Regulation of sulfate metabolism in C4 plants

Ausma, Ties

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Summary

This dissertation addresses how C_4 photosynthesis affects the regulation of sulfate uptake and reduction at the whole plant level. Sulfur is an essential macronutrient for the proper physiological functioning of plants (**chapter 1**). The element is present in the amino acids cysteine and methionine, which are part of proteins. Sulfur is also part of other compounds, including compounds that crucially determine the taste, smell, and color of plant products. Plants usually acquire sulfur as sulfate via the root. After its uptake by specific transporters, sulfate is reduced to sulfide and subsequently incorporated in cysteine. Cysteine constitutes a central molecule in sulfur metabolism from which proteins and other sulfur-containing compounds are synthesized.

It is important to analyze how plants metabolize sulfate into cysteine, because the suboptimal availability of sulfur limits crop yield and quality in countries across the world, including the Netherlands. Plant sulfur research contributes to improving sulfur fertilizer levels and the identification of breeding targets for the efficient uptake and use of sulfate. It is particularly relevant to perform sulfur research on plants that have C_4 photosynthesis [e.g., maize (*Zea mays*) and sorghum (*Sorghum bicolor*)], since these plants may increasingly be utilized in agriculture. C_4 plants have suitable traits for increasing both agricultural productivity and sustainability. Compared to plants that have C_3 photosynthesis, C_4 plants can grow with a high productivity, while using water and nitrogen efficiently. Thus, this dissertation analyzed the consequences of C_4 photosynthesis for the regulation of sulfate uptake and reduction at the whole plant level.

Chapter 2, however, first underlines the importance of sulfur for plants. It describes the effects of sulfur, nitrogen, and phosphorus limitation on floral traits. Sulfur deficiency decreased floral display size, caused aberrant flower shapes, and altered the size and chemical composition of pollen. Furthermore, sulfur deprivation reduced yellow flower colors, though it did not affect purple, red, and white flower colors. The reduction in yellow flower color could be attributed to a decreased synthesis of violaxanthin, lutein, and other carotenoids. The impacts of sulfur deprivation on floral traits were specific to sulfur deprivation, since nitrogen and phosphorus deprivation hardly affected floral traits. Clearly, sulfur deficiency negatively affects the production of floral displays. The aberrant floral traits associated with sulfur limitation reduce the plant's attractiveness to pollinators, which likely has repercussions for plant reproduction and pollinator fitness. Consequently, sulfur deprivation may hamper (agro)ecosystem functioning.

The fumigation of plants with atmospheric hydrogen sulfide (H_2S) is a powerful tool for obtaining insight into the whole plant regulation of sulfate uptake and reduction. **Chapter 3** reviews the impacts of atmospheric H_2S on plants. H_2S is a gas with a distinct rotten egg smell that is present at high levels in several regions (e.g., in volcanic regions and in regions with polluting industry or agriculture). Plants absorb atmospheric H_2S via stomates in the foliage. Although H_2S is phytotoxic above a plant-specific threshold concentration, plants can also benefit from H_2S presence. Plants can directly use H_2S as sulfur source for cysteine synthesis and they can even grow with H_2S as sole sulfur source (*viz.* in the absence of a sulfate supply to the root). Whereas there is no relation between the rate of H_2S metabolism and the H_2S susceptibility of a plant, there may be a strong relation between the rate of H_2S and sulfate metabolism. If plants from the C_3 genus *Brassica* were grown with H_2S in the atmosphere and sulfate in the rhizosphere, H_2S absorbance downregulated sulfate uptake and reduction. The nature of this downregulation has been analyzed. These analyses indicated that H_2S fumigation provides insights into how the rate of sulfate uptake and reduction are tuned to the plant's sulfur demand at the whole plant level. Therefore, this dissertation used H_2S fumigation to study the regulation of sulfate metabolism.

In **chapter 4,** the C_3 plant barley (*Hordeum vulgare*) was fumigated with atmospheric H_2S to assess if the whole plant regulation of sulfate metabolism varies between C_3 *Brassica* and barley. The data showed that different C_3 plants respond differently to H_2S fumigation. For instance, sulfate deprivation enhanced the sulfate uptake capacity (*viz.* the activity of the sulfate uptake transporters). In barley, this increase was alleviated by H_2S fumigation, whereas in *Brassica* it was not. Thus, the whole plant regulation of sulfate metabolism profoundly varies among C_3 plants.

In **chapters 5 and 6,** the C_4 plant maize was fumigated with H_2S to analyze if C_4 photosynthesis affects the regulation of sulfate metabolism. Chapter 5 outlines the impacts of H_2S fumigation on the stomatal aperture dynamics of maize. The interpretation of the impacts of H_2S fumigation on enzyme activities may depend on the extent to which H_2S affects these dynamics (as the dynamics may, hypothetically, affect the extent of H_2S absorbance). However, the stomatal aperture dynamics of maize were not affected by exposure to a subtoxic concentration of atmospheric H_2S. H_2S fumigation did not affect leaf area, stomatal density, stomatal resistance, and transpiration rate of plants, meaning that H_2S

exposure did not affect the transpiration rate per stoma. Thus, in contrast to general assumptions, H_2S is not a gaseous signal molecule that controls stomatal aperture dynamics. Chapter 6 describes the data on the impact of H_2S on sulfate uptake and reduction in maize. Comparing the impacts of atmospheric H_2S in maize with those in C_3 plants and C_4 sorghum indicated that C_4 photosynthesis does not require a distinct whole plant regulation of sulfate uptake and reduction.

Chapter 7 presents the first findings from a comparative study on C_3 and C_4 *Panicum*, *Cleome*, and *Atriplex* species. These first findings suggest that although the whole plant regulation of sulfate uptake and reduction does not feature a C_3-C_4 pattern, C_4 plants feature higher cysteine and glutathione levels compared to C_3 plants (glutathione is a tripeptide that functions as an overflow of excessively absorbed sulfur). Potentially, these higher levels are caused by higher sulfide production rates, since C_4 plants also exhibited a higher APS reductase activity (a key-regulating enzyme in sulfate reduction) than C_3 plants. Thus, the capacity for sulfate reduction may be higher in C_4 plants. Surprisingly, the comparison also indicated that C_4 plants may have higher phosphate levels than C_3 plants. C_4 photosynthesis may therefore have broad implications for the mineral nutrition of plants.

From the dissertation, I conclude that plants profoundly differ in the whole plant regulation of sulfate uptake and reduction, but that C_3 and C_4 photosynthesis are not associated with distinctive forms of this regulation. To further appreciate the whole plant regulation of sulfate metabolism and variation therein, the nature of the pathways that govern sulfate uptake and reduction should be analyzed. In this context, the molecular pathways that control the expression of the sulfate uptake transporters and APS reductase have been tremendously clarified. However, in **chapter 8**, I argue that these molecular pathways have limited significance in determining the whole plant regulation of sulfate metabolism. I synthesize that there is no correlation between the expression and activity of sulfate metabolism enzymes. Physiological processes should thus significantly determine how much sulfate a plant metabolizes. I recommend to characterize these processes and I propose experiments that will increase our understanding of the regulation of sulfate metabolism and hence improve the cultivation of C_4 and other plants.