Silica Distribution on the Husk Epidermis at Different Parts of the Panicle in Rice (Oryza sativa L.) Determined by X-ray Microanalysis

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Key words: Epidermis, Husk, Oryza sativa L., Panicle, Silica, X-ray microanalysis.

Compared with other crops, rice (Oryza sativa) accumulates considerable amounts of silica on the surface of its husk or leaf (Takahashi et al., 1990) and numerous studies on the benefits of Si have been conducted in rice and other plant taxa (Yoshida et al., 1959; Jones et al., 1967; Epstein, 1994, 1999; Savant et al., 1997). Although silica is not considered necessary for plant growth or production, the cuticle-silica layer of epidermis helps rice plants maintain erect leaves, reduce transpiration and protects the plants from disease and insect pests (Iwata et al., 1961; Maxwell et al., 1972; Agarie et al., 1992, 1998; Ma, 2004).

Ueno et al. (2005) found that silica deficiency in the cell walls of stomatal apparatus increased evaporative water loss from the rice leaves. Water loss from the panicle of rice is primarily controlled by the cuticle-silica layer on the epidermis of the husk. Given that rice husks, unlike leaves, lack stomata and thus have no dynamic water conservation mechanism, glumes and anthers are subjected to severe desiccation, which contributes to reduced flowering and high sterility of the panicles (Ekanayake et al., 1989, 1993). Therefore, the water status of the panicles needs to be analyzed in detail to determine the mechanism of rice sterility. The transpiration rate and silica concentration/distribution on the epidermis of the husk are the two main factors that determine the water states of the panicle, because silica is considered to suppress the transpiration from the husk.

Silica is transported by the transpiration stream and is deposited at places where transpiration occurs. Consequently, the distribution of silica in the rice plant may indicate specific sites of high transpiration rates or water loss (Dikeman et al., 1981; Balasta et al., 1989). Soni et al. (1973) reported that silica distribution on the epidermis of the rice husk was uneven and observed heavy silica deposition on the papillae, which presented on the margins of the husk. However, differences in silica concentration/distribution on the epidermis of husks in relation to their position in the panicle have not been reported.

The distribution of Si can be determined by X-ray microanalysis. For example, X-ray microanalysis revealed the deposition of silica in the outer walls or leaf epidermis in rice (Lux et al., 1999), sorghum (Lux et al., 2002), and bamboo (Lux et al., 2003).

The objective of the present study was to investigate the differences in silica concentration/distribution on the epidermis of husks at different parts of the rice panicle at the flowering stage using scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) microanalysis.

Materials and Methods

1. Plant material and growth conditions

Three weeks after germination, rice (Oryza sativa L. cv. Akitakomachi) seedlings with three expanded leaves were transplanted to 3.5-L plastic pots with three expanded leaves were transplanted to 3.5-L plastic pots (3 seedlings/pot). Twelve seedlings were grown hydroponically in an artificially illuminated chamber (MIR-553, Sanyo Electric Biomedical Co. Ltd., Tokyo, Japan). The culture solution was the same as that of Mae et al. (1981) and was renewed every seven days. Although the silica concentration of the culture solution was not adjusted, it was approximately 7 mg L⁻¹. Day/night temperature was maintained at 35 ± 1/28 ± 1°C under a 12-h day/12-h night cycle. Relative humidity was maintained at 50 ± 10 % and the photosynthetic photon flux density at the top of the plant was adjusted to 1040 µmol m⁻² s⁻¹ by changing the height of the lamps throughout the experiment.

Rice husk samples were obtained from the tips of the primary rachis at the upper and lower parts of the panicle when the panicle headed completely from the

Received 6 September 2005. Accepted 11 November 2005. Corresponding author: N. Takahashi (aa27062@mail.ecc.u-tokyo.ac.jp, fax+81-3-5841-8172).
leaf sheath. In this study, the flowering stage was about 70 days after germination. The silica distribution on the epidermis of the rice husk was determined by the SEM/EDX analysis.

2. Measurements by SEM/EDX

For SEM/EDX, the dried rice husks obtained from the upper and lower parts of the panicle were cut in half with a razor blade. These samples were mounted on aluminum specimen stubs with a double-stick carbon tape and dried in a vacuum oven for 24 h (EYELA VOS-300SD, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) before being coated with platinum-palladium using an ion-sputter-coater (E-1030, Hitachi Co. Ltd., Tokyo, Japan). The relative concentration of silica was determined using a field emission type SEM (S-4000, Hitachi Co. Ltd., Tokyo, Japan) equipped with an EDX microanalyzer (EMAX-5770X, Horiba Ltd., Kyoto, Japan). The working distance was 15 mm, accelerating voltage was 6 kV and emission current was 10 µA for the analysis by SEM/EDX. For observations of silica distribution on the epidermis, six sections of epidermis at the center of the rice husk were used (Fig. 1). The size of each section was approximately 1140 µm × 840 µm. The relative concentration and distribution of silica in each section was determined using a quantitative analysis program attached to the EDX microanalyzer and EDX mapping analysis, respectively. The scanning time was 200 s for the quantitative analysis and 500 s for the mapping analysis. The relative silica concentration was calculated from the sum of Si, C, and O, and determined by comparing the sum to the peak of Pt as internal standard using the ZAF program attached to the EDX microanalyzer.

3. Statistical analysis

Four panicles were collected from the same plant, and husks from the upper and lower parts of each panicle were used for the SEM/EDX analysis. Differences in the relative silica concentration on the husk epidermis between the upper and the lower parts of the panicle were statistically analyzed using t-test with JMP software.

Results and Discussion

1. Silica distribution on the epidermis of the rice husk

In this study, the rice husks from the upper and lower parts of the panicle were similar in size and shape. Silica distribution on the husk epidermis increased gradually from the basal to apical parts of the husk (Fig. 2). The difference in the relative silica concentration between the basal and apical parts of the husk was approximately 9 % in the upper part of the panicle and 12 % in the lower part of the panicle.

Figure 3 shows the silica mapping images on the epidermis of the husk examined by EDX microanalysis. The number of trichomes increased from the base to the tip of the husk. Heavy deposition of silica was observed on the papillae and trichomes and these results coincided with the report by Soni et al. (1973), who attributed this uneven deposition to the heavier
accumulation of silicon on the papillae. In the present study, the uneven deposition was striking particularly on the husks at the lower part of the panicle (Figs. 3.3 and 3.4). Sangster et al. (1983a) also showed that bract hairs of *Phalaris canariensis* contained some silicon prior to growth extension. Our results demonstrated that the silica deposition on the epidermis increased from the base to the tip of the husk with increasing number of trichomes. The uneven deposition of silica on the husk epidermis may be caused by the heavy silica deposition on the trichomes.

Baba (1955) reported that accelerated transpiration promoted translocation of silica, and increased its accumulation in leaf blades in the rice plants. In the rice leaf, silica deposition on the epidermis increased from the base to the tip of the leaf and this suggested that water loss from the leaf might be uneven (Takeoka et al., 1984). These findings also suggested that the location of transpiration or water loss on the epidermis might be uneven in the rice husk.

2. **Silica distribution on the epidermis of the husk at different parts of the panicle**

The relative concentrations of silica on the
epidermis of husks at the upper part of the panicle were significantly \( p < 0.05 \) at a, d, e and f; \( p < 0.01 \) at b and c) higher than those at the lower part of the panicle (Fig. 2). In each of the corresponding epidermal sections of a husk, the difference in the relative silica concentrations between the upper and lower parts of the panicle was approximately 10\%. Mapping images also showed that silica concentrations were higher on the epidermis of husks at the upper part of the panicle than on that of those at the lower part of the panicle, as shown in Figs. 3.2 and 3.4.

One explanation for this finding is that the rate of transpiration from the husks at the upper part of the panicle is higher than that at the lower part of the panicle for some as yet undetermined reason. Another possibility is that the difference in silica deposition is due to difference in the time of emergence from the leaf sheath between the upper and the lower parts of the panicle. Sangster et al. (1983b) reported that emergence of the inflorescence from the leaf sheath is a phase of critical importance in the silicification in the *Phalaris canariensis*. Consequently, the difference in silica deposition on the husk epidermis may have been brought about by differences in the amount of transpiration; husks at the upper part of the panicle transpired more than those at the lower part and hence increased silica deposition. The second hypothesis would be more likely since we cannot explain the increased transpiration rate at the upper part of the panicle.

Tsuda et al. (2000) reported that the decrease in silica deposition on rice husks correlated well with sterility and the occurrence of white heads. This can be attributed to the excessive water loss from the panicle (O’Toole et al., 1984). Our results showed that there were differences in the silica concentration/distribution even in a panicle depending on the site of the husk in the panicle, and consequently, that variations in sterility may thus exist within one panicle. Further research on the correlation of silica distribution with the extent and occurrence of sterility in a panicle is required to elucidate the detailed mechanisms underlying rice sterility.

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