Whole plant regulation of sulfate uptake and distribution in cabbage

Koralewska, Aleksandra Dominika

Chapter 1.

General introduction

Uptake, distribution and assimilation of sulfate in plants

Sulfate taken up by the roots is a primary sulfur source for plants. Sulfate is actively transported into root cells across the plasma membrane through a proton/sulfate co-transport ($3H^+/SO_4^{2-}$) mediated by sulfate transporters and driven by a proton gradient generated by ATPases (Clarkson *et al.* 1993; Davidian *et al.* 2000; Hawkesford and Wray 2000; Hawkesford 2003, 2007; Hawkesford and De Kok 2006). Subsequently sulfate is transported into the stele where it is loaded into the xylem and distributed to the shoot. Sulfate has to be reduced to sulfide before it is further metabolized into cysteine, the precursor and sulfur donor for the majority of other organic sulfur compounds present in plants (Hell 1997; De Kok *et al.* 2002a; Saito 2004; Hawkesford and De Kok 2006; Fig. 1). Root plastids contain all enzymes of the assimilatory sulfate reduction pathway, but the major proportion of the sulfate is reduced in the chloroplasts in the shoot (Droux 2004; Saito 2004), since in herbaceous species and crops plants the shoot to root ratio is generally higher than 2 (Zhao *et al.* 2008). Prior to its reduction, sulfate is activated to adenosine 5'-phosphosulfate (APS) catalyzed by ATP sulfurylase, subsequently APS is reduced to sulfite by APS reductase and the sulfite formed is reduced to sulfide by sulfite reductase. The reduction of APS by APS reductase is considered to be one of the rate-limiting steps in the assimilation of sulfur in plants. Activity of APS reductase is the lowest from the enzymes of sulfate reduction pathway and it has a fast turnover rate (Brunold 1990, 1993; Leustek and Saito 1999; Kopriva and Koprivova 2003, 2004). In the last step catalyzed by *O*-acetylserine(thiol)lyase sulfide is incorporated into the amino acid skeleton of *O*-acetylserine and cysteine is produced (Hell 1997, 2003; Droux 2004; Saito 2004). Sulfate that is not directly assimilated may be stored in the vacuole (Davidian *et al.* 2000; Hawkesford and Wray 2000; Hawkesford 2003, 2007; Hawkesford and De Kok 2006).

In addition to sulfate taken up by the roots, plants are able to utilize foliarly absorbed sulfur gases, *viz.* H_2S, SO_2, and atmospheric levels of ≥ 0.05 µl l^{-1} may contribute substantially to plant sulfur nutrition (De Kok *et al.* 2007). It has been established that foliarly absorbed H_2S is directly metabolized with high affinity into cysteine and subsequently into other organic sulfur compounds (De Kok *et al.* 1997, 2000, 2002b, 2007). The rate

of H_2S metabolism into cysteine determines internal (mesophyll) resistance of the shoot to H_2S and its deposition (De Kok and Tausz 2001). H_2S exposure may result in a down-regulation of sulfate uptake by the root (Westerman *et al.* 2000, 2001a,b; Durenkamp *et al.* 2007).

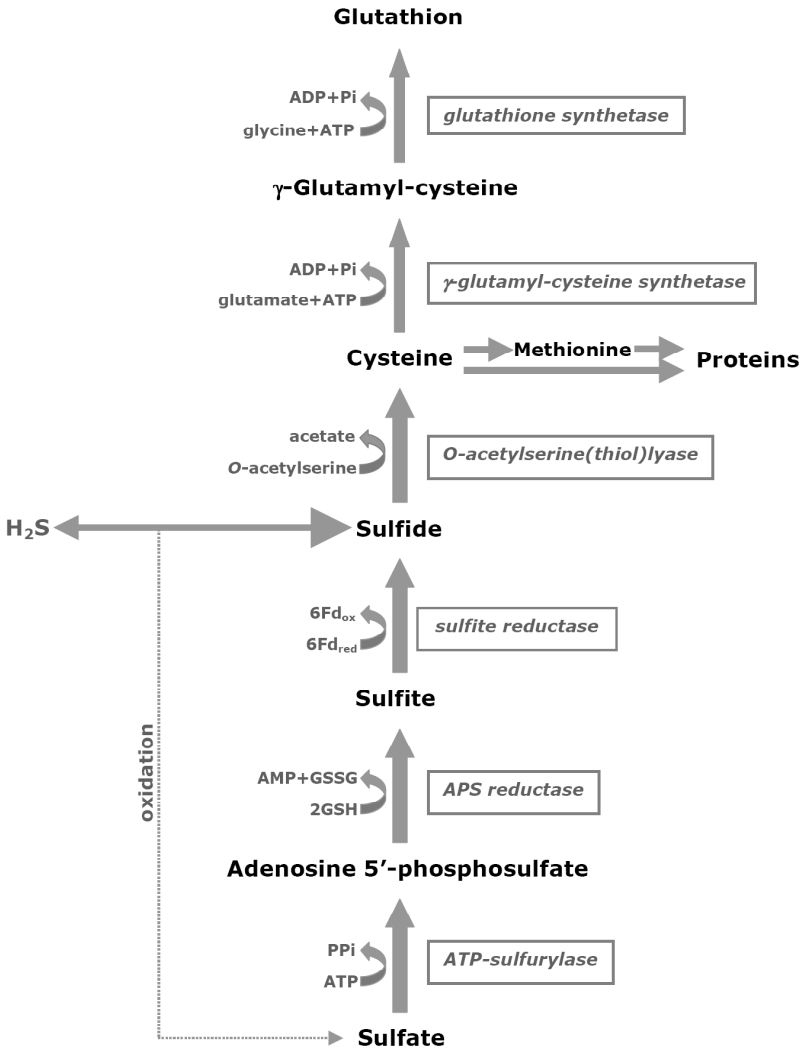

Fig. 1. Sulfate reduction and assimilation, and atmospheric H_2S metabolism in plants (adapted from De Kok *et al.* 2007). APS reductase, adenosine 5'-phosphosulfate reductase; Fd_{red}, Fd_{ox}, reduced and oxidized ferredoxin; GSH, GSSH, reduced and oxidized glutathione.

Sulfate transporters in plants

Sulfate transporters are transmembrane proteins and on basis of amino acid sequences of sulfate transporters the secondary structure of 12 membrane-spanning domains (MSD) and a sulfate transport/antisigma-factor antagonists domain (STAS domain) at their C-terminal region was derived (Hawkesford and Smith 1997; Hawkesford 2003). The STAS domain possesses a conserved loop with a potentially phosphorylated conserved serine residue, which may be involved in regulation. Shibagaki and Grossman (2004) have shown the importance of the STAS domain for facilitating localization of the sulfate transporters to the plasma membrane and its influence on the kinetic properties of these proteins. However, studies on the STAS domain in *Arabidopsis thaliana* sulfate transporter Sultr1;2 revealed that truncation of this domain results in loss of the transporter ability, whereas the targeting to the plasma membrane was correct. The hypothesis that the STAS domain of Sultr1;2 is involved in protein-protein interactions, which could control sulfate uptake in plants, was proposed (Rouached *et al.* 2005).

Distinct sulfate transporters are involved in the uptake and distribution of sulfate in plants. The sulfate transporter gene family has been classified in up to 5 different groups according to their cellular and subcellular expression and possible functioning (Davidian *et al.* 2000; Hawkesford and Wray 2000; Hawkesford 2003, 2007; Buchner *et al.* 2004b); 12 possible sulfate transporter genes have been isolated from *Brassica oleracea*, which on basis of sequence analysis are classified into 4 different groups (Buchner *et al.* 2004a; Fig. 2). Three different sulfate transporters have been characterized within the Group 1 transporters. Sultr1;1 and Sultr1;2 are high affinity sulfate transporters responsible for primary sulfate uptake by the roots and localized in the epidermis, cortex and root hairs (Takahashi *et al.* 2000; Shibagaki *et al.* 2002; Yoshimoto *et al.* 2002, 2007). They have an apparent K_m for sulfate around 10 µM (Smith *et al.* 1995, 1997), similar to that observed for whole root uptake (Leggett and Epstein 1956; Clarkson *et al.* 1983). Sultr1;2 appears also to be involved in the transport of selenate (El Kassis *et al.* 2007). The third Group 1 transporter (Sultr1;3) is involved in phloem loading of sulfate (Yoshimoto *et al.* 2003).

The so-called low affinity Group 2 sulfate transporters, with an apparent K_m for sulfate > 100 μM are involved in distribution of sulfate in the plant (vascular transport; Hawkesford 2003; Hawkesford *et al.* 2003b). The Sultr2;1 is localized in the xylem parenchyma and pericycle cells of roots and in the xylem and parenchyma cells of shoot. Sultr2;2 is localized in the root phloem and leaf vascular bundle sheath cells (Takahashi *et al.* 2000).

Five putative sulfate transporters belong to the less well characterized Group 3 (Hawkesford *et al.* 2003b; Buchner *et al.* 2004a). In *Arabidopsis* the Sultr3;1, Sultr3;2 and Sultr3;3 sulfate transporters are localized in the leaves (Takahashi *et al.* 2000; Hawkesford *et al.* 2003b), whereas in *Brassica oleracea* the Sultr3;2 was exclusively present in the root and Sultr3;3 in leaf, stem and root tissue (Buchner *et al.* 2004a). Moreover, in *Brassica oleracea* Sultr3;4 was present only in the stem and Sultr3;5 in the root (Buchner *et al.* 2004a). The role of this group of sulfate transporters is not well understood and until now enhanced activity of Sultr2;1 and increased sulfate uptake capacity were demonstrated for Sultr3;5 co-expressed with Sultr2;1 (Kataoka *et al.* 2004a).

Two Group 4 sulfate transporters have been identified in *Arabidopsis* and *Brassica*, and only one in rice and wheat (Hawkesford 2008). These transporters are localized in the tonoplast and may function in the vacuolar efflux of sulfate (Kataoka *et al.* 2004b; Hawkesford 2007, 2008).

The Group 5 transporters are quite distinct from the other sulfate transporter Groups, since they do not posses the STAS domain (Hawkesford 2003). Two Group 5 transporters have been identified in *Arabidopsis* and rice, and one in *Brassica* (Hawkesford 2003, 2008). In *Brassica napus* Sultr5;1 is present in the root, stem and leaves and is localized in the tonoplast (Hawkesford *et al.* 2003b; Parmar *et al.* 2007). In *Arabidopsis*, transporter AtSultr5;2, appears to be a high affinity molybdenum transporter and has been renamed MOT1 (molybdenum transporter 1; Tomatsu *et al.* 2007; Baxter *et al.* 2008).

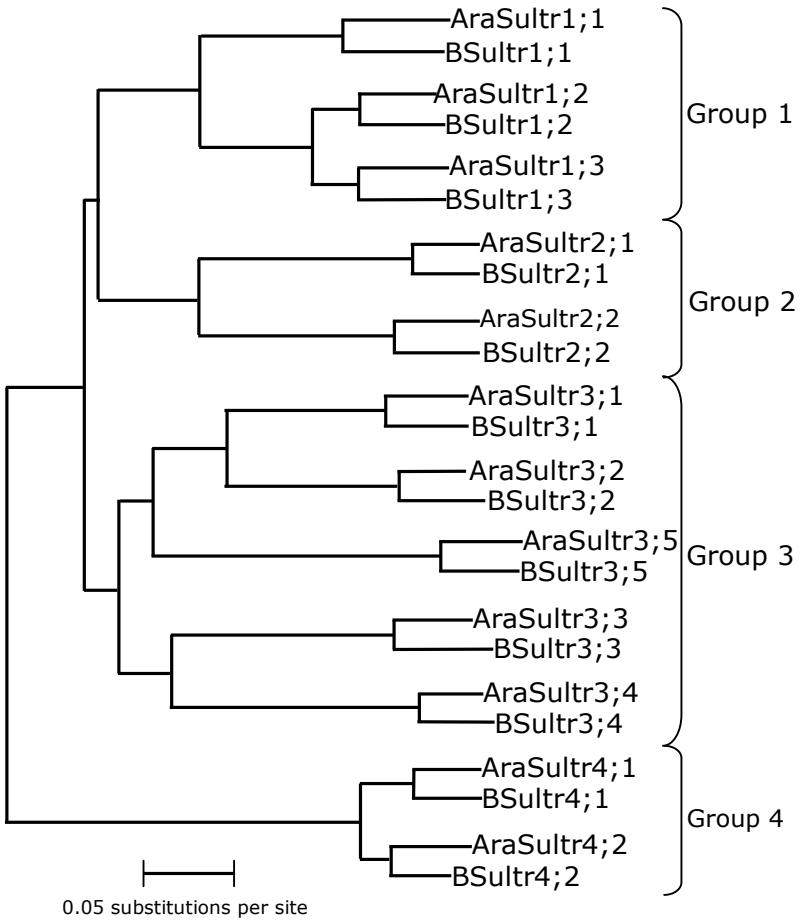

Fig. 2. Phylogenetic neighbour-joining tree the coding cDNAs of the *Arabidopsis* and *Brassica oleracea* sulfate transporter family (Buchner *et al.* 2004a).

Regulation of sulfate transporters

Expression and activity of sulfate transporters are generally regulated by the sulfur status of the plant (Hawkesford 2000, Hawkesford *et al.* 2003a,b; Hawkesford and De Kok 2006). In *Brassica* the expression of Group 1, 2 and 4 sulfate transporters was up-regulated upon sulfate deprivation, whereas that of Group 3 and Sultr5;1 was not affected (Buchner *et al.* 2004a; Parmar *et al.* 2007). Likewise the activity of the sulfate transporters was up-regulated upon sulfate deprivation.

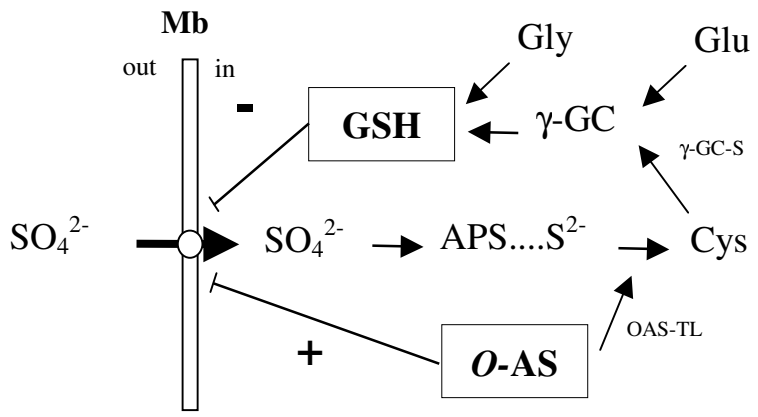

Fig. 3. Proposed model of the regulation of sulfate uptake and the signals therein involved (adapted from Davidian *et al.* 2000). Glutathione (GSH) and *O*-acetylserine (*O*-AS) have been shown to have respectively an inhibitory and a stimulatory effect on sulfate transport and on the expression of genes encoding sulfate transporters. OAS-TL, *O*-acetylserine(thiol)lyase; γGC-S, γ-glutamyl-cysteine synthetase.

The signal transduction pathway in the regulation of the uptake and distribution of sulfate in plants and the role of shoot to root signaling therein involved are still ambiguous. The regulation may occur at the transcriptional, translational and allosteric level (Hawkesford and Wray 2000; Hawkesford and De Kok 2006). It was observed that an up-regulated sulfate uptake was accompanied by a decreased content of thiols and sulfate, which led to the model of substrate repression of expression and activity (Hawkesford *et al.* 2003a,b). The products of sulfate assimilation (sulfate, sulfide, cysteine, glutathione) under sufficient sulfur supply would act as repressors in the process of negative feedback inhibition controlling the sulfate uptake (Fig. 3). Upon sulfate deprivation a decreased level of repressors and therefore a de-repression (up-regulation) of sulfate uptake would occur. In addition, the positive regulatory effect of OAS (*O*-acetylserine), which would accumulate as a consequence of a lack of sulfide supply to produce cysteine, on sulfate transporter expression and activity was proposed (Hawkesford and Smith 1997; Davidian *et al.* 2000; Hell and Hillebrand 2001; Brunold *et al.* 2003; Hawkesford *et al.* 2003a,b; Fig. 3).

In general the shoot is the major sink for sulfur and the necessity of demand-driven signaling from the shoot to root has been proposed (Lappartient and Touraine 1996). Glutathione, the end product of sulfur assimilation, essential in the storage and transport of reduced sulfur, was suggested as an important inter-organ signal compound of the sulfur status from the shoot to the root (Lappartient *et al.* 1999; Davidian *et al.* 2000; Herschbach *et al.* 2000; Vauclare *et al.* 2002).

Aim and outline of the thesis

The aim of the present study was to get more insight into whole plant regulation of sulfate uptake and distribution and the possible signal transduction pathways therein involved. Despite the current knowledge on the regulation of sulfate transporters at the gene/cellular level, insight in regulation of the uptake and distribution of sulfur at the whole plant level, e.g. shoot to root and source to sink signaling is still largely unclear. Many questions remain to be resolved: i) how the overall uptake and distribution of sulfate in the plants is controlled by the sulfur demand for growth, ii) what is the significance of shoot to root signaling between sulfate assimilation and uptake, iii) which metabolites are involved in the signal transduction pathway at cellular and interorgan level, and iv) what is the significance of expression *versus* activity of the sulfate transporters.

Two *Brassica* species, curly kale (*Brassica oleracea* L.) and Chinese cabbage [*Brassica pekinensis* (Lour.) Rupr.], were selected to study the modulation of expression and activity of the sulfate transporters by the level of varying pedospheric/atmospheric sulfur nutrition and plant sulfur status. *Brassica* belongs to Brassicaceae (the mustard family; Schranz *et al.* 2006). Curly kale and Chinese cabbage are characterized by a high growth rate and a high sulfur requirement (De Kok *et al.* 2000; Westerman *et al.* 2001a; Castro *et al.* 2003, 2004; Yang *et al.* 2006a,b). On the base of sequence comparison and phylogenetic analysis Buchner *et al.* (2004a) identified and characterized 12 different sulfate transporters in curly kale (*Brassica oleracea*) what made this plant species an interesting candidate to study regulation of expression and activity of sulfate transporters.

Chapter 2 describes the material and methods used in the present study. In Chapter 3 the impact of low sulfate concentrations in the root environment on the regulation of the expression and activity of the sulfate transporters and the distribution of sulfur was studied in curly kale. In Chapter 4 the effect of sulfate re-supply on the expression and activity of the sulfate transporters in sulfur-deficient curly kale was examined. In Chapter 5 the patterns of regulation of Sultr1;1 and Sultr1;2 sulfate transporters as affected by sulfate deprivation and H_2S exposure were studied. In Chapter 6 the significance of the shoot sink capacity in the regulation of expression and activity of the sulfate transporters was evaluated. In Chapter 7 whole plant regulation of sulfate uptake and distribution in cabbage is discussed.