# Analytical Electron Microscopy and Atomic Force Microscopy Reveal a Physical Mechanism of Silicon-Induced Rice Resistance to Blast

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## ABSTRACT

Locations of silicon accumulation in rice leaves and its possible association with resistance to rice blast were investigated by analytical electron microscopy and atomic force microscopy. A blast-susceptible cultivar, Jinmi, and partially resistant cultivars, Hwaseong and Suwon345, were grown under a hydroponic culture system with modified Yoshida's nutrient solution. Electron-dense silicon layers were frequently found beneath the cuticle in epidermal cell walls of silicon-treated plants. Increasing levels of silicon were detected in the outer regions of epidermal cell walls. Silicon was present mainly in epidermal cell walls, middle lamella, and intercellular spaces within subepidermal tissues. Furthermore, silicon was prevalent throughout the leaf surface with relatively small deposition on stomatal guard cells in silicon-treated plants. Force-distance curve measurements revealed relative hardness and smaller adhesion force in silicon-treated plants (18.65 uN) than control plants (28.39 uN). Moreover, force modulation microscopy showed higher mean height values of elastic images in silicon-treated plants (1.26 V) than in control plants (0.44 V), implying the increased leaf hardness by silicon treatment. These results strongly suggest that silicon-induced cell wall fortification of rice leaves may be closely associated with enhanced host resistance to blast.

## INTRODUCTION

Silicon is the second most abundant element on the earth. It is present in plants in amounts equivalent to those of certain macronutrients such as calcium, magnesium, and phosphorus. As a major inorganic constituent of higher plants, silicon is considered to be important for normal plant growth and development. Rice (*Oryza sativa* L.) shows the greatest uptake of silicon in the family Gramineae, accumulating silicon at 10 to

15% on a dry weight basis. Silicon has been reported to benefit rice in a number of ways; i) increasing canopy photosynthesis, ii) increasing resistance to pathogens and pests, and iii) improving water use efficiency, etc. However, the mechanisms by which silicon reduces disease development have not been well established. The objectives of this study were to i) examine ultrastructural modification of rice leaves after silicon applications, ii) localize silicon deposition, and iii) determine a physical mechanism of silicon-induced resistance to blast at the cellular level.

## MATERIALS AND METHODS

**Rice growth under a hydroponic culture system.** Three rice cultivars were used in this study; i) Jinmi as a susceptible cultivar to leaf blast and ii) Hwaseong and Suwon345 as partially resistant cultivars to the disease. Rice plants were grown under a hydroponic culture system with modified Yoshida's nutrient solution with 0, 50, 100, and 200 ppm of silicon.

**Analytical electron microscopy.** Rice leaves were sampled and processed for transmission and scanning electron microscopy. Silicon deposition sites were determined using an energy-dispersive X-ray spectrometer. Several points or areas of the leaves were randomly selected and probed for characteristic X-ray emission to identify the elemental composition of the specimens.

**Atomic force microscopy.** Topographic imaging over $1.5 \times 1.5$ $um^2$ scan areas and force-distance curve measurements were made under ambient conditions using silicon cantilevers. In addition, force modulation microscopy was performed to acquire elastic images of rice leaves under ambient conditions.

## RESULTS

**Localization of silicon deposition in rice leaves.** X-ray microanalysis indicated that silicon was present in epidermal cell walls, middle lamellae, and intercellular spaces of silicon-treated rice plants (Fig. 1). X-ray counts of silicon increased as probe positions progressed from the inner side of epidermal cell walls outward, showing the highest count in the electron-dense outermost region of cell walls.

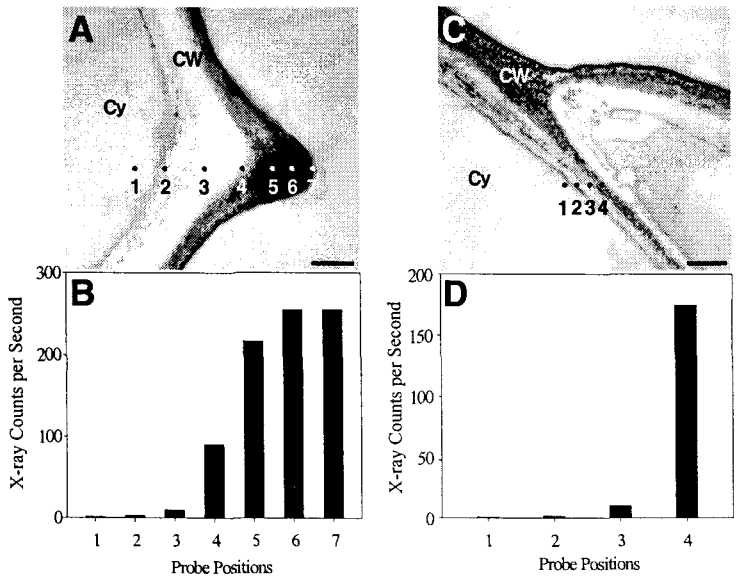
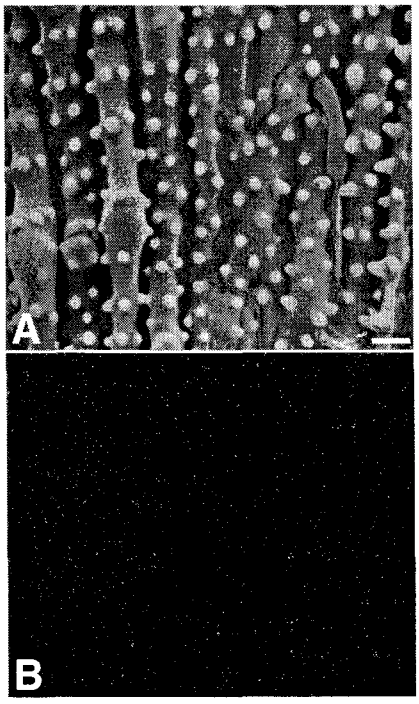



**Fig. 1.** Transmission electron micrographs and X-ray spectra of silicon-treated (200 ppm) Jinmi leaves. **A,** An epidermal cell wall (CW) with a wart-like protuberance (WP). Numbers = probe positions for X-ray emission. Cy = cytoplasm. Bar = 1 ㎛. **B,** X-ray counts of silicon at the probe positions in 1A. **C,** Epidermal cells. Note the electron-dense middle lamella (arrow) between the two cells. Bar = 0.5 ㎛. **D,** X-ray counts of silicon at the probe

**Localization of silicon deposition on rice leaves.** X-ray mapping of silicon on the surface of rice leaves showed that X-ray counts of silicon, which were imaged as white dots, were observed from the leaf surface of silicon-treated plants (Fig. 2). Silicon was present throughout the leaf surface with apparently low silicon X-ray counts around stomatal guard cell areas.



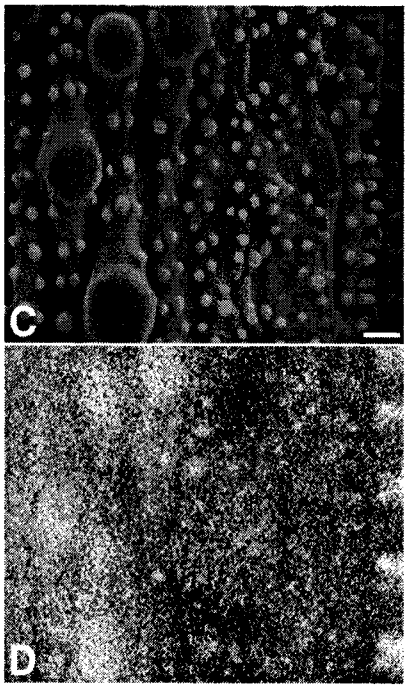



**Fig. 2.** Scanning electron micrographs and silicon X-ray maps of Jinmi leaves. **A,** Leaf epidermis of a control plant. Bar = 10 ㎛. **B,** An X-ray map of silicon of 2A. X-ray counts of silicon (white dots) were hardly observed. **C,** Leaf epidermis of a silicon-treated (200 ppm) plant. Bar = 10 ㎛. **D,** An X-ray map of silicon of 2C.

**Atomic force microscopy.** Topographic imaging of rice leaves showed surface morphology with height variations (Fig. 3A and B). Upon approach of a control plant, long-range repulsion was detected, and a downward curvature was observed upon retraction (Fig. 3C). On a silicon-treated plant, the cantilever experienced repulsion at a much shorter distance upon approach, and relatively short-range adhesion forces were detected upon retraction than on a control plant (Fig. 3D).

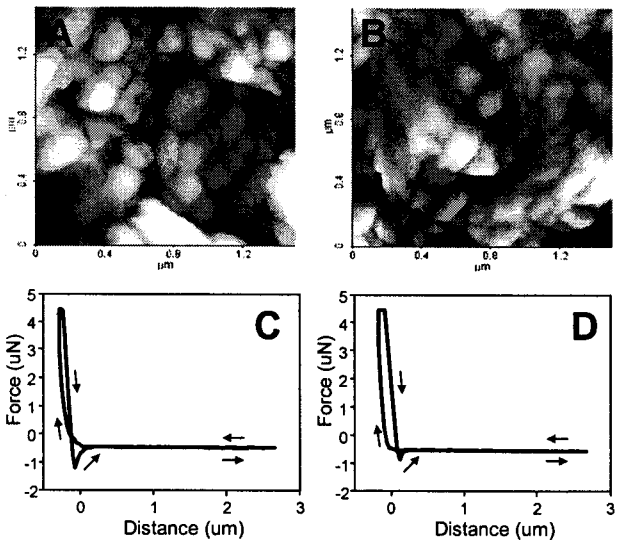



**Fig. 3.** Atomic force micrographs and force-distance curves of rice leaves. Topographic images of leaf epidermis of a control (**A**) and a silicon-treated (**B**) Suwon345 at the 8-leaf growth stage. Force-distance curves of leaf epidermis of a control (**C**) and a silicon-treated (**D**) Suwon345 at the same growth stage.

## DISCUSSION

Analytical electron microscopy in this study provided evidence that silicon was evidently deposited in epidermal cell walls, middle lamellae, and intercellular spaces within subepidermal tissues of silicon-treated rice leaves. An epidermal cell wall of silicon-treated rice plants was frequently composed of two distinct layers; an outer electron-dense silicon layer and an inner electron-translucent layer often having thin electron-dense silicon layers embedded in cellulose microfibrils. It is likely that silicon was incorporated into cell walls as silicon-aromatic ring associations between lignin and carbohydrate in rice leaves. These organo-silicon compounds in epidermal cell walls have been suggested to play a role in limiting lesion expansion, which may reduce the infection efficiency (the number of sporulating lesions) and inoculum production for secondary infection cycles of *M. grisea* in silicon-treated rice plants.

Atomic force microscopy revealed the direct indications as to the functions of silicon in rice leaves. The long-range repulsion force experienced on the control plant may be attributed to the compression of the cell wall. On the other hand, the much shorter range observed in the silicon-treated plant would reflect a harder epidermis. The fact that no significant deviation from linearity was seen in the contact region on the silicon-treated plant indicates that the leaf surface was not deformed by the approaching cantilever.

In conclusion, these results suggest that silicon accumulation on leaf surface and in epidermal cell walls, middle lamellae, and intercellular spaces can limit fungal penetration and invasion by acting as a physical barrier. Even after occasional penetration, fungal cell-to-cell movement or invasion to neighboring tissues may be limited by silicon aggregates found in middle lamellae and intercellular spaces of silicon-treated rice plants. Plant responses to silicon applications are of multi-component nature, and appear to be involved in a variety of biological phenomena of the plants. Further elucidation of biomineralization of silicon and the implication with enhanced disease resistance will clarify our understanding of the nature and role of silicon in plants.

## REFERENCES

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