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Biological, Biochemical, and Chemical Studies of Silica in Plants

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abstract

Background Botanists and materials scientists have established the presence of silica in plant cell walls, but the mechanisms by which plants transport silicon and regulate its polymerization, as well as the functions played by silica in situ, remain poorly known.

Recent Advancement Recent research into silicification processes has uncovered a Si transporter that requires energy to function, Si as a physiologically active element that triggers natural defense systems, and the ways by which silica alleviates abiotic toxicities. The significance of the environment in silica creation is currently poorly understood, but essential to a complete knowledge of silica formation in vivo. The interactions between the biomolecules and silica and the consequences of their presence on the mineralized structures generated are shown by the results of in-vitro investigations of the impact of cell-wall components associated with polymerized silica on mineral formation. Scope This Botanical Briefing explains how plants take in, store, and use Si, as well as the role biomolecules play when integrated into model systems of silica polymerization and where this line of inquiry is headed next.

Key words: Sulfuric acid, biosilicification, resistance to stress, silicon transport, and silica.

INTRODUCTION

Naturally occurring silicon (Si) exists in a wide variety of chemical compounds. Plants may absorb silicon (Si) from the soil if it is present as silicic acid [$\text{Si}(\text{OH})_4$] (or its ionized form, $\text{Si}(\text{OH})_3\text{O}_2^-$, which predominates at pH. 9) (Sommer et al., 2006). Although silicic acid concentrations in soils are typically between 0.1 and 0.6 mM (Epstein, 1994), we are unaware of any biosilicification events occurring in soil. With the exception of the early-diverging Equisetaceae (Chen and Lewin, 2004), this is not often considered a component important to the life cycle of plants. Si is present in plants in concentrations ranging from 0.1 to 10% (103-105 mg kg⁻¹; dry weight basis), which is comparable to or even exceeds that of some macronutrients and is found in certain algae and diatoms (discovered in 1969) (Epstein, 1994). Si is the sole nutrient that is not detrimental when gathered in excess, therefore plants lacking Si tend to be structurally weaker and more prone to growth, development, and reproductive disorders (Epstein, 1999). The purpose of this Botanical Briefing is to provide an overview of Si uptake and its function as a buffer against biotic and abiotic stress, as well as to shed light on how studies of the surrounding environment of plant cells are shedding light on the role of chemical influences in the formation of polymeric silica in plants. The element silicon is represented by the symbol Si, which is frequently used as a shorthand for unspecified silicon compounds. Silicic acid, also known as orthosilicic acid, is the primary component of silicas and has the chemical

formula $\text{Si}(\text{OH})_4$. just the most basic form of silica. Synthesized from $\text{Si}(\text{OH})_4$, silica is an amorphous, hydrated, and often polymerized substance.

OCCURRENCE AND FORM

Si occurs in the plant as a consequence of its absorption from the soil in the form of soluble $\text{Si}(\text{OH})_4$ or $\text{Si}(\text{OH})_3\text{O}_2^-$ and its subsequent, directed polymerization. Extensive investigation of Si absorption in plants has been performed, although the capacity of a plant to absorb Si varies substantially across species (0.1 - 10% of shoot dry weight) (e.g. Simpson and Volcani, 1981; Takahashi et al., 1990; Hodson et al., 2005). There is more evidence of Si buildup in monocotyledonous plants, but this is not the case for dicotyledonous plants. The families Poaceae, Equisetaceae, and Cyperaceae have plants with high Si accumulation (.4% Si), the families Cucurbitales, Urticales, and Commelinaceae have plants with intermediate Si accumulation (2-4% Si), and most other species indicate minimal accumulation. Most plant orders, including liverworts, horsetails, clubmosses, mosses, angiosperms, gymnosperms, and ferns, have decreasing Si concentrations in their shoots (Hodson et al., 2005). One plant can have widely varying Si accumulation in its various parts; for example, the Si content of rice ranges from 0.5 g kg⁻¹ in refined rice to 50 g kg⁻¹ in rice bran to 130 g kg⁻¹ in rice straw to 230 g kg⁻¹ in rice hulls to 350 g kg⁻¹ in rice joints (the part of the grain that connects to the stem) (Van Hoest, 2006).

These levels also stand in stark contrast to the tens of g kg⁻¹ found in oat and wheat straw. Phytoliths, also called silica bodies, are generated upon Si absorption by the plant and are found in the cell walls and lumina of certain plant cells (Prychid et al., 2004).

Because its relationship to other cell-wall components including polysaccharides, lignins, and proteins is incompletely understood (Perry and Lu, 1992). Pectins, which are found in abundance in the cell-wall environment, are rich in galacturonic acid residues cross-linked by the co-ordination of Ca ions, while cellulose, the simplest polysaccharide, provides the framework for the matrix (Fry, 2004). As the silica phase forms, its solubility and binding capacities may be affected by the biochemical milieu (Perry and Keeling-Tucker, 1998).

ROLE OF SILICA IN STRESS RELIEF

Many fertilizers now include silicates since the presence of Si in plants has been shown to reduce a wide variety of abiotic and biotic stressors. It has not been completely explained how Si provides protection, however it has been suggested that it may have a function in the body's physical and/or biochemical defenses. It has been investigated if silica's deposition acts as a physical barrier to penetration and decreases sensitivity to enzymatic destruction by fungal infections (Yoshida et al., 1962). Whether or whether this enhanced strength is adequate to explain the protective benefits reported is a matter of contention (Fauteux et al., 2005). One possible reason for silicon's protective properties is because it is a physiologically active element that may set off several defense mechanisms. The phenomenon was first observed in cucumbers: Plants exposed to Si have elevated levels of enzymes that combat fungal infections, including chitinases, peroxidases, polyphenol oxidases, and flavonoid phytoalexins (Che'rif et al., 1994; Fawe et al., 1998). More research by Be'langer et al. (2003) on wheat blast and Rodrigues et al. (2003) on rice blast found that these species were also capable of inducing similar biologically active defense agents, such as increased production of glycosylated phenolics and antimicrobial products like diterpenoid phytoalexins in response to silica. The production of a proline-rich protein and the presence of silica at the site of attempted penetration were shown to provide additional resistance to infection in experiments with cucumber leaves after fungal infection (Kauss et al., 2003). This protein's C-terminus had an abundance of lysine and arginine residues, which were hypothesized to catalyze the deposit of silica in a targeted area.

The mechanisms by which Si exerts its protective benefits against metal toxicity, salinity, drought, and temperature stressors are currently being studied (Liang et al., 2007).

The accumulation of Zn as a silicate (Neuman and zur Nieden, 2001); decreased lipid peroxidation and increased enzymatic (e.g. superoxide dismutase; SOD) and non-enzymatic (e.g. ascorbate) anti-oxidants (against Mn toxicity) (Shi et al., 2005); and increased release of phenolics with strong chelating ability for Al tolerance are all proposed (Kidd et al., 2001). Increased drought resistance due to the use of "Si" may reduce leaf heat-load, resulting from reduced transpiration (Epstein, 1999) and the existence of silicified structures in plants, indicated an efficient cooling mechanism, allowing for increased plant tolerance to high temperatures (Wang et al., 2005). Increased levels of antioxidant enzymes like superoxide dismutase (SOD) and catalase, which protect membranes from oxidative stress, have been linked to salt stress resistance (Zhu et al., 2004; Moussa, 2006).

TRANSPORT

While Takahashi et al. (1990) postulated three mechanisms of uptake—active, passive, and rejective—for silica, it soon became clear that plants' capacities to collect silica varied widely. Different mechanisms of silicic acid uptake and transport have been deduced from more research on Si absorption in various plant species. In order to investigate how rice (a recognized Si accumulator) accumulates silicic acid, Ma and coworkers have employed mutants deficient in silicic acid absorption.

We discovered that rice absorbs Si from solution through two distinct transport pathways. Root cortical cells absorb silicic acid from the external solution thanks to a low-affinity transporter (Lsi1) localized on the lateral roots (Ma and Yamaji, 2006). Lsi1, the offending gene, has been localized to chromosome 2; it has been shown to have five exons and four introns; and it codes for a protein of 298 amino acids. The projected membrane protein has several features with aquaporins, including six transmembrane domains and two Asn-Pro-Ala (NPA) motifs. Particularly, alanine 132 was identified as a key position because its alteration led to different protein conformation. Although Lsi1 expression is constitutive and most prevalent in the roots, it is regulated by silicic acid availability, with a steady supply of silicic acid having a suppressive effect on expression. Injection of the cDNA encoding Lsi1 into *Xenopus laevis* oocytes boosted silicic acid transport, and the transporter was found on distal cells of the exodermis and endodermis (Ma and Yamaji, 2006). Rice's Lsi1 gene is not related to a silicic acid transport gene family in the marine diatom *Cylindrotheca fusiformis* (Hildebrand et al., 1998). Cucumbers and tomatoes, two other plants studied for their silicic acid absorption, are moderate and poor accumulators, respectively. Although a transporter with the same affinity for silicic acid as Lsi1

was identified to facilitate silicic acid absorption from the environment in both species, changes in transporter density on the lateral roots were found to account for the differential in Si uptake. While passive diffusion is responsible for xylem loading in cucumbers and tomatoes, a second transporter in rice is responsible for Si loading (Mitani and Ma, 2005).

Solution nuclear magnetic resonance (NMR) methods were used in a sophisticated study of silicic acid absorption in wheat (Casey et al., 2003). This allowed the researchers to identify the molecular species containing Si. Soluble Si concentrations in xylem exudate collected after shoot removal were determined after developing seedlings were grown in an aqueous growth medium containing 98.7 at.% ^{29}Si . Si was detected by a colorimetric technique, and the specific species were determined via solution state ^{29}Si -NMR spectroscopy. Over the course of the 90-minute sample period, the highest concentration of dissolved silicate was 8 mM, which is much higher than the solubility limit of silicic acid. NMR analyses of the early exudates, however, showed only monomeric and dimeric silicic acid, although at slightly varying quantities. It is unfortunate that ^{29}Si -NMR spectroscopy was not used to investigate the subsequent exudates (nor by ^1H - or ^{13}C -NMR spectroscopy, which would have indicated whether organic components were present).

BIOGENIC SILICA STRUCTURES: MOLECULES TO MATERIALS

Ornate hierarchically patterned biosilicas may be accumulated, stored, and processed by plants, diatoms, and sponges. In an aqueous setting, at atmospheric pressure and temperatures between 4 and 40 °C, organisms create silica from under-saturated solutions of silicic acid; this production, totaling to gigatons per year, much outweighs that produced industrially. There has been extensive research and discussion about the mechanisms by which this can be accomplished in living things, but it is clear that the organic environment, which includes a wide variety of proteins, carbohydrates, lipids, metal ions, and (in plants) phenolic compounds, is likely to play a fundamental role (Perry and Lu, 1992; Harrison, 1996). Orthosilicic acid, a mildly acidic monomer, is silica's simplest form.

molecule (pK_a 9.8) made up of Si tetrahedrally coordinated to four hydroxyl groups (Iler, 1979) that may be found ubiquitously in soil at low quantities (a few mg kg^{-1}). This is the form of Si that plants may take up and use in the processes outlined above. At concentrations above around 100–200 mg kg^{-1} , polycondensation

ENVIRONMENTAL INFLUENCES ON SILICA POLYMERISATION

Cell-wall extracts of rice shoots have been studied for the presence of Si-binding compounds (Inanaga and Okasaka, 1995). Phenol–carbohydrate complexes (obtained after treatment with cellulase) and lignin–carbohydrate complexes (extracted with dimethyl sulfoxide) subjected to

processes cause the following: the polymerization of monomers into stable nuclei of critical size; the development of nuclei into spherical particles; and the aggregation of particles into branching chains or structural motifs (Fig. 1). (Perry et al., 2003). Silica particles interact with their surroundings, such as the plant cell wall, when they reach a size of 1 to 3 nm (as observed in nature) and have a surface negative charge. Condensation of silica depends on several variables such as temperature, pH, concentration of silicic acid, and presence of other ions, tiny molecules, and polymers (Iler, 1979) nevertheless the materials that shape are always constructed

composed of SiO_4 tetrahedra with varying bond lengths between the Si atoms and the oxygen atoms. Materials are amorphous at the nanometer scale (Mann and Perry, 1986). Silica from various species or precipitation settings may vary substantially in density, hardness, solubility, viscosity, and composition because of the hydroxyl groups they contain and the reaction environment in which the mineral develops (Perry et al., 2003).

There is a lot of variety amongst plant groups in terms of the silica structures created and where they are located. Silica is deposited in a 2.5 mm layer directly under the cuticle layer in the Poaceae, with a Si–cuticle double layer seen in the rice leaf blades. Silica cells, which are found on vascular bundles, and silica bodies, which are found on bulliform cells of rice leaves, are the two main types of silicified structures in the Poaceae family. Cell silicification occurs not only in the leaf blades, but also in the epidermis and vascular tissue of the stem, leaf sheath, and hull (Prychid et al., 2004). As was previously mentioned, the quantity of silica found in various rice plant components varies widely. Multiple studies (Kaufman et al., 1971; Perry and Fraser, 1991; Holzhu ter et al., 2003) have discovered that the silicified structures of *Equisetum* manifest themselves on the epidermal surface of the complete cell wall as distinct knobs and rosettes, which are themselves coated with spicules (Fig. 2). The thickness of this silica surface layer varies depending on where in the plant you look; in the stem, it measures 3–7 mm, while in the leaf, it measures 0.2–1.0 mm. Microstructural studies of plant hairs from the Poaceae (Perry et al., 1984), *Equisetaceae* (Perry and Fraser, 1991), and nettle stinging hairs (Hughes, 1989) revealed similar microstructural forms, including globular, fibrous, and sheet-like structures, with the distribution of these ultrastructural motifs depending on the anatomical region studied. Particle size distribution for certain structural motifs is limited in the silicas generated in biological systems.



FIG. 1. The polymerization of monomeric silicic acid to form larger silica particles proceeds through various condensation reactions with dimers, oligomers and aggregates as intermediates.

Si, if present, co-eluted with phenolic acids, lignin, and carbohydrates in the chromatographic eluates. However, conclusive proof for Inanaga and Okasaka's claim that Si is coupled with the other species present is still lacking. There is a close relationship between the silica phase and the organic matrix, as shown in the silica phase of the early-diverging higher plants *Equisetum telmateia* and *Equisetum arvense* as well as hairs from the lemma of the grass *Phalaris canariensis* (Harrison, 1996; Perry and Keeling-Tucker, 1998, 2003). Different chemical procedures for extracting and analyzing the organic matrix, with varied degrees of success in oxidizing the plant cell wall, showed interesting chemical profiles. The most readily extracted material was high in glycine (20 mol.%), hydroxylated amino acids (25 mol.%), and acidic amino acids (25 mol.%). Extraction number two had a substantial

Proline substantially replaced glycine, but serine and threonine were reduced. High concentrations of lysine,

proline, and aliphatic amino acids were found in a third extract, and the rise in lysine was maintained. It has been postulated that this increasingly fundamental element has a role in controlling the nucleation of silica (Perry and Keeling-Tucker, 1998, 2003). To some extent, the cell-wall extracts' composition is similar to that of the highly cationic proline-rich protein obtained from systemically resistant cucumber plants (Kauss et al., 2003).

Several model systems have been developed to observe the silicification processes in vitro, thanks to research into the mechanisms by which Si polymerization occurs in vivo and the influence of the surrounding environment. Salts of silicates (such as diluted and buffered Na_2SiO_3 ; Coradin and Livage, 2001), salts of Si complexes (such as potassium silicate, sodium silicate, and sodium silicate hydrate), and other compounds may all serve as precursors to silicic acid.

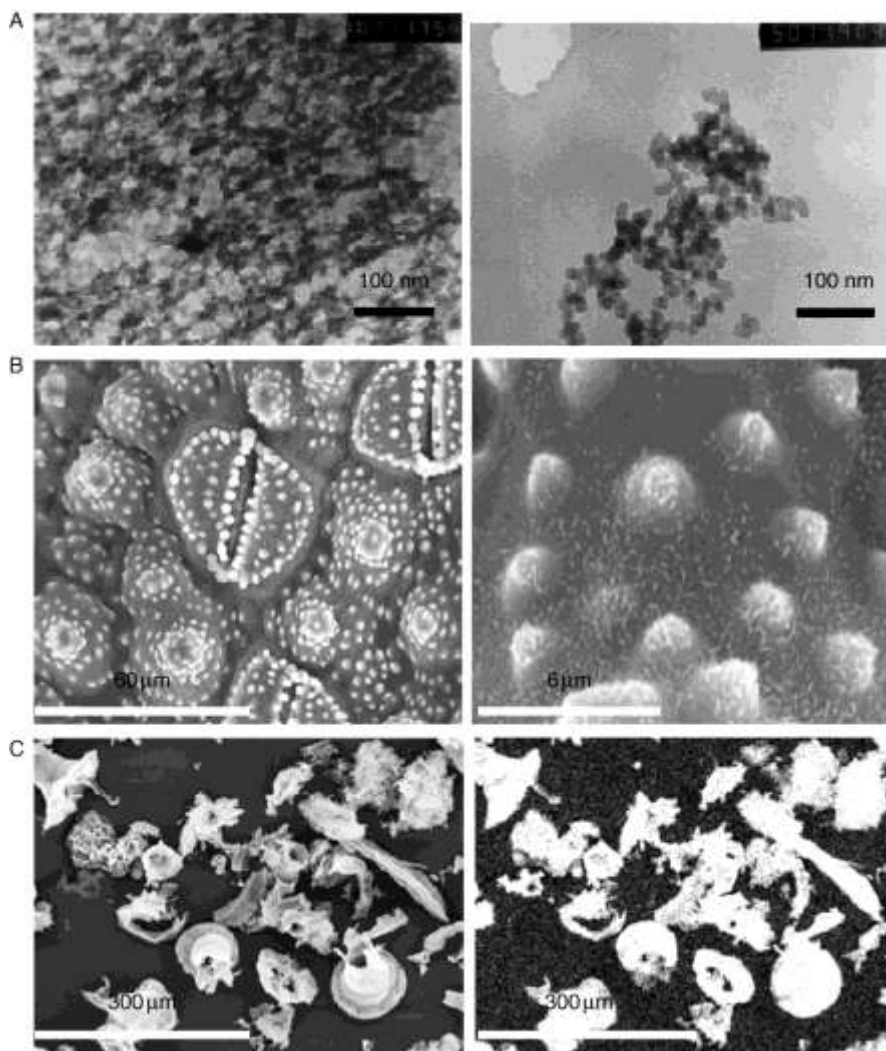


FIG. 2. Electron micrographs of silica structures from plants. (A) Gel-like (left) and globular silica (right) from the early-diverging plant *Equisetum arvense* while the silica structures are yet in their infancy. Mature *E. arvense* stomata (B), encircled by pilules covered in rosettes; the observed specimens contain 0.1% w/w C, with the balance being silica; left, top surface; right, lower surface. Leaf material from *Cucurbita* (marrow) plants that has been acid-digested to remove organic matter (right-hand image shows the Si elemental map).

In chemistry, $K_2[Si(C_6H_4O_2)_3]$ is the formula for silicon catecholate.

xH_2O ; (Perry and Lu, 1992), alkoxyasilanes (such as $Si(OCH_3)_4$ (TMOS) and $Si(OCH_2)_4$ (TEOS)), and alkylsilanes (such as $CH_3Si(OCH_3)_3$; Cha et al., 1999; Zhou et al., 1999). Incomplete breakdown to monomeric silicic acid; the inclusion of methanol, ethanol, or catechol as breakdown products; very rapid kinetics that preclude extensive examination of kinetics (alkoxyasilanes) (silicate solutions). The silicon catecholate complex (solubility up to about 100 mM) can be brought to and maintained at

(A) roughly physiological pH (approx. 7) for long periods of time without the need for additional buffers, and the system exhibits relatively slow reactions, making it possible to easily monitor the earliest stages of the condensation process, particle growth, etc. Si concentrations in solution have been measured using colorimetric techniques [molybdenum yellow and blue (the latter being more sensitive to low amounts)], atomic absorption, and inductively coupled plasma analysis (Iler, 1979; Perry, 2003). Care must be taken in the preparation of the reagent solutions to ensure that the pH is optimized to 'stop' reactions (i.e. pH must be 1 - 2) and that the 'Si' species then measured result from the breakdown of specific Si-containing oligomers only, making colorimetric methods the most appropriate if concentration changes over time are to be monitored (e.g. a 10-min delay between the addition of the two required reagents results in analysis of Si present in monomers or dimers only). Larger oligomers may be degraded by waiting for longer delay periods. Particle sizes, aggregate properties, porosity and surface area characteristics may all be determined by analyzing the produced materials using techniques like dynamic light scattering, gas adsorption, and electron microscopy.

THE EFFECT OF BIOLOGICALLY RELEVANT SOLUTION ADDITIVES

Further understanding of the chemical mechanisms involved in silicification in vivo may be gained by study of the polymerization process in vitro. Examining the chemical interactions between Si and functional groups in the production of well-defined hierarchical silica structures is made possible by using a model system of Si polymerization and the impacts of biomolecules taken from plant systems and chemically "simpler" substances. Cellulose, a significant component of cell walls, has only a little impact on reaction kinetics but does influence particle aggregation into enormous thin sheets, suggesting that there is some interaction between the organic and inorganic phases (Perry and Lu, 1992). The smallest oligosaccharides that could control particle development and aggregation were glucose tetramers (Harrison and Lu, 1994). Though there has been a wealth of research on proteins, peptides, and amino acids, very little has been done with plant protein isolates. The effects of *Equisetum telmateia* isolates and *Phalaris canariensis* plant hairs on yeast growth have been evaluated. These materials were produced by acid breakdown of the cell wall and solubilization of the protein-containing materials with buffered HF treatment. aggregate formation, particle enlargement, and polymerization (Perry and Keeling-Tucker, 1998, 2003). Early on in the reaction, the bio-derived material was shown to accelerate the condensation process, create smaller-diameter particles, and organize into unique silica structures.

Peptides and amino acids have been used in complementary in-vitro research (e.g. Coradin and Livage, 2001; Belton et al., 2004). Using buffered solutions of diluted sodium silicate across a wide pH range, Coradin and Livage investigated the impact of four amino acids (lysine, serine, aspartic acid, and proline) and their homopeptides (approximately 50 amino-acid units for poly-L-lysine, poly L-aspartic acid, and poly-L-proline and approximately 130 amino-acid units for poly-L-serine). The generation of silica is influenced by both the kind of side-chain and the length of the homopeptide. Electrostatic interactions between positively charged amino-acid side chains and negatively charged silica species had the greatest impact for positively charged side-chains. Acceleration of the early-stage reaction kinetics is positively correlated with the amino acids' isoelectric points (pI), and the hydrophobicity of the side-chain is correlated with the surface area of the silica material produced. Rates of oligomer formation and aggregate development, in addition to decreased silica surface area, were observed with addition of L-lysine homopeptides of increasing length (n 1-5) to the model reaction system. Peptides, especially the tiny positively charged ones, are believed to be able to connect particles to create larger structures.

In addition to these macromolecules, plants also contain a wide variety of Polyamines such as spermine, spermidine, and putrescine, which are sometimes found conjugated to

phenolic acids, are studied in model reaction systems. Condensation and particle aggregation were both sped up by the addition of the simple alkyldiamine putrescine (Belton et al., 2005a). Aggregation was shown to be length-dependent for the polyamines spermidine and spermine. These findings were confirmed by analyzing many synthetically manufactured homologues, despite the fact that only two naturally occurring polyamines were studied (Belton et al., 2005b).

FUTURE ASPECTS

To get a complete picture of silica production in vivo, it's important to examine the conditions under which it takes place. Due to their close relationship with the plant's genetic code, cell-wall proteins may be studied with relative ease. Amino acid sequence and structure may be extracted, characterized, and then correlated to proteins with established activities before being used as a starting point for further investigation.

the role of these proteins in the in vitro production of silica. Alternatively, molecular biology techniques like subtractive cDNA libraries or microarrays [as performed by Fauteux et al. (2006) to identify the defense-related genes expressed in *Arabidopsis* following Si treatment] may be used to identify the genes involved for Si polymerization. These molecular techniques should make it easy to get rid of proteins that serve regulatory roles but aren't directly involved in silica synthesis.

Without a clear genetic connection, molecular methods are inefficient for analyzing the more abundant carbohydrate phase. High-pH anion-exchange chromatography, mass spectrometry, and nuclear magnetic resonance (NMR) are some of the tried and true methods for analyzing and quantifying cell-wall polysaccharides. Glycosylated proteins are more challenging to extract and analyze due to the delicate oligosaccharide component, which necessitates the use of non-aggressive methods. As was just discussed, several model systems may be used to examine how isolated biomolecules affect silica polymerization.

Extracting and purifying the biomolecules of interest in the controlled polymerization of silica inside the plant is a time-consuming and labor-intensive operation. To do this, it may be helpful to standardize methods that investigate the plant system as a whole, dissecting the system into its constituent parts and studying them individually. Researchers would be better able to prevent altering the biomolecule from its state in vivo by using specified biochemical approaches for this sort of investigation [for which Fry (1988) is an outstanding source].

In conclusion, it's likely that there are many functions and responsibilities that 'Si' may perform among higher plants that we are unaware of. It is also plausible to assume that if studies of plant development were redone, in which 'Si'

was previously neglected, the element would be shown to be involved in a variety of cellular activities. Improving crop yields and plant resilience to disease might result from a better grasp of how the 'element' influences plant development in many different kinds of higher plants.

reference

The authors of the study were Be'langer RR, Benhamou N, and Menzies JG in 2003. Strong cytological evidence for silicon's involvement in wheat's resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*). *Plant Disease* 93:402-412

D. Belton, G. Paine, S. V. Patwardhan, and C. C. Perry. 2004. Amino acids and lysine oligomers in silicification: a step toward understanding (bio)silicification. Pages 2231–2241 in the *Journal of Materials Chemistry*, volume 14.

Belton, David, S. V. Patwardhan, and C. C. Perry. 2005a. Electrostatic interactions and the hydrophobic effect are the means by which putrescine homologues regulate silica development. *The Journal of Chemical Communications* 27: 3475-3477.

As of 2005b, Belton DJ, Patwardhan SV, and Perry CC. Customized silicas may be made from spermine, spermidine, and its equivalents. In the *Journal of Materials Chemistry*, Volume 15, Pages 4629–4638.

Reference: Casey WH, Kinrade SD, Knight CTG, Rains DW, Epstein E. 2003. Complexes of water-soluble silicates in wheat (*Triticum aestivum* L.). *The plant cell and environment* 27: 49–52.

Morse, DE; Chmelka, BF; Shimizu, K; Christiansen, SC; Zhou, Y; Stucky, GD; Cha, JN; 1999. Polymerization of silica and silicenes may be controlled in vitro by filaments and subunits of silicatein extracted from a marine sponge. This study was published in the *Proceedings of the National Academy of Sciences* 96:361–365.

As of 1969, the work of Chen C-H and Lewin J. *Equisetum arvense* may benefit from silicon as a nutrient. *The Canadian Journal of Botany*, 47, pp. 125–131.

1994: Che'rif M, Asselin A, Be'langer RR. Soluble silicon induces defense responses against *Pythium* spp. in cucumber roots, *Phytopathology* 84: 236–242.

Coradin, T., and Livage, J. The polymerization of silicic acid and the influence of certain amino acids and peptides. This article may be found in *Colloids and Surfaces B: Biointerfaces*, Volume 21, Issue 3, Pages 329–336.

According to Epstein (1994). What makes silicon so unusual in plant biology. *Science, Proceedings of the 91st Meeting*: 11–17.

E. Epstein. 1999. Silicon. *Plant Molecular and Physiological Biology: An Annual Review* 50: 641–664.

Be'langer RR, Menzies JG, Re'mus-Borel W, and Fauteux F. 2005. Protecting plants against fungal pathogens is a key function of silicon. *Journal of Bacteriology* 249:1–6.

In 2006, Fauteux F, Chain F, Belzile F, Menzies JG, and Be'langer RR published a study on the topic. Silicon's protective function in the *Arabidopsis* powdery mildew patho-system. 103, p. 17554–17559 of the *Proceedings of the National Academy of Sciences of the United States of America*.

To cite this article: Fawe A, Abou-Zaid M, Menzies JG, Be'langer RR. 1998. Accumulation of flavonoid phytoalexins in cucumbers through silicon mediation. *Journal of Phytopathology* 1988(8):396-401.

Fry SC. 1988. Chemical and metabolic analysis of the developing plant cell wall. Longman Scientific & Technical; Harlow.

Fry SC. 2004. Methods for tracing the histories of wall polymers in live plant cells; primary cell wall metabolism. 161(9):641-675 in the *New Phytologist*.

The presence of protein inside plant silicas provides evidence for the existence of protein-containing macromolecules, Harrison CC. 1996. 41, *Phytochemistry*, pages 37–42.

Polymer controlled silicification: in vivo and in vitro experiments. (1994) Harrison CC, Lu Y. See pages 151–158 in volume 14 of the *Bulletin of the Institute of Oceanography*.

Research by Hildebrand, Dahlin, and Volcani (1998). Sequences, expression analyses, and the discovery of homologs in other diatoms comprise the characterization of a gene family encoding silicon transporters in *Cylindrotheca fusiformis*. To be found in *Molecular and General Genetics* 260:480-486.

According to Hodson MJ, White PJ, Mead A, and Broadley MR (2005). Different plant families have different silicon ratios. The 96th issue of the *Annals of Botany* covers the years 1027-1046.

Holzhu ter, Gerber, and Narayanan, 2003. *Equisetum arvense* silica structure. This study was published in *Analytical and Bioanalytical Chemistry* 376, pages 512-517.

1989 Hughes NP. Biomineralization processes have been studied in both in vivo and in vitro settings. Theses for a doctoral degree written at Oxford.

R.K. Iler. 1979. Silica's chemical composition. John Wiley & Sons, Inc., New York.

Inanaga, S., and Okasaka, A. 1995. Compounds in the rice shoot cell wall that bind calcium and silicon. Specifically, 103–110, which is the page

range for the journal *Soil Science and Plant Nutrition*.

Ghosheh, N. S., Schmid, R., Bigelow, W. C., and P. B. Kaufman. 1971. Examining silica in the epidermal cells of *Equisetum* using an electron microprobe. This article appears in the 58th issue of the *American Journal of Botany*. Pages 309–316.

Researchers Kauss, Seehaus, Franke, Gilbert, Dietrich, and Kroger published their findings in 2003. Plants with systemic resistance, like cucumbers, produce a silica-depositing protein that is highly cationic and rich in proline. 87–95 in *The Plant Journal*, volume 33.

Kidd P. S., M. Llugany, B. Gunse, and J. Barcelo. 2001. The significance of root exudates in three maize varieties' resistance to aluminum and to silicon-induced amelioration of aluminum toxicity (*Zea mays* L.). 52(9):1339–1352 in the *Journal of Experimental Botany*.

Mechanisms of silicon-mediated relief from abiotic stressors in higher plants: a review. Liang Y, Sun W, Zhu Y-G, Christie P. 2007. *The Journal of Pollution Research* 147:422–428. Ma, J.-F., and N. Yamaji. 2006. Higher plant silicon absorption and accumulation. As seen in *Trends in Plant Science*, volume 11, issue 3, pages 392–397.

Mann, S., and C. C. Perry. 1986. Characteristics of the structure of naturally occurring silica. Symposium 121 of the Ciba Foundation: 40–58. To cite this entry: Mitani, N., and Ma, J.-F. The mechanisms through which various plant species absorb silicon. 56:1255–1256 of the *Journal of Experimental Biology*.

H. R. Moussa. 2006. The effect of silicon fertilization on salt-stressed maize's physiological response (*Zea mays* L.). *The International Journal of Agriculture and Biology* 8(1993):293–297.

Dr. David Neuman and Dr. Ursula zur Nieden. 2001. The ability of higher plants to tolerate silicon and heavy metals. *Journal of Phytochemistry* 56:685–692.

Reference: Perry, CC. 2003. The process by which living things take up and mineralize silica is called silicification. There were reviews in the journal *Reviews in Mineralogy and Geochemistry*, volume 54, pages 291 to 327.

1991. Perry CC, Fraser MA. Ultrastructure and silica deposition in the cell wall of *Equisetum arvense*: the role of cell wall structures and flow regulation in biosilicification. *The Royal Society of London, Philosophical Transactions B* 334, pp. 149–157.

To cite: Perry CC, Keeling-Tucker T. 1998. a crystalline form of silica made by evaporating water from a solution containing bioextracts contained inside silica at room temperature. In

the journal *Chemical Communications*, page numbers 2587 and 2588 may be found in issue 23.

CC Perry & T. Keeling-Tucker 2003 Modeling the precipitation of colloidal silica using *Equisetum telmateia* biosilica extracts. *The Journal of Colloid and Polymer Science* 281(4-5):652–664.

Y. Lu and C. C. Perry. Cellulose's influence on polymerization and aggregation regulation during silica preparation from silicon complexes, *Faraday Trans.* 88, pp.2915–2921.

1984. Perry CC, S. Mann, and R.J.P. Williams. Analysis of the structure and composition of silicified grass macrohairs extracted from the lemma. *Proceedings of the Royal Society of London B* 222:427–438; *Phalaris canariensis* L.

2003. Perry CC, Belton D, and Shafran K. Biosilica research includes looking at their structure, chemical principles, current models, and potential developments. *Journal of Cellular and Molecular Medicine* 33:269–299.

According to Prychid CJ, Rudall PJ, and Gregory M. (2004). Silica bodies in monocotyledons: a systematic and biological study. In: *The Botanical Review*, 69, 3, pp. 377–440. The authors of this study are Rodrigues FA, Benhamou N, Datnoff LE, Jones JB, and Be'langer RR. Silicon-mediated rice blast resistance: ultrastructural and cytochemical features. *Phytopathology* 93:535–546.

2005. Shi, Q., Bao, Z., Z., He, Y., Q., and Yu, J. The antioxidant enzymes superoxide dismutase and ascorbate peroxidase play a role in how silicon reduces Mn toxicity in *Cucumis sativus*. *The Journal of Phytochemistry* 66:1551–1559.

Volcani, B.E., and TL Simpson (eds). 1981. *Cell membranes and other biological structures made of silicon*. Springer, New York.

Sommer, Michael, David Kaczorek, Yakov Kuzyakov, and Jonathan Breuer. 2006. A look at how silicon accumulates and moves through soils and landscapes. Referenced in 169:310–329 of the *Journal of Plant Nutrition and Soil Science*.

It was published in 1990 by Takahashi E, Ma J-F, and Miyake Y. The potential role of silicon as a plant-essential element. Replies to Issues Raised in Volume 2 of the *Journal of Agricultural and Food Chemistry*, Pages 99–122.

S. Yoshida, Y. Ohnishi, and K. Kitagishi. 1962. Silicon Histochemistry III in a Rice Plant. The epidermis has a cuticle and a silica layer underneath it. *Science of the Soil and Nutrition of Plants* 8(1):1–5.

Published in: Van Hoest PJ. Improving the quality of rice straw with the use of silica and

other treatments. *Science and Technology of Animal Feed* 130: 137–171. In 2005, Wang L, Nie Q, Li M, Zhang F, Zhuang J, Yang W, et al. Structures made of biosilica, which are used to cool plant leaves, emit heat in the mid-infrared spectrum quite efficiently. *Journal of the American Physical Society* 87(19): 194105.

Y. Zhou, K. Shimizu, J. N. Cha, G. D. Stucky, and D. E. Morse. 1999. For silicatein a to effectively catalyze the formation of polysiloxanes, it must have very precise hydroxyl and imidazole functions. *Worldwide Edition of Angewandte Chemistry* 38:780-782.

2004. Zhu, Z., Wei, G., Li, J., Qian, & Yu, J. Cucumbers that have been exposed to high levels of salt have their leaf antioxidant enzyme activity reduced, although silica may help (*Cucumis sativus* L.). 167, pp. 527-533, of *Plant Science*.