Sulphur systems biology—making sense of omics data

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Abstract

Systems biology approaches have been applied over the last two decades to study plant sulphur metabolism. These 'sulphur-omics' approaches have been developed in parallel with the advancing field of systems biology, which is characterized by permanent improvements of high-throughput methods to obtain system-wide data. The aim is to obtain a holistic view of sulphur metabolism and to generate models that allow predictions of metabolic and physiological responses. Besides known sulphur-responsive genes derived from previous studies, numerous genes have been identified in transcriptomics studies. This has not only increased our knowledge of sulphur metabolism but has also revealed links between metabolic processes, thus indicating a previously unexpected complex interconnectivity. The identification of response and control networks has been supported through metabolomics and proteomics studies. Due to the complex interlacing nature of biological processes, experimental validation using targeted or systems approaches is ongoing. There is still room for improvement in integrating the findings from studies of metabolomes, proteomes, and metabolic fluxes into a single unifying concept and to generate consistent models. We therefore suggest a joint effort of the sulphur research community to standardize data acquisition. Furthermore, focusing on a few different model plant systems would help overcome the problem of fragmented data, and would allow us to provide a standard data set against which future experiments can be designed and compared.

Keywords: Arabidopsis, metabolomics, plant systems, proteomics, sulphur metabolism, systems biology, transcriptomics.

Introduction

Regulation of cellular processes in plants occurs as the result of developmental programmes or the necessity to respond to external signals. Regulation does occur at all tiers of information conversion from DNA to physiology. Epigenetics, histone and chromatin modifications, regulation of transcription, of translation, of protein stability, and at the level of enzyme activity, all modify the response flow. At the next layer, communications between cells and tissues coordinate the responses through hormones and long-distance signals to attain the physiological responses necessary for survival and propagation.

Advances in technical capabilities for measuring large numbers of biochemical compounds (Fiehn et al., 2000) have allowed high-throughput analyses to be performed on biological materials, producing large increases in the quantities of data obtained. The aim of data cataloguing and the definition of systems biology as we consider it here (Klipp et al., 2016) is to measure all available components and functions of a cell or tissue, or at least as many as possible, and to use mathematical modelling to understand the underlying network and cooperativity. The intention of this approach is to yield a holistic understanding of a system by capturing all its parts at a
given state or condition. This additionally includes system-wide responses that, based on our current understanding, may seemingly be unrelated to sulphur nutrition, and which would be missed in targeted approaches. The ultimate goals are to identify candidate genes, elucidate gene functions, and understand physiological processes (Fiehn et al., 2000; Stitt and Fernie, 2003). Systems biology should also aim to look at the dynamics of the response of a system over time or in relation to developmental states.

One of the first examples of ‘omics’ approaches was DNA sequencing, which eventually yielded the sequence information of whole organisms, for example Arabidopsis thaliana (The Arabidopsis Genome Initiative, 2000), thus laying the grounds for the area of genomics. There were obviously precursors of high-throughput analyses when sequence information was not yet available, such as amplified fragment length polymorphism (AFLP) mapping (Howarth et al., 2005). Over the last two decades, this has been followed by a wide variety of new, upcoming omics-technologies aimed at determining the various functional entities in a cell, namely DNA, RNA, metabolites, proteins, enzyme activities, and flux dynamics. Improvements in technical approaches continue to provide data even more efficiently and faster, for example high-throughput sequencing technologies such as RNA sequencing (RNA-seq) (Weber, 2015). Coupled to this is an increasing need for improved bio-statistical approaches, generally termed bioinformatics, to catalogue and analyse the data being generated (Rhee et al., 2006).

Of these ‘omics’ approaches, metabolomics analyses provide the most complex data sets because the analytes are constituted of chemical compounds with a huge range of molecular masses and diverse physicochemical properties (such as hydrophobicity and ionic strengths) and have to be extracted and analysed using multiple methods (Fiehn et al., 2000; Stitt and Fernie, 2003; Watanabe et al., 2018). In contrast, DNA and RNA in genomics and transcriptomics are composed of only four different, closely related chemicals, nucleotides, with only a few modifications that add only slightly to their complexity. The initial concept was to use unbiased approaches to capture the metabolic state of a system, that is without challenging the plant with, for example, a stress such as sulphate-deficient conditions (Roessner et al., 2000; Kusano et al., 2007). Soon however, environmental challenges were applied or different developmental states compared in order to obtain more informative data. Compared to classical differential screening approaches, systems biology produces a multitude of data in order to obtain a holistic response pattern rather than concentrating on individual parts of metabolism. An iterative analytical phase has now been reached where the results of omics-based screening approaches that have identified candidate genes are being subjected to (for example) reverse genetics for a further round of validation again by omics approaches (Aarabi et al., 2016). This is still the classical reductionist approach, but with the intent of analysing the system in a holistic manner. The eventual goal of systems approaches with respect to sulphur metabolism is to unravel gene function and to generate a network scheme and a model for plant sulphur metabolism that allows predictive biology.

In this review, we concentrate on the systems biology of plant sulphur metabolism (‘sulphur-omics’) in Arabidopsis, especially on transcriptome, metabolome, and proteome studies. These approaches began in the early years of this century with the first papers on transcriptomics using DNA arrays (Hirai et al., 2003; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003). We try to answer the question as to whether ‘omics’ approaches have provided us with a better understanding, and we discuss what needs to be done in the future to further enhance our knowledge. Finally, we ask whether we are already at a level of understanding to bring about agronomical improvements in terms of sulphate use efficiency, crop quality, and production of medicinal compounds.

What is the status of sulphur-omics?

Conceptually, systems approaches are designed to provide as much and as unbiased data as possible. These data provide initial information on the subject of investigation, here plant sulphur metabolism. For data validation, an iterative cycle of analytical procedures is necessary (Fig. 1A), aimed at understanding the molecular and physiological processes regulating plant sulphur metabolism and homeostasis. The initial results are then analysed in an iterative process using systems or more targeted approaches to yield a better understanding of the system, for example by employing altered conditions or mutants of selected candidate genes identified through forward and reverse genetics.

Transcriptomics of Arabidopsis with respect to sulphur metabolism

Arabidopsis is well established as a valuable model plant system (Scholl et al., 2000). The release of its genome sequence (The Arabidopsis Genome Initiative, 2000) increased its value as a research tool and boosted approaches aimed at deciphering holistic rather than individual plant responses to particular conditions. Such systems approaches have been applied to study plant sulphate metabolism, with the aim of systematically unravelling the molecular responses. While early analyses focussed on gene and metabolite responses of sulphate uptake, reduction, and assimilation processes, systems approaches have sought to examine the connections and interplay within the system as a whole. This has been based on the inherent assumption that a response to (e.g.) sulphate starvation will not only affect sulphur metabolism per se but also other interconnected and downstream processes. It is obvious that in this context different parts of plants, such as roots, leaves, or seeds, will show both general and also specific responses. Furthermore, developmental aspects have to be taken into account when analysing plant responses to sulphate withdrawal or resupply over time. Transcriptomics studies as part of sulphur systems biology were pioneered by Hirai et al. (2003), Nikiforova et al. (2003), and Maruyama-Nakashita et al. (2003) using Arabidopsis. A transcriptome analysis of sulphur (S-) deprived Arabidopsis seedlings was performed for different durations of S starvation.
(A) Systems biology experimental flow chart

Fig. 1. Schematic representation of sulphur systems biology. (A) Experimental flow chart. Raw data is acquired by employing “omics” technologies to results from plants exposed to altered S availability and by conducting targeted experiments. These data are then analysed by various data-mining steps such as annotation of genes, metabolites, or proteins. External knowledge is included for data mining. Bioinformatics analyses, again with the use of external knowledge, help to sort the data and to identify significant changes or correlations. Data interpretation leads to the identification of candidate genes, proteins, or processes, which results in the generation of hypotheses or models to explain the observed responses. These models need to be validated in an iterative analysis, for example using transgenic approaches, mutants, or altered conditions, i.e., the identified candidates are subjected to a second analytical cycle. Eventually, data interpretation should result in the formulation of a theory that explains the aspects of S metabolism that have been investigated, and thus our knowledge of plant S metabolism is increased. This knowledge may then be exploited for plant breeding and the generation of new crop varieties. Crop quality validation and field testing might employ the same analytical circle. (B) Identification of the function of SDI genes. An example of an experimental flow chart focusing on SDI genes using an iterative research cycle. AFLP, amplified fragment length polymorphism; GLS, glucosinolate; indOX, inducible overexpression; KO, knockout; OAS, O-acetylserine; OX, overexpression; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; QTL, quantitative trait locus; SDI, sulfur deficiency-induced; SERAT, serine acetyltransferase; slm1, sulfur limitation1; SNP, single nucleotide polymorphism; TF, transcription factor.
in order to address the development of the response over time (Nikiforova et al., 2003). In addition, Arabidopsis seedlings were treated with O-acetylserine (OAS), the immediate precursor of cysteine biosynthesis (Hirai et al., 2003). OAS accumulates upon S deprivation and early research considered it as an S-starvation signal (Saito, 2000; Hopkins et al., 2005), which was indeed subsequently demonstrated (Hubberten et al., 2012a). Due to the technical limitations at the time, these
pioneering studies were performed on macro-arrays, each comprising about 10,000 random cDNAs (Hirai et al., 2003; Nikiforova et al., 2003), or using Affymetrix 8K chips with probes for ~8000 genes (Maruyama-Nakashita et al., 2003). The differentially expressed genes that were identified included some that were already known to be responsive to S status, such as sulphate transporters (Smith et al., 1997; Hawkesford, 2000), which confirmed the validity of the approach. More interestingly, information on novel genes was obtained. Thus, alongside known genes, these early studies provided a catalogue of genes that as yet had no assignment of their function in response to S-deficient growing conditions.

The number of transcriptome studies that have been conducted on Arabidopsis is still quite low with only 14 in total (Table 1). It is justifiable to include arrays of plants exposed to selenium (Van Hoewyk et al., 2008) as it acts as a competitor with sulphur, thus mimicking S deprivation. Among related Brassicaceae species a sulphate starvation study was performed on rapeseed (Buhtz et al., 2008, 2010). Despite the fact that rapeseed has a high requirement for sulphate (Girondé et al., 2008), there is a lack of transcriptomics studies on this subject. With respect to Arabidopsis, the tissues and conditions investigated in the early studies were already quite diverse (Table 1). This provided a wealth of information, but it made comparisons between studies difficult as each experiment was based on very specific conditions with respect to the sulphate levels applied and/or the tissues examined. For example, the tissues studied in response to S-deprivation included whole seedlings grown on agar plates (Nikiforova et al., 2003), seedlings separated into leaves and roots (Hirai et al., 2003; Maruyama-Nakashita et al., 2003, 2005, 2006), and developing seeds (Higashi et al., 2006). Subsequent studies have examined whole seedlings exposed to S deprivation in submerged seedling cultures followed by resupply in order to score for recovery processes (Bielecka et al., 2015), hydroponically grown root tissues exposed to S deprivation and separated into fractions of various cell types (Iyer-Pascuzzi et al., 2011), and studies where S deprivation has been one factor among other combined stresses (Barciszewska-Pacak et al., 2015; Forieri et al., 2017). Sulphate starvation has been used as a condition to investigate phloem-specific micro-RNAs in rapeseed (Buhtz et al., 2010). Although only a subset of the phloem RNA fraction was analysed, results regarding the regulatory function of miRNA-395 were substantiated in further studies employing Arabidopsis (Kawashima et al., 2009, 2011). Sulphate metabolism in response to acid rain conditions has been investigated, with high inputs of S under low pH conditions (Liu et al., 2014). Acid rain is an ecological and a health problem in many countries due to combustion of fossil fuels releasing SO2. In North America and Europe, SO2 emissions have been successfully reduced over recent decades due to legislative measures that have regulated industrial and domestic use of fossil fuels. However, this has consequently reduced sulphur inputs into agro-ecological systems, which in turn has triggered research into its agricultural impact (Haneklaus et al.,

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Table 1. Transcriptome analyses related to sulphur metabolism

| Experiment | Species | Tissue | Type | ID | References |
|------------|---------|--------|------|----|------------|
| S          | Arabidopsis | Seedling | Macroarray | GSE30100 | Nikiforova et al. (2003) |
| S          | Arabidopsis | Leaf, Root | Macroarray | GSE30009 | Maruyama-Nakashita et al. (2003) |
| S          | Arabidopsis | Leaf, Root | Affymetrix 8K Chip | GSE30098 | Hirai et al. (2003) |
| S          | Arabidopsis | Leaf, Root | Agilent oligo microarray | E-MEXP-211 | Higashi et al. (2006) |
| S          | Arabidopsis | Root | GeneChip ATH1 | GSE4555 | Maruyama-Nakashita et al. (2005) |
| S          | Arabidopsis | Seed | GeneChip A-AFFY-2 | E-ATMX-1 | Maruyama-Nakashita et al. (2006) |
| S          | Arabidopsis | Root | GeneChip ATH1 | GSE4555 | Maruyama-Nakashita et al. (2006) |
| S          | Arabidopsis | Root cell types | GeneChip ATH1 | GSE30166 | Iyer-Pascuzzi et al. (2011) |
| S          | Arabidopsis | Root cell types | GeneChip ATH1 | GSE64972 | Bielecka et al. (2015) |
| S          | Arabidopsis | Seedling | GeneChip ATH1 | GSE66999 | Barciszewska-Pacak et al. (2015) |
| S          | Oilseed rape | Leaf | Illumina HiSeq 2000 | GSE81347 | Aarabi et al. (2016) |
| S          | Arabidopsis | Leaf, Root | GeneChip ATH1 | GSE93048 | Dong et al. (2017) |
| S          | Arabidopsis | Root | GeneChip 1.1 ST | GSE77602 | Forieri et al. (2017) |
| S          | Arabidopsis | Root | GeneChip 1.1 ST | GSE77602 | Forieri et al. (2017) |
| S          | Arabidopsis | Root | GeneChip 1.1 ST | GSE77602 | Forieri et al. (2017) |
| S          | Arabidopsis | Leaf, Root, Phloem | LC Sciences dual colour | GSE20263 | Buhtz et al. (2010) |
| +Se        | Arabidopsis | Leaf, Root | GeneChip ATH1 | GSE9311 | Van Hoewyk et al. (2008) |
| +O2        | Arabidopsis | Seedling | GeneChip ATH1 | GSE9311 | Branco-Price et al. (2008) |
| +acid rain S | Arabidopsis | Leaf | GeneChip ATH1 | GSE52487 | Liu et al. (2014) |
| +acid rain S | Arabidopsis | Root | GeneChip Array | E-MEXP-1415 | Howarth et al. (2008) |
| +acid rain S | Arabidopsis | Root | GeneChip Array | E-MEXP-1694 | Bo et al. (2014) |
| +acid rain S | Arabidopsis | Root | GeneChip Array | GSE61679 | Gupta et al. (2017) |
| +acid rain S | Arabidopsis | Grain | Illumina HiSeqTM PE125/PE1 | E-MTAB-1782 | Yu et al. (2018) |
| +acid rain S | Arabidopsis | Grain | NimbleGen microarray | E-MTAB-1782 | Dai et al. (2015) |
| +acid rain S | Arabidopsis | Grain | NimbleGen microarray | E-MTAB-1920 | Vincent et al. (2015) |
With increasing depositions of data related to S metabolism, including data on species other than Arabidopsis (Table 1), such approaches will have a greater impact on the generation of hypotheses. New candidate genes and biochemical processes interconnected to plant S metabolism will be identified as a result of these systems-based and targeted approaches. It is a matter of ongoing debate, probably driven by individual research interests, as to whether only ‘robust’ processes that occur under a variety of conditions and in various plants are relevant or whether ‘specific’ responses that occur under only certain conditions are the most meaningful for improving our understanding of plant sulphur physiology.

While initial high-throughput analyses can lead to the generation of hypotheses (Nikiforova et al., 2003, 2005a, 2005b), these need to be tested experimentally for further validation (Fig. 1A). Cataloguing alone is insufficient to develop knowledge of processes and, eventually, to exploit them for plant breeding and crop production. Validation efforts necessarily need to employ all levels of molecular biology and bioinformatics-based approaches in an iterative manner (Hoefer and Watanabe, 2017). An example is the investigation of predicted hub genes (Nikiforova et al., 2005a) through a mutational approach (Falkenberg et al., 2008). Three transcription factors, IAA13, IAA28, and ARF-2 (ARF1-Binding Protein), in a network responsive to S deprivation have been identified as being connected to multiple downstream and upstream interactors, and thus constitute hubs, making it likely that they represent important genes (Mähler et al., 2017). Falkenberg et al. (2008) subsequently showed that these transcription factors indeed play a role in controlling certain aspects of plant sulphate metabolism, and thus validating the assumption that identification of correlole network hubs is a tool that can be used to identify relevant target genes—in this case linking S deprivation to auxin signalling. In fact, IAA28 may constitute the link between auxin signalling, S starvation, and alterations in root development (Rogg et al., 2001; Falkenberg et al., 2008; De Rybel et al., 2010), although this remains to be demonstrated functionally. A link to auxin had been postulated previously (Nikiforova et al., 2005a). A further example is the identification of the functional roles of sulfur deficiency induced 1 (SDI1) and SDI2 (Fig. 1B). An AFLP study on wheat identified SDIs as being strongly responsive S-deprivation genes (Howarth et al., 2005) and they were also identified in early macroarray studies on S-deprived and OAS-treated Arabidopsis (Hirai et al., 2003; Nikiforova et al., 2003). However, the function of the SDI genes was not clear from these initial studies. A combination of a bioinformatics approach to OAS-related responses (Hubberten et al., 2012a) and a mutational approach coupled with transcriptomics and metabolomics analyses (Aarabi et al., 2016) revealed that SDI1 and SDI2 interact through protein–protein binding with a previously described transcription factor, MYB28. Upon S deprivation in Arabidopsis, this binding down-regulates MYB28 transcription and consequently reduces the biosynthesis of glucosinolates (Gigolashvili et al., 2007b; Sonderby et al., 2007). In functional terms, this may divert S resources from secondary to primary metabolism. Interestingly, Hubberten et al. (2012a) additionally revealed a group of OAS-responsive genes

2003; Menz and Seip, 2004). From the molecular perspective, several transcriptome datasets on wheat in relation to responses to S nutrition may be the primary resource for studying the effects of sulphur inputs (Table 1).

A common feature of all systems biology approaches is that they yield vast amounts of data (Kopra et al., 2015). Hence, statistical methods have had to be developed or adapted to deal with this (Klipp et al., 2016; Xia, 2018). In the context of sulphur systems biology, such methods were already being applied to the early transcriptomics data sets. Especially when attempting to correlate transcriptomics and metabolomics data (Nikiforova et al., 2005b), it was inevitably necessary to apply bioinformatics approaches in order to allow data interpretation and the development of models (Hirai et al., 2004; Hirai and Saito, 2004; Nikiforova et al., 2004, 2005a). Results are often displayed as correlation networks (Nikiforova et al., 2005a).

This kind of approach is aimed at filtering the data to remove the ‘noise’ of variability associated with gene expression and metabolite contents, and in doing so to highlight differences that are statistically significant (Massonnet et al., 2010).

One constraint of systems approaches such as transcriptomics, proteomics, or metabolomics is the fact that even if concentration differences per se are determined, they may not represent changes in activities of relevant proteins or enzymes, or of metabolite fluxes. An example of a situation where transcriptomics would not reveal an important gene is the transcription factor sulfur limitation1 (SLIM1, AT1G73730), which has been identified through genetic screening of Arabidopsis mutants (Maruyama-Nakashita et al., 2006) and has been shown to control a major part of the S-starvation response (Kawashima et al., 2011; Wawrzyńska and Sirko, 2014). As far as current data suggest, SLIM1 itself is not, or is only marginally, transcriptionally regulated upon S deprivation. EIN3 (AT3G20770), a major factor involved in ethylene signalling, has been shown to modulate SLIM1 binding activity to its target gene promoters (Wawrzyńska and Sirko, 2016). As the authors suggest, this probably interferes with the S deficiency-dependent induction of target genes by SLIM1. However, they do not exclude the possibility that further regulators might be involved in shaping the response to S deprivation. To unravel the complexity of the regulation of plant S metabolism it is therefore obvious that despite the wealth of data provided by systems approaches, targeted analyses need to be combined in order to reveal the cellular and physiological responses to S deprivation (Fig. 1A).

Deposition of systems biology results in databases allows data to be revisited when new knowledge is available, such as improved gene annotation, and this can not only confirm initial assumptions but also provide novel information (Fig. 1A; Nikiforova et al., 2005a; Hoefer and Watanabe, 2017). Recently, Henríquez-Valencia et al. (2018) have conducted a comparative meta study using existing data sets together with novel bioinformatics approaches. This led to the identification of transcription factor networks that provide new candidate genes for sulphate research that would not otherwise have been identifiable in individual experimental set-ups. This also highlights the need for further transcriptomics studies to be provided to the scientific community to advance our knowledge.
that are co-regulated under various conditions, termed OAS-cluster genes. Co-regulated expression hints at the existence of common upstream regulatory control mechanisms, which would be worth investigating.

**Transcription factors of Arabidopsis related to sulphur metabolism**

The responses of plant S metabolism to changes in the availability of sulphate are well described (Davidian and Kopriva, 2010). In terms of regulation, several candidate genes have been identified (Table 2). ‘Omics’ approaches do not usually identify post-transcriptional or post-translational modifications unless they are specifically designed to indicate modifications such as persulphidation (Aroca et al., 2017) or DNA methylation (Huang et al., 2016). Proteomics approaches are suited to identify protein modifications but the number of such studies on responses to S deprivation is low, even for Arabidopsis. Even less information is available regarding the signal molecules that induce the S-deprivation response. Results that indicate the involvement of phosphorylation originate from targeted and not from systems analyses, except for the potential involvement of sucrose non-fermenting-1-related protein kinases

| Transcription factor | AGI Code     | Regulation                        | References                                      |
|----------------------|--------------|-----------------------------------|------------------------------------------------|
| SLIM1                | AT1G73730    | S response, S metabolism          | Maruyama-Nakashita et al. (2006)                |
| HY5                  | AT5G11260    | S assimilation (APR)              | Lee et al. (2011); Koprivova and Kopriva (2014) |
| MYB28                | AT5G061420   | Aliphatic glucosinolate           | Celenza et al. (2005); Bielecka et al. (2015)  |
| MYB29                | AT5G07690    | Aliphatic glucosinolate           | GigoIashvili et al. (2007a); Hirai et al. (2007); Sønderby et al. (2007); Malitsky et al. (2008); Davidian and Kopriva (2010) |
| MYB76                | AT5G07700    | Aliphatic glucosinolate           |                                                |
| MYB34                | AT5G60890    | Indolic glucosinolate             |                                                |
| MYB51                | AT1G18570    | Indolic glucosinolate             |                                                |
| MYB122               | AT1G74080    | Indolic glucosinolate             |                                               |
| SD1                  | AT5G48850    | Glucosinolate (MYB)               | Aarabi et al. (2016)                           |
| SD2                  | AT1G04770    | Glucosinolate                     |                                                |
| NF-YA2               | AT3G05680    | Development, S, N, P responses    | Henríquez-Valencia et al. (2018)               |
| RVE2                 | AT5G37260    | Germination, Circadian rhythm     |                                                |
| MSA1 (SHM7)          | AT1G83670    | S-adenosylmethionine              | Huang et al. (2016)                            |
| PHR1                 | AT4G28610    | S, P responses, Sulphate shoot-to-root flux | Gojon et al. (2009); Rouached et al. (2011); Pant et al. (2015); Falkenberg et al. (2008) |
| IAA28                | AT5G25890    | Auxin signalling, lateral root    |                                                |
| IAA13                | AT2G33310    | Auxin signalling, embryonic root  |                                                |
| ARF1-ARF2            | AT5G62010    | Auxin signalling, plant ageing    |                                                |
| OBP2 (DOR)           | AT1G07640    | Glucosinolate                     |                                                |
| Calmodulin binding IQD protein (IQD1) | AT3G06710 | Glucosinolate                     |                                                |
| miRNA395             | AT3G06370    | Sulphate transporter (SULTR2;1), Sulphur assimilation (ATPS) | Kawashima et al. (2009, 2011); Buhtz et al. (2010); Liang et al. (2012) |
| EIN3                 | AT3G20770    | Ethylene signalling, SLIM1        | Wawrzyńska and Sirko (2016)                    |
| ARF12                | AT1G34310    | Auxin response, root development  | Bielecka et al. (2015)                         |
| ARR16                | AT2G40670    | Cytokinin signalling, root        |                                                |
| ATAF1 (NAC)          | AT1G01720    | Abscisic acid biosynthesis        |                                                |
| CO-like Yabby        | AT1G73870    | Auxin homeostasis                 |                                                |
| DREB A-4             | AT2G44940    | –                                 |                                                |
| HAT14 (HB)           | AT5G06710    | –                                 |                                                |
| MADS                 | AT4G33960    | –                                 |                                                |
| MYB9                 | AT5G16770    | Suberin in seed coat              |                                                |
| MYB31                | AT1G74650    | –                                 |                                                |
| MYB45                | AT3G48920    | –                                 |                                                |
| MYB52                | AT1G17950    | Secondary cell wall               |                                                |
| MYB53                | AT5G65230    | Lateral root                      |                                                |
| MYB54                | AT1G73410    | Secondary cell wall               |                                                |
| MYB71 (MYB306)       | AT3G24310    | –                                 |                                                |
| MYB75 (PAP1)         | AT1G66650    | Anthocyanin                       |                                                |
| MYB93                | AT1G34670    | Lateral root                      |                                                |
| Trihelix             | AT3G10040    | Hypoxia response                  |                                                |
| WRKY56               | AT1G64000    | –                                 |                                                |
| ZAT12 (C2H2)         | AT5G59820    | Abiotic/oxidative stress          |                                                |
| ZAT6 (C2H2)          | AT5G04340    | S&P response, root development    |                                                |
(SNRKs; Iyer-Pascuzzi et al., 2011) that is suggested based on transcriptomics of different root cell types in Arabidopsis starved of sulphate, nitrate, and phosphate. OAS has been considered as a potential signal and evidence has accumulated to substantiate its signalling function (Saito, 2000; Hirai and Saito, 2004; Hubberten et al., 2012a, 2012b; Arabi et al., 2016). But exactly how OAS is sensed is still unknown as neither the receptor nor the signal transduction chain has yet been identified, although SDII and SDI2 seem to be induced by OAS. In addition to OAS, several other intermediates of the sulphate assimilation pathway have been suggested as signals, including sulphate (Rouached et al., 2005), sulphite (Brychkova et al., 2013; Naumann et al., 2018), sulphide with its role in persulphidation (Ma et al., 2015; Aroca et al., 2018), glutathione (GSH), and cysteine. The problem is that the sulphate assimilation pathway reacts to changes in any of its metabolite concentrations with correlated changes of other metabolites of the pathway, making it difficult to discern individual effects. For OAS, this could be experimentally resolved by expressing a serine acetyltransferase (SERAT) gene under control of an inducible promoter, and by the finding that OAS is possibly related to stress-induced reactive oxygen species (ROS) that are induced under conditions where no further changes of the S-containing metabolites are detected (Hubberten et al., 2012a). Receptors have not been identified in transcriptomics studies. A mutational approach might provide this information, but although such an approach did identify SLIM1 (Maruyama-Nakashita et al., 2006) as a transcription factor (TF) that controls certain parts of the sulphate starvation response, it did not identify a receptor of S-containing metabolites. Various hormones have been generally implicated in regulating aspects of sulphate metabolism (Falkenberg et al., 2008; Amtmann and Blatt, 2009; Gojon et al., 2009; Rubio et al., 2009; Wawrzynska and Sirko, 2016) but their exact involvement remains still elusive. A cytokinin receptor, CRE1/AHK4 (cytokinin response 1/Arabidopsis histidine kinase 4), has been identified and suggested to play a role in the regulation of sulphate uptake (Maruyama-Nakashita et al., 2004). This receptor has been previously determined to modulate phosphate starvation responses by inhibiting phosphate transporter expression and to down-regulate sulphate uptake in roots under conditions with sufficient P and S supply (Gojon et al., 2009). The TF phosphate starvation response1 (PHR.1) is also known to be associated with control of sulphate metabolism as shoot-to-root sulphate transport is affected in phr1 mutants and the accumulation of sulphoquinovosyl diacylglycerol (SQDG) decreases about 2-fold relative to the wild-type under P-deprived conditions (Rouached et al., 2011; Pant et al., 2015). These findings indicate the existence of crosstalk between P and S metabolism (Gojon et al., 2009).

A comparative study dedicated to identifying TFs that respond to sulphate deprivation and resupply but not to N or P starvation (Bielecka et al., 2015) yielded several candidates (Table 2). In the same study a set of known TFs, in particular those related to aliphatic and indolic glucosinolate biosynthesis (MYB28, 29, 76, 34, 51, 122), were correlated with the expression of glucosinolate pathway genes. Furthermore, TFs shown to be responsive to S availability included those regulating anthocyanin biosynthesis, mainly MYB75 (PAP1, production of anthocyanin pigment1) together with a set of TFs probably controlled by PAP1, namely MYB90 (PAP2), MYB113, and MYB114 for anthocyanin; TT8 (TRANSPARENT TESTA 8), bHLH, TTG1 (TRANSPARENT TESTA GLABRA1), WD40, and TTG2 (TRANSPARENT TESTA GLABRA2), and WRKY for flavonoids. Such a link to anthocyanin and flavonoid biosynthesis is obvious as S-starved plants (in common with N- and P-starved plants) display accumulation of redish pigments in leaves (Nikiforova et al., 2005b; Walff-Zottele et al., 2010). Although induced by S deprivation, these TFs might be also be part of a more general stress rescue system (Whitcomb et al., 2014). Most of the TFs suggested by Bielecka et al. (2015) have not yet been validated through further analyses, but they provide a valuable data resource for future research. Unravelling the signals, the receptors, the signalling cascade, the TFs, and other regulators that control plant S metabolism is an ongoing challenge.

**Metabolomics of Arabidopsis with respect to sulphur metabolism**

The sum of all the metabolites in a cell or tissue is referred to as the metabolome. The number of metabolomics studies dedicated to the response to S deprivation or S resupply in Arabidopsis is low (Table 3). At the level of primary metabolite composition, we postulate that most plants will share related responses as primary metabolites constitute those metabolic pathways that are present in all plants to serve the basic functions of life (Pichersky and Gang, 2000). Thus, it is justifiable to compare S-related metabolome studies even between different species (Fig. 1A; Table 3). These studies display common changes in the form of reductions of tissue levels of sulphate, thios, and other S-containing metabolites, while other metabolites such as OAS accumulate (Nikiforova et al., 2003, 2004, 2005a, 2005b; Hirai et al., 2004; Maruyama-Nakashita et al., 2006). They also show unpredicted effects on metabolite composition, for example reduction in chlorophyll, protein, and RNA contents, and accumulation of N-rich compounds, such as allantoin, asparagine, glutamine, and putrescine. Flavonoids accumulate as an effect of MYB75 expression, as indicated by the reddish colour of leaf tissues. With respect to secondary metabolites, more caution has to be exercised in drawing conclusions; for example, there is a reduction in glucosinolate content upon sulphate deprivation in Brassicaceae species such as Arabidopsis and rapeseed, but not in other plants where these compounds are not present (Gigolashvili et al., 2007a, 2007b, 2008; Hirai et al., 2007).

It can be argued that metabolomics only hints at affected pathways and does not provide detailed information with respect to proteins or genes that are actually involved in modulating the metabolic composition. The metabolite composition can be assumed to reflect the integration of all the transcription, translation, enzyme activity, and flux dynamics that are relevant to all previous regulatory responses and biochemical steps. As such, metabolomics data contain information on how a plant adapts to a given stress such as S deprivation. Shifts in metabolic patterns might provide information on the pathways affected, but very little on the individual genes or enzymes involved in generating the overall output. The accumulation of intermediates that are otherwise not present in the
The authors concluded by calling for a re-evaluation of sulphur metabolism. However, several of these novel compounds are not yet annotated. Whether low-concentration S compounds can indeed exert effects on plant metabolism needs to be evaluated. A particular case is the identification of health-promoting compounds in garlic and onion (Nakabayashi et al., 2013, 2016; Nakabayashi and Saito, 2017) (Table 3) where heavy-isotope labelling has helped to identify the relevant compounds and pathways. Secondary metabolite analysis mostly provides information within a plant family; however, the methods presented in these studies are applicable to other species and are thus helpful for sulphur systems biology.

**Proteomics**

For Arabidopsis, only a few studies are available that have been dedicated to the determination of proteome changes in leaves or roots upon S deprivation (Table 4). A combined transcriptomics and proteomics study on Arabidopsis seeds that investigated the response to S deprivation (Higashi et al., 2006)
corroborated the shift of storage proteins in favour of those containing less S amino acids by blocking C-terminal degradation of low-S 12S globulins and reducing the amount of S-rich 2S albumins. Similar findings were also found in seed proteomics studies of wheat (Grove et al., 2009) and rapeseed (D’Hooghe et al., 2014) under S deprivation, allowing the assumption that this constitutes a general response. However, the molecular mechanisms and regulatory control of this response still remain to be elucidated. In addition, these studies revealed that S-responsive genes such as sulphate transporters and APS reductase were induced in seeds under S limitation together with several genes related to ROS protection, indicating the presence of ROS stress in S-deprived seeds. The genes identified overlapped with those found in earlier studies on Arabidopsis exposed to S stress (Hirai et al., 2003; Nikiforova et al., 2003). It was hypothesized that ROS accumulation might be the result of reduced thiol availability in the S-deprived seeds, and that seeds possess mechanisms to counteract these effects to maintain viability. Extended S starvation in rapeseed has been shown to impair viability and germination ability (D’Hooghe et al., 2013, 2014). These proteomic studies of rapeseed exposed to S limitation combined with metabolomics studies on leaves (D’Hooghe et al., 2013) and seeds (D’Hooghe et al., 2013, 2014) corroborate the findings described for Arabidopsis, with additional changes in the seed lipid composition in favour of long fatty-acid chains and impairment of photosynthesis in the leaves. In addition, these studies have also indicated a link with ethylene and jasmonate metabolism, as has been described for Arabidopsis (Nikiforova et al., 2003; Wawrzynska et al., 2015; Wawrzyńska and Sirko, 2016). As such, proteomics studies in other Brassicaceae species might compensate for the lack of studies on Arabidopsis. A recent study focussed on the gasotransmitter H$_2$S and L-cysteine desulphydrase 1 (DES1) used a specialized proteomics approach to identify persulphidated proteins in Arabidopsis (Aroca et al., 2017, 2018). Persulphidation is believed to affect a variety of biological functions, including stress responses and carbon metabolism, and displays a new type of regulation that is assumed to counteract the nitrosylation-mediated response of the gasotransmitter NO that presumably acts on the same proteins. We anticipate a detailed systems biology-based study on the effects of persulphidation being carried out in the future. A proteomics study on the Tibetan alpine plant Lamiophlomis rotata revealed that H$_2$S as an important player in adaptation to high-altitude stresses, and linked S metabolism to this adaptation process and to oxidative stress (Ma et al., 2015). Proteomics studies in response to heavy metal stresses have not provided much novel information with respect to S proteomics per se. Specific heavy metal stresses that have been examined include exposition to cadmium (reviewed in Villiers et al., 2011; Bagheri et al., 2017) of various plants and tissues such as Arabidopsis leaves (Semane et al., 2010), poplar leaves and roots (Kieffer et al., 2009), and roots of Brassica juncea (Alvarez et al., 2009). In addition, chromium (Yildiz and Terzi, 2016) and arsenite expositions (Dixit et al., 2015) have been investigated. However, these studies are still relevant as they relate to S metabolism, because GSH and phytochelatin synthesis are essential to detoxify metal ions and/or alleviate the consequent effects of ROS.

The analysis of persulphidation in plants of the des1 mutant provides an example of a repeated analytical cycle employing ‘omics’ analysis as illustrated in Fig. 1A. Another example of the approach illustrated in the figure is that in the initial phase, ‘omics’ studies were conducted on wild-type plants exposed to S deprivation (Table 1) and then in an iterating analytical cycle, mutants were analysed as a result of targeted research efforts (Aroca et al., 2017) or from candidate genes (e.g. SDI1 and SDI2; Fig. 1B) identified from previous ‘omics’ studies (Howarth et al., 2005; Aarabi et al., 2016).

### Bioinformatics of Arabidopsis with respect to sulphur metabolism

Sulphur systems biology has developed over the last two decades to become a widely used analytical approach in research on S physiology. Right from the beginning, the sheer amount of data produced by ‘omics’ studies made it difficult to identify relevant information. Hence, early in the development sulphur

### Table 4. Proteome analyses related to sulphur metabolism

| Experiment | Species        | Tissue            | Type                        | References                     |
|------------|----------------|-------------------|-----------------------------|--------------------------------|
| –S         | Arabidopsis    | Seed              | 2DE, MALDI-TOF, LC-MS/MS    | Higashi et al. (2006)          |
|            | Oliseed rape   | Leaf              | 2DE, ESI LC-MS/MS           | D’Hooghe et al. (2013)         |
| –S         | Brassica napus | Seed              | 2DE, ESI LC-MS/MS           | D’Hooghe et al. (2014)         |
| –S, +Cd    | Spinach        | Leaf              | SDS-PAGE                    | Bagheri et al. (2017)          |
| +Cd        | Arabidopsis    | Leaf              | 2DE, ESI LC-MS/MS           | Semane et al. (2010)           |
| –S         | Poplar         | Leaf, Root        | 2DE, MALDI-TOF/TOF          | Kieffer et al. (2009)          |
| S          | Brassica juncea| Root              | 2DE, nano-LC-MS/MS          | Alvarez et al. (2009)          |
| S, +As (III)| Rice           | Leaf              | 2DE, MALDI-TOF/TOF          | Dixit et al. (2015)            |
| H$_2$S     | Arabidopsis    | Leaf              | 2DE, MALDI-TOF/TOF          | Aroca et al. (2017)            |
|            | Lamiophlomis rotata | Leaf         | 2DE, MALDI-TOF/TOF            | Ma et al. (2015)               |

2DE, two-dimensional gel electrophoresis; ESI LC-MS/MS, electrospray ionization liquid chromatography with tandem mass spectrometry; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; nano-LC-MS/MS, nanoscale liquid chromatography coupled to tandem mass spectrometry; SDS-PAGE, polyacrylamide gel electrophoresis.
systems biology; bioinformatics approaches were employed to determine correlations among responses and to deduce correlation networks (Nikiforova et al., 2003, 2005a; Hirai and Saito, 2004; Hirai et al., 2004; Ohta et al., 2008; Usadel et al., 2009; Obayashi and Kinoshita, 2010). Approaches such as gene-to-gene correlations, gene-to-metabolite correlations, clustering, principal component analyses, and batch-learning self-organizing maps (BL-SOM) helped to organize data into relevant units and aided the generation of hypothesis, and hence the identification of candidate genes or processes (Fig. 1A). Examples of the successful use of BL-SOM are the identification of the involvement of S metabolism in the biosynthesis of medicinally active compounds in plants (Rai et al., 2017), the identification of a correlation between S metabolism and the MYB transcription factor PAP1 that controls anthocyanin biosynthesis (Hirai et al., 2005), and the functional elucidation of SD11 and SD12 (Aarabi et al., 2016) (Fig. 1B). Obviously, an important aspect is the clear definition of the experimental question, and the experimental and applied biostatistical approaches that are taken: without these, data interpretation will remain incomplete and unsatisfactory. A statistical challenge is the gap between the number of samples, such as the conditions or plant genotypes, and the much greater number of determined values, such as genes, metabolites, proteins, or other data. To resolve this challenge, dimensionality reduction methods have been developed and are widely applied to ‘omics’ data (Steinfath et al., 2008; Weckwerth, 2008).

Another factor to consider for successful S systems biology is standardization of experiments and data (Salek et al., 2015). This mainly concerns to the experimental side: how plants are grown, how material is processed, and how values are measured. As demonstrated by recent studies (Kopriva et al., 2015; Henríquez-Valencia et al., 2018), the available S transcriptome data have been obtained from plants grown under different conditions and various tissues and developmental stages have been used. There is still an overlap between these studies that can indicate the most robust responders, usually those with the biggest increments in change of expression or metabolite content between samples (Kopriva et al., 2015). Among them, an NADPH oxidoreductase (AT1G75280) has been speculated but not proven to act as an isoformolinoid reductase (IFR) that is active in anthocyanin/flavonoid biosynthesis to provide ROS-protection capacity under S-deprived, and hence GSH-deprived, conditions (Nikiforova et al., 2003). This may well link to the correlation of PAP1 with sulphate metabolism under S-deprived conditions discussed earlier (Hirai et al., 2005). Another example is the identification of the OAS-cluster genes (Hubberten et al., 2012a), which correlate various conditions where OAS is accumulated in tissues with a set of co-expressed genes. Here, the link between these diverse conditions and S metabolism is not yet established. Thus, bioinformatics in support of sulphur systems biology has proven successful as early findings have laid the basis for later detailed research (e.g. OAS-cluster genes) or have provided candidate genes (e.g. IFR) whose function in plant S metabolism can be further examined.

An important problem is that subtle differences in (e.g.) gene expression might be difficult to identify. For example, the transcriptional changes of TFs are usually low, with thresholds often involving changes of only 1.5- or 2-fold, which may scarcely be above background variation (Nikiforova et al., 2003; Maruyama-Nakashita et al., 2006; Bielecka et al., 2015; Forieri et al., 2017). Differences in data generated by different laboratories also exist, even when methods are standardized. Massonnet et al. (2010) organized a number of independent laboratories to conduct the same experiment using similar genotypes and standardized growth conditions with leaf phenotypes, transcriptomics, and metabolomics as the output, or with material produced in one lab being distributed to the others for analysis. But despite this standardization differences in the data produced were still observed. The variations were suggested to be attributable to variability in the plants and sample handling (i.e. human factors) as well as to slight differences in growth conditions (light quality, temperature, and water). Moreover, comparability of S deprivation is further complicated as this is a dynamic process and is dependent on the specific level of deprivation that is applied (Whitcomb et al., 2014; Henríquez-Valencia et al., 2018). It would be helpful for the advancement of our knowledge of S metabolism if an agreement could be reached on standard conditions, plant lines, and procedures, and on a more systematic and complete catalogue of the systems response to distinct S deprivation and resupply conditions. This would be helpful as a blueprint on which to base future experiments using other cultivars, conditions, or mutants, the results of which could then be compared back to this master data set. Such a blueprint has been provided in the case of senescence (Watanabe et al., 2013). The data that are currently available do still allow meta-analysis of the S transcriptome and can yield suggestions for novel TFs that potentially play roles in S metabolism. For example, Henríquez-Valencia et al. (2018) deduced a network of known and putatively correlated regulators and TFs, which in particular suggested that NF-YA2 (AT3G05690, nuclear transcription factor Y subunit A-2) and RVE2 (AT5G37260, reveal2) act as upstream regulators of the S deprivation response (Table 2). NF-YA2 and RVE2 display connections to five S-related response modules. NF-YA2 is induced by S depletion and has been shown to be associated with the regulation of several developmental processes, such as flowering and leaf and root system architecture, and to respond to depletions of N and P. RVE2 is reduced by S depletion and has been shown to be associated with germination and control of the circadian rhythm. Network analysis has suggested that IAA28 (AT5G25890) acts as a regulator in the S-starvation response network, although its expression is not markedly changed upon S depletion of seedlings (Nikiforova et al., 2003, 2005a, 2005b; Falkenberg et al., 2008; Hoeefgen and Watanabe, 2017).

Conclusions and outlook

Sulphur systems biology has provided novel information, especially with respect to fundamental research findings. It is an inherent feature of transcriptomics approaches that they cannot identify when regulation occurs post-transcriptionally or even post-translationally, for example through changes of enzyme activities or regulatory properties. An example is the first TF identified to control S metabolism, SLIM1 (Maruyama-Nakashita et al., 2003, 2005a, 2005b; Henríquez-Valencia et al., 2018).
et al., 2006), which is not (or hardly) transcriptionally altered upon S deprivation. SLIM1 instead seems to be modulated by a protein–protein interaction with EIN3, a positive regulator in the ethylene response pathway (Wawrzynska and Sirko, 2016). Thus, systems biology studies provide a certain subset of information, usually based on differential accumulation of molecules. Hence, integrating targeted analyses or agronomic data is necessary to gain a holistic understanding of the system (Fig. 1A).

Sulphur systems approaches have already served to build models that have allowed novel candidate genes to be identified and confirmed and, in the subsequent iterative process of applying targeted and non-targeted analyses of omics-derived candidates, have allowed further details to be uncovered (Fig. 1A, B).

As S moieties are a key determinant of a vast number of bio-molecules and biochemical processes, it is not surprising that systems approaches have highlighted processes that seemingly appear to be unrelated to *bona fide* S assimilation and the biosynthesis of primary S–containing metabolites. Sulphur-omics is a rich source of candidate genes, many of them still awaiting detailed examination. The huge flood of data that has been produced through sulphur systems biology approaches has led to results that have helped to elucidate plant S physiology. Systems biology will continue to support the identification of novel genes and the validation of candidate genes as a standard tool of molecular biology.

What might be required to increase our knowledge beyond what has currently been achieved? A more systematic analysis of plants, especially of the model Arabidopsis, exposed to defined conditions of S availability would be helpful to provide a blueprint and to correlate future research findings to a master data set. The current systems biology data available for Arabidopsis are derived from only a limited number of conditions, genotypes, and developmental time-points (Tables 1, 3, 4). Even when considering other plant species, the database is only marginally greater. In the case of proteomics, the data set available is insufficient. There is not only a need for cataloging changes in protein contents, but also for changes in protein activities and protein modifications, such as phosphorylation, persulfidation, and glutathionylation. A recent study on protein persulphidation provides an example of what is urgently needed (Aroca et al., 2017). Likewise, flux analyses at a systems level are generally missing and would help in the construction of consistent functional networks. This does not necessarily simply mean that more data is better data, but that the S research community should agree on standards, on approaches, and on data storage and exchange. We would even propose a joint research effort between different laboratories despite the redundancy of data it might include. A broader and reliable database would be a good resource for future bioinformatics attempts to deduce relevant conclusions. A debate needs to be had as to whether fragmentation of data should be avoided by concentrating research on model systems such as Arabidopsis or whether analysis of multiple plant species with diverse biology would eventually provide a better understanding. With ever-improving sequence technologies there are hardly any restrictions with regards to the availability of genome sequence data, and resources that provide data on genetic variability are available for many crop species, such as rapeseed, rice, and wheat, besides the Arabidopsis model system (Scholl et al., 2000).

In the case of bioinformatics, there is a need to improve data interpretation and model building in order to close the gap between bioinformatics and the biological interpretation of data. The identification of relevant candidates, pathways, and processes is still knowledge-driven rather than being provided in an objective manner by bioinformatics prediction tools. Studies such as a recent analysis of network topologies and their relation to stability against mutational variation (Mähler et al., 2017) might be helpful for sulphur systems biology. Further, bioinformatics studies and systems biology studies should aim at understanding dynamic processes over time rather than just the current snapshot view of S metabolism.

Sulphur is not an isolated entity within the biochemistry of a plant. It is instead interactive, cross-influencing and being influenced by numerous other processes, the foremost of which are the links to ROS tolerance and detoxification, and the interplay with other mineral nutrient ions (Kopriva and Rennenberg, 2004; Kruse et al., 2007; Forieri et al., 2013; Zuchi et al., 2015). Aspects such as photosynthesis (Nikiforova et al., 2005b; Wulff-Zottele et al., 2010; Naumann et al., 2018) and seed protein quality (Galili and Höfgen, 2002; Galili et al., 2005) are also worth considering in greater detail within this context.

With regards to the application of sulphur systems biology for agronomy, there are no reports yet in relation to new varieties released to the market. However, systems biology has become a standard element in the analytical toolbox for crop research (Langridge and Fleury, 2011; Reynolds and Langridge, 2016; Heyneke et al., 2017; Casartelli et al., 2018). Application of knowledge originating from plant S research needs to take into account agricultural procedures such as fertilization regimes, cropping systems, soil parameters, and water availability. The specific needs of certain crops for S supply also have to be considered, for example the high demand of rapeseed (Bloem et al., 2004). Sulphate supply is also considered necessary for legume nodule functioning (Krusell et al., 2005) and root–mycorrhiza interactions (Sieh et al., 2013). Not least, S metabolism is dependent on nutrient interactions under natural conditions (Zuchi et al., 2015; Forieri et al., 2017).

We have reviewed sulphur systems biology and looked separately at transcriptomics, metabolomics, proteomics, and bioinformatics. However, systems biology inherently integrates all existing information from genotype to phenotype. We are convinced that integration of the results of all ‘omics’ technologies and of classical biochemical, physiological, and agronomical experiments will eventually lead to breakthrough results (Fig. 1A).

Systems biology is not about ‘omics’ technologies but about a holistic view of the highly complex biological system of the plant and trying to capture its function through understanding all (or at least as many) parts as possible using integrative approaches, i.e. high-throughput ‘omics’ determinations supported by targeted approaches. The ultimate goal of Sulphur-omics is to understand the underlying network scheme, to model it mathematically, and to use this for predictions—at the cellular, organ, and whole-plant level. Sulphur systems biology has the chance to provide a showcase for nutrient systems biology.
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