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The Sulfate Assimilation Pathway in Higher Plants: Recent Progress Regarding Multiple Isoforms and Regulatory Mechanisms

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Abstract

The sulfur atom is an essential nutrient for living organisms because it plays a central role in protein folding, enzyme catalysis and maintenance of the redox status of cells. Microorganisms and plants can synthesize organic sulfur compounds, including cysteine, methionine and glutathione, from inorganic sulfur compounds such as sulfates. In contrast, animals utilize organic sulfur compounds that are mainly synthesized by plants. In the last decade, many genes whose products are involved in sulfate assimilation have been isolated from higher plants, and it has been revealed that there exist multiple isoforms for each step. The different properties of isoforms has been examined for sulfate transporters, which serve at the first step of sulfate assimilation, and for *O*-acetylserine(thiol)lyases, which catalyze the incorporation of sulfide into cysteine. Currently, however, it is not clear why plants developed multiple forms with similar catalytic properties.

Mechanisms of regulation of the sulfate assimilation pathway have also attracted much attention, since metabolites such as cysteine and glutathione affect related enzymatic activity or corresponding gene expression. The precursor of cysteine, *O*-acetylserine, is a key compound in maintaining the balance between sulfate and nitrate assimilation in higher plants. In addition to nutrition, sulfate assimilation is important for conferring tolerance against environmental stresses including heavy metals. Genetic engineering of components of the sulfate assimilation pathway is a useful approach to generate useful plants for agriculture and phytoremediation.

Importance of sulfur

Sulfur, essential for all living organisms, is defined as the macroelement. An adequate concentration in dry tissue is 0.1 % in plants, while those for carbon and nitrogen, which are structural component of biomolecules, are 45 % and 1.5 %, respectively (Raven *et al.*, 1999). Most organic sulfur occurs as cysteine and methionine in protein (Anderson 1990). Cysteine can make a disulfide bond with another cysteine molecule under oxidized conditions and can be restored with reduction. This allows cysteine residues to function in protein folding and catalytic activity, especially in metalloenzymes, in which the amino acid acts as the metal ligand (Leustek *et al.*, 2000). Methionine is commonly involved in regulation of enzymatic activity. The sulfur of methionine is vulnerable to oxidation and can readily form a methionine sulfoxide. If methionine of a protein is converted to methionine sulfoxide, the protein is reversibly inactivated (Leustek *et al.*, 2000). The third major organic sulfur compound is glutathione, which is enzymatically synthesized from glutamate, cysteine and glycine and regulates intracellular redox homeostasis through reversible change between the thiol form (GSH) and the disulfide form (GSSG). Glutathione is directly involved in detoxification of heavy metals and xenobiotics, and also serves as a stress signal and developmental trigger (Leustek *et al.*, 2000).

By using inorganic sulfur, plants and microorganisms are able to synthesize organic sulfur compounds as mentioned above. In contrast, animals are unable to utilize inorganic sulfur, and they need to uptake organic sulfur mostly from plants. Consequently, the sulfur assimilation pathway of plants is crucial not only for plants themselves but also for animal health and nutrition (Leyh 1993).

Environmental problems due to sulfur

After the Industrial Revolution, atmospheric sulfate aerosols have increased substantially as a result of burning of fossil fuels. Sulfate aerosols can act as cloud condensation nuclei, and are thought to cool the surface of the Earth through increasing cloudiness and scattering solar radiation (Buchanan *et al.*, 2000), acting as one of cause of acid rain. This latter causes acidification of soil, resulting in aluminum dissociation, which inhibits root elongation and nutrient uptake (Kochian 1995). In order to solve such environmental problems, it has been proposed that genetically modified plants be created, which can eliminate sulfurous pollutants because of a large capacity for sulfate assimilation.

In recent decades, air pollution has become issue generating enormous public interest, and considerable progress has been achieved in reducing emissions of sulfur into the atmosphere. However, since in some areas, including Europe, industry is one of the main sources of inorganic sulfur for arable land, decrease of atmospheric sulfate may be accompanied by reduction in the sulfur content of crops (Howkesford 2000). Extreme deficiency of sulfate causes decrease of photosynthetic capacity and results in a decrease of total yield (Lencioni *et al.*, 1997). Mild sulfate deficiency may not affect crop growth and yield, but it can change the ratio of sulfur to nitrogen. For example, storage proteins of legume and cereal plants grown under sulfate sufficient conditions contain high amounts of sulfur amino acids. With sulfate deficiency, plants accumulate another type of storage proteins, which contain low sulfur amino acids (Galyer and Sykes 1985; Zhao 1999). Such changes in protein composition account for deterioration in quality and nutrient value. Appropriate application of fertilizer is one of the solutions for sulfate deficiency. However, this approach is not in itself enough to resolve the problem with respect to the efficiency of utilization of sulfur by crops and the run off from the rhizosphere. Genetic modification of the sulfate assimilation pathway may be able to improve the capacity for uptake, storage and translocation of sulfur compounds in plants. For this purpose, it is necessary to understand enzymatic properties and expression patterns of the proteins involved, together with their regulatory mechanisms.

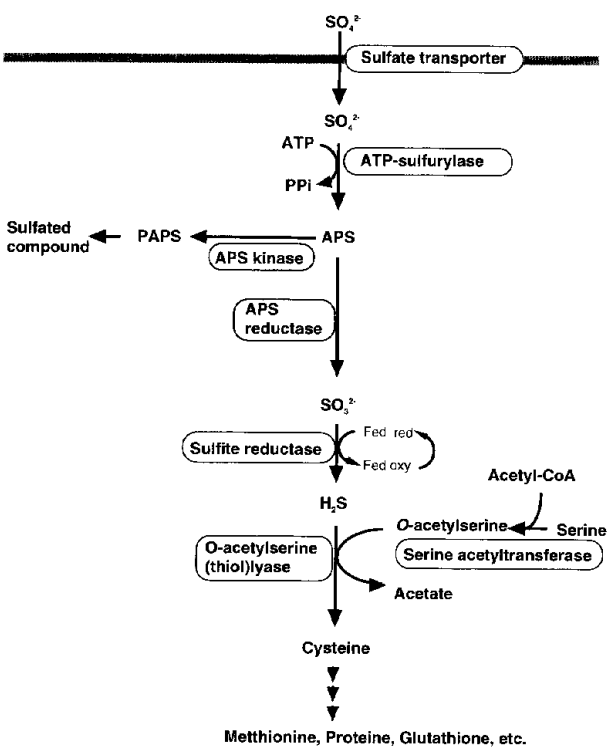

Fig. 1 Schematic diagram of sulfate assimilation in higher plants.

Sulfate assimilation pathway

The sulfate assimilation pathway of higher plants is illustrated in **Fig. 1**. It is initiated by uptake of sulfate by roots from the soil, proton/sulfate symporters in plasma membranes being responsible for this step. Sulfate is then further transported into plastids, where reduction and most of the assimilation processes take place, and into vacuoles, acting as stockrooms. In the plastids, sulfate is activated by adenosine triphosphate (ATP) to form adenosine 5'-phosphosulfate (APS) in a reaction catalyzed by ATP sulfurylase, since sulfate is relatively inert. APS is a branch point intermediate, which can be employed in both reduction and sulfation reactions. APS receives two electrons from glutathione in a reaction catalyzed by APS reductase to form sulfite, which is then further reduced with six electrons from ferredoxin by sulfite reductase. The resultant sulfide can subsequently be utilized for synthesis of cysteine from serine in a two-step process involving a complex of serine acetyltransferase and *O*-acetylserine (thiol) lyase (OAS-TL). This complex is called cysteine synthase. The first step catalyzed by serine acetyltransferase is acetylation of serine in the presence of acetyl-CoA to form *O*-acetylserine (OAS). The second is the formation of cysteine by OAS-TL from sulfide and OAS, with release of acetic acid. The two-step reaction system exists in cytoplasm and plastids, as well as mitochondria, although it is not clear why it is needed in each compartment. Cysteine is further utilized to synthesize methionine, proteins, glutathione and other molecules (Saito 2000; Leustek *et al.*, 2000).

Multiple isoforms in the sulfate assimilation pathway

Many enzymes involved in the sulfate assimilation pathway have now been purified from several plant species, and their enzymatic properties determined. Genes encoding these enzymes were also isolated from several higher plants, mainly from Arabidopsis, in recent years. In contrast to microorganisms, which have at most two isoforms for each step, Arabidopsis has at least three isoforms for most steps (**Table 1**). In particular, eleven and nine genes encoding sulfate transporters and OAS-TL, respectively, are predicted in Arabidopsis genome.

It can be speculated that each isoform has a distinct expression pattern and/or enzymatic properties in accordance with temporal and spatial variation in functions. Perhaps multiple isoforms are necessary to respond to distinct demands for reduced sulfur in each organ or each intracellular compartment. Studies on differential roles among isoforms have been performed with sulfate transporters and OAS-TLs. Here we describe recent advances in a survey of properties of isoforms of sulfate transporters and of OAS-TLs.

Sulfate transporters — Approximately 20 genes encoding sulfate transporters have been isolated from various plant species, including eleven from Arabidopsis. Heterologous expression experiments using yeast mutants, in which genes encoding SUL1 sulfate transporters were deleted, indicated that them to encode proton/sulfate symporters (Smith *et al.*, 1995, 1997). Twelve membrane-spanning domains (MSDs), conserved in many cation/solute symporters, were predicted from all amino acid sequences of these gene products (Smith *et al.*, 2000). Plant sulfate transporters are classified into 4 groups based on phylogenic analysis of amino acid sequences (Takahashi *et al.*, 2000) (**Fig. 2**). They differ from each other in affinity for sulfate, organ specificity and response to sulfur status in plants, although not all isoforms have been analyzed as yet. Group 1 proteins show a high-affinity for sulfate (Km = 3~12 μM) and are found mainly in roots (Smith *et al.*, 1995, 1997, Takahashi *et al.*, 2000). Transcript levels of *Sultr1;1*, a representative member of group 1, increase over 20-fold in roots when Arabidopsis seedlings are subjected to sulfate deficient stress, and decrease to the initial levels upon release from this stress (Takahashi *et al.*, 2000). The accumulation profile of *Sultr1;1* transcripts is consistent with sulfate uptake activity of roots in response to sulfate deficiency. Promoter analysis of *Sultr1;1* revealed it to be expressed in lateral root caps, root hairs and the epidermis and cortex of roots. Sulfate transporters of group 2 show rather low-affinity (Km > 0.1 mM) for sulfate, and are found in both roots and above ground tissues (Smith *et al.*, 1995; Takahashi *et al.*, 1996, 1997). In this

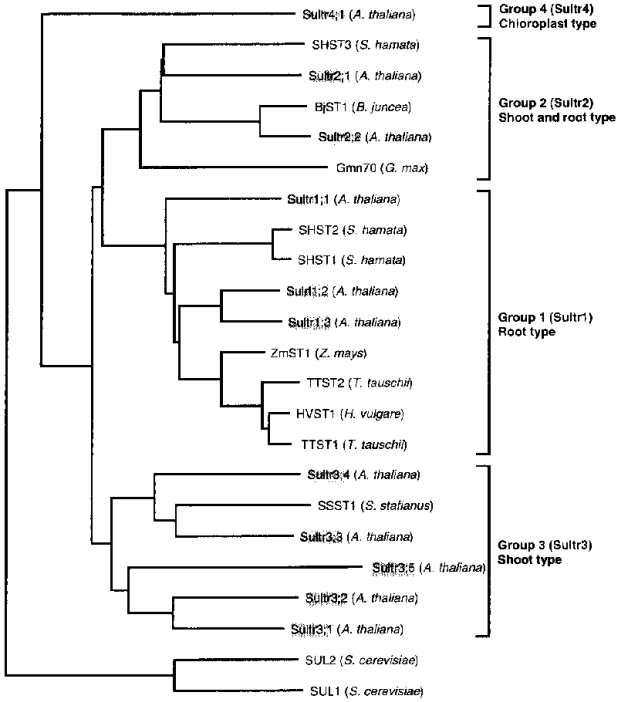

Fig. 2 A phylogenic tree inferred from the amino acid sequences of various sulfate transporters. Amino acid sequences were analyzed using the ClustalX program (Thompson *et al.*, 1997) and the tree was obtained using the NJplot program (Perriere and Gouy 1996). Sequences were from: Sultr1;1 (AB018695, *A. thaliana*), Sultr1;2 (AB042322, *A. thaliana*), Sultr1;3 (AB049624, *A. thaliana*), Sultr2;1 (AB003591, *A. thaliana*), Sultr2;2 (D85416, *A. thaliana*), Sultr3;1 (D89631, *A. thaliana*), Sultr3;2 (AB004060, *A. thaliana*), Sultr3;3 (AB023423, *A. thaliana*), Sultr3;4 (AT3g15990, *A. thaliana*), Sultr3;5 (AT5g19600, *A. thaliana*), Sultr4;1 (AB008782, *A. thaliana*), SHST1 (X82255, *Stylosanthes hamata*), SHST2 (X82256, *S. hamata*), SHST3 (X82454, *S. hamata*), HVST1 (X96431, *Hordeum vulgare*), ZmST1 (AF016306, *Zea mays*), BjST (AJ6223495, *Brassica juncea*), TTST1 (4850270, *Triticum tauschii*), TTST2 (4850272, *T. tauschii*), SSST1 (X96761, *Sporobolus stafianus*), Gmn70 (D13505, *Glycine max*), SUL1 (NP_009853, *S. cerevisiae*), SUL2 (NP_013193, *S. cerevisiae*). Classification of sulfate transporter family is indicated at the right side by brackets. SUL1 and SUL2 of *S. cerevisiae* are set as an outgroup. All Arabidopsis sequences are indicated in shadedboxes.

Table 1. The list of all isoforms involved in sulfate assimilation of Arabidopsis

Name	Protein code[a]	Gene name(accession no.)
Sulfate transporter	AT1g22150	*sultr1;3*[b] (AB049624)
	AT1g23090	*sultr3;3*[b] (AB023423)
	AT1g77990	*sultr2;2*[b] (D85416)
	AT1g78000	*sultr1;2*[b] (AB042322)
	AT3g12520	*sultr4;1*[b] (AB008782)
	AT3g15990	*sultr3;4*[b]
	AT3g51900	*sultr3;1*[b] (D89631)
	AT4g02700	*sultr3;2*[b] (AB004060)
	AT4g08620	*sultr1;1*[b] (AB018695)
	AT5g10180	*sultr2;1*[b] (AB003591)
	AT5g19600	*sultr3;5*[b]
ATP sulfurylase	AT1g19920	*met3-1*(X79210),*ASA1*(U40715),*APS2*(U06276,U59737)
	AT3g22890	*APS1* (AF198964,U05218)
	AT4g14680	*APS3* (U59738,U05218)
	AT5g43780	*APS4* (AJ012586)
APS kinase	AT2g14750	*ATK* (U05238),*akn*(X75782)
	AT3g03900	
	AT4g39940	*akn2* (AF043351)
	AT5g67520	
APS reductase	AT1g62180	*APR2* (U56921), *PRH43*(U53866), *APSR*(AF023167)
	AT4g04610	*APR 1*(U43412), *PRH19*(U53864)
	AT4g21880	*APR 3*(U56922), *PRH26*(U53865)
sulfite reductase	AT5g04590	sir(Z49217)
O-acetylserine(thiol)lase	AT2g43750	*Bsas2;1*[c] (X80377,X81698)
	AT3g03630	*Bsas5;1*[c] (XB003041)
	AT3g04940	*Bsas4;2*[c] (AB024284,AJ011603)
	AT3g22460	*Bsas1;2*[c] (AJ011976)
	AT3g59760	*Bsas2;2*[c] (X81973)
	AT3g61440	*Bsas3;1*[c] (AB024283,AJ010505)
	AT4g14880	*Bsas1;1*[c] (X84097,X80376,X81697)
	AT5g28020	*Bsas4;1*[c] (AB024283,AJ011044)
	AT5g28030	*Bsas4;3*[c]
Serine acetyltransferase	AT1g55920	*SAT-p*[d] (Z34888,L42242)
	AT2g17640	*SAT-106*(AF112303)
	AT3g13110	*SAT-m*[d](X80938,U22964,L78443,X82888)
	AT4g35640	
	AT5g56760	*SAT-c*[d](U05238),akn(X75782)

[a] Protein codes are from MIPS date base

[b,c,d] Gene names were refferred to the reports of Takahashi *et al.*, (2000), Hatzfeld *et al.*, (2000) and Noji *et al.*, (1998), respectively.

group, there are 2 isoforms, *Sultr2;1* and *Sultr2;2*, in Arabidopsis, which are well analyzed with regard to their expression patterns (Takahashi *et al.*, 2000). *Sultr2;1* is present in root caps, central cylinders and the vascular system of above ground tissues. Transcript levels in roots, subjected to sulfur deficient stress, specifically increased up to 9-fold in comparison with controls (Takahashi *et al.*, 1997).

Sultr2;2 is expressed in root phloems and leaf vascular bundle cells and its transcription also increases when seedlings are exposed to sulfur deficiency (Takahashi *et al.*, 2000). Group 3 consists of Sultr3;1, Sultr3;2 and Sultr3;3 from Arabidopsis, but their identification is based on their homology to other sulfate transporters (Yamaguchi *et al.*, 1997; Takahashi *et al.*, 1999b). Transcripts are only detected in above ground tissues and they do not respond to sulfate deficient stress. An additional 2 genes, which show high similarity with sulfate transporters of group 3, have been found in the Arabidopsis genome. Group 4 has only one member , Sultr4;1, which was isolated by homology search with the sulfate transporter from *Synechocystis* sp. PCC6803 (Takahashi *et al.*, 1999a). Its function has yet to be identified, although it has a signal sequence for plastid targeting at the N-terminus and a fusion protein of this N-terminus signal sequence and GFP was transported to plastids. Transcripts of *Sultr4;1* are detected in both above ground tissues and roots, and increase in the former under sulfate deficiency conditions. Since reduction of sulfate to sulfide mainly occurs in chloroplasts, Sultr4;1 may function in transport of sulfate from the cytosol to chloroplasts. Based on the described properties, physiological roles of sulfate transporters have been proposed (Takahashi *et al.*, 2000). High-affinity sulfate transporters of group 1 located at the surface of roots, would appear to play a central role in uptaking sulfate from soil. Sulfate transport from the root tissues to the above ground tissues is performed by low-affinity sulfate transporters of group 2 through the central cylinder of roots or vascular bundles of the above ground tissues. Sulfate translocation to plastids may be performed by the sulfate transporters of group 4, while group 3 forms may be involved in loading of leaf cells, phloem loading and transfer of nutrients to meristems and storage tissues (Smith *et al.*, 2000), although further analysis is necessary to clarify this point. Although plant cells are known to store sulfate in vacuoles (Anderson 1990), sulfate transporters which translocate sulfate across the tonoplast has not been isolated from any plants.

O-acetylserine(thiol)lyases - OAS-TL enzymes from various plants are diverse, with multiple isoforms capable of synthesizing cysteine in different intracellular compartments (Saito 2000). To date, more than 25 cDNAs have been registered as OAS-TLs in the GenBank, 9 of which are from Arabidopsis, although not all of them were confirmed to have OAS-TL activity. The complexity of OAS-TL isoforms is considered to be due to enzymatic properties (Hatzfeld *et al.*, 2000; Warrilow and Hawkesford 2000; Yamaguchi *et al.*, 2000). They require a cofactor, pyridoxal-5'-phophate (PLP), for activity, and are therefore classified into the β-family of PLP-dependent enzymes (Hayashi 1995). This latter also contains forms that catalyze synthesis of β-substituted alanine, such as cyanoalanine (Braunstein and Goryachenkova 1984). OAS-TL and β-cyanoalanine synthase (CASase), respectively, have been reported to possess β-cyanoalanine synthase activity and OAS-TL activity to some extent (Warrilow and Hawkesford 1998). For example, purified CASase of cocklebur exhibits OAS-TL activity, and its N-terminus amino acid sequence shows a high homology to cytosolic OAS-TL (Maruyama *et al.*, 1998). It is conceivable that reported genes for OAS-TL encode not only OAS-TL but also a large range of PLP-dependent β-family enzymes. Despite insufficient functional analysis, these enzymes are classified as belonging to the β-substituted alanine synthase (BSAS) family based on their enzymatic properties and primary structures (Hatzfeld *et al.*, 2000). Group 1 consists of cytosolic OAS-TLs including the Bsas1;1 and Bsas1;2 reproted for Arabidopsis. Bsas1;1 has a high affinity for OAS (Km = 1.2 mM), compared with other isoforms, while Bsas1;2 is currently thought to be a pseudogene because its cDNA contains an intron sequence and an in-frame stop codon (Jost *et al.*, 2000; Hatzfeld *et al.*, 2000). Group 2 consists of organellar OAS-TLs including Bsas2;1 and Bsas2;2 of Arabidopsis with a high affinity for OAS (Km < 1.0 mM) (Jost *et al.*, 2000). Bsas2;1 is localized in chloroplasts and Bsas2;2 in mitochondria (Hesse *et al.*, 1999). A cDNA for group 3 was initially isolated as OAS-TL by PCR of a preparation from spinach (Saito *et al.*, 1994). Bsas3;1 of Arabidopsis and CysC of spinach are localized in mitochondria, showing low OAS-TL, but high CASase activity (Takahashi and Saito 1996; Yamaguchi *et al.*, 2000; Hatzfeld *et al.*, 2000). This is consistent with the previous observation that CASase activity is highest in plant mitochondria (Manning 1988). Indeed, a mutant of Arabidopsis, in which *Bsas3;1* was disrupted by T-DNA insertion, was found to have only 20 % of the CASase activity of the wild type, indicating that Bsas3;1 catalyzes β-cyanoalanine synthesis (Yamaguchi *et al.*, unpublished result). Group 4 contains Bsas4;1 and Bsas4;2, the former with relatively and the latter low OAS-TL activity. Neither of them show CASase activity (Yamaguchi *et al.*, 2000). An additional gene, *Bsas4;3*, with the highest identity to *Bsas4;1*, has been found in the Arabidopsis genome. *Bsas4;1* and *Bsas4;3* are lo-

cated in tandem on chromosome 5, indicating that duplication might have occured during evolution. Members of group 5 and 6 have been reported as OAS-TLs solely based on amino acid sequence homology (Nakamura *et al.*, 1997, 1999). Our preliminary experiment, however, showed that Bsas5;1 possesses neither OAS-TL nor CASase activity (Yamaguchi *et al.*, unpublished result). It is probable that members of group 5 and 6 catalyze β-elimination or β-replacement, because of their high homology with OAS-TL and conservation of the PLP binding site. It has in fact been proposed that diverse PLP-dependent enzymes have evolved to substrate-specific enzymes from a reaction-specific ancestor (Mehta and Christen 2000). This may be applicable to the plant BSAS family, but whether or not BSAS enzymes other than OAS-TLs impact on sulfate assimilation remains to be determined.

Regulation of sulfate assimilation

Regulatory mechanisms are indispensable for homeostasis, facilitating and repressing sulfate uptake and reduction under sulfate-limiting and sulfur-rich conditions, respectively. The rate of sulfate assimilation is known to be controlled by allosteric regulation of enzymatic activity (Hawkesford 2000). Furthermore, analyses using bacteria and yeast, which have regulatory molecules named CysB and Met4, respectively, indicated that the sulfate assimilation pathway is coordinately controlled at the transcriptional level as well (Schmidt and Jäger 1992; Marzluf 1997).

In higher plants, regulatory mechanisms for sulfate assimilation are more complicated than in unicellular organisms because sulfur requirements vary depending on the organs and on the developmental stage. It is established that regulation of sulfate assimilation is tightly associated with nitrate assimilation (Anderson 1990). Under sulfate and nitrate-sufficient conditions, cysteine inhibits cytosolic SATase allosterically, whereas plastidic and mitochondrial SATase forms are not affected (Noji *et al.*, 1998). Such an inhibition mechanism is analogous to that found in *E. coli* and *Salmonella typhimurium* (Schmidt and Jäger 1992). Reduced sulfur compounds including cysteine and glutathione repress uptake, activation and reduction of sulfate (Hawkesford 2000). Under sulfate-sufficient and nitrate-limiting conditions, the concentration of sulfide increases on decrease of OAS availability for cysteine synthesis. Sulfide also represses uptake, activation and reduction of sulfate (Hawkesford 2000). Under sulfate-deficient conditions, transcript levels of *Sultr1;1* and *Sultr2;1* (sulfate transporters) and of *APR1* (APS reductase) were found to increase up to 20-, 9- and 5.5-fold in roots, respectively, in comparison with control samples (Takahashi *et al.*, 1997, 2000). Transcript levels of *SATp* encoding plastidic SATase became elevated up to 3.5-fold in leaves with sulfate-deficiency

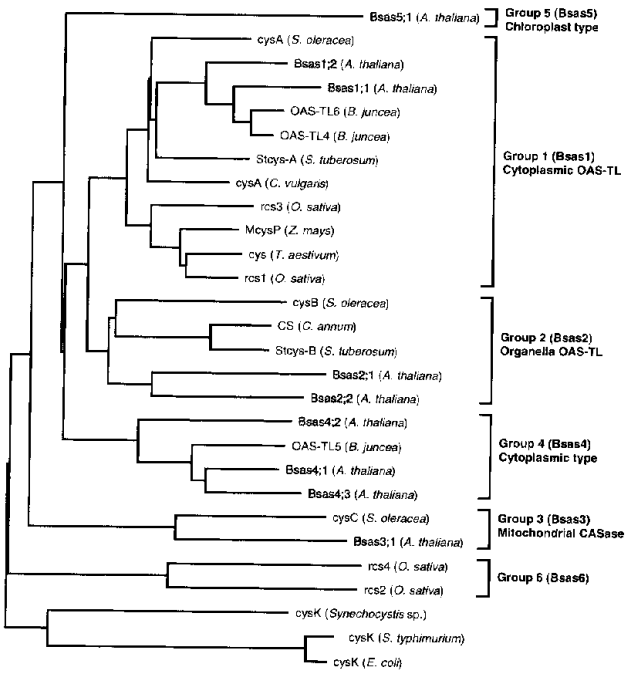

Fig. 3 A phylogenic tree inferred from the amino acid sequences of various β-substituted alanine synthases (BSAS). Amino acid sequences were analyzed using the ClustalX program (Thompson *et al.* 1997) and the tree was obtained using the NJplot program (Perriere and Gouy 1996). Sequences were from: Bsas1;1 (X81697, *A. thaliana*), Bsas1;2 (AJ011976, *A. thaliana*), Bsas2;1 (X 81698, *A. thaliana*), Bsas2;2 (X81973, *A. thaliana*), Bsas3;1 (AB024282, *A. thaliana*), Bsas4;1 (AB024283, *A. thaliana*), Bsas4;2 (AB024284, *A. thaliana*), Bsas4;3 (AT5g28030, *A. thaliana*), Bsas5;1 (AB003041, *A. thaliana*), cysA (D10476, *Spinacia oleracea*), cysB (D14722, *S. oleracea*), cysC (D37963, *S. oleracea*), rcs1 (AF073695, *Oriza sativa*), rcs2 (AF073696, *O. sativa*), rcs3 (AF073697, *O. sativa*), rcs4 (AF073698, *O. sativa*), OAS-TL4 (Y10845, *B. juncea*), OAS-TL5 (Y10846, *B. juncea*), OAS-TL6 (Y10847, *B. juncea*), CS (X64874, *Capsicum annum*), Stcys-A (AF044173, *Solanum tuberosum*), Stcys-B (AF044172, *S. tuberosum*), cysA (D28777, *Citrullus vulgaris*), McysP (X85803, *Z. mays*), cys (D13153, *Triticum aestivum*), cysK (M21451, *E. coli*), cysK (M21450, *S. typhimurium*), cysK (slr1842, *Synechocystis sp.*). Classification of BSAS family is indicated at the right side by brackets. Bacterial OAS-TLs are set as an outgroup. All Arabidopsis sequences are indicated in shadedboxes.

(Takahashi *et al.*, 1997). Under sulfate- and nitrate-deficient conditions, however, transcripts of *APR1* did not increase (Yamaguchi *et al.*, 1999). It has been proposed that OAS is a signal molecule for sensing sulfur/nitrogen balance, because sulfate-limiting and nitrate-sufficient conditions cause its accumulation (Hawkesford 2000). Application of OAS facilitates transcription of sulfate transporter gene in barley as observed in *E. coli* and *S. typhimurium* (Smith *et al.*, 1997). This transcriptional activation was partially repressed by application of reduced sulfur compounds. A high level of OAS disrupts OAS-TL/SATase complexes, in which only SATase shows sufficient activity, resulting in prevention of further OAS synthesis (Droux *et al.*, 1998; Hawkesford 2000). Although metabolites such as OAS and cysteine have been identified as signal molecules for regulation of sulfate assimilation, regulatory components which link signal molecules to transcriptional activation have yet to be isolated form higher plants.

Sulfate assimilation and stress tolerance

The sulfate assimilation pathway is important not only for nutrition but also for the stress response in plants. Transcript levels of some of the genes involved may increase when plants are subjected to salt, wounding or heavy metal stresses (Schäer *et al.*, 1998; Barroso *et al.*, 1999; Harada *et al.*, 2000). Transgenic tobacco overexpressing bacterial SATase, for example, shows increased resistance to oxidative stress (Blaszczyk *et al.*, 1999). Overexpression of rice cytosolic OAS-TL in tobacco is associated with resistance to cadmium (Choi *et al.*, 2001). This is in line with the fact that plants require more cysteines in order to synthesize glutathiones and/or phytochelatins to tolerate environmental stresses. Glutathione is one of the most efficient scavengers of peroxides arising through oxidation processes, also playing an important role in detoxification of xenobiotics and toxic compounds by targeting them into vacuoles (May *et al.*, 1998; Gutierrez-Alcala *et al.*, 2000). Phytochelatin, which consists of γ-glutamyl-cysteine dipeptide repeats followed by a terminal glycine, detoxifies heavy metals by chelation (Cobbett 2000). These findings open the possibility of introducing genetic modifications to improve plant tolerance to environmental stresses and also for phytoremediation purposes.

Conclusions

Molecular techniques have enabled isolation of genes involved in the sulfate assimilation pathway and analysis of their expression patterns. However, there is little information available concerning the molecular mechanisms responsible for sensing and transmitting information on the sulfur status of plants so that sulfur distribution is regulated in response to demands. The elucidation of these mechanisms should lead us to effective and feasible practical applications.

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