

Proceedings of the International Plant Sulfur Workshop

Luit J. De Kok
Malcolm J. Hawkesford
Silvia H. Haneklaus
Ewald Schnug *Editors*

Sulfur Metabolism in Higher Plants - Fundamental, Environmental and Agricultural Aspects

 Springer

Proceedings of the International Plant Sulfur Workshop

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Editors

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Editors

Luit J. De Kok
Laboratory of Plant Physiology
Groningen Institute for Evolutionary
Life Sciences
University of Groningen
Groningen, The Netherlands

Malcolm J. Hawkesford
Plant Biology and Crop Science
Department
Rothamsted Research
Harpenden, Hertfordshire, UK

Silvia H. Haneklaus
Federal Research Centre for Cultivated
Plants
Institute for Crop and Soil Science,
Julius Kühn-Institut (JKI)
Braunschweig, Germany

Ewald Schnug
Federal Research Centre for Cultivated
Plants
Institute for Crop and Soil Science,
Julius Kühn-Institut (JKI)
Braunschweig, Germany

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Heinz Rennenberg

Foreword: There Is (Almost) No Way to Escape from Sulfur in Plant Research

This Sulfur Workshop in Plants series was initiated during a sabbatical stay in Groningen in 1988 as a result of discussions between Ineke Stulen, Luit De Kok, and myself. Therefore, it was logical that the first Sulfur Workshop on plants was held in Groningen in 1989. At this time, plant research on sulfur was largely focused on consequences of atmospheric pollution with SO₂ for plant growth and development, and studies on other aspects of sulfur metabolism in plants were not well developed (e.g., Rennenberg 1982) compared to studies in mammals (Meister and Anderson 1983). It is the merit of the highly successful first workshop on “Sulfur Nutrition and Sulfur Assimilation in Higher Plants” (subsequently abbreviated “Sulfur Workshop”) held in Groningen that the door became wide open for studies on sulfur metabolism of plants including fundamental and applied aspects. In particular, it was the European plant science community that took advantage of this situation and soon played a leading role in this area of research.

When the first Sulfur Workshop was held in Groningen, I already could look back to more than a decade of studies on sulfur in plants. In my diploma and PhD thesis, I had worked on glutathione production in tobacco suspension cultures, a system that subsequently became recognized as a useful tool for in-depth analyses of glutathione synthesis and degradation in plants (Bergmann and Rennenberg 1993). At this time, it was also established that plants are not only a sink for atmospheric sulfur compounds but are also able to emit volatile sulfur compounds into the atmosphere (Rennenberg 1991). This new view of a bidirectional flux of sulfur between plants and the atmosphere initiated numerous studies on sulfur metabolism in terrestrial and aquatic plants that included volatile products.

Despite the multitude of valuable results obtained by studies with tissue cultures, it became obvious that sulfur compounds undergo long-distance transport (Bonas et al. 1982) and that regulation from the cellular scale-up to the seasonal dynamics of sulfur in plants requires studies at the whole-plant level (Herschbach and Rennenberg 1997; Herschbach et al. 2012). The significance of such studies was

fueled by the generation of transgenic poplar plants with modified glutathione synthesis and reduction capacity (Noctor et al. 1998). With these tools, sulfur in plants entered the era of molecular biology. Molecular research on sulfur became soon extended to the analyses of sulfate transporters (Smith et al. 1997; Hawkesford et al. 2003) and the cross talk of sulfur metabolism with nitrogen and carbon metabolism (Kopriva and Rennenberg 2004) that constitute important areas of plant research until today. The current view on molecular studies has changed dramatically from the initial approaches that were focused on analyses of transcription of a set of enzymes and transporters: today, it is generally accepted that the characterization of metabolic processes and metabolic cross talk requires more than quantification of the transcriptome and largely relies on an integrative view on mRNA, protein, and metabolite abundances, as well as metabolite fluxes (Rennenberg and Herschbach 2014; Kalloniati et al. 2015).

Transgenic poplars with modified glutathione synthesis and reduction capacity became a useful tool to analyze the role of sulfur metabolism in the compensation of abiotic and biotic stress. The multiple stress compensation reactions relying on sulfur metabolism include reactive oxygen species (ROS) scavenging, heavy metal detoxification, and hypersensitive responses to pathogen attack (Noctor et al. 1998; Foyer and Rennenberg 2000; Peuke and Rennenberg 2006; Rennenberg and Herschbach 2012; He et al. 2015). Among the stress factors studied, the consequences of SO₂ exposure of plants turned out to be of particular complexity, but surprisingly, this was only recognized in recent years (Hänsch et al. 2007). As a radical, SO₂ can mediate ROS formation that requires reduced sulfur in the form of glutathione for scavenging in the Foyer-Halliwell-Asada cycle (Foyer and Rennenberg 2000); if involved in ROS formation in the apoplastic space, SO₂ can interact with lignin formation and requires scavenging by peroxidase activity for ROS homeostasis (Hamisch et al. 2012); as an essential intermediate, sulfite derived from SO₂ interacts with assimilatory sulfate reduction and, as a product of this pathway, also with sulfur nutrition (Rennenberg 1984; Takahashi et al. 2011; Herschbach et al. 2012). Even subsequent to oxidative and reductive SO₂ detoxification (Hamisch et al. 2012), the detoxification products, i.e., sulfate and sulfide, will interact with signaling processes at the cellular and whole-plant level (Leitner et al. 2009; Lisjak et al. 2010; García-Mata and Lamattina 2013; Hancock and Whiteman 2014; Calderwood and Kopriva 2014). This was indicated already by early H₂S fumigation studies with different plant species (De Kok et al. 1991; Herschbach et al. 1995a, b, 2000) but only recently connected to the consequences of SO₂ exposure (Hamisch et al. 2012). In the research area of sulfur-mediated signaling, the proposed role of sulfate as a root-to-shoot signal controlling stomatal aperture upon drought (Malcheska et al. 2017) provides a new notion, why excess sulfur in the form of sulfate has to be sequestered in the vacuole but also needs to be mobilized from this pool under particular environmental conditions, processes that were already observed in early studies on sulfur in plants (Rennenberg 1984).

Over the years, I made several attempts to escape from sulfur, e.g., by focusing on N and P nutrition, on radiatively active biogenic trace gases in the atmospheres,

and recently on plant carnivory. At the end, (almost) all of these attempts ended up with studies on sulfur in plants. Even for plant carnivory, sulfur metabolism turned out to be of pivotal importance. In the Venus flytrap, a plant that actively catches its animal prey with snap traps, the prey is digested in hermetically closed traps by the release of an acidic, sulfur-rich enzyme cocktail from the gland-based secretory system on the inner surface of the traps (see Fasbender et al. 2017 and literature cited therein). The production of the sulfur-rich hydrolytic enzyme cocktail requires stimulation of thiol synthesis from assimilatory sulfate reduction during prey digestion (Scherzer et al. 2017). Thus, whatever process in plants I found interesting to study (almost) always ended up to be connected to sulfur. Therefore, finally, sulfur in plants accompanied me from my diploma, PhD, and habilitation thesis to my position as research associate at the University of Cologne and the DOE Plant Research Laboratory at Michigan State University in East Lansing, and to my professor positions at the University of Cologne, the Technical University of Munich, the Fraunhofer Institute of Atmospheric Environmental Research, and the University of Freiburg up to my retirement in 2017.

The broad range of different aspects of sulfur metabolism could only be studied in more than 40 years of my research activities in collaboration with numerous colleagues and friends, including, among many others (in alphabetical order), Ludwig Bergmann, Christian Brunold, Luit J. De Kok, Manolis Flemetakis, Christine Foyer, Dieter Grill, Robert Hänsch, Rainer Hedrich, Rüdiger Hell, Stanislav Kopriva, Ralf R. Mendel, Andrea Polle, Winfried Rauser, Kazuki Saito, Andreas Weber, and Marcus Wirtz that in several cases stayed in my group for a period of time. In addition, work on sulfur in plants in my group would not have been possible without additional strong partners such as Cornelia Herschbach, Jürgen Kreuzwieser, and Monika Eiblmeier who have accompanied me in my research at the University of Freiburg until today. It is time to thank them all for creating such a fruitful and pleasant working atmosphere.

Institut für Forstwissenschaften
Universität Freiburg
Freiburg, Germany

Heinz Rennenberg

References

- Bergmann L, Rennenberg H (1993) Glutathione metabolism in plants. In: De Kok LJ, Stulen I, Rennenberg H, Brunold C, Rauser W (eds) Sulfur nutrition and sulfur assimilation in higher plants. SPB Academic Publishing, The Hague, pp 109–123
- Bonas U, Schmitz K, Rennenberg H, Bergmann L (1982) Phloem transport of sulfur in *Ricinus*. *Planta* 155:82–88
- Calderwood A, Kopriva S (2014) Hydrogen sulfide in plants: from dissipation of excess to signaling molecule. *Nitric Oxide* 41:72–78

- De Kok LJ, Rennenberg H, Kuiper PJC (1991) The internal resistance in spinach shoots to atmospheric H₂S deposition is determined by metabolism. *Plant Physiol Biochem* 29:463–470
- Fasbender L, Maurer D, Kreuzwieser J, Kreuzer I, Schulze WX, Kruse J, Becker D, Alfarraj S, Hedrich R, Werner C, Rennenberg H (2017) The carnivorous Venus flytrap uses prey derived amino acid carbon to fuel respiration. *New Phytol* (in press)
- Foyer CH, Rennenberg H (2000) Regulation of glutathione synthesis and its role in abiotic and biotic stress defense. In: Brunold C, Rennenberg H, De Kok LJ, Stulen I, Davidian J-C (eds) *Sulfur nutrition and sulfur assimilation in higher plants: molecular, biochemical and physiological aspects*. Paul Haupt, Bern, pp 127–153
- García-Mata C, Lamattina L (2013) Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. *Plant Sci* 201–201:66–73
- Hamisch D, Randewig D, Schliesky S, Weber APM, Geffers R, Herschbach C, Rennenberg H, Mendel RR, Hänsch R (2012) Impact of SO₂ on *Arabidopsis thaliana* transcriptome in wildtype and sulfite oxidase knock-out plants analyzed by RNA deep sequencing. *New Phytol* 196:1074–1085
- Hancock JT, Whiteman M (2014) Hydrogen sulfide and cell signaling: team player or referee? *Plant Physiol Biochem* 78:37–42
- Hänsch R, Lang C, Rennenberg H, Mendel RR (2007) Significance of plant sulfite oxidase. *Plant Biol* 9:589–595
- Hawkesford MJ, Buchner P, Hopkins L, Howarth JR (2003) The plant sulfate transporter family: specialized functions and integration with whole plant nutrition. In: Davidian J-C, De Kok LJ, Stulen I, Hawkesford MJ, Schnug E, Rennenberg H (eds) *Sulfur transport and assimilation in plants: regulation, Interaction, Signaling*. Backhuys Publishers, Leiden, pp 1–10
- He J, Li H, Ma C, Zhang Y, Polle A, Rennenberg H, Cheng X, Luo Z-B (2015) Over-expression of γ -glutamylcysteine synthetase mediates changes in cadmium influx, allocation, and detoxification in poplar. *New Phytol* 205:240–254
- Herschbach C, Rennenberg H (1997) Sulfur nutrition of conifers and deciduous trees. In: Rennenberg H, Eschrich W, Ziegler H (eds) *Trees – Contributions to modern tree physiology*. Backhuys Publishers, Leiden, pp 293–311
- Herschbach C, De Kok LJ, Rennenberg H (1995a) Net uptake of sulphate and its transport to the shoot in tobacco plants fumigated with H₂S or SO₂. *Plant Soil* 175:75–84
- Herschbach C, De Kok LJ, Rennenberg H (1995b) Net uptake of sulfate and its transport to the shoot in spinach plants fumigated with H₂S or SO₂: does atmospheric sulfur affect the ‘inter-organ’ regulation of sulfur nutrition? *Bot Acta* 108:41–46
- Herschbach C, van der Zalm E, Schneider A, Jouanin L, De Kok LJ, Rennenberg H (2000) Regulation of sulphur nutrition in wildtype and transgenic poplar overexpressing γ -glutamylcysteine synthetase in the cytosol as affected by atmospheric H₂S. *Plant Physiol* 124:461–473
- Herschbach C, Gessler A, Rennenberg H (2012) Long-distance transport and plant internal cycling of N- and S-compounds. *Progr Bot* 73:161–188
- Kallonati C, Krompas P, Karalius G, Udvardi MK, Rennenberg H, Herschbach C, Flemetakis M (2015) Nitrogen-fixing nodules as a new strong source of reduced sulfur trigger global changes in sulfur metabolism in *Lotus japonicus*. *Plant Cell* 27:2384–2400
- Leitner M, Vandelle E, Gaupels F, Bellin D, Belledonne M (2009) NO signals in the haze – nitric oxide signalling in plant defense. *Curr Opin Plant Biol* 12:451–458
- Lisjak M, Srivastava N, Teklic T, Civale L, Lewandowski K, Wilson I, Wood ME, Whiteman M, Hancock JT (2010) A novel hydrogen sulphide donor causes stomatal opening and reduces nitric oxide accumulation. *Plant Physiol Biochem* 48:931–935
- Malcheska F, Ahmad A, Badoo S, Ludwig-Müller J, Kreuzwieser J, Randewig D, Hedrich R, Hell R, Wirtz M, Herschbach C, Rennenberg H (2017) Drought enhanced xylem sap sulfate

- closes stomata by affecting ALMT12 and guard cell ABA synthesis. *Plant Physiol*, revised submitted
- Meister A, Anderson ME (1983) Glutathione. *Annu Rev Biochem* 52:711–760
- Noctor G, Arisi A-CM, Jouanin L, Kunert KJ, Rennenberg H, Foyer CH (1998) Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. *J Exp Bot* 49:623–647
- Peuke A, Rennenberg H (2006) Heavy metal resistance and phytoremediation with transgenic trees. In: Fladung M, Ewald D (eds) *Tree transgenesis-recent developments*. Springer Publishers, Heidelberg, pp 137–155
- Rennenberg H (1982) Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry* 21:2771–2781
- Rennenberg H (1984) The fate of excess sulfur in higher plants. *Annu Rev Plant Physiol* 35:121–153
- Rennenberg H (1991) The significance of higher plants in the emission of sulfur compounds from terrestrial ecosystems. In: Sharkey T et al (eds), *Trace gas emissions by plants*. Academic, New York, pp 217–265
- Rennenberg H, Herschbach C (2012) Sulfur compounds in multiple compensation reactions of abiotic stress responses. In: De Kok LJ, Tausz M, Hawkesford MJ, Höfgen R, McManus MT, Norton RM, Rennenberg H, Saito K, Schnug E, Tabe L (eds) *Sulfur metabolism in plants: mechanisms and application to food security and responses to climate change*. Springer, Berlin, pp 203–215
- Rennenberg H, Herschbach C (2014) A detailed view on sulphur metabolism at the cellular and whole plant level illustrates challenges in metabolite flux analyses. *J Exp Bot* 65:5711–5724
- Scherzer S, Shabala L, Hedrich B, Fromm J, Bauer H, Munz E, Jakob P, Al-Rascheid K, Kreuzer I, Becker D, Eiblmeier M, Rennenberg H, Shabala S, Bennett M, Hedrich R, Neher E (2017) Insect haptic-electrical stimulation of Venus flytrap triggers exocytosis in gland cells. *Proc Natl Acad Sci U S A*, submitted
- Smith FW, Hawkesford MJ, Ealing PM, Clarkson DT, Vanden Berg PJ, Belcher AR, Warrilow AGS (1997) Regulation of expression of a cDNA from barley roots encoding a high affinity sulphate transporter. *Plant J* 12:875–884
- Takahashi H, Kopriva S, Giordano M, Saito K, Hell R (2011) Sulfur assimilation in photosynthetic organisms; molecular functions and regulations of transporters and assimilatory enzymes. *Annu Rev Plant Biol* 62:157–184

Preface

The International Plant Sulfur Workshop series was initiated in order to bring together scientists from various research disciplines and to discuss all aspects of sulfur metabolism, from molecular biology, biochemistry, and physiology to ecology and agriculture. The first workshop in the series entitled “Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Fundamental Environmental and Agricultural Aspects” was held in Haren, the Netherlands, 1989. The following workshops were held in Garmisch-Partenkirchen, Germany, 1992; Newcastle upon Tyne, United Kingdom, 1996; Wengen, Switzerland, 1999; Montpellier, France, 2002; Kisarazu, Chiba, Japan, 2005; Warsaw, Poland, 2008; Creswick, Victoria, Australia, 2010; and Freiburg-Munzingen, Germany, 2014. Contents of the respective proceedings are included in this volume.

This proceedings volume contains a selection of invited and contributed papers of the 10th Jubilee Plant Sulfur Workshop, which was held in Goslar, Germany, from September 1 to 4, 2015. During this workshop, the outcome of the previous workshops was summarized, and the still existing gaps and prospects for future research were highlighted by a selection of speakers who have significantly contributed to plant sulfur research during the last 25 years.

We are delighted to dedicate this volume to our dear colleague Heinz Rennenberg from the University of Freiburg, Germany, who together with Ineke Stulen, Christian Brunold, and Luit J. De Kok initiated the workshop series and furthermore was involved in the organization and issuing of proceedings volumes of all previous plant sulfur workshops. In addition, he has significantly contributed to the understanding of the regulation of uptake and assimilation of sulfur and the significance of sulfur metabolites in stress

tolerance of higher plants over more than four decades thus leaving a durable “sulfur footprint”.

Groningen, The Netherlands
Braunschweig, Germany
Harpenden, UK
Braunschweig, Germany

Luit J. De Kok
Silvia H. Haneklaus
Malcolm J. Hawkesford
Ewald Schnug

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Contents

Part I Overview Papers

Sulfate Transport in Plants: A Personal Perspective	3
Malcolm J. Hawkesford	
Quo Vadis Sulfur Investigation?: 25 Years of Research into Plant Sulfate Reduction	13
Stanislav Kopriva	
Expression Profile of the Serine Acetyltransferase (<i>SERAT</i>) and <i>O</i>-Acetylserine (thiol)lyase (<i>OASTL</i>) Gene Families in <i>Arabidopsis</i>	31
Mutsumi Watanabe and Rainer Hoefgen	
Elucidating the Effects of Higher Expression Level of Cystathionine γ-Synthase on Methionine Contents in Transgenic <i>Arabidopsis</i>, Soybean and Tobacco Seeds	39
Hagai Cohen, Yael Hacham, Ifat Matityahu, and Rachel Amir	
Biosynthesis of <i>S</i>-Alk(en)yl-L-Cysteine Sulfoxides in <i>Allium</i>: Retro Perspective	49
Naoko Yoshimoto and Kazuki Saito	
The Effect of Sulfur Nutrition on Glucosinolate Patterns and Their Breakdown Products in Vegetable Crops	61
Britta Pitann, Carolin Heyer, and Karl H. Mühling	
Prospects for Agricultural Sulfur Research	75
Silvia H. Haneklaus, Elke Bloem, and Ewald Schnug	

Part II Research Papers

DMSP: Occurrence in Plants and Response to Salinity in <i>Zea mays</i>	87
Ties Ausma, Marko Kebert, Jacqueline Stefels, and Luit J. De Kok	

Impact of Atmospheric H₂S, Salinity and Anoxia on Sulfur Metabolism in <i>Zea mays</i>	93
Ties Ausma, Saroj Parmar, Malcolm J. Hawkesford, and Luit J. De Kok	
Salinity Influences Single Glucosinolate Content in the Halophyte <i>Lepidium latifolium</i>	103
Christian Boestfleisch, Johann Hornbacher, Annekathrin Rumlow, and Jutta Papenbrock	
The Application of S⁰-Coated Fertilizer to Durum Wheat Crop	115
Styliani N. Chorianopoulou, Georgios I. Saridis, Petros Sigalas, Miltos Margetis, Dimitris Benardos, Haris Mavrogiannis, and Dimitris L. Bouranis	
Re-assessing Systems Biology Approaches on Analyzing Sulfate Metabolism	123
Rainer Hoefgen and Mutsumi Watanabe	
Combining Isotope Labelling with High Resolution Liquid Chromatography-Tandem Mass Spectrometry to Study Sulfur Amino Acid Metabolism in Seeds of Common Bean (<i>Phaseolus vulgaris</i>)	135
Jaya Joshi, Justin B. Renaud, Mark W. Sumarah, and Frédéric Marsolais	
Investigations on the Current Sulfur and Sulfate Intake of Cattle in Germany: Are There Any Risks for a Consumption Exceeding Recommended Upper Limits?	145
Josef Kamphues, Anne Dohm, F. von und zur Mühlen, and Petra Wolf	
Manganese Toxicity Hardly Affects Sulfur Metabolism in <i>Brassica rapa</i>	155
Mariana I. Neves, Dharmendra H. Prajapati, Saroj Parmar, Tahereh A. Aghajanzadeh, Malcolm J. Hawkesford, and Luit J. De Kok	
Localization of Sulfate Uptake and pH Changes at Sulfur-Deprived Roots of Intact <i>Brassica pekinensis</i> Seedlings by Using H⁺-Selective Microelectrodes	163
Martin Reich, Marten Staal, Luit J. De Kok, and J. Theo M. Elzenga	
Evidence for Regulation of the Iron Uptake Pathway by Sulfate Supply in S-Deprived Maize Plants	175
Georgios I. Saridis, Styliani N. Chorianopoulou, Panagiotis Katinakis, and Dimitris L. Bouranis	
The Sulfur Pathway and Diagnosis of Sulfate Depletion in Grapevine	181
Sílvia Tavares and Sara Amâncio	

Impact of Sulfate Deprivation and H₂S Exposure on the Metabolites of the Activated Methyl Cycle in Chinese Cabbage 191
Mei-Hwei Tseng, Chao-Kai Yang, C. Elisabeth E. Stuiver, Chiu-Ching Chang, and Luit J. De Kok

Sulfate Transporters Involved in Cd-Induced Changes of Sulfate Uptake and Distribution in *Arabidopsis thaliana* 199
Chisato Yamaguchi and Akiko Maruyama-Nakashita

A Glycine-Rich Protein Encoded by Sulfur-Deficiency Induced Gene Is Involved in the Regulation of Callose Level and Root Elongation 207
Anna Znój, Katarzyna Zientara-Rytter, Paweł Sęktas, Grzegorz Moniuszko, Agnieszka Sirko, and Anna Wawrzyńska

Titles and Content of the Proceedings of the International Plant Sulfur Workshops (1990–2009) 215

Index 239

Part I
Overview Papers

Sulfate Transport in Plants: A Personal Perspective

Malcolm J. Hawkesford

Abstract Early key research milestones for sulfate transport in plants include the first description of kinetics of sulfate uptake into plant roots (Leggett and Epstein, *Plant Physiol* 31:222–226, 1956), nutritionally regulated sulfate uptake into plants (Clarkson et al., *J Exp Bot* 34:1463–1483, 1983), and the first gene for a plant sulfate transporter (Smith et al., *Proc Natl Acad Sci U S A* 92:9373–9377, 1995a). Since then a well-described gene family encoding putative sulfate transporters has been characterized in multiple species, initially most notably in *Arabidopsis* but subsequently for a number of other models or important crops (examples: Brassica, wheat, rice, poplar and Medicago, see Buchner et al., *Genome* 47:526–534, 2004a; Buchner et al., *Plant Physiol* 136:3396–3408, 2004b; Buchner et al., *Mol Plant* 3:374–389, 2010; Kumar et al., *Plant Signal Behav* 10:e990843, 2015; Dürr et al., *Plant Mol Biol* 72:499–517, 2010; Gao et al., *Planta* 239:79–96, 2014). Regulation of expression has been well documented and this regulation is both a useful marker of sulfur-nutritional status and a model for the elucidation of control pathways. The complexity of the gene family in relation to functional, regulatory and spatial distribution indicates an apparent whole plant management system for sulfur, coordinated with growth and demand and interacting with nutrient availability. In addition to sulfate, there is direct involvement of this transporter family in the uptake and accumulation of both selenate and molybdate, with clear consequences for nutritional quality. Is the story now complete almost 60 years since the first transport description and 20 years since the first sulfate transporter gene isolation, and a plethora of research projects and publications? Do we know how sulfur is acquired and appropriately distributed within the plant? Do we know the critical signals that control these processes? Are we even sure that these processes are coordinated? This review documents research progress and assesses to what extent the key questions have been addressed.

M.J. Hawkesford (✉)

Plant Biology and Crop Science Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

e-mail: malcolm.hawkesford@rothamsted.ac.uk

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Introduction

All plants require sulfur for growth and for land plants is most is acquired from the external environment as sulfate. For land plants this from the soil via the roots. Typically concentrations are low and often extremely variable. Thus, transport needs to be active to facilitate uptake against a concentration gradient, specific for sulfate and regulated to optimize uptake to growth and ensure optimal energy utilization in this process. For vascular plants transport is not only across a single membrane at the soil-root interface but also across many other plasma membranes to facilitate distribution, across the chloroplast membrane to the site of reduction and also across the tonoplast to allow transport in and out of the vacuole for the transient storage of excess sulfate taken up.

Progress on the understanding of plant sulfate transporters has been substantial and reported in successive volumes of the Sulfur Workshop series, with key landmark papers from a number of groups being published throughout this period. Some early key milestones in the development of the plant sulfate transporter research field are illustrated as a timeline in Fig. 1.

The first suggestion for active absorption was in an analysis of whole plant uptake of sulfate into barley roots. An enzyme based description of affinities and competition by selenate but not nitrate or phosphate unequivocally demonstrated the activity of a transmembrane ion transporter (Leggett and Epstein 1956). It would be 40 years before the molecular components would be identified in plants (Smith et al. 1995b). Prior to this key elements of regulation by de-repression (that is induction upon starvation) were described in a topical legume (Clarkson et al. 1983) and suggestion for involvement of a metabolite linking N and S metabolism, namely *O*-acetylserine (OAS), was described in maize (Clarkson et al. 1999). The importance of OAS as a regulator of gene expression for a cluster of genes has been described, separating S-related and other regulation (Hubberten et al. 2012, 2015). Mechanistic evidence for transport being driven by proton gradients was obtained in a duckweed (Lass and Ullrich-Eberius 1984).

The first substantial progress on the identification of sulfate transporters genes was inevitably for bacteria (Ohta et al. 1971; Sirko et al. 1990), fungi and yeast (Ketter et al. 1991; Smith et al. 1995b; Cherest et al. 1997), mammalian systems (Schweinfest et al. 1993; Hästbacka et al. 1994; Silberg et al. 1995) and finally in plants (Kouchi and Hata 1993; Smith et al. 1995a, 1997 ; Takahashi et al. 1996). Similarities in the sequence of many of the genes, some not identified as sulfate transporters was first noted by Sandal and Marcker (1994). Much of this work has been reviewed in previous volumes in this series: Kredich 1993 (bacteria); Thomas et al. 1997 (yeast); Davidian et al. 2000, Hawkesford et al. 2003, Buchner et al. 2010, Hawkesford 2012 (plants) and elsewhere (Markovich 2001) for mammalian transporters. These transporters are now recognised to be part of a large family of transmembrane ion transporters known as SulP (see also Price et al. 2004).

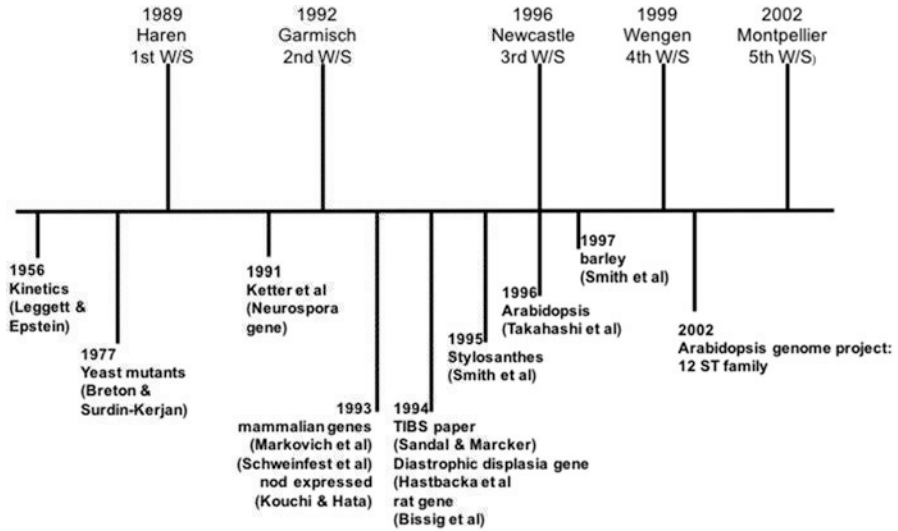


Fig. 1 Key early milestones in the development of the understanding of sulfate transport in plants placed in relation to the first 5 Sulfur Workshops

A Family of Sulfate Transporters

In a series of papers predominantly from the Takahashi group but with notable contributions from a number of others including the Davidian group it became apparent that a gene family of up to 14 genes encoded a group of related proteins in *Arabidopsis* (Takahashi et al. 1996, 1997; Vidmar et al. 2000). Similar gene families were subsequently identified in *Brassica* (Buchner et al. 2004b), in rice (Kumar et al. 2015), poplar (Dürr et al. 2010), *Medicago* (Gao et al. 2014) and in wheat (Buchner et al. 2004a).

Phylogenetic analysis of plant sulfate transporter sequences indicates discrete clades within the family (Fig. 2) and it has been proposed that these align with discrete functions and that within clades there may be some functional redundancy (Hawkesford 2003). In summary, Group 1 represents high affinity types responsible for up take into the cell, particularly in the roots, and are subject to nutritional regulation. Group 2 are lower affinity, less regulated and distributed throughout the plant. Group 3 are somewhat more enigmatic (see below), Group 4 are uniquely tonoplast located and responsible to vacuolar efflux. Group 5 are the most distantly related to the rest of the family and the 2 members are quite distinct from each other, lack a STAS domain and remain something of a puzzle. They seem to be involved in Mo accumulation, perhaps transport and as such have been name mot1 and mot2 (Tomatsu et al. 2007; Baxter et al. 2008; Gasber et al. 2011).

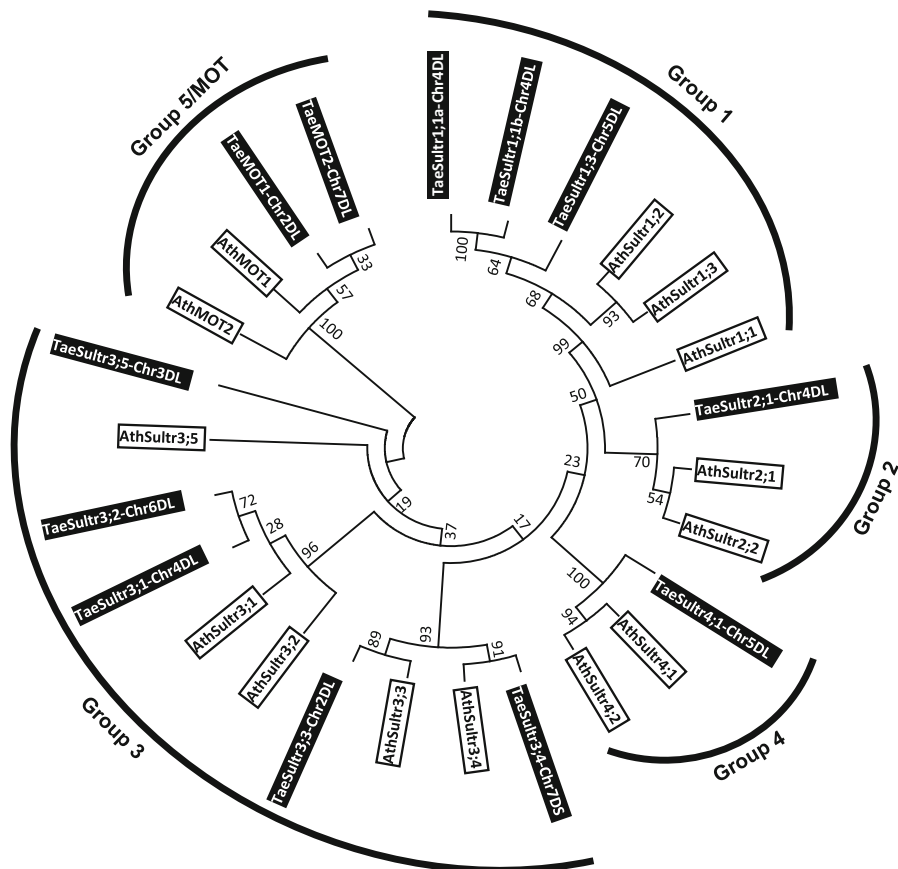


Fig. 2 Phylogenetic relationship of the wheat and *Arabidopsis* sulfate transporter gene families: Neighbour-Joining Tree (Mega 6, Tamura et al. 2013) from Multiple Alignment (ClustalX V.2.1, Larkin et al. 2007) of coding cDNAs of the *Triticum aestivum* cv. Chinese spring D-genome (*white bold – black highlighted*) and *Arabidopsis thaliana* (*square framed white highlighted*) sulfate transporter gene family. The bootstrap values, expressed as percentage, were obtained from 1000 replicate trees (Courtesy of Peter Buchner)

The Transporter Itself: Recent Structural Insights

Early analysis of the amino acid sequences of the transporter was suggestive of 12 transmembrane domains, based on hydrophobicity plots and occurrence of charged amino acids (Clarkson et al. 1993; Smith et al. 1995a; Takahashi et al. 1996). More recent analysis of transporters in the same superfamily (Sulp/SLC26 family) combining both topology mapping of for example the BicA transporter (see Price and Howitt 2014) and for prestin, homology modelling, molecular dynamics simulations and cysteine accessibility scanning are strongly supportive of a complex 14 transmembrane model (Gorbunov et al. 2014). In this analysis a

3-dimensional model has been derived which also proposes a central cavity as the substrate-binding site, midway in an anion permeation channel. Features of this cavity are almost certainly involved in substrate specificity and could potentially be modified to further increase selectivity, for example between sulfate and selenate, opening up the potential for designer crops.

An additional feature of members of this family is the STAS domain (Aravind and Koonin 2000; Rouached et al. 2005). Activity is totally dependent upon its presence and it is strongly suggested that it is involved in protein:protein interaction regulating activity, probably involving phosphorylation of a threonine residue.

The question of whether the transport acts as a monomer or oligomer is of interest and it has been suggested that heterodimers are required for activity or may have an import regulatory role. Maximal sulfate uptake and growth were obtained when a Group 3 transporter was co-expressed with a Group 2 transporter from *Arabidopsis* in yeast complementation approach, suggestive of the activity of a heterodimer (Kataoka et al. 2004a). No activity of the Group 3 when expressed alone was seen in this study. In contrast Group 3 transporter isolated from *Lotus* root nodules was able to complement a yeast mutant when expressed by itself (Kruszell et al. 2005) indicating some variability for this oligomer requirement.

Specificity for Sulfate, Selenate and Molybdate

The non-specificity of the transporter was exploited in early studies, particularly with yeast, to obtain sulfate transporter-less mutants by harassing the toxic nature of oxyanion analogues of sulfate, particularly selenate but also chromate (Breton and Surdin-Kerjan 1977; Smith et al. 1995b). Selenate has also been applied as a selection agent for the isolation of *Arabidopsis* mutants by several groups (see for example, Shibagaki et al. 2002).

As the anions sulfate, selenate and molybdate are all transported by the same transporters, it is not surprising that their respective accumulations in plant tissues are connected. Analysis of what grain from mildly sulfate deficient plots at Rothamsted showed a remarkable accumulation of Se and Mo (Shinmachi et al. 2010; Stroud et al. 2010). The S-deficient plots had a reduced grain yield and reduced grain S-content, both decreasing by about 10%, but several-fold increases in Se and Mo content. This could be partially explained by the observed induction of sulfate transporters in the roots of these field-grown plants, increasing uptake, and a more favourable ration of selenate and molybdate relative to sulfate in the soil solution. Whilst Se generally flows the distribution of sulfate in term of redistribution during grain filling and in relation to storage protein distribution in the grain, some enriched sub-cellular regions were indicative of specific accumulation on non-protein Se, possibly in vacuoles (Moore et al. 2010). Mo was less efficiently remobilized to the grain than Se during grain filling indicating either a fixation of the mineral in the vegetative tissue or a limitation to its later transportation (Shinmachi et al. 2010; Stroud et al. 2010).

Where Now?

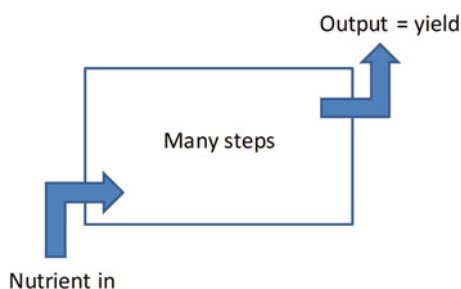
Much has been determined about the nature of sulfate transporters in plants, not only in model species but also in crops. A knowledge of the regulation and properties of the transporters helps explain many physiological phenomena and some agronomic responses of crops. The question remains of how may this aid in breeding better genotypes or in informing agronomic treatments.

A previously stated ideotype for optimum S use involves uptake and storage during fluctuating supply, effective remobilization upon demand and appropriate partitioning to ensure healthy and nutritious crops (Hawkesford 2012). Breeding or biotechnology may help deliver such germplasm and the acquired knowledge is an essential prerequisite for such developments. Sulfur will always be required for crop growth so effective capture and utilization are worthy targets.

Acquisition is an important issue. Certainly the adaptation of de-repression will aid scavenging, but only in conjunction with root proliferation. Prospects for improving efficiency of uptake are limited, although constitutive uptake and over-accumulation, followed by storage and effective remobilization remains one key strategy. In relation to this strategy, challenges still exist in the understanding of movement of sulfate within the plant from organ to organ, distribution within specific tissues and finally within individual cells between organelles. It is still unclear as to how S moves into and out of the chloroplast, the key point of entry into the biosynthetic pathway. One reports indicated a chloroplast localizing isoform of the family (Takahashi et al. 1999) but this remains to be corroborated. Clearer is the involvement of Group 3 transporters in release of sulfate from vacuoles, a key storage site (Kataoka et al. 2004b).

David Clarkson proposed the idea of a black box (Fig. 3) in a foreword to the proceedings of the 3rd Workshop (Clarkson 1997). Substantial progress has been made in determining detail within this box since then, but the question of how to improve sulfur nutrient use efficiency remains. Sulfur is required for growth and health, for resistance to biotic and abiotic stresses, and contributes to nutritional properties of food and feed. Decreasing requirements is unlikely to be an option, optimizing agronomic inputs remains the key practical approach, although in the future this may be complemented with plants optimized genetically for specific qualities. Some investigations into natural variation in *Arabidopsis* have been made

Fig. 3 Clarkson's Black Box (Clarkson 1997). A modified representation of the simple representation of nutrient use efficiency, but inevitably complex in the detail



(Loudet et al. 2007) but there has been little investigation in crop plants and this is a key area for development.

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References

- Aravind L, Koonin EV (2000) The STAS domain – a link between anion transporters and antisigma-factor antagonists. *Curr Biol* 10:R53–R55
- Baxter I, Muthukumar B, Park HC, Buchner P, Lahner B, Danku J, Zhao K, Lee J, Hawkesford MJ, Guerinot ML, Salt DE (2008) Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (MOT1). *PLoS Genet* 4(2):e1000004. doi:10.1371/journal.pgen.1000004
- Bissig M, Hagenbuch B, Stieger B, Koller T, Meier P (1994) Functional expression cloning of the canalicular sulfate transport system of rat hepatocytes. *J Biol Chem* 269:3017–3021
- Breton A, Surdin-Kerjan Y (1977) Sulfate uptake in *Saccharomyces cerevisiae*: biochemical and genetic study. *J Bacteriol* 132:224–232
- Buchner P, Prosser IM, Hawkesford MJ (2004a) Phylogeny and expression of paralogous and orthologous sulphate transporter genes in diploid and hexaploid wheats. *Genome* 47:526–534
- Buchner P, Stuiver CEE, Westerman S, Wirtz M, Hell R, Hawkesford MJ, De Kok LJ (2004b) Regulation of sulfate uptake and expression of sulfate transporter genes in *Brassica oleracea* as affected by atmospheric H₂S and pedospheric sulfate nutrition. *Plant Physiol* 136:3396–3408
- Buchner P, Parmar S, Kriegel A, Carpentier M, Hawkesford MJ (2010) The sulfate transporter family in wheat: tissue-specific gene expression in relation to nutrition. *Mol Plant* 3:374–389
- Cherest H, Davidian J-C, Thomas D, Benes V, Ansoerge W, Surdin-Kerjan Y (1997) Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. *Genetics* 145:627–635
- Clarkson D (1997) Foreword: Sulphate transport and its regulation: a personal view. In: Cram WJ, De Kok LJ, Stulen I, Brenhold C, Rennenberg H (eds) Proceedings of the 3rd workshop on sulphur metabolism in higher plants, Newcastle., Backhuys, Leiden, pp xi–xiii
- Clarkson DT, Smith FW, Vanden Berg PJ (1983) Regulation of sulphate transport in a tropical legume, *Macroptilium atropurpureum*, cv Siratro. *J Exp Bot* 34:1463–1483
- Clarkson DT, Hawkesford MJ, Davidian J-C (1993) Membrane and long distance transport of sulfate. In: De Kok LJ, Stulen I, Rennenberg H, Brunold C, Rauser WE (eds) 2nd workshop on sulfur metabolism in higher plants, Garmisch. SPB Publishing, The Hague, pp 3–20
- Clarkson DT, Diogo E, Amancio 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
- Davidian JC, Hatzfeld Y, Cathal N, Tagmount A, Vidmar JJ (2000) Sulfate uptake and transport in plants. In: Brunhold C, Rennenberg H, De Kok LJ, Stulen I, Davidian JC (eds) 4th workshop on sulfur nutrition and sulfur assimilation in higher plants, Wengen. Paul Haupt, Bern, pp 19–40
- Dürr J, Bücking H, Mult S, Wildhagen H, Palme K, Rennenberg H, Dittengou F, Herschbach C (2010) Seasonal and cell type specific expression of sulfate transporters in the phloem of *Populus* reveals tree specific characteristics for SO₄²⁻ storage and mobilization. *Plant Mol Biol* 72:499–517
- Gao Y, Tian Q, Zhang WH (2014) Systemic regulation of sulfur homeostasis in *Medicago truncatula*. *Planta* 239:79–96
- Gasber A, Klaumann S, Trentmann O, Trampczynska A, Clemens S, Schneider S, Sauer N, Feifer I, Bittner F, Mendel RR, Neuhaus HE (2011) Identification of an Arabidopsis solute