Proceedings of the International Plant Sulfur Workshop

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Sulfur Metabolism in Plants

Mechanisms and Applications to Food Security and Responses to Climate Change

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Luit J. De Kok, Heinz Rennenberg, and Malcolm J. Hawkesford

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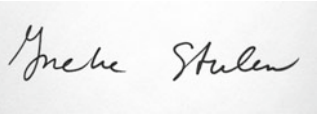

Preface

This proceedings volume contains the invited and a selection of the contributed papers of the 8th International Workshop on Sulfur Metabolism in Higher Plants, which was held at the Department of Forest and Ecosystem Science, University of Melbourne, Water Street, Creswick, Victoria 3363, Australia, from November 22 to 27, 2010. The meeting was co-organized by the University of Melbourne (Australia), the University of Groningen (The Netherlands), Massey University, Palmerston North (New Zealand), the International Plant Nutrition Institute, Horsham (Australia), and CSIRO Plant Industry, Canberra (Australia). The content of the volume shows that the understanding of sulfur metabolism in plants and the interaction of the environment are rapidly progressing. This volume covers various aspects of the regulation of sulfate uptake and assimilation in plants, from a cellular to a whole plant level, and additionally emphasizes interactions with other minerals. Moreover, the significance of sulfur metabolism in biotic and abiotic stress responses, in food security and quality, and in relation to interactions with global change factors is discussed in detail.

We are pleased to dedicate this book to A/Professor Ineke Stulen, Laboratory of Plant Physiology, University of Groningen, The Netherlands. She was one of the initiators of the Sulfur Workshop series in 1989 and was a member of the Organizing Committee of all succeeding workshops.

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Sulfur Metabolism – Food Security and Environmental Interactions

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

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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):

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

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).

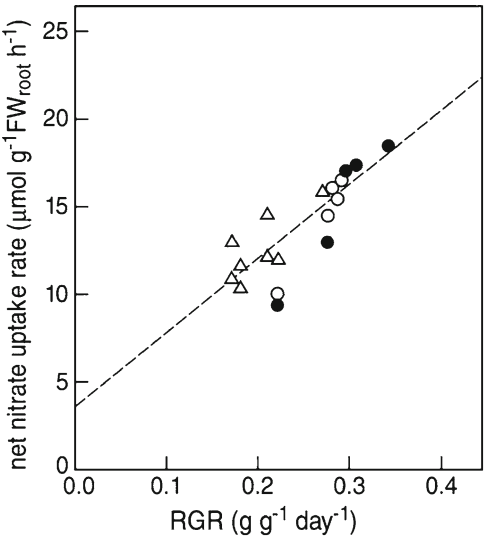

Fig. 1 Relationship between net nitrate uptake rate in *Spinacia oleracea* L. (Data from Ter Steege et al. 1998, 1999) and *Plantago major* L. (Fonseca et al. 1997), grown in nutrient solution with unlimited access to nitrate. *Spinacia* (Δ); *Plantago*, grown at an ambient (○) or elevated (•) atmospheric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

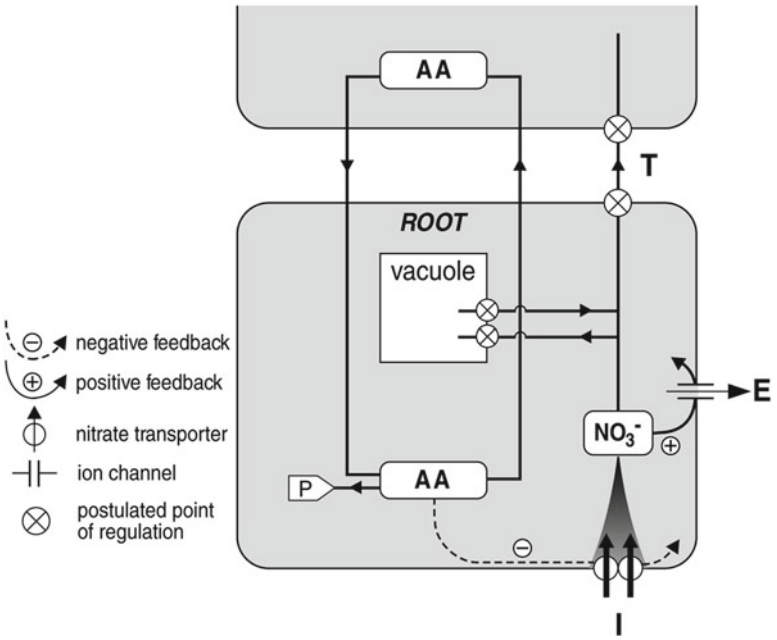

Fig. 2 Localization and regulation of processes of uptake, translocation and storage of nitrate in plant roots. *I* nitrate influx, *E* nitrate efflux, *T* nitrate translocation, *AA* amino acids, *P* protein (Adapted from Ter Steege 1996)

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.

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Part I
Sulfur Metabolism – Mechanisms

Sulfate Uptake and Assimilation – Whole Plant Regulation

Malcolm J. Hawkesford

Abstract Sulfur remains an important issue on the agenda for crop plant nutrition. In addition to avoidance of sulfur deficiency, which will impact on yield and quality, there are requirements for adequate fertilization of crops for resistance to biotic and abiotic stresses. Equally importantly, there are clear consequences for efficient nitrogen utilization and there are interactions with micronutrient acquisition (selenium and molybdenum). Substantial advances have been made at the cellular level, dissecting the signal transduction pathways linking cellular nutritional status with expression of sulfur regulated genes and pathways. However cellular processes need to be placed in the context of whole plant regulation of sulfur uptake and assimilation, which encompasses developmental, spatial and environmental factors, and which facilitates optimum growth and fecundity (and yield in the case of crops) with available sulfur supply. During development, adequate sulfur must be acquired for optimum growth, ideally with any excess being sequestered into re-mobilizable temporary stores. As the plant develops, efficient utilization of sulfur will require organ to organ transfer, and additionally degradation pathways, metabolic inter-conversions and multiple trans-membrane and vascular tissue mediated transport steps for both inorganic and organic sulfur compounds. For crops, efficient transfer of sulfur to harvested sink tissues and its incorporation into protein are important agronomic traits. Insufficient sulfur to meet the demand for growth results in a number of plant responses, targeted at optimising uptake and use of available sulfur. Notable early and specific responses are the up-regulation of transporters and key steps of the assimilatory pathways in sulfur-deficient tissues, and the allocation of resources to stimulate growth of root tissues compared to the shoots.

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These responses involving root proliferation and transporter functionality are adaptations to improve pedospheric sulfur acquisition. A long standing question has concerned the existence and nature of inter-organ signals of nutrient status. It is possible that local sulfur availability, coupled with intrinsic cell specific programmed function, is sufficient to mediate local gene and pathway expression, influence organ responses and effect whole plant sulfur management without inter organ signals. Developmental cues will influence organ specific pathways, most clearly demonstrated in processes of leaf senescence and associated nutrient remobilization. Conversely, the recognition of possibly mobile phloem located miRNAs may be indicative of long distance regulatory mechanisms. Similarly, root proliferation will almost certainly have a hormonal basis.

Introduction

With respect to whole plant regulation of sulfur uptake and assimilation, the key questions to consider include: what regulation occurs, when is this regulation important, what signals are involved to initiate/control the regulation, what transduction pathways are required, how is cellular regulation integrated at the whole plant level and ultimately at the ecosystem level, and finally what are the consequences for agriculture? The primary function of regulatory mechanisms is to manage fluxes of sulfur in response to developmental and environmental cues (Hawkesford and De Kok 2006). The goal for the plant is to optimise the use of available sulfur to match the demands for growth and development, and for resistance to stress. Under limiting conditions this focuses on survival and reproduction. For a crop, management of sulfur is essential for both yield and quality (Zhao et al. 1999).

There has been substantial progress in the elucidation of signal transduction pathways at the cellular and gene level (see the chapter “Molecular and Cellular Regulation of Sulfate Transport and Assimilation”, this volume), although the key links between metabolism and the transduction pathways remain elusive. Regulation at the level of the whole plant is at least the sum of cellular regulation integrated throughout the whole plant, and comprises both spatial and temporal components, possible inter organ signaling, and a co-ordination with developmental processes.

Key Processes

Regulated processes extend from flux control in branches of the sulfur assimilatory pathways to whole plant distribution of sulfur pools. The uptake of sulfate into plant roots has long been known to be regulated (induced during sulfur-deficiency, repressed at adequate supply), and this has been shown to be due to the direct regulation (certainly transcriptional (Smith et al. 1995a, 1997) but also possibly post transcriptional control (Yoshimoto et al. 2007) and post translational control

as evidenced by the presence of the STAS (sulfate transporter and anti-sigma antagonist) domain (Aravind and Koonin 2000; Shibagaki and Grossman 2004; Rouached et al. 2005) of sulfate transporters (STs) at the plasma membrane of root cells. The transport of sulfate is catalyzed by members of a transporter family (SulP), which for example comprises 14 genes in *Arabidopsis*: these may be subdivided into five groups (Hawkesford 2003), with respectively, high and low affinities, undetermined function, vacuolar efflux transport function and a specific involvement in Mo accumulation. Expression of the genes for many of these transporters (particularly Groups 1, 2 and 4) is influenced by sulfur-nutritional status and development and the differential expression and activity of the whole family will contribute significantly to distribution, storage and remobilization of sulfate and other oxyanion pools (see below).

In wheat or barley root tissues, the major Group 1 ST is up-regulated (transcript abundance and transporter activity as measured by root uptake capacity) under conditions of limiting sulfur and re-repressed upon re-supply (Smith et al. 1997; Buchner et al. 2010). In *Arabidopsis* and Brassica the situation is complicated by the occurrence of two related transporters having differential patterns of expression: both are more highly expressed under sulfur-limiting conditions but one (Sultr1;1) is highly induced from a situation of little or no expression (hence very many-fold), whilst the other (Sultr1;2) shows expression increased around a twofold, with a background level comparable to the induced state of Sultr1;1.

Substantial regulation of the assimilatory pathway is evident (see Chap. 3, Yoshimoto and Saito, this volume, and Fig. 1), facilitating coordination with C and N metabolism (and hence growth), and enabling partitioning between primary assimilation and sulfur-containing amino acid biosynthesis or to secondary metabolites such as glucosinolates. Substantial allosteric or gene expression regulation of ATP sulfurylase, APS reductase (APR) and APS kinase as well as the occurrence of compartment-specific isoforms of these enzymes facilitates biochemical partitioning of sulfur between different branches of the assimilatory pathway (*e.g.* (Vauclare et al. 2002; Mugford et al. 2009)).

An important coordination with C/N metabolism occurs at the level of cysteine biosynthesis, with the cysteine synthase complex (serine acetyltransferase (SAT) and *O*-acetylserine(thiol)lyase (OASTL)) acting as both a sensor and a regulator (Hell et al. 2002), mediated by a reversible association/dissociation of the complex. SAT is active when associated with OASTL, but inactive when dissociated. As the dissociation is promoted by excess OAS, the complex effectively senses both OAS and sulfur availability, and self regulates further OAS production accordingly. Thus OAS is a signal mediating between substrate availability and flux. OASTL, which is in excess, will always catalyze synthesis of cysteine given availability of OAS and sulfide.

The induction/repression of STs and initial steps of the assimilatory pathway was assumed initially to be a negative feedback controlling gene expression, mediated by downstream products of the assimilatory pathway (*e.g.* cysteine or glutathione, see Fig. 1). Subsequently a model was proposed in which OAS mediates the up-regulation of ST gene (and others) expression (also shown on Fig. 1). This was proposed by analogy with the prokaryotic model, for example the *cysB* mediated

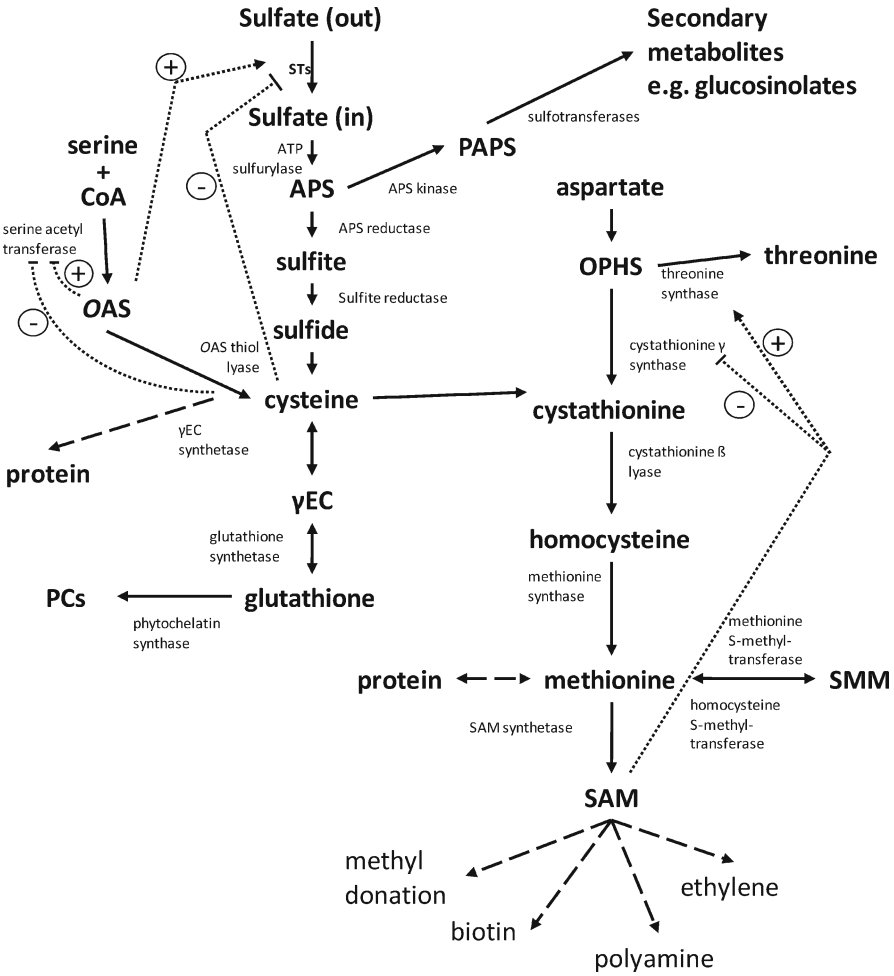

Fig. 1 Cellular regulation: S metabolism is part of a metabolic web (Modified from (Hawkesford et al. 2006)). *Solid lines* represent metabolite pathways (*dashed*=multiple steps), *dotted lines* represent possible feedback loops, which may be allosteric or may act via control of gene expression. *ST* sulfate transporter, *APR* APS reductase, *OAS O*-acetyl serine, *APS* adenosine 5′-phosphosulfate, *PAPS* 3′-phosphoadenosine5′-phosphosulfate, *OPHS O*-phosphohomo-serine, *SAM S*-adenosyl methionine, *SMM S*-methylmethionine, *PCs* phytochelatins

regulation in *Salmonella typhimurium* and *Escherichia coli* (Kredich 1993) in which OAS bound to cysB protein facilitates transcription, whilst sulfide is inhibitory. Evidence to support such a mechanism in plants was initially obtained by OAS feeding experiments to young seedlings grown in hydroponics, which showed enhanced ST expression in roots, with consequent high levels of reduced sulfur compounds (cysteine and glutathione) which might have been expected to mediate repression (Smith et al. 1997). A similar induction by OAS was seen in potato, and

furthermore, transgenic over-expression of serine acetyltransferase (SAT), which modestly enhanced endogenous root OAS levels, also resulted in induction of a high affinity ST in roots (Hopkins et al. 2005). However a sulfur-deprivation treatment (up to 8 days) eventually leading to a huge OAS accumulation, only resulted in a small transitory increase in ST expression, soon after the removal of the external sulfur supply and before there was any OAS accumulation. In spite of these anomalies, regulation mediated by OAS or thiol compounds remains a viable model, but critically is not yet proven in plants. Alternatively sulfate may be a candidate as a signal as tissue sulfate contents show an inverse relationship with measured specific mRNA pools for STs (Buchner et al. 2010). Another candidate may be sulfide as copper exposure induces ST and APR expression in Chinese cabbage (*Brassica pekinensis)*, with one explanation being an interference with the induction/repression signal transduction pathway, possibly by binding sulfide (Shahbaz et al. 2010).

Combining pedospheric sulfur deprivation experiments with atmospheric supply of hydrogen sulfide indicated, for *Brassica napus* and *Brassica pekinensis*, that foliar absorbed hydrogen sulfide is a viable source of sulfur to meet demands for growth, however little repression of induced root STs was observed. Shoot to root communication was concluded to be inefficient (Buchner et al. 2004; Koralewska et al. 2008).

Whereas in barley and wheat there appear to be good correlations between transport activity, gene expression and tissue sulfate concentrations (Smith et al. 1997; Buchner et al. 2010), this is not always the case. Large changes in mRNA abundance are often not matched by large changes in transporter capacity. Upon S resupply in *Brassica oleracea*, expression of BolSultr1;1 (as indicated by mRNA abundance) is transiently repressed but then remains high, decreasing only after a number of days; transport activity also remains high in spite of tissue sulfate and thiol levels being restored to control (sulfur-replete) levels (Koralewska et al. 2009). Although it appears that sometimes there is no correlation between activity, gene expression and potential regulatory metabolites, all of these studies are severely limited by the imprecise spatial resolution of the tissue sampling, usually separating little more than shoots and roots.

Translating cellular responses up to whole plant responses to sulfur limitation is dominated by the concept of demand driven regulation (Lappartient and Touraine 1996; Lappartient et al. 1999). Demand may be defined at the cellular or whole plant level, and will depend on growth rate and ‘extra’ demands imposed by stress resistance mechanisms involving sulfur-containing compounds; sulfur nutritional status will be the balance of supply and demand (Fig. 2). Demand at the cellular level modifies metabolite levels, which in turn triggers the changes in gene expression, and which leads to modified pathway or transporter activity. As internal pools are depleted in cells or organs, a ‘signal’ is effectively propagated, without the need for a long distance signaling molecule. Confounding this simplistic model is the recent description of a micro RNA (miR395) which has a likely role in ensuring turnover of mRNAs for key sulfur pathways genes, including a sulfate transporter (2:1) and ATPS; it is possible that this may be phloem-mobile and could facilitate a long-distance signal function (Kawashima et al. 2009; Liang et al. 2010).

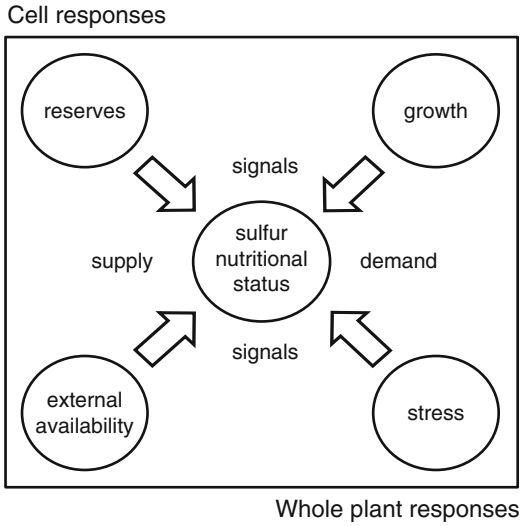

Fig. 2 Local or systemic regulation? The sulfur nutritional status will depend on both supply (external and internal pools) and demand (from growth and responses to stresses requiring S-containing compounds); cell responses involve changes in gene expression and modified fluxes through biochemical pathways which have a net impact on whole plant fluxes of sulfur, modified biomass partitioning and ultimately development. Whole plant regulation may be viewed as an extension of regulatory processes at the single cell or may involve signals between cells distributed throughout the plant

Coordination During Development

Sulfate transporter (ST) expression in relation to the management of plant sulfur reserves has been examined in detail in *Arabidopsis* (Yoshimoto et al. 2002; Kataoka et al. 2003, 2004a, b, c; Kataoka and Takahashi 2005), *Brassica* (Buchner et al. 2004; Parmar et al. 2007; Koralewska et al. 2008, 2009) and wheat (Buchner et al. 2010; Shinmachi et al. 2010). Cell specific expression data has been determined in *Arabidopsis* but is limited for the other species, and whilst many similarities exist between the different species (allowing straightforward assumptions of functional homologues), specific peculiarities are apparent, for example wheat has no close homologue of Atsulr1;2. In addition, the simplistic assumption of individual STs being expressed in a cell or organ specific manner is inadequate and almost all transporters are expressed in all tissues at some point in development, albeit at quite different levels and with differential responses to sulfur-nutrition (Buchner et al. 2010).

Sulfate is taken up to meet the needs for growth and metabolism throughout development, and that which is in excess of current demand is stored in vacuoles; generally it is assumed that cytoplasmic sulfate concentrations do not vary greatly. The vacuolar sulfate pools represent the tissue sulfate pools that respond to periods when demand outstrips supply, for example due to depletion in the pedosphere or unavailability caused by limiting water supply. However pre-programmed patterns