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Active silicon uptake by wheat

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Abstract

The absorption of Si by wheat, *Triticum aestivum* L. 'Yecora Rojo,' followed Michaelis–Menten kinetics over a concentration range of 0.004–1.0 m*M*. K_m and V_{max} were determined using linear transformations and the calculated curve fitted the data closely. The absorption resulted in accumulation ratios of 200/1 or more. In keeping with that finding, this study also demonstrated that Si uptake by wheat is under metabolic control, being severely restricted by dinitrophenol (DNP) and potassium cyanide (KCN). Silicon uptake by wheat was not significantly affected by phosphate ions, but the chemical analog Ge exerted a direct competitive effect on Si uptake, and *vice versa*.

Introduction

Silicon is the second most prevalent element in the earth's crust and in soils. Although the main reservoir of mineral elements is the solid phase of the soil, the immediate source from which plant roots absorb silicon and other mineral elements is the soil solution (Epstein and Bloom, 2005). In soil solutions Si is normally present at concentrations ranging from about 0.1 to 0.6 m*M*, existing primarily as orthosilicic acid (H_4SiO_4) at pH levels found in most agricultural soils (Knight and Kinrade, 2001).

Silicon plays important roles in agronomic plants. It often minimizes lodging, enhances resistance to diseases and pests, affords protection against Al and heavy metal toxicities, promotes orientation of leaves toward the sun in such a way as to maximize light interception and hence, photosynthesis, affects the quality of plant fibers, influences the absorption and transport of several mineral elements, and performs many other functions in the life of plants (Epstein, 1999, 2001; Epstein and Bloom, 2005, Ma and Takahashi, 2002). Also, physical abnormalities have been quantitatively determined in wheat deprived of Si (Rafi et al., 1997). So significant is the role of Si for so many plant species and for so many situations that it has been categorized as a 'quasi-essential' element (Epstein, 1999; Epstein and Bloom, 2005).

Quantitatively, the absorption of Si by plants is of major significance in the biogeochemical cycling of this element (Basile-Doelsch et al., 2005), its delivery to watersheds (Derry et al., 2005), and eventually, the oceans (Conley, 2002).

In spite of its important roles in the growth and development of many plants, the absorption and translocation of Si have been studied predominantly in rice, which accumulates Si to a greater extent than do other crop species, as has been known for a long time; see the classical review by Jones and Handreck (1967), and, for grain crops only, the paper by Tamai and Ma (2003). There is, however, clear-cut evidence that,

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like rice, wheat accumulates Si (Casey et al., 2003; Jarvis, 1987; Rafi and Epstein, 1999). In this paper we extend the evidence on the absorption of Si by wheat in terms of kinetics and metabolism.

Methods

Experimental conditions

Plant material

Wheat plants, *Triticum aestivum* L. 'Yecora Rojo,' were cultured, using techniques similar to those described earlier (Casey et al., 2003). The nutrient solution was the same and details are presented in Table 1 in that publication. Briefly, the plants, supported by a plastic sleeve, or tube, were grown in 18-L plastic containers. Demineralized water was used throughout; Si was not detectable (<4 μmol L^{-1}). The plant support system is described and shown in a drawing presented by Casey et al. (2003). The entire assembly was supported above the solution and water was added when necessary. Wheat seeds were sterilized with hypochlorite (Aslam et al., 1997) before planting, soaked in demineralized water for 24 h and transplanted to the growth support system. The seedlings, once established, were thinned to two per tube. These setups were placed in a controlled environment chamber with a 14/10 h, day/night cycle at 24/18 °C. The photosynthetic photon flux started at 300 μmol m^{-2} s^{-1} and was gradually raised to 1000 μmol m^{-2} s^{-1} for the last 14 days of growth. The nutrient solutions were maintained at a pH of approximately 6.2 during the growing period. At the time of the experiment plants were well developed with approximately 14 fully developed heads and 38 tillers per tube.

Experimental protocol

For all experiments, the plants were pretreated in 18-L plastic containers containing 0.5 mM $CaSO_4$ and 0.02 m*M* KNO_3 solution for 24 h prior to the start of the experiment. For the experiments proper, the uptake solution volumes were 8 L. The standard uptake solutions were 0.5 m*M* $CaSO_4$, 0.02 m*M* KNO_3, and Si and Ge at the designated levels. Silicate was added as basic Na_2SiO_3 and the pH adjusted to 5.8–6.2 with 1.0 N HCl prior to addition of the other nutrients. The Si at this pH is present as silicic acid (H_4SiO_4). Previous experiments (Casey et al., 2003) demonstrated that wheat plants exposed to H_4SiO_4 translocated primarily monosilicic acid in the xylem.

Absorption periods were initiated by transferring the plants from the pretreatment solution to the 8-L experimental solutions; the plants were maintained in the controlled growth chamber under the conditions described above. Twenty-milliliter samples of solution were taken at intervals for up to 5 h and saved for analysis. At the end of the experiment the plant roots were excised, rinsed thoroughly with demineralized water and blotted to remove excess moisture. Fresh weights were determined. The roots were then dried for 48 h at 73 °C, after which dry weights were taken. The volume of the experimental solution was determined at the end of the experiment and used to assess the transpiration rate during the absorption period and to correct the concentration of the depleted solution. The volumes of the samples withdrawn during the experiments were taken into account.

Uptake studies

Silicon uptake in relation to competition, inhibitors, concentration, and the uptake over time were determined by following Si losses from the experimental solutions (Goyal and Huffaker, 1986; Rafi and Epstein, 1999). To evaluate the influence of metabolic inhibitors on the uptake and translocation of Si in intact plants, wheat plants grown in the absence of Si were exposed to 0.10 mM Si under the following conditions. 2,4-Dinitrophenol (DNP) and potassium cyanide (KCN) were added to solutions at an initial level of 0.1 mM. Si uptake was determined for 3 h.

Interference by mineral elements was also determined. Phosphate (initial concentrations 0.1 and 0.5 mM) was added to solutions at 0.1 mM Si and Si uptake was determined over a 3 h period. Mutual interference between Ge and Si was examined by adding Ge and Si alone and in combination to solutions and following Ge and Si depletion for 5 h. Silicon and Ge were added at a concentration of 0.3 mM initially. Silicon and Ge uptake rates were determined by measuring depletion at 30 min intervals. Because Si and Ge were depleted at different rates, the solutions did not have the same concentrations of these elements at each sampling time. Data were fitted

to either Michaelis–Menten by linear regression or by quadratic equations. (All r^2 values were greater than 0.99.) To compare the total uptake of Ge and Si, it was necessary to fit the uptake curves with respect to the concentration of these two elements and then calculate the sum of Si and Ge uptake at specific concentrations.

Analysis

Samples removed from the experimental solutions were analyzed for Si or Ge by the heteropoly-blue method, using a standard kit purchased from Hach Company (Hach Company, Loveland, CO, USA). In the Hatch method (No. 8282), citric acid is used to suppress interference by phosphate. Because Si and Ge mutually interfere in the colorimetric analysis, Si and Ge were determined by ICP analysis in those solutions in which both were present (Thermo Advantage ICAP, Standard Methods for the Examination of Water and Waste Water, 20th Ed. Am. Pub Health Assoc.). Agreement between the colorimetric and ICP methods was excellent. The depletion in Si and Ge concentrations in the experimental solution represents net absorption of Si and Ge by the plant. The uptake of Si and Ge was expressed as μmol gfw^{-1} of root. Specific experimental protocols are described for individual experiments.

Results and discussion

The measurement of uptake by determining the Si concentration of the experimental solution at intervals has been used before. See for example (for wheat) Jarvis (1987) and Rafi and Epstein (1999). In the present investigation the intervals between samplings of the experimental solutions were on the order of minutes. In view of the total lifespan of the plants these measurements yielded essentially instantaneous absorption rates.

Figure 1 illustrates the Si uptake rate, determined by the depletion method, over a range of concentrations from 0.004 to 1 mM. The uptake rate shows saturation kinetics. The kinetic constants were a V_{max} of 4.49 μmol gfw^{-1} roots h^{-1} and a K_m of 0.086 mM. The calculated curve in Figure 1 was plotted using the Michaelis–Menten equation. The response curve is typical of several such experiments, all of which gave similar results. The response curve is very similar to earlier results reported for the high-affinity mechanism 1 of K^+ absorption (Epstein et al., 1963) and for absorption of many elements since then (Epstein, 1973; Epstein and Bloom, 2005).

Interestingly, the apparent affinity of the Si transporters in wheat as reflected in the K_m values is higher in wheat ($K_m = 0.086$ mM) than in rice ($K_m = 0.32$) as reported by Tamai and Ma (2003), the ratio of the affinities being 3.7/1 (wheat/rice). In another study, Ma et al. (2004) reported a value of 0.15 mM, but the results are not closely comparable because those reported by Ma et al. (2004) refer, not to absorption on the basis of root fresh weight, but the concentration of Si in the symplastic solution in root tips, possibly not representative of most root tissue. In any event, even that value indicates a lower affinity for Si in rice than in wheat.

Previous studies have shown that Si concentrations in the xylem exudate of wheat exceeded the external concentration by more than 400/1 (Casey et al., 2003). To evaluate the uptake and translocation of Si in intact plants, wheat plants grown in the absence of Si were exposed to 0.10 mM Si for 3 h. The roots contained 3.57 μmol gfw^{-1}, and the shoots, 10.74. These data indicate a metabolically

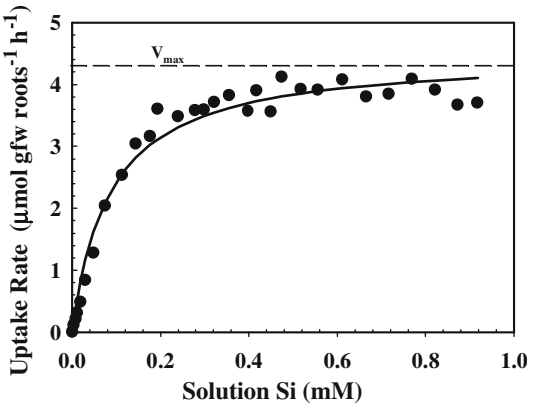

Figure 1. Uptake of Si by wheat plants as a function of Si concentrations. The plants were transferred to an uptake solution containing 1.0 mM Na_2SiO_3 (adjusted to pH 6.0), 0.5 mM $CaSO_4$ and 0.02 mM KNO_3. For 5 h, 25-mL samples of the solutions were taken at intervals for subsequent determination of Si concentrations. The decrease in Si concentration represented uptake by the plants and was expressed on a root fresh weight basis in this and all other figures. The data were mathematically transformed to linear functions and regression analyses were used to determine the kinetic constants. The curve was calculated from the Michaelis–Menten equation using the kinetic constants.

mediated transport and translocation system for Si in wheat, supporting and extending earlier evidence to that effect (Rafi and Epstein, 1999).

It has been demonstrated that Si uptake is affected by metabolic inhibitors. In rice, Okuda and Takahashi (1962) reported a significant inhibiton of Si uptake by NaCN and DNP, and recently Tamai and Ma (2003) used several inhibitors to characterize the Si transport in rice. In the present study with wheat, metabolic inhibitors also limited Si uptake. The results are presented in Figure 2. In the presence of 0.1 m*M* KCN the rate of Si uptake was approximately 50% that of the control. In the presence of 0.1 m*M* DNP uptake of Si was approximately 10% of that of the control. These data, together with those on the high Si accumulation ratios, are evidence for a metabolic role in Si uptake by wheat. The dramatic and rapid effect of DNP demonstrates the critical need for oxidative phosphorylation in providing energy for Si uptake. Accumulation ratios observed in the translocation system and the effect of metabolic inhibitors are consistent with, though they do not prove, operation of a proton-silicate transport mechanism (Casey et al., 2003).

A number of mineral elements affect Si uptake by plants. To name a few, interactions have been observed between Si and Ca, Mn, phosphate, and most specifically, Ge (Azam and Volcani, 1981). In this study we evaluated the potential interactions of Si with phosphate and Ge. Figure 3 illustrates the effect of phosphate on Si uptake by wheat plants. Phosphate added at even five times the Si concentration had no significant effect on Si absorption. These results contrast with those reported for rice by Ma and Takahashi (2002) to the effect that uptake of silicon is affected by phosphate in that species.

Other mineral elements are known to affect Si uptake by plants. Specifically, Ge has been found to inhibit Si uptake by both lower and higher forms of plant life (Azam and Volcani, 1981). Interaction between Si and Ge was therefore investigated in this study. Germanium and Si uptake was measured by depletion from solution. The uptake of these two elements, separately and in combination, was evaluated. The data for the individual elements are shown in Figure 4. The two elements were present initially at a concentration of 0.3 m*M*. Germanium uptake was less than that of Si at concentrations above 0.05 m*M*; however, at lower concentrations (<0.05 m*M*) the uptake rates for Si and Ge were similar. Qualitatively the response curves for Si and Ge were

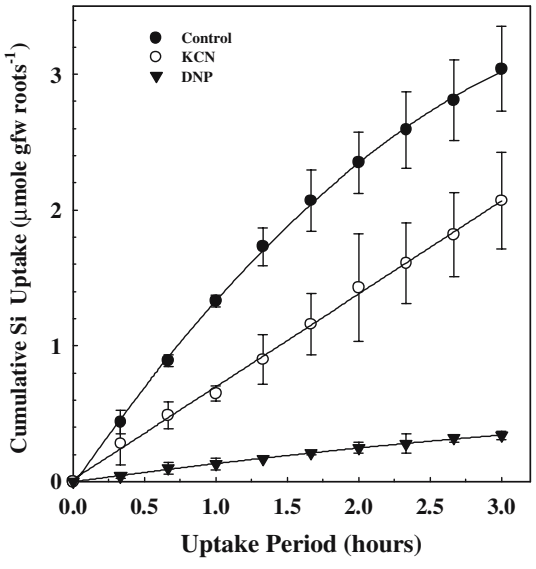

Figure 2. The effect of metabolic inhibitors on Si uptake by wheat plants. The solutions and experimental protocol were the same as in Figure 1 except that the initial Si concentration was 0.1 mM. Potassium cyanide and DNP were added at the initiation of the uptake period at 0.1 mM. The experiment was run for 3 h and samples taken every 20 min. The bars represent the SE of the means of two replicates.

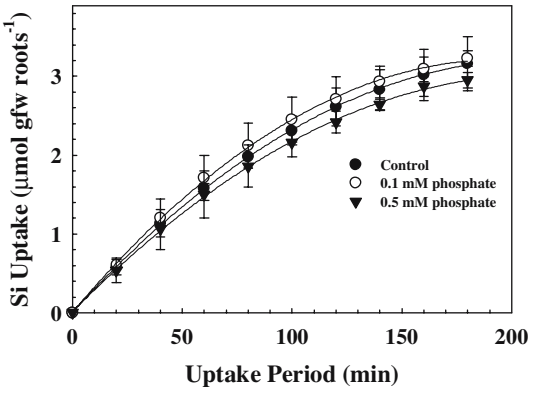

Figure 3. The effect of phosphate on Si uptake by wheat plants. Phosphate was added at 0.1 and 0.5 mM; the initial Si concentration was 0.1 mM. The experiment was run for 3 h and samples were taken every 20 min. The bars represent the SE of the means of two replicates. All other conditions and experimental protocol were as described for Figure 1.

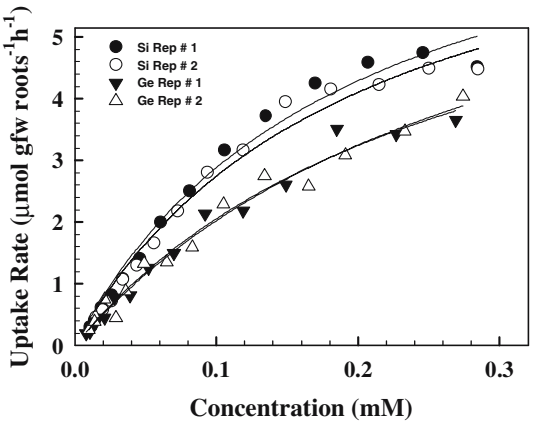

Figure 4. The uptake of Si and Ge by wheat plants as a function of concentration. All other conditions and conventions as in Figure 1 except that the solutions were sampled for 5 h every 30 min. Germanium was added as GeO_2 and the pH adjusted to 6.0. The samples were analyzed for either Ge or Si.

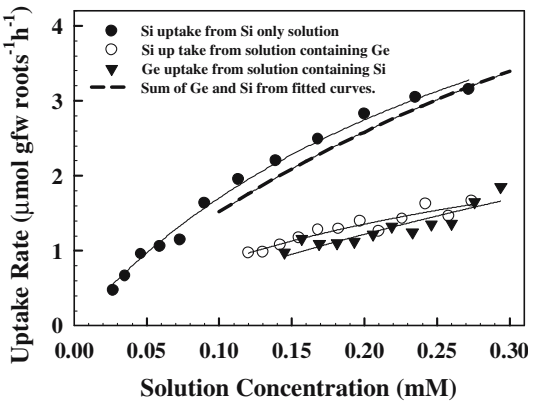

Figure 5. The uptake of Si and Ge by wheat plants. All experimental conditions were similar to those in the experiment shown in Figure 4 except that Si and Ge were added together in one treatment. The other treatment was Si only, used as the control. In the Si + Ge treatment analyses of both Si and Ge were done on the same sample taken from the combined treatments. The uptake rates of Ge and Si were summed and that rate was very similar to that of the control (Si only).

alike. To determine the interaction between Si and Ge, Si uptake was measured in the presence and absence of Ge, and Ge uptake in the presence of Si (see Figure 5). The uptake rates of Ge and Si were both reduced by the presence of the other element. Since these elements interfere with each other's colorimetric determination when the elements are both present in the solution, the concentrations were determined by ICP. As already mentioned, agreement between the ICP method and the colorimetric method for Si and Ge was very good. If the uptake rates of the two elements in the presence of the other are added the total uptake of both is nearly the same as Si uptake in the absence of Ge. This indicates competition between the two elements in their absorption.

The present results amplify those of Rafi and Epstein (1999) and Casey et al. (2003). In the former study the authors found that wheat plants accumulate Si to the point where it could no longer be detected in the solution. Casey et al. (2003) used Si -29 nuclear magnetic resonance spectroscopy (NMR) and found that only monosilicic acid and some disilicic acid were present in the xylem exudate; there was no evidence of organosilicic complexes. Subsequently, Ma et al. (2004) also used the Si-29 MNR technique in a study of Si uptake in rice. They likewise found no evidence of organosilicic complexes.

In conclusion, the absorption of Si by wheat plants shows a concentration dependence obeying Michaelis–Menten kinetics up to an external Si concentration of 1.0 mM. Silicon absorption is interfered with by metabolic inhibitors and is unaffected by phosphate but competitively depressed by Ge. Similarities to, and differences from Si absorption by rice are discussed. The results are in keeping with the conclusion by Rafi and Epstein (1999) and Casey et al. (2003) that the fundamentals of Si absorption by wheat, rice and other species are similar, the differences being matters of degree.

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