

FINAL REPORT

**Plant Physiological Aspects of Silicon
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Plant Physiological Aspects of Silicon

Introduction

The element silicon, Si, represents an anomaly in plant physiology (Epstein, 1994, 1999b).

Plants contain the element in amounts comparable to those of such macronutrient elements as phosphorus, calcium, magnesium, viz. at tissue concentrations (dry weight basis) of about 0.1-10%, although both lower and higher values may be encountered. In some plants, such as rice and sugarcane, Si may be the mineral element present in largest amount.

In much of plant physiological research, however, Si is considered a nonentity. Thus, not a single formulation of the widely used nutrient solutions includes Si. Experimental plants grown in these solutions are therefore abnormally low in their content of the element, being able to obtain only what Si is present as an unavoidable contaminant of the nutrient salts used, and from the experimental environment and their own seeds.

The reason for the astonishing discrepancy between the prominence of Si in plants and its neglect in much of the enterprise of plant physiological research is that Si does not qualify as an "essential" element. Ever since the introduction of the solution culture method in the middle of the last century (Epstein, 1999a, b) it has been found that higher plants can grow in nutrient solutions in the formulation of which Si is not included. The only exceptions are the Equisitaceae (horsetails or scouring rushes), for which Si is a quantitatively major essential element.

But “essential” or not, by an overly rigid definition of the term (Epstein, 1999 a,b), there is ample evidence that Si plays important roles in plant biology (Epstein, 1999 b). Many plants grown under Si deprivation are structurally weak, grow abnormally, tend to lodge, are unusually sensitive to abiotic stresses such as metal toxicities, and exceptionally prone to be ravaged by diseases and pests. It must be kept in mind that plants grow in a medium containing Si and absorb it: the +Si condition is the norm, or “control.”

The object of the research of this project was to demonstrate that Si-replete plants differ from Si-deprived ones and that the latter are therefore to an extent “experimental artifacts” (Epstein 1994, 1999 b). The research was to “concentrate on describing mechanical and biochemical features.” It was successful in the former and partially successful in the latter feature. The mechanical properties of plant cell walls are of paramount importance in plant growth and development, and in the interactions between plants and their environment, including gravity, wind, microorganisms, and insects. Having produced the first quantitative evidence of mechanical differences between cell walls (specifically, trichomes, or “hairs”) of Si-deprived and Si-replete plants, we believe this project to have succeeded in our stated purpose, viz. “to test the hypothesis that plants grown in conventional (-Si) solution culture differ demonstrably from plants grown in the presence of...it in the substrate” (Grant Application, p. 2-3). Finally, we report unprecedented findings on the absorption of Si by wheat plants.

Materials and Methods

Plant materials

We experimented with wheat, *Triticum aestivum*, 'Yecora Rojo,' and rice, *Oryza sativa*, 'CM 201'. Time and resources did not allow us to include tomato, *Lycopersicon esculentum*, except for a few preliminary explorations. The bulk of the effort was devoted to wheat because contrary to our initial expectations, Si-replete and Si-deprived wheat plants differed more in their physical characteristics than did rice; hence our concentration on the former.

Solution culture

The culture solution was basically a modified Hoagland solution. It had the following composition (+Si solution), in $\text{mmol} \cdot \text{L}^{-1}$. KNO_3 : 3, $\text{Ca}(\text{NO}_3)_2$: 2, $\text{NH}_4\text{H}_2\text{PO}_4$: 1, MgSO_4 : 0.5, Na_2SiO_3 : 0.5, Fe-EDTA : 0.025, micronutrients other than Fe: half the concentration of modified Hoagland solution as given in Epstein (1972).¹ In making up this solution, the Na_2SiO_3 is added to the water first; the pH is then lowered to 5.5 with HCl. Only then are the nutrient salts added one by one, with constant stirring. This procedure keeps ions such as PO_4^{-3} and SO_4^{-2} from precipitating out. The -Si solutions contained $1 \text{ mmol} \cdot \text{L}^{-1}$ NaCl to equal the Na^+ concentration in the +Si solutions. (Chloride at this concentration had no detectable effect.)

¹ Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley and Sons, New York.

The plants were grown in 100-L tanks, normally four plants per tank. The solutions were aerated. After an initial low-light regime, conditions in the controlled environment chambers were as follows. Photoperiod: 12 h; photosynthetic photon flux: 1000-1100 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; temperature: 24/18°C; and relative humidity: 50/70%.

Other aspects of the methods used will be discussed in connection with the relevant experiments, as will exceptions or variations in the procedures.

Principal Experiments and Results

Growth and inorganic plant nutrition.

We find that two of our experimental species, wheat, *Triticum aestivum* 'Yecora Rojo,' and rice, *Oryza sativa* 'CM 201,' differ in their responses to the silicon status of their solution culture media.

As for wheat, the height, biomass, and other indices of growth did not differ in +Si and -Si cultures. There was, however, a pronounced difference in their inorganic nutrition, +Si plants having for the most part lower tissue levels of K, Ca, and Zn.

For rice, the results were different. On both a fresh weight and dry weight basis, the +Si plants produced more biomass than the -Si ones (29% and 22% more, respectively). The content of several inorganic nutrients, however, was lower in the +Si plants, as was the case in wheat. The

Si content itself was much higher in both +Si wheat and rice plants than in -Si ones, from 1% to several percent (dry weight basis) in the +Si plants as against a small fraction of 1% in the -Si ones.

Cell wall biochemistry

Pyrolysis GC-MS analysis of cell wall material was done. The data set was reduced by selecting mass chromatograms representing the following constituents: polysaccharides, H, G, and S-type lignins, short-chain hydrocarbon fragments, levoglucosan, alkyl-substituted aromatic marker (via toluene), general carbohydrate marker (via benzene), and carbohydrate + aromatic marker (via phenol). For the first five constituents, the total area of the mass chromatograms were used, while area of the specific peak was used for latter four. Literally hundreds of other markers can also be extracted from the data set, but we concentrated on these general ones at this stage.

For wheat, there were no obvious trends in +Si vs. -Si grown plants, although some subtle differences could be discerned, but these would require many more replicates to confirm, since the differences were small. On the other hand, the first rice experiment showed trends between +Si vs. -Si grown plants 2 weeks and 4 weeks old, so this experiment was repeated with modifications, including a longer (47 days) period of growth. There were no clear trends in the roots, youngest and intermediate leaves, or the stems, but there were considerable differences in the oldest leaves between +Si vs. -Si; the -Si tissues had consistently higher polysaccharide, H-, G-, and S-lignin content, ranging from 200-600% higher. Initial findings of large differences in

the levels of levoglucosan (much higher ones in the -Si than the +Si leaves of rice) have not been confirmed in subsequent experiments.

Physical properties of wheat leaves and awns

Wheat leaves and awns bear trichomes, hook-like extensions of the epidermis, the hooks pointing “upwards”, toward the tips of these organs. The leaf edges and awns of wheat plants grown in conventional nutrient solutions (-Si) felt smooth, but those of plants in +Si solutions quite rough. We invoked differences in the silicon content of the trichomes as the cause of this differential roughness. We have quantified these differences in experiments with heads of wheat, i.e., measuring the roughness of the awns. The friction force needed to cause heads of +Si wheat to slide down an inclined plane is significantly greater than the corresponding value for -Si heads; see Table 1 of the enclosed paper (Rafi *et al.*, 1997), in which these experiments are described in detail. This is, to the best of our knowledge, the first quantitative determination of differences in the mechanical properties of +Si and -Si plant materials done *in vivo*. By the same token, these results confirm our hypothesis concerning the artifactual nature of silicon deprived plants.

The stiffness of the trichomes that makes for their rough feel is due to the deposition of solid, amorphous silica, $\text{SiO}_2 \cdot n\text{H}_2\text{O}$, or “opal phytoliths.” X-ray microanalysis combined with scanning electron micrographs clearly showed the presence of phytoliths in the trichomes of Si-replete wheat plants, and their absence (i.e. undetectability) in those of Si-deprived plants.

As for rice, the differences that were so pronounced in wheat were also present, but to a lesser degree.

The results of this investigation have been published; see Rafi et al. (1997), under Publications.

Silicon transport

In the investigation just referred to, concerning the role of Si in the physical properties of trichomes, the following observation was made. When wheat plants had grown to near-maturity (heads fully developed but still green) without a supply of Si, their awns were smooth, as noted. When the plants were then transferred to +Si solutions (0.5 mM), the awns became rough within two days. This roughness being consistently concomitant with the presence of silica phytoliths, the conclusion emerged that within two days of exposure of the roots to Si, Si had been absorbed by the roots, transferred through the conducting elements to the shoots and into the trichomes, and solidified there in the form of opal phytoliths.

This evidence for rapid Si transport prompted a series of experiments. In these experiments the depletion of Si by four wheat plants from 100 L culture solutions containing initially Si at 0.5 mM was followed as a function of time. Whether initially grown in +Si or -Si solutions, nearly mature plants (heads fully developed but still green) absorbed Si at rates on the order of $2 \mu\text{mol} \cdot \text{g}^{-1} \text{ root fresh wt} \cdot \text{h}^{-1}$. These rates of Si uptake are of the same order of magnitude as those of macronutrient elements such as K and P.

The virtual equality of the rates of Si absorption by Si-preloaded and unpreloaded plants suggests the absence of an effective feedback mechanism that would coordinate growth, Si content, and Si transport, the latter therefore continuing unabated.

The results of this investigation of Si transport have been submitted for publication (Rafi and Epstein, 1999). Additional results are being prepared for publication.

Publications

Epstein, E. 1994. The anomaly of silicon in plant biology. Proc. Natl. Acad. Sci. USA 91:11-17.¹

Epstein, E. 1995. Daniel I. Arnon: photosynthesis, inorganic plant nutrition, solutions, and problems. Photosynthesis Res. 46:37-39.

Rafi, M.M., Epstein, E., and Falk, R.H. 1997. Silicon deprivation causes physical abnormalities in wheat (*Triticum aestivum* L.). J. Plant Physiol. 151:497-501.

Epstein, E. 1997. Grass roots science: a fifty-year personal perspective. *In: Radical Biology: Advances and Perspectives on the Function of Plant Roots.* H.E. Flores, J.P. Lynch, and D. Eissenstat, eds. American Society of Plant Physiologists, Rockville, MD, p 3-12.

Rafi, M.M. and Epstein, E. 1999. Silicon transport in wheat (*Triticum aestivum* L.). Submitted.

¹ Supported by a previous DOE grant.

Epstein, E. 1999a. The discovery of the essential elements. *In: Discoveries in Plant Biology*, Vol. 3, S.D. Kung and S.F. Yang, eds. World Scientific Publishing, Singapore. In press.

Epstein, E. 1999b. Silicon. *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 50. In press.

Meetings, recognition

Our work has drawn attention. I was invited to participate in the DOW Corning Workshop on the Biochemistry & Molecular Biology of Silicon, La Jolla, California, May 5-8, 1996, and gave a presentation. I also presented results of our research at the Sixth International Symposium on Genetics and Molecular Biology of Plant Nutrition, Elsinore, Denmark, August 17-21, 1998.

The journal *BioScience* took note of our research in a "BioBrief": Silicon and plants: the lost connection. (*BioSci.* 47:552. 1997).

Our research was responsible for my being asked to prepare a review on silicon for Annual Review of Plant Physiology and Plant Molecular Biology (see Publications, Epstein, E. 1999b, in press).

Partly on account of the importance of silicon to rice, I was asked to join the Editorial Board of *Soil Science and Plant Nutrition*, published by the Japanese Society of Soil Science and Plant Nutrition.

To sum up, I believe that we have conducted a successful project under this grant. Specifically, the importance of silicon in plants and their structural or mechanical features have been thoroughly documented, the latter in quantitative, physical terms – a first. The thesis that silicon-replete plants are the norm will gain increased acceptance, and the omission of silicon from experimental media will receive the critical scrutiny it deserves.