Cloning and characterization of a root specific high-affinity sulfate transporter from *Arabidopsis thaliana*[1]

Joseph John Vidmar[2,3], Abderrahmane Tagmount[2,4], Nicole Cathala, Bruno Touraine, Jean-Claude Eugène Davidian*

Biochimie et Physiologie Moléculaire des Plantes, UMR 5004, ENSA-Montpellier/INRA/CNRS/UM2, 34060 Montpellier Cedex 1, France

Received 25 February 2000; received in revised form 11 May 2000

Edited by Marc Van Montagu

Abstract ***Hst1At*** **(accession number AB018695) was identified from the** ***Arabidopsis thaliana*** **sequencing project on BAC T3F12, and the corresponding cDNA was isolated by reverse transcription-PCR. Southern blot analysis reveals a single copy of this gene. The cDNA encodes a root specific sulfate transporter of 649 amino acids. Heterologous expression of** ***hst1At*** **in a sulfate transport deficient yeast mutant shows that this gene encodes a high-affinity transport system ($\sim$2 μM). The transcript relative abundance increases in roots in response to sulfate deprivation, which correlated with increased root SO_4^{2-} influx capacity. These patterns were reversed upon sulfate addition to the medium and were accompanied by an increased glutathione level in roots. Feeding plants with cysteine or glutathione led to similar responses. Using buthionine sulfoximine, an inhibitor of glutathione synthesis, we show that glutathione rather than cysteine controls** ***hst1At*** **expression.**

Key words: Sulfate transport; Root expression; High-affinity transporter; Yeast functional complementation; Glutathione; *Arabidopsis thaliana*

1. Introduction

Plant growth and development rest on the uptake of inorganic ions from soil solution by roots and subsequent assimilation. Plants have devised strategies to ensure demand-driven control of nutrient acquisition [1] despite spatial and temporal variations of ion availability in soil. These adaptive strategies include (i) various membrane carrier systems, which allow nutrient acquisition at a large range of external concentrations; (ii) reallocation of resources for increased root growth when nutrients are limiting, (iii) increased uptake capacity in response to decreased ion availability. This later modification of root transport capacity is thought to be mediated by an increase in the relative abundance of high-affinity transport systems, which are able to remove efficiently the ion from the external solution in the micromolar range. In higher plants, the transport of sulfate has been mainly studied at the physiological level, until the first molecular data presented by Smith et al. [2]. In plants as well as many lower organisms, sulfate transport is mediated by both high- and low-affinity systems [3] [4]. Both act as thermodynamic active processes which are thought to be driven by proton motive force, through a H^+/SO_4^{2-} co-transport [5].

In *Arabidopsis thaliana* sequencing database, seven putative clones have homology to sulfate transporters already isolated from other plant species [2,6]. To date, *ast56* and *ast68* have been characterized [7,8]. The clone *ast68* has been shown to restore growth of the *Saccharomyces cerevisiae* mutant YSD1 when grown on media containing 100 μM sulfate [9]. The two *Arabidopsis* cDNAs have high sequence similarity with *shst3*, a sulfate transporter from *Stylosanthes hamata* with a K_m for sulfate of $\sim$100 μM [2]. Sulfate transporters (*shst1*, *shst2*, *shst3*, *hvst1*, *ast56* and *ast68*) have been shown to be regulated by sulfur status of the plant [2,8,10], which parallels known physiological responses [11,12]. Changes in transcript level and sulfate uptake are thought to be mediated by the release of negative feedback, which would involve sulfate or a product of the sulfate assimilation pathway: L-cysteine, L-methionine, glutathione [6,10,13]. In this paper, we report the cloning and functional characterization of an *A. thaliana* cDNA encoding a root specific high-affinity sulfate transporter (*hst1At*).

2. Materials and methods

2.1. Plant material

Plants of *A. thaliana* ecotype Columbia were hydroponically grown in sterile vessels as described previously [14], except that macronutrients were supplied as 1 mM KH_2P0_4, 500 μM NH_4NO_3, 500 μM $Ca(NO_3)_2$, 250 μM $MgSO_4$ (sucrose and micronutrients as previously). Sulfur-free solution was prepared using $Mg(NO_3)_2$ instead of $MgSO_4$.

2.2. Preparation of RNA and cDNA synthesis

Total RNA was extracted using the modified guanidinium HCl procedure according to [15]. Messenger RNA was isolated using the Quickprep micro mRNA isolation kit (Pharmacia) according to the manufacturer's instructions. 5 μg of mRNA was used for first strand synthesis of cDNA using 0.5 nmol of oligo$(dT)_{15}$ (Boehringer Mannheim) and Superscript II reverse transcriptase (Life Technologies). The first strand cDNA was then used as template for polymerase chain amplification using two gene specific oligonucleotides HAST5 and HAST3 (5′-ATCATGTCCGGGACTATTAATCCCC and 5′-TTAAGTTTGTTGCTCAGCCACTTC, respectively).

2.3. DNA cloning

The PCR products were cloned into pCR2.1 (Invitrogen) and used for transformation of the bacterial strain DH5α resulting in plasmid pCRhst1. For yeast expression studies, cDNA insert from pCRhst1

*Corresponding author. Fax: (33)-467525737.
E-mail: davidian@ensam.inra.fr

[1] During the course of this work, a cDNA (accession number AB018695, Takahashi et al.) identical to *hst1At*, named Ast101, was cloned independently.

[2] These authors contributed equally to this work.

[3] Present address: Department of Botany, University of British Columbia, Vancouver, BC, Canada.

[4] Present address: Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.

0014-5793/00/$20.00
PII: S0014-5793(00)01615-X

was released by digestion with *Hin*dIII and *Not*I, then directionally cloned in the *Hin*dIII–*Not*I sites of the shuttle vector pYES2 (Invitrogen). All the plant cDNAs used in this study were cloned in the same pYES2 vector. Yeast *SUL1* gene encoding a high-affinity sulfate transporter was inserted in the *Hin*dIII site of the vector pEMBLYe23 [16].

2.4. Northern blot analysis and reverse transcription (RT)-PCR

RNA was analyzed as previously described [10]. A 2.2 kb *Eco*RI fragment from PCR product clone pCRhst1 was radioactively labeled with [α-^{32}P]dCTP using Prime-A-Gene kit (Promega) and used for hybridization experiments. For expression control levels of total RNA, the *Xho*I fragment of plasmid pEF1 which contains the *EF1α* cDNA was used as a probe. The oligonucleotides used for analyzing by RT-PCR the transcript abundance of the genes encoding glutathione synthetase (*GSH2*) and the sulfate transporter (*HST1At*) are the following: Ini-*gsh2* 5′-TTCCAGACCCTAAAACTCTGAGAAATC, Rev-*gsh2* 5′-CTCTTGTACACTCCCTTCTTTTTCGAC and Dir-*hst1* 5′-TTGCTTCAAGTGACGAGACCAAGAAC, Stop-*hst1* 5′-TGCTCAGCCACTTCCGTAGAACAAAC.

2.5. Physiological measurements

^{35}S-Sulfate influx was measured by short-term labeling experiments [17]. Thiols were extracted by grinding in 0.1 N HCl, 1 mM Na_2-EDTA, 0.1 g insoluble polyvinylpyrrolidone. The suspension was centrifuged at 18 000×*g* for 10 min at 4°C, and the supernatant was removed for thiols analysis. Thiols were determined using reverse-phase high performance liquid chromatography after reduction and derivatization with monobromobimane (Calbiochem, La Jolla, CA, USA) [18]. Aliquots from standard solutions (1 μM Cys, 1 μM γGluCys and 10 μM GSH) were submitted to the protocol. Derivatized compounds were separated on a Hypersil ODS 5 μm 250×4.6 mm column eluted with a gradient of 0.25% (v/v) acetic acid in water (pH 3.9) and methanol, and detected fluorimetrically (Jasco 821-FP).

2.6. Yeast transformation and growth

The *S. cerevisiae* yeast mutant strain, YSD1 (*MATα*, *his3, leu2, ura3, sul1*), defective in its sulfate transport capacity [9] has been kindly provided by Dr. M.J. Hawkesford, (IACR-Rothamsted, UK). Plasmids were introduced into the yeast using a lithium chloride/polyethyleneglycol procedure as described by Ito et al. [19]. The ability of each clone encoding a putative sulfate transporter to complement the YSD1 mutation was evaluated by analyzing the capacity of the yeast mutant to grow at 28°C, on synthetic solid media which contained added sulfate as a unique sulfur source [20]. The required auxotrophy factors, and the appropriate sugar source were added when needed as described below. Short-term ^{35}S-sulfate influx was measured in yeast, as extensively described previously [16].

3. Results

3.1. Cloning and identification of the hst1At cDNA encoding a root specific high-affinity sulfate transporter

Search for sulfate transporters in the *Arabidopsis* database (http://genome-www2.stanford.edu/cgi-bin/AtDB) resulted in the appearance of an uncharacterized sulfate transporter (T3F12.7) on BAC T3F12 representing a portion of the long arm of chromosome 4. Gene specific primers HAST5 and HAST3 were fabricated directed at the 5′ and 3′ coding regions, start and stop codons, respectively. Messenger RNA isolated from roots of 24 h S-starved *Arabidopsis* plants was used as template for RT-PCR. The analysis of PCR amplicon by gel electrophoresis demonstrated the presence of a single specific band at approximately 2.0 kb. The PCR product was isolated from the gel and subcloned into pCR2.1 (Invitrogen). We designated this cDNA clone *hst1At* for high-affinity sulfate transporter 1 *A. thaliana*. Southern blot analysis under high stringency reveals one copy of this member of sulfate transporters in the *Arabidopsis* genome (data not shown). The deduced amino acid sequence of the protein encoded by *hst1At* contains 649 amino acids. The predicted polypeptide has a relative molecular weight of 70.6 kDa and a p*I* of 9.65. It has the characteristics of membrane protein with 12 membrane spanning domains (TMpred at BCM launcher, Baylor College of Medicine, TX, USA). The phylogenetic analysis (Darwin, ETHZ) obtained by comparing amino acid sequences of the known plant sulfate transporter demonstrated that HST1At is closely related to the transporters SHST1, SHST2 and HVST1 [2,6], with a similarity of 85–87%. RT-PCR of mRNA isolated from roots, and of a mixture of aerial portions of plants (leaves, stems, flowers, silica), demonstrated a specific product for the root tissue (Fig. 1A), suggesting that *hst1At* is expressed specifically in roots whatever the nutritional sulfur status of the plant. Under the same sulfate star-

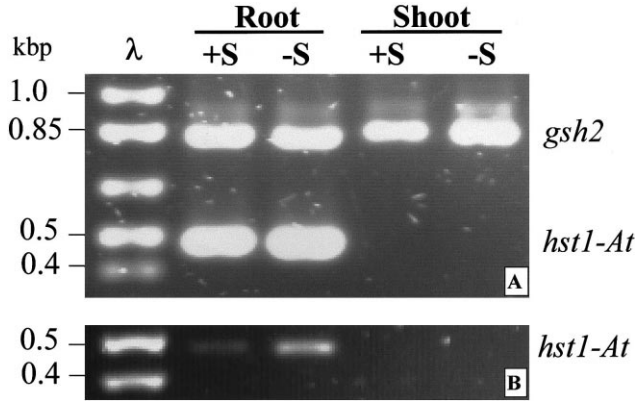


Fig. 1. RT-PCR of *hst1At*. RNA was isolated from 5 week old *Arabidopsis* plants grown in hydroponic conditions in presence of 200 μM sulfate (+S) or starved for 48 h (−S). RNA was extracted from roots, and a mixture of aerial portions of the plants (shoot) comprising leaves, stems, inflorescences and silica. *gsh2* encodes an *A. thaliana* glutathione synthetase. Lane λ represents the DNA size markers (kb). A and B are the same samples amplified with two PCR conditions, respectively 35 and 15 cycles.

Table 1
Responses of SO_4^{2-} influx, *hst1At* and *ast68* transcripts to Cys and glutathione, in relation to the changes in glutathione content in roots

	Treatment				
	BSO	SO_4^{2-}	Cys	Cys+BSO	GSH
$[GSH]_{root}$ (μmol g^{-1} FW)	0.14 ± 0.03	0.40 ± 0.04	0.80 ± 0.11	0.14 ± 0.03	1.00 ± 0.28
SO_4^{2-} influx (μmol h^{-1} g^{-1} FW)	0.68 ± 0.07	0.16 ± 0.03	0.07 ± 0.02	0.69 ± 0.06	0.06 ± 0.02
hst1At mRNA[a] (relative level)	100	31	11	111	10
ast68 mRNA[a] (relative level)	100	26	15	106	21

Hydroponically grown *Arabidopsis* plants were S-deprived for 48 h then treated for 24 h by: 1 mM BSO; 0.25 mM $MgSO_4$; 1 mM Cys; 1 mM Cys plus 1 mM BSO; 1 mM glutathione (GSH). Results for SO_4^{2-} influx and glutathione content in roots are means of eight replicates ± S.E.M.

[a]Transcript relative abundances are expressed as percentage of the BSO level taken as 100.

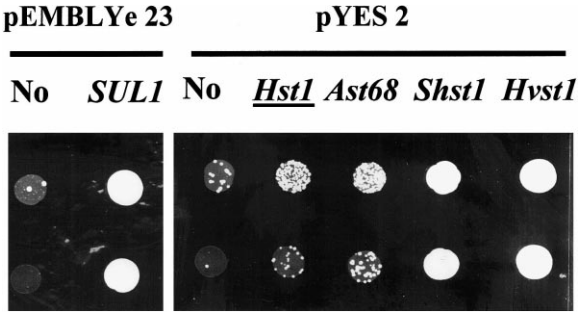


Fig. 2. Drop test for yeast mutant YSD1 complementation. Yeasts were grown at 28°C in synthetic liquid medium supplemented with 0.1 mM homocysteine thiolactone as a sole sulfur source and the appropriate auxotrophy factors. After having reached an absorbance of ca. 1.0 unit, the cells were collected, washed with water several times, and dropped onto agarose synthetic medium containing 20 μM sulfate and the appropriate auxotrophy factors [16]. Media onto which yeast strains were transformed with the plasmid pEMBLYe23 or pYES2 contained respectively glucose or galactose (10 g l^{-1}). The lower line drops contained the same transformed strains as the upper one, but diluted 10 times before their deposit. The photograph was taken after 48 h of growth. *Hst1* (*hst1At Arabidopsis*, this work), *ast68* (*Arabidopsis* [8]), *Hvst1* (Barley [6]), *shst1* (*Stylosanthes* [2]), *SUL1* (yeast [9,16]).

vation conditions, the increase in *hst1At* transcript level in roots is evident when appropriate RT-PCR protocols (15 cycles) are used (Fig. 1B). The control clone *gsh2* which encodes an *A. thaliana* glutathione synthetase is expressed in both root and aerial parts, comprising leaves.

3.2. Heterologous expression of hst1At in a yeast strain defective in sulfate transport

The growth of the yeast mutant YSD1 rescued by the plasmid pYES2-*hst1At* was restored in synthetic liquid media (not shown) as well as solid media containing ammonium sulfate (20 μM) as the sole sulfur source (Fig. 2), in presence of 10 g l^{-1} galactose. No growth was observed for the mutant transformed with one of the vectors alone (pYES2 or pEMBLYe23). We confirmed that several genes encoding sulfate transporters are able to complement the YSD1 mutant: (i) a *S. cerevisiae* gene which had been shown to encode one of the two high-affinity yeast sulfate transporters *sul1* [9,16], (ii) two plant cDNA encoding high-affinity sulfate transporters, *hvst1* and *shst1* cloned respectively from *Hordeum vulgare* [6] and from *S. hamata* [2], (iii) an *A. thaliana* cDNA (*ast68*) thought to encode a sulfate transporter with a lower affinity for sulfate [8]. The two *Arabidopsis* cDNAs seem to be less efficient to restore growth of the YSD1 mutant compared to *shst1* and *hvst1* clones (Fig. 2). The sulfate uptake rate by the yeast strain mutant YSD1 complemented by the two *Arabidopsis* cDNAs (*hst1At* and *Ast68)* was measured for 2 min in media containing ^{35}S-labeled sulfate at concentrations ranging from 1.0 to 400 μM (Fig. 3). Sulfate uptake showed typical Michaelis–Menten saturation for *hstAt* and *ast68* with calculated mean V_{max} values of 76 and 33 μmol min^{-1} (10^9 cells)$^{-1}$, respectively. The calculated apparent K_m values for sulfate

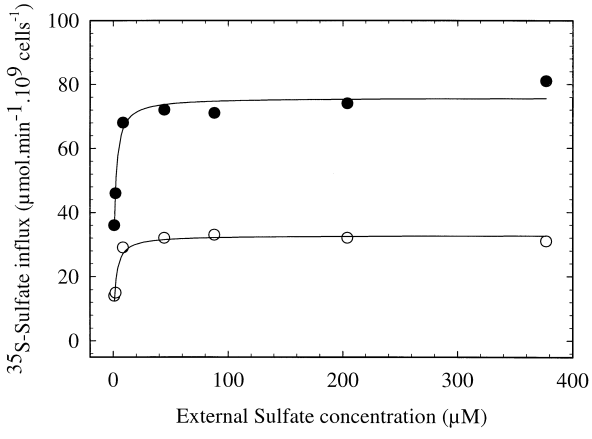
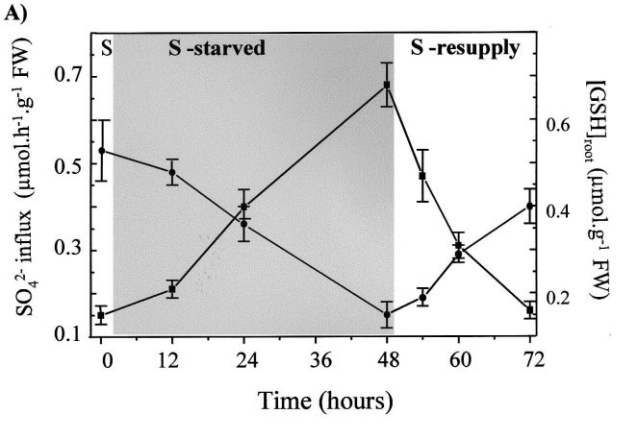


Fig. 3. Kinetic analysis of sulfate uptake by complemented yeast mutant YSD1 as function of external sulfate concentration. For each sulfate concentration, sulfate influx was measured at 28°C for 3 min (within the initial linear rate of uptake). Measurements were started by adding sulfate at the appropriate concentrations and the radioelement ($Na_2^{35}SO_4$) at a constant specific radioactivity, in presence of galactose, and at pH 5.0 [16]. K_m and V_{max} values were determined both by non-linear regression. Symbols (●) and (○) for *hst1At* and *ast68*, respectively. Each result is the mean of three replicates.



C)

	+S	(-S) starvation				S -resupply			
Time (h)	0	6	12	24	48	3	6	12	24
ast68	3	4	6	16	100	39	12	8	1
hst1At	18	34	41	94	100	50	24	22	13

Fig. 4. Responses of SO_4^{2-} influx and *hst1At* transcript to S starvation and SO_4^{2-} re-supply, in relation to the changes in glutathione content in roots. *Arabidopsis* plants were grown on complete liquid nutrient solution for 3 weeks, with S supplied as 250 μM SO_4^{2-}, and then transferred on S-free solution for periods of time as indicated. The S-re-supplied plants were transferred on S-free solution for 48 h prior to the experiment, then transferred on SO_4^{2-} solution for periods of time, as indicated. (A) (■) SO_4^{2-} influx and (●) glutathione content in roots (eight replicates ± S.E.M.). (B) Northern blot analysis of *hst1At*, *ast68* and *EF1α*. (C) Quantification of *hst1At* and *ast68* transcript levels, normalized using *EF1α*, and expressed as percentage of the transcript relative abundance in 48 h sulfur-starved roots. Quantification was done using Storm phosphoimager (Molecular Dynamics).

for *hst1At* and *ast68* are 1.5 and 5 μM, respectively, which suggests that both clones encode high-affinity sulfate transporters.

3.3. Effect of S status on hst1At mRNA abundance, sulfate influx and thiol levels

Arabidopsis plants, previously grown under conditions of sufficient S supply (250 μM SO_4^{2-}), were transferred to a S-free solution for time periods varying from 3 to 48 h. The duration of culture prior to S starvation varied so that tissues were harvested from plants of the same age (3 weeks) and at the same time of the day. Northern blot analysis showed that the relative abundance of *hst1At* transcripts peaked between 24 and 48 h upon SO_4^{2-} withdrawal from nutrient solution (Fig. 4B,C). In comparison, the *ast68* transcript level peaked at 48 h. In parallel, $^{35}SO_4^{2-}$ influx increased by 4.5-fold and glutathione level in roots declined by 70% (Fig. 4A). Re-supplying SO_4^{2-} to plants previously starved of S for 48 h led to a decline in the abundance of *hst1At* and *ast68* mRNA, a decrease in SO_4^{2-} influx, and an increase in glutathione level in roots (Fig. 4). Using Northern blot analysis, *hst1At* transcript was not detected in aerial parts under either S-sufficient or S-starved conditions (data not shown) which agreed with RT-PCR data presented in Fig. 1.

In order to investigate the relations between glutathione and the down-regulation of *hst1At*, plants starved of S for 48 h were exposed to treatments in which both cysteine and glutathione, alternatively glutathione only, accumulated. For this purpose, cysteine was supplied to plants with or without buthionine sulfoximine (BSO), an inhibitor of γ-glutamyl-cysteine synthetase [21] which prevents the synthesis of glutathione. Cysteine plus glutathione treatments had a similar effect as SO_4^{2-} re-supply, but induced a more substantial decline in *hst1At* and *ast68* transcripts accumulation and SO_4^{2-} influx, associated to a stronger effect on internal glutathione concentration (Table 1). In presence of BSO, the down-regulation of *hst1At* and *ast68* mRNA's abundance and of SO_4^{2-} influx by cysteine were released.

4. Discussion

Plant sulfate transporter cDNAs were first isolated from *S. hamata* [2] by functional complementation of a yeast mutant disrupted in the yeast high-affinity sulfate transport system encoded by the *sul1* gene. At present, a number of genes have been identified as sulfate transporters in *A. thaliana* by EST and genomic sequencing projects. Functional evidence has been obtained through heterologous expression in yeast mutant YSD1 [9], of cDNAs encoding SO_4^{2-} transporters, SHST1, SHST2, SHST3 [2], HVST1 [6] and AST68 [7], which have been shown to restore growth on limiting SO_4^{2-} conditions.

The analysis of phylogenetic relationship between all known and putative plant SO_4^{2-} transporters showed that HST1At is more closely related to SHST1, SHST2 and HVST1 [22]. HST1At shares with these members a common root localization feature (Fig. 1) [2,6], and a very low apparent K_m value for sulfate (< 10 μM). AST68, which has been shown to be expressed in both roots and shoots [8], belongs to an other sulfate transporter group which includes SHST3 with a higher apparent K_m value for sulfate (< 100 μM) [2]. For *ast68*, the calculated apparent K_m value for sulfate (1.5 μM) (Fig. 3) can explain the capacity of the complemented YSD1 mutant to grow at a low sulfate concentration (20 μM, Fig. 2). The calculated apparent K_m values for sulfate of the two clones *hst1At* and *ast68* are very close and therefore belong to the so called high-affinity range. The three times higher V_{max} value calculated for HST1At compared to AST68 (Fig. 3), which reveals a higher sulfate transport efficiency of the former in yeast, could be due to a better targetting of the expressed HST1At protein to the yeast plasma membrane leading to a higher density of functional transporters on this membrane. Such a unidirectional ^{35}S-sulfate influx difference did not permit to observe between the two clones any advantage for growth neither on synthetic solid media (Fig. 2), nor in synthetic liquid media (data not shown). This would suggest that sulfate uptake rate would not be a limiting factor for the growth of complemented yeast mutant. Previous results have shown that ATP sulfurylase overexpression in tobacco plants failed to improve growth [23]. Both observations suggest that regulation steps might operate beyond these two first steps of the sulfur metabolic pathway.

Hst1At is up-regulated in plants grown without sulfur source for periods of several hours to a few days, and conversely rapidly down-regulated when SO_4^{2-} was re-supplied to sulfur-starved plants (Fig. 4B). This resembles observations reported for other genes encoding SO_4^{2-} transporter or ATP sulfurylase [2,8,10,23]. In plants grown in similar conditions, the time-scale responses to changes in sulfur availability occurred earlier for *hst1At* than *ast68* (Fig. 4C), suggesting that these two genes are differently regulated. The changes in the abundance of *hst1At* mRNA paralleled the changes of SO_4^{2-} influx measured in a 250 μM SO_4^{2-} solution (Fig. 4A), suggesting that this gene encodes a transport system that has a major role in SO_4^{2-} uptake at relatively a low concentration range. These *hst1At* up- and down-regulation patterns were respectively accompanied by the decrease and increase of glutathione content in root tissues. Using BSO, we have shown that the changes in *hst1At* mRNA level specifically correlate the accumulation of glutathione, but not cysteine, from which it is synthesized (Table 1). The same type of regulation is observed for *ast68* (Table 1 and [10]). This supports the hypothesis that glutathione would have a role in the sulfur signaling processes involved in the demand-driven control of SO_4^{2-} acquisition by plant roots.

Acknowledgements: We like to thank Drs. K. Saito (Chiba University, Japan), M.J. Hawkesford (BBSRC Rothamsted, UK) and P. Ullmann and L. Gondet (IBMP, Strasbourg University, France) for providing yeast expression plasmids *ast68*, *shst1* and *gsh2*, respectively. Postdoctoral positions of J.J.V. and A.T. were respectively supported by French Ministère des Affaires Etrangères and Institut National de la Recherche Agronomique.

References

[1] Imsande, J. and Touraine, B. (1994) Plant Physiol. 105, 3–7.
[2] Smith, F.W., Ealing, P.M., Hawkesford, M.J. and Clarkson, D.T. (1995) Proc. Natl. Acad. Sci. USA 92, 9373–9377.
[3] Datko, A.H. and Mudd, S.H. (1984) Plant Physiol. 75, 466–473.
[4] Yildiz, F.H., Davies, J.P. and Grossmann, A.R. (1994) Plant Physiol. 104, 981–987.
[5] Lass, B. and Ullrich-Eberius, C.I. (1984) Planta 161, 53–60.
[6] Smith, F.W., Hawkesford, M.J., Ealing, P.M., Clarkson, D.T., Van den Berg, P.J., Belcher, A.R. and Warrilow, A.G.S. (1997) Plant J. 12, 875–884.

[7] Takahashi, H., Sasakura, N., Noji, M. and Saito, K. (1996) FEBS Lett. 392, 95–99.
[8] Takahashi, H., Yamazaki, M., Sasakura, N., Watanabe, A., Leustek, T., De Almeida Engler, J., Engler, G., Van Montagu, M. and Saito, K. (1997) Proc. Natl. Acad. Sci. USA 94, 11102–11107.
[9] Smith, F.W., Hawkesford, M.J., Prosser, I.M. and Clarkson, D.T. (1995) Mol. Gen. Genet. 247, 709–715.
[10] Lappartient, A.G., Vidmar, J.J., Leustek, T., Glass, A.D.M. and Touraine, B. (1999) Plant J. 18, 89–95.
[11] Clarkson, D.T., Smith, F.W. and Van den Berg, P.J. (1983) J. Exp. Bot. 34, 1463–1483.
[12] Clarkson, D.T., Hawkesford, M.J., Davidian, J.C. and Grignon, C. (1992) Planta 187, 306–314.
[13] Herschbach, C. and Rennenberg, H. (1994) J. Exp. Bot. 45, 1069–1076.
[14] Touraine, B. and Glass, A.D.M. (1997) Plant Physiol. 114, 137–144.
[15] Lobréaux, S., Massenet, M. and Briat, J.-F. (1992) Plant Mol. Biol. 19, 563–575.
[16] Cherest, H., Davidian, J.-C., Thomas, D., Benes, V., Ansorge, W. and Surdin-Kerjan, Y. (1997) Genetics 145, 627–635.
[17] Lappartient, A.G. and Touraine, B. (1996) Plant Physiol. 111, 147–157.
[18] Schupp, R., Schatten, T., Willenbrink, J. and Rennenberg, H. (1992) J. Exp. Bot. 43, 1243–1250.
[19] Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol. 153, 163–168.
[20] Cherest, H. and Surdin-Kerjan, Y. (1992) Genetics 130, 51–58.
[21] Griffith, O.W. (1982) J. Biol. Chem. 257, 13704–13712.
[22] Vidmar, J.J., Schjoerring, J.K., Touraine, B. and Glass, A.D.M. (1999) Plant Mol. Biol. 40, 883–892.
[23] Hatzfeld, Y., Cathala, N., Grignon, C. and Davidian, J.-C. (1998) Plant Physiol. 116, 1307–1313.