Glutathione-Mediated Regulation of ATP Sulfurylase Activity, \( \text{SO}_4^{2-} \) Uptake, and Oxidative Stress Response in Intact Canola Roots

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The dual role of glutathione as a transducer of S status (A.G. Lappartient and B. Touraine [1996] Plant Physiol 111: 147–157) and as an antioxidant was examined by comparing the effects of S deprivation, glutathione feeding, and \( \text{H}_2\text{O}_2 \) (oxidative stress) on \( \text{SO}_4^{2-} \) uptake and ATP sulfurylase activity in roots of intact canola (Brassica napus L.). ATP sulfurylase activity increased and \( \text{SO}_4^{2-} \) uptake rate severely decreased in roots exposed to 10 mM \( \text{H}_2\text{O}_2 \), whereas both increased in S-starved plants. In split-root experiments, an oxidative stress response was induced in roots remote from \( \text{H}_2\text{O}_2 \) exposure, as revealed by changes in the reduced glutathione (GSH) level and the GSH/oxidized glutathione (GSSG) ratio, but there was only a small decrease in \( \text{SO}_4^{2-} \) uptake rate and no effect on ATP sulfurylase activity. Feeding plants with GSH increased GSH, but did not affect the GSH/GSSG ratio, and both ATP sulfurylase activity and \( \text{SO}_4^{2-} \) uptake were inhibited. The responses of the \( \text{H}_2\text{O}_2 \)-scavenging enzymes ascorbate peroxidase and glutathione reductase to \( \text{S} \) starvation, GSH treatment, and \( \text{H}_2\text{O}_2 \) treatment were not to glutathione-mediated \( S \) demand regulatory process. We conclude that the regulation of ATP sulfurylase activity and \( \text{SO}_4^{2-} \) uptake by \( S \) demand is related to GSH rather than to the GSH/GSSG ratio, and is distinct from the oxidative stress response.

In higher plants the activity of root uptake systems is regulated so that the total intake of a nutrient depends on the plant’s need for this element rather than on its concentration in the rooting medium. Experimentally, this behavior is translated into a specific increase of ion uptake rate in plants recovering from hours to days of deficiency of a single element, as has been observed for \( K^+ \) (De la Guardia et al., 1985; Siddiqui and Glass, 1987), \( \text{H}_2\text{PO}_4^- \) (Clarkson and Scarratt, 1982; Drew and Saker, 1984; Lee, 1993), \( \text{NO}_3^- \) (Lee and Rudge, 1986; Bowman et al., 1989; Lee, 1993), and \( \text{SO}_4^{2-} \) (Clarkson and Saker, 1989; Hawkesford and Belcher, 1991; Hawkesford et al., 1993; Lee, 1993; Lappartient and Touraine, 1996). This demand-driven regulation of uptake rate is a fundamental feature contributing to the ability of terrestrial plants to maintain nutrient homeostasis in cells of their various tissues and organs despite frequent variations in the root environment. These demand-driven regulatory processes in root activity appear to be controlled by shoots via phloem-translocated signals, as has been described for \( \text{NO}_3^- \) uptake (Mason and Touraine, 1994; Touraine et al., 1994). In the case of \( \text{SO}_4^{2-} \), enhancement of \( \text{SO}_4^{2-} \) uptake in a given root was observed when other roots were deprived of \( S \) while the root under observation was continuously fed with \( \text{SO}_4^{2-} \) (Clarkson et al., 1983; Lappartient and Touraine, 1996). Both \( \text{SO}_4^{2-} \) uptake and ATP sulfurylase are probably repressed under normal conditions and are derepressed when the \( S \) requirement increases. It has been proposed that the signal responsible for the repression of \( \text{SO}_4^{2-} \) uptake is either internal \( \text{SO}_4^{2-} \) concentration (Datko and Mudd, 1984; Lass and Ullrich-Eberius, 1984) or one of the products of its reduction, such as glutathione (Herschbach and Rennenberg, 1991).

We have previously shown that the \( \text{SO}_4^{2-} \) uptake rate and ATP sulfurylase activity in roots of intact canola correlated negatively with glutathione concentrations in the roots and sieve sap that fed them (Lappartient and Touraine, 1996). Furthermore, feeding roots with exogenous glutathione resulted in an inhibition of \( \text{SO}_4^{2-} \) uptake (Herschbach and Rennenberg, 1994) and ATP sulfurylase activity (Lappartient and Touraine, 1996); similar results were obtained by supplying this compound to roots via the phloem translocation pathway (Lappartient and Touraine, 1996). Overall, these data favor the hypothesis that glutathione could act as a phloem-translocated signal responsible for the regulation of \( \text{SO}_4^{2-} \) uptake and ATP sulfurylase activity in roots.

Glutathione is widely believed to protect against oxidative stress by maintaining cellular redox potential. This thiol compound may react directly with active oxygen species, protect protein thiol groups, form mixed disulfides with proteins, or be involved in enzymatic detoxification of \( \text{H}_2\text{O}_2 \) (for review, see De Kok and Stulen, 1993). Indirect evidence suggests that tissue glutathione concentrations increase under oxidative stress conditions. For instance, the accumulation of glutathione in the leaves of a catalase-
deficient barley mutant was increased 5- to 10-fold when plants grown in an atmosphere enriched in 0.5% CO₂ were subsequently placed in air, whereas in similar conditions wild-type barley plants exhibited no change in leaf glutathione (Smith et al., 1984). Similarly, feeding catalase inhibitors to illuminated, excised shoots of barley, soybean, and tobacco plants resulted in an increase in glutathione content, which was determined to be GSSG (Smith, 1985). An accumulation of glutathione was also observed in low-light-grown wheat seedlings transferred to high-light conditions (Mishra et al., 1995). The activities of enzymes involved in the H₂O₂-scavenging pathway were also observed to increase under various stress conditions (Gossett et al., 1994; Kampfenkel et al., 1995; Mishra et al., 1995; Knörzer et al., 1996).

In summary, the function of glutathione in plants appears to be dual: regulation of S nutrition and defense against oxidative stress. This paper therefore questions the specificity of the demand-driven control of SO₄²⁻ uptake and ATP sulfurylase activity versus oxidative stress responses. For this purpose, we compared the effect of nutritional stress (S starvation and GSH supply) and oxidative stress (H₂O₂ in the nutrient solution) on various root parameters, indicative either of the control exerted on S nutrition (SO₄²⁻ influx and ATP sulfurylase activity) or of oxidative stress responses (GSH and GSSG pools and ascorbate peroxidase and glutathione reductase activities). Because SO₄²⁻ influx in roots may be affected by H₂O₂ in a nonspecific way due to possible oxidation of plasma membranes, our investigations used two different procedures, referred to as whole-root and split-root experiments. In the latter experiments, the effect of a treatment application on a part of the root system was recorded in other roots that were isolated from the exogenous treatment solution. In such an experiment, the responses observed cannot be ascribed to a direct effect of H₂O₂ on root membranes. On the contrary, considering the vascular connections in the whole plant, the circulation of some compound in phloem sap is necessarily involved as a transducing step.

**MATERIALS AND METHODS**

Canola (Brassica napus L. cv Drakkar) plants were hydroponically grown for 21 d as described previously (Lappartient and Touraine, 1996). The culture was carried out in a chamber in which temperature and RH were set at 25°C and 70%, respectively, during the 14-h light period, and at 20°C and 75%, respectively, during the 10-h dark period. Fluorescent lamps provided a PPFD of 400 μmol m⁻² s⁻¹ at shoot level during the daytime. The basic nutrient solution contained 2 mM KNO₃, 1 mM (Ca(NO₃)₂), 1 mM MgSO₄, 1 mM KH₂PO₄, 100 μM NaFeEDTA, 50 μM KCl, 30 μM H₂BO₃, 5 μM MnCl₂, 1 μM CuCl₂, 1 μM ZnCl₂, and 100 mM (NH₄)₂SO₄.

**Treatments**

Fresh nutrient solution was given within the 24 h preceding the start of the experiments. One of three different treatments was applied: S starvation, H₂O₂ treatment, or GSH treatment. Control plants were continuously fed with the culture solution. S starvation was effected by transferring plants to a solution in which MgCl₂ was substituted for MgSO₄ for 0 to 72 h. For the H₂O₂ treatment the culture solution was supplemented with 10 mM H₂O₂ for 0 to 8 h. In preliminary experiments we noticed that the ATP sulfurylase activity of canola roots responded to 1 to 10 mM external H₂O₂ within a few hours. For the purpose of comparing these responses to those of S starvation, we decided to induce oxidative stress using 10 mM H₂O₂ to maximize the effects of H₂O₂ treatment. The treatment was applied for 8 h prior to ATP sulfurylase extraction or SO₄²⁻ uptake rate measurement to achieve maximum response levels without visually detecting toxic symptoms. For the GSH treatment, the culture solution was supplemented with 1 mM GSH for 0 to 36 h. It has been confirmed that by the end of our experiments (24–36 h), the major part (approximately 85%) of the glutathione added to the nutrient solution was still in its reduced form (GSH). As mentioned above, two series of experiments were performed, with treatments being applied either to the entire root system (whole-root experiments) or to about two-thirds of the root system (split-root experiments). In the split-root experiments SO₄²⁻ uptake and ATP sulfurylase activity were assayed in the nontreated roots.

**Enzymatic Activities**

Fresh root tissues were ground rapidly at 4°C in a buffer consisting of 10 mM Na₂EDTA, 20 mM Tris-HCl (pH 8.0), 2 mM DTT, and approximately 0.01 g/mL insoluble PVP using a 1:4 (w/v) tissue:buffer ratio. The homogenate was strained through gauze and centrifuged at 20,000g for 10 min at 4°C. The supernatant (crude extract) was used for in vitro enzymatic assays.

ATP sulfurylase activity in crude extracts was measured using the molybdate-dependent formation of pyrophosphate, as described previously (Lappartient and Touraine, 1996).

For ascorbate peroxidase and glutathione reductase activity measurements, crude extracts were purified on Sephadex G-25 (PD10 column, Pharmacia). Ascorbate peroxidase activity was determined in a reaction mixture containing 135 μM H₂O₂, 330 μM ascorbic acid, 20 mM Tris-HCl (pH 8.0), and 2 mM Na₂EDTA. Oxidation of ascorbic acid was measured at 285 nm. Measurement of glutathione reductase activity involved GSSG-dependent oxidation of NADPH. The reaction mixture contained 500 μM GSSG, 20 mM Tris-HCl (pH 8.0), and 2 mM Na₂EDTA. Reactions were initiated by the addition of 120 μM H₂O₂, and the rate of NADPH oxidation was monitored at 340 nm.

**Sulfate Uptake**

Sulfate influx in roots was estimated from the rate of ³⁵S incorporation over 5 min. The labeled solution was identical to the basic nutrient solution except that the SO₄²⁻ concentration was 0.5 mM. At the onset of the loading period, ³⁵S-labeled Na₂SO₄ (Amersham) was added to the...
uptake solution (specific radioactivity, approximately 5.2 MBq/mmol). Roots were separated from shoots, rinsed for three consecutive 20-s periods in chilled, unlabeled 0.2 mM CaSO₄ solution, and blotted. Root fresh weights were recorded and tissues were digested in 0.1 N HCl for 1 h (20 mL g⁻¹ fresh weight). Radioactivity was determined by liquid scintillation counting (460-C Tri-Carb, Packard, Meriden, CT) and corrected for quenching. Radioactivity translocated to shoots was always negligible (never exceeding 2% of the total activity recovered in the whole plant), so these values are not presented.

**Thiol Determination**

Leaves and roots were frozen in liquid nitrogen immediately after harvesting. Thiolos were extracted by grinding 1 to 2 g fresh weight in 4 mL of a mixture containing 0.1 N HCl, 1 mM Na₂EDTA, and approximately 0.1 g of insoluble PVP. The suspension was centrifuged at 18,000 g for 10 min at 4°C, and the supernatant was removed for analysis.

Thiols from tissue extracts were determined with reverse-phase HPLC after reduction with DTT and derivatization with monobromobimane (Calbiochem) according to the method of Schupp et al. (1992). Using this methodology individual thiols were determined as the sum of their reduced and oxidized forms. This procedure measures precisely the equivalent concentration of the reduced form, either that actually present, as in the extract solution, or that issued from the reduction of the oxidized form extracted from the tissues. The irreversible binding of N-ethylmaleimide on free thiol groups prior to the reduction, derivatization, and chromatography steps was used to specifically determine oxidized thiol compounds (e.g., GSSG but not GSH). Again, not the actual concentration of GSSG, but that of GSH released by the reduction of GSSG was thus measured.

Prior to reduction, 200-μL aliquots were treated with 300 μL of 0.2 M 2-(N-cyclohexylamino)ethanesulfonic acid (pH 9.3) and 150 μM N-ethylmaleimide for 10 min (GSSG determination), whereas similar aliquots received 300 μL of 0.2 M 2-(N-cyclohexylamino)ethanesulfonic acid (pH 9.3) without N-ethylmaleimide. Reduction by 50 μL of 3 mM DTT was carried out in darkness at room temperature for 1 h. For derivatization, 10 μL of 30 mM monobromobimane was added. After 15 min at room temperature in darkness, the reaction was stopped by the addition of 440 μL of 5% acetic acid. Aliquots from standard solutions (10 μM Cys, 10 μM γGluCys, and 100 μM GSH) were submitted to the same reduction and derivatization protocol. Derivatized compounds were separated on a 5-μm, 250- x 4.6-mm column (Hypersil, ODS, Aldrich), and eluted with a gradient of 0.25% (v/v) acetic acid in water (pH 3.9) and methanol. Monobromobimane derivatives were detected fluorometrically (model 821-FP, Jasco, Hachioji City, Japan) at 480 nm (emission) and 380 nm (excitation). The recovery ratios of thiols in the tissue extracts were 92, 93, and 95% for Cys, γGluCys, and GSH, respectively, as determined using internal standards. For comparison purposes, we chose to express data on thiols as equivalent S concentrations rather than to calculate actual concentrations of the reduced and oxidized forms. In practice, the GSSG values given are twice the actual concentration of GSSG, and for total glutathione, values are the concentration of GSH plus twice the concentration of GSSG.

**RESULTS**

**Effects of H₂O₂ on ATP Sulfurylase Activity and SO₄²⁻ Uptake Rate**

In whole-root experiments, treatment with 10 mM H₂O₂ for 8 h resulted in a stimulation of the activity of ATP sulfurylase extracted from roots, but an inhibition of SO₄²⁻ influx (Fig. 1). In split-root experiments, H₂O₂ also inhibited SO₄²⁻ influx in the roots not directly exposed to H₂O₂. In contrast, no change in the activity of ATP sulfurylase extracted from those roots was detected within the 8-h experimental period.

**Effects of H₂O₂, S Starvation, or External GSH on Glutathione Pools**

Tissue analysis from H₂O₂-treated roots (whole-root experiments) revealed a decreased concentration of total glutathione in roots (Fig. 2A), as was also observed in roots from S-starved plants (Fig. 2C). In both cases, GSH concentration declined and GSSG concentration increased. The increase in GSSG, however, did not compensate for all of the GSH lost, which is the reason for the decrease in total

![Figure 1](https://www.plantphysiol.org)
The treatment period prior to harvest.

Treatments were as follows: H$_2$O$_2$, 10 mM GSH for 24 h resulted in a large increase in the total glutathione concentration in roots (Fig. 2E), but no significant change in the GSH/GSSG ratio was observed (GSH-GSSG, 1 mM GSH added to nutrient solution). Note that the ordinate scales in E and F differ from those in C, and GSH, 1 mM GSH added to the nutrient solution. All measurements were in 21-d-old plants, regardless of the length of the treatment period prior to harvest. As mentioned in the text, both GSH and GSSG concentrations are expressed as equivalent S concentrations (hence, the total concentration of glutathione is the concentration of GSH plus twice the concentration of GSSG, and GSH/GSSG is one-half the ratio of the actual concentrations of GSH and GSSG). Note that the ordinate scales in E and F differ from those in A, B, C, and D. Data are means ± SD of five replicates. FW, Fresh weight.

glutathione. Consequently, the GSH/GSSG ratio was dramatically modified by both treatments when applied to the whole root system. Quantitative differences in the relative proportions of GSH and GSSG in roots, however, were evident: whereas only traces of GSH were found in roots of plants fed with 10 mM H$_2$O$_2$ for 4 h (Fig. 2A), one-fourth of the initial GSH content was still present in roots of 72-h S-starved plants (Fig. 2C). Feeding whole roots of plants 1 mM GSH for 24 h resulted in a large increase in the total glutathione concentration in roots (Fig. 2E), but no significant change in the GSH/GSSG ratio was observed (GSH-treated plants, 6.0 ± 2.0; control plants, 7.9 ± 1.4).

Figure 2 reveals a few differences between whole-root and split-root plants in specific changes in GSH and GSSG concentrations. First, internal GSH concentration was almost zero when whole roots were treated with H$_2$O$_2$ for 4 h (Fig. 2A), whereas one-fourth of the initial root concentration remained when H$_2$O$_2$ was applied to split roots for 4 h (Fig. 2B). Accordingly, the increase in GSSG concentration was lower in split-root experiments than in whole-root experiments. After an 8-h treatment, however, there were no significant differences between the root GSH and GSSG concentrations of plants from whole-root and split-root experiments. Second, both GSH and GSSG concentrations decreased after 72 h of S starvation in split-root experiments (no change in GSH/GSSG ratio; Fig. 2D), whereas GSH concentration declined and GSSG concentration increased when whole roots were starved of S for the same period of time (decline in GSH/GSSG ratio; Fig. 2C).

Effects of Oxidative and Nutritional Stresses on the Activities of Ascorbate Peroxidase and Glutathione Reductase

Treatments with 10 mM H$_2$O$_2$ (Fig. 3) and 1 mM GSH (Fig. 4) both stimulated ascorbate peroxidase activity. Despite the difference in the experiments' time scales, GSH was seemingly able to promote much higher ascorbate peroxidase activities than H$_2$O$_2$. S deprivation also increased ascorbate peroxidase activity (Fig. 5), but to a lesser extent than the H$_2$O$_2$ treatment. Furthermore, the response was delayed by at least 24 h, and the activity decreased again between the 2nd and the 3rd d of culture on S-free solution. The resupply of SO$_4^{2-}$ resulted in a further decrease of ascorbate peroxidase activity, which reached a level lower than in control plants within 1 d.

Glutathione reductase activity showed an inverse response to ascorbate peroxidase activity on H$_2$O$_2$ treatment (Fig. 3). Moreover, the time-course patterns of these responses were different: whereas the maximum inhibition of glutathione reductase activity was obtained within 2 h, the stimulation of ascorbate peroxidase activity was only evident in plants treated with H$_2$O$_2$ for 2 h or more. Conversely, GSH enhanced both glutathione reductase and ascorbate peroxidase activities, although not to the same extent (Fig. 4). Furthermore, the effect of GSH on glutathione reductase activity was delayed by 12 h at least and

Figure 2. Effect of H$_2$O$_2$ (A and B), S starvation (C and D), and GSH (E and F) treatments on glutathione pools in the roots of intact canola plants. Plants were grown and treated according to the whole-root (A, C, and E) or the split-root (B, D, and F) procedure, as in Figure 1. Treatments were as follows: H$_2$O$_2$, 10 mM H$_2$O$_2$ added to nutrient solution for 4 or 8 h as indicated; -S, feeding with a nutrient solution without S-starved plants (Fig. 2C). Feeding whole roots of plants 1 mM GSH for 24 h resulted in a large increase in the total glutathione concentration in roots (Fig. 2E), but no significant change in the GSH/GSSG ratio was observed (GSH-GSSG, 1 mM GSH added to nutrient solution). Note that the ordinate scales in E and F differ from those in A, B, C, and D. Data are means ± SD of five replicates. FW, Fresh weight.

Figure 3. Time-course responses of the activities of ascorbate peroxidase and glutathione reductase to H$_2$O$_2$ treatment. Canola plants were grown as in Figure 1. H$_2$O$_2$ (10 mM) was supplied at time zero. Enzyme activities were assayed in vitro on crude root extracts. Data are means ± SD of five replicates. prot, Protein.
Figure 4. Time-course responses of the activities of ascorbate peroxidase and glutathione reductase to GSH treatment. Canola plants were grown as in Figure 1. Glutathione was supplied to plants as 1 mM GSH at time zero. Enzyme activities were assayed in vitro on crude root extracts. Data are means ± SD of five replicates. prot, Protein.

seemed transitory. Transferring plants to a S-free solution resulted in a regular decline in glutathione reductase activity throughout the 3-d experiment (Fig. 5). Conversely, supplying SO₄²⁻ to the roots of S-deficient plants resulted in the partial recovery of glutathione reductase activity (approximately 20% of the activity measured in plants continuously fed with SO₄²⁻ was recovered in the roots starved of S for 3 d within 24 h of SO₄²⁻ resupply).

DISCUSSION

We previously reported that the increases in ATP sulfurylase activity and SO₄²⁻ uptake rate in S-starved canola roots are correlated with a loss of tissue glutathione (Lappartient and Touraine, 1996). The ATP sulfurylase activity also increased in roots supplied with 10 mM H₂O₂ (Fig. 1), and this activity was negatively correlated with internal glutathione levels (Fig. 2A). These similarities between oxidative stress and S starvation, therefore, are consistent with the hypothesis that glutathione is responsible for the observed stimulation of ATP sulfurylase activity. H₂O₂, however, failed to stimulate ATP sulfurylase activity in the roots remote from the site of application (Fig. 1, split root). The oxidative stress, however, is likely to induce a variety of responses, including shifts in antioxidant levels (especially glutathione) and changes in enzymatic activities, following a sequence of events that differs from those induced by decreased or increased S status. In this context, the combination of various glutathione-mediated or glutathione-independent effects may have hidden the stimulation of ATP sulfurylase activity linked to decreased internal glutathione concentration when oxidative stress was applied in a split-root experiment.

In most studies of canola, SO₄²⁻ influx in roots increased or decreased together with ATP sulfurylase activity. This was the case in roots from S-deficient and GSH-fed plants, both in whole-root and split-root experiments (Lappartient and Touraine, 1996). In contrast, H₂O₂ inhibited SO₄²⁻ influx in both whole-root and split-root experiments, whereas ATP sulfurylase activity increased or remained unchanged, respectively (Fig. 1). Considering the whole-root experiment alone, the possible oxidation of the plasma membrane of root cells, which in turn would nonspecifically affect ion uptake, provides a feasible explanation for SO₄²⁻ influx inhibition. However, the observation that a similar response pattern was observed in roots that did not experience exogenous H₂O₂ (split-root experiment) leads us to dismiss this possibility. Nevertheless, the failure of H₂O₂ treatment to stimulate ATP sulfurylase activity in the split-root experiments is explained by the fact that SO₄²⁻ is likely to be inhibited by oxidative stress independent of glutathione pool variations, which would hide its expected stimulation by the decreased glutathione level. Oxidative-stress-like responses, therefore, cannot account for the demand-driven regulation of ATP sulfurylase activity and SO₄²⁻ uptake.

To investigate further the relationship between nutritional and oxidative stresses in canola roots, the activity of two enzymes involved in antioxidative defense, ascorbate peroxidase and glutathione reductase, have been studied for their responses to the treatments used here. Foyer et al. (1991) reported a decrease in glutathione reductase activity in tobacco leaves treated with methyl viologen (an H₂O₂ provider) in the light. Consistent with this report, H₂O₂ led to reduced glutathione reductase activity in

Figure 5. Time-course responses of the activities of ascorbate peroxidase and glutathione reductase to S starvation. Canola plants were grown as in Figure 1 and transferred to S-free solution (−S) for 1 to 3 d, or to S-free solution and then back to complete solution (+S) for 1 d, prior to harvest. All measurements were in 21-d-old plants, regardless of the length of the treatment period. Enzyme activities were assayed in vitro on crude root extracts. Data are means ± SD of five replicates. prot, Protein.
canola roots (Fig. 3). Conversely, ascorbate peroxidase activity appeared to be stimulated by this treatment. Similar response patterns of each activity were noted in plants fed with S-free solutions (Fig. 5), although the time courses cannot be compared. Quantitative changes in activities, however, highlighted differences between their responses to either stress. Although ascorbate peroxidase activity stimulation in 48- to 72-h S-starved roots and 6-h H$_2$O$_2$-treated roots were similar (2- to 2.4-fold), the activity of glutathione reductase was inhibited 4-fold after 2 to 6 h of H$_2$O$_2$ treatment, versus an inhibition of 15-fold or more by 48 to 72 h of S starvation. In contrast to these response patterns, both enzymes displayed higher activities in GSH-treated roots (1 mM GSH for 24 h resulted in 50- and 2-fold increases in ascorbate peroxidase and glutathione reductase activities, respectively; Fig. 4). Comparing the three different treatments applied, the activity of glutathione reductase thus changed in the opposite direction than ATP sulfurylase activity. In contrast, the activities of ATP sulfurylase and ascorbate peroxidase varied either in the same direction under S starvation or direction than ATP sulfurylase activity. In the case of ATP sulfurylase and ascorbate peroxidase, no correlation between the activity of the H$_2$O$_2$-scavenging enzymes studied and the level of S demand as sensed by the internal glutathione level. Finally, no general pattern is evident that would support the hypothesis that the responses of ATP sulfurylase activity to changes in S demand may result from an oxidative stress, as was proposed for the response to iron excess (Kampfenkel et al., 1995).

Considering that two forms of glutathione, GSH and GSSG, coexist in plant tissues, four parameters are putative candidates for the demand-driven control of SO$_4^{2-}$ influx and ATP sulfurylase activity: the concentration of total glutathione, the concentration of GSH, the concentration of GSSG, and the glutathione redox state (GSH/GSSG ratio). In normal culture conditions, glutathione was predominantly recovered as GSH. The GSH/GSSG ratio in the leaves of barley, soybean, and tobacco (Smith, 1985), and in Nicotiana plumbaginifolia supplied with an excess of iron (Kampfenkel et al., 1995). Likewise, feeding roots with a SO$_4^{2-}$-free culture solution (whole-root experiments) induced a shift in root glutathione pools due to opposite variations of GSH and GSSG concentrations (Fig. 2C). In both cases, whole-root experiments suggest that ATP sulfurylase activity is negatively correlated with total glutathione, the GSH pool, and the GSH/GSSG ratio, whereas it is positively correlated with the GSSG pool.

Split-root experiments show that the above conclusion is not general. In the case of S starvation, there was no shift in reduced and GSSG pools in roots that remained supplied with the complete nutrient solution (Fig. 2D), which exhibited concomitant decreases in GSH and GSSG concentrations. This indicates that the increase in GSSG concentration in roots fed with the solution lacking SO$_4^{2-}$ (whole-root experiments; Fig. 2C) was not the cause of enhanced ATP sulfurylase activity upon S starvation. Furthermore, it is unlikely that this activity is regulated according to the glutathione redox state.

Consistent with the hypothesis that internal glutathione exerts a control over ATP sulfurylase activity in roots, glutathione supplied as GSH inhibited this activity (Lappartient and Touraine, 1996). The ability of plant cells to take up GSH has been demonstrated using photoheterotrophic and heterotrophic tobacco cell suspensions (Rennenberg et al., 1988; Schneider et al., 1992). Furthermore, the same studies showed that GSH uptake in heterotrophic cells, which are considered a model system for root cells, is unaffected by external SO$_4^{2-}$. Adding GSH to our SO$_4^{2-}$-containing nutrient solution was, therefore, expected to affect internal glutathione concentration. Indeed, roots treated with 1 mM glutathione exhibited a large increase in glutathione concentration (Fig. 2E). The reduced activity of ATP sulfurylase suggests that increased internal glutathione concentration mimicked a reduction in the level of S demand, and that, conversely, decreased internal glutathione concentration was involved in the stimulation of both activities by S starvation (Lappartient and Touraine, 1996).

In contrast to what was observed in roots of S-starved plants, root GSH and GSSG concentrations were not differentially changed in GSH-fed roots (Fig. 2E). As a consequence, the redox state of glutathione remained essentially unchanged, indicating that this compound may act as a repressor of ATP sulfurylase activity and SO$_4^{2-}$ uptake independent of any oxidative stress. Split-root experiments showed that phloem-translocated glutathione exerted a negative control on root ATP sulfurylase activity, just like the GSH supplied directly to the roots (Lappartient and Touraine, 1996). Furthermore, the profile for the glutathione pools in whole and split roots in 24-h GSH-treated plants were not significantly different (Fig. 2, E and F). Combining the data on internal glutathione pools from each treatment (H$_2$O$_2$ supply, S starvation, GSH supply within whole or split roots) reveals that ATP sulfurylase activity and SO$_4^{2-}$ uptake increases are correlated with a decrease in tissue GSH concentration and total glutathione concentration, whereas no consistent relationships were observed with tissue GSSG concentration and the redox state of glutathione.

In summary, our study shows that even if there are similarities between oxidative stress and S nutritional stress, the demand-driven regulation and oxidative stress responses of ATP sulfurylase activity are independently exerted. This is even more obvious for SO$_4^{2-}$ uptake, since SO$_4^{2-}$ influx was differentially affected by oxidative and nutritional stresses. Moreover, the changes in glutathione pools (GSH and GSSG) and, subsequently, in glutathione...
redox state under the various treatments applied (S starvation, GSH, and H$_2$O$_2$) are not consistent with the hypothesis that oxidative-stress-like responses would be involved in the control exerted by glutathione on ATP sulfurylase activity. Furthermore, the differences in the response patterns of ascorbate peroxidase and ATP sulfurylase activities support the conclusion that ATP sulfurylase activity is independently regulated by each stress. Finally, either GSH or total glutathione (which is present mainly as GSH), but not GSSG or the glutathione redox state, is responsible for the demand-driven regulation of SO$_4^{2-}$ influx and ATP sulfurylase activity in roots.

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**LITERATURE CITED**


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