Foreword: Exploring Interactions Between Sulfate and Nitrate Uptake at a Whole Plant Level

Ineke Stulen and Luit J. De Kok

Abstract Nitrogen and sulfur are essential for crop growth and quality, because both are needed for amino acid and protein synthesis. The organic N/S ratio on a molar basis is usually about 20. Plants, therefore, must have mechanisms to coordinate sulfur and nitrogen uptake and assimilation so that appropriate proportions of sulfur containing and other amino acids are available for protein synthesis. Experiments with vegetable crop plants grown at non-limiting nutrient supply showed that the uptake rates of nitrate and sulfate by the root are related to the growth rate of the plant. Reduced nitrogen and sulfur compounds as glutamine, glutathione and *O*-acetyl-L-serine, and/or nitrate and sulfate, might act signal molecules in regulation of the uptake of nitrate and sulfate. However, there is no evidence for a direct linkage between the uptake of nitrate and sulfate.

Introduction

In general most soils contain sufficient sulfur to cover the requirements of plants, whereas nitrogen is often limiting for plant growth. Nitrogen in soil is available in various forms, but in agriculture mostly nitrate and some ammonium are the main forms taken up by the root and used as source for growth (Miller and Chapman 2011). Sulfate taken up by the root appears to be the major sulfur source for growth (Hawkesford and De Kok 2006; Zhao et al. 2008; Haneklaus et al. 2007; De Kok et al. 2011). However, in industrialized areas atmospheric sulfur deposition may

---

I. Stulen • L.J. De Kok (✉)
Laboratory of Plant Physiology, University of Groningen,
P.O. Box 11103, 9700 CC Groningen, The Netherlands
e-mail: l.j.de.kok@rug.nl

L.J. De Kok et al. (eds.), *Sulfur Metabolism in Plants*, Proceedings of the International Plant Sulfur Workshop 1, DOI 10.1007/978-94-007-4450-9_1,

contribute to a significant extent to the sulfur fertilization of plants (De Kok et al. 2007, 2009, 2011), whereas nitrogen fertilization by atmospheric nitrogen deposition is limited (Wellburn 1990).

Nitrate and sulfate need to be reduced prior to their incorporation into various essential organic nitrogen and sulfur compounds. The uptake and assimilation of sulfur and nitrogen are strongly interrelated, since the major proportion of the reduced nitrogen and sulfur in plants is incorporated into amino acids and subsequently into proteins (Stulen and De Kok 1993; De Kok et al. 2011). The synthesis of cysteine from *O*-acetylserine and sulfide is a major reaction in the direct coupling between nitrogen and sulfur metabolism in the plant (Brunold 1993). Cysteine plays a key role in the synthesis of organic sulfur compounds; it is incorporated into proteins and the tripeptide glutathione and it is used as the sulfur compound for the synthesis of the essential amino acid methionine (Giovanelli 1990). Proteins contain both sulfur and non-sulfur amino acids and for this reason the availability of nitrogen and sulfur interacts with the utilization of nitrogen and sulfur for proteins and plant growth. Plants maintain their nitrogen and sulfur content in proteins within a certain range (Stulen and De Kok 1993) and the organic N/S ratio is generally around 20 on a molar basis (Stulen and De Kok 1993; Haneklaus et al. 2007). Plants, therefore, must have mechanisms to coordinate the uptake and reduction of sulfate and nitrate so that appropriate proportions of both sulfur containing and other amino acids are available for protein synthesis.

Nitrogen and sulfur research has mainly been focused on elucidating the pathways and characterizing the transporters and enzymes involved in their uptake and assimilation and their subsequent incorporation into organic compounds, from the molecular to the crop yield level. Although both laboratory and agronomic data indicate N/S interactions in metabolism, growth and plant composition, it is not known whether a direct mutual regulation as for instance proposed by Reuveny et al. (1980) based on experiments with isolated plant cell model systems growing under extreme nutrition conditions (nitrogen and sulfate deprivation), really occurs in whole plants in steady state grown under well-controlled nutrient conditions. Experiments on the effect of changes in nutrient supply and various environmental conditions have been performed from the molecular, biochemical and physiological to crop yield level. However, the question to what extent the measured changes in parameters as expression of the transporters and enzymes are of physiological significance in a whole plant context is not often addressed.

This foreword briefly evaluates the physiological mechanisms involved in the regulation of nitrogen and sulfur uptake, and the regulatory control of the coordination, on a whole plant level, based on results of experiments performed in our research group during our long cooperation.

Nitrate and Sulfate Uptake in Relation to Plant Growth Rate

The uptake of nutrients by the roots is generally adapted/in tune with the plant's need for growth (Hawkesford and De Kok 2006; Zhao et al. 2008; Haneklaus et al. 2007; De Kok et al. 2011). For plants in the vegetative phase, grown at non-limiting nutrients, the nutrient flux (N_{flux}) needed per gram plant biomass produced with time can be calculated as follows (Haneklaus et al. 2007; Zhao et al. 2008; De Kok et al. 2011):

$$N_{flux} = N_{content} \times \text{RGR}$$

where the N_{flux} is expressed as μmol g^{-1} plant day^{-1}, $N_{content}$ is the total nutrient content of the plant (μmol g^{-1} plant) and RGR is the relative growth rate of the plant during the growth period under investigation (g g^{-1} day^{-1}). RGR can be calculated by linear regression from the ln transformed weight data (Hunt 1982) or by an exponential fit of the weight data (Poorter 1989).

The uptake of nitrate and sulfate by the root are active processes and driven by a proton gradient maintained by a proton ATPase, mediated by transporter proteins. Distinct nitrate transporter groups have been characterized and plants contain inducible (iHATS) and constitutive (cHATS) high affinity nitrate transporters and constitutive low affinity nitrate transporters (LATS) (Touraine 2004; Miller and Chapman 2011). Likewise, different sulfate transporter proteins are involved in the uptake and distribution of sulfate in the plant, which may contain 12–14 different transporters classified in up to five different groups according to the possible functioning (Hawkesford and De Kok 2006; De Kok et al. 2011).

In experiments with *Spinacia oleracea* L. and *Plantago major* L. net nitrate uptake rate (NNUR) was measured in combination with RGR and plant nitrogen content. NNUR can be expressed on a plant weight basis (μmol g^{-1} plant day^{-1}) or a root weight basis (μmol g^{-1} root day^{-1}). Plants with a relatively low root weight ratio (RWR, root weight/plant weight) have a relatively high uptake rate on a root weight basis. These experiments showed that the measured NNUR was in accordance with the plant nitrogen flux, and therefore of physiological significance (Ter Steege et al. 1998, 1999; Fonseca et al. 1997). There was a linear relationship between RGR and measured NNUR, if plants were grown in non-limiting nutrient solution under the same environmental conditions and with similar plant nitrogen content and root weight ratio (Fig. 1). Part of the *Plantago* plants was grown at elevated $CO_{2,}$ which resulted in an increase in RGR at the time of the measurement, Fig. 1 shows that NNUR in these species is closely linked to the RGR of the plant. Apparently, the NNUR is a well-regulated process, under the control of an internal regulating mechanism, which adjusts the NNUR to the nitrogen need of the plant, as determined by RGR and total plant nitrogen content (Touraine et al. 1994; Ter Steege et al. 1998, 1999).

There has been a long debate on the role of nitrate influx and efflux in the control of NNUR by roots under steady-state conditions. A double labeling design, with both ^{13}N- and ^{15}N-nitrate, made it possible to study the contribution of both fluxes to the regulation of NNUR rate in spinach (Ter Steege et al. 1998). These experiments showed that nitrate influx and efflux together regulate NNUR by roots, thereby providing a flexible and sensitive nitrate uptake system (Ter Steege et al. 1998, 1999; Miller and Chapman 2011; Fig. 2). It is unclear to what extent efflux of sulfate (*e.g.* via sulfate selective anion channels) has significance in the regulation of the net sulfate uptake by roots is still an open question (De Kok et al. 2011).

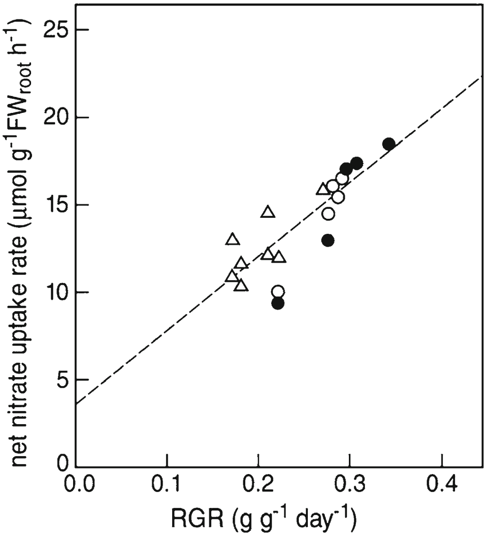

Fig. 1 Relationship between net nitrate uptake rate in *Spinacia oleracea* L. (Data from Ter Steege et al. 1998, 1999) and *Plantago major* L. (Fonseca et al. 1997), grown in nutrient solution with unlimited access to nitrate. *Spinacia* (Δ); *Plantago*, grown at an ambient (○) or elevated (•) atmospheric CO_2 concentration of 350 and 700 µl l^{-1}, respectively

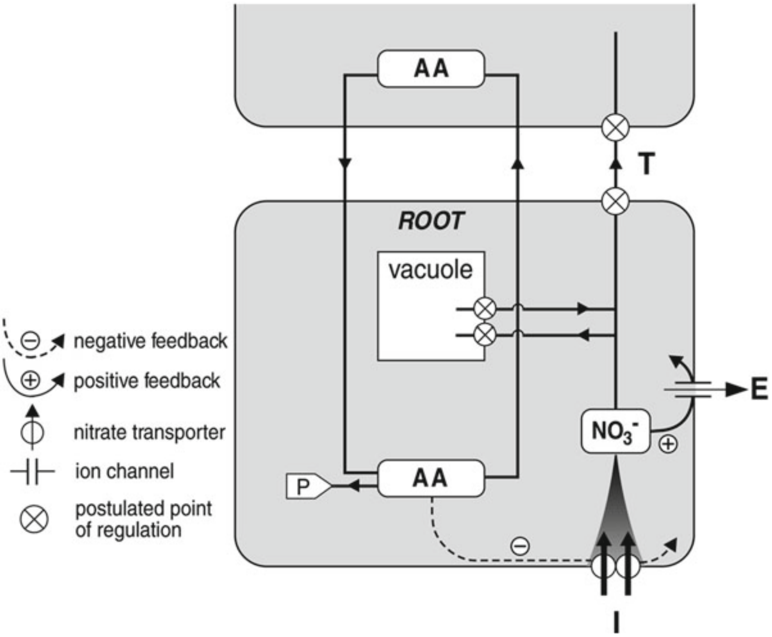

Fig. 2 Localization and regulation of processes of uptake, translocation and storage of nitrate in plant roots. *I* nitrate influx, *E* nitrate efflux, *T* nitrate translocation, *AA* amino acids, *P* protein (Adapted from Ter Steege 1996)

Regulation of Nitrate Uptake – Comparison with Sulfate Uptake

The regulation of activity of the nitrate and sulfate transporters may be controlled at a transcriptional, translational and/or post-translational level (*e.g.* activation/deactivation; Hawkesford and De Kok 2006; De Kok et al. 2011). However, the expression and activity of the nitrate and sulfate transporters are differently regulated. Sulfur-deprived plants are characterized by a high expression and uptake capacity of the sulfate transporters (Westerman et al. 2000; Buchner et al. 2004; Hawkesford and De Kok 2006; Koralewska et al. 2008, 2009), whereas nitrate-deprived plants, show a lag phase in nitrate uptake capacity, related to an induction phase of the nitrate transporter proteins (Clarkson 1986).

It has been postulated that a shoot-derived signals down-regulate nitrate and sulfate uptake by negative feedback control at the level of the nitrate transporter proteins (Touraine et al. 1994; Ter Steege 1996; Ter Steege et al. 1999) and sulfate transporter proteins (Hawkesford and De Kok 2006). At present glutamine seems to be the most likely signal molecule for the regulation of nitrate influx (Touraine et al. 1994). Nitrate efflux might offer a mechanism for rapid reactions to increased cytoplasmic root nitrate concentrations, and nitrate itself might act as signal molecule (Ter Steege 1996; Hawkesford 2011). The signal transduction pathway involved in the regulation of the uptake sulfate uptake it still largely unsolved; it might be signaled or mediated by sulfate itself or products of the assimilatory reduction pathway (*e.g.* H_2S, cysteine or glutathione; Hawkesford and De Kok 2006; De Kok et al. 2011). Moreover, the cysteine precursor *O*-acetyl-L-serine (OAS) is thought to play an important role in the induction of sulfate uptake (Clarkson et al. 1999). However, the majority of plant cells, including root cells, have the capacity to both reduce and assimilate nitrate and sulfate, presumably facilitating local signaling of nitrate and sulfate uptake at a cellular level, which makes it difficult to separate local signaling at a local cellular root level from signaling at an integrated tissue *viz.* shoot to root level (Hawkesford and De Kok 2006; De Kok et al. 2011). Besides, it remains obscure to what extent changes concentrations of potential signal compounds and expression of the nitrate and sulfate transporters, both determined at the whole organ level, provides sufficient insight into the actual regulatory control of the sulfate uptake at the root cellular level (De Kok et al. 2011).

There is apparently no direct linkage between the uptake of nitrate and sulfate in roots. Sulfate deprivation of *Brassica* resulted in a decreased growth and nitrate uptake rate, whereas the expression and activity if the sulfate transporters rapidly increased (Westerman et al. 2000; Buchner et al. 2004; Yang et al. 2006; Koralewska et al. 2008, 2009). When sulfate-sufficient *Brassica* plants were exposed to atmospheric H_2S, both nitrate uptake rate and RGR were unaffected, while the sulfate uptake was decreased (Westerman et al. 2000, 2001). Exposure of *Brassica* to atmospheric NH_3 resulted in a downregulation of the nitrate uptake, whereas the uptake of sulfate remained unaffected (Castro et al. 2006). If *Brassica* plants were exposed to elevated Cu^{2+} concentrations in the root environment, it resulted in both a decrease in plant growth and nitrate uptake, however, the sulfate uptake was increased (Shahbaz et al. 2010).

The latter was probably due to a direct interference of the Cu with the signal transduction pathway involved in the regulation of the expression and activity of the sulfate transporters (Shahbaz et al. 2010).

Conclusions

Plants maintain their nitrogen and sulfur content within a certain range, since both are essential for synthesis of proteins. Protein synthesis requires inorganic carbon, and reduced nitrogen and sulfur. Co-ordination of the assimilatory reduction pathways of nitrate and sulfate is therefore necessary, so that appropriate proportions of both sulfur containing and other amino acids are available for protein synthesis. This might implicate a mutual regulation of the nitrate and sulfate uptake by the root. However, from our studies it is evident that changes in nitrate uptake rate are related to changes in growth, and that there is no direct linkage between the uptake of nitrate and sulfate.

References

Brunold C (1993) Regulatory interactions between sulfate and nitrate assimilation. In: De Kok LJ, Stulen I, Rennenberg H, Brunold C, Rauser WE (eds) Sulfur nutrition and assimilation in higher plants: regulatory, agricultural and environmental aspects. SPB Academic Publishing bv, The Hague, pp 61–75

Buchner P, Stuiver CEE, Westerman S, Wirtz M, Hell R, Hawkesford MJ, De Kok LJ (2004) Regulation of sulfate uptake and expression of sulfate transporter genes in *Brassica oleracea* as affected by atmospheric H_2S and pedospheric sulfate nutrition. Plant Physiol 136:3396–3408

Castro A, Stulen I, Posthumus FS, De Kok LJ (2006) Changes in growth and nutrient uptake in *Brassica oleracea* L. exposed to atmospheric ammonia. Ann Bot 97:121–131

Clarkson DT (1986) Regulation of the absorption and release of nitrate by plant cells: a review of current ideas and methodology. In: Lambers H, Neeteson JJ, Stulen I (eds) Physiological, ecological and applied aspects of nitrogen metabolism in higher plants. Martinus Nijhoff Publishers, Dordrecht, pp 3–28

Clarkson DT, Diogo E, Amâncio S (1999) Uptake and assimilation of sulphate by sulphur deficient *Zea mays* cells: the role of *O*-acetyl-L-serine in the interaction between nitrogen and sulphur assimilatory pathways. Plant Physiol Biochem 37:283–290

De Kok LJ, Durenkamp M, Yang L, Stulen I (2007) Atmospheric sulfur. In: Hawkesford MJ, De Kok LJ (eds) Sulfur in plants – an ecological perspective. Springer, Dordrecht, pp 91–106

De Kok LJ, Yang L, Stuiver CEE, Stulen I (2009) Negative vs. positive functional plant responses to air pollution: a study establishing cause-effect relationships of SO_2 and H_2S. In: Legge AH (ed) Air quality and ecological impacts: relating sources to effects, 9th edn, Developments in environmental sciences. Elsevier B.V, Amsterdam/Boston/London, pp 121–134

De Kok LJ, Stulen I, Hawkesford MJ (2011) Sulfur nutrition in plants. In: Hawkesford MJ, Barraclough P (eds) Molecular and physiological basis of nutrient use efficiency in crops. Wiley-Blackwell, Chichester/Ames, pp 295–309

Fonseca FG, Bowsher CG, Stulen I (1997) Impact of elevated CO_2 on nitrate reductase transcription and activity in leaves and roots of *Plantago major*. Physiol Plant 100:940–948

Giovanelli J (1990) Regulatory aspects of cysteine and methionine synthesis. In: Rennenberg H, Brunold C, De Kok LJ, Stulen I (eds) Sulfur nutrition and sulfur assimilation in higher plants; fundamental, environmental and agricultural aspects. SPB Academic Publishing, The Hague, pp 33–48

Haneklaus S, Bloem E, Schnug E, De Kok LJ, Stulen I (2007) Sulfur. In: Barker AV, Pilbeam DJ (eds) Handbook of plant nutrition. CRC Press, Boca Raton, pp 183–238

Hawkesford MJ (2011) An overview of nutrient use efficiency and strategies for crop improvement. In: Hawkesford MJ, Barraclough P (eds) Molecular and physiological basis of nutrient use efficiency in crops. Wiley-Blackwell, Chichester/Ames, pp 5–19

Hawkesford MJ, De Kok LJ (2006) Managing sulfur metabolism in plants. Plant Cell Environ 29:382–395

Hunt R (1982) Plant growth curves. The functional approach to plant growth analysis. E. Arnold Publ, London

Koralewska A, Stuiver CEE, Posthumus FS, Kopriva S, Hawkesford MJ, De Kok LJ (2008) Regulation of sulfate uptake, expression of the sulfate transporters Sultr1;1 and Sultr1;2, and APS reductase in Chinese cabbage (*Brassica pekinensis*) as affected by atmospheric H_2S nutrition and sulfate deprivation. Funct Plant Biol 35:318–327

Koralewska A, Buchner P, Stuiver CEE, Posthumus FS, Kopriva S, Hawkesford MJ, De Kok LJ (2009) Expression and activity of sulfate transporters and APS reductase in curly kale in response to sulfate deprivation and re-supply. J Plant Physiol 166:168–179

Miller AJ, Chapman N (2011) Transporters involved in nitrogen uptake and movement. In: Hawkesford MJ, Barraclough P (eds) Molecular and physiological basis of nutrient use efficiency in crops. Wiley-Blackwell, Chichester/Ames, pp 193–210

Poorter H (1989) Plant growth analysis: towards a synthesis of the classical and the functional approach. Physiol Plant 75:237–244

Reuveny Z, Dougall DK, Trinity P (1980) Regulatory coupling of nitrate and sulfate assimilation pathways in cultured tobacco cells. Natl Acad Sci USA 77:6670–6672

Shahbaz M, Tseng MH, Stuiver CEE, Koralewska A, Posthumus FS, Venema JH, Parmar S, Schat H, Hawkesford MJ, De Kok LJ (2010) Copper exposure interferes with the regulation of the uptake, distribution and metabolism of sulfate in Chinese cabbage. J Plant Physiol 167:438–446

Stulen I, De Kok LJ (1993) Whole plant regulation of sulfur metabolism: a theoretical approach and comparison with current ideas on regulation of nitrogen metabolism. In: De Kok LJ, Stulen I, Rennenberg H, Brunold C, Rauser WE (eds) Sulfur nutrition and assimilation in higher plants: regulatory, agricultural and environmental aspects. SPB Academic Publishing bv, The Hague, pp 77–91

Ter Steege MW (1996) Regulation of nitrate uptake in a whole plant perspective. Thesis, University of Groningen, The Netherlands

Ter Steege MW, Stulen I, Wiersema PK, Paans AJM, Vaalburg W, Kuiper PJC, Clarkson DT (1998) Growth requirement for N as a criterion to assess the effects of physical manipulation on nitrate uptake fluxes in spinach. Physiol Plant 103:181–192

Ter Steege MW, Stulen I, Wiersema PK, Posthumus F, Vaalburg W (1999) Efficiency of nitrate uptake in spinach: impact of external nitrate concentration and relative growth rate on nitrate influx and efflux. Plant Soil 208:125–134

Touraine B (2004) Nitrate uptake by roots – transporters and root development. In: Amâncio S, Stulen I (eds) Nitrogen acquisition and assimilation in higher plants. Kluwer Academic, Dordrecht, pp 1–34

Touraine B, Clarkson DT, Muller B (1994) Regulation of nitrate uptake at the whole plant level. In: Roy J, Garnier E (eds) A whole plant perspective on carbon-nitrogen interactions. SPB Academic Publishing, The Hague, pp 11–30

Wellburn AR (1990) Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers. New Phytol 115:395–429

Westerman S, De Kok LJ, Stulen I (2000) Interaction between metabolism of atmospheric H_2S in the shoot and sulfate uptake by the roots of curly kale (*Brassica oleracea* L.). Physiol Plant 109:443–449

Westerman S, Stulen I, Suter M, Brunold C, De Kok LJ (2001) Atmospheric H_2S as sulfur source for *Brassica oleracea*: consequences for the activity of the enzymes of the assimilatory sulfate reduction pathway. Plant Physiol Biochem 39:425–432

Yang L, Stulen I, De Kok LJ (2006) Impact of sulfate nutrition on the utilization of atmospheric SO_2 as sulfur source for Chinese cabbage. J Plant Nutr Soil Sci 169:529–534

Zhao FJ, Tausz M, De Kok LJ (2008) Role of sulfur for plant production in agricultural and natural ecosystems. In: Hell R, Dahl C, Knaff DB, Leustek T (eds) Sulfur metabolism in phototropic organisms. Springer, New York, pp 425–443