Phyton (Horn, Austria)	**Vol. 45**	Fasc. 1	45–50	30. 6. 2005

Interactions Between Atmospheric and Pedospheric Sulfur Nutrition: Impact of Short-Term H_2S Exposure on the Uptake and Distribution of ^{35}S-Sulfate in Roots and Shoots of Curly Kale (*Brassica oleracea* L.)

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With 1 Figure

Received December 9, 2004

Key words: Curly kale, H_2S, labeled sulfur, organic sulfur, inorganic sulfur.

Summary

WONISCH A., WEIDNER W., TAUSZ M., WESTERMANN S., DE KOK L. J. & Grill D. 2005. Interactions between atmospheric and pedospheric sulfur nutrition: Impact of short-term H_2S exposure on the uptake and distribution of ^{35}S-sulfate in roots and shoots of curly kale (*Brassica oleracea* L. *Brassicaceae*). – Phyton (Horn, Austria) 45 (1): 45–50, 1 figure. – English with German summary.

Curly kale (*Brassica oleracea* L., *Brassicaceae*) was exposed to 0 and 400 nl l^{-1} H_2S and simultaneously transferred to a 25 % Hoagland nutrient solution containing ^{35}S-sulfate for 24 h. H_2S exposure hardly affected the contents of the different sulfur pools, but resulted in a decrease in the level of ^{35}S in the plants and in the organic sulfur fraction of both roots and shoots. Evidently, H_2S already induced a partial down-regulation of sulfate uptake by the root within 24 h and the plants start to transfer from sulfate to H_2S as sulfur source for the synthesis of organic sulfur compounds.

Zusammenfassung

WONISCH A., WEIDNER W., TAUSZ M., WESTERMANN S., DE KOK L. J. & GRILL D. 2005. Wechselwirkungen von atmosphärischer und pedosphärischer Schwefelver-

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sorgung: Der Einfluss von H_2S auf die Aufnahme und den Transport von radioaktiv markiertem Schwefel in Spross und Wurzeln von Krauskohl (*Brassica oleracea* L). – Phyton (Horn, Austria) 45 (1): 45–50, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Pflänzchen von *Brassica oleracea* L. *(Brassicaceae)* wurden in einer 25 % Hoagland Nährlösung, die radioaktiv markierten Schwefel enthielt, für 24 Stunden mit 400 nl l^{-1} H_2S begast. Die H_2S-Behandlung hatte kaum Einfluss auf die Schwefelmenge der unterschiedlichen Schwefelpools (organischer, anorganischer Schwefelpool). Allerdings zeigte sich im Vergleich zu den Kontrollpflanzen ein verringerter Anteil von radioaktiv markiertem Schwefel, und zwar sowohl in den Pflänzchen insgesamt als auch im organischen Schwefelpool der Sprosse und Wurzeln. Dies deutet darauf hin, dass bei einer 24-stündigen Einwirkung von 400 nl l^{-1} H_2S die Sulfataufnahme reduziert wird und die Pflanzen zur atmosphärischen Schwefelquelle H_2S wechseln, um ihren organischen Schwefel zu synthetisieren.

Introduction

In today's environment plants may have to deal with sulfur sources from the atmosphere (SO_2 and H_2S), which may cause significant changes in plant growth and development. H_2S is actively taken up by plant shoots and is directly incorporated into cysteine and subsequently into other organic compounds (De Kok 1990, De Kok & al. 1997, 1998). It has been observed that curly kale is able to use both sulfate and atmospheric H_2S as a sulfur source for growth (De Kok & al. 1997, 1998). H_2S exposure did not substantially affect the total sulfur content of curly kale, demonstrating that the utilization of the different sulfur sources is highly regulated and in tune with the sulfur requirement for growth (Westerman & al. 2000, 2001a,b). This is in contrast to studies with SO_2 as sulfur source for curly kale. SO_2 exposure resulted in increased content of organic and inorganic sulfur in curly kale (Wonisch & al. 2003). In curly kale that H_2S exposure may result in a down-regulation of both the sulfate uptake by the root and its reduction in the shoots. When plants were exposed to $\geq$ 200 nl l^{-1} H_2S, levels sufficient to meet the plant sulfur requirement for growth, the sulfate uptake was down-regulated maximally after 3 days of exposure. Furthermore, the activity of adenosine 5'-phosphosulfate reductase, one of the major enzymes of the sulfate reduction pathway, was already substantially decreased after 1 day of exposure (Westerman & al. 2001a,b). When curly kale was exposed to 400 nl l^{-1} H_2S and grown on a nutrient solution containing ^{35}S-sulfate for 6 days it was obvious from the distribution of the labeled sulfur that the major proportion of the metabolized atmospheric H_2S was incorporated into the protein fraction.

This study was designed to complement these results by measuring ^{35}S-label directly in the sulfur fractions collected from an HPLC analysis upon short-term H_2S exposure and ^{35}S-sulfate nutrient solution. The in-

teraction between metabolism of the two different sulfur sources and the sulfur distribution within the different sulfur pools is evaluated.

Materials and Methods

Curly kale (*Brassica oleracea* L., cv Bornick F1, Nickerson-Zwaan, The Netherlands) was germinated and grown on a 25% Hoagland nutrient solution for 3 weeks (see for details WESTERMAN & al. 2000). At the start of the experiment plants were transferred to a ^{35}S-sulfate labeled 25% Hoagland nutrient solution with specific activity of 4.34 MBq mmol^{-1} sulfate. Plants were subsequently exposed to 400 nl l^{-1} H_2S for 24 h in cylindrical stainless steel fumigation cabinets with a polycarbonate top (as described by STUIVER & al. 1992). Day and night temperature were 23/18 °C, relative humidity 65% and the photon fluence rate 250–270 µmol m^{-2} s^{-1} (within the 400–700 nm range) for 12 h day^{-1}. After removing the labeled solution roots were washed three times in 250 ml of demineralized water. Nine plants for each treatment (3 plants for each treatment, control, H_2S) were sampled, divided into shoots and roots (3 plants for one sample mixture, respectively) and immersed immediately in liquid nitrogen. The plant material was lyophilized and pulverized under liquid nitrogen in a dismembrator. The plant powder was stored at –25 °C in humidity-proof plastic vials before it was subjected to HPLC analysis. Sulfate content was measured by an isocratic HPLC-method described in TAUSZ & al. 1996. The determination of the total sulfur content was carried out on plant dry matter, which was combusted in oxygen atmosphere over hydrogen peroxide. The formed sulfate was measured by HPLC analysis as described above. Organic sulfur was calculated as the difference of total S and sulfate. For estimation of the radioactivity of the organic and inorganic sulfur fractions, the injected samples were collected with a fraction collector and measured in a liquid scintillation counter (Counter Quantulus 1220–001, Wallac Oy Finland, Spectrum Analysis Program V2.12M).

Figures show medians ± median deviations best suited for small sample sizes. Differences between controls and H_2S exposed plants were evaluated by Mann-Whitney test using exact probabilities for small sample sizes (Sachs 1992).

Results and Discussion

From previous studies it is obvious that upon prolonged exposure curly kale is able to transfer from pedospheric sulfate to atmospheric H_2S as a sulfur source for growth (DE KOK & al. 1997, 1998, WESTERMAN & al. 2000, 2001a,b). Atmospheric H_2S decreased the uptake of sulfate by the roots, depressed the reduction of sulfate in the shoots and changed incorporation of and distribution ^{35}S-sulfate taken up by roots into the various fractions in roots and shoots upon prolonged exposure (WESTERMAN & al. 2000, 2001a,b).

A 24 h exposure of curly kale to 400 nl l^{-1} H_2S did not significantly affect the contents of the organic sulfur and sulfate fractions in both shoots and roots. On a dry weight basis, shoots of control plants contained 405 ± 11 µmol sulfate g^{-1}, 172 ± 10 µmol g^{-1} organic S. Shoots of H_2S exposed plants 455 ± 30 µmol g^{-1} sulfate and 161 ± 38 µmol g^{-1} organic S.

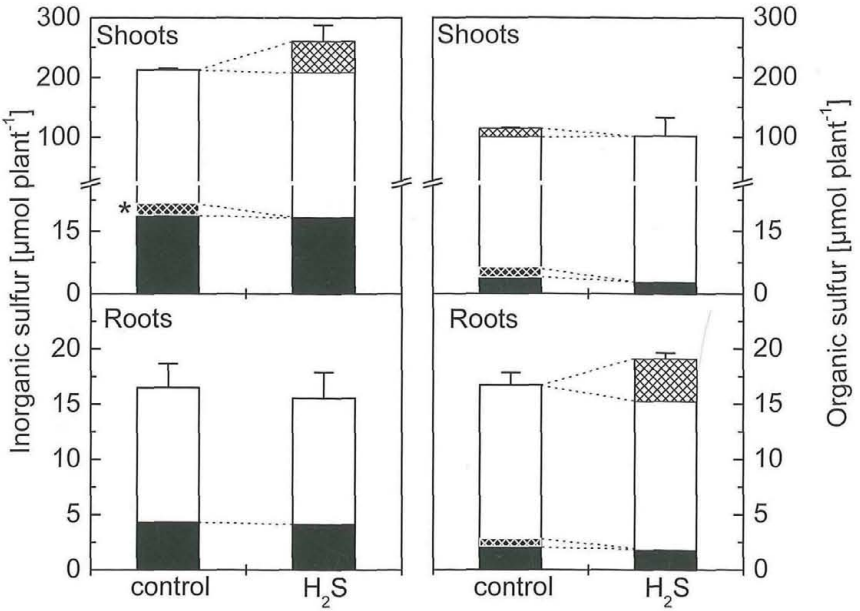

Fig. 1. Organic and inorganic sulfur fractions in curly kale (*Brassica oleracea* L.) shoots and roots after fumigation with 400 nl l^{-1} H_2S for 24 h. Sulfur originating from ^{35}S-sulfate taken up from nutrient solution (■) and sulfur from non-labeled sources (□), i.e. from H_2S or internal S cycling. Cross-hatched signatures indicate changes relative to control (0 nl l^{-1} H_2S). Columns show medians, error bars show median deviation, asterisks mark significant ($p<0.05$) changes. Shoot fresh weights were 6.6 ± 0.6 (controls) and 6.7 ± 0.6 (H_2S exposed) g $plant^{-1}$, plant dry weights were 0.6 ± 0.1 (controls) and 0.6 ± 0.1 g $plant^{-1}$ (H_2S) at the end of the experiment. Shoot/root ratios were 5.8 ± 0.2 (controls) and 5.8 ± 0.1 (H_2S exposed) on a fresh weight basis, and 8.1 ± 0.4 (controls) and 7.6 ± 0.5 (H_2S exposed) on a dry weight basis. Significant differences in plant weights were not observed.

Roots of control plants showed 223 ± 4 µmol g^{-1} sulfate and 216 ± 1 µmol g^{-1} organic S , those of H_2S fumigated plants 210 ± 10 µmol g^{-1} sulfate and 233 ± 25 µmol g^{-1} organic S. Differences were not significant. Concentrations of total ^{35}S from the nutrient solution were significantly higher in shoots of control plants (51 ± 2 µmol g^{-1} dwt versus 36 ± 1 in H_2S treated plants, $p<0.05$). In roots, difference was not significant (89 ± 1 µmol g^{-1} dwt versus 79 ± 11 in H_2S treated plants).

Fig. 1 shows S fractions on a whole plant basis. The total level of ^{35}S in H_2S-exposed plant was significantly lower than that in non-exposed plants (28 ± 4 versus 34 ± 1 µmol $plant^{-1}$, $p<0.05$, compare Fig. 1), demonstrating that H_2S exposure already resulted in a down-regulation of sulfate

uptake within 24 h. In accordance with WESTERMAN & al. 2000, 2001a, significantly decreased levels of ^{35}S in shoot sulfate fractions (Fig. 1) point toward a reduced sulfate import and/or reduced use of imported sulfate for S reduction and incorporation. There was a tendency toward a decreasing level of ^{35}S in the total organic sulfur fraction of both shoots and roots, which showed that curly kale already started to transfer from pedospheric sulfate to atmospheric sulfide as sulfur source for growth. Albeit not significant, it supports previous observations by WESTERMAN & al. 2000 that the greater proportion of the metabolized atmospheric H_2S in curly kale is directly incorporated into the insoluble fraction (mainly proteins). Furthermore, the decrease in ^{35}S-sulfur in the organic sulfur fraction in roots upon H_2S exposure – albeit also not significant in this study – supports the previous observation that curly kale roots are dependent on the shoots for their organic sulfur supply (WESTERMAN & al. 2000).

Acknowledgements

The authors gratefully acknowledge financial support by the Austrian Science Foundation (P13285-BIO) and COST Action 829.

References

DE KOK L. J. 1990. Sulfur metabolism in plants exposed to atmospheric sulfur. - In: RENNENBERG H., BRUNOLD C., DE KOK L. J. & STULEN I. (Eds.), Sulfur nutrition and sulfur assimilation in higher plants; fundamental, environmental and agricultural aspects, pp. 111–130. – SPB Academic Publishing, The Hague.

— , — & STULEN I. 1998. The impact of elevated levels of atmospheric H_2S on plants. – In: DE KOK L. J. & STULEN I. (Eds.), Responses of plant metabolism to air pollution and global change, pp. 51–63. – Backhuys Publishers, Leiden.

— , — , RUBINIGG M., WESTERMAN S. & GRILL D. 1997. Impact of atmospheric sulfur deposition on sulfur metabolism in plants: H_2S as sulfur source for sulfur deprived *Brassica oleracea* L. – Bot. Acta 110: 411–419.

SACHS L. 1992. Angewandte Statistik. 7. Aufl. – Springer Berlin Heidelberg New York.

STUIVER C. E. E., DE KOK L. J. & KUIPER P. J. C. 1992. Freezing tolerance and biochemical changes in wheat shoots as affected by H_2S fumigation. – Plant Physiol. Biochem. 30: 47–55.

TAUSZ M., DE KOK L. J., STULEN I. & GRILL D. 1996. Physiological responses of Norway spruce trees to SO_2 and elevated CO_2 concentrations. – J. Plant Physiol. 148: 362–367.

WESTERMAN S., WEIDNER W., DE KOK L. J. & STULEN I. 2000. Effects of H_2S exposure on ^{35}S-sulfate uptake, transport and utilization in curly kale. – Phyton (Austria) 40: 293–302.

— , STULEN I., SUTER M., BRUNOLD C. & DE KOK L. J. 2001a. Atmospheric H_2S as sulphur source for *Brassica oleracea*: Consequences for the activity of the enzymes of the assimilatory sulfate reduction pathway. – Plant Physiol. Biochem. 39: 425–432.

— , BLANKE-KALFF M. M. A., DE KOK L. J. & STULEN I. 2001b. Sulfate uptake and utilization by two varieties of *Brassica oleracea* with different sulfur need as affected by atmospheric H_2S. – Phyton (Austria) 41: 49–62.

WONISCH A., WEIDNER W., TAUSZ M., WESTERMAN S., DE KOK L. J. & GRILL D. 2003. Effects of SO_2 exposure on sulfur distribution in curly kale (*Brassica oleracea* L.) investigated by ^{35}S labeled nutrient solution. – In: DAVIDIAN J.-C., GRILL D., DE KOK L. J., STULEN I., HAWKESFORD M. J., SCHNUG E. & RENNENBERG H. (Eds.), Sulfur transport and assimilation in plants: regulation, interaction, signaling, pp. 359–361. – Backhuys Publishers, Leiden.