Plant sulfur nutrition: From Sachs to Big Data

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Abbreviations: APS, adenosine 5′-phosphosulfate; PAPS, 3′phosphoadenosine 5′-phosphosulfate; QTL, quantitative trait locus

Together with water and carbon dioxide plants require 14 essential mineral nutrients to finish their life cycle. The research in plant nutrition can be traced back to Julius Sachs, who was the first to experimentally prove the essentiality of mineral nutrients for plants. Among those elements Sachs showed to be essential is sulfur. Plant sulfur nutrition has been not as extensively studied as the nutrition of nitrogen and phosphate, probably because sulfur was not limiting for agriculture. However, with the reduction of atmospheric sulfur dioxide emissions sulfur deficiency has become common. The research in sulfur nutrition has changed over the years from using yeast and algae as experimental material to adopting Arabidopsis as the plant model as well as from simple biochemical measurements of individual parameters to system biology. Here the evolution of sulfur research from the times of Sachs to the current Big Data is outlined.

All plants require 16 elements for life: carbon, oxygen and hydrogen in carbon dioxide and water, and 13 mineral nutrients: nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, copper, zinc, manganese, molybdenum, boron, and chlorine. The idea that plants obtain their nutrition from soil can be dated back to Aristoteles, but it was generally believed that the main nutrient is water.1 At the end of 17th century Woodward showed that water indeed stimulates plant growth, but the dirtier the water is, the better the plants grow, which led for the first time to appreciation of soil as the source of plant nutrition.2 The recognition of the importance of mineral elements for plants has culminated in the 19th century in the work of 2 German scientists, who can be considered the founders of plant nutrition research.3 Justus von Liebig formulated the Law of the Minimum, which states the principle that growth is limited not by all single nutrients were left out of the solution, that Sachs experimentally determined that N, P, K, Ca, Mg, S, and Fe are indeed essential for plants. He also showed that plants do not need ammonium as suggested by Liebig, but grow well with nitrate as nitrogen source.4 The use of hydroponics revolutionized plant nutrition research as it allowed precise qualitative and quantitative formulation of the individual nutrients and so enabled to study their effects on plant physiology. One important aspect of using hydroponics is also the comparability of results between different laboratories, which is impossible to guarantee using local soil. Interestingly, after Sachs showed that iron is essential for plant growth in 1865, the next nutrients, Mn and B, were put on the list of essential elements only in the 1920s.5

The Way to the Textbook Pathway

Among the nutrients shown by Sachs to be essential is sulfur. Sulfur as a nutrient is similar to nitrogen and phosphorus in that it can be taken up and utilized in many forms, sulfate, sulfur dioxide, hydrogen sulfide, cysteine, and other organic sulfur compounds. The major form of sulfur used by plants is sulfate, which enters a complex network of metabolic reactions to produce the variety of metabolites essential for life. In plants sulfur is found in the amino acids cysteine and methionine, and so in proteins, in many co-factors and prosthetic groups, peptides such as glutathione, in sulfolipids, sulfated polysaccharides, and many secondary metabolites, e.g., glucosinolates and alliins.6 Metabolism of sulfur compounds has been extensively investigated, the enzymes and genes at least of the primary pathways have been identified, and the regulation of these pathways described, even if the molecular mechanisms are still largely unknown.7

The ways this information has been derived have of course changed dramatically from the times of Sachs. The questions, the methods, the experimental objects, all have been changing.
throughout the history. However, something remained: a certain lack of urgency in the sulfur research, many questions on sulfur has been approached only after they were solved for nitrogen, phosphate, or potassium. This is true for the effects of sulfur deficiency in the 1930s as well as, e.g., the cloning of genes for specific transporters in 1990s. Possibly, the reason has been the abundance of sulfur in the environment due to industrial pollution, which contributed to the view that sulfur is not limiting. This has been recognized very early, already in 1920s it was shown that rainwater in industrial areas contains large concentrations of sulfur. Consequently, in the 1930s it was shown that plants can assimilate atmospheric sulfur dioxide. However, with the reduction in air pollution from 1980s the atmospheric sulfur deposits were diminished and sulfur deficiency has become a problem for agriculture, primarily for oilseed rape farming. Another contributing factor to the common occurrence of sulfur deficiency has been the increased use of high definition fertilizers, as older formulations were often contaminated with sulfur since, e.g., traditional production of superphosphate used sulfuric acid. This in turn triggered the interest in sulfur research and sulfate deficiency became one of the best studied environmental conditions by systems biology approaches.

The first questions connected with plant sulfur research were those aimed to find the nature of the sulfur source used by plants. This research led to uncovering of the sulfur cycle in soil and of a large number of bacteria using redox reactions of sulfur to gain energy. Sulfate has been established as the major source of sulfur already by Sachs, but other sources such as cysteine, sulfate and atmospheric sulfur dioxide were also shown to support plant growth. These experiments were facilitated by the use of radioactive $^{35}$S isotope of sulfur, which allowed to measure specific uptake, but also to identify compounds into which the sulfur is incorporated. The initial knowledge on pathways of sulfate assimilation has been however obtained by studying bacteria, fungi, yeast, and algae. From today’s point of view it is almost unbelievable that the first dedicated review of sulfate metabolism in Annual Reviews of Plant Physiology in 1962 is based from some 90% on results derived from bacteria, yeast, or fungi. Indeed, the greatest impact on the initial understanding of sulfur metabolism had experiments with Salmonella, Escherichia coli, and yeast. Analyses of Neurospora and Aspergillus mutants provided knowledge on the synthesis of methionine from cysteine, but led also to wrong concepts, e.g., on an inorganic reduction pathway or on an essential role of thiosulfate as intermediate.

The pathway of sulfate reduction started to get in the right shape after discovery of 3’phosphoadenosine 5’-phosphosulfate (PAPS) as the active sulfate and ATP sulfurylase as the enzyme catalyzing the entry of sulfate into metabolism. Soon it has been established that sulfate is first activated to PAPS, then reduced to sulfite and sulfite is reduced to sulfide. Interestingly, the description of the enzymes and intermediates has been achieved through synthesis of data from animal, yeast, and bacterial systems. Therefore the pathway has been seen as universal for organisms reducing sulfate, including plants.

The general agreement of plant sulfate assimilation with the yeast pathway has been initially corroborated by work on algae, particularly Chlorella pyrenoidosa, and only later by experiments with higher plants. The reaction intermediates have been confirmed and the enzymatic activities have been found in Chlorella, therefore, the yeast (and bacterial) pathway has been accepted in plant physiology textbooks. Later on, detailed analyses of the individual reaction steps, however, suggested some important alterations and the exact mechanism of plant sulfate reduction has been a matter of controversy for a long time. Firstly, adenosine 5’-phosphosulfate (APS) and not PAPS has been shown to be the intermediate in sulfate reduction in algae and plants and then sulfite bound to a carrier has been identified among the reaction products after reduction of APS. This controversy has been resolved only relatively recently (around 2000) by describing the mechanisms of APS reductase from Lemma minor. Interestingly, the genes encoding APS reductase and all other enzymes have been identified and described before this clarification of the pathway. However, the full pathway of sulfate assimilation in plants has been resolved only when sulfate oxidase has been identified in plants in 2001. When the whole sulfur metabolism is considered, including secondary metabolites, it is possible that new components will be found, as, e.g. glucosinolate transporter has been identified only in 2012.

The Path to Understanding Regulation of Sulfur Metabolism

The lack of consensus about the pathway, however, did not prevent the investigations of its regulation. Initially the responses of sulfate uptake or accumulation of S-containing compounds to changes in environment have been measured. Light was shown to increase sulfate uptake of Chlorella and sucrose induced the uptake in the dark. The well-known regulations of sulfate uptake and assimilation by sulfate deficiency and reduced sulfur compounds have been described already in the 50s and 60s. The studies of regulation document best the changes in plant nutrition science over time, starting from measurements of growth through the autoradiographs of whole plants after using $^{35}$S as a tracer, to the deciphering of molecular mechanisms. Together the older studies, however, set sulfur metabolism well in the context of general plant physiology, showing the interdependence with carbon and nitrogen, regulation by sulfur sources, light, sucrose, etc. The investigations were continued in the 1960s and 70s by studies of individual enzymes and the regulation of their activities which significantly contributed to understanding of the control of the pathway. Importantly, the possibility of using $^{35}$S tracer enabled measurements of metabolic fluxes, giving a much better understanding of the effects of the changed environment on the pathway. The next big step has been the entry of molecular biology in sulfur research, starting in plants with cloning of O-acetylserine thiolylase, a component of cysteine synthase complex from spinach in 1993. This enabled comprehensive studies of regulation of sulfate assimilation by analysis of transcript and protein levels, enzyme activities, metabolites, and the fluxes. These experiments confirmed the initial findings.
of the links, e.g., between sulfate and nitrate assimilation, and enabled to identify key regulatory steps and mechanisms. A number of studies showed the importance of APS reductase in control of the pathway, which has been later confirmed by quantitative genetics. Analysis of Arabidopsis mutants revealed that also other components of the pathway contribute to the control, such as ATP sulfurylase and sulfite reductase. The investigations of single genes, gene families, or several different components of the pathway enabled to characterize the regulation of sulfate metabolism, to identify important genes and signals, and to point to links with other metabolic pathways. They, however, did not allow to appreciate how deeply sulfur metabolism is embedded in plant primary and secondary metabolism, as enabled by the –omics technologies. Sulfate deficiency was one of the early environmental conditions studied and one that contributed significantly to development of methods to analyze global expression data as well as global metabolite data. Hirai et al. and Nikiforova et al. combined transcriptome and metabolome data from sulfur deficient plants in the first comprehensive networks. These first big data were important to show the coordinated regulation of most genes of the pathway, to find links to new metabolic pathways, e.g., synthesis of jasmonate, and to identify new unknown genes highly responsive to sulfate deficiency. In the pioneering work the combined metabolite and transcript networks helped to define the processes underlying response to sulfur starvation and to predict functions of unknown genes and integrate them into pathway of glucosinolate synthesis. In the meantime, numerous –omics experiments have been performed with a large number of conditions and genotypes and the data deposited in public repositories. These big data can be used to learn about the pathway of interest even from untargeted experiments, e.g., showing a circadian regulation of numerous sulfate assimilation genes. Although such –omics data per se are purely descriptive, they can be used to dissect mechanisms of regulation, as shown in the confirmation of signaling function of O-acetylserine. Large scale analyses also revealed that in Arabidopsis, sulfate assimilation and glucosinolate synthesis genes are preferentially expressed in bundle sheath-like cell layers around the veins, a finding with possibly important consequences of our understanding of control of sulfur metabolism on the whole plant level. With a further development of in silico tools, the vast amount of information available will certainly further improve our knowledge of mechanisms of regulation, either directly or through underpinning new experimental approaches.

About the same time as systems biology, another field of science intersected with plant sulfur research, plant genetics. Similar to systems biology, genetic approaches are unbiased and can find completely new connections between metabolic pathways. Two types of approaches are particularly suited and both were employed for investigations of sulfur metabolism. Firstly, several genetic screens were used to identify components of the sulfate deficiency signaling. From several reports, the identification of SULFUR LIMITATION1 (SLIM1) as a transcription factor responsible for regulation of large number of genes by sulfur limitation has made the largest impact on sulfur research. Although other genes were found which when disrupted, affected response to sulfur limitation, none of these could be firmly put into the signaling pathway. SLIM1 controls the increase in sulfate uptake, reduction in glucosinolate synthesis, and induction of microRNA miR395 in sulfate limited plants, but not the increase in expression of APS reductase. Another screen resulted in defining a role for LONG HYPOCOTYL5 (HY5) in regulation of APS reductase by several environmental conditions, but not sulfate deficiency.

Alternative approach exploits natural variation among Arabidopsis (and other species) accessions. QTL analyses have been long exploited for finding genes responsible for variation in diverse traits in crop plants. In sulfur metabolism, QTL mapping was first used to assess control of glucosinolate content in oilseed rape and Arabidopsis. As in many traits, the genes underlying the QTLs encode metabolic genes, e.g., a variation in methylthioalkylmalate synthase controls the ratio between long-chain and short-chain aliphatic glucosinolates. In primary assimilation, QTL analysis confirmed the importance of APS reductase for control of the pathway, since the APR2 isoform was found to control sulfate content in Arabidopsis Bay-0 × Shahdara population. Interestingly, second QTL from the same population has been cloned as the gene for ATPS1 isoform of ATP sulfurylase, the enzyme preceding APS reductase in the pathway. The large increase in Arabidopsis genomics resources enabled to step from 2-parent recombinant populations into wider sampling of genetic diversity. These genome wide association (GWA) analyses were instrumental in finding that the gene annotated and considered as sulfate transporter SULTR5;1 actually functions as molybdenum transporter, and that APR2 is more broadly associated with variation in sulfate and total sulfur content.

What Next?

Obviously, sulfur research had undergone a very long way from the times of Sachs and his confirmation of essentiality of sulfur for plants to the vast amount of information about sulfur metabolism and its regulation available today. However, many questions are still open. The first fundamental question, whether all genes of sulfur metabolism have been identified, has to be answered with no. Despite the importance of vacuolar storage of sulfate, the transporter importing it into the vacuoles is unknown so far. On the other hand, from the 21 identified sulfotransferases in Arabidopsis only for half the substrates and functions are known. Probably even worse is the situation with transcription factors controlling regulation of sulfur metabolism, only a few have been described and even for the known ones the mechanisms of action are not known. When sulfur containing metabolites are taken into account, the situation does not get any different: a large number of unknown compounds have been found in Arabidopsis, and many more natural products might be expected in other plant species. Describing this metabolite diversity will require improved sensitivity of the analytical tools, as many of these
diseases.66 However, in this rapidly changing scientific landscape we should not forget that many basic every day techniques have been exciting new discoveries decades before. Hydroculture developed by Julius Sachs can serve as an excellent example and memento.

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