

## BOTANICAL BRIEFING

# Silica in Plants: Biological, Biochemical and Chemical Studies

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- **Background** The incorporation of silica within the plant cell wall has been well documented by botanists and materials scientists; however, the means by which plants are able to transport silicon and control its polymerization, together with the roles of silica *in situ*, are not fully understood.
- **Recent Progress** Recent studies into the mechanisms by which silicification proceeds have identified the following: an energy-dependent Si transporter; Si as a biologically active element triggering natural defence mechanisms; and the means by which abiotic toxicities are alleviated by silica. A full understanding of silica formation *in vivo* still requires an elucidation of the role played by the environment in which silica formation occurs. Results from *in-vitro* studies of the effects of cell-wall components associated with polymerized silica on mineral formation illustrate the interactions occurring between the biomolecules and silica, and the effects their presence has on the mineralized structures so formed.
- **Scope** This Botanical Briefing describes the uptake, storage and function of Si, and discusses the role biomolecules play when incorporated into model systems of silica polymerization as well as future directions for research in this field.

**Key words:** Silica, biosilicification, stress resistance, silicon transport, silicic acid.

## INTRODUCTION

Silicon (Si) in a chemically combined form is ubiquitous in nature. The Si content of soils can vary dramatically from <1 to 45 % dry weight (Sommer *et al.*, 2006), and its presence in the form of silicic acid [Si(OH)<sub>4</sub>] (or its ionized form, Si(OH)<sub>3</sub>O<sup>-</sup>, which predominates at pH > 9) allows its uptake by plants. Silicic acid is generally found in soils at concentrations ranging from 0.1 to 0.6 mM (Epstein, 1994) but, to our knowledge, there has been no evidence of the occurrence of biosilicification reactions in the soil. Although not traditionally thought of as an element essential to the life cycle of plants, with the exception of the early-diverging Equisetaceae (Chen and Lewin, 1969), certain algae and diatoms, Si is found in plants at concentrations ranging from 0.1 to 10 % (=10<sup>3</sup>–10<sup>5</sup> mg kg<sup>-1</sup>; dry weight basis), an amount equivalent to, or even exceeding, several macronutrients (Epstein, 1994). Plants deprived of Si are often weaker structurally and more prone to abnormalities of growth, development and reproduction and it is the only nutrient which is not detrimental when collected in excess (Epstein, 1999). This Botanical Briefing aims to cover aspects of Si uptake and its role as an alleviator of biotic and abiotic stress, and also to provide insight into how investigations into the surrounding plant cell-wall environment are helping to elucidate the role of chemical influences in the formation of polymeric silica in plants. ['Si' is the symbol for the element silicon and is also used as a generic term when the nature of the silicon compound(s) is not being specified. Si(OH)<sub>4</sub> is silicic acid, more correctly named orthosilicic acid; it is the fundamental building block of silicas and is

itself the simplest silica. Silica is amorphous, hydrated and usually polymerized material produced from Si(OH)<sub>4</sub>.]

## OCCURRENCE AND FORM

The occurrence of Si within the plant is a result of its uptake, in the form of soluble Si(OH)<sub>4</sub> or Si(OH)<sub>3</sub>O<sup>-</sup>, from the soil and its controlled polymerization at a final location. However, the ability of a plant to accumulate Si varies greatly between species (0.1–10 % of shoot dry weight) and extensive analysis of Si uptake in plants has been carried out (e.g. Simpson and Volcani, 1981; Takahashi *et al.*, 1990; Hodson *et al.*, 2005). Si accumulation has been found to a greater extent, but not exclusively, in monocotyledonous plants. Plants of the families Poaceae, Equisetaceae and Cyperaceae show high Si accumulation (>4 % Si), the Cucurbitales, Urticales and Commelinaceae show intermediate Si accumulation (2–4 % Si), while most other species demonstrate little accumulation. Si concentrations of shoots tend to decline in the order liverworts > horsetails > clubmosses > mosses > angiosperms > gymnosperms > ferns (Hodson *et al.*, 2005). Different parts of the same plant can show large differences in Si accumulation, a good example being the variation from 0.5 g kg<sup>-1</sup> in polished rice, 50 g kg<sup>-1</sup> in rice bran, 130 g kg<sup>-1</sup> in rice straw, 230 g kg<sup>-1</sup> in rice hulls to 350 g kg<sup>-1</sup> in rice joints (found at the base of the grain) (Van Hoest, 2006). These concentrations are also in distinct contrast to those found for oat and wheat straw, which are at a levels of tens of g kg<sup>-1</sup>.

On Si uptake into the plant, phytoliths or silica bodies are formed which infill the cell walls and lumina of certain plant cells (Prychid *et al.*, 2004); however, the nature

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of its association with cell-wall components including polysaccharides, lignins and proteins is not fully understood (Perry and Lu, 1992). The cell-wall environment is a highly complex matrix composed of cellulose and more complex polysaccharides such as hemicelluloses and pectins, the latter being rich in galacturonic acid residues cross-linked by the co-ordination of Ca ions (Fry, 2004). This biochemical environment may be capable of modifying the solubility and binding abilities of the silica phase as it develops (Perry and Keeling-Tucker, 1998).

#### ROLE OF SILICA IN STRESS RELIEF

The presence of Si in plants has been found to alleviate many abiotic and biotic stresses, leading to the incorporation of silicates into many fertilizers. How Si is able to exert such a protective effect has yet to be fully elucidated although roles including providing a physical and/or biochemical defence system have been proposed. The deposition of silica as a physical barrier to penetration and reduction in the susceptibility to enzymatic degradation by fungal pathogens has been examined (Yoshida *et al.*, 1962). However, there is debate as to whether this increased strength is sufficient to explain the protective effects observed (Fauteux *et al.*, 2005). An alternative explanation for silicon's protective role is as a biologically active element capable of triggering a broad range of natural defences. This was first demonstrated in cucumbers: Si-treated plants demonstrated enhanced activity of chitinases, peroxidases, polyphenol oxidases and flavonoid phytoalexins, all of which may protect against fungal pathogens (Chérif *et al.*, 1994; Fawe *et al.*, 1998). Further investigation of these defence mechanisms by Bélanger *et al.* (2003) and Rodrigues *et al.* (2003), studying wheat and rice blast, respectively, indicated that these species were also capable of inducing similar biologically active defence agents, including increased production of glycosylated phenolics and antimicrobial products such as diterpenoid phytoalexins in the presence of silica. Experiments performed on cucumber leaves following fungal infection showed that further resistance to infection is acquired by expression of a proline-rich protein together with the presence of silica at the site of attempted penetration (Kauss *et al.*, 2003). The C-terminus of this protein contained a high density of lysine and arginine residues proposed to catalyse the localized deposition of silica at the site of vulnerability.

In addition, metal toxicity, salinity, drought and temperature stresses can be alleviated by Si application (Liang *et al.*, 2007) and the means by which Si exerts these protective effects is still under investigation. Metal toxicities including Mn, Cd, Al and Zn have been studied and the proposed mechanisms for the action of Si include the accumulation of Zn as a silicate (Neuman and zur Nieden, 2001); reduction of lipid peroxidation and increased enzymatic (e.g. superoxide dismutase; SOD) and non-enzymatic antioxidants (e.g. ascorbate) against Mn toxicity (Shi *et al.*, 2005); and increased release of phenolics with strong chelating ability for Al tolerance (Kidd *et al.*, 2001). Drought tolerance brought about by the application of 'Si' may

result from decreased transpiration (Epstein, 1999) and the presence of silicified structures in plants suggested a reduction of leaf heat-load, providing an effective cooling mechanism and thereby improving plant tolerance to high temperatures (Wang *et al.*, 2005). The resistance to salt stress has been found to be due to the enhancement of enzymes such as SOD and catalase, preventing membrane oxidative damage (Zhu *et al.*, 2004; Moussa, 2006).

#### TRANSPORT

Plants differ in their abilities to accumulate silica and initially Takahashi *et al.* (1990) proposed three methods of uptake: active, passive and rejective. Further examination of Si uptake in several different plant species has resulted in different modes of silicic acid uptake and transport being elucidated. Ma and colleagues have used mutants defective in silicic acid uptake to examine the means by which silicic acid is accumulated in rice, a known Si accumulator.

The uptake of Si from solution by rice was found to be the result of two different transport mechanisms. A low-affinity transporter (*Lsi1*) found on the lateral roots is responsible for the uptake of silicic acid from the external solution to the root cortical cells (Ma and Yamaji, 2006). The gene responsible, *Lsi1*, has been mapped to chromosome 2, contains five exons and four introns, and encodes a 298-amino-acid protein. The predicted membrane protein has high homology with the aquaporins, including six transmembrane domains and two Asn-Pro-Ala (NPA) motifs. In particular, alanine 132 was found to be a critical residue as substitution resulted in altered conformation of the protein. The expression of *Lsi1* is constitutive and occurs predominantly in the roots; however, it is regulated by silicic acid availability with constant silicic acid supply resulting in decreased expression. The transporter has been localized on the distal cells of exodermis and endodermis, and injection of the cDNA encoding *Lsi1* into *Xenopus laevis* oocytes resulted in increased silicic acid transport (Ma and Yamaji, 2006). No homology of the *Lsi1* gene from rice has been found with a gene family responsible for silicic acid transport in the marine diatom *Cylindrotheca fusiformis* (Hildebrand *et al.*, 1998). Other plants investigated for their uptake of silicic acid are cucumber and tomato, moderate and low-level accumulators, respectively. For both species the uptake of silicic acid from the environment was found to be mediated by a transporter with the same affinity for silicic acid as *Lsi1*, but differences in transporter density on the lateral roots were detected, accounting for the variation in Si uptake. A second transporter has also been identified in rice which is responsible for xylem loading of Si, but in cucumber and tomato plants xylem loading occurs by passive diffusion (Mitani and Ma, 2005).

An elegant investigation of silicic acid uptake in wheat has been performed (Casey *et al.*, 2003) using solution nuclear magnetic resonance (NMR) techniques to identify the molecular species containing Si. Developing seedlings were provided with an aqueous growth medium that included 98.7 at. %  $^{29}\text{Si}$  and the xylem exudate collected after excision of the shoots was measured for soluble

Si by using a colorimetric method; the precise species were identified by solution state  $^{29}\text{Si}$ -NMR spectroscopy. The maximum concentration of dissolved silicate recorded in the 90-min sampling period was 8 mM, well above the solubility limit of silicic acid. However, NMR studies of the earliest exudates only detected monomeric and dimeric silicic acid although the concentrations detected by the two methods varied somewhat. It is a great pity that the later exudates were not examined by  $^{29}\text{Si}$ -NMR spectroscopy (nor by  $^1\text{H}$ - or  $^{13}\text{C}$ -NMR spectroscopy, which would have indicated whether organic components were present).

### BIOGENIC SILICA STRUCTURES: MOLECULES TO MATERIALS

Plants, diatoms and sponges are capable of accumulating, storing and processing Si to create ornate hierarchical patterned biosilicas. The production of silica by organisms is carried out in an aqueous environment from under-saturated solutions of silicic acid, at atmospheric pressure and with temperatures ranging between 4 and 40 °C, and this production, amounting to gigatons per annum, vastly outweighs that produced industrially. The methods by which this can be achieved in organisms have been the subject of immense study and debate; however, it is apparent that the organic environment, including a vast range of proteins, carbohydrates, lipids, metal ions and (in plants) phenolic compounds, is likely to play a fundamental role (Perry and Lu, 1992; Harrison, 1996). The simplest form of silica is the monomer orthosilicic acid, a weakly acidic molecule ( $\text{pK}_a$  9.8) composed of Si tetrahedrally co-ordinated to four hydroxyl groups (Iler, 1979) that is found ubiquitously in soil at low concentrations (a few  $\text{mg kg}^{-1}$ ). It is in this form that plants are able to take up Si via the processes described above. Where the concentration is in excess of 100–200  $\text{mg kg}^{-1}$ , polycondensation reactions lead to: polymerization of monomers to form stable nuclei with critical size; growth of nuclei to form spherical particles; and aggregation of particles to form branched chains or structural motifs (Fig. 1) (Perry *et al.*, 2003). As the silica particles grow and approach 1–3 nm in size (as found in nature), they carry a surface negative charge and these particles then interact with the local environment such as the plant cell wall. Many different factors affect the process of silica condensation, including the concentration of silicic acid, the temperature, the pH, and the presence of other ions, small molecules and polymers (Iler, 1979) but in all cases the materials that form are built up

from  $\text{SiO}_4$  tetrahedra with variable Si–O–Si bond angles and Si–O bond distances. The materials are amorphous at the 1-nm length scale (Mann and Perry, 1986). They contain hydroxyl groups and, according to the reaction environment in which the mineral forms, silica from different organisms or precipitation conditions can vary greatly with regard to density, hardness, solubility, viscosity and composition (Perry *et al.*, 2003).

The silica structures formed and their localization show great variation between individual plant families. In the Poaceae, silica is deposited as a 2.5- $\mu\text{m}$  layer immediately beneath the cuticle layer with an Si–cuticle double layer being found in the leaf blades of rice. The silicified structures in the Poaceae can be subdivided into silica cells, dumb-bell-shaped cells located on vascular bundles and silica bodies found on bulliform cells of rice leaves. However, the silicification of cells is not restricted to the leaf blades and silicified cells are also found within the epidermis and vascular tissue of the stem, leaf sheath and hull (Prychid *et al.*, 2004). As already discussed, the amount of silica present in the different parts of the rice plant is very variable. The silicified structures of *Equisetum*, examined by a number of researchers (Kaufman *et al.*, 1971; Perry and Fraser, 1991; Holzhüter *et al.*, 2003), are found on the epidermal surface of the entire cell wall primarily as discrete knobs and rosettes, which are themselves covered in spicules (Fig. 2). The thickness of this silica surface layer is dependent upon the location within the plant: thicknesses of 3–7  $\mu\text{m}$  and 0.2–1.0  $\mu\text{m}$  are observed in the stem and leaf, respectively. Characterization of the silica ultrastructures found in plant hairs from an example of 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 being dependent on the anatomical region studied. The silicas formed in biological systems show a narrow particle size distribution for specific structural motifs.

### 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 sulphoxide) subjected to

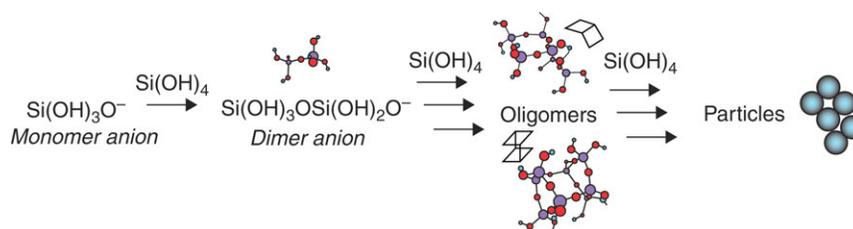


FIG. 1. The polymerization of monomeric silicic acid to form larger silica particles proceeds through various condensation reactions with dimers, oligomers and aggregates as intermediates.

chromatography produced eluates in which any Si present co-eluted with phenolic acids, lignin and carbohydrate in the void volume. Inanaga and Okasaka proposed that Si is combined in some way with the other species present although definitive evidence is yet to be provided. Examination of the silica phase of the early-diverging higher plants *Equisetum telmateia* and *Equisetum arvense* along with hairs from the lemma of the grass *Phalaris canariensis* has revealed an intimate association between the silica phase and the organic matrix (Harrison, 1996; Perry and Keeling-Tucker, 1998, 2003). Extraction and examination of the organic matrix by chemical methods varying in their ability to oxidize the plant cell wall revealed distinctive chemical compositions. The most easily extracted material was rich in hydroxylated amino acids (25 mol. %), acidic amino acids (25 mol. %) and glycine (20 mol. %). The second extract contained much

lower levels of serine and threonine, higher levels of lysine, and glycine was largely replaced by proline. The increase in lysine continued in the third extract and high levels of proline and aliphatic amino acids were also observed. This increasingly basic material has been speculated to be involved in the regulation of silica nucleation (Perry and Keeling-Tucker, 1998, 2003). The composition of the cell-wall extracts in some ways mirrors that of the strongly cationic proline-rich protein isolated from systemically resistant cucumber plants (Kauss *et al.*, 2003).

Investigation into the modes by which Si polymerization proceeds *in vivo* and the effects of the surrounding environment has led to the development of several laboratory-based model systems to monitor the processes of silicification *in vitro*. A variety of silicic acid precursors, including salts of silicates (e.g. diluted and buffered  $\text{Na}_2\text{SiO}_3$ ; Coradin and Livage, 2001), salts of Si complexes (e.g. potassium

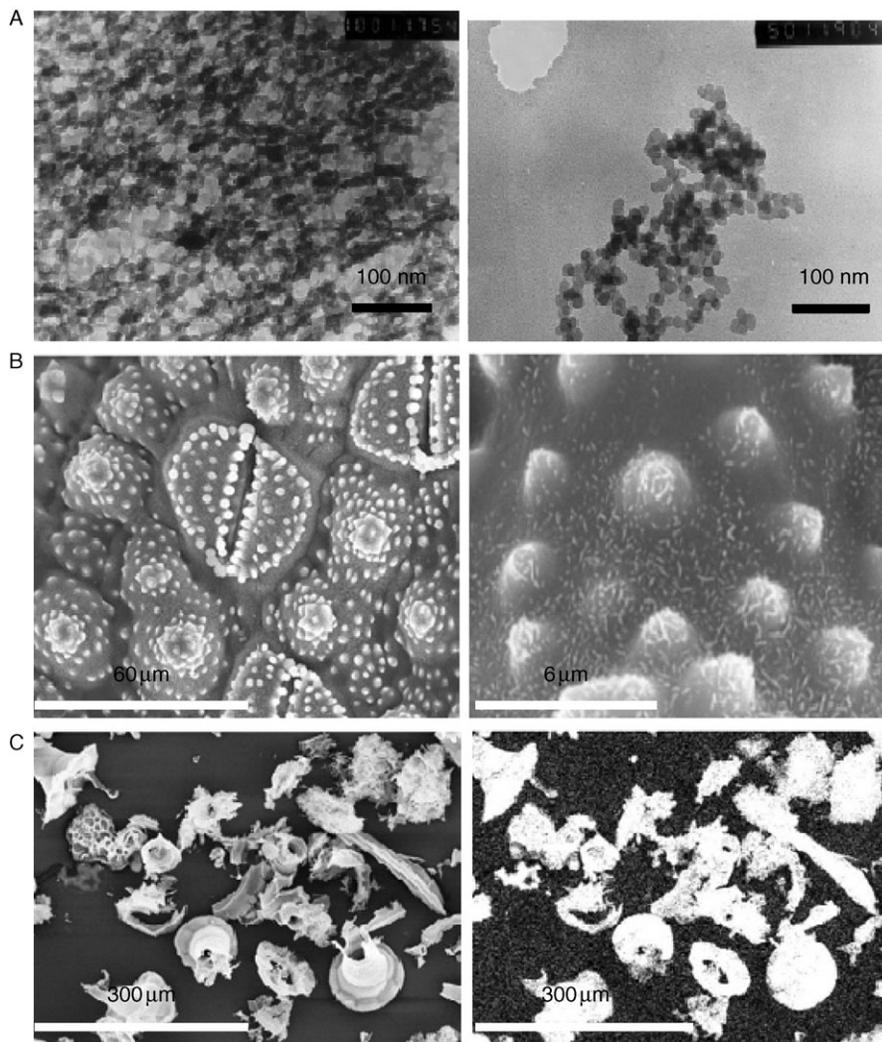


FIG. 2. Electron micrographs of silica structures from plants. (A) Gel-like (left) and globular silica (right) from the early-diverging plant *Equisetum arvense* at very early stages of development of the silica structures. (B) Stomata of mature *E. arvense* surrounded by pilulae encrusted with rosettes; the observed specimens contain 0.1% w/w C, with the remainder being silica; left, upper surface; right, lower surface. (C) Inorganic material from acid-digested *Cucurbita* (marrow) leaves (right-hand image shows the Si elemental map).

silicon catecholate,  $K_2[Si(C_6H_4O_2)_3] \cdot xH_2O$ ; (Perry and Lu, 1992), alkoxy silanes (e.g.  $Si(OCH_3)_4$  (TMOS) and  $Si(OC_2H_5)_4$  (TEOS)) and alkylsilanes (e.g.  $CH_3Si(OCH_3)_3$ ; Cha *et al.*, 1999; Zhou *et al.*, 1999), have been examined. All silicic acid precursor systems have their disadvantages: the presence of methanol, ethanol or catechol as breakdown products; very fast kinetics preventing detailed analysis of kinetics (alkoxy silanes); or incomplete breakdown to monomeric silicic acid (silicate solutions). The silicon catecholate complex (solubility up to about 100 mM) can be taken to and maintained at roughly physiological pH (approx. 7) for long periods of time without the need for additional buffers and the system exhibits relatively slow reactions thereby allowing the early stages of the condensation process as well as particle growth, etc., to be readily monitored. Colorimetric methods [molybdenum yellow and blue (the latter being more sensitive to low concentrations)], atomic absorption and inductively coupled plasma analysis are all methods that have been used to measure Si concentrations in solution (Iler, 1979; Perry, 2003). The colorimetric methods are most appropriate if changes in concentration with time are to be monitored but 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 (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). Longer delay times allow larger oligomers to break down. Analysis of the materials formed is possible by a number of methods, including dynamic light scattering, gas adsorption and electron microscopy in order to determine particle sizes, aggregate characteristics, and porosity and surface area characteristics.

#### THE EFFECT OF BIOLOGICALLY RELEVANT SOLUTION ADDITIVES

Investigating the polymerization process *in vitro* allows the further elucidation of chemical processes involved in silicification *in vivo*. The effects of biomolecules extracted from plant systems and chemically 'simpler' compounds within a model system of Si polymerization allow the examination of chemical interactions between Si and functional groups in the formation of well-defined hierarchical silica structures. Cellulose, although a major cell-wall polymer, has little effect on reaction kinetics but affects the aggregation of particles into large thin sheets, indicating some degree of interaction between the organic and inorganic phases (Perry and Lu, 1992). Tetramers of glucose were the smallest oligosaccharides able to regulate particle growth and aggregation (Harrison and Lu, 1994). Studies on proteins, peptides and amino acids have been more extensive although few experiments have been conducted using protein-containing isolates from plants. Isolates from *Equisetum telmateia* and plant hairs from *Phalaris canariensis* obtained by acid digestion of the cell wall followed by solubilization of the protein-containing materials by buffered HF treatment have been assessed for their effects on

polymerization, particle growth and aggregation (Perry and Keeling-Tucker, 1998, 2003). Acceleration of the condensation process, the formation of smaller-diameter particles and organization into unusual silica structures at early stages of reaction were observed, indicating that the bio-derived material has an influence early in the silica formation process.

Complementary *in-vitro* studies using peptides and amino acids have also been performed (e.g. Coradin and Livage, 2001; Belton *et al.*, 2004). Coradin and Livage studied the effect of four amino acids (lysine, serine, aspartic acid and proline) and their homopeptides (approx. 50 amino-acid units for poly-L-lysine, poly L-aspartic acid and poly-L-proline and approx. 130 amino-acid units for poly-L-serine) on silica formation using buffered solutions of diluted sodium silicate over a range of pH values; Belton *et al.* (2004) explored the effect of 11 amino acids (lysine, arginine, asparagine, glutamine, threonine, serine, tyrosine, glycine, alanine, glutamic acid and proline) together with homopeptides of glycine (1–5 amino-acid units) and lysine (1–5 amino-acids units) on silica formation from solutions containing monomeric silicic acid derived from an Si-catecholate complex. The nature of the side-chain and the length of homopeptide both affect the silica formation process. Positively charged side-chains showed the largest effect via electrostatic interactions between positively charged amino-acid side chains and negatively charged silica species. The isoelectric point (pI) of the amino acids correlates positively with acceleration of the early-stage reaction kinetics, whereas the hydrophobicity of the side-chain correlates with the surface area of the silica material formed. The addition of L-lysine homopeptides of increasing length ( $n=1-5$ ) to the model reaction system showed increases in rates of oligomer formation and aggregate growth, together with reduced silica surface area as  $n$  increased. The small positively charged peptides are thought to be able to bridge particles to produce extended structures.

Other macromolecules found in plants that have been examined in model reaction systems are the polyamines spermine, spermidine and putrescine, which are sometimes found conjugated to phenolic acids. Putrescine, a simple alkyldiamine, was able to increase the rate of condensation and the rate of particle aggregation (Belton *et al.*, 2005a). The longer-chain polyamines, spermidine and spermine, influenced aggregation according to molecule length. Although only two natural polyamines were examined, these conclusions were verified by the analysis of a number of synthetically produced homologues (Belton *et al.*, 2005b).

#### FUTURE ASPECTS

In order fully to understand silica formation *in vivo*, it is necessary to study the environment in which silica formation occurs. Cell-wall proteins are most easily examined owing to their direct link to the plant genetic code. One approach includes extraction and characterization of amino-acid sequence and structure; correlation to proteins with defined functions, if known; followed by exploration

of these proteins in the formation of silica *in vitro*. An alternative approach is to determine the genes responsible for Si polymerization by use of molecular biology techniques such as subtractive cDNA libraries or microarrays [as carried out by Fauteux *et al.* (2006) to determine the defence-related genes expressed in Arabidopsis after treatment with Si]. The use of such molecular approaches should allow the rapid elimination of proteins involved in general regulatory functions rather than silica formation.

Analysis of the more abundant carbohydrate phase is more difficult because of the lack of a direct genetic link, making molecular techniques ineffective. The only current approach is the careful extraction of cell-wall polysaccharides with their analysis and quantification being performed by well-established techniques such as high-pH anion-exchange chromatography, mass spectroscopy and NMR. The extraction and analysis of glycosylated proteins is more difficult because of the sensitive nature of the oligosaccharide component and requires the use of non-aggressive techniques. The effect of the isolated biomolecules on silica polymerization can be tested in a range of model systems as described above.

Examination of the biomolecules involved in the controlled polymerization of silica within the plant is a complex process requiring their careful extraction and purification. This may best be achieved through standardization of techniques designed to look at the plant system as a whole, allowing each different component to be isolated and examined in its natural state. The use of defined biochemical techniques for this type of study [for which Fry (1988) is an excellent source] would help researchers to avoid methods of extraction and purification that result in the alteration of the biomolecule from its state *in vivo*.

In summary, we probably do not know all the functions and roles that 'Si' may play within higher plants. It is also reasonable to suggest that if plant growth studies where 'Si' has previously been ignored were revisited it is likely that the element in some form will be found to be involved in a range of cellular processes. Understanding of the effect of the 'element' on plant growth may lead to improved yields and resistance to disease for a wide range of higher plants.

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