time, and no clear difference was observed among other three cultivars, except for the Mg content of Nipponbare which was significantly lower than the other cultivars after 20 DAH. The concentrations of the examined elements were also highest in Arborio except for Mg concentrations after 20 DAH. However, except for Ca, the differences in the concentrations between Arborio and the other three cultivars were not so large compared with those in their contents.

#### Contents and concentrations of Si and other elements in brown rice

The lowest content of Si per brown rice was found at heading and it increased gradually until maturity in all the cultivars (Fig. 3). This tendency was most remarkable for Arborio which accumulated 0.02 mg of Si in the brown rice until maturity, while the other three cultivars accumulated less than 0.005 mg Si. Irrespective of the cultivars, the Si content in the brown rice at maturity was less than one twentieth of that in the hull. At every sampling time, Si content per brown rice was highest in Arborio and no significant difference was found among the other three cultivars. Different from the Si content per brown rice, only a slight increase was observed for the Si concentrations in the brown rice of all the cultivars (Table 3). Further, the remarkable difference in the Si concentration between Arborio and the other three cultivars was not observed, and Tadukan showed the highest Si concentration at maturity.

Contrary to the decreasing pattern in the hulls, the contents of N, P, K, Ca, and Mg in the brown rice were lowest at heading and gradually increased until maturity, except for Nipponbare (Fig. 3 and Table 3). In the case of Nipponbare, slight decreases were observed between 30 DAH and at maturity. From heading to maturity, the largest increase in the content of these elements was recorded for Arborio and the smallest was for Tadukan. The N concentration in the brown rice was almost in the similar level from 10 DAH to maturity. Although the large fluctuations were not observed for the concentrations of P and Mg as well as N, slight increases were found for the P concentrations between 10 and 20 DAH except for Nipponbare, and for the Mg concentrations between 20 and 30 DAH of Tadukan and Nipponbare. In the case of K and Ca, the concentrations decreased steeply between 10 and 20 DAH and then very slightly until maturity. At the same sampling time, the contents of the examined elements were always highest in Arborio, followed by Nanjing 11 or Nipponbare, and were lowest in Tadukan. However, the differences between Arborio

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and the other three cultivars were more remarkable compared with those among the other three cultivars, especially at the late stage of the grain filling period. While the concentrations of N, P, and K were also highest in Arborio, those of Ca and Mg were highest in Tadukan at every sampling time.

Element	Cultivar	10 DAH	20 DAH	30 DAH	Maturity	
		g/kg dry weight				
Si	Tadukan	0.46	0.46	0.53	0.64	
	Nanjing 11	0.36	0.41	0.42	0.47	
	Nipponbare	0.33	0.32	0.38	0.57	
	Arborio	0.43	0.43	0.54	0.58	
LSD(0.05)			0.14			
Ν	Tadukan	13.1	14.4	14.5	14.8	
	Nanjing 11	13.55	14.95	15.75	14.6	
	Nipponbare	11.75	12.25	13.2	12.7	
	Arborio	16.15	16.5	17	16.55	
LSD(0.05)			2.06			
Р	Tadukan	2.77	3.2	3.39	3.42	
	Nanjing 11	3.1	3.73	3.83	3.79	
	Nipponbare	2.5	2.52	2.94	2.62	
	Arborio	3.39	3.86	3.95	3.87	
LSD(0.05)			0.39			
K	Tadukan	3.99	2.92	2.97	2.83	
	Nanjing 11	5.05	3.92	3.77	3.41	
	Nipponbare	4.96	2.82	2.68	2.34	
	Arborio	6	4.05	3.95	3.61	
LSD(0.05)			0.39			
Ca	Tadukan	0.3	0.23	0.22	0.22	
	Nanjing 11	0.26	0.21	0.2	0.19	
	Nipponbare	0.27	0.16	0.16	0.15	
	Arborio	0.26	0.17	0.17	0.15	
LSD(0.05)			0.04			
Mg	Tadukan	1.19	1.19	1.38	1.45	
	Nanjing 11	1.55	1.53	1.59	1.63	
	Nipponbare	1.23	1.18	1.36	1.22	
	Arborio	1.43	1.39	1.43	1.33	
LSD(0.05)			0.17			

Table 3. Concentrations of Si and other elements in brown rice during the grain filling period.

## Morphological characteristics and Si distribution on hull

By using SEM, the existence of trichomes and dentate protuberances arranged regularly in rows paralleled with longitudinal axis was confirmed on the outer surface

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around the tip area of the hull for all of the cultivars. The number of trichomes was higher in Arborio and Nanjing 11, followed by Tadukan and Nipponbare (Plate 1). The length of trichomes was longer in Arborio, medium in Nanjing 11 and short in Tadukan. Mapping of Si on the outer surface of the lemma revealed heavy deposition of Si on the dentate protuberances on the abaxial surface of hull, and relatively a few amounts of Si were present in the trichomes.

It is well known that large amounts of Si absorbed by the rice plants are deposited mainly in the leaves and hulls. Yoshida et al. (1962) estimated the average amounts of Si in the rice hull and in the leaf sheath as 7.1% and 4.7%, respectively. The same authors reported that heavy deposition of silicon in the inflorescence bracts/husk especially on the abaxial surface was evident (Yoshida et al. 1962). Furthermore, electron probe microanalysis of lemma and palea of rice has revealed heavy deposition of Si associated with the abaxial surface (Soni and Parry, 1973; Balasta et al. 1989; Hodson and Sangstar, 1989). Consistent with these previous reports, our study showed that the amount of accumulated Si was much higher in the hull than in the brown rice irrespective of the cultivars of different grain size and that the accumulation pattern was similar both in the hull and in the brown rice. By the analysis of Si distribution by EPMA and SEM images, it was also confirmed for all of the cultivars that Si was heavily deposited on the dentate rectangular protuberances in the abaxial surface of the hull. However, the difference in the amount of the deposited Si among the cultivars was not found clearly. On the other hand, the higher amount of Si was detected on the dentate rectangular protuberances than in the trichomes although Soni and Perry (1973) reported that a considerable amount of silicon existed in the trichomes. It seemed necessary to conduct qualitative analysis by EPMA in order to evaluate the amounts of Si properly.

The results of present study indicated that the Si content and concentration in the hull of Arborio (javanica type) were significantly higher than those of indicia and japonica type. These different amounts of deposition of Si on the abaxial surface of the hull among the cultivars were supposed to be one of the reasons for the different weight of hull in each cultivar.

The increase of Si content in the hull of Arborio during the grain filling period was more remarkable than that of Si concentration. Furthermore, the Si concentration of the hull of Tadukan (indica type) was comparable to that of Arborio, despite the low Si content. Taking into consideration of these facts, the high amount of Si in the hull of Arborio might be accomplished by the high number of silica cells on the large grain surface rather than the increased deposition of Si in each silica cell. In addition, SEM image of the hull surface of Arborio revealed the number and the length of the trichome were higher than those of the other cultivars. Most of the absorbed Si by plants remains in the apoplast and is deposited after water evaporation at the termini of the transpiration stream (Epstein, 1994; Marschner, 1995). Since the trichomes have a large surface area, the translocation and the accumulation of Si in the hull of Arborio might be enhanced after water evaporation compared with the other cultivars with less or short trichomes.

In the present study, it was indicated that, except for Ca, both of the content and concentration of all the macro elements in the hull decreased until maturity, and this tendency was negatively related to the accumulation of Si in the hull. Contrary to these decrements, the content of these elements in the brown rice increased with time although the changes in the concentrations were slight except that the concentrations of K and Ca decreased at early stage of the grain filling period. These results

suggested that the heavy accumulation of Si in the hull was the cause of the enhanced accumulation of the other elements in the brown rice. Lu *et al.* (2013) reported the elemental distribution of Ca was more in hull (63.8%) than brown rice (36.2%) and this was vice versa for K (67.9% for brown rice). They also showed that the local distribution of K was highly concentrated in the embryo and Ca was preferentially

The results of this study indicated that the decreases of the N and P contents in the hull and the increase of these elements were most remarkable for Arborio. As mentioned already, the highest content and the concentration of Si in the hull was observed for this cultivar throughout the grain filling period. Therefore, it was supposed that lower transpiration caused by the heavy accumulation of Si on the hull enhanced the translocation of the other elements into the brown rice. In addition, the Ca concentration in the hull of Arborio was significantly higher than that of the other cultivars throughout the grain filling period, and that in the brown rice was lower. These results suggest that some of the accumulated Si by Arborio were co-precipitated with Ca in the hull.

## Conclusion

In summary, the present study indicated that the amount of Si in the hull of Arborio was higher than that of Tadukan, Nanjing 11 and Nipponbare. The results also suggested that the hull with the high accumulation of Si might enhance the translocation of other elements such as N and P from the hull into the brown rice. Further, the different amounts of deposition of Si on the abaxial surface of the hull among the cultivars were supposed to be one of the reasons for different weight of the hull in each cultivar. The fact that the hull of the javanica type Arborio showed high Si content and concentration could be utilized for the production of soil amendments with high Si contents, and could help to breed high yielding rice cultivars.

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Status of Nutrient Elements in Rice Grain in Relation to Silicon Accumulation Pattern During Grain Filling



Fig. 1. Dry weight of a hull (A) and a brown rice (B) of different cultivars at different

days after heading (DAH). Bar indicates the LSD value for crop-stage 4 variety at 5%.



Fig 2. Contents of Si and other elements in the hull of different cultivars during grain filling period. Bar indicates the LSD (0.05) value for crop-stage  $\times$  variety.





Fig. 3. Contents of Si and other elements in the brown rice of different cultivars during grain filling period. Bar indicates the LSD(0.05)value for crop-stage  $\times$ 

variety.



Plate 1. Secondary electron images (left side) and corresponding X-ray distribution of Si (right side) on the adaxial surface of the hulls (lemma) of different cultivars,

(A) Tadukan (B) Nanjing 11 (C) Nipponbare and (D) Arborio.