Got silicon? The non-essential beneficial plant nutrient

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Research on a possible nutritional role for the element silicon has been hampered by the diverse beneficial effects that it has on monocots and dicots, and the subsequent difficulties in focusing studies on a single genetic model system. Although deemed a non-essential nutrient for the majority of plants, the benefits of silicon include increasing pest and pathogen resistance, drought and heavy metal tolerance, and the quality and yield of agricultural crops. Although the pathways and molecular mechanisms by which silicon is absorbed and deposited in plants are still unclear, recent progress has been achieved through the use of rice mutants that are deficient in silicon uptake. Additionally, the application of electron-energy-loss spectroscopy (EELS) allows one to determine the composition of silica deposits conclusively. Thereby shedding light upon the role of silicon in heavy metal tolerance. With the complete sequence of the genomes for a dicot (*Arabidopsis*) and a monocot (rice) available for large-scale genetic analysis, the future bodes well for a more complete understanding of the biological role of silicon and its mode of transport into and through plants.

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Abbreviations
EELS electron-energy-loss spectroscopy
EST   expressed sequence tag
GR1   GERMANIUM RESISTANT1

Introduction

Silicon is the second most prevalent element within the soil. Although deemed a non-essential nutrient for the majority of plants, silicon uptake by plants provides many benefits such as improved pest and pathogen resistance [1,2], drought [3] and heavy-metal tolerance [4••], and agricultural crop quality and yield [5]. As the effects and benefits of silicon absorption vary from species to species, and are usually only noted under conditions of biotic and abiotic stress, a comprehensive view of silicon plant biology and its role in plant health has not been formed. Recent reviews [6••,7•] have noted advances in dissecting the roles of silicon in plants, but our knowledge of silicon metabolism in higher plants lags behind that in other organisms, such as diatoms [8,9]. Recent research touching upon silicon uptake and deposition has, however, initiated the first examination of the genetic basis of silicon absorption and deposition pathways in plants.

Although abundant, silicon is never found in a 'free' form and is always combined with other elements, usually forming oxides or silicates. Silicon is absorbed by plants in the form of uncharged silicic acid, $Si(OH)_4$, and is ultimately irreversibly precipitated throughout the plant as amorphous silica (SiO_2–nH$_2$O; also referred to as 'opal', silica gel, or phytoliths in higher plants). Therefore, although silicon is plentiful, most sources of silicon are insoluble and not in a plant-available form. Typical concentrations of silicic acid in soil solution range from 0.1 to 0.6 mM [10]. Soils that have low silicon concentrations are commonly amended with silicon compounds to increase the quality and quantity of some agricultural crops, such as rice and sugarcane [5]. In addition, silicon supplementation in the form of external foliar treatments has proven to increase the pathogen resistance of plant species that do not take up silicon efficiently [11,12]. Silicon is an element that does not cause severe injury to plants when present in excess and can provide multiple benefits [7•].

Silicon accumulation and uptake

Past theories on silicon absorption have considered silicon uptake either as a passive event that coincides with the uptake of water or as an active form of nutrient recruitment. This divergence of opinion may stem from the varied relationship of silicon with different plant species. In a study of more than 500 plant species [7•], divisions were formed to group the high-, intermediate-, and non-silicon accumulators. The groupings were based upon measurements (on a dry weight basis) of silicon and the silicon-to-calcium ratio in plant tissue, and illustrate how silicon accumulation varies widely between species. There are some general trends in silicon accumulation: moncots tend to be high accumulators and dicots poor accumulators. There are, however, exceptions to these trends and silicon accumulation can vary substantially even among ecotypes of the same species [13].

After analyzing more than 147 species for silicon content, different modes of silicon uptake (active, passive and rejective) were suggested to account for accumulator, intermediate and excluder groups, respectively [14]. It should be noted that these modes do not refer or correlate

to a molecular uptake mechanism, such as a channel, pump or carrier, but are based upon measurements of silicon content and transpiration rates relative to a preset baseline. A silicon content of 0.5% (the average for the species tested) was set as the baseline for intermediate (or passive) uptake of silicon, assuming a transpiration coefficient of 500 and growth on soil containing 0.35 mM silicon [7•]. Silicon contents of more than 0.5% suggested that the plants were using an active-uptake or sequestration mechanism to acquire silicon. These uptake groupings, together with evidence from past studies [15,16], counter the theory that silicon is passively absorbed with water. As the translocation of silicon is generally favored by transpiration, and it is unclear how this relationship may alter or regulate the uptake mechanism, the complex relationship between silicon and water uptake will require further study.

Recently, progress has been made in determining the mechanisms by which silicon is absorbed into the plant apoplast and symplast. Silicon transport in plants is much more complex than in other silicon-utilizing organisms, such as unicellular diatoms, because of the additional modes of transport needed to enable long-distance transport across specialized tissues and compartments from the roots to stomata. Rice was an early choice of model plant for studies of silicon transport because, as typifies a monocot, it is a high accumulator of silicon. Silicon uptake in rice is not affected by transpiration but is inhibited by metabolic inhibitors, such as 2,4-dinitrophenol, iodoacetate and 2,4-dichlorophenoxyacetic acid (2,4-D), and by the respiratory inhibitor NaCN. Owing in part to these findings, and because of the recent completion of rice genome sequencing [17,18], this model system remains the focus of studies on silicon uptake.

Initial work on silicon uptake focused on identifying the location of this process. Ma et al. [19••] used rice root mutants, one defective in the formation of root hairs (RH2) and a second defective in the formation of lateral roots (RM109), to demonstrate that silicon is actively taken up through the lateral roots but not through the root hairs. Subsequent analysis of F_2 populations from RM109 × wildtype crosses confirmed that silicon uptake correlates with the presence of lateral roots. Although specific transport systems have been identified in root hairs, including potassium transport channels in tomato [20] and potassium–sodium co-transport channels in wheat [21], the experiments by Ma et al. [19••] appear to eliminate root hairs as a site of silicon uptake.

Ma et al. [22••] went on to identify a rice mutant that is defective in active silicon uptake. If confirmed, this mutation would be the first known to cause a specific deficiency in silicon uptake. The mutation appears to act through disruption of a putative uptake pathway rather than by causing a defect in gross morphological struc-

ture(s). Rice mutants that are deficient in silicon uptake were identified through a screen using germanium (Ge), an analog for silicon. Thousands of plants could be visually assayed for Ge uptake in this screen because although Ge appears to be fully able to substitute for silicon during uptake, it causes low-level toxicity *in planta* that gives symptoms of brown spotting on rice leaves. Significantly, Ma et al. [22••] identified rice mutants that were insensitive to Ge, and they further characterised the GR1 mutant, which showed the greatest resistance to Ge. Although the silicon uptake of GR1 mutants is considerably lower than that of wildtype plants, the mutants did not differ from the wildtype in root morphology, shoot phenotype, or in the uptake of other nutrients such as potassium and phosphorus. Interestingly, silicon uptake is not completely absent in GR1 mutants or in wildtype plants that have been treated with metabolic inhibitors, suggesting that silicon uptake may occur by more than one mechanism. Even so, this study is noteworthy, because although the GR1 phenotype could have a variety of causes, including a mutated promiscuous transporter (which imports silicon in addition to its primary nutrient), analysis of GR1 will identify an uptake route for silicon.

With rice genome mapping nearing completion and with the GR1 mutants already known, it appears that the pathway for silicon uptake in rice could be elucidated shortly. Although a family of silicon transporters has been identified in diatoms [23–25], no similar transporter has yet been identified in plants. Analysis of the rice genome has not detected any putative protein that correlates with the known diatom silicon transporters. In fact, whether plants specifically gather this nonessential nutrient remains controversial. Recently, microarray chips composed of close to 9000 rice expressed sequence tags (ESTs) have been used to analyze the gene expression of rice grown in the presence or absence of silicon. Analysis of these arrays showed that 1% of the ESTs displayed a significant (two-fold difference) change in expression in response to silicon [26]. Although these unidentified ESTs may be important candidates for future study, it is not clear if these differences are direct or indirect effects of silicon nutrition.

Silicon uptake and accumulation inside the cytoplasm and vacuoles of plant cells has also been studied [27,28•]. It has been suggested recently that higher-molecular-weight silicon complexes could be transported into the vacuoles through a novel endocytotic process [28•].

Silicon deposition
Despite an abundance of studies on the site and shape of silica depositions within plants, no molecular mechanism for this deposition has been characterized. Depositions of opal occur throughout the plant in cell walls, cell lumens, tricombs, intracellular spaces, roots, leaves and

reproductive organs. It has been thought that these depositions primarily occur through evapotranspiration [29], a hypothesis that is partially based on the fact that the common locations of opal coincide with major evapotranspiration sites. There is, however, some evidence that plant macromolecules participate in forming an organic matrix for silica deposition [30–32]. Such molecules have already been identified in other organisms that deposit silica [33–35].

Through the use of electron-energy-loss spectroscopy (EELS), researchers are now able to determine the composition of silica deposits for the first time, and the progress made by Neumann and co-workers is noteworthy in this regard [4••,28•,36]. Using EELS and other techniques [4••,28•,36–38,39•], components of some silica deposits have been shown to be precipitates of silicon and zinc, or silicon and aluminum. It is thought that this co-precipitation of heavy metals and silicon is part of the mechanism that allows plants to ameliorate heavy metal toxicity. Silicon may have additional roles in increasing tolerance of aluminum: silicon-treated maize plants release fifteen times more phenolics than untreated maize plants [40]. These flavonoid-phenolics (i.e. catechin and quercetin) have a strong Al-chelating ability and may provide heavy-metal tolerance in plants.

Phytoliths are silica deposits that retain genus- or species-specific morphological characteristics in higher plants. Recently, phytoliths have received increased attention because of the application of phytolith analysis in archeological research. Unique and specific deposits have been noted in Equisitaceae (horsetails), Coniferophyta (gymnosperms), and Magnoliophyta (angiosperms; including moncots and dicots) [41]. Interestingly, in addition to its archeological uses, phytolith analysis has been utilized in food science to test for the adulteration of durum wheat by dilution with bread wheat [42]. Work by Piperno *et al.* [43] and Dorweiler *et al.* [44] examined the genetic basis of silica deposition. In the dicot *Cucurbita*, phytolith deposition was correlated with the presence of a mutant locus called *Hard rind* (*Hr*) [43]; whereas in the monocot maize, phytolith deposition appeared to be linked to the *teosinte glume architecture1* (*tga1*) locus [44]. In both plant species, silification appeared to be linked to loci that are involved in lignification. However, silicic acid has a strong affinity to the organic polyhydroxyl compounds, which participate in the synthesis of lignin [45,46]. It is difficult to conclude therefore whether the silification and lignification processes are correlated or whether increases in lignin synthesis concurrently increase silification.

Silicon, plant metabolism and defenses
To date, no evidence has been provided to show that silicon plays a catalytic role in metabolic processes. Some speculation has surrounded the intriguing discovery of silicon complexes that involve polyols, which may suggest

that silicon has a biological role [47], but this evidence has recently been disputed [48].

A model system using cucumber that is infected with powdery mildew has been studied intensely, and has demonstrated that silicon may play a role in plant disease resistance. Studies have shown that plants treated with silicon produce increased amounts of phytoalexins in the form of inactive glyosylated precursors [49]. Subsequent infection of these silicon pre-treated plants by powdery mildew then caused these compounds to be activated, thereby inducing fungal cell death. To date, the induction of plant defenses by silicon has not been shown in other dicots or in any moncots.

Conclusions
There is no dispute that silicon can provide a survival benefit for a variety of species. Research on this element could identify the silicon uptake pathway and allow for the transgenic alteration of crop plants, thereby introducing the benefits of silicon uptake into new species. Delineation of the mechanisms by which silicon uptake is regulated has just begun in earnest. Recent progress in the field has yielded a candidate gene(s) whose mutation causes a deficiency in silicon uptake, which should allow researchers to clarify at least one uptake mechanism. A large collection of 'reverse genetic' gene knockouts is already available for *Arabidopsis* and will be available for rice in the near future [50]. It will be interesting, therefore, to screen the silicon content of tens of thousands of gene-knockout mutants to identify those genes that are involved in silicon biology. The identification of putative silicon transporters and the control of their expression will accelerate the pace of research in plant 'siliconology'. Owing to the recent strides in the field, it is reasonable to be optimistic and to expect that these mechanisms will become more fully understood soon. The controversy regarding silicon uptake and deposition and its role in plant health should also be resolved soon.

Acknowledgements

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