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Effects of Nitrate Supply Site on Selenite Uptake by Rice Roots

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Selenite uptake by rice roots is regulated by many factors. The goal of this paper was to study the effects of the nitrate supply site on selenite uptake by rice roots. Using the excised-root experiment system, we found that, due to anion−anion interactions, nitrate can partly block the selenite uptake by rice roots by local action (a corporate supply of nitrate and selenite in the same portion). Using a split-root experiment system (whole-plant level), we found that (1) nitrate can also partly block selenite uptake by rice (roots and shoots) by local action; and that (2) under long-distance action (separate supply of nitrate and selenite in two different portions of the root), nitrate can partly facilitate selenite uptake by rice (roots and shoots), probably by regulating root glutathione content. Thus, our results suggest that nitrate can inhibit or facilitate selenite uptake by rice roots depending on the nitrate supply site.

KEYWORDS: Glutathione; nitrate; rice; selenite; uptake

INTRODUCTION

Selenium (Se) is an essential micronutrient for many organisms, including animals and humans (*1*−*4*). A number of diseases of significant human concern are Se deficiency syndromes, such as Keshan disease, Kashin−Beck disease, and endemic liver cancer. In China, a wide low-Se belt stretches from Heilongjiang Province in the far northeast to Yunnan Province along China's southern border. Thus, Se deficiency remains a very serious nutritional and health problem in China (*5*). The National Research Council has established a recommended dietary allowance (RDA) of Se for humans, amounting to 55 and 70 μg day^{-1} for men and women, respectively (*1*). However, in China, human Se intake is only 26 to 32 μg day^{-1}, with intake in some regions under 10 μg day^{-1} (*5*). People usually obtain Se from plant foods, among which rice (*Oryza sativa* L.) is the most important staple food in China (*6*). According to a China Nutrition Society report, daily rice consumption should range from 300 to 500 g day^{-1} (*5*). Thus, Se uptake by rice plants plays a significant role in the food chain for satisfying human nutrition requirements.

Selenium is a very active element, having several inorganic forms, Se^{2-}, Se^0, SeO_4^{2-}, SeO_3^{2-}, in soils. Of these, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are available to plants (*7*). Selenate is known to be taken up by plant roots via a high-affinity sulfate transporter (*8*). However, less is known about the mechanisms of selenite uptake by plants (*3*). Research has shown that selenite uptake by plant roots is not metabolically dependent (*9*). However, low temperature and respiratory inhibitors suppress selenite uptake by plants (*10*). Recent results have also suggested that selenite uptake by wheat was inhibited by metabolic inhibitors and enhanced by phosphorus deficiency (*11*). Therefore, selenite uptake by plants requires further investigation.

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Rice primarily takes up selenite, which is the dominant form of available Se in paddy soils (*12*). Our previous results showed that iron plague outside roots affected selenite uptake by rice plants grown in solution culture (*13*). We also found that pH affected the selenite uptake by rice and that selenite in the form of H_2SeO_3 can enter rice roots through aquaporins (*14*). Some other studies reported that Se inhibits nitrogen uptake by plants (*15*, *16*). On the other hand, nitrate was found to inhibit selenite uptake in the unicellular green alga *Chlamydomonas reinhardtii* (*17*). However, the mechanism of how nitrate inhibits selenite uptake by plants (especially higher plants) is unclear.

Ammonium is the main nitrogen source for rice plants growing under anaerobic paddy conditions, but in aerobic and upland soils, nitrate is also a major nitrogen source. Rice plants can grow well with both mixed- and single-source nitrate and ammonium supplies (*18*, *19*). Due to oxygen secretion by rice roots, some amount of nitrate may also exist around the rice rhizosphere (*20*). In addition, nitrate can act as a signal molecule and regulate nutrient uptake by plants (*21*). Uptake of nutrients by plant roots relies not only on transporters located in the plasma membranes of root cells but also on powerful signal transduction systems to adjust the nutrient uptake and utilization of the whole plant (*21*−*23*). As mentioned above, some studies have focused on the regulation of selenite uptake by plants. However, very few have studied in detail the regulation of nitrate on selenite uptake by rice plants. Therefore, in the present study, we analyzed the differential regulation of local and long-distance controls by nitrate in the selenite uptake of rice using both excised-root and split-root experiments. A further objective was to examine the physiological mechanism of nitrate-regulated selenite uptake by rice, which has so far remained unclear.

MATERIALS AND METHODS

Plant Material and Experimental Conditions. According to our previous results (*13*, *14*), Xiushui 48, a high-Se rice (*Oryza sativa* L.) cultivar (Se content in this rice cultivar was over 74 μg kg^{-1}), was chosen as

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the experimental material. After surface sterilizing with 2% NaClO (sodium hypochlorite), rice seeds were soaked in distilled water in darkness for 24 h and then transferred to an incubator with temperature set at 35 °C. After rice seeds germinated and radicles grew to 2−3 cm, uniform rice seedlings were transferred to a nylon mesh and then planted in a hydroponic system inside a growth chamber with a 14 h light period (photosynthetically active radiation of 300 μmol m^{-2} s^{-1}). The temperature was 24 °C during the day and 18 °C at night; the relative humidity was 67%. Rice seedlings were cultured with half-strength Kimura nutrient solution (*24*) which, 3 days later, was changed to full strength. The 10-day-old rice seedlings at uniform size were transplanted to boxes containing 18 L of full nutrient solution. The solution pH was adjusted to 5.5 every day using dilute KOH and HCl, and the solution was renewed every 3 days. Some uniform rice plants were harvested after the treatments had been imposed for 15 days. Excised-root experiments were then performed according the method of Mohammed et al. (*25*). Rice roots were excised at the base of the shoots and used in the following excised-root absorption experiments. In addition, some uniform rice plants were chosen for split-root experiments. Each independent experiment had three replicates, and each replicate contained three rice plants. In addition, independent experiments were repeated at least twice under the same conditions (biological repeats).

Excised-Root Experiments. About 0.3 g fresh weight of excised roots of Xiushui 48 was placed in a beaker containing 100 mL of S-free nutrient solutions for 30 min, and then transferred to an absorption solution containing 2.89 μM (500 μg L^{-1}) Na_2SeO_3 solutions with different KNO_3 concentrations (0.03, 0.3, 1, 3, 10, or 30 mM). The pH of the absorption solution was then adjusted to 5.5 using dilute KOH and HCl. After absorption for 3 h, the excised roots were first rinsed with an ice-cold solution containing 5 mM MES, 0.5 mM $CaCl_2$, and 0.5 mM K_2SO_4 (pH 5.5) and then immersed in the same solution for 15 min to remove the adsorbed Se on the root surfaces. The roots were then blotted dry and analyzed for Se content.

Split-Root Experiments. As described above, some uniform rice plants were selected and transported into the split-root box, which was divided into two isolated compartments of identical size. Each compartment could contain 5 L of nutrient solution. The split-root box was covered with a lid containing holes in the middle into which rice plants could be placed.

To evaluate this split-root experiment system, we designed three treatments (T1, T2, and T3). In the T1 treatment, no rice plants were placed in the split-root box. In the T2 treatment, rice plants were placed in the split-root box. All rice roots were divided into two parts, and each part was dipped into the solutions. In the T3 treatment, rice plants were placed in each compartment and rice roots were not divided into two parts. In all three treatments, one compartment of the split-root box contained full-strength Kimura nutrient solution, and the other compartment contained full-strength Kimura nutrient solution with 5 μM selenite. After 48 h, the solutions in each compartment were collected and analyzed for Se content.

We also designed three treatments (S1, S2, and S3) to analyze the contents of Se, nitrate, and glutathione (GSH) in rice plants. In all three treatments (S1, S2, and S3), rice plants were placed in the split-root box. Furthermore, all rice roots were divided into two parts, and each part was dipped into the solutions. In the S1 treatment, one compartment of the split-root box contained full-strength Kimura nutrient solution, and the other compartment contained full-strength Kimura nutrient solution with 5 μM selenite. In the S2 treatment, one compartment contained full-strength Kimura nutrient solution, and the other compartment contained full-strength Kimura nutrient solution with 5 μM selenite and 1 mM nitrate. In the S3 treatment, one compartment contained full-strength Kimura nutrient solution with 1 mM nitrate, and the other compartment contained full-strength Kimura nutrient solution with 5 μM selenite. After 48 h, rice roots and shoots in each compartment were collected and analyzed for Se, nitrate, and GSH contents.

Measurement of Se in Rice Plants. Measurement of Se in rice plants followed the method described by Zhang et al. (*14*). All glassware used in the experiments was first soaked in 10% HCl overnight to avoid contamination, then rinsed in deionized water and dried by heating. In all experiments, roots or shoots were separated at harvest, washed three times with deionized water, and dried at 60 °C. Preweighed samples of ground roots or shoots were placed in glass digestion tubes. A 5 mL mixture of HNO_3 and $HClO_4$ (4:1 in v/v) was added to each tube, and the mixture was allowed to digest overnight at room temperature. The tubes were heated to 150 to 165 °C for 2 h; 2.5 mL of acid mixture was then added, and the tubes were continuously heated until the solutions became clear. After cooling, 2.5 mL of 6 M HCl was added to the tubes, and the tubes were maintained at 100 °C for 1 h to reduce Se(VI) to Se(IV). The digests were diluted with deionized water to a final volume of 25 mL. The Se concentrations in the digested samples were determined by hydride generation flame atomic fluorescence spectrometry (BRAIC AF-610A, Beijing, 1999). All chemical reagents used were guaranteed superpure grade.

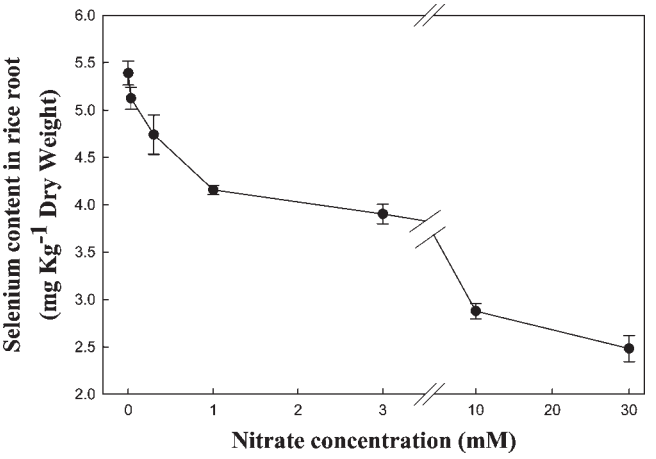

Figure 1. Effect of different nitrate concentrations on selenite uptake by rice roots at pH 5.5 in the excised-root experiments. Approximately 0.3 g fresh weight of excised roots of Xiushui 48 were placed in an absorption solution (pH 5.5) containing 2.89 μM (500 μg L^{-1}) Na_2SeO_3 solution with different KNO_3 concentrations (0.03, 0.3, 1, 3, 10, or 30 mM). After absorption for 3 h, the excised roots were blotted dry and analyzed for Se content. The data were subjected to analysis of variance and post hoc comparisons with Duncan's multiple range test at $p < 0.05$ using SPSS version 13.0.

Determination of Nitrate in Rice Plants. The nitrate concentration of roots and shoots was determined colorimetrically by the nitration of salicylic acid according to the method of Cataldo et al. (*26*). For tissue analysis, 100 mg of fresh plant tissue (root or shoot) was frozen in liquid nitrogen, pulverized, and added to 1 mL of deionized water. The suspension was incubated at 45 °C for 1 h and then centrifuged at 5000*g* for 15 min. The supernatant was utilized for nitrate quantitation.

Determination of Glutathione Content. Concentrations of GSH were spectrophotometrically determined with an enzyme-recycling assay at 412 nm (*27*). The assay was based on the sequential oxidation of glutathione by 5,5′-dithiobis-(2-nitrobenzoic acid) and reduction by NADPH in the presence of known amounts of glutathione reductase (GR).

Statistical Analysis. Data were subjected to analysis of variance, and post hoc comparisons were performed with Duncan's multiple range test at $p < 0.05$. The statistical software program used was SPSS version 13.0.

RESULTS AND DISCUSSION

Effect of Nitrate on Selenite Uptake by Rice Roots in the Excised-Root Experiments. As shown in **Figure 1**, different nitrate concentrations (0.03, 0.3, 1, 3, 10, or 30 mM) significantly inhibited Se uptake by rice roots at pH 5.5 in the excised-root experiments ($p < 0.05$). Also, Se content of rice roots shows a close negative correlation to nitrate concentration ($R^2 = 0.7252$). Our previous results showed that, under pH 5.5, selenite is the dominant Se form and may actively enter rice roots (*14*). Therefore, these results show that nitrate inhibited selenite uptake by rice roots in the excised-root experiments. Using the unicellular green alga *C. reinhardtii*, Morlon et al. (*17*) also showed that increasing nitrate and sulfate (anionic macronutrient) concentration induced significant inhibition of selenite uptake. Thus, our results and the results of Morlon et al. (*17*) suggest that, due to anion−anion interactions, nitrate, an anionic macronutrient, inhibits the uptake of selenite (anionic element) in

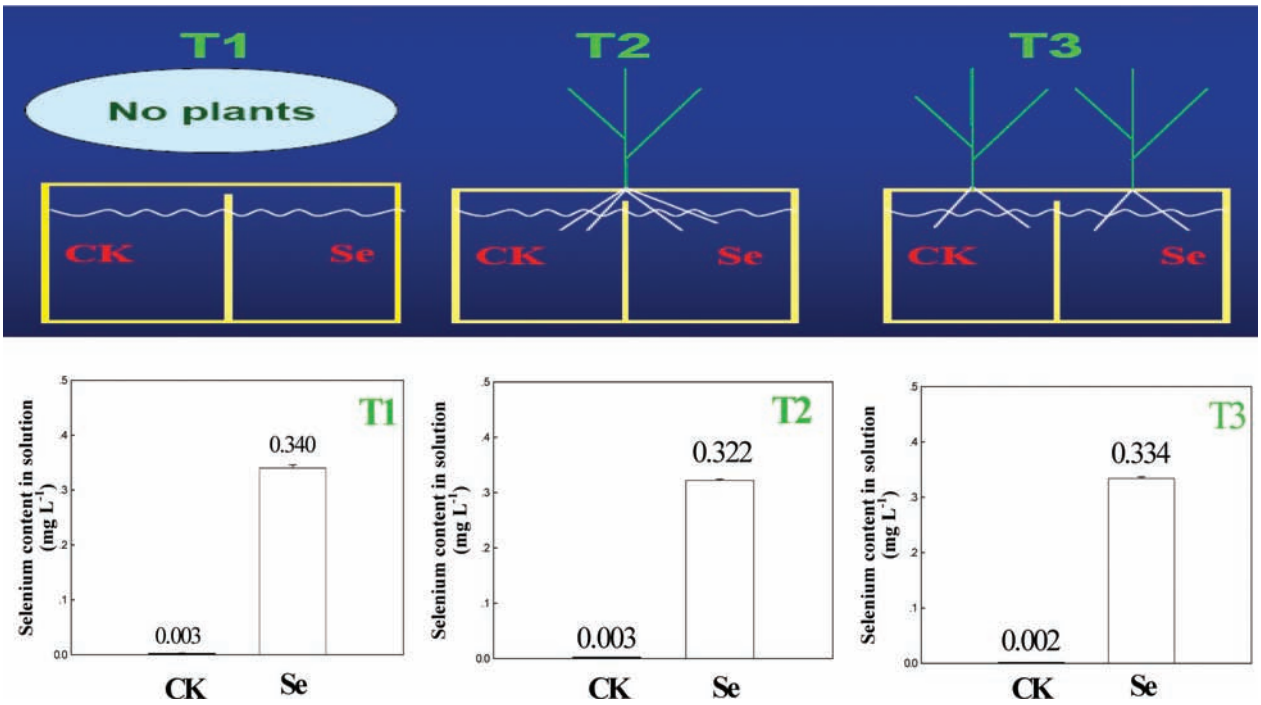

Figure 2. Evaluation of the split-root experiment system. To evaluate our split-root experiment system, we designed three treatments (T1, T2, and T3). In all three treatments, one compartment of the split-root box contained full-strength Kimura nutrient solution, and the other compartment contained full-strength Kimura nutrient solution with 5 μM selenite. After 48 h, the solution in each compartment was collected and determined for Se content. The data were subjected to analysis of variance and post hoc comparisons with Duncan's multiple range test at $p < 0.05$ using SPSS version 13.0.

plants, probably by competing for the selenite transport site. In addition, our results showed that 1 or 3 mM nitrate almost repressed the uptake of selenite in rice roots by 70%. Therefore, further studies were carried out with 1 mM nitrate. In the excised-root experimental systems, nitrate inhibited selenite uptake by rice roots. Root nitrate uptake is ultimately regulated by signals transported from the shoots to the roots (*22*), and selenite uptake by roots was influenced by shoots in the whole-plant experimental systems. Thus, what is the property of selenite uptake in rice roots control by nitrate in the whole-plant level? The split-root experimental system is a good system with which to research nutrient uptake, transport, and regulation (*21*−*23*). Thus, a split-root experimental system (whole-plant level) was designed to investigate the effect of nitrate on selenite uptake by rice roots.

Evaluation of the Split-Root Experiment System. Plant roots can volatilize Se. Volatile Se may enter the nutrient solution without Se and influence the results of split-root experiments. Thus, evaluation of the split-root system was needed in this study. We designed three treatments (T1, T2, and T3) (see Materials and Methods for details). **Figure 2** shows that no significant differences in the Se content of the normal nutrient solution (CK) were observed among the three treatments (T1, T2, or T3) ($p < 0.05$). Also, there were no significant differences in the Se content of the nutrient solution with 5 μM selenite (Se) among the three treatments (T1, T2, and T3) ($p < 0.05$). These results show that volatile Se does not enter the nutrient solution without Se and does not influence the results of split-root experiments. Therefore, the split-root experiments in our study were reliable.

Effect of Nitrate on Selenite Uptake by Rice (Root or Shoot) in the Split-Root Experiments. To investigate the regulation of nitrate on selenite uptake by rice roots at the whole-plant level, a refined experimental system (split-root system), in which the roots of rice plants were split into two parts, was set up (**Figure 3**). This allowed separate supplies of nitrate or selenite to different portions of the root of a single rice plant. We also designed three treatments (S1, S2, and S3) in this split-root experiment (see Materials and Methods for details). As shown in **Figure 3**, the root Se content in the right portion of rice roots grown under S2 treatment (corporate supply of nitrate and selenite) was significantly lower than that in the right portion of rice roots grown under S1 treatment (supply of single selenite). In addition, **Figure 4** shows that the shoot Se content of rice plants grown under S2 treatment was also significantly lower than that of rice plants grown under S1 treatment ($p < 0.05$). The above results support the finding that nitrate can inhibit selenite uptake by rice roots in the excised-root experiments. However, **Figure 3** shows that the root Se content in the right portion of rice roots grown under S3 treatment (separate supply of nitrate or selenite) was significantly higher than that in the right side of rice roots grown under S1 treatment (supply of single selenite). In addition, **Figure 4** shows that the shoot Se content of rice plants grown under S3 treatment was also significantly higher than that of rice plants grown under S1 treatment ($p < 0.05$). Taken together, these results suggest that nitrate can partly block selenite uptake by rice roots by local action (corporate supply of nitrate and selenite in the same portion), while nitrate can partly facilitate the selenite uptake of rice roots by long-distance action (separate supply of nitrate and selenite in the two different portions). Regulation of root nutrient uptake systems is known to ultimately depend on global signals, transported from the shoot and integrating the demand of the whole plant. In addition, nitrate can act as a signal molecule and regulate nutrient uptake in plants (*21*). Thus, these results also show that nitrate may be a signal regulating selenite uptake by rice roots. However, what are the mechanisms of this phenomenon? Can nitrate regulate selenite uptake in rice roots by itself, or is adjustment for other factors necessary?

Nitrate Content of Rice (Root or Shoot) in the Split-Root Experiment. To investigate whether nitrate can regulate selenite uptake in rice roots by itself, the nitrate content in rice roots or

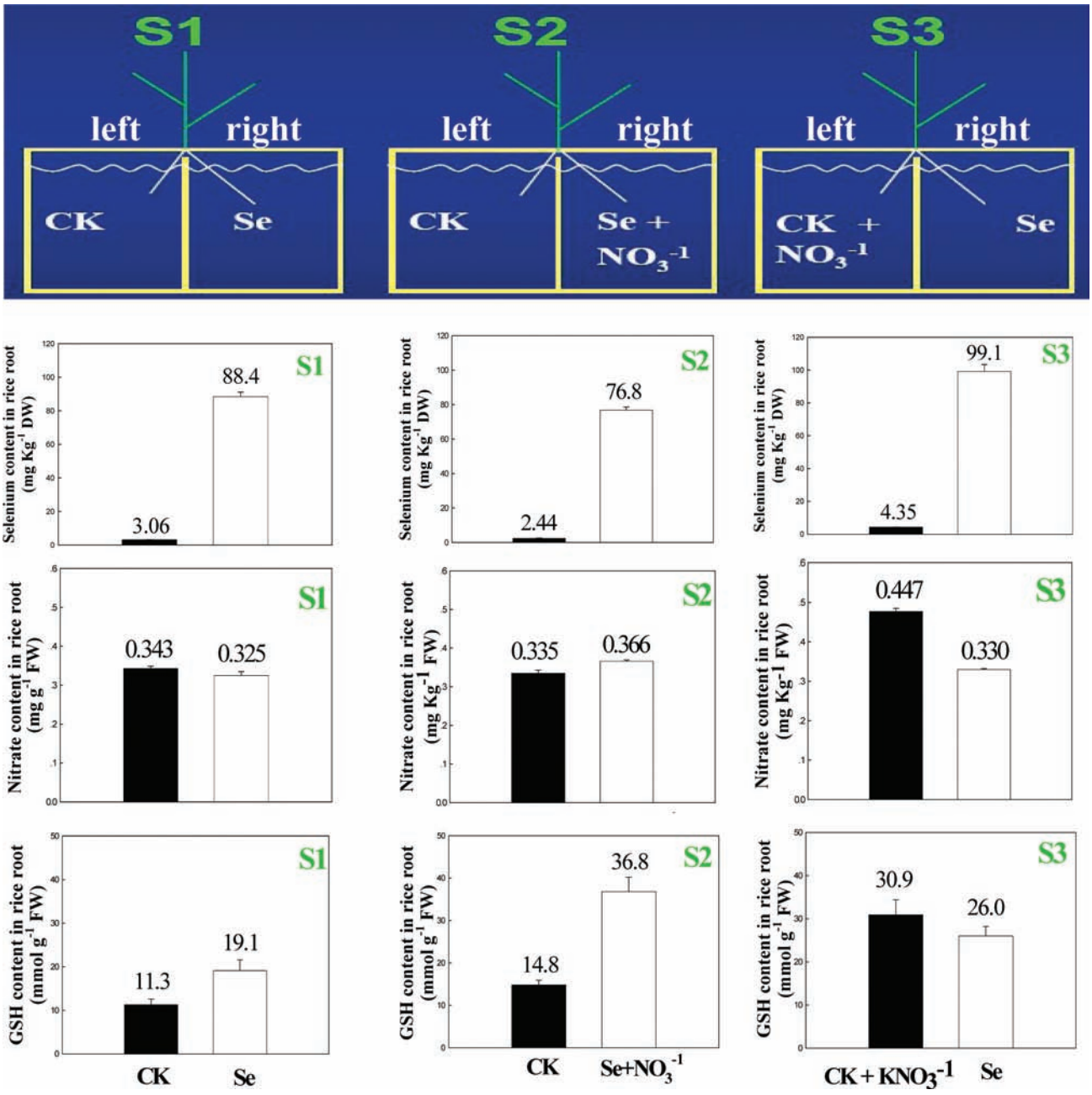

Figure 3. Selenium, nitrate, and GSH contents of rice roots in the split-root experiments. We designed three treatments (S1, S2, and S3) to analyze the contents of Se, nitrate, and GSH in rice roots. The data were subjected to analysis of variance and post hoc comparisons with Duncan's multiple range test at $p < 0.05$ using SPSS version 13.0. CK: Kimura nutrient solution. Se: Kimura nutrient solution with 5 μM selenite. Se + NO_3^{-1}: Kimura nutrient solution with 5 μM selenite and 1 mM nitrate. CK + NO_3^{-1}: Kimura nutrient solution with 1 mM nitrate.

shoots was analyzed in the split-root experiments. No significant differences in the nitrate content of the right portion of rice roots were observed among the three treatments (S1, S2, or S3) ($p < 0.05$). Furthermore, there were no significant differences in the nitrate content of rice shoots among the three treatments (S1, S2, and S3) ($p < 0.05$). However, the nitrate content of the left portion of rice roots in S3 was significantly higher than that of the left portion of rice roots in S1 or S2. The probable reason for this difference is that 1 mM nitrate was added in the left nutrient solution of rice roots in the S3 treatment, unlike in the left nutrient solutions of the S1 or S2 treatments. These results suggest that nitrate regulates selenite uptake in rice roots, but probably not by itself.

GSH Content of Rice (Root or Shoot) in the Split-Root Experiment. Some studies have shown that GSH can facilitate selenite uptake by nonenzymatically reducing selenite to selenocysteine in plants (*28*). Our results also indicate that GSH addition can greatly increase selenite uptake by rice roots (data not shown). In addition, many studies have found that nitrate regulates numerous biological factors involved in plant growth and development (*22*, *29*). To investigate whether nitrate can regulate selenite uptake in rice roots by other factors such as GSH, the GSH content in rice roots and shoots was analyzed in the split-root experiments (**Figure 3**). Although no significant differences in the GSH content of rice shoots were observed among the three treatments (S1, S2, or S3), the GSH content in the right portion of rice roots grown under the S2 treatment or in the left portion of rice roots grown under the S3 treatment was significantly higher than that in the other portions of rice roots grown under the three treatments ($p < 0.05$). These results show that nitrate addition

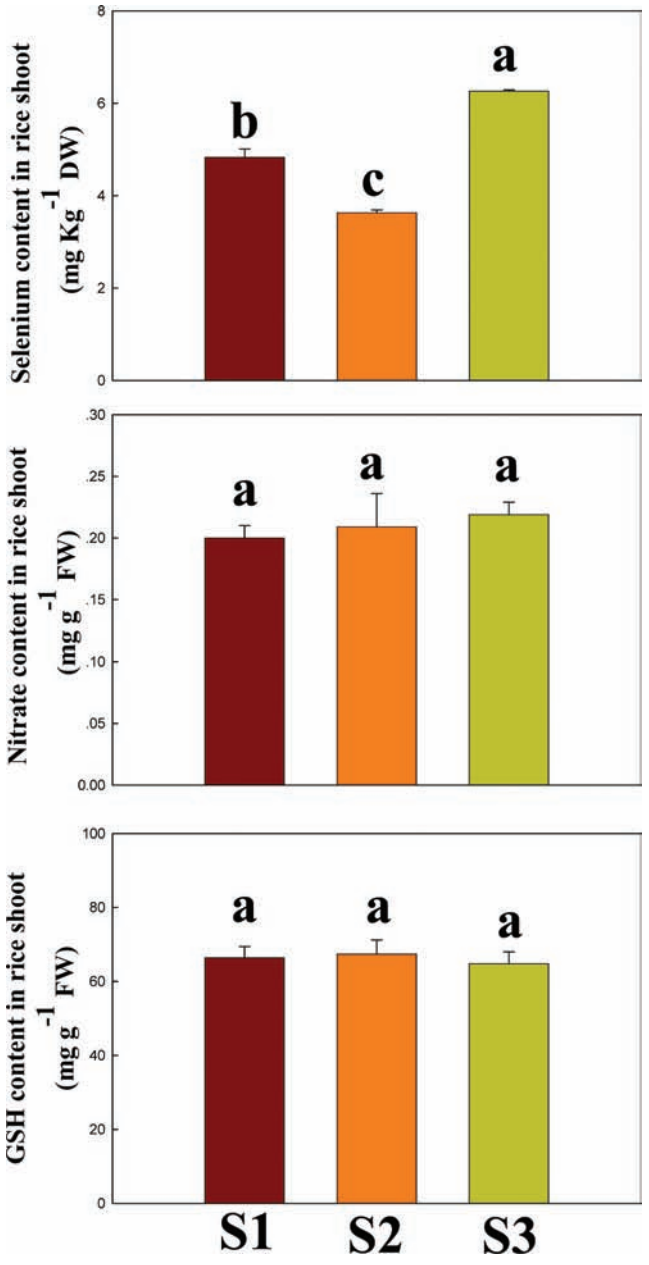

Figure 4. Selenium, nitrate, and GSH contents in rice shoots in the split-root experiment. Under the same split-root system (see **Figure 3** for details), we designed three treatments (S1, S2, and S3) to analyze the contents of Se, nitrate, and GSH in rice shoots. The data were subjected to analysis of variance and post hoc comparisons with Duncan's multiple range test at $p < 0.05$ using SPSS version 13.0.

improved the GSH content in rice roots. In addition, the GSH content in the right portion of rice roots grown under the S3 treatment was significantly higher than that in the right portion of rice roots grown under the S1 treatment ($p < 0.05$). This result indicates that the addition of nitrate to one portion of rice roots improved the GSH content in the other portion of rice roots. Although the GSH content in the right portion of rice roots grown under the S2 treatment was significantly higher than that in right portion of rice roots grown under the S1 or S3 treatment, the Se content in the right portion of rice roots grown under the S2 treatment was significantly lower than that in the right portion of rice roots grown under the S1 or S3 treatment ($p < 0.05$). The probable reason is the fact that nitrate partly blocks selenite uptake by rice roots by local action (corporate supply of nitrate and selenite in the same portion). However, the GSH and Se contents in the right portion of rice roots grown under the S3 treatment were significantly higher than those in the right portion of rice roots grown under the S1 treatment. Therefore, when nitrate or selenite was separately supplied to two different portions of rice roots, nitrate partly facilitated selenite uptake by rice roots probably by regulating root GSH content.

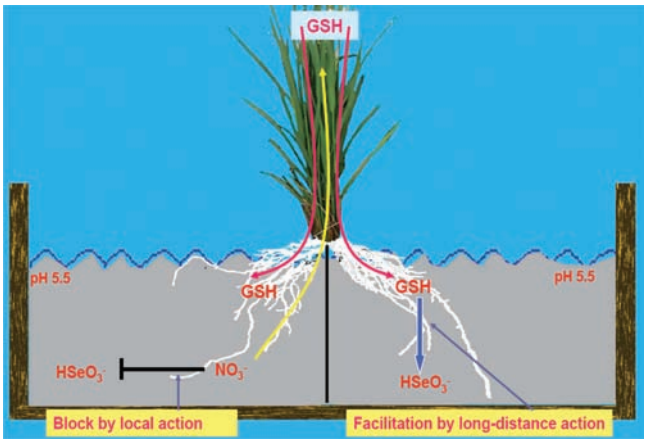

Figure 5. A proposed action mode for nitrate-regulated selenite uptake in rice plants. On one hand, nitrate can partly block selenite uptake by rice roots by local action (corporate supply of nitrate and selenite). On the other hand, under long-distance action (separate supply of nitrate or selenite), nitrate can partly facilitate selenite uptake by rice roots, probably by regulating root GSH content.

A Proposed Action Mode for Nitrate-Regulated Selenite Uptake in Rice Plants. Taken together, using the excised-root or split-root experiments (**Figure 5**), our results suggest that, on one hand, nitrate can partly block selenite uptake by rice roots by local action (corporate supply of nitrate and selenite). On the other hand, under long-distance action (separate supply of nitrate or selenite), nitrate can partly facilitate selenite uptake by rice roots probably by regulating root GSH content. Thus, based on the fact that nitrate can either inhibit or facilitate selenite uptake by rice roots depending on the nitrate supply site, to improve selenite uptake efficiency of rice plants, separate supplies of selenite and nitrate fertilizers in paddy rice soils should be utilized in the future. However, clarifying the actual roles and complexity of nitrate in altering selenite uptake by rice roots remains a challenging goal for future research.

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