

[Short Report]

Silica Deposition in Cell Walls of the Stomatal Apparatus of Rice Leaves

Osamu Ueno* and Sakae Agarie**

(*Plant Physiology Department, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan;

**Faculty of Agriculture, Saga University, Honjo, Saga 840-8502, Japan)

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The rice plant is known to accumulate large amounts of silica. Silica has beneficial effects on the growth and development of rice plants, conferring mechanical stability and disease resistance. In the absence of silica, dry matter production and grain yield are significantly reduced (Winslow et al., 1997; Agarie et al., 1998a; Epstein, 1999; Prychid et al., 2004 and references cited therein).

Silica also reduces transpiration in leaves. The cuticular conductance of leaves is greater in plants grown without silica than in those grown with silica (Agarie et al., 1998b). Accumulation of silica in the epidermis is considered a major factor responsible for reduction of cuticular evaporation (Yoshida et al., 1962a, b). However, silica reduces transpiration through stomatal pores, as well as reducing cuticular evaporation (Agarie et al., 1998b). Interestingly, the sensitivity of leaf conductance to changing light and humidity differs between rice plants grown with and without silica: plants grown without silica show delayed responses of stomata (Agarie et al., 1998b), and the stomatal response to blue light differs between plants grown with and without silica (Agarie et al., 1999). These findings imply that some mechanical alterations in the stomatal cell walls caused by silica deficiency may result in the aberrant movement of stomata. However, the cell walls of rice leaf stomata have not yet been fully studied in relation to silica deposition.

Therefore, we investigated whether there is a structural difference in the cell walls of the stomatal apparatus of leaves between rice plants grown with and without silica.

Materials and Methods

Rice plants (*Oryza sativa* L. cv. 'Koshihikari') were grown hydroponically in nutrient solution with silica (100 ppm SiO₂) and without silica. The procedure for hydroponic culture and the growth conditions were as described previously (Agarie et al., 1998a). The uppermost, fully expanded leaves on the main culms of

plants grown for 50 days were used for the experiment. The middle portion of the leaf blades was fixed in glutaraldehyde and post-fixed in osmium tetroxide. After dehydration through an acetone series, the leaf samples were embedded in Spurr's resin, as described by Ueno (2001). Ultrathin cross-sections were cut with a diamond knife, double-stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (Hitachi H-7000, Tokyo, Japan). Ultrathin sections on nickel grids were treated with a 2.5% (v/v) solution of hydrogen fluoride to remove the silica, as described by Agarie et al. (1998a).

Results and Discussion

In the leaf blades of the rice plants grown with silica, all cell walls of the guard cells, except for the anticlinal walls adjacent to the subsidiary cells, had electron-dense deposits (Figs. 1A, B). The deposits formed a layer beneath the cuticle, but often had a multilayered appearance (Fig. 1B). The ledges of guard cells also accumulated deposits. In the subsidiary cells, the outer periclinal walls and the region of the inner periclinal walls adjacent to the guard cells also had the same electron-dense deposits (Figs. 1A, B). As previously reported (Agarie et al., 1998a), the outer periclinal walls and the outer half of the anticlinal walls of ordinary epidermal cells accumulated large amounts of electron-dense deposits (data not shown). The layers of electron-dense deposits in both the guard and subsidiary cells were thinner than those in the ordinary epidermal cells (data not shown, but see Fig. 5A of Agarie et al., 1998a). In addition, the mortar (bulliform) cells and the epidermal cells adjacent to the sclerenchyma were often filled with large amounts of electron-dense deposits, which are known as opaline silica bodies (data not shown). There was no difference between the adaxial and abaxial epidermes in the localization pattern of electron-dense deposits in the stomatal apparatus and the ordinary epidermal cells.

In the leaf blades of the rice plants grown without

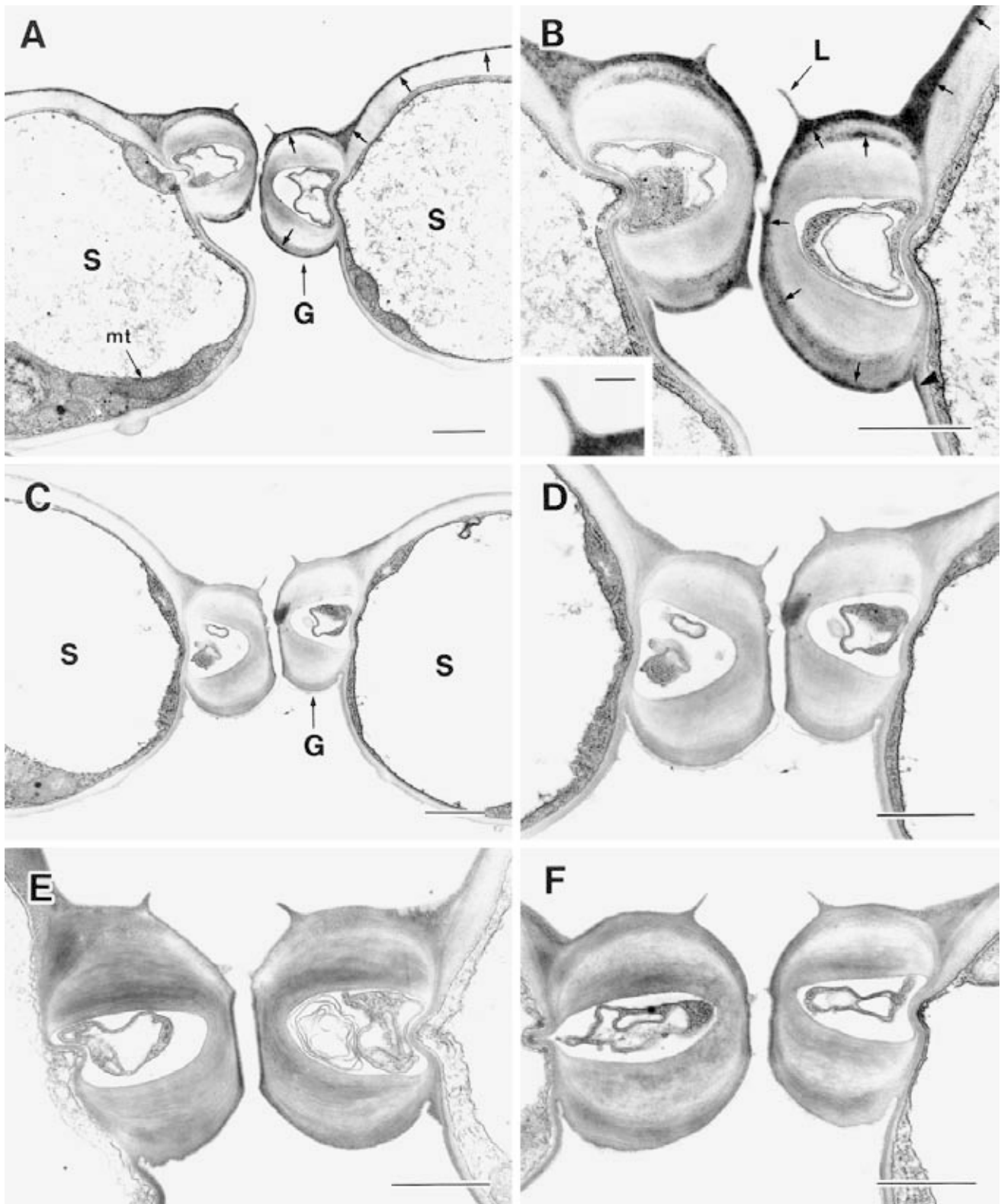


Fig. 1. Cross-sections through the stomatal apparatus of rice leaves. (A) and (B), Stomatal apparatus of a rice plant grown with silica. Unlabeled arrows show electron-dense deposits. The arrowhead in (B) shows electron-dense deposits in the inner periclinal walls of the subsidiary cell, adjacent to the guard cell. The inset in (B) is an enlargement of the ledge. Cuticle is seen in the outermost region of the cell wall. (C) and (D), Stomatal apparatus of a rice plant grown without silica. (E) and (F), Stomatal apparatus of rice plants with (E) and without silica (F) treated with hydrogen fluoride to remove silica. In (E), electron-dense deposits have almost completely disappeared from the cell walls. No change is apparent in (F). All bars $1\ \mu\text{m}$ except inset in (B), $0.2\ \mu\text{m}$. G, guard cell; L, ledge; mt, mitochondrion; S, subsidiary cell.

silica, no electron-dense deposits were observed in the cell walls of the subsidiary or guard cells (Figs. 1C, D) or of the ordinary epidermal cells (data not shown, but see Fig. 5B of Agarie et al., 1998a). No opaline silica bodies were detectable in the epidermal cells. Apart from the presence or absence of electron-dense deposits, no other structural difference was found in the cell walls of leaf cells between the rice plants grown with and without silica. It is noted that plasmolysis was almost always observed in the cytosol of the guard cells of both rice plants under our fixation condition.

When hydrogen fluoride solution was applied to the leaf sections of plants grown with silica, the electron-dense deposits in the cell walls and the opaline silica bodies almost completely disappeared (Fig. 1E). When hydrogen fluoride solution was applied to the leaf sections of plants grown without silica, no structural change occurred in the cell walls (Fig. 1F), indicating that this chemical treatment does not cause any cellular damage except for removal of the electron-dense deposits.

Most previous studies on silica deposition in plants used a scanning electron microscope in conjunction with energy-dispersive X-ray microanalysis (e.g., Kaufman et al., 1985; and reviewed in Prychid et al., 2004). Although this method shows the intercellular pattern of silica distribution, it is not fine enough to show the precise localization of silica deposition, such as that within cell walls in a given cell. A recent study on leaves of *Sasa veitchii* (Poaceae) by this method revealed that the stomatal apparatus accumulates silica, but the exact intracellular localization was unclear (Motomura et al., 2004). On the other hand, Sakai et al. (1979) examined ultrathin sections of the stomata of sugarcane with a transmission electron microscope. In the cell walls of the guard and subsidiary cells, they found electron-dense deposits similar to those we found in rice leaves, and identified these deposits as silica by using X-ray analysis. Our present study shows that these deposits can be removed by treatment with hydrogen fluoride. Thus, it is clear that the electron-dense deposits observed in the stomatal apparatus and other epidermal cells of rice plants are silica. We confirmed the same deposition in rice plants grown normally in soil (data not shown).

The physical properties of plants are determined to a large extent by those of their cell walls. In rice plants, silica is involved in cell rigidity (Agarie et al., 1998a; Epstein, 1999). It is deposited as solid amorphous silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). It appears that silica is biochemically inert, but that it is associated with cell wall components such as polysaccharides and proteins (Epstein, 1999). During stomatal movement, turgor fluctuations are transmitted to the wall polymers, causing large changes in the volume and surface area of guard cells (Willmer et al., 1996). In grasses, subsidiary cells are also involved in the stomatal mechanism (Willmer et al., 1996). Therefore, it seems that the silica in the cell walls of the stomatal apparatus strongly affects the cell's mechanical and physical properties. At present there is no evidence indicating that silica may be involved in the signaling pathway of stomatal movement (Outlaw, 2003). Thus, our results suggest that silica deficiency would cause the aberrant movement of stomata in rice leaves by changing the physical and mechanical properties of the cell walls. Silica deposition also suppresses cuticular evaporation from the epidermal surfaces of rice leaves (Agarie et al., 1998b). Thus, silica deficiency in the cell walls of the stomatal apparatus may increase evaporative water loss from the surfaces, upsetting the osmotic regulation required to generate turgor differences in the guard cells.

References

- Agarie, S. et al. 1998a. *Plant Prod. Sci.* 1 : 89-95.
Agarie, S. et al. 1998b. *Plant Prod. Sci.* 1 : 96-103.
Agarie, S. et al. 1999. *Plant Prod. Sci.* 2 : 232-234.
Epstein, E. 1999. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50 : 641-664.
Kaufman, P.B. et al. 1985. *Ann. Bot.* 55 : 487-507.
Motomura, H. et al. 2004. *Ann. Bot.* 93 : 235-248.
Outlaw, W.H. 2003. *Crit. Rev. Plant Sci.* 22 : 503-529.
Prychid, C.J. et al. 2004. *Bot. Rev.* 69 : 377-440.
Sakai, W.S. et al. 1979. *Ann. Bot.* 44 : 245-248.
Ueno, O. 2001. *J. Exp. Bot.* 52 : 1003-1013.
Willmer, C. et al. 1996. *Stomata*, 2nd Ed., Chapman and Hall, London.
Winslow, M.D. et al. 1997. *Plant Soil* 188 : 239-248.
Yoshida, S. et al. 1962a. *Soil Sci. Plant Nutr.* 8 (1) : 36-41.
Yoshida, S. et al. 1962b. *Soil Sci. Plant Nutr.* 8 (2) : 1-5.