Annals of Botany **75**: 605–607, 1995

The Detection of the Accumulation of Silicon in *Phalaenopsis* (Orchidaceae)

TIAN-SU ZHOU

Gunma Center, Agribusiness Division, Sapporo Breweries Ltd. 37-1, Kizaki, Nitta, Gunma, Japan 370-03

Received: 8 December 1992 Accepted: 25 September 1994

Small particles along the veins of leaves in *Phalaenopsis* contain silicon. The silica bodies are spherical in shape and 5–30 μm in diameter. In the *in vitro* cultured plantlets, they grow differently in size, depending on the developmental stage of the plantlets and the concentration of silicon added to the medium. The growth of the silica body was increased by increasing the concentration of $CaSiO_3$ from 0·01 to 0·5 mg l^{-1} and was maximized from 0·5 to 1·0 mg l^{-1}. In the medium with 1·0 mg l^{-1} $CaSiO_3$, they grew to a size larger than that of the greenhouse plants after 6 months in culture. The sensitivity of the growth of the silica bodies to the environmental concentration of silicon was then suggested to be a useful indicator for studying the uptake of silicon in plants.

Key words: *Phalaenopsis*, Orchidaceae, silica body, stegmata.

INTRODUCTION

The accumulation of silicon in various plants has been investigated in past years. Silicon is deposited in plants as hydrated amorphous silica ($SiO_2.nH_2O$) and is necessary for the normal development of many plants (Jones and Handreck, 1967; Miyake and Takahashi, 1978). The silica bodies vary in shape and distribution depending on the species (Metcalfe, 1963) and have a taxonomic value.

In many orchid plants, the silica bodies were observed as longitudinal rows along the veins of the leaves. They were spherical or conical in shape depending on the species. The morphological characteristics and the systematical usage of silica bodies in orchids had been described by Møller and Rasmussen (1984), Rasmussen (1986) and Dressler and Cook (1988). This paper describes the substance and growth of silica bodies in *Phalaenopsis*.

MATERIAL AND METHODS

Both the greenhouse plants and the *in vitro* cultured plantlets of a *Phalaenopsis* hybrid, Orchid World, were used for the following analyses and examinations.

Scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDXA)

The foliar veins from several tens of leaves of some 4-year-old plants grown in our greenhouse were digested in hot 12% KOH for 1 h and ground (in a pestle and mortar) in water. The resulting homogenate was filtered through different sizes of nylon mesh (five steps from 0·5 mm to 40 μm) into a small tube. The deposit of the silica bodies was washed with water repeatedly until the purified silica bodies (checked with light microscopy) were obtained and then stored in absolute acetone before examination.

For photographing, a few microlitres of the purified silica bodies were mounted on an aluminium post and coated with gold. The photographs were carried out at 15 kV with an SEM (Hitachi S-4000). For elemental analysis, the purified silica bodies were mounted on an aluminium post without coating and examined with the above-mentioned SEM fitted with an EDXA (Kevex, Super dry) system. The operating voltage was 25 kV and the counting time was 100 s. The result of the elemental analysis was presented as X-ray spectra and plotted with an x–y recorder.

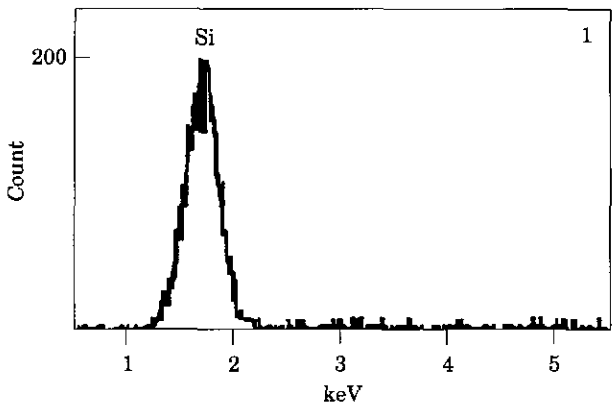
Light microscopy

Leaves from plants grown in the greenhouse were hand sectioned, and those from the young plantlets grown *in vitro* were bleached with 5% sodium hypochlorite solution and cleared in saturated chloral hydrate. Both materials were observed and photographed by differential interference microscopy (Nickon Labophot). The diameters of the silica bodies were measured in millimetres from the amplified photographs from the light microscopy and then converted to the actual length in micrometres.

In vitro *cultures*

For examining the growth of silica bodies in the leaves of the *in vitro* plantlets, protocorm like bodies (PLBs) derived from our tissue culture system were cultured on the modified Vacin and Went medium (mVW) (Tanaka, 1987) with 0·0, 0·01, 0·05, 0·1, 0·3, 1·0 and 3·0 mg $^{-1}$ of calcium silicate ($CaSiO_3.xH_2O$) added. The 0·0 mg l^{-1} of $CaSiO_3$ medium was used as the control (the basal mVW medium was added with 15% coconut water, the only natural substance, and the Si level was not absolutely zero). Thirty PLBs were cultured together in a plastic bottle in triplicate for each concentration. The cultures were kept at 25 °C with a 16 h photoperiod of 500 lx. Transplantations were carried out

0305-7364/95/060605+03 $08.00/0



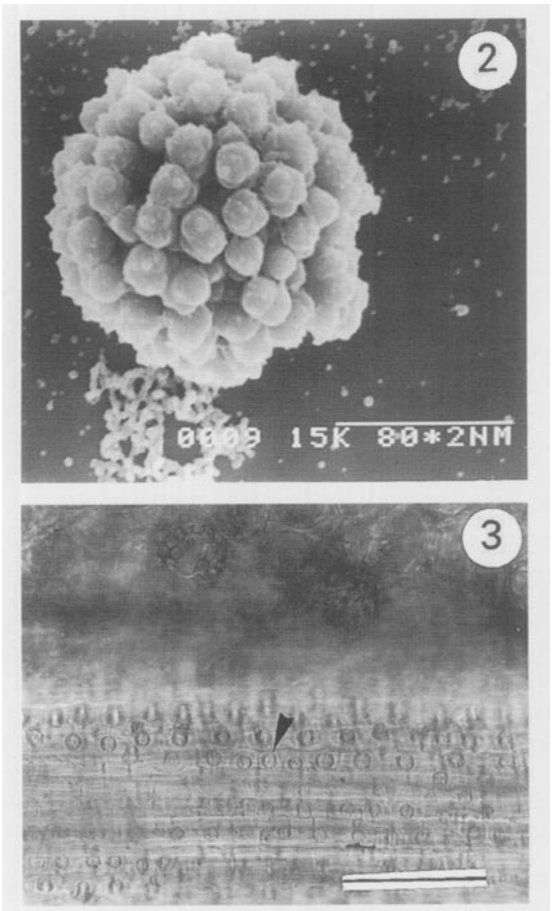


FIG. 1. Energy dispersive X-ray analysis spectra of the small particles in *Phalaenopsis*. The tallest peak is at 1·74 keV and represents K_a silicon (Si). The vertical scale is the count. The horizontal scale is in keV.

FIG. 2. The single silica body isolated from the leaf of *Phalaenopsis*. Bar = 8 μm.

FIG. 3. The surface view of a leaf from a greenhouse plant, showing a single vein on which the silica-bodies are arranged in rows. Bar = 190 μm.

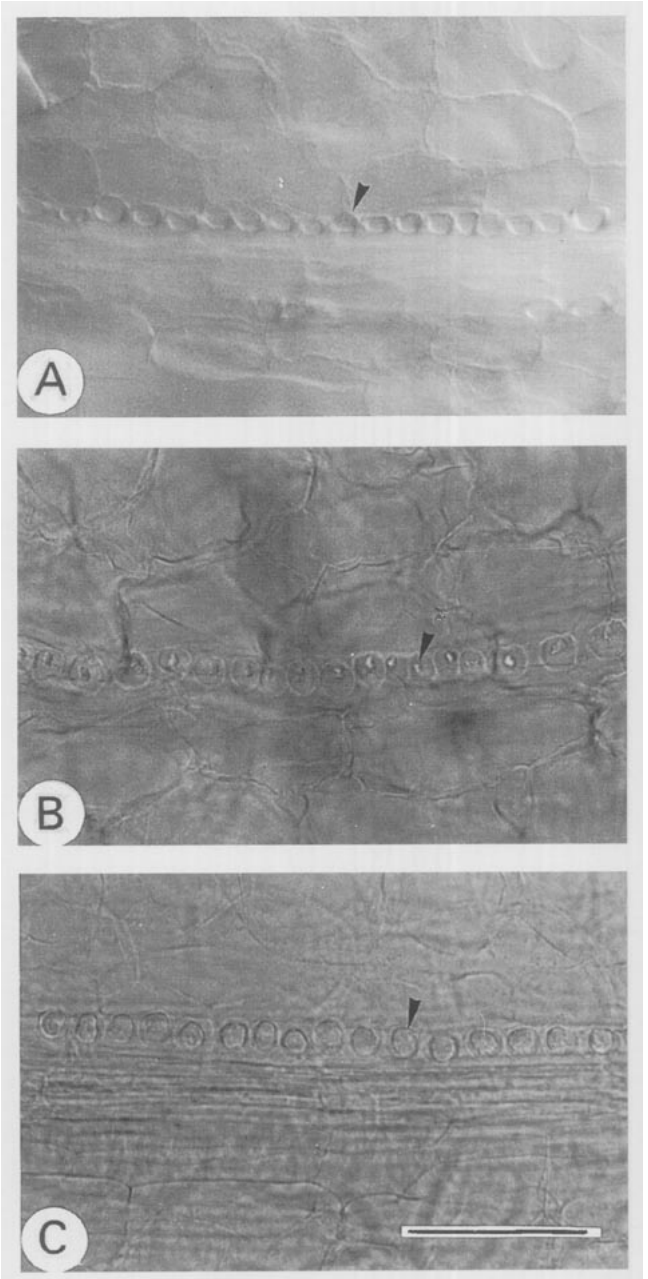


FIG. 4. The growth of silica bodies in the leaves of plantlets cultured on the mVW medium with 1·0 mg l^{-1} $CaSiO_3$ added. A, The leaf of a plantlet cultured at the first month. Stegmatal cells (arrowhead) are in the longitudinal row without silica bodies in them. B, The leaf of a plantlet cultured at the second month. The growth of a silica body (arrowhead) is observed. C, The leaf of a plantlet cultured at the fourth month. Silica bodies grow into a large size. Bar = 100 μm.

monthly. Samples were taken after 1 month of culture, following a 2-month interval. For each sample, five plantlets were collected from one bottle, with 15 plants total for each concentration. The five plantlets were sampled from the minimum to the maximum in leaf length for one bottle. The sampled leaves were treated and examined as mentioned above.

RESULTS AND DISCUSSION

Particles along the veins of leaves in orchid plants have been suggested to be silica bodies based on their resistance to high temperature and to hydrochloric acid (Møller and Rasmussen, 1984). However, the truth has remained unknown. Our elemental analyses of the purified particles

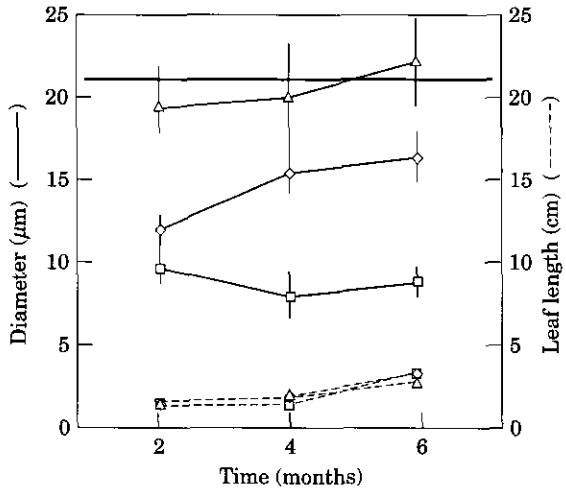


FIG. 5. The growth of silica bodies and the elongation of leaves during the culture of plantlets on the mVW medium with 0 (□), 0·1 (◇) and 1·0 (△) mg l^{-1} $CaSiO_3$ added. Each measurement of diameter presents the average of 150 silica bodies (Bar = s.d.). Each measurement of leaf length presents the average of five samples of leaves. The block line indicates the average diameter of 135 silica bodies from the greenhouse plants.

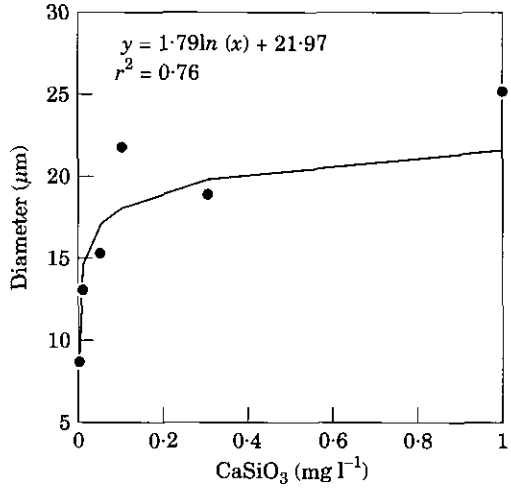


FIG. 6. Diameters of silica bodies in plantlets cultured on the mVW medium with 0·0, 0·01, 0·05, 0·1, 0·3 and 1·0 mg l^{-1} $CaSiO_3$ at the third month. Each measurement presents the average of 150 silica bodies. The curve presents the result of the correlation (in the figure) between the diameter of the silica body (y) and the concentration of the $CaSiO_3$ (x) by the method of least squares.

indicated that they contain a high level of silicon, i.e. there is only one peak on the spectra (Fig. 1); it appears at 1·74 keV on the horizontal axis, and this position is typical of K_α silicon. Particles in *Phalaenopsis* are spherical in shape (Fig. 2) and are called a 'spherical silica body' (Møller and Rasmussen, 1984; Rasmussen, 1986).

Silica bodies have occurred as longitudinal rows along foliar veins (Fig. 3) and exist individually in small silica cells (stegmata) (Fig. 4). At the early stages of development, the leaves of plantlets often have stegmatal cells without silica bodies in them (Fig. 4A). An obvious sign of silica bodies is observed after culturing PLBs for 1 or 2 months, after which PLBs develop into plantlets with leaves about 1·0 cm in length (Fig. 4B). Silica bodies continue to grow on media with $CaSiO_3$ added (Fig. 4C) but do not continue growing on the control medium as in Fig. 4B.

During a 6-month culture of the plantlets, silica bodies grew gradually in diameter on media with 0·1 and 1·0 mg l^{-1} of $CaSiO_3$ but did not continue growing on the control medium (Fig. 5). Those on the medium with 1·0 mg l^{-1} $CaSiO_3$ grew to sizes larger than those on medium with 0·1 mg l^{-1} $CaSiO_3$ at each month and were even larger than those in the greenhouse plants after 6 months in culture. A positive relationship was found between the growth of silica bodies and the concentration of $CaSiO_3$ in the range of 0·01 to 1·0 mg l^{-1}. The growth tended to be maximized from 0·5 to 1·0 mg l^{-1} (Fig. 6). Meanwhile, plantlets grew from 1·0 to 3·0 cm in leaf length on each medium. There was not an obvious difference among the plantlets grown on the different media. Also, there was not an obvious difference between the leaf length and the size of the silica body on a medium at the same developmental stage.

Silicon was reported to be taken up in the form of $Si(OH)_4$, and deposited as $SiO_2.nH_2O$ in plants (Jones and Handreck, 1967). The usual way to detect the uptake of silicon in plants was to compare the concentration of silicon in the medium and the percent of dry weight (Lewin and Reimann, 1969). The above-mentioned results indicate that the uptake of silicon in plants can be detected by measuring the growth of the silica bodies. This is expected to be helpful in further studies involving the uptake of silicon in orchid plants.

ACKNOWLEDGEMENTS

I thank Dr N. Hara, Emeritus Professor of the University of Tokyo, for the helpful comments and suggestions for this manuscript. I also thank Mr M. Kohgo, Microbiology Dept., Brewing Research Laboratories, Sapporo Breweries Ltd., for his assistance with the electron microscopy and energy-dispersive X-ray analysis results and Mr H. Machita and Miss M. Takayama for their help with various aspects of this work.

LITERATURE CITED

Dressler RL, Cook SL. 1988. Conical silica bodies in *Eria javaniva*. *Lindleyana* **3**: 224–225.

Jones LHP, Handreck KA. 1967. Silica in soil, plants and animals. *Advances in Agronomy* **19**: 107–149.

Lewin J, Reimann BEF. 1969. Silicon and plant growth. *Annual Review of Plant Physiology* **20**: 289–304.

Metcalfe CR. 1963. Comparative anatomy as a modern botanical discipline: With special reference to recent advances in the systematic anatomy of monocotyledons. *Advances in Botanical Research* **1**: 101–147.

Miyake Y, Takahashi E. 1978. Silicon deficiency of tomato plant. *Soil Science and Plant Nutrition* **24**: 175–189.

Møller JD, Rasmussen H. 1984. Stegmata in Orchidales: character state distribution and polarity. *Botanical Journal of the Linnean Society* **89**: 53–76.

Rasmussen H. 1986. An aspect of orchid anatomy and adaptationism. *Lindleyana* **1**: 102–107.

Tanaka M. 1987. Studies on the clonal propagation of *Phalaenopsis* through *in vitro* culture. *Memoirs of Faculty of Agriculture, Kagawa University* **49**: 1–85.