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Low Levels of H_2S May Replace Sulfate as Sulfur Source in Sulfate-Deprived Onion

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Summary

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Onion (*Allium cepa* L.) was exposed to low levels of H_2S in order to investigate to what extent H_2S could be used as a sulfur source for growth under sulfate-deprived conditions. Sulfate deprivation for a two-week period resulted in a decreased biomass production of the shoot, a subsequently decreased shoot to root ratio and an increased dry matter content in shoot and roots. Furthermore, it resulted in decreased contents of total sulfur, sulfate and organic sulfur and in a decreased sulfate to total sulfur ratio. Symptoms of sulfur deficiency disappeared upon simultaneous exposure to relatively low levels of H_2S (0.05, 0.1 and 0.15 μl l^{-1}), which showed that H_2S could be used as a sulfur source for growth. H_2S exposure even resulted in a slightly increased biomass production in sulfate-sufficient plants. The observed accumulation of sulfate and organic sulfur upon H_2S exposure in both sulfate-sufficient and sulfate-deprived plants is discussed.

Introduction

Sulfur is an essential element for plant growth, although amongst other elements it is present in minor quantities only (0.03-2 mmol g^{-1} dry weight; DE KOK & al. 2002a). Sulfur deficiency results in a loss of plant growth, fitness and resistance to environmental stress and pests (DE KOK & al. 2002c). Sulfate taken up by the roots is used as the principal sulfur source for growth. The uptake, transport and subcellular distribution of sulfate are mediated by specific sulfate transporter proteins (HAWKESFORD 2003, BUCHNER & al. 2004). Prior to its incorporation into organic compounds, sulfate needs to be reduced to sulfide, a process which

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primarily takes place in the chloroplast (SAITO 2004). Sulfide is subsequently incorporated into cysteine, from which most other organic sulfur compounds like methionine, glutathione and secondary sulfur compounds can be synthesized. The main proportion of sulfur is generally present in the protein fraction as cysteine and methionine residues, where it is highly significant in the structure, conformation and function (DE-KOK & al. 2002a).

Onion (*Allium cepa* L.) and related *Allium* species may contain high amounts of secondary sulfur compounds (γ-glutamyl peptides and alliins). These compounds presumably have cysteine as precursor, and γ-glutamyl peptides are thought to act as intermediates in the biosynthesis of alliins (LANCASTER & SHAW 1989, RANDLE & LANCASTER 2002). Upon cellular disruption, alliins (present in the cytosol) are degraded by the enzyme alliinase (present in the vacuole), which results in the formation of a wide range of degradation products (BLOCK 1992). It is mainly these degradation products that are responsible for the specific odor and taste of onions and for their health benefits (GRIFFITHS & al. 2002).

Atmospheric sulfur gases, viz. H_2S and SO_2, are potentially phytotoxic but can also be used as sulfur source for growth, especially when the sulfate supply to the roots is limited (DE KOK & al. 2002a,b,c). H_2S is taken up via the stomates and can directly be metabolized into cysteine. The rate of uptake is dependent on the stomatal conductance and on the internal resistance viz. the rate of incorporation of the absorbed sulfide into cysteine (STUIVER & DE KOK 2001, DURENKAMP & DE KOK 2002, DE KOK & al. 2002b). Exposure of onion to high levels of H_2S-(≥ 0.3 μl l^{-1}) resulted in an accumulation of sulfate and non-protein organic sulfur compounds (possibly γ-glutamyl peptides and/or alliins) in the shoot (DURENKAMP & DE KOK 2002, 2004, DURENKAMP & al. 2005).

In *Brassica oleracea* H_2S could be used as sulfur source and an atmospheric level as low as 0.075 μl l^{-1} was nearly sufficient to cover the sulfur requirement for growth of plants upon sulfate deprivation (BUCHNER & al. 2004). In this species, there was a good coordination between the uptake and metabolism of atmospheric H_2S in the shoot and the uptake of sulfate by the root and upon exposure to levels exceeding the sulfur requirement for growth the total sulfur content of the plants was hardly affected (WESTERMAN & al. 2000, 2001, DE KOK & al. 2002c).

In this paper, onion was exposed to low levels of H_2S (0.05, 0.1 and 0.15 μl l^{-1}) in order to investigate to what extent H_2S could be used as a sulfur source for growth under sulfate-deprived conditions.

Material and Methods

Seeds of onion (*Allium cepa* L. cv. Nerato F1; Nickerson-Zwaan, Made, The Netherlands) were germinated in vermiculite in a climate-controlled room. Two-week-old seedlings were transferred to 30 l tanks (12 plants per set, 20 sets per tank) containing a 25% Hoagland nutrient solution, pH 5.9, and grown for 11 days. Day and night temperatures were 20 and 17 °C, respectively, with a relative humidity of 60-70%. The photoperiod was 14 hours at a photon fluence rate of 250-300 μmol m^{-2} s^{-1} (PAR 400-700 nm).

Seedlings were transferred to stainless steel containers filled with a 25% Hoagland nutrient solution (pH 5.9; with 0 (-S) or 0.5 (+S) mM sulfate), placed in 150 l cylindrical stainless steel cabinets with a polycarbonate top and exposed to 0, 0.05, 0.1 or 0.15 µl l^{-1} H_2S (DURENKAMP & DE KOK 2002). Day and night temperatures were 19 and 16 °C, respectively, relative humidity was 40-50% and the photoperiod was 14 hours at a photon fluence rate of 280-350 µmol m^{-2} s^{-1} (PAR 400-700 nm). For studies on sulfate deprivation (-S), $MgCl_2$ replaced $MgSO_4$ in the Hoagland nutrient solution and their chloride salts replaced all micronutrient salts.

For determination of sulfate and total sulfur, shoot and roots were dried at 80 °C, pulverized in a Retsch Mixer-Mill (type MM2, Haan, Germany) and determined as described before (DURENKAMP & DE KOK 2002). The organic sulfur content was calculated by subtracting the sulfate from the total sulfur content.

Data were statistically analyzed using an unpaired Student's *t*-test.

Results

When onion was sulfate-deprived for two weeks, biomass production of the shoot and shoot to root ratio were significantly reduced and dry matter content was increased in both shoot and roots (Table 1).

Growth of sulfate-deprived onion, when simultaneously exposed to relatively low concentrations of H_2S (0.05, 0.1 and 0.15 µl l^{-1}), was quite similar to that under sulfate-sufficient conditions. However, the decrease in shoot to root ratio upon sulfate deprivation was unchanged upon exposure to 0.05 and 0.1 µl l^{-1} H_2S, whereas at higher H_2S levels intermediate values between those for sulfate-deprived and sulfate-sufficient plants were observed (Table 1). In sulfate-sufficient plants a two-week exposure to 0.05 - 0.15 µl l^{-1} H_2S even resulted in a slight stimulation of shoot and root biomass production (Table 1).

A two-week sulfate deprivation resulted in a substantial decrease in the total sulfur content and in hardly detectable levels of sulfate in both shoot and roots of onion (Fig. 1). The decrease in the organic sulfur content upon sulfate deprivation was less pronounced than the decrease in the sulfate content, which resulted in a decreased sulfate to total sulfur ratio. H_2S exposure resulted in an increase in the total sulfur, sulfate and organic sulfur content of the shoot in both sulfate-deprived and sulfate-sufficient plants, which depended on the H_2S level (Fig. 1). Upon exposure to 0.15 µl l^{-1} H_2S, the organic sulfur content in shoots of sulfate-deprived plants was equal to that of the control plants (+S, 0 µl l^{-1} H_2S). The increase in the total sulfur content of the shoot in sulfate-sufficient plants was less pronounced in the second week when compared to the first week of H_2S exposure. Exposure to 0.15 µl l^{-1} H_2S resulted in a slight but not significant increase in the total sulfur content of roots under sulfate-deprived conditions, which could mainly be attributed to an increase in the organic sulfur content (Fig. 1). Sulfur contents in roots of sulfate-sufficient plants were not affected by H_2S exposure.

Table 1. Impact of sulfate nutrition and H_2S exposure on growth of onion (*Allium cepa* L.). 25-day-old seedlings were grown in 25% Hoagland nutrient solution with 0 (-S) or 0.5 (+S) mM sulfate and exposed to 0, 0.05, 0.1 or 0.15 µl l^{-1} H_2S for two weeks. Data on fresh weight of shoot and roots (g), shoot to root ratio and dry matter content of shoot and roots (%) represent the mean of three measurements with 24 (7 days) or 12 (14 days) plants in each (± SD). Initial values for fresh weight of shoot and roots, shoot to root ratio and dry matter content of shoot and roots at the start of the experiment were 0.28 ± 0.01 and 0.14 ± 0.00 g, 1.99 ± 0.05 and 6.61 ± 0.04 and 4.45 ± 0.04 %, respectively. Different letters indicate significant differences between treatments ($P<0.05$, Student's *t*-test).

	0.00 µl l^{-1} H_2S		0.05 µl l^{-1} H_2S		0.10 µl l^{-1} H_2S		0.15 µl l^{-1} H_2S	
	- S	+ S	- S	+ S	- S	+ S	- S	+ S
7 days								
Fresh weight shoot	0.62 ± 0.02^{a}	0.65 ± 0.07^{ab}	0.63 ± 0.03^{a}	0.68 ± 0.04^{ab}	0.65 ± 0.02^{ab}	0.69 ± 0.07^{ab}	0.67 ± 0.06^{ab}	0.69 ± 0.03^{b}
Fresh weight root	0.26 ± 0.00^{cd}	0.23 ± 0.01^{ab}	0.26 ± 0.01^{cd}	0.24 ± 0.01^{bc}	0.28 ± 0.03^{cd}	0.25 ± 0.02^{abd}	0.26 ± 0.01^{d}	0.23 ± 0.00^{a}
Shoot to root ratio	2.39 ± 0.05^{a}	2.84 ± 0.29^{cd}	2.43 ± 0.03^{ab}	2.78 ± 0.10^{c}	2.33 ± 0.08^{a}	2.79 ± 0.15^{c}	2.60 ± 0.14^{bc}	3.03 ± 0.10^{d}
DMC shoot	6.04 ± 0.05^{e}	5.92 ± 0.09^{ac}	5.89 ± 0.07^{ab}	5.95 ± 0.06^{bc}	5.98 ± 0.04^{bc}	6.01 ± 0.01^{e}	5.83 ± 0.05^{a}	5.91 ± 0.08^{ab}
DMC root	3.97 ± 0.09^{ad}	4.00 ± 0.05^{abc}	3.91 ± 0.05^{a}	4.04 ± 0.12^{ad}	4.02 ± 0.07^{bd}	4.07 ± 0.02^{d}	3.88 ± 0.11^{ab}	4.09 ± 0.06^{cd}
14 days								
Fresh weight shoot	1.06 ± 0.10^{a}	1.33 ± 0.08^{b}	1.37 ± 0.13^{bc}	1.70 ± 0.26^{cd}	1.40 ± 0.16^{bd}	1.57 ± 0.12^{cd}	1.51 ± 0.23^{bd}	1.62 ± 0.14^{d}
Fresh weight root	0.43 ± 0.07^{ab}	0.40 ± 0.02^{a}	0.53 ± 0.03^{cd}	0.53 ± 0.08^{bd}	0.59 ± 0.07^{d}	0.48 ± 0.04^{bc}	0.53 ± 0.11^{abd}	0.46 ± 0.03^{b}
Shoot to root ratio	2.49 ± 0.23^{abc}	3.34 ± 0.13^{de}	2.57 ± 0.09^{b}	3.24 ± 0.23^{ce}	2.37 ± 0.09^{a}	3.25 ± 0.06^{cd}	2.91 ± 0.29^{c}	3.55 ± 0.20^{e}
DMC shoot	6.64 ± 0.17^{e}	6.16 ± 0.01^{bd}	6.21 ± 0.09^{bd}	6.00 ± 0.03^{a}	6.30 ± 0.12^{d}	6.21 ± 0.15^{bcd}	6.02 ± 0.08^{ac}	5.98 ± 0.21^{abc}
DMC root	4.66 ± 0.17^{e}	4.06 ± 0.14^{ab}	4.29 ± 0.13^{bcd}	4.21 ± 0.12^{abc}	4.30 ± 0.09^{cd}	4.27 ± 0.11^{bc}	4.09 ± 0.04^{a}	4.22 ± 0.06^{bc}

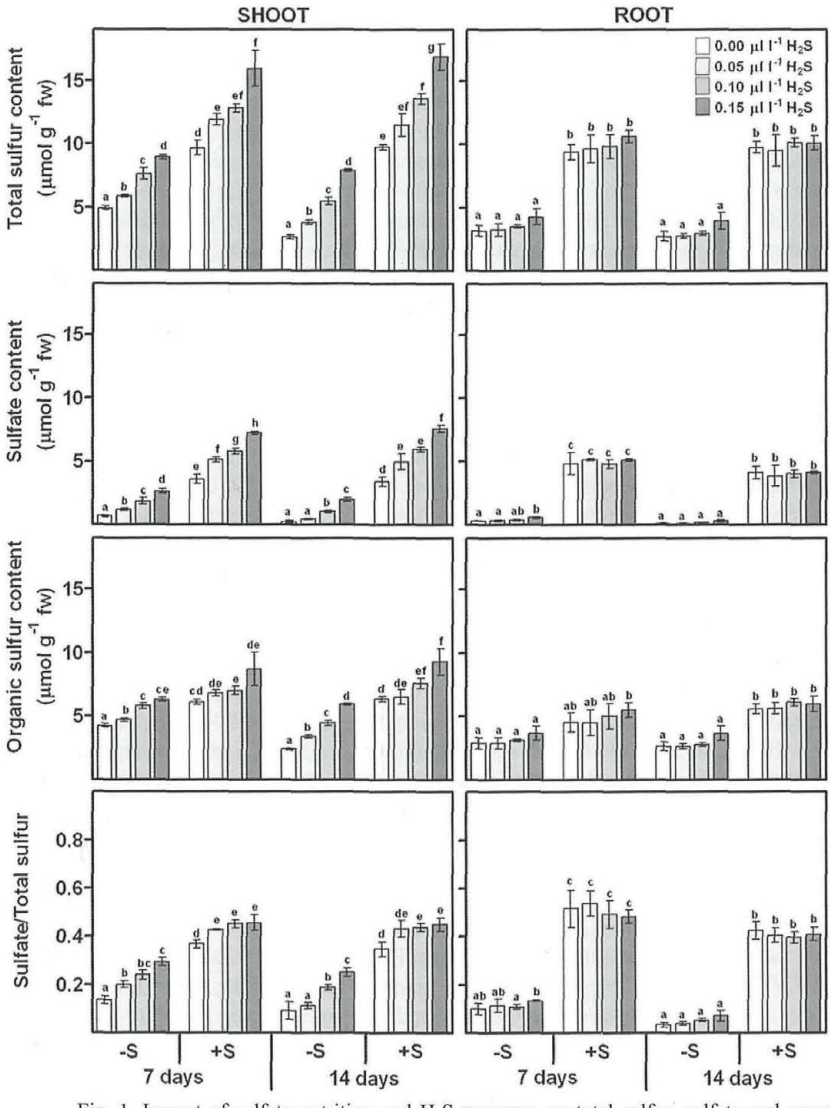

Fig. 1. Impact of sulfate nutrition and H_2S exposure on total sulfur, sulfate and organic sulfur content and sulfate to total sulfur ratio in shoot and roots of onion (*Allium cepa* L.). 25-day-old seedlings were grown in 25% Hoagland nutrient solution with 0 (-S) or 0.5 (+S) mM sulfate and exposed to 0, 0.05, 0.1 or 0.15 µl l^{-1} H_2S for two weeks. Data (µmol g^{-1} fw) represent the mean of three measurements with 24 (7 days) or 12 (14 days) plants in each (± SD). Initial values for total sulfur, sulfate and organic sulfur content and sulfate to total sulfur ratio at the start of the experiment were 9.8 ± 0.2, 3.1 ± 0.2 and 6.7 ± 0.1 µmol g^{-1} fw and 0.31 ± 0.02 for shoot and 9.4 ± 0.2, 4.0 ± 0.2 and 5.4 ± 0.3 µmol g^{-1} fw and 0.42 ± 0.03 for roots, respectively. Different letters indicate significant differences between treatments ($P<0.01$, Student's *t*-test).

Discussion

In general, sulfur deficiency has a pronounced impact on plant growth and metabolism. Biomass production is severely reduced mainly in the shoot, which results in a decrease in the shoot to root ratio (BUCHNER & al. 2004). An increase in the dry matter content is usually observed upon sulfur deficiency due to an accumulation of soluble sugars and starch (DE KOK & al. 1997, STUIVER & al. 1997). Onion is a rather slow growing species with a relative growth rate of approx. 0.1 g g^{-1} fw day^{-1} (DURENKAMP & DE KOK 2002). Therefore, sulfate deprivation for one week only resulted in minor symptoms of sulfur deficiency (Table 1, DURENKAMP & DE KOK 2004). After two weeks, however, plants evidently became sulfur deficient as demonstrated by a reduced biomass production and an increased dry matter content (Table 1). When plants were sulfate deprived and simultaneously exposed to relatively low concentrations of H_2S (0.05, 0.1 and 0.15 µl l^{-1}), biomass production and dry matter content were unaltered compared to control plants, which showed that H_2S could be used as a sulfur source for growth in onion (Table 1, BUCHNER & al. 2004, DURENKAMP & DE KOK 2004). Low levels of H_2S even resulted in a stimulation of growth in sulfate-sufficient plants (Table 1, DURENKAMP & DE KOK 2002). This phenomenon was observed in several other species, but could not be explained (THOMPSON & KATS 1978, DE KOK & al. 1983). A stimulation of growth was also observed in *Brassica oleracea* upon exposure to non-toxic levels of NH_3 (CASTRO & al. 2005). Although biomass production was unaltered upon H_2S exposure in sulfate-deprived plants compared to control plants, shoot to root ratio at 0.05 and 0.1 µl l^{-1} H_2S was comparable to that under sulfate-deprived conditions, whereas at $\geq$ 0.15 µl l^{-1} intermediate values were observed (Table 1, DURENKAMP & DE KOK 2004). This correlated well with the slightly increased sulfur content in the roots at $\geq$ 0.15 µl l^{-1} H_2S in sulfate-deprived plants (Fig. 1, DURENKAMP & DE KOK 2004), which suggested that the sulfur status of the roots partly determined the shoot to root ratio, i.e. the distribution of carbohydrates between shoot and roots, in sulfate-deprived onion.

Sulfate deprivation obviously resulted in a decrease in the content of total sulfur, sulfate, organic sulfur, thiols and sulfolipids (Fig. 1, DE KOK & al. 1997, STUIVER & al. 1997, BUCHNER & al. 2004, DURENKAMP & DE KOK 2004, KOPRIVA & al. 2004). The fast decrease in the sulfate content was due to continued growth, as well as remobilization of sulfate and its assimilation into organic sulfur compounds, leading to a decrease in the sulfate to total sulfur ratio (Fig. 1, BLAKE-KALFF & al. 1998). This also showed that the organic sulfur content is a better parameter to describe the sulfur requirement for growth than the total sulfur content (CASTRO & al. 2003), since growth of the plants was hardly affected by the fast decrease in the sulfate content. Symptoms of sulfur-deficiency presumably occur as the result of the breakdown of proteins for the liberation of organic sulfur and/or a halted formation of proteins, due to a lack of sulfur. A halted formation of proteins also results in increased contents of nitrate and free amino acids (DE KOK & al. 1997, STUIVER & al. 1997, PROSSER & al. 2001, BUCHNER & al. 2004).

H_2S levels of ≥ 0.1 µl l^{-1} should be sufficient to cover the organic sulfur requirement for growth of most plant species, including onion (DURENKAMP & DE KOK 2004). Indeed, higher H_2S levels (0.15 µl l^{-1}, Fig. 1) resulted in a slight accumulation of organic sulfur in the roots of sulfate-deprived plants, and the organic sulfur content in the shoot was equal to that of sulfate-sufficient conditions. The accumulation of organic sulfur, at least in shoots of sulfate-sufficient plants, was due to an increase in non-protein (secondary) sulfur compounds, e.g. γ-glutamyl peptides and alliins (DURENKAMP & DE KOK 2002, 2004, DURENKAMP & al. 2005). Even low levels of H_2S, below the sulfur requirement for growth, resulted in an increase in the sulfate content in shoots of sulfate-deprived plants. Sulfate accumulation under sulfate-deprived conditions could be the result of direct oxidation of H_2S and/or degradation of accumulated (secondary) sulfur compounds (DURENKAMP & DE KOK 2004).

Sulfur deprivation severely affects the distribution of sulfur in leaves and bulbs of onion (RANDLE & al. 1995, MCCALLUM & al. 2002, BLOEM & al. 2004), resulting in a decrease in sulfate and a relative increase in secondary sulfur compounds. Although secondary sulfur compounds were not directly determined in our study, it is assumed that they contribute significantly to the organic sulfur pool in onion. The increase in the sulfate to total sulfur ratio upon H_2S exposure in shoots of sulfate-deprived plants (Fig. 1), might very well reflect a partial degradation of secondary sulfur compounds, i.e. γ-glutamyl peptides and/or alliins, into sulfate (DURENKAMP & DE KOK 2004). The increased activity of alliinase, the enzyme responsible for the initial degradation of alliins, suggests a possible remobilization of secondary sulfur compounds upon sulfur deprivation (LANCASTER & al. 2000). Although decreasing contents of alliins were observed in ^{35}S-uptake studies in sulfur-deprived onion, an endogenous role of alliinase (without damaging the cell), has never been observed (RANDLE & LANCASTER 2002).

DURENKAMP & DE KOK 2004 showed that the increase in the total sulfur content upon H_2S exposure for a one-week period depended on the H_2S level and the duration of the exposure. However, after two weeks this increase was less pronounced (Fig. 1), which could have been caused by a proportional decrease in H_2S uptake (due to a proportionally decreased leaf area ratio, i.e. leaf area per gram plant), a decrease in sulfate uptake by the roots (WESTERMAN & al. 2000) and/or by a release of volatile (secondary) sulfur compounds (KANDA & TSURUTA 1995).

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