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Effect of H_2S Exposure on ^{35}S-Sulfate Uptake, Transport and Utilization in Curly Kale

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With 2 figures

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Summary

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When *Brassica oleracea* L. was exposed to 0.2 µl l^{-1} H_2S the sulfate uptake measured during a dark or light period was decreased to the same extent. Both the xylem loading and the net sulfate uptake rate were decreased by 42% after 6 days of exposure to 0.4 µl l^{-1} H_2S. This suggested that the xylem loading was not the limiting factor in the uptake of sulfate by the roots. When *Brassica oleracea* L. was exposed to 0.4 µl l^{-1} H_2S and grown on a nutrient solution containing ^{35}S-sulfate, the specific radioactivity of the labeled S of the insoluble fraction (mainly proteins) of the shoot and roots was decreased more than the specific radioactivity of the labeled S of the soluble fraction (mainly sulfate). This demonstrated that the major proportion of the metabolized atmospheric H_2S was incorporated into proteins. The change in partitioning of the labeled sulfur upon H_2S exposure was similar in the roots compared to the shoot, which suggested that the roots were dependent on the shoot for their organic sulfur supply.

Zusammenfassung

WESTERMAN S., WEIDNER W., DE KOK L. J. & STULEN I. 2000. Einfluss von H_2S Begasung auf ^{35}S-Sulfataufnahme, Transport und Verwertung in Grünkohl. – Phyton (Horn, Austria) 40 (2): 293–302, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Sobald *Brassica oleracea* L. 0.2 µl l^{-1} H_2S ausgesetzt wurde, sank die Sulfataufnahme sowohl während der Dunkel- als auch während der Lichtperiode im gleichen Ausmass. So verminderte sich das Xylem loading und die Nettosulfataufnahmerate nach 6 Tagen Begasung mit 0.4 µl l^{-1} um 42%. Dies weist daraufhin, dass das Xylem loading nicht den begrenzenden Faktor bei der Aufnahme von Sulfat durch die Wurzeln darstellt. Bei der Begasung von *Brassica oleracea* L. mit 0.4 µl l^{-1} H_2S und gleichzeitigem Wachstum auf einer Nährlösung, die ^{35}S-Sulfat enthielt, sank die spezifische Radioaktivität des markierten S in der unlöslichen Fraktion (hauptsächlich Proteine) des Sprosses und der Wurzeln stärker als die spezifische Radioaktivität des markierten S in der löslichen Fraktion (hauptsächlich Sulfat). Dies zeigt, dass die Hauptmenge an umgesetzten gasförmigen H_2S in Proteine eingebaut wurde. Der Wechsel in der Aufteilung von markiertem Schwefel nach H_2S Begasung war ähnlich in den Wurzeln wie in den Sprossen, was darauf hindeutet, dass die Wurzeln in ihrer Versorgung mit organischem Schwefel von den Sprossen abhängig sind.

Introduction

Shoots form an active sink for atmospheric H_2S, which is taken up via the stomates, metabolized with high affinity into cysteine and subsequently incorporated into other organic sulfur compounds (De Kok 1989, De Kok & al. 1991, 1998). Atmospheric H_2S can be used as the sole sulfur source for growth (De Kok & al. 1997, 1998).

The sulfate uptake by the roots and its loading into the xylem may be reduced upon H_2S exposure (Brunold & Erismann 1974, Herschbach & al. 1995a,b, De Kok & al.1997, 1998). It was suggested that the xylem loading site is most sensitive to a change in the sulfur nutritional status of the plant (Herschbach & al. 1995a,b). The uptake of sulfate into roots is mainly a metabolic process mediated by transporter proteins and appears to be strongly related to the sulfur nutritional status of the plant, with sulfate, *O*-acetyl serine, or thiol compounds being the most likely regulators (Clarkson & al. 1993, Hawkesford & Smith 1997). When curly kale (*Brassica oleracea* L.) was exposed to 0.2 µl l^{-1} H_2S, a level sufficient to meet its sulfur demand for growth, it resulted in a partial (maximal 50%) repression in sulfate uptake by the roots (De Kok & al. 1997, 1998, Westerman & al. 2000). When plants were grown with H_2S as the sole source of sulfur the total organic sulfur content of plants was 40% of that of the plants grown at ample sulfate supplied to the roots (De Kok & al. 2000). The total sulfur content of the latter plants, however, was not substantially affected upon prolonged exposure to H_2S (De Kok & al. 2000).

Whether the proportion of the sulfate taken up by the roots that was incorporated into the organic sulfur pool was replaced by sulfur absorbed from the atmosphere was investigated in the present study. The partitioning of ^{35}S-sulfur in the organic and inorganic fraction was measured in the shoot and roots of curly kale upon exposure to a H_2S level that was suffi-

cient to meet its organic sulfur demand. Furthermore it was investigated whether the decreased sulfate uptake occurring upon H_2S exposure was affected by a transfer of plants from dark to light, and it was studied how the loading of sulfate in the xylem was regulated upon H_2S exposure.

Materials and Methods

Seeds of curly kale (*Brassica oleracea* L., cv. Bornick F1 (Nickerson-Zwaan, The Netherlands)) were germinated in vermiculite in a climate-controlled room. Day and night temperatures were 22 and 18 °C, respectively, the relative humidity was 65 ± 5% and the photoperiod was 12 h at a photon fluence rate of 250 μmol m^{-2} s^{-1} (within the 400–700 nm range). In all studies 12-day-old seedlings were transferred to a 25% Hoagland nutrient solution (30-l tanks, 60 plants per tank). Exposure to H_2S took place in cabinets after transfer of the plants to 12-l stainless steel containers (19.5 × 15.0 × 45.0 cm) filled with 25% Hoagland nutrient solution at 0.5 mM sulfate.

Plants were exposed to 0, 0.2 or 0.4 μl l^{-1} H_2S in 150-l cylindrical stainless steel fumigation cabinets (diameter 0.6 m) with a polycarbonate top, as described by Stuiver & al. 1992. Day and night temperatures were 22 and 16 °C (± 1 °C), respectively, relative humidity was 55 ± 5% and the photoperiod was 14 h at a photon fluence rate of 200–250 μmol m^{-2} s^{-1} (within the 400–700 nm range).

For measurements on the sulfate and nitrate uptake during a light or dark period, 3 plants were placed on vessels containing exactly 1 l of freshly prepared 25% Hoagland nutrient solution at 0.5 mM sulfate. At the start of the uptake measurements and after the 12-h dark period, the 12-h light period or after 24 h, a sample was taken from the nutrient solution after adjusting the nutrient solution to the original volume. The anions were determined by HPLC after separation on an Iono-Spher A anion exchange column (250 × 4.6 mm; Chrompack, Middelburg, The Netherlands) and 25 mM potassium biphthalate (pH 4.3), containing 0.02% NaN_3, was used as a mobile phase. The flow rate was 1 ml min^{-1}; detector temperature was kept at 25 °C by a waterbath (Maas & al. 1986). The anion uptake was calculated as the difference in ion content (μmol) between samples taken at the start and after 12 or 24 h of exposure, divided by the root fresh weight (g) after 12 or 24 h, and expressed as μmol g fresh $weight^{-1}$ h^{-1}.

H_2S uptake by plant shoots was measured in cylindrical 5 l cuvettes, over four parallel measurements as described by De Kok & al. 1997. The H_2S uptake and transpiration were derived from the inlet and outlet port of the cuvette, air flow through the cuvette and total shoot weight of the exposed plants, and calculated according to De Kok & al. 1991.

^{35}S-sulfate uptake and transport to the shoot were initiated by transferring 3 plants to vessels containing exactly 1 l of freshly prepared 25% Hoagland nutrient solution at 0.5 mM sulfate containing 44.5 pmol ^{35}S-sulfate (ICN biomedicals, Zoetermeer, the Netherlands). The specific radioactivity of the 25% Hoagland nutrient solution was 5.5–6.5 MBq/mol SO_4. The ^{35}S-sulfate uptake and its transport to the shoot was terminated after 4 h, 24 h and 6 d. For this purpose roots were washed three times in 250 ml of demineralized water. Plants were then divided into roots and shoots, weighed and dried at 85 °C in a drying oven.

Analysis of labeled S in the samples was performed according to Schulte & al. 1998 and Herschbach & al. 1995a. Dried material was powdered in a mortar. Sulfate

and water-soluble organic compounds were extracted in 0.1 N HCl. The dried material was placed in 0.1 N HCl at a ratio of 1:30 to 1:80 w/v and placed in a waterbath for 15 min at 95 °C. From each sample two aliquots of 500 µl were transferred to 4 ml plastic vials (Pony vials, Packard, Groningen, The Netherlands) and 4 ml of scintillation fluid (Ultima Gold XR, Packard, Groningen, the Netherlands) was added. Radioactivity was counted in a liquid scintillation counter (Tri-Carb 2000 CA, Packard Instrument Company, Meriden, USA) at counting efficiencies of 99±6% without correction for quenching.

For determination of the total amount of labeled S incorporated, aliquots of 40–80 mg powdered dried material were weighed into 20 ml glass scintillation vials (LSC vials, Packard, Groningen, The Netherlands). 400 µl of H_2O_2 was added to the samples which were then placed at 40 °C in a drying oven for bleaching for 5 days. The bleached samples were exposed to 1 ml of tissue solubilizer (Soluene-350, Packard, Groningen, the Netherlands), and incubated at room temperature for 24 h. 200 µl of isopropanol was added and the samples were bleached overnight after addition of 300 µl of 30% H_2O_2. Thereafter, 15 ml of scintillation fluid (Ultima Gold XR, Packard, Groningen, the Netherlands) was added and radioactivity was counted in a liquid scintillation counter (Tri-Carb 2000 CA, Packard Instrument Company, Meriden, USA) at counting efficiencies of 85–93% with correction for quenching.

The labeled S associated with the insoluble fraction was calculated by determining the difference between the total labeled S and the labeled S associated with the soluble fraction.

The specific radioactivity of the plant tissue was determined from the ratio of the labeled S and the total S content. For the calculations it was assumed that the total sulfur content in the H_2S exposed plants was similar to that of the non-exposed plants as has been observed previously (De Kok & al. 2000). The total S content was derived from the measurements on the net sulfate uptake rate and the RGR, and was calculated according to the following formula:

$$S_{content}\ (\mu mol\ g^{-1}\ plant) = {SO_4}^{2-}{}_{uptake\ rate}\ (\mu mol\ g^{-1}\ root\ day^{-1}) / RGR\ (g\ g^{-1}\ day^{-1}) \times R_{weight}\ (g\ root) / P_{weight}\ (g\ plant)$$

RGR represents the relative growth rate calculated on a fresh weight basis, ${SO_4}^{2-}{}_{uptake\ rate}$, the sulfate uptake rate expressed on a root fresh weight basis and $S_{content}$ the total plant tissue sulfur content.

Results and Discussion

Effect of H_2S exposure on the sulfate uptake by the roots during dark or light

Previous studies showed that upon exposure of curly kale to 0.2 µl l^{-1} H_2S, a level sufficient to meet the sulfur demand for growth, the sulfate uptake by the roots was partially reduced (maximal 50%) and the sulfate assimilation was down-regulated (Westerman & al. 2000). The rate of sulfate assimilation is in general higher in the light than in the dark (Brunold 1993). The uptake rate of H_2S is high in the light and relatively low in the dark, since at low H_2S levels the stomatal conductance is limiting the uptake rate of H_2S (De Kok & al. 1989). Whether the sulfate uptake by the roots was regulated in a different way in the light compared to the dark

Table 1.

The H_2S uptake rate and transpiration rate in the dark or light. 12-day-old seedlings of curly kale were transferred to plastic pots filled with soil (1 plant per pot) and grown in the climate controlled room for 10 days. The humidity was 53±4% during light and 44±2% during darkness and the temperature 23 °C during light and 18 °C during darkness. Data are expressed on a shoot fresh weight basis and represent the mean of 4 measurements (±SD).

	H_2S uptake rate (μmol g fresh weight^{-1} h^{-1})	Transpiration rate (mmol g fresh weight^{-1} h^{-1})
Light	0.36±0.04	41.5±3.2
Dark	0.20±0.03	15.2±3.4

upon H_2S exposure and in this way resulted in a partial reduction of the sulfate uptake was investigated in the present study.

Upon exposure of curly kale plants to 0.2 μl l^{-1} H_2S the transpiration rate was 2-fold lower in the dark as compared to that in the light. At the same time a 2-times lower uptake rate of H_2S was observed (Table 1). In the non-exposed curly kale plants the uptake of sulfate and nitrate measured in the dark was similar to that measured in the light and the 24-h period (Table 2). Upon exposure of curly kale to 0.2 μl l^{-1} H_2S for 1 week the uptake of sulfate was decreased by 50% when measured over the light period and by 60% when measured over the dark period (Table 2). There is generally a diurnal variation in the uptake of ions by the roots, with higher rates in the light than in the dark. However, the response of the ion uptake to a transfer from light to darkness varies between plant species (CLARKSON & al. 1993, MACDUFF 1988, MACDUFF & al. 1997). In addition to

Table 2.

The response of the sulfate and nitrate uptake during a dark or light period upon H_2S exposure. 12-day-old seedlings were grown for 10 days on a 25% Hoagland nutrient solution and were thereafter exposed to 0.2 μl l^{-1} H_2S at a photoperiod of 12 h. The uptake of sulfate and nitrate was measured over a 12-h-light period, a 12-h-dark period or the whole 24-h period on the eighth day after start of exposure to 0.2 μl l^{-1} H_2S. Data are expressed on a root fresh weight basis and represent the mean of 3 measurements with 3 plants in each (±SD).

	H_2S (μl l^{-1})	SO_4^{2-} uptake (μmol g fresh weight^{-1} h^{-1})	NO_3^- uptake (μmol g fresh weight^{-1} h^{-1})
Light	0	3.1±0.7	23.6±4.0
	0.2	1.6±0.2	20.0±0.8
Dark	0	2.2±0.6	17.4±3.0
	0.2	0.8±0.1	14.0±2.4
Light+dark	0	2.5±0.4	19.5±1.9

transpiration related effects, the uptake of sulfate and movement into the xylem is strongly affected by the sulfur nutritional status of the plant (CLARKSON & al. 1993). The present data showed that the sulfate uptake was reduced to the same extent upon H_2S exposure when measured in the dark or light period (Table 2). Apparently, the net sulfate uptake is not affected by a change in the uptake rate and metabolization of H_2S and remains repressed continuously in H_2S exposed in curly kale.

Effect of H_2S on xylem loading of sulfate

The sulfate taken up by the roots has to be loaded into the xylem before it is transported to the shoot via the transpiration stream (CLARKSON & al. 1993). In response to H_2S in spinach the xylem loading site appeared to be more sensitive than the net uptake rate of sulfate and the relative proportion of sulfate that was transported to the shoot was decreased upon H_2S or SO_2 exposure (HERSCHBACH & al. 1995a,b). When curly kale was exposed for 4 h to 0.4 µl l^{-1} H_2S, the net sulfate uptake rate and the transport of sulfate to the shoot were not yet significantly decreased (Fig. 1). After 24 h the net sulfate uptake rate was decreased by 27% and after 6 d by 42%. The sulfate transport to the shoot was decreased to the same

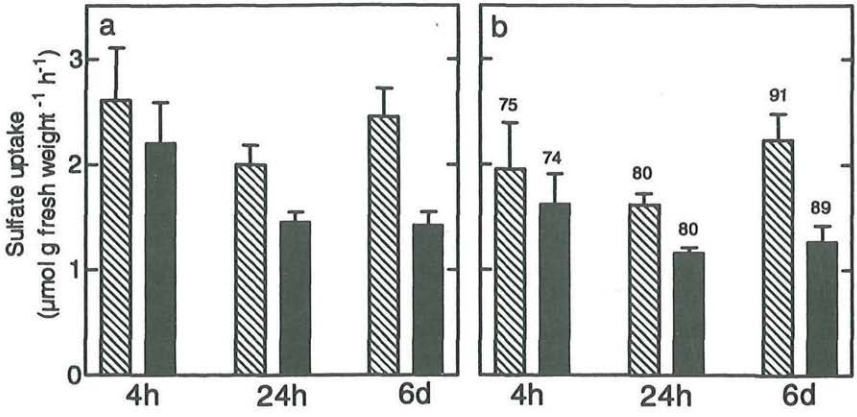

Fig. 1. The impact of H_2S on the net sulfate uptake rate and sulfate transport to the shoot. 10-day-old seedlings were grown for 4 weeks on a 25% Hoagland nutrient solution and were thereafter exposed to H_2S for 4 h or 24 h or 10-day-old seedlings were grown for 3 weeks on a nutrient solution and were thereafter exposed for 6 days to H_2S. The plants were exposed to 0 (shaded bars) and 0.4 µl l^{-1} H_2S (filled bars). The sulfate uptake rate (a) and the absolute proportion of sulfate transported to the shoot (b) were calculated on a root fresh weight basis and measured over a 4-h, 24-h or 6-day exposure period. The relative proportion of sulfate that was transported to the shoots (%) is indicated above the bars. Data represent the mean of 3 measurements with 3 plants in each (±SD).

extent as the net sulfate uptake rate (Fig. 1). The relative proportion of sulfate taken up that was transported to the shoot remained unaffected by H_2S exposure. The presented results suggested that in curly kale repression of the sulfate uptake occurring upon H_2S exposure is independent of sulfate loading into the xylem.

The effect of H_2S exposure on the partitioning of labeled S taken up by the roots

When plants are exposed to elevated levels of H_2S, the proportion of H_2S that is taken up by the plants is rapidly incorporated into organic compounds. This generally results in an accumulation of thiol compounds such as cysteine or glutathione (BUWALDA & al. 1993, 1994). The thiol fraction however comprises only a small proportion (about 2%) of the organic reduced sulfur and the major proportion of the metabolized H_2S ends up in the proteins (STULEN & DE KOK 1993). Curly kale is able to grow with H_2S as the sole sulfur source and upon exposure to a H_2S level sufficient for growth the sulfate uptake was decreased (DE KOK & al. 1997). The total sulfur content was not substantially affected upon prolonged exposure to a range of H_2S levels (DE KOK & al. 2000). This indicates that plants used sulfur from the atmosphere instead of sulfate taken up by the roots for its organic sulfur need for growth. Matching the supply of sulfur in the form of pedospheric or atmospheric sulfur to the sulfur need for growth, therefore, appeared to be regulated nicely. The effect of exposure to a H_2S level sufficient to cover the organic sulfur need on the partitioning of labeled sulfur taken up by the roots in the insoluble (mostly proteins) and soluble fraction (mostly sulfate) was investigated in the present study.

Curly kale was placed on a solution labeled with ^{35}S-sulfate for 6 d and was exposed to 0 or 0.4 µl l^{-1} H_2S. This level of H_2S is sufficient to supply the plant with an ample amount of sulfur for growth and was shown to have only a slightly negative effect on plant growth upon a two-week exposure period (DE KOK & al. 1997). The total amount of ^{35}S-sulfate taken up remained unaffected after 4 h of exposure to 0.4 µl l^{-1} H_2S, but was decreased by 25% after 24 h and by 40% after 6 days in the H_2S exposed plants (Fig. 2). The proportion of labeled S that was incorporated into the soluble fraction of the shoot and the roots was increased compared to the non-exposed plants after 24 h and 6 d of exposure. This was associated with a decreased proportion of labeled S that was incorporated into the insoluble fraction upon H_2S exposure, which was more pronounced in the shoot than in the roots (Fig. 2). The specific radioactivity of the labeled sulfur of the insoluble fraction of the shoot and roots of the H_2S exposed plants was decreased by 64 and 50%, respectively, compared to the non-exposed plants. The specific radioactivity of the labeled sulfur of the soluble fraction was also decreased upon H_2S exposure, although it was less pronounced, by 37 and 21% in the shoot and roots, respectively (Table 3).

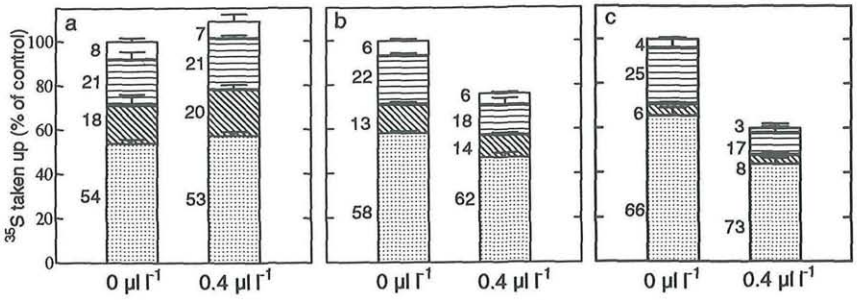

Fig. 2. The impact of H_2S on the partitioning of labeled sulfur in the plant. 10-day-old plants grown for 4 weeks on a 25% Hoagland nutrient solution were thereafter exposed to 0 or 0.4 µl l^{-1} H_2S for 4 h (a) or 24 h (b), or seedlings were grown for 3 weeks on a nutrient solution and were thereafter exposed to 0 or 0.4 µl l^{-1} H_2S for 6 days (c). Total bar graph heights indicate total labeled sulfur taken up (% of control). The percentage of the total labeled sulfur taken up that was incorporated in the soluble fraction of the shoot (dotted bar segments) and roots (shaded bar segments) or in the insoluble fraction of the shoot (striped bar segments) and roots (white bar segments) are indicated to the left of the bar segment. Data represent the mean of calculations of 3 measurements with 3 plants in each (±SD).

These data indicated that the major proportion of metabolized H_2S was ending up in the insoluble fraction. Even though the level of H_2S was sufficient for the organic sulfur need for growth, a substantial proportion of the sulfate taken up by the roots had entered the insoluble fraction and part of the metabolized H_2S was incorporated in the soluble pool. This suggests that the sulfate taken up by the roots that was incorporated into the organic sulfur fraction was not completely replaced by sulfur taken up from the atmosphere. Previous studies showed however, that the uptake of

Table 3.
The specific radioactivity (GBq mol^{-1}) of the ^{35}S-sulfur associated with the soluble and insoluble fraction of the shoot and roots. 10-day-old seedlings were grown on a 25% Hoagland nutrient solution for 3 weeks and thereafter placed in the fumigation cabinets and exposed 0 or 0.4 µl l^{-1} H_2S for 6 days. The RGR was 0.18 g g^{-1} day^{-1} and the root and plant fresh weight were 1.0 and 5.3 g, respectively. Data represent the mean of calculations on 3 measurements with 3 plants in each (±SD).

	0 µl l^{-1} H_2S	0.4 µl l^{-1} H_2S
Shoot		
Soluble fraction	2.28±0.07	1.43±0.17 (63)
Insoluble fraction	0.88±0.19	0.32±0.10 (36)
Roots		
Soluble fraction	1.36±0.06	1.08±0.07 (79)
Insoluble fraction	0.86±0.11	0.43±0.11 (50)

sulfate by the roots and the activity of enzymes involved in reduction of sulfate were maximally decreased after 3 days and 1 day of exposure to H_2S, respectively. The partly decreased specific acitivity of labeled sulfur of the insoluble pool and the decrease of the specific activity of labeled sulfur of the soluble pool may therefore in part be explained by the delay of adjustment of the supply of sulfur in the form of pedospheric or atmospheric sulfur to the sulfur need for growth.

Roots have the capability to reduce sulfur and cultured roots can grow on a medium containing sulfate as the sole sulfur source (BRUNOLD & SUTER 1989, HAWKESFORD & BELCHER 1991). The activity of enzymes involved in reduction of sulfate remained unaffected in the roots upon H_2S exposure, whereas in the shoot sulfate reduction was inhibited (WESTERMAN & al. in preparation). In the presence of a H_2S level that was sufficient for growth still a substantial amount of sulfate was taken up by the roots (DE KOK & al. 1997). This suggests that the roots have the potential to assimilate sulfur independently from the shoots, transporting sulfur to the shoots which exceeded the incorporation into the organic fraction of the roots. The present data showed, however, that the change in specific radioactivity of the labeled sulfur of the organic and inorganic fraction was similar in the roots compared to the shoot (Table 3). This indicated that in curly kale the roots were dependent on the shoot for their organic sulfur supply.

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