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Sulfate and Thiol Levels in Roots and Shoot of Sulfur-Deprived Spinach Plants as Affected by High Pedospheric Sulfate Levels

By

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Summary

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Sulfur-deprivation of spinach resulted in a reduced growth, a decreased shoot/root ratio and an increase in dry matter content. The content of sulfur, thiols and soluble proteins was strongly decreased, whereas that of nitrate and free amino acids was increased. When sulfur-deprived plants were transferred to a nutrient solution containing a strongly elevated sulfate level (10 mM), it resulted in gradual but rapid increase in the sulfate content, simultaneously in both roots and shoot. The sulfate accumulation was accompanied by a rapid increase in thiol and cysteine content, illustrating that a proportion of the sulfate taken up in sulfur-deprived plants was rapidly reduced and assimilated into cysteine and its metabolites. During the first hours after the transfer the actual sulfate uptake exceeded a rate of 0.2 μmol g^{-1} fresh weight plant h^{-1}. In sulfur-sufficient plants a transfer to 10 mM sulfate solely resulted in a increase in sulfate content in the roots, whereas the shoot sulfate content and the plant thiol content remained unaffected. The transfer of excised roots of sulfur-deprived spinach plants to 10 mM sulfate also resulted in an increase in both the sulfate and thiol content, which demonstrated that excised roots of sulfur- deprived plants were able to reduce and assimilate sulfate. The sulfate and thiol content of roots of excised sulfur-sufficient plants were hardly affected by a transfer to 10 mM sulfate.

Introduction

Sulfate is usually the sulfur source for plant growth, which is after its uptake by the roots loaded into the xylem and transported to the shoot, where it is

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reduced in the chloroplast and subsequently metabolized into cysteine (BRUNOLD 1990). The major proportion of the reduced sulfur is incorporated as cysteine and methionine into proteins (GIOVANELLI 1990). Part of the reduced sulfur is present in low molecular non-protein thiols, however, its proportion is generally less than 2% of the total organic sulfur fraction (DE KOK & STULEN 1993). Presumably the roots are largely dependent on the shoot for its reduced sulfur supply (BRUNOLD 1990). The reduced sulfur may be transported as glutathione via the phloem to the roots, where it is in part degraded in order to make the reduced sulfur available for protein synthesis (RENNENBERG & LAMOUREUX 1990). The pool of thiols in plant tissue is very dynamic and may strongly be affected by physiological and environmental factors, though glutathione is the pre-dominant thiol-compound present in most species (BERGMANN & RENNENBERG 1993, DE KOK & STULEN 1993). If regulation of the sulfate uptake by the roots is bypassed and foliar tissue is directly exposed to excessive sulfur, oxidized or reduced, then not only the size but also the composition of the thiol pool may alter and strongly enhanced levels of cysteine and γ-glutamyl-cysteine (the latter compound pre-dominantly in the dark) may be present (BUWALDA & al. 1988, 1990, 1993, DE KOK 1990, DE KOK & STULEN 1993, DE KOK & al. 1998, POORTINGA & DE KOK 1997).

Sulfur deprivation may have a decisive impact on the sulfate transporters and sulfate-reducing enzymes, which activities in generally increase (CLARKSON & al. 1993, CLARKSON 1997, HAWKESFORD & SMITH 1997, LAPPARTIENT & al. 1997). In the present paper the impact of high pedospheric sulfate levels on the content of sulfate and thiols in sulfur-deprived spinach plants and excised roots was studied in order to get more insight into regulatory aspect of changes in the size and composition of the thiol pool and the possible significance of the roots in the reduction of sulfate.

Materials and Methods

Spinach (*Spinacia oleracea* L. cv. Subito) was sown in sterilized soil. The seeds were germinated in a climate-controlled room at a day and night temperature of 23 and 20 °C, respectively. The relative humidity was 63 ± 3%; the photoperiod was 12 hours and the photon fluence rate 350 μmol m^{-2} s^{-1} (within the 400-700 nm range). The seedlings were watered daily with tap water. After 7-10 days the seedlings were transferred to 25% strength Hoagland nutrient solution (30 l tanks, 60 plants per tank) at a sulfate concentration of 0.27 μM (sulfur-deprived) or 0.5 mM sulfate (sulfur-sufficient). Iron was added as Fe_3EDTA. In the Hoagland solution containing 27 μM sulfate, $MgSO_4$ was replaced by $MgCl_2$. The nutrition solutions were refreshed weekly. After two weeks plants or excised roots (the shoot was cut off with a razor blade and the cutting was covered with wax) were transferred to a 25 % strength Hoagland solution containing 10 mM $MgSO_4$ and plants/roots were harvested after a 3 to 48 h exposure. The content of the pigments was determined as described by LICHTENTHALER 1987. Sulfate, nitrate and amino acids were extracted and estimated as described before by DE KOK & al. 1997. The water-soluble non-protein thiols and cysteine were extracted in sulfosalicylic acid and their contents were measured as described by DE KOK & al. 1988. The soluble protein content was determined according to the method of BRADFORD 1976.

Results and Discussion

For studies on thiol metabolism as affected by pedospheric sulfur nutrition, spinach plants were grown at both sulfur-sufficient (0.5 mM sulfate) and sulfur-deprived (0.27 μM sulfate) conditions. The impact of sulfur deprivation on growth and metabolite content was quite similar to that observed in other plant species (DE KOK & al. 1997, STUIVER & DE KOK 1997, STUIVER & al. 1997). Biomass production of shoot and roots of spinach was strongly reduced after two weeks of sulfur deprivation (Table 1). During this period the relative growth rate (RGR) of the sulfur-sufficient and sulfur-deprived plants was 22 and 15% day^{-1}, respectively (on a fresh weight basis). This reduction in growth was accompanied by an increase in dry matter content. Shoot growth was more affected than that of roots by sulfur deprivation resulting in a decrease in the shoot/root ratio. The shoot/root ratios (on a fresh weight basis) of sulfur-sufficient and the sulfur-deprived plants were 5.4 and 3.0, respectively. Sulfur-deprived spinach developed sulfur-deficiency symptoms after one week, visible by yellowing of the leaves and after two weeks the contents of chlorophyll and carotenoids were decreased by more than 70% (Table 1). Furthermore, sulfur deprivation resulted in strongly decreased levels of sulfate, water-soluble non-protein thiols and soluble proteins and increased levels of nitrate and free amino acid (Table 1). In sulfur-sufficient and sulfur-deprived plants the proportion of cysteine in both shoot and roots accounted for about 14 and 30 % of the total thiols, respectively (Fig. 1). In several species the turnover rate of sulfate in the vacuole, the compartment wherein the major proportion of the measurable sulfate appears to be present, was too slow to meet the sulfur demand even at deprived conditions (CRAM 1990, STUIVER & DE KOK 1997, STUIVER & al. 1997). Likewise, shoot and roots of sulfur-deprived spinach still contained substantial amounts of sulfate, even though severe deficiency symptoms already had developed.

When sulfur-deprived spinach plants were transferred to a 25% strength Hoagland solution containing 10 mM sulfate, it resulted in gradual but rapid increase in the sulfate content, simultaneously in both roots and shoot (Fig. 1). During the 2 days of exposure, the rate of sulfate accumulation was approx. 0.1 μmol g^{-1} fresh weight plant h^{-1} and the sulfate content exceeded that observed in sulfur-sufficient plants. When sulfur-sufficient plants were transferred to 10 mM sulfate it only resulted in an increase in sulfate content of the roots, though to a lesser extent than that observed in roots of sulfur-deprived plants (Fig. 1). The sulfate content of the shoot was not affected by the transfer to 10 mM sulfate.

The accumulation of sulfate in sulfur-deprived plants was accompanied by a rapid increase in both total thiol and cysteine contents, illustrating that a proportion of the sulfate taken up in sulfur-deprived plants was rapidly reduced and assimilated into cysteine and its metabolites. During the first hours after transfer of sulfur-deprived plants the rate of thiol accumulation/synthesis was approx. 0.1 μmol g^{-1} fresh weight plant h^{-1}. Evidently, during the first hours after the transfer to 10 mM sulfate the actual sulfate uptake rate was higher than 0.2 μmol g^{-1} fresh weight plant h^{-1}, which exceeded even the sulfur need of sulfur-sufficient plants

growing at a RGR of 22% day^{-1} (STULEN & DE KOK 1993). The rapid accumulation of sulfate and thiols in sulfur-deprived plants may be explained by an increased sulfate uptake capacity by the roots upon sulfur-deprivation

Table 1. Growth and metabolite content of *Spinacia oleracea* L. as affected by sulfur nutrition. Plants were grown on a 25% strength Hoagland solution containing 0.27 µM (sulfur-deprived) or 0.5 mM sulfate (sulfur-sufficient) for two weeks. Fresh weight represents the mean of 48 measurements (± SD). Dry matter content and content of metabolites represent the mean of 3 or 6 (thiols) measurements on 3 plants in each (± SD). The initial fresh weight of the shoots and roots of the seedlings was 0.09 ± 0.02 and 0.07 ± 0.01 g, respectively (n = 60).

	SHOOTS		ROOTS	
	0.27 µM sulfate	0.5 mM sulfate	0.27 µM sulfate	0.5 mM sulfate
Fresh weight (g)	0.93 ± 0.36	2.81 ± 0.82	0.31 ± 0.13	0.52 ± 0.18
Dry matter content (%)	8.3 ± 0.4	5.4 ± 0.2	7.3 ± 0.4	5.6 ± 0.8
Chlorophyll (µg g^{-1} FW)	257 ±91	986 ± 190		
Chlorophyll a/b	3.31 ± 0.10	3.45 ± 0.16		
Chlorophyll/Carotenoids	3.41 ± 0.21	4.12 ± 0.13		
Sulfate (µmol g^{-1} FW)	0.60 ± 0.01	1.32 ± 0.07	0.42 ± 0.07	1.09 ± 0.02
Nitrate (µmol g^{-1} FW)	103 ± 9	35 ± 7	54 ± 9	24 ± 4
Thiols (µmol g^{-1} FW)	0.136 ± 0.007	0.314 ± 0.024	0.173 ± 0.007	0.343 ± 0.020
Amino acids (µmol g^{-1} FW)	94.0 ± 4.4	11.7 ± 0.1	57.5 ± 5.9	12.1 ± 0.9
Soluble proteins (mg g^{-1} FW)	2.17 ± 0.03	4.26 ± 0.49	2.01 ± 0.17	3.14 ± 1.00

(CLARKSON & al. 1993, HAWKESFORD & al. 1997). The proportion of cysteine in the thiol fraction decreased from about 30 % to 15 %, directly after the onset of the transfer. Thiol and cysteine accumulation in roots and shoot was maximal after 24 and 6 hours after the transfer to10 mM sulfate and their contents were 2- and 1.5-fold higher than that in sulfur-sufficient plants, respectively. The thiol and cysteine content in sulfur-sufficient plants were not affected by the transfer to 10 mM sulfate. Exposure of plants to H_2S or SO_2 not solely resulted in enhanced thiol levels, it also affected the composition of the thiol pool. Plant may contain extreme high levels of cysteine and γ-glutamyl-cysteine (the latter compound predominantly in the dark) in the shoots (BUWALDA & al. 1988, 1990, 1993, DE KOK 1990, DE KOK & STULEN 1993, DE KOK & al. 1998, POORTINGA & DE KOK 1997).

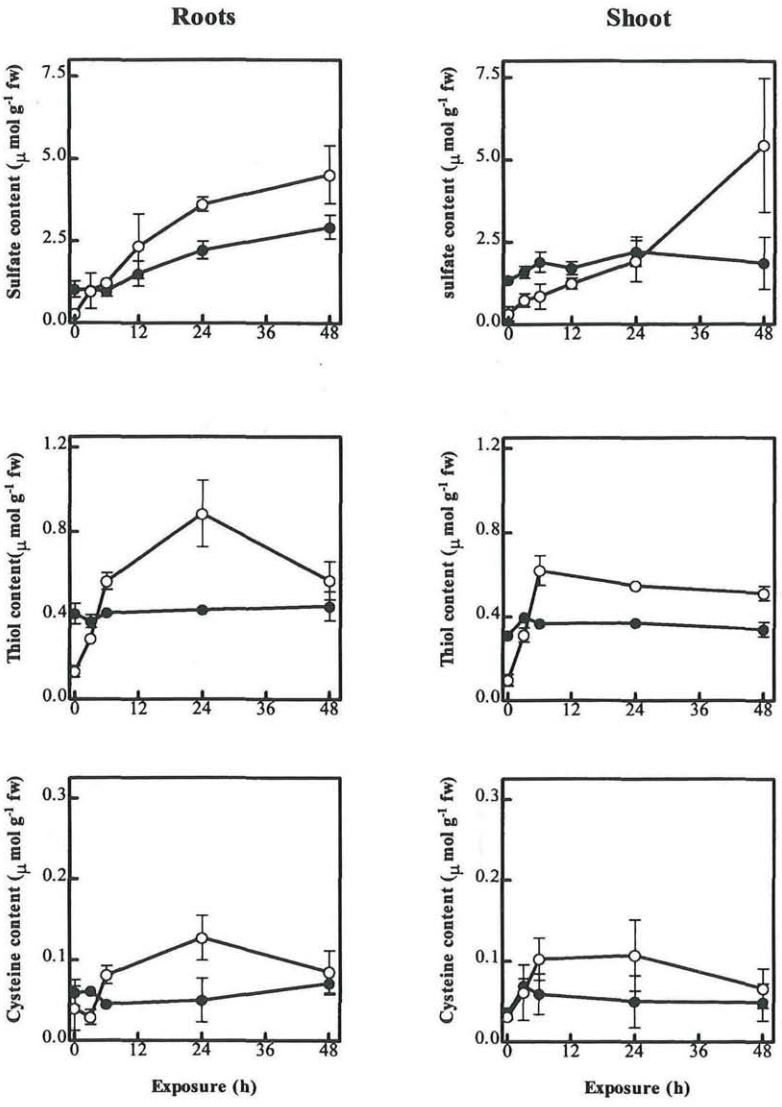

Fig. 1. The effect of 10 mM sulfate on the sulfate, thiol and cysteine content of the roots and shoot of sulfur-deprived (open circles) and sulfur-sufficient (closed circles) spinach. Plants grown for two weeks on 25 % strength Hoagland containing 0.27 μM (sulfur-deprived) and 0.5 mM (sulfur-sufficient). See for data on growth and metabolite contents Table 1. The data represent the mean of 3 measurements on 3 plants in each (± SD).

A similar change in the size and composition of thiol pool occurred when sulfate was directly supplied to foliar tissue (De Kok & Kuiper 1986, De Kok & al. 1988,

POORTINGA & DE KOK 1995). The present results reconfirm that changes in the composition of the thiol pool only occur when the regulation of the sulfate uptake by the roots and its transport to the shoot are by-passed.

Under normal conditions roots largely depend on the shoot for their reduced sulfur supply, even though roots contain all enzymes of the assimilatory sulfur pathway (BRUNOLD 1990, RENNENBERG & LAMOUREUX 1990). However, this may be different under sulfur-deprivation, which may consequence in a higher activity of sulfate-reducing enzymes, particularly of that in the roots (CLARKSON & al. 1997, LAPPARTIENT 1997). If sulfur-deprived roots would solely depend on the shoot for reduced sulfur one would expect that after the transfer to 10 mM sulfate the accumulation of thiols in the shoot would precede that in the roots. However, from the present results it is evident that within the first 6 hours after the transfer the rates of accumulation of thiols in roots and shoot were quite similar. This indicated that part of the sulfate taken up by sulfur-deprived plants was directly reduced and further assimilated into thiol compounds in the roots. The latter is supported by the observations in experiments with excised roots, which showed quite similar results as observed in intact plants (Fig. 2). Transfer of excised roots of sulfur-deprived spinach plants to 10 mM sulfate also resulted in an increase in sulfate content. However, contrary to the observations with roots of intact sulfur-deprived plants the accumulation already reached a maximum within 6 hours after the transfer. In addition, there was a substantial increase in the thiol content of excised roots of sulfur-deprived plants. The latter clearly demonstrated that excised roots of sulfur-deprived plants were able to reduce and assimilate the sulfate taken up. The sulfate and thiol content of roots of excised sulfur-sufficient plants were not affected by a transfer to 10 mM sulfate.

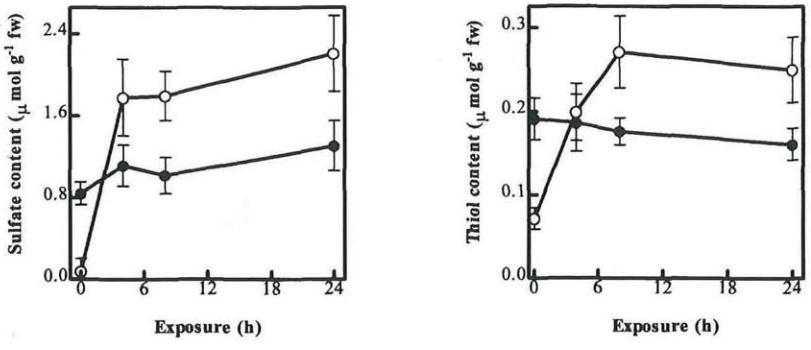

Fig. 2. The effect of 10 mM sulfate on the sulfate and thiol contents of excised roots of sulfur-deprived (open circles) and of sulfur-sufficient (closed circles) spinach. Plants were grown for two weeks on 25 % Hoagland containing 0.27 μM (sulfur-deprived) and 0.5 mM sulfate (sulfur-sufficient). See for data on growth and metabolite contents Table 1. The data represent the mean of 6 measurements on roots of 6 plants in each (± SD).

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