Appendix S1. Expression analysis by qRT-PCR of wheat genes involved in sulfur metabolism.

Total RNA was extracted using the TRIzol reagent (Invitrogen) according to manufacturer's instructions. The resulting RNA was treated with RNase-free DNase I (Promega) according to the manufacturer's protocol. Following digestion, nucleotides were removed from RNA using a G50 Sepharose buffer exchange column (Amersham). Absence of genomic DNA contamination in DNase I-treated samples was checked by PCR using three different primer pairs designed to amplify intron sequences of PDI-like genes (TaPDIL1-1, TaPDIL5-1 and TaPDIL7-1) located, in the chromosome homeologous groups 4, 5 and 2, respectively (d'Aloisio et al., 2010). If a single DNase treatment did not remove completely interfering genomic DNA, a second DNase incubation was performed to eliminate any detectable DNA. Synthesis of cDNA from the DNase I treated RNA was only performed when the genomic control amplifications scored negative. RNA concentration and integrity were checked with a UV/VIS spectrophotometer Lambda 3B (Perkin Elmer) before and after DNase I digestion. The quality of RNA samples was also assessed by electrophoresis on 1% formaldehyde agarose gels. First-strand cDNA was synthesized from 3 µg of RNA by the Expand TM Reverse Transcriptase (ROCHE) and the resulting cDNA was diluted 1/5 for qRT-PCR analyses.

A set of candidate genes previously evaluated for their expression stability in different wheat tissues (Paolacci *et al*., 2009) were initially selected to identify the most suitable reference genes in our experimental conditions. The six candidate reference genes encoded the following proteins: ADP-ribosylation factor (Unigene Cluster Ta2291; DFCI contig TC278558), Ubiquinol-cytochrome C reductase (Unigene Cluster Ta4045; DFCI contig TC350975), Cell division control protein belonging to the AAA-superfamily of ATPases (Unigene Cluster Ta54227; DFCI contig TC308517), S-adenosylmethionine decarboxylase (Unigene cluster Ta53919; DFCI contig TC320440), RNase L inhibitor-like protein (Unigene cluster Ta2776; DFCI contig TC278756), GABA-receptor-associated protein (Unigene cluster Ta54963; DFCI contig TC304353).

Specific primer pairs were designed for cloned cDNAs and for the selected reference genes using the Beacon Designer 6 software (STRATAGENE) and imposing the following stringent criteria: TM of 55°C ± 2°C, PCR amplicon length between 60 and 280 bp, primer length of 20 ± 2 nt, and 40 to 60% guanine-cytosine content. Primers (Table S3) were also designed within the 3' end region of each sequence to encompass all potential splice variants and to ensure equal RT efficiencies.

Quantitative RT-PCR analyses were performed using an Mx3000PTM real time PCR system with Brillant SYBR green QPCR master mix (STRATAGENE), according to manufacturer's protocols, in 25 µl reaction volumes containing 1 µl of each fivefold diluted cDNA and 150 nM forward and reverse primers. No template and RT-minus controls were run to detect contamination, dimer formation and presence of genomic DNA. Standard curves based on five-points, corresponding to a fivefold dilution series (1:1-1:625) from pooled cDNA, were used to compute the PCR efficiency of each primer pair. PCR efficiency (E) is given by the equation $E= (10^{[-1/m]} -1) \times 100$ (Radonic *et al*., 2004), where m is the slope of linear regression model fitted over log-transformed data of the input cDNA concentration versus Ct values according to the linear equation $y=m*\log(x) + b$. The thermal profile comprised three segments: 1) 95°C for 10 min; 2) 40 cycles of 30 s denaturation at 95°C, 1 min annealing at 55°C and 30 s extension at 72°C (amplification data collected at the end of each extension step); 3) dissociation curve consisting of 1 min incubation at 95°C, 30 s incubation at 55°C, a ramp up to 95°C. Three biological replicates, resulting from three different RNA extractions, RT and qRT-PCR reactions, were used in quantification analysis; three technical replicates were analysed for each biological replicate.

Only primer pairs generating a sharp peak by melting curve analysis (without unspecific products or primer-dimer artefacts) and showing efficiencies between 90 and 110% and R^2 values (coefficient of determination) calculated for standard curves higher than 0.997 were selected for expression analysis of the genes of interest and of the candidate reference genes. The specificity of the

amplicons was also checked by electrophoresis in 2% agarose gel and by sequencing of the PCR products to confirm that their sequences corresponded to the target genes.
The number of cycles (Ct) at which the amplification-corrected normalized fluorescence (dRn) for each reaction crossed the threshold value was exported to Excel (Microsoft) for further analyses. Raw C_t values were transformed to relative quantities using the delta-C_t formula $Q=E^{\Delta Ct}$, where E is the efficiency of the primer pair used in the amplification of a particular gene and ΔC_t is the difference between the sample with the lowest C_t (highest expression) from the data set and the C_t value of the sample in question.
The expression stability of the six candidate reference genes was evaluated by the software program NormFinder (a Microsoft Excel Add-in available on the Internet) according to the author's recommendations (Andersen *et al*., 2004). The best combination of two genes recommended by NormFinder was that of RNase L inhibitor-like protein and Ubiquinol-cytochrome C reductase, with a stability value significantly lower than that of the most stable gene (RNase L inhibitor-like protein) taken alone, indicating a more reliable normalization than that based on the single most stable gene. Therefore, the expression data of the genes of interest were normalized using the geometric average of the two reference genes RNase L inhibitor-like protein and Ubiquinol-cytochrome C reductase and their normalized relative values given as mean value +/- SD (Standard deviation). Standard deviations on normalized expression levels were computed according to the geNorm user manual (geNorm manual, update July 8, 2008). Relative expression levels of the genes of interest were referred to that of a calibrator set to the value one, which was represented by the treatment with the lowest expression. Statistical significance of the differences was evaluated by oneway ANOVA followed by the Tukey test.

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Appendix S2. Cloning and characterization of wheat genes involved in sulfur metabolism

Most components of sulfate uptake and assimilation in plants are encoded by multiple genes organised in small gene families (Kopriva et al., 2009); this is partly due to the localization of several metabolic steps in multiple cellular compartments. However, the composition of the gene families is more complex than simply organellar versus cytosolic forms and it seems to depend from the species considered (Kopriva et al., 2009; Davidian and Kopriva, 2010). Up to now, a systematic study on the diversity and roles of several members of these gene families has been carried out only in Arabidopsis (reviewed in Lewandoska and Sirko, 2008; Kopriva et al., 2009; Hell and Wirtz, 2011; Takahashi et al., 2011). Thus the identification of cDNA sequences of wheat genes involved in sulfate uptake and assimilation was based on the BLAST search of DFCI wheat gene index (TaGI, version 12) and NCBI databases using the available Arabidopsis sequences of genes coding for high affinity transporters and enzymes involved in sulfate assimilation and reduction (Table S1). Ten sequences retrieved from these two databases referred to distinct non-homeologous genes and were considered suitable for cloning full-length cDNAs in durum wheat. The identified non-redundant sequences were used as templates for isolating by RACE the corresponding 5' and 3' extensions, subsequently validated by sequence analysis. Full-length cDNAs of ten genes were cloned by RT-PCR of RNA from roots and shoots of durum wheat cv Svevo using specific primers designed in the 5' and 3' untranslated regions (Table S2). The ten isolated full-length cDNAs correspond to transcripts of one of the two homoeologous genes of tetraploid wheat (AABB genome).

Three transcribed genes encoding high affinity sulfate transporters were identified in Arabidopsis genome, whereas in wheat EST databases we identified only two transcribed sequences, whose corresponding cDNAs were cloned from durum wheat and named *TdSultr1.1* and *TdSultr1.3* (Table 1), according to Buchner et al. (2010). The ORF of *TdSultr1.1* included 1989 bp and encoded a protein of 662 aa, whereas the ORF of *TdSultr1.3* comprised 1971 bp encoding 656 aa; their deduced amino acid sequences had an identity of 74% and shared an identity ranging between 68 and 75% with the polypeptides encoded by the three corresponding genes of Arabidopsis (Table 1 in the paper).

Arabidopsis contains four ATPS isoforms compared to two genes in most other analysed species, including spinach, potato, rice, sorghum, *Brachypodium* (Kopriva et al., 2009 and our unpublished results). Although ATPS activity is present in the plastids as well as in the cytosol of several plant species, only spinach and potato contain two ATPS isoforms specific to cytosol and plastids, whereas all the other identified ATPS genes encode proteins with putative plastid targeting peptides (Kopriva et al., 2009). The two cloned ATPS cDNA sequences from durum wheat, *TdATPSul1* and *TdATPSul2*, contained ORFs of 1449 and 1410 bp, corresponding to polypeptides of 482 and 469 aa, respectively (Table 1). Analysis of the deduced amino acid sequences using Target P1.1 and ChloroP 1.1 indicated that the TdATPSul1 and TdATPSul2 proteins have cloroplast/plastid transit peptides of 44 and 37 aa, respectively, at their N-terminal region. Comparison of the deduced protein sequences revealed that TdATPSul2 is more similar to APS1, APS3 and APS4 from Arabidopsis (identity of about 72%), whereas TdATPSul1 showed the highest identity (72.8%) with APS2 (Table 1).

In Arabidopsis APR is encoded by a small gene family with three different isoforms, whereas in grass species like rice, maize, sorghum and *Brachypodium* it is encoded by a single gene (Patron et al., 2008; Kopriva et al., 2009 and our unpublished results). Accordingly, in wheat EST databases we identified only a single non-homoeologous transcribed sequence, whose corresponding full-length cDNA was cloned from durum wheat (Table 1). *TdAPR* has an ORF of 1386 bp coding for a protein of 461 aa and, based on sequence analysis using Target P1.1 and ChloroP 1.1, it includes a choloroplast/plastid transit peptide sequence at its N-terminus. This is consistent with the known localization of the enzyme in Arabidopsis (Rotte and Leustek, 2000) and the localization of the sulfate assimilation pathway to plastids (reviewed in Takahashi et al., 2011). TdAPR possesses two

domains characteristic of most APRs from higher plants: a C-terminal PAPS reductase-like domain and an N-terminal thioredoxin-like domain. TdAPR shares from 71.6 to 73.5% amino acid sequence identities with the three Arabidopsis APR isoforms (Table 1).

Sulfite reductase (SiR) is the only component of sulfate assimilation metabolism which is encoded by a single gene in Arabidopsis, whereas the rice genome contains two and the poplar genome three copies (Kopriva et al. 2009). BLAST search using the single sequence from Arabidopsis allowed us to identify a single non-homoeologous transcribed sequence in the wheat ESTs databases, which was used as template to clone the corresponding full-length cDNA sequence from durum wheat (Table 1). In agreement with these results we identified a single SiR gene in the genome of different grass species, such as maize, sorghum and *Brachypodium* (data not shown). *TdSiR* encoded a protein of 635 aa, which was predicted to contain a choloroplast/plastid transit peptide sequence at the N-terminus of 44 aa and showed 76% amino acid sequence identity with SIR from Arabidopsis (Table 1).

In Arabidopsis, there are nine *OASTL*-like genes (Table S1), including *OASA2* (*Bsas1;2*; At3g22460), a pseudogene which is transcribed but encodes a truncated, non-functional protein (Jost et al., 2000). The three most highly expressed genes encode the major isoenzymes in the cytosol, plastids and mitochondria: OASA1 (At4g14880, Bsas1;1), OASB (At3g22460, Bsas2;1), and OASC (At2g43750, Bsas2;2), respectively. These three proteins and the low expressed cytosolic OASD1 (At3g04940, Bsas4;2) and OASD2 (At5g28020, Bsas4;1) proteins are enzymatically true OASTLs, although the involvement of the last two proteins in the cysteine synthesis *in vivo* have not currently been determined (Hell and Wirtz, 2011 and Takahashi et al., 2011). Thus, in order to identify the wheat genes coding for the enzymatically true OASTL in the BLAST searches we used only the sequences of the Arabidopsis genes encoding the three major OASTL isoforms: OASA1, OASB and OASC. These searches allowed the identification of only two putative expressed sequences in wheat ESTs databases, whose corresponding full-length cDNAs were cloned from durum wheat (*TdOASTL1* and *TdOASTL2* in Table 1). *TdOASTL1* possessed an ORF of 1179 bp coding for a protein of 392 aa, which showed the highest identity (76.8%) with the plastidic isoform OASB from Arabidopsis (Table 1). Moreover, the analysis of the deduced amino acid sequences using Target P1.1 and ChloroP 1.1 indicated that the protein TdOASTL1 possesses a cloroplast/plastid transit peptide of 66 aa at their N-terminal region. On the other hand, the protein encoded by *TdAOSTL2* (326 aa) showed higher amino acid sequence identity with the cytosolic isoform OASA1 (81.6%) than with the plastidic (AOSB) or mitochondrial (AOSC) isoforms from Arabidopsis (Table 1). In agreement with the homology results, the analysis of the deduced amino acid sequences using Target P1.1 and ChloroP 1.1 indicated that the most probable localization of the TdAOSTL2 protein was the cytosol, because it did not carry chloroplast transit peptide or mitochondrial targeting peptide sequences. It is noteworthy that a database search using the three Arabidopsis major AOASTL isoforms indicated that all the sequenced genomes of grasses (rice, maize, sorghum and *Brachypodium*) contain only two homologous genes, whose encoded proteins were predicted to be localized in plastids and cytoplasm (data not shown).

In Arabidopsis five genes (*SAT1-5*) encode SAT enzymes for OAS synthesis (Table S1). BLAST searches in wheat EST databases using the sequences of the five Arabidopsis genes allowed us to identify only two distinct transcribed sequence, whose corresponding full-length cDNAs were cloned from durum wheat (Table 1). The ORFs of the two cDNA sequences *TdSAT1* and *TdSAT2* consisted of 954 and 1014 bp, corresponding to polypeptides of 317 and 337 aa, respectively. Sequence comparison indicated that TdSAT1 and TdSAT2 are likely orthologous to the Arabidopsis cytosolic isoforms SAT5 and SAT2/SAT4, respectively (Table 1). Moreover, the analysis of the deduced amino acid sequences using Target P1.1 and ChloroP 1.1 indicated that the most probable localization of the TdSAT1/2 proteins was the cytosol, because they were predicted not to possess any chloroplast transit peptide or mitochondrial targeting peptide sequences. Approaches using RT-PCR with degenerate primers homologous to plastidial and mitochondrial

SAT type genes based on sequences derived from other monocotyledonous and dicotyledonous plant species were not able to amplify the corresponding transcripts from roots and shoots of durum wheat cv Svevo.

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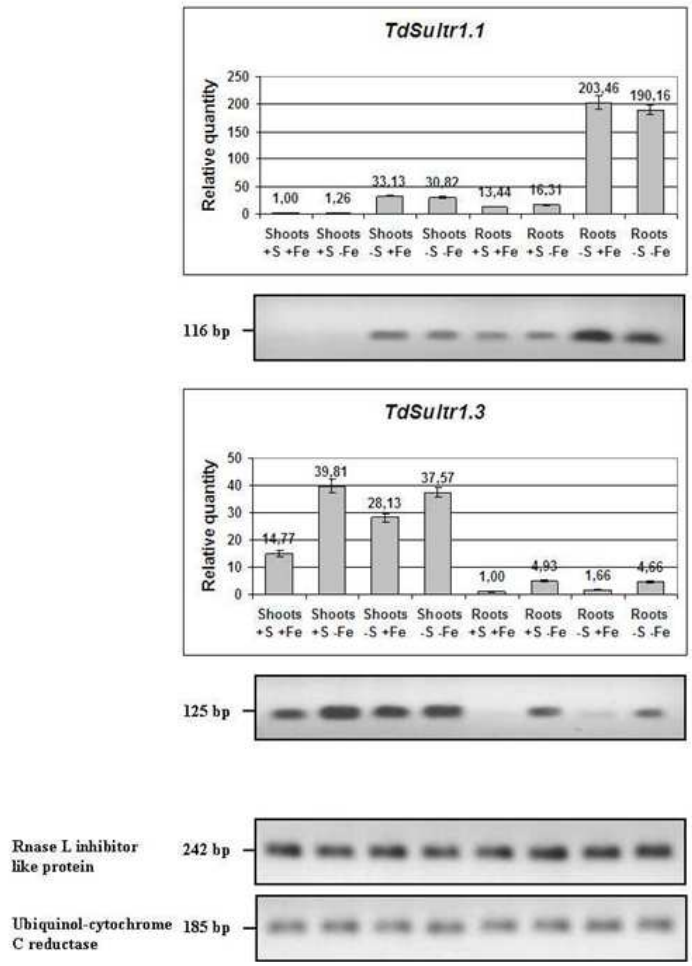

Fig. S1. Relative expression levels by quantitative real time RT-PCR (qRT-PCR) and semi-quantitative RT-PCR of the genes encoding durum wheat high-affinity sulfate transporters (*TdSultr1.1 and TdSultr1.3*) in shoots and roots of 11 days old seedlings of cv Svevo grown with or without 100 μM FeIII-EDTA, at two S (sulfur) levels in the nutrient solution (+S=1.2 and -S=0 mM sulphate). In qRT-PCR, the 24 cDNA pools (three biological replicates and eight seedling samples including shoots and roots) were tested in triplicate and normalized using the geometric average of the relative expression of the two reference genes Ubiquinol-cytochrome C reductase (Unigene Cluster Ta4045; DFCI contig TC350975) and RNase L inhibitor-like protein (Unigene cluster Ta2776; DFCI contig TC278756). The relative expression levels in different samples of each sulfate transporter gene were referred to that a calibrator set to the value one, which was represented by the treatment with the lowest expression. Normalized values of relative expression of each gene are given as average + SD.

In semi-quantitative RT-PCR the transcripts of the genes encoding Ubiquinol-cytochrome C reductase and RNase L inhibitor-like protein were amplified as controls and the sample products (5 μl) of the control and analysed genes were collected after 28 and 25 PCR cycles, respectively, and analysed on 1.5% agarose gels.

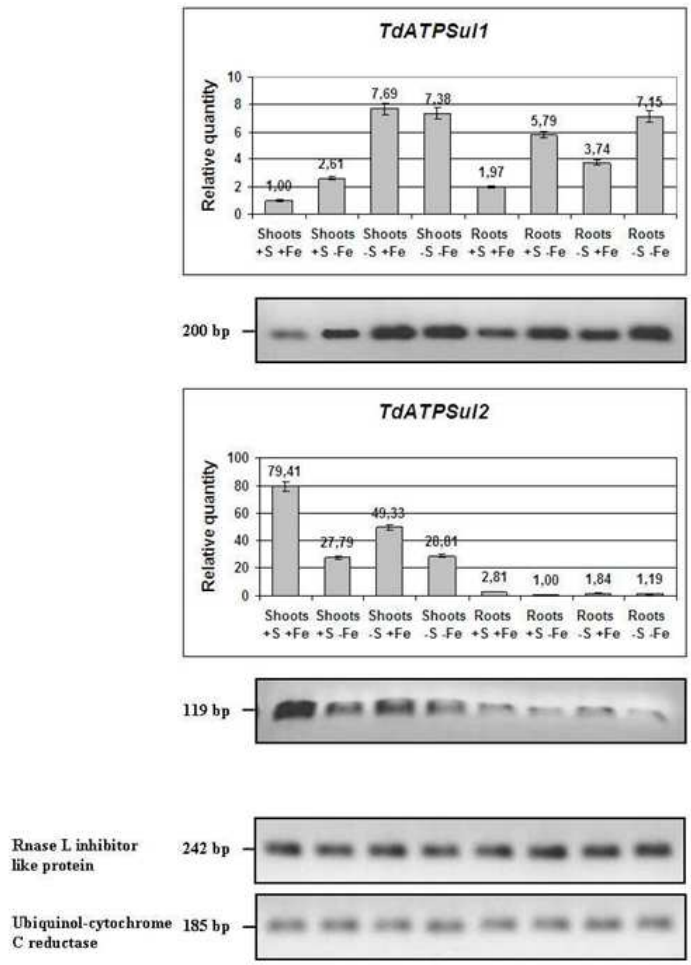

Fig. S2. Relative expression levels by quantitative real time RT-PCR (qRT-PCR) and semi-quantitative RT-PCR of the genes encoding durum wheat ATP sulfurylase (*TdATPSul1* and *TdATPSul2*) in shoots and roots of 11 days old seedlings of cv Svevo grown with or without 100 μM FeIII-EDTA, at two S (sulfur) levels in the nutrient solution (+S=1.2 and -S=0 mM sulphate). In qRT-PCR, the 24 cDNA pools (three biological replicates and eight seedling samples including shoots and roots) were tested in triplicate and normalized using the geometric average of the relative expression of the two reference genes Ubiquinol-cytochrome C reductase (Unigene Cluster Ta4045; DFCI contig TC350975) and RNase L inhibitor-like protein (Unigene cluster Ta2776; DFCI contig TC278756). The relative expression levels in different samples of each ATP sulfurylase gene were referred to that a calibrator set to the value one, which was represented by the treatment with the lowest expression. Normalized values of relative expression of each gene are given as average + SD.

In semi-quantitative RT-PCR the transcripts of the genes encoding Ubiquinol-cytochrome C reductase and RNase L inhibitor-like protein were amplified as controls and the sample products (5 μl) of the control and analysed genes were collected after 28 PCR cycles and analysed on 1.5% agarose gels.

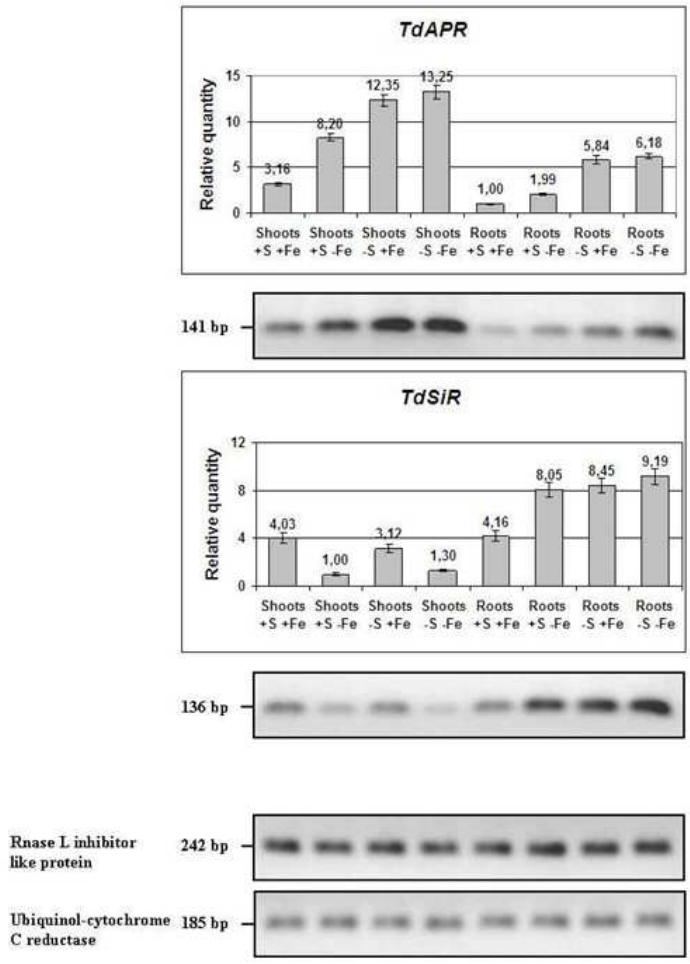

Fig. S3. Relative expression levels by quantitative real time RT-PCR (qRT-PCR) and semi-quantitative RT-PCR of the genes encoding durum wheat adenosine 5-phosphosulfate reductase (APR) (*TdAPR*) and sulfite reductase (*TdSiR*) in shoots and roots of 11 days old seedlings of cv Svevo grown with or without 100 μM FeIII-EDTA, at two S (sulfur) levels in the nutrient solution (+S=1.2 and -S=0 mM sulphate). In qRT-PCR, the 24 cDNA pools (three biological replicates and eight seedling samples including shoots and roots) were tested in triplicate and normalized using the geometric average of the relative expression of the two reference genes Ubiquinol-cytochrome C reductase (Unigene Cluster Ta4045; DFCI contig TC350975) and Rnase L inhibitor-like protein (Unigene cluster Ta2776; DFCI contig TC278756). The relative expression levels in different samples of each of the two genes were referred to that a calibrator set to the value one, which was represented by the treatment with the lowest expression. Normalized values of relative expression of each gene are given as average + SD.

In semi-quantitative RT-PCR the transcripts of the genes encoding Ubiquinol-cytochrome C reductase and RNase L inhibitor-like protein were amplified as controls and the sample products (5 μl) of the control and analysed genes were collected after 28 PCR cycles and analysed on 1.5% agarose gels.

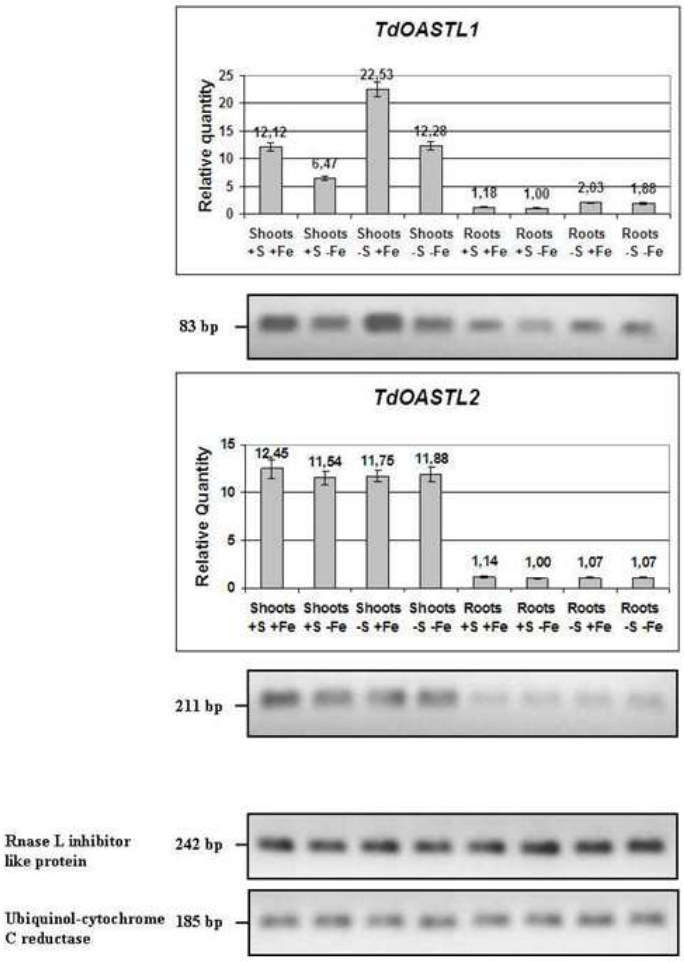

Fig. S4. Relative expression levels by quantitative real time RT-PCR (qRT-PCR) and semi-quantitative RT-PCR of the genes encoding durum wheat *O*-acetylserine (thiol) lyases (*TdOASTL1* and *TdOASTL2*) in shoots and roots of 11 days old seedlings of cv Svevo grown with or without 100 μM FeIII-EDTA, at two S (sulfur) levels in the nutrient solution (+S=1.2 and -S=0 mM sulphate). In qRT-PCR, the 24 cDNA pools (three biological replicates and eight seedling samples including shoots and roots) were tested in triplicate and normalized using the geometric average of the relative expression of the two reference genes Ubiquinol-cytochrome C reductase (Unigene Cluster Ta4045; DFCI contig TC350975) and RNase L inhibitor-like protein (Unigene cluster Ta2776; DFCI contig TC278756). The relative expression levels in different samples of each OASTL gene were referred to that a calibrator set to the value one, which was represented by the treatment with the lowest expression. Normalized values of relative expression of each gene are given as average + SD.

In semi-quantitative RT-PCR the transcripts of the genes encoding Ubiquinol-cytochrome C reductase and RNase L inhibitor-like protein were amplified as controls and the sample products (5 μl) of the control and analysed genes were collected after 28 PCR cycles and analysed on 1.5% agarose gels.

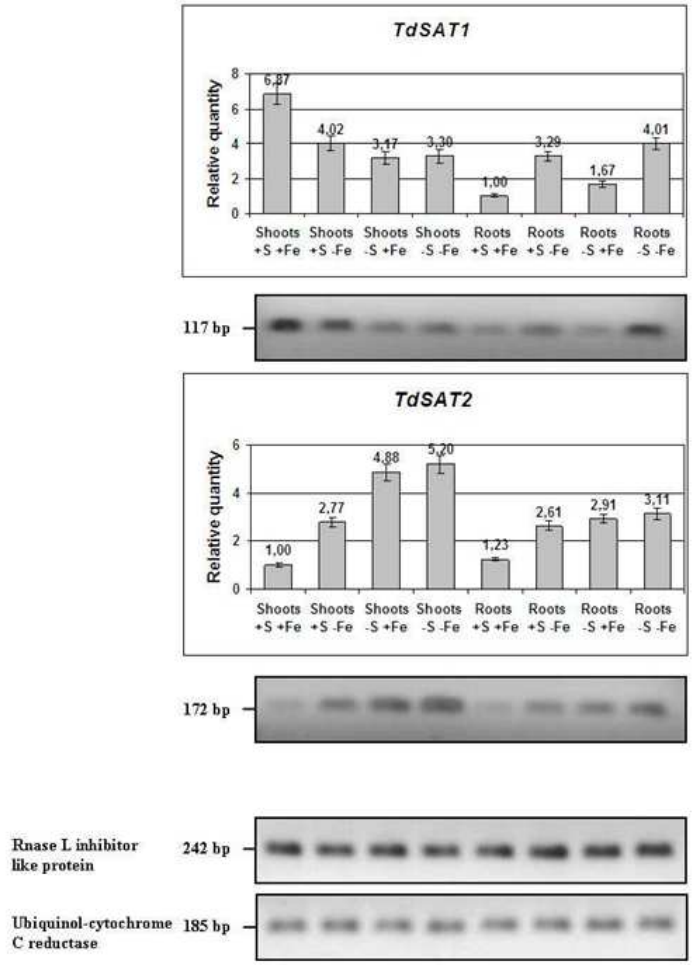

Fig. S5. Relative expression levels by quantitative real time RT-PCR (qRT-PCR) and semi-quantitative RT-PCR of the genes encoding durum wheat serine acetyltransferase (*TdSAT1* and *TdSAT2*) in shoots and roots of 11 days old seedlings of cv Svevo grown with or without 100 μM FeIII-EDTA, at two S (sulfur) levels in the nutrient solution (+S=1.2 and -S=0 mM sulphate). In qRT-PCR, the 24 cDNA pools (three biological replicates and eight seedling samples including shoots and roots) were tested in triplicate and normalized using the geometric average of the relative expression of the two reference genes Ubiquinol-cytochrome C reductase (Unigene Cluster Ta4045; DFCI contig TC350975) and RNase L inhibitor-like protein (Unigene cluster Ta2776; DFCI contig TC278756). The relative expression levels in different samples of each SAT gene were referred to that a calibrator set to the value one, which was represented by the treatment with the lowest expression. Normalized values of relative expression of each gene are given as average + SD.
In semi-quantitative RT-PCR the transcripts of the genes encoding Ubiquinol-cytochrome C reductase and RNase L inhibitor-like protein were amplified as controls and the sample products (5 μl) of the control and analysed genes were collected after 30 PCR cycles and analysed on 1.5% agarose gels.

Table S1. Arabidopsis genes involved in sulfur metabolism identified according to TAIR database(http://www.arabidopsis.org/index.jsp), Lewandoska and Sirko (2008) and Hell and Wirtz (2011).

Protein function	Gene name	Genome Locus	NCBI mRNa REF SEQ	NCBI Protein REF SEQ
High affinity sulfate Transporters (SULTR)	*SULTR1;1*	At4g08620	NM_1169631	NP_192602
	SULTR1;2	At1g7800	NM_106449	NP_565166
	SULTR1;3	At1g22150	NM_102065	NP_564159
ATP sulfurylase (ATPS) EC 2.7.7.4	*APS1 (ATP-sulfurylase 3)*	At3g22890	NM_113189	NP_188929
	APS2 (ATP-sulfurylase 1)	At1g19920	NM_101847	NP_564099
	APS3 (ATP-sulfurylase 2)	At4g14680	NM_117550	NP_193204
	APS4	At5g43780	NM_123745	NP_199191
APS reductase (APR) EC 1.8.99.2	*APR1*	At4g04610	NM_116699	NP_192370
	APR2	At1g62180	NM_104899	NP_176409
	APR3	At4g21990	NM_118320	NP_193930
Sulfite reductase (Sir)	*SIR*	At5g04590	NM_120541	NP_196079
O-Acetylserine (thiol)-Lyase (OAS-TL) EC 2.5.1.47	*OASA1 (Bsas1;1)*	At4g14880	NM_117574	NP_193224
	OASB (Bsas2;1)	At2g43750	NM_129937	NP_181903
	OASC (Bsas2;2)	At3g59760	NM_115838	NP_191535
	ATCYSC1 (Bsas3;1)	At3g61440	NM_116009	NP_191703
	ATCYSD1 (Bsas4;2)	At3g04940	NM_111366	NP_566243
	ATCYSD2 (Bsas4;1)	At5g28020	NM_122685	NP_198154
	OAS-like (Bsas4;3)	At5g28030	NM-122686	NP_198155
	CS26 (Bsas5;1)	At3g03630	NM_111234	NP_187013
	OAS-like (Bsas1;2)	At3g22460	NM_113145	NP_188885
Serine acetyltransferase (SAT) EC 2.3.1.30	*SAT1 (Atserat2;1)*	At1g55920	NM_104470	NP_175988
	SAT2 (AtSerat3;1)	At2g17640	NM_127318	NP_565421
	SAT3 (AtSerat2;2)	At3g13110	NM_112150	NP_187918
	SAT4 (AtSerat3;2)	At4g35640	NM_119729	NP_195289
	SAT5 (AtSerat1;1)	At5g56760	NM_125059	NP_200487

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Table S2. Primer pairs used for the isolation of the full-length cDNA of wheat genes involved in sulfur metabolism

Gene	Forward primer	TM (°C)	Reverse Primer	TM (°C)
TdSultr1.1	CCATCTGCACATAGGCACA	59.2	TTTGCACGAACTATGCCG	59.4
TdSultr1.3	CGATCACAGGAAGCACCGT	62.3	AAGCCACATCATATTGCGCC	63.2
TdATPSul1	TCGGTCCGTCGAACACCTA	62.6	CCCTGCGTTCACCATTCCT	63.4
TdATPSul2	CCAAATCGTCGACGGGAAG	63.8	CCTTATTGGCACCACAAGCAC	62.7
TdAPR	CATTCAGCGGCCGGTAAT	61.9	CCTTTGTCTCCAACATTTCCG	61.8
TdSir	TACATCACTCACGCTCGCC	61.0	GACAGGTTTCCTCCTCCTCTC	59.3
TdOASTL1	AAAGGAAGGAAGGAAGGATGG	60.8	GCGTAGGTCAAGCGTTCGT	61.4
TdOASTL2	GGGAGGATCACTTGGCTGT	60.1	GGGATTTGGCTTTGGAGAAC	60.8
TdSAT1	CAGCAATCCATCCATCCCT	60.8	GGTTGCTCAGCTGAAGAAACA	60.6
TdSAT2	AACCTAGATACGTCTCCCGC	58.3	CAGGCCCAACTGCATAGTC	59.3

Table S3. Primer pairs used in qRT-PCR analyses. Primer pairs efficiencies and R^2 values (coefficient of determination) calculated for standard curves (5-fold dilution series from pooled cDNAs) in the three biological replicates (I, II and III), and characteristics of the corresponding amplicons are also reported.

Gene	Forward primer	Reverse primer	Amplicon lenght (bp)	Amplicon Tm (°C)	Efficiencies			R^2		
					I	II	III	I	II	III
TdSultr1.1	5'-CTATGAGACAGCCTACCTTATATG -3'	5'-CAACCGCAATGAGCAAGC-3'	116	72.8	92.6	92.4	92.6	1.000	0.999	0.998
TdSultr1.3	5'-ATGTCAGTGGTGGTTCTTC-3'	5'-ATTGCTGCTTCGTAGTCC-3	125	74.2	104.5	107.6	103.8	0.997	0.997	0.996
TdATPSul1	5'- GCCATCCAACAGAGAAGAG-3'	5'-CGTATCGTATGCTGCTACC-3'	119	74.6	96.3	95.6	96.4	0.999	0.999	1.000
TdATPSul2	5'-CTGTTCTTCTTCTCCATCCA -3'	5'-AGCATTTATACGAGCCTTAGC-3'	200	76.2	92.4	92.1	92.2	0.999	0.999	0.998
TdAPR	5'- GCAGACGGCGAGCAGAAG-3'	5'-GAAGGCGAGGAGGGATTGG-3'	141	81.3	102.0	100.5	101.8	0.999	0.999	0.999
TdSiR	5'-GAGGAGTCTGTAGTGGTGAGGATAAC-3'	5'- AGGTGGTCTGGTTTGGTGTTCC -3'	136	77.2	92.7	92.5	93.2	0.999	0.999	1.000
TdOASTL1	5'-GCCCAGAGACCCGAGAAC-3'	5'-AACAGCACCGACGACAGG-3'	83	77.5	100.4	100.5	100.8	0.997	0.998	0.998
TdOASTL2	5'- GTGTGGAGCCTACCGAGAG-3'	5'- GCAGCAGCACCAGAAGAG-3'	211	75.4	96.4	95.9	96.2	0.998	0.999	1.000
TdSAT1	5'-GTCTCCATCCTCCACCAC-3'	5'-GCACATCTATCAGCACCAC -3'	172	83.6	92.3	92.7	92.0	0.997	0.998	0.997
TdSAT2	5'-AAGGAACACGGAGATAGACACC-3'	5'- AAGGGAACCAGCGGCAATC-3'	117	75.9	94.2	93.1	94.6	0.999	0.999	0.998
Protein AAA-superfamily ATPases	5'-CAAATACGCCATCAGGGAGAAC-3'	5'-CGCTGCCGAAACCACGAGAC-3'	227	80.4	97.2	98.4	97.6	0.997	0.999	0.999
ADP-ribosylation factor	5'-GCTCTCCAACAACATTGCCAAC-3'	5'-GCTTCTGCCTGTCACATACGC-3'	165	73.7	101.8	99.8	100.8	0.997	0.996	0.997
Rnase L inhibitor like protein	5'-CGATTCAGAGCAGCGTATTGTT-3'	5'-AGTTGGTCGGGTCTCTTCTAAA-3'	242	73.5	96.0	94.4	95.8	1.000	0.999	1.000
Ubiquinol-cytochrome C reductase	5'-CCTGCCCCGTACAACCTTGAG-3'	5'-CACCGTTGCGATAGTCCTGAAA-3'	185	74.9	94.0	94.2	94.0	1.000	0.999	1.000
S-adenosylmethionine decarboxilase	5'-GGCTGGACAAGAAGAAGG-3'	5'-ATGGATGGTGGAGACGTC-3'	191	78.7	101.0	101.8	101.4	0.998	0.997	0.998
GABA-receptor-associated protein	5'-AGGAGAACAAGGACGAGGAC-3'	5'-AGGAGGCATTCAGAGCGATTG-3'	111	75.5	101.2	101.6	101.0	0.997	0.998	0.997