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Sulphate-uptake rate under the influence of exogenous supply of various S-metabolites

Thirty-day-old Arabidopsis thaliana plants, grown on sulphur-sufficient medium, were transferred to sulphur-deficient solution for 3 days. These were treated with 250 μM each of cysteine (T2), methionine (T3), glutathione (T4), O-acetyl serine (T5) and -S (T1) and sulphate-uptake rate was measured at 2, 4, 6, 8, 10 and 12 h of incubation in MES-1.5 mM SO₄²⁻ solution through sulphate-depletion method. The results on the effect of various treatments (T1-T5) on the sulphate-uptake rate are depicted in Fig. 5. Irrespective of treatments, the rate of sulphate uptake increased up to 8 h of incubation. No further increase in the uptake rate was observed. The maximum sulphate uptake rate was observed with T1 treatment. Minimum sulphate uptake rate was observed in control plants (+S). Compared with T1, the uptake rate was lesser when treated with various metabolites. The maximum reduction in the uptake rate was observed with glutathione treatment (T4), which resulted in 44-47% reduction in comparison with T1. Besides, T2 reduced the uptake rate by 33-38% and T5 reduced it by 8-15% over T1. The uptake rate at T4 was almost similar to the control, whereas T3 was similar to T5.
Fig. 5. Sulphate uptake rate of *A. thaliana* at various treatments of S-metabolites. Values are means of three independent replicates. SE is shown by vertical bars.
Transcriptional expression level of high affinity sulphate transporter by exogenous supply of various S-metabolites

Expression of AtSultr1;1 in the root of Arabidopsis thaliana was observed in response to various sulphur metabolite treatments which were used for analyzing the sulphate-uptake rate. The expression was remarkably changed under the treatments of various metabolites (Fig. 6). A rapid induction in the expression level was observed in all the plants as soon as these were exposed to S-metabolites. Significant differences in expression level were observed under various metabolite treatments. The highest expression was found in the T1 treated plants. Expression level was significantly reduced in the plants treated with T4, when compared to T1 treated plants.
Fig. 6. Relative expression level of high-affinity sulphate transporter, AtSultr1;1 in root of S-starved Arabidopsis thaliana under the treatments of various metabolites. The mRNA contents were determined by real time RT-PCR. Means of independent triplicates samples and SD values (n =3) are indicated. S-deprived plants were transferred to the uptake medium containing 250 µM each of cysteine (T2), methionine (T3), glutathione (T4), OAS (T5) and sulphate (T6). One set of plants was without sulphate. The mRNA contents are indicated as relative values against T1 treatment.
Activities and transcriptional expression level of S-assimilatory enzymes under short-term exogenous supply of S-metabolites

Thirty-day-old *A. thaliana* plants, grown on the sulphur-sufficient medium, were transferred to sulphate-deficient solution. In place of sulphate, various S-metabolites viz., cysteine (T2), methionine (T3), glutathione (T4) and O-acetyl serine (T5) were added. One set of plants, maintained with sulphate, served as control (T1). Activities of S-assimilatory enzymes were measured at 1, 3, 5 and 7 days after addition of S-metabolites, and comparison was made with the control. The data are presented in figures (7-10). At the 1-day of treatment, there was no significant change in the activities of S-assimilatory enzymes, when compared with the control (Fig. 7). Sulphur-metabolites treatments for 3, 5 and 7 days caused significant reduction in the activities of S-assimilatory enzymes. However, the level of reduction in the enzyme activity varied with the type of metabolite and days of treatments. After 3 days of treatments, the activity of ATP Sulphurylase was reduced by 5%, 22%, 32% and 3% over the control when treated by cysteine, methionine, glutathione and O-acetyl serine respectively. Corresponding reductions were 45%, 45%, 55% and 3% respectively, in APS-reductase activity; 9%, 16%, 25% and 5%, respectively, in sulphite reductase activity and 9%, 15%, 25% and 3%, respectively, in the O-acetyl serine (thiol) lyase activity (Fig. 8). S-metabolites treatments for 5 days caused further reduction in the activities of S-assimilatory enzymes. The reduction was 7%, 13%, 27%, 3% in ATP-sulphurylase activity, 54%, 54%, 44%, 3% in APS-reductase activity, 12%, 14%, 24%, 11% in sulphite-reductase activity and 13%, 18%, 31%, 5% in O-
acetyl serine (thiol) lyase activity by the treatment of cysteine, methionine, glutathione and O-acetyl serine, respectively, when compared with control (Fig. 9). The treatments of S-metabolites for 7 days reduced the ATP sulphurylase activity by 8%, 17%, 23% and 6%, APS-reductase activity by 20%, 29%, 35% and 18%, sulphite reductase activity by 4%, 4%, 6% and 1%, and O-acetyl serine (thiol) lyase activity by 10%, 8%, 16% and 5% with the treatments of cysteine, methionine, glutathione and O-acetyl serine, respectively (Fig. 10). Relative expression levels of genes of S-assimilatory enzymes (ATPS, APR, SiR and OAS-TL) were measured with respect to control. The relative expression pattern followed the same trend as observed above for activities of these enzymes under the influence of exogenous supply of S-metabolites (Fig. 11-14).
Fig. 7. Activities of S-assimilatory enzymes in the leaves of 30-day-old *A. thaliana* at 1 day treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.
Fig. 8. Activities of S-assimilatory enzymes in 30-day-old *A. thaliana* at 3 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.
Fig. 9. Activities of S-assimilatory at 5 days of treatments of S-metabolites. Values are means of three enzymes in 30-day-old A. thaliana replicates. Bars indicate SE.
Fig. 10. Activities of S-assimilatory enzymes in 30-day-old A. thaliana at 7 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.
Fig. 11. Relative expression level of ATPS, APR, SiR and OAS-TL in leaves of 30-day-old Arabidopsis thaliana under the treatments of various metabolites for 1 day. The mRNA contents were determined by real time RT-PCR. Means of independent triplicates samples and SD values (n =3) are indicated as vertical bars. S-deprived plants were transferred to the nutrient medium containing 250 µM each of cysteine, methionine, glutathione and OAS. One set of plants was sulphate (Control). The mRNA contents are indicated as relative values against control treatment.
Fig. 12. Relative expression level of ATPS, APR, SiR and OAS-TL in leaves of 30-day-old *Arabidopsis thaliana* under the treatments of various metabolites for 3 days. The mRNA contents were determined by real time RT-PCR. Means of independent triplicates samples and SD values (n =3) are indicated as vertical bars. S-deprived plants were transferred to the nutrient medium containing 250 µM each of cysteine, methionine, glutathione and OAS. One set of plants was sulphate. The mRNA contents are indicated as relative values against control treatment.
**Fig. 13.** Relative expression level of ATPS, APR, SiR and OAS-TL in leaves of 30-day-old *Arabidopsis thaliana* under the treatments of various metabolites for 5 days. The mRNA contents were determined by real time RT-PCR. Means of independent triplicates samples and SD values (n =3) are indicated as vertical bars. S-deprived plants were transferred to the nutrient medium containing 250 µM each of cysteine, methionine, glutathione and OAS. One set of plants was sulphate. The mRNA contents are indicated as relative values against control treatment.
Fig. 14. Relative expression level of ATPS, APR, SiR and OAS-TL in leaves of 30-day-old Arabidopsis thaliana under the treatments of various metabolites for 7 days. The mRNA contents were determined by real time RT-PCR. Means of independent triplicates samples and SD values (n =3) are indicated as vertical bars. S-deprived plants were transferred to the nutrient medium containing 250 µM each of cysteine, methionine glutathione and OAS. One set of plants was sulphate. The mRNA contents are indicated as relative values against control treatment.
Endogenous level of S-metabolites in the leaves under the influence of exogenous supply of S-metabolites

Sulphate content in the leaves of *A. thaliana* varied remarkably under the influence of sulphur metabolite treatments for 1, 3, 5 and 7 days (Fig. 15-19). Sulphate content increased continuously with days of treatments of the exogenous supply of sulphate. With the treatment of cysteine and methionine, there was no significant change in sulphate content in the leaves at 1, 3, 5 and 7 days. Contrary to this, the treatments of glutathione and O-acetyl serine resulted in significant decline in the level of sulphate in the leaves of *A. thaliana* with increase in the days of treatments. The treatment of glutathione reduced the content of sulphate by 15%, 42% and 59% at 3, 5 and 7 days of treatments respectively, when compared with the level of sulphate at day 1. The decline in sulphate level was 38%, 65% and 91% by the treatment of OAS for 3, 5 and 7 days respectively as compared with the level of sulphate at day 1 with this treatment. Exogenous supply of sulphur-metabolites for 1, 3, 5, and 7 days affected the level of cysteine in the leaves of *A. thaliana* (Fig. 16) but the effect was dissimilar. Treatments of sulphate, cysteine and GSH enhanced the level of cysteine in the leaves with increase in the days of treatments. OAS and methionine caused no significant effect. No significant change in the endogenous level of methionine was observed under the influence of exogenous supply of S-metabolites (Fig. 17). However, the level of glutathione in the leaves increased by the exogenous supply of S-metabolites for 5 and 7 days (Fig 18). Similarly, the level of OAS in the leaves
was increased by the exogenous supply of S-metabolites for 5 and 7 days (Fig. 19).

**Fig. 15.** Endogenous level of sulphate in the leaves of 30-day-old *A. thaliana* at 1, 3, 5 and 7 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.
Fig. 16. Endogenous level of cysteine in the leaves of 30-day-old *A. thaliana* at 1, 3, 5 and 7 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.

Fig. 17. Endogenous level of methionine in the leaves of 30-day-old *A. thaliana* at 1, 3, 5 and 7 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.
Fig. 18. Endogenous level of glutathione in the leaves of 30-day-old *A. thaliana* at 1, 3, 5 and 7 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.

Fig. 19. Endogenous level of O-acetyl serine in the leaves of 30-day-old *A. thaliana* at 1, 3, 5 and 7 days of treatments of S-metabolites. Values are means of three replicates. Bars indicate SE.
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Change in the activities of S-assimilatory enzymes during the life cycle of A. thaliana

Activity of S-assimilatory enzymes in the leaves was analyzed during various developmental stages of plants under the control and treated conditions. In the control plants, the activity of sulphur assimilatory enzymes was the maximum in youngest plants (30 days old) and declined progressively during development (Fig. 20-23). Activities of ATP sulphurylase, OAS-TL and SiR showed a linear, 3-fold decline between 30 and 61 d after germination. The activity of APS Reductase increased from 14-30 days, the activity stabilized at 30-47 days, sharply declined at 47-61 days and finally became constant at 61-67 days. Thus, activities of all enzymes declined approximately 3-fold during the development, but the decline in APS reductase was much more rapid than in other enzymes. The S-metabolites treatments caused decline in the activities of these enzymes. The maximum effect was observed with the treatment of glutathione throughout the developmental stages of plants.
Fig. 20. Activity of ATP sulphurylase in the leaves of Arabidopsis thaliana at various phenological stages under the treatments of various S-metabolites. The values are means of independent triplicates samples. Vertical bars show SE (n=3).

Fig. 21. Activity of APS Reductase in the leaves of Arabidopsis thaliana at various phenological stages under the treatments of various S-metabolites. The values are means of independent triplicates samples. Vertical bars show SE (n=3).
Fig. 22. Activity of Sulphite Reductase in the leaves of *Arabidopsis thaliana* at various phenological stages under the treatments of various S-metabolites. The values are means of independent triplicates samples. Vertical bars show SE (n =3).

Fig. 23. Activity of O-acetyl serine thiol lyase in the leaves of *Arabidopsis thaliana* at various phenological stages under the treatments of various S-metabolites. The values are means of independent triplicates samples. Vertical bars show SE (n =3).
ORGAN-SPECIFIC ACTIVITY OF S-ASSIMILATORY ENZYMES

Organ-specific activity of S-assimilatory enzymes (ATP sulphurylase, APR reductase, sulphite reductase and OAS-TL) in 60-day-old Arabidopsis thaliana is depicted in Figs. 24, 25, 26 and 27. As shown in the previous figure, enzymatic activity was the maximum in vegetative stage, declined during flowering stage, and further declined during silique stage. Among plant organs, the activity of all the S-assimilatory enzymes was highest in leaves, followed by flowers. The ATP-sulphurylase activity was minimum in the shoot, while APS reductase activity was minimum in the root. Sulphite reductase activity was minimum in the silique and OAS-TL activity was minimum in the stem (Fig. 24). Endogenous level of various S-metabolites in different plant organs of 60-day-old Arabidopsis plant is shown in Fig. 25. The content of cysteine, methionine, glutathione and OAS was the maximum in flowers followed by the leaves and the shoot. The minimum content of S-metabolite was reported in roots.

Activities of enzymes involved in assimilatory sulphate reduction were determined at different stages of leaf maturity. ATP-sulphurylase activity declined slightly, but not significantly, from 15.2 nmol ATP mg⁻¹ protein min⁻¹ in developing leaves and to 14.6 nmol ATP mg⁻¹ protein min⁻¹ in old leaves. APS reductase activity was the maximum in young leaves. Sulphite reductase activity declined slightly from 2.8 nmol cysteine mg⁻¹ protein min⁻¹ in young developing leaves to 2.6 nmol cysteine mg⁻¹ protein min⁻¹ in old mature leaves. O-acethylserine (thiol) lyase activity was similar in all the leaf-
development stages (Fig. 26). The endogenous level of various S-metabolites in the young, 70% expanded, mature and old leaves of 60-day-old *A. thaliana* is depicted in Fig. 27. Level of S-metabolite was maximum in young leaves and minimum in mature leaves.

![Graphs showing activities of S-assimilatory enzymes](image)

**Fig. 24.** Activities of S-assimilatory enzymes in various organs of 60-day-old *A. thaliana*
Fig. 25. Endogenous level of various S-metabolites in various organs of 60-day-old A. thaliana
Fig. 26. Activities of S-assimilatory enzymes in young developing, 70% expanded, mature and old leaves of 60-day-old *A. thaliana*.
**Fig. 27.** Endogenous level of various S-metabolites in young developing, 70% expanded, mature and old leaves of 60-day-old *A. thaliana*