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Silica deposition in plants: scaffolding the mineralization

Nerya Zexer[1,#,*], Santosh Kumar[2,3,#,*] and Rivka Elbaum[4,*]

[1]Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA, [2]Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, K. K. Birla Goa Campus, Zuarinagar 403726, Goa, India, [3]School of Biotechnology, Kalinga Institute of Industrial Technology, Bhubaneswar 751024, Odisha, India and [4]R. H. Smith Institute of Plant Sciences and Genetics in Agriculture, Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot 7610001, Israel

**For correspondence. E-mail neryazexer@gmail.com, santosh.kumar@kiitbiotech.ac.in or rivka.elbaum@mail.huji.ac.il*

[#]Equal contribution

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• **Background** Silicon and aluminium oxides make the bulk of agricultural soils. Plants absorb dissolved silicon as silicic acid into their bodies through their roots. The silicic acid moves with transpiration to target tissues in the plant body, where it polymerizes into biogenic silica. Mostly, the mineral forms on a matrix of cell wall polymers to create a composite material. Historically, silica deposition (silicification) was supposed to occur once water evaporated from the plant surface, leaving behind an increased concentration of silicic acid within plant tissues. However, recent publications indicate that certain cell wall polymers and proteins initiate and control the extent of plant silicification.
• **Scope** Here we review recent publications on the polymers that scaffold the formation of biogenic plant silica, and propose a paradigm shift from spontaneous polymerization of silicic acid to dedicated active metabolic processes that control both the location and the extent of the mineralization.
• **Conclusion** Protein activity concentrates silicic acid beyond its saturation level. Polymeric structures at the cell wall stabilize the supersaturated silicic acid and allow its flow with the transpiration stream, or bind it and allow its initial condensation. Silica nucleation and further polymerization are enabled on a polymeric scaffold, which is embedded within the mineral. Deposition is terminated once free silicic acid is consumed or the chemical moieties for its binding are saturated.

Key words: Cell wall, lignin, phytoliths, silica, silicic acid, Siliplant1.

INTRODUCTION

Silicon (Si) in the form of silicates and silicic acid is widely abundant in soils and thus plant roots are continuously exposed to the mineral. Silicon uptake from soil has been investigated in different plant species, mainly from the Poaceae and Cucurbitaceae families, which are considered high accumulators of Si (Hodson *et al*., 2005). Silicon uptake by roots is possible in its dissolved form as monosilicic acid ($Si(OH)_4$) (Casey *et al*., 2004; Ma *et al*., 2006; Mitani *et al*., 2008). Two types of Si transporter, named low silicon, were found in plants. The first type (Lsi1, Lsi6) encodes an aquaporin, which allows passive diffusion of silicic acid across the plasma membrane and functions as an influx transporter (Mitani *et al*., 2008). The second type (Lsi2, Lsi3) is an H^+ antiporter, exporting silicic acid from cells (Ma and Yamaji, 2015). The corresponding functions of the Lsi1 channel and Lsi2 H^+ antiporter and their arrangement in root tissues facilitate the uptake of silicic acid from the soil solution (Sakurai *et al*., 2015). Although this is the established conception of Si uptake in plants, some researchers challenge the notion of active Si uptake and view the passage of silicic acid molecules through Lsi proteins as incidental, arguing that these proteins are not Si-specific (Exley *et al*., 2020).

Silicon uptake, transport and the presence of Si-selection sites in the plant body can be elucidated by Si isotope fractionation study. Three points of metabolic interference affect the Si isotope fractionation signature in banana, namely, the entry to the root at the root endodermis, xylem loading and xylem unloading (Opfergelt *et al*., 2006). The Si-transport system in rice includes Si transporters in three similar locations (Ma, 2010; Yamaji *et al*., 2015): absorption into the exodermis and endodermis symplast via Lsi1; unloading the endodermis cells and transfer to the apoplastic volume of the xylem via Lsi2; and exiting the leaf xylem via Lsi6, and finally unloading to the apoplastic parenchyma volume at node I, leading to the panicle and flag leaf via specialized localization of Lsi6, Lsi2 and Lsi3 at the node vascular bundles (Yamaji *et al*., 2015). This arrangement of passive and active transport over a hydrophobic apoplastic barrier effectively concentrates silicic acid above its saturation concentration (Sakurai *et al*., 2015). The Si transport model in rice is partially conserved in other grasses, as well as in squashes (Mitani *et al*., 2011).

The distribution of Si to the mesophyll and epidermis is not well understood in any species. After unloading, silicic acid is assumed to move passively with water, and to polymerize to solid hydrated silica as a result of the water loss during evapotranspiration. Silica deposits mainly as part of cell walls in the

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shoot, as a silicified layer beneath the cuticle, in specialized cells of the epidermis (most commonly in silica cells, papillae and silica hairs of the culms, leaves and inflorescence bracts of grasses) and in root endodermis (Yoshida *et al*., 1962; Parry and Smithson, 1964; Sangster *et al*., 2001; Kumar *et al*., 2017*b*; Hodson, 2019). This is achieved by the condensation of silicic acid molecules to form a hydrated biosilica mineral ($SiO_2 \cdot nH_2O$), also called biogenic opal. In two cases we showed that silicic acid polymerizes into silica even without transpiration, i.e. in sorghum epidermal silica cells and root endodermis, forming silica aggregates (Kumar *et al*., 2017*a*; Soukup *et al*., 2020).

In this review, we will first discuss the distribution of soluble Si in the plant apoplast and symplast. We argue that silicic acid is mostly localized at the apoplast, where it polymerizes into biogenic silica. We further review the current knowledge on plant scaffolds, whether proteinaceous or polysaccharidic in nature, enhancing silica deposition. We discuss the possible chemical environment in cell walls and varied biogenic polymers that induce silica polymerization *in vitro* and may also act *in vivo*. Published evidence demonstrates unequivocally the existence of specialized proteins that induce silicification. In addition, hemicellulose, callose and lignin carry hydroxyl groups that may stabilize silicic acid. Our model suggests that the cell wall polymers must be modified by positively charged chemical moieties in order to induce silicic acid condensation and silica sedimentation at predefined locations.

SYMPLASTIC AND APOPLASTIC SILICON

The uptake from the soil solution involves two transporters (Lsi1 and Lsi2) that allow silicic acid into the symplast of root cortex and endodermis (Mitani-Ueno and Ma, 2021). Silicon is detected in vacuoles of wheat endodermis (Hodson and Sangster, 1989). Rice, cucumber and tomato roots concentrate symplastic Si from the growth solution (Mitani and Ma, 2005). Root symplastic silicic acid is mostly transported to the stele and can be mapped to xylem vessels in freeze-substituted wheat roots (Hodson and Sangster, 1989). The majority of silicic acid moves with the apoplastic water transpiration stream to the shoot. Some silica is associated with starch grains in potato tubers (Khalil and Duncan, 1981) and maize cell culture (Nissan *et al*., 2019), indicating its symplastic location. Silica binds to starch possibly through the silanol groups of silicic acid connecting to hydroxyl groups of the starch sugar units. However, the bonds are weak as Si is washed out when maize cells grown in high-Si medium are transferred to low-Si medium (Nissan *et al*., 2019). Silicic acid may be taken up by cells via endocytosis. Vesicles containing Si were detected by energy-dispersive X-ray spectroscopy in leaves of tobacco and *Arabidopsis* (Neumann and Figueiredo, 2002) and in maize cell culture (Nissan *et al*., 2019). Silica is deposited in the lumen of a few plant cell types (Hodson, 2016; Kumar *et al*., 2017*b*). The lumen silicification may happen after cell death, in which case the mineral possibly traps degraded cellular constituents (Hodson, 2019). Nonetheless, Si deposits were identified only outside and not within the protoplast of *Adiantum raddianum*, in the outer layer of the primary cell wall (Leroux *et al*., 2013) and specific cell wall layers in stomata of sugarcane (Sakai and Thom, 1979). In orchard grass (*Dactylis glomerata*), silica accumulates in the interface between the cytoplasm and cell wall while the protoplast is devoid of solid silica (Laue *et al*., 2006). Similarly in sorghum silica cells, silicification occurs at the interface between the cell membrane and primary wall, and is tightly controlled by a viable protoplast (Kumar *et al*., 2017*a*; Kumar and Elbaum, 2018).

The association of silicic acid movement in plants with the global transpiration stream and the correlation between organ silicification and its transpiration indicate that most of the silicic acid is present in the apoplast (Jones and Handreck, 1967; Rosen and Weiner, 1994; Euliss *et al*., 2005). In accordance with this, a large part of silica deposition occurs in the cell walls (Davis, 1987). Such mass transport with no apparent membrane partitioning should result in deposits that are not well controlled. Indeed, silica depositions form at the surface of plants, as part of the cuticle and distal (outer) epidermis cell walls (Yoshida *et al*., 1962; Kim *et al*., 2002; Pierantoni *et al*., 2017; Vandegeer *et al*., 2021) and cast into micro-morphologies that characterize cell walls (Jones *et al*., 1963). As a result of the intimate contact between silica and cell walls, their digestibility as animal feed is reduced (Van Soest, 2006).

From the examples cited above, it is quite clear that Si in plants can exist in both the symplast and the apoplast; however, most of the Si exists in the apoplastic solution and its concentration in the apoplast of grasses may exceed its solubility level (Casey *et al*., 2004; Mitani *et al*., 2005; Soukup *et al*., 2020). A persisting question is how the silicic acid stays in solution and does not form catastrophic deposits that plug the xylem. The excessive concentration of silicic acid in the sap is transient, and when extracted out of the plant body it is reduced to a solubility level of around 2.0 mM within hours (Mitani *et al*., 2005). This suggests an *in planta* mechanism that stabilizes monosilicic acid. It was suggested that the high negative pressures in the xylem may act to suppress silicic acid condensation (Exley, 2015). Likewise, hydroxyl moieties in the cell walls may bind to silicic acid silanols and increase the saturation solubility of silicic acid (Preari *et al*., 2014). An interesting finding in this context is the ectopic deposition of silica in the mesophyll cell walls of a rice mutant that is defective in hemicellulose synthesis (Kido *et al*., 2015), suggesting a role for hemicellulose in stabilizing silicic acid in the apoplastic sap of the wild type rice leaves.

SILICA IN THE CELL WALL

Silica is complexed into the cell wall, and may mineralize the middle lamella of epidermis cells as well as several hypodermal cell walls (Davis, 1987; Peleg *et al*., 2010). Nonetheless, at least in some species, silica deposits are abundant in specific cells, such as papillae, hairs and silica cells. In these epidermal features, silica deposits earlier in the lifetime of a leaf (Hughes *et al*., 1988; Motomura *et al*., 2004, 2006; Kumar *et al*., 2017*a*). In *Arabidopsis*, the base of trichomes is silicified (Brugiére and Exley, 2017; Kulich *et al*., 2018). We identify accumulations of silica in leaf trichomes of tomato (Fig. 1). This demonstrates that even in Si non-accumulators, such as tomato and *Arabidopsis*, some silicic acid is taken up and directed to specific epidermal structures. The mineral may localize to the

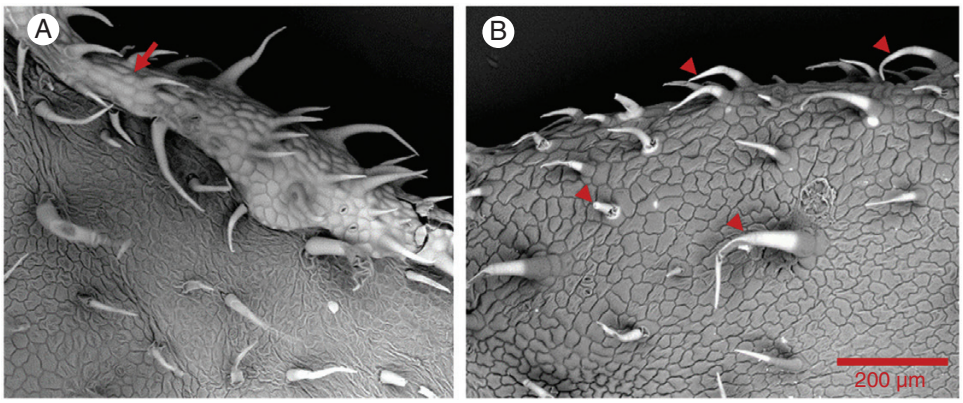

Fig. 1. Silica deposited in tomato, which is a Si non-accumulator. Scanning back-scattered electron micrograph showing in high brightness features containing high concentrations of silicon. Leaf margin (A; indicated by arrow) and epidermal trichomes (B; indicated by arrowheads) are mineralized by silica (courtesy of G. Haint).

base or tip of the hair cell wall (Mustafa *et al.*, 2018; Ensikat and Weigend, 2021), indicating a control over silica deposition within the cell wall.

A possible way to direct the mineralization to these specialized loci is the presence of a chemical scaffold with high affinity to silicic acid that would incorporate the polymerizing silica. This would result in a composite material, made of the mineral and the scaffolding entities. Another strategy would be nucleating mineralization by catalysis of the condensation reaction of two Si-OH moieties that would further expand to form silica (Fig. 2). In the process of biomineralization, the organic molecules acting as scaffold are trapped, and eventually integrate into the forming biomineral. This integration changes the properties of the inorganic mineral precursor and possibly helps the mineral in its biological function (Addadi and Weiner, 2014). In any case, the specific binding and polymerization of silicic acid would lower its local concentration, creating a localized silicic acid sink and leading to its mass transport to the deposition site.

MOIETIES POLYMERIZING SILICA

What could serve as a scaffold or nucleation for silica formation? Since a large part of silica in plants is deposited in the cell wall, cell wall polymers are the obvious suspects for facilitating silicic acid condensation and polymerization. Monosilicic acid in solutions above saturation spontaneously reacts to form oligomeric silicic acid, preferentially cyclic species. These oligomers serve as stable nuclei that grow to form nano-colloids at the expense of smaller oligomers. Further aggregation of the colloids may result in sedimentation of silica or silica gel, depending on pH and salts leading to a low or high fraction of water and silanol groups, respectively (Iler, 1979; Icopini *et al.*, 2005). However, in concentrations that are only a few times the saturation concentration, the first stage of oligomerization may take hours and longer (Iler, 1979). This stability is suggested to increase with the availability of protons, blocking the reactive Si-O$^-$ groups (Skordalou *et al.*, 2020; Icopini *et al.*, 2005). Similarly, hydroxyl and other H-bond-forming residues may stabilize super-saturation, by binding hydroxyl groups on the soluble mono- and disilicic acid molecules. The stabilization is

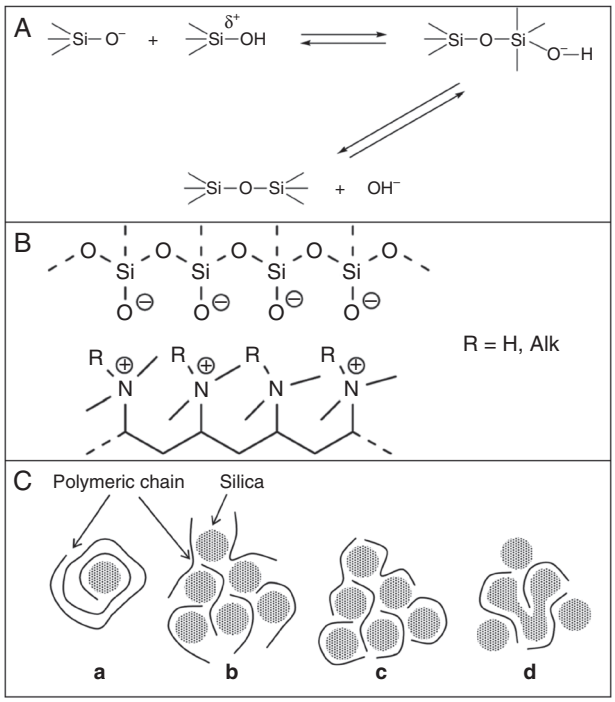

Fig. 2. Silica formation from silicic acid solution. (A) Nucleophilic attack of an ionized silanol (siloxyl) on a silicic acid molecule resulting in disilicic acid. The reaction is slow under neutral pH even under supersaturation. The rate of similar condensation reactions is increased in polysilicic acid, which stabilizes the siloxyl group. Further condensation leads to cyclic structures that evolve to silica colloids. (B) The condensation reaction may be catalyzed by positively charged amine residues that stabilize transition states in the condensation. (C) Cell wall polymers may catalyse the condensation and also template the forming mineral. Schematic representation of different silica–polymer composites. (a) Long polymeric chain stabilizes siliceous particle. (b–d) Relatively short polymeric chains that give multiparticle aggregates, soluble (b) or insoluble (c and d). Reproduced from Annenkov *et al.* (2017) with permission from the Royal Society of Chemistry.

cooperative, and increases in polymers carrying neighbouring H-bonding sites, like polyethylene glycol (Preari *et al.*, 2014). Sites for H-bond formation are abundant in cell wall polymers, including polysaccharides, pectin and lignin. Therefore, it is

not surprising that all major cell wall polymers are suggested to affect silica deposition (Lux *et al*., 2020). Two effects may be assigned to the plant polymers: stabilization of soluble silicic acid species versus enhanced condensation and polymerization. High ionic strength and H-bonding reduce the polymerization activity, while amines (Hare and Pfaendtner, 2022) in the presence of a moiety carrying a negative charge (Zhai *et al*., 2022), specifically phosphate ions (Adiram-Filiba *et al*., 2020), are especially effective in catalysing the production of biosilica. This points to two contrasting roles for proteins and for polysaccharides in silica deposition in plants. We will explain the two scenarios one by one.

PEPTIDES & PROTEINS

Peptide and protein-based mineralization has been discovered across diverse organisms (Kumar *et al*., 2021). Nonetheless, our knowledge on *in planta* interaction between silicic acid and proteins is rather limited. Strong evidence of the presence of proteins inside plant silica derived from higher plants was reported from *Equisetum telmateia* and *Phalaris canariensis* (Harrison, 1996). Branches of *E. telmateia* and lemma tissues of *P. canariensis* were treated with concentrated acids to remove the cellular cytoplasmic content and the cell wall. The remaining insoluble material, largely containing silica, was dissolved using buffered aqueous solutions of HF and the non-dialyzed fraction was analysed for the presence of different amino acids and monosaccharides. High percentages of proline–glutamic acid (PE), proline–lysine (PK) or serine–aspartic acid–glycine (SDG) residues were found in the non-dialysable fraction. These residues were intricately associated with plant silica and could be recovered only upon its dissolution. Based on these results, the author (Harrison, 1996) hypothesized that highly charged residues (lysine and glutamic acid) in combination with the rigid backbone containing abundance of proline might be involved in nucleating silica growth *in planta*. Evidence of carbohydrate was also found in the intra-silica extract of *Phalaris*; however, it was not possible to conclude whether the carbohydrate fraction resulted from glycosylated proteins or mixtures of peptides and carbohydrates (Harrison, 1996). Later, it was found that organic matter isolated from silicified phytoliths contains peptides that are differentially glycosylated. These peptides are connected by disulfide bonds, whose reduction results in fragmentation of the glycoproteins (Elbaum *et al*., 2009). While proteins/peptides are obviously trapped within plant silica structures, their sequence and ability to scaffold silica *in vitro* or *in planta* remained elusive. So far, only two proteins with a confirmed role in scaffolding silica have been reported in higher plants. The first protein is involved in the reinforcement of plant cell walls upon fungal infection (Kauss *et al*., 2003) while the other is involved in the lumen silicification of specialized leaf epidermal cells known as silica cells (Kumar *et al*., 2020).

Silica deposits during fungal infection

In order to establish fungal infection in plants, the fungal spores must make entry inside the plant tissues either through natural openings or wounds, or by damaging the plant cell wall locally at the site of infection (Knogge, 1996). One of the strategies adopted by plants to avert such infection is cell wall reinforcement by depositing insoluble silica at the site of attempted fungal penetration (Coskun *et al*., 2019). A proline-rich protein (PRP1) involved in systemic acquired resistance was reported in cucumber (Kauss *et al*., 2003). PRP1 is inducible upon mimicking fungal infection using synthetic elicitors suggestive of its role in plant defence against fungal pathogens. PRP1 consists of 92 amino acids (~10.8 kDa) and is presumably a secretory protein. The C-terminal of PRP1 has six tandem repeats of a conserved sequence rich in proline, lysine and tyrosine. Antibodies were raised against a short peptide sequence from PRP1 rich in positively charged amino acids, termed pep1. The antibodies cross-react with cell wall polypeptide extracted from plants stimulated by an analogue of fungal effectors. Pep1 precipitates solid silica *in vitro* from metastable solution of silicic acid within seconds. Another peptide with overall the same amino acid composition but the amino acid sequence scrambled in such a way that at least one amino acid exists between two positively charged residues also precipitates silica to the same extent as pep1. It is therefore concluded that the silica precipitation activity of the peptide results from its positive charge density rather than its amino acid sequence. Based upon the inducible nature of PRP1 by mimicking infection and the *in vitro* silica precipitation activity of pep1 peptide, the authors postulated that PRP1 is involved in plant defence against fungal infection by reinforcing the cell wall at the attempted fungal penetration site using silica (Kauss *et al*., 2003).

Silica in epidermal silica cells

The only known example of tightly controlled cell lumen silicification occurring in viable cells on a proteinaceous template is that of silica cells. Silica cells occur over the veins in both the adaxial and abaxial leaf epidermal cell layer in grasses (Kaufman *et al*., 1969; Fahn, 1995). Silicification in silica cells of sorghum leaves is brought about by a protein named Siliplant1 (Slp1). Slp1 has an N-terminal signal sequence followed by seven repeat units consisting of at least two of the three domains, namely domain-A (His-, Asp-rich), domain-B (Pro-, Lys-rich) and domain-C (Pro-, Thr- and Tyr-rich). During silica cell maturation, the cells synthesize and store Slp1 molecules in vesicles. When silica cells are ready to silicify, they secrete Slp1 into their apoplast, which comes in contact with supersaturated silicic acid and immediately leads to silica precipitation (Kumar *et al*., 2020). Silicification is supported as long as silica cells are viable. Depositing silica in the apoplast leads to a thickening silica wall that constricts the cytoplasmic volume. The shrinking lumen supports a viable protoplast which is connected to neighbouring cells through plasmodesmata (Kumar *et al*., 2017*a*). Ultimately all silica cells undergo programmed cell death irrespective of their silicification status (Kumar and Elbaum, 2018). Transient overexpression of Siliplant1 results in ectopic silica deposition in all epidermal cell types in sorghum (Fig. 3). A small peptide derived from the Siliplant1 sequence also precipitated silica *in vitro* from metastable silicic acid solution (Adiram-Filiba *et al*., 2020; Kumar *et al*., 2020). These results suggest that Siliplant1 expression is sufficient to derive silicification in the presence of supersaturated silicic acid in the

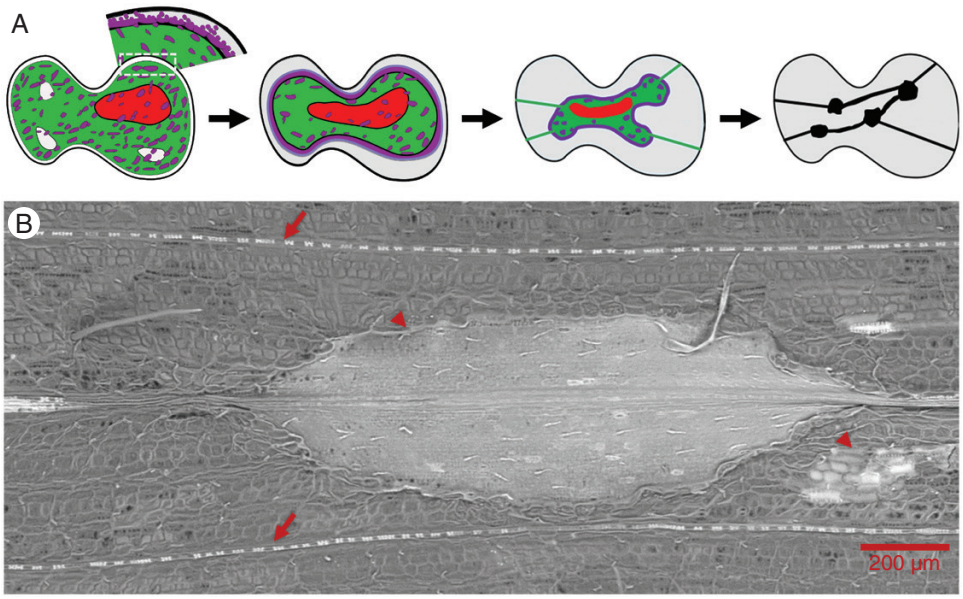

FIG. 3. Activity of Siliplant1 at the apoplast of sorghum leaves. (A) Silica cell at the active silicification zone. Left to right: Siliplant1 (purple) is exported to the apoplastic space (inset), catalysing the polymerization of the supersaturated silicic acid and forming a silica secondary cell wall (grey) that reduces the cytoplasmic volume (green) and nucleus (red). Finally, silica cells undergo programmed cell death. (B) Ectopic silica deposition in sorghum leaf overexpressing Siliplant1. Arrows point to rows of normal silica cells that are silicified similar to wild-type plants. Arrowheads point to epidermal regions silicified under the activity of transiently overexpressed Siliplant1. All epidermal cell types are heavily silicified. Reproduced with permission from Kumar *et al.* (2020).

apoplast, although post-translational modifications, if any, may aid in the silicification process.

POLYSACCHRIDES & PECTIN

Silica isolated from plants carries morphological features on the micrometre scale that are identical to the cell wall features. This suggests that the cell wall polymers connect to the mineral and template its deposition (Jones *et al.*, 1963). Polymers of sugar molecules may interact with silica through arrays of hydroxyls that can stabilize silanol (Si-OH) and siloxyl (SiO^-) groups. Three groups of cell wall polysaccharides are reported to affect plant silicification. (1) Cellulose, the mechanical component of the walls, which is made of long linear poly(1–4) β-glucose chains. Cellulose forms submicron-long crystalline regions connected by less ordered domains. These rods, termed microfibrils, are very stiff and provide the wall with its resistance to longitudinal extension (Niklas, 2004). (2) Hemicellulose and callose are non-crystalline polysaccharides that cross-link the cellulose microfibrils. Hemicellulose is mainly made of xylan [poly(1–4) β-xylose] and (gluco)mannan [poly(1–4) β-mannose] modified by varied mono- and oligosaccharides and hydroxycinnamic acid (Kumar *et al.*, 2016). In grasses and related families, a special type of mixed linkage (1–3,1–4) β-glucan (MLG) is abundant in primary cell walls (Vogel, 2008). Callose is a poly(1–3) β-glucose that deposits in damaged mature walls to form gel-like plugs of disordered mass (Schneider *et al.*, 2016). (3) Pectin is a gelatinous acidic polymer that also cross-links the cell wall polymers. It is made of linear poly-arabinose/galactose chains with blocks of galacturonic and glucuronic acid and other modifications. The acidic groups can bind water molecules and hydrate significantly to form a gel. Cross-linking of two pectin chains may be facilitated through calcium ions that are coordinated by the acidic groups or borate ions covalently bound to apiose groups (Peaucelle *et al.*, 2012; Funakawa and Miwa, 2015).

Cellulose organizes nano-silica particles

Cellulose microfibrils attract colloids of silica *in vitro*. Perry and Lu (1992) precipitated silica colloids of 1–5 nm from a saturated silicic acid solution. With addition of cellulose powder, the mineral colloids formed sheets, suggesting that the cellulose microfibrils can template the spontaneously forming mineral particles. Organization of silica nanoparticles by cellulose fibrils occurs in grasses (Perry *et al.*, 1987; Nakamura *et al.*, 2021). Scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDX) mapping in rice leaves and husks demonstrates a silica layer between the cuticle and epidermis distal cell walls. A matrix of cellulose microfibrils in this region forms in young tissues. Later, silica particles of 50–80 nm nucleate and grow, possibly together with a proteinaceous material, within the cellulose mesh (Nakamura *et al.*, 2021).

Ferulated hemicellulose co-localizes with silica deposits

Analysis of silica and cell wall deposition in lemma epidermal hairs in canary grass (*Phalaris canariensis*) suggests variation in the morphology of silica nanoparticles with variation in the hemicellulose composition. Specifically, when cellulose and xylan deposit, silica particles of ~15 nm arrange in sheets, possibly reflecting the layered structure of cellulose. With a shift to deposition of MLG, the silica particles grow

to ~40 nm (Perry *et al*., 1987). Since cell walls of grasses and horsetail are rich in MLG as well as silica, MLGs were suggested to play a role in silica deposition (Fry *et al*., 2008). Interestingly, reduction in MLG biosynthesis leads to silica deposition in mesophyll of rice leaves, whereas in wild-type leaves silica localizes to the epidermis (Kido *et al*., 2015). This may indicate a role for the MLG in inhibiting the deposition of silica rather than nucleating it. The reciprocal effect of Si on MLG and cell wall composition in general was tested in *Brachypodium* defective in Si uptake. In comparison with wild-type plants, the MLG fraction decreases in young and increases in mature straw of low-Si *Brachypodium*. This, together with other changes in the cell wall composition, indicates that the reduction in Si affects broad aspects of plant biology (Głazowska *et al*., 2018). A striking variation in cell wall composition was the reduction in ferulated hemicellulose that cross-links the polysaccharidic matrix with lignin. In parallel, lignin structure rather than composition was altered in low-Si *Brachypodium* (Głazowska *et al*., 2018). In sorghum root endodermis, silica may aggregate together with arabinoxylan modified by ferulic acid (Soukup *et al*., 2017). The aggregates form in locations of low lignification. These spots form regardless of silica availability to the root, and would bind silicic acid after root maturation and in detached root segments (Lux *et al*., 2003; Soukup *et al*., 2017).

Direct link of hemicellulose and callose with silica

Silicon maps to the first-deposited cell wall in fibre-like epidermis cells of the fern *Adiantum raddianum*. Spatial distribution of polysaccharides co-localizes pectic arabinan with silica deposits (Leroux *et al*., 2013). Similarly, silica is co-localized with hemicellulose and pectin in horsetail (*Equisetum hyemale*) stems (Gierlinger *et al*., 2008). However, the wall is naturally built of cellulose and other polysaccharides that may influence silicification. Therefore, the detection of a covalent bond between hemicellulose or pectin and silica marks a breakthrough in understanding silica deposited in cell walls (He *et al*., 2013). The simplified system of rice cells grown in suspension allowed the detection of a Si-O-C bond, which prevails in extracted hemicellulose. About 60 % of the silica extracts together with hemicellulose, suggesting that some cell wall silica is covalently bound to hemicellulose (He *et al*., 2015).

Callose is chemically similar to hemicellulose and both polymers are extracted together (Fan *et al*., 2020). In mature cells, callose is incorporated into cell walls as part of the plant reaction to environmental stresses. It blocks cell-to-cell communication and increases cell wall mechanical stability (Piršelová and Matušíková, 2013). Under benign conditions silica and callose co-localize in horsetail, *Arabidopsis* and rice (Waterkeyn *et al*., 1981; Law and Exley, 2011; Brugiére and Exley, 2017; Guerriero *et al*., 2018). In *Arabidopsis*, callose deposition at the base of leaf trichomes is necessary for silica deposition in the same location, suggesting that callose acts as a matrix for silica polymerization (Kulich *et al*., 2018). However, in wheat infected by powdery mildew (*Blumeria graminis*), callose deposits that form in association with the fungal invasion are not co-localized with silica deposits (Bélanger *et al*., 2003). This indicates that callose may template silica formation only under certain physiological conditions or in certain species.

In conclusion, silica nanoparticles that form within the cellulosic cell walls are organized in layers between the microfibrils. Unordered polysaccharides, most probably callose and ferulated hemicellulose, and proteinous moieties may catalyse silicic acid condensation and silica polymerization within this matrix.

SILICA – LIGNIN INTERACTIONS

After cellulose, lignin is the most abundant natural polymer, and can make up to 30 % of plant cell walls. Unlike the polysaccharides cellulose and hemicellulose, lignin is a phenolic heteropolymer composed of 4-hydroxyphenylpropanoids. This hydrophobic polymer functions in mechanically stiffening plants and in waterproofing certain tissues. Lignin forms mostly during secondary cell wall synthesis, which takes place when cells reach maturity and cease expansion. Three main building blocks called monolignols (coniferyl, *p*-coumaryl and sinapyl alcohols) are oxidized to monolignol radicals and couple together in cell walls to form the lignin polymer (Meents *et al*., 2018; Dixon and Barros, 2019).

Silica deposits are negatively correlated to lignin deposits, suggesting that silica replaces lignin as a stiffening agent (Raven, 1983; Goto *et al*., 2003; Schoelynck *et al*., 2010; Suzuki *et al*., 2012; Yamamoto *et al*., 2012). Surprisingly, mechanical measurements indicate no influence of silica on tissue stiffness in *Equisetum* (Speck *et al*., 1998) and wheat awns (Zancajo *et al*., 2020), suggesting that, at least in these cases, silica may not play a mechanical role. Further, the silica–lignin negative correlation is not universal; a rice mutant was identified having lower concentrations of lignin in parallel to silica (Ishimaru *et al*., 2008) and indication of a common genetic control for lignification and silicification was observed in the development of *Cucurbita* fruit rind (Piperno *et al*., 2002). In rice, silica was found to be mostly associated with Klason-extracted lignin, constituting ~80 % of the total silica (Pan *et al*., 2017), and silicified structures are associated with lignified tissues (Inanaga and Okasaka, 1995; Zhang *et al*., 2013; Fleck *et al*., 2015). The literature is inconclusive, indicating that the silica–lignin relationship is not based on a simple chemical interaction.

Lignin has highly intricate chemistry. Diverse linkage of monolignols, variable proportions of the different subunits and the inclusion of non-canonical subunits may affect the properties of the polymer (Meents *et al*., 2018). Using a multimodal imaging approach, silica distribution in sorghum leaves was found to correlate with lignin in the epidermis, while no silica was detected in heavily lignified xylem vessels (Zancajo *et al*., 2022). This suggests that only lignin with specific characteristics contributes to silica deposition. Further support for this can be found in roots. Silica deposition in rice roots occurs predominantly in the exodermis, endodermis and sclerenchyma fibres and not in xylem cell walls (Lux *et al*., 2020), despite all being vastly lignified tissues. In primary roots of sorghum, silicification is initiated in endodermis cell walls (Fig. 4). Specifically, silica aggregates form as part of the lignified tertiary inner tangential wall of these cells in a spotted pattern (Sangster and Parry, 1976; Lux *et al*., 2020). In this case, onset of silicification

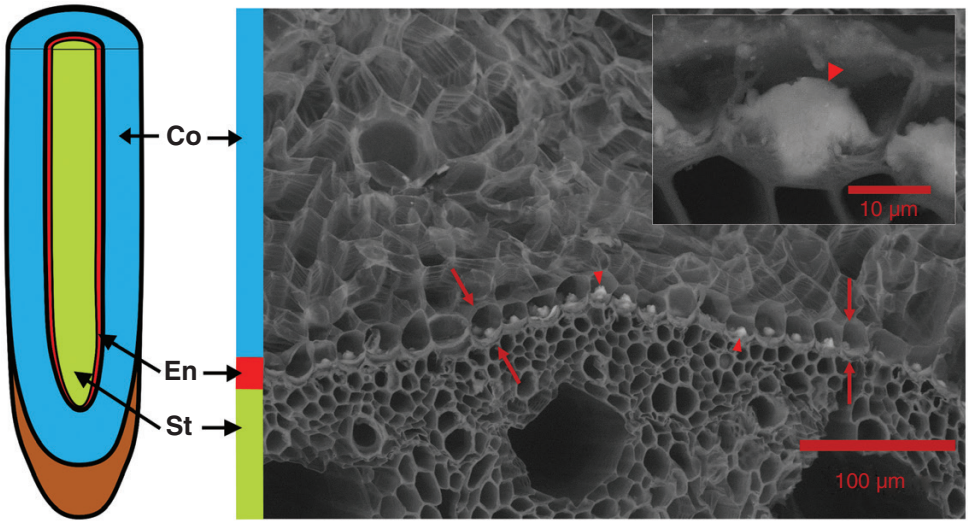

FIG. 4. Silica aggregates at the endodermis of sorghum adventitious root. (Left) Diagram demonstrating root cortex (Co; blue), endodermis (En; red) and stele (St; green). (Right) Silica aggregates (white particles, arrowheads) form at the endodermis (cell layer between two arrows). Inset: close-up of an endodermis cell showing the aggregate (arrowhead) being an integral part of the inner tangential cell wall.

is dependent on the formation of a specific lignin-like material. The deposition of this material is independent of silicification, but its patterning along the endodermal cell walls determines where silica aggregation is initiated (Fig. 5) (Soukup *et al.*, 2017; Zexer and Elbaum, 2020). Moreover, enhancing the formation of this lignin causes excess aggregation of silica, while restricting its deposition eliminates the aggregation (Zexer and Elbaum, 2022). This last example demonstrates micron-scale control over silicification by the deposition of modified lignin that is chemically distinct from adjacent lignin in the same cell wall layer.

The reverse effect, where availability of Si leads to increased lignin formation, has been reported in multiple developmental contexts. In wheat plants infected with powdery mildew, deposition of phenolic compounds is a major cytochemical effect in Si-supplemented plants (Bélanger *et al.*, 2003). Likewise, Si availability enhances the development of the endodermal diffusion barrier in both rice and maize (Fleck *et al.*, 2011, 2015; Lukačová *et al.*, 2013), and silicic acid availability promotes synthesis of lignin and lignin precursors in wound healing of potato tubers (Han *et al.*, 2022).

Due to the heterogeneity of lignin and the overall complex nature of the plant cell wall composite, *in vivo* studies of Si–lignin interactions have proved challenging. Traces of ferulic acid and arabinoxylan were found in isolated silica aggregates of sorghum roots, and were hypothesized to interact with lignin to produce a silica deposition scaffold (Soukup *et al.*, 2017). Based on shifts in lignin autofluorescence under elevated pH conditions, the authors suggested that ferulic acid moieties in the silica aggregates are tied at the nucleation sites via ether bonds. The negatively charged silicic acid may be stabilized through hydrogen bonding with hydroxyl groups of the ferulic acid or other lignin aromatic residues (Soukup *et al.*, 2014, 2017). Based on further SEM and Raman spectroscopic analysis, we found the cell wall at the silica aggregation sites to be denser than the surrounding cell wall (Fig. 6). These sites are enriched with aromatic carbonyls but not with hemicellulose–ferulic acid complexes (Fig. 5E). While ferulic acid-containing complexes are associated with biogenic silica in sorghum endodermis, they are not part of the silica nucleation scaffold (Zexer and Elbaum, 2020, 2022). The contradictions demonstrated by these examples highlight the difficulties in correctly measuring and interpreting the results of such *in planta* studies of Si–lignin interactions.

In vitro production of lignin-like polymers using peroxidase and monolignols (usually coniferyl alcohol) is a common model to study lignification (Nakamura *et al.*, 2006). Adding silicic acid to synthetic lignin results in precipitation of silica. The silica particles are of varied sizes between 50 and 250 nm, depending on the concentration and composition of the artificial lignin polymer (Fang *et al.*, 2003). Natural lignin extracted from plants also causes silicic acid polymerization into silica (Fang and Ma, 2006). Freshly synthesized coniferyl lignin catalyses the formation of silica, but lignin monomers do not. Infrared absorptions of the silanol bond suggest a Si-OH binding to the lignin, forming silica–lignin particles (Soukup *et al.*, 2020). In a recent study, addition of silicic acid to the *in vitro* lignin reaction led to structural changes in the resulting polymer. It was suggested that Si binds to coniferyl alcohol dimers via electrostatic interactions, producing a less homogeneous and more compact polymer (Radotić *et al.*, 2022). This observation may support the aforementioned denser cell wall found at silica aggregation sites in sorghum roots (Fig. 6).

Complexing of Si and lignin in plant tissues is ubiquitous and a hallmark feature of grass cell walls. Furthermore, Si supplementation induces biosynthesis of lignin, among other phenolic compounds (Fleck *et al.*, 2011; Rivai *et al.*, 2022). The examples of Si–lignin interactions are plentiful, and some point to a key role of lignin in nucleating Si polymerization and scaffolding the growth of the mineral. In these cases, lignin with unique chemical features is involved. However, the chemical nature of these interactions remains obscure. Possibly, there is no single type of interaction that takes place between silica and lignin in plants. While at least some electrostatic

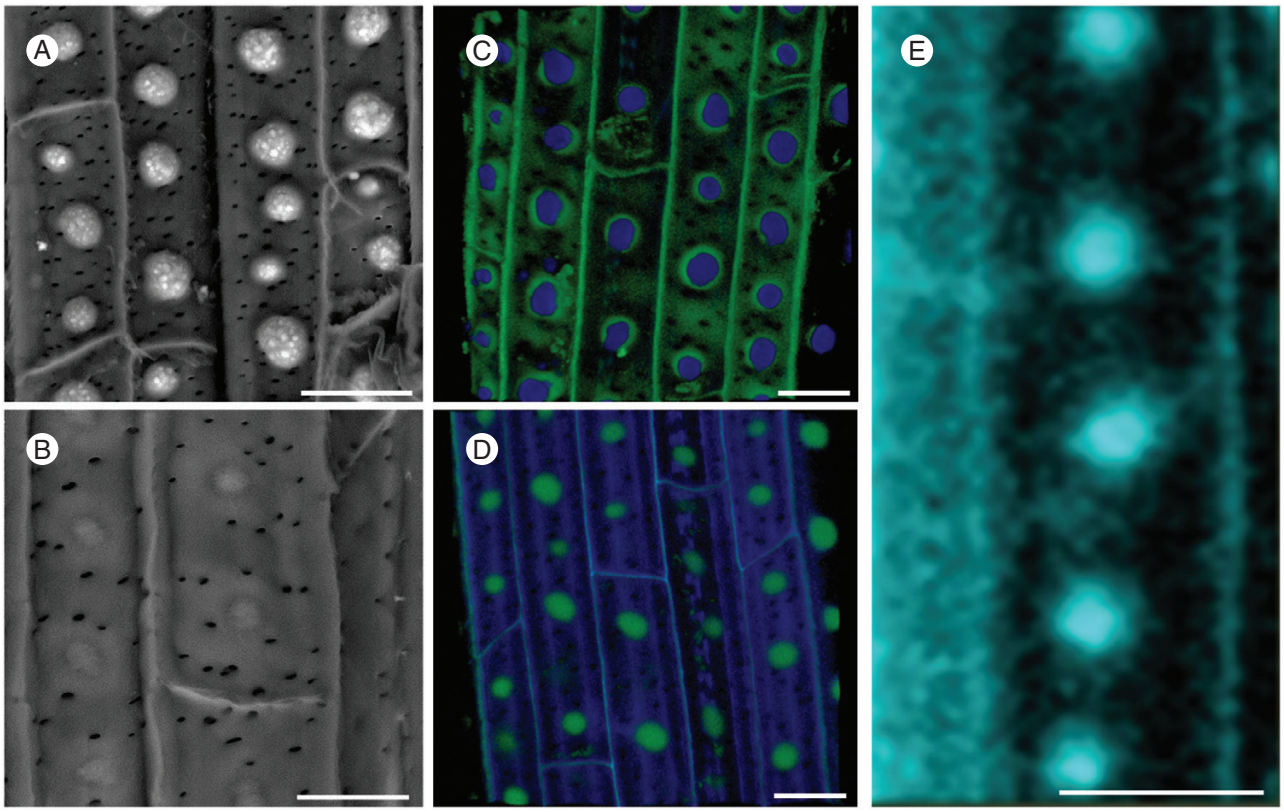

FIG. 5. Micro-imaging of silica formation in sorghum primary roots demonstrating the patterning of modified lignin that patterns silica aggregation. (A) SEM imaging of silica aggregates (white) on the background of the endodermis inter-tangential cell wall (dark background). (B) Similar SEM imaging taken from roots of Si-deprived plants. Autofluorescence of the endodermis cell wall of roots grown (C) with and (D) without Si supplementation. Silica aggregates in (C) fluoresce in blue, while silica nucleation sites deprived of Si in (D) fluoresce in green. This shift in fluorescence hints at a change in the chemistry of cell wall phenolic materials following silicification. (E) Raman spectroscopy of the endodermis cell wall of roots deprived of Si. Mapping of the spectral signal at 1660–1775 cm^{-1}, assigned to aromatic carbonyls, recreates the spotted pattern of Si nucleation sites and suggests that lignin modification by carbonyl groups may nucleate silica deposition. All scale bars represent 10 μm. The figure is reproduced with permission from Zexer and Elbaum (2020, 2022).

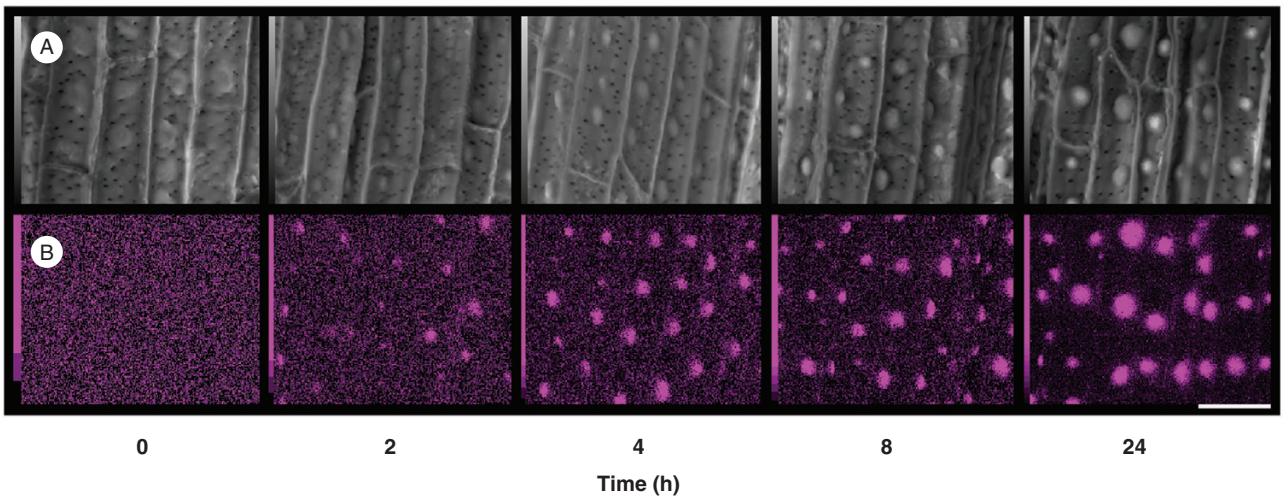

FIG. 6. Time course of Si aggregate formation over 24 h using SEM and EDX. Roots grown in Si-deprived medium were transferred to Si-rich medium and Si aggregation was monitored by (A) SEM backscattered electrons and (B) EDX. Before exposure to Si, bright structures can be identified by SEM (0 h, A). These structures contain no measurable Si (0 h, B). After 2 h, first signs of Si aggregation are detected using both SEM and EDX. Silicification continues until reaching saturation after 24 h. Scale bar represents 10 μm. The figure is reproduced with permission from Zexer and Elbaum (2020).

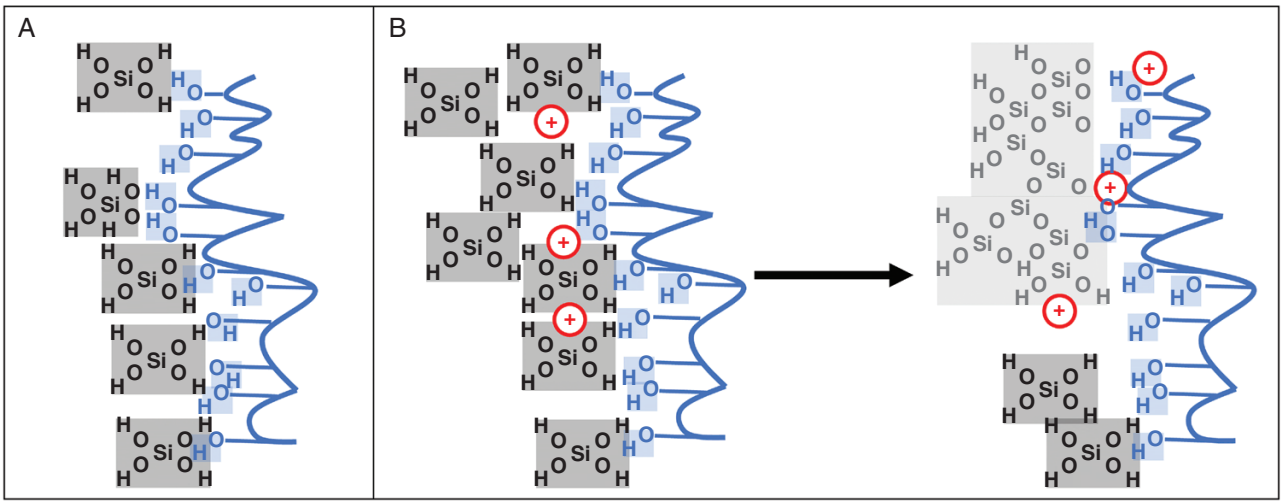

FIG. 7. Suggested model to explain supersaturation and deposition of silicic acid in the apoplast. (A) Stability of silicic acid (black) at supersaturation may be established through H-bonding to hydroxyl groups of a cell wall polymer (blue), as suggested for polyethylene glycol (Preari *et al*., 2014). (B) Once chemical moieties carrying positive charge (red) are introduced, silica deposition (grey) could be catalysed. Positive charge may originate from proteins similar to Siliplant1.

bonds connect silica to lignin, the complex structure and heterogeneity of the polymer makes it difficult to pinpoint specific chemistry. Future research must combine *in planta* and *in vitro* studies to elucidate the specific moieties that participate in Si–lignin complexing.

TEMPLATING OR CATALYSIS OF SILICIC ACID CONDENSATION?

Many biological materials interact with silicic acid *in vitro* (Annenkov *et al*., 2017). This fact most likely results from the nature of silicon oxide and silicon hydroxide chemistry, depending on the formation of hydrogen bonding between the silanol (Si-OH) groups of silica, poly- and monosilicic acid, and the hydroxyl, phosphate and amine groups of biological materials (Currie and Perry, 2007; Mathé *et al*., 2013; Emami *et al*., 2014). Some interaction was detected with almost all components of plant cell walls, regardless of their presence in the cell walls of silicifying or non-silicifying species. This indicates that the cell wall is an excellent matrix that can both template silica formation and act to prevent silica precipitation from a supersaturated sap. Plants may avoid extensive silicification by moving the sap quickly and under low pressure during transpiration. Night-time may be the period for the plant to catalyse silica precipitation in sink locations (Blackman, 1969) and thus overcome the suggested tendency of supersaturated silicic acid to precipitate when transpiration is stopped. At least some parts of the biomineral in the lumen silica deposits (silica cells) have <10 % organic material, which is rich in nitrogen (Alexandre *et al*., 2015). This suggests a protein similar to Slp1 that catalyses the condensation of silicic acid without being incorporated into the mineral, possibly during the night. The local reduced concentration of monosilicic acid would result in diffusion of more silicic acid molecules to add to the growing mineral.

Still we do not know how silicic acid is polymerized in specific locations within the cellulosic cell walls. Apparently, in this case the organic matter percentage in the mineral is as large as the organic cell wall trapped within it (Hodson, 2019). Interestingly, negative phosphate ions are required for the catalysis of silica by polyamine (Adiram-Filiba *et al*., 2020). This suggests that both positively charged and negatively charged moieties are necessary for the silicic acid condensation reaction (Zhai *et al*., 2022). Since cell walls carry negatively charged -O^- groups, there could be involvement of positively charged moieties, e.g. lysine groups present on Siliplant1 or similar proteins. Overexpression of Siliplant1 in sorghum leaves catalyses ectopic silica precipitation in all epidermal cell types (Kumar *et al*., 2020), supporting this mode of action. In this case, the cell wall polymers would supply the required negatively charged moieties in the catalysis of silica precipitation by the positively charged lysines (Fig. 7). Such a model may also explain the high stability of silicic acid in the sap, so long as the positively charged moieties are not available (Preari *et al*., 2014).

Possibly, positive charges of metal cations such as sodium, potassium and calcium could be locally exported to the cell wall matrix and induce silica precipitation in specific epidermal cell walls (Hodson and Bell, 1986). This would explain silica formation in long cells neighbouring non-silicified stomata cells. To test this model, we need to map local charged ions and metal ion transporters in silicifying tissues. Obviously, more experimental data are required to understand plant silica mineralization. Our review highlights the need to combine simplified and complex systems, including the chemistry of silicic acid in solutions, cell and tissue culture, hydroponics growth that allows the control over plant mineral nutrition feeding, and mutations of candidate genes in whole-plant experiments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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