Transcriptional profiling of an *Fd-GOGAT1/GLU1* mutant in *Arabidopsis thaliana* reveals a multiple stress response and extensive reprogramming of the transcriptome

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**Abstract**

**Background:** Glutamate plays a central position in the synthesis of a variety of organic molecules in plants and is synthesised from nitrate through a series of enzymatic reactions. Glutamate synthases catalyse the last step in this pathway and two types are present in plants: NADH- or ferredoxin-dependent. Here we report a genome wide microarray analysis of the transcriptional reprogramming that occurs in leaves and roots of the *A. thaliana* mutant *glu1-2* knocked-down in the expression of *Fd-GOGAT1* (*GLU1*; *At5g04140*), one of the two genes of *A. thaliana* encoding ferredoxin-dependent glutamate synthase.

**Results:** Transcriptional profiling of *glu1-2* revealed extensive changes with the expression of more than 5500 genes significantly affected in leaves and nearly 700 in roots. Both genes involved in glutamate biosynthesis and transformation are affected, leading to changes in amino acid compositions as revealed by NMR metabolome analysis. An elevated glutamine level in the *glu1-2* mutant was the most prominent of these changes. An unbiased analysis of the gene expression datasets allowed us to identify the pathways that constitute the secondary response of an *FdGOGAT1/GLU1* knock-down. Among the most significantly affected pathways, photosynthesis, photorespiratory cycle and chlorophyll biosynthesis show an overall downregulation in *glu1-2* leaves. This is in accordance with their slight chlorotic phenotype. Another characteristic of the *glu1-2* transcriptional profile is the activation of multiple stress responses, mimicking cold, heat, drought and oxidative stress. The change in expression of genes involved in flavonoid biosynthesis is also revealed. The expression of a substantial number of genes encoding stress-related transcription factors, cytochrome P450 monoxygenases, glutathione S-transferases and UDP-glycosyltransferases is affected in the *glu1-2* mutant. This may indicate an induction of the detoxification of secondary metabolites in the mutant.

**Conclusions:** Analysis of the *glu1-2* transcriptome reveals extensive changes in gene expression profiles revealing the importance of Fd-GOGAT1, and indirectly the central role of glutamate, in plant development. Besides the effect on genes involved in glutamate synthesis and transformation, the *glu1-2* mutant transcriptome was characterised by an extensive secondary response including the downregulation of photosynthesis-related pathways and the induction of genes and pathways involved in the plant response to a multitude of stresses.

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Background

Nitrogen is an important nutrient for plants and a limiting factor in plant development. It is mainly in the form of nitrate through nitrate transporters that nitrogen is taken up by plants. Nitrate is first reduced to nitrite and subsequently to ammonium through the action of nitrate reductase (EC 1.7.1.1) and nitrite reductase (EC 1.7.7.1), respectively. Glutamine synthetase (EC 6.3.2.1) catalyses the incorporation of this ammonium into glutamate and thereby producing glutamine. Subsequently, glutamate synthase transfers the amide amino group of glutamate to 2-oxoglutarate, yielding two molecules of glutamate. Production of glutamate is a key point in the synthesis of a variety of organic molecules, such as nucleic acids, amino acids and secondary metabolites (for review: [1]; Figure 1).

Besides the function in primary nitrogen assimilation, the GS/GOGAT pathway plays a central role in the reassimilation of ammonium produced by photorespiration (for review: [2]). Photorespiration is a photosynthesis-related pathway where O$_2$ is taken up and CO$_2$ is released due to the oxygenation of ribulose-1,5-biphosphate (RuBP) catalysed by RuBP carboxylase/oxygenase [3].

Plants possess two forms of glutamate synthase, which are both localized in plastids. One uses NADH as electron donor and is commonly called NADH-GOGAT (EC 1.4.1.14; GOGAT for “glutamine oxoglutarate aminotransferase”). The other one uses ferredoxin as electron donor and is called Fd-GOGAT (EC 1.4.7.1) (for review: [4]). In Arabidopsis thaliana, NADH-GOGAT is encoded by a single gene (At5g53460) whereas Fd-GOGAT is encoded by two genes, previously called GLU1 (Fd-GOGAT1, At5g04140) and GLU2 (Fd-GOGAT2, At2g41220) [5]. The two genes encoding Fd-GOGAT in A. thaliana show contrasting patterns of expression, with Fd-GOGAT1 expression being highest in leaves, whereas Fd-GOGAT2 is mostly expressed in roots [5,6]. Total glutamate synthase activity in A. thaliana is to a very large extent due to ferredoxin-dependent glutamate synthase, Fd-GOGAT1 contributing most [5,7]. Fd-GOGAT1 and Fd-GOGAT2 expression is also regulated differently: light causes a dramatic increase in Fd-GOGAT1 whereas Fd-GOGAT2 expression is not or only slightly affected. Similarly, Fd-GOGAT1 but not Fd-GOGAT2 expression is induced by sucrose [5]. Both Fd-GOGATs are localized to plastids but a mitochondrial localisation of Fd-GOGAT1 has also recently been shown [8].

Plants deficient in Fd-GOGAT activity have been described in A. thaliana, under several names (gls [9], gltS [7], gls [5], glu1 [8]), and in other species such as barley and tobacco [10,11]. A chlorotic phenotype and a lethal phenotype under photosynthetic conditions, which indicate the importance of glutamate synthase in the respiratory pathway, are characteristic for Fd-GOGAT mutants [5,9].

The aim of the study was to characterise the transcriptional reprogramming that occurs in an A. thaliana mutant named glu1-2, knocked-down in the expression of Fd-GOGAT1 (GLU1; At5g04140) and to relate this to metabolic and phenotypic changes observed for this mutant.

We report here the genome-wide transcriptional analysis by microarray and the metabolic profiling by NMR spectroscopy of in vitro grown glu1-2 mutant plantlets. These analyses identified substantial reprogramming of several pathways and processes in the mutant. These include primary and secondary nitrogen assimilation, leading to changes in the levels of certain amino acid, and photosynthesis related processes. The mutant was also affected in flavonoid biosynthesis and exhibited extensive transcriptional changes indicating the induction of multiple stress responses.

Results and Discussion

Phenotype of the glu1-2 mutant

An A. thaliana mutant presenting a T-DNA insertion in the Fd-GOGAT1 (GLU1, GLS1; At5g04140) gene was used in the present study. This mutant will hence be referred to as glu1-2 mutant hereafter. Under the in vitro growth conditions that were used in the present study, glu1-2 mutant plants exhibited a moderate

![Figure 1 The central role of glutamine/glutamate in plant metabolism. The central role of glutamine/glutamate in plant metabolism is represented schematically. Plain arrows indicate the enzymatic reactions catalysed by glutamine synthetase (1) and glutamate synthase (2). Dashed arrows represent pathways that feed into or are affected by these two key amino acids. The representation is inspired by Forde and Lea [48].](http://www.biomedcentral.com/1471-2164/11/190)
chlorotic phenotype and reduced growth compared to wild-type Col-0 plants (Figure 2).

Global overview and comparison of gene datasets that are affected in the Fd-GOGAT1 mutant leaves and roots
Changes in gene expression in leaves and roots of 18 day old in vitro grown A. thaliana glu1-2 plantlets were analysed using a genome wide microarray approach. This analysis showed that the expression of a high number of genes was affected in the glu1-2 mutant. Only genes whose expression was identified as being significantly changed at P = 0.01 were retained.

With 5615 genes whose expression were significantly affected (either induced or repressed) in leaves versus 687 genes in roots, there was an 8-fold difference in the number of affected genes between the two organs (Table 1). This was most likely due to the fact that Fd-GOGAT1, as opposed to Fd-GOGAT2 and NADH-GOGAT, has a much lower expression in roots than in leaves, which will be discussed later on. Some probes hybridizing to genes encoded by mitochondrial and chloroplastic genomes were found in the dataset of genes downregulated in leaves of the glu1-2 mutant. Within each organ the ratio between number of induced and the number of repressed genes was only slightly biased towards induction (1.09 in leaves and 1.27 in roots). When comparing the change fold in expression between the glu1-2 mutant and the wild-type, the log₂ ratio of

| Organ | Change in expression | Number of genes |
|-------|---------------------|----------------|
| leaf  | increased           | 2957           |
| leaf  | reduced             | 2708*          |
| root  | increased           | 384            |
| root  | reduced             | 303            |

Table 1 Overview of genes differentially expressed between the glu1-2 mutant and the wild-type mutant

*This number includes genes from mitochondrial and plastid genomes.

Figure 2 Chlorotic phenotype of glu1-2 mutant plants. Picture showing the chlorotic phenotype of eighteen-day old glu1-2 mutants (left) compared to Col-0 wild-type plants (right) grown in vitro.
affected genes in leaves varied between 6.41 and -5.73 whereas that in roots was much more moderate with a variation between 1.71 and -2.61 (Additional file 1).

In glu1-2 mutant leaves, 2865 of the 2957 (almost 97%) induced genes were exclusively affected in this tissue (Figure 3). The 92 genes whose expression was also affected in roots, were almost equally distributed between induced (50) and repressed (42). genes. Among the 2708 genes repressed in leaves, 2540 (almost 94%) were only affected in leaves. Of the remaining 168 genes that are repressed in leaves and affected in roots, there is a clear bias towards induction. In glu1-2 roots, 228 out of 384 (over 59%) induced genes and 199 out of 303 (over 65%) repressed genes were exclusively affected in this tissue. Among the remaining genes, which were also affected in leaves, the induced ones were more prevalent than the repressed ones, irrespective of whether they were up- or down-regulated in leaves.

Analysis of overrepresented gene ontologies and affected pathways among affected genes in the glu1-2 mutant indicates a reprogramming of several biological processes

As seen above, a large number of genes are affected in the glu1-2 mutant, notably the leaves, which indicates that a profound transcriptional reprogramming takes place. The schematic representation by Mapman [12] illustrates to what extent different cellular processes and metabolic pathways are affected in the glu1-2 mutant (Figure 4). In order to identify the most relevant ones, an unbiased analysis of affected gene ontologies (GOs) and pathways was performed.

An analysis of overrepresented GO terms of the “biological process” classification using GOstat [13] (P = 0.01 level with FDR/Benjamini correction) on the different glu1-2 transcriptional datasets was performed. The results of this analysis are shown in detail in Additional file 2 and summarised below.

When this analysis is applied to the genes whose expression is affected in glu1-2 leaves, not discriminating between induced and repressed ones, 54 overrepresented GO_biological_process terms are identified. When the datasets are separated into genes induced or repressed in glu1-2 leaves, 73 and 19 overrepresented GO terms are identified respectively. In comparison, only 13 GO_biological_process terms are overrepresented among the affected (induced or repressed) genes in glu1-2 roots. Analysis on glu1-2 root-induced and -repressed genes separately identifies 34 and 0 overrepresented GO_biological_process terms, respectively. When combining expression patterns from leaves and roots 2 GO_biological_process terms are overrepresented among affected genes, while the subset of genes downregulated in leaves and upregulated in roots reveals three additional overrepresented GO_biological_processes. Two terms are overrepresented among genes induced in both organs of the glu1-2 mutant (Additional file 2). Hence, GOstat identifies in total 124 unique GO_biological_process terms (confounded levels) that are overrepresented in the glu1-2 transcriptional profile changes (Additional file 2). Analysis with two further algorithms, PathExpress [14] and GeneBins [15], gave overlapping results to the ones obtained by GOstat as to which biological processes and pathways are affected in the glu1-2 mutant. These results are therefore not further detailed in the text but are shown in Additional files 3 and 4.

Visualisation of the networks of GO terms that are enriched in the glu1-2 mutant versus wild-type, using the Cytoscape [16] plug-in ClueGO [17], illustrates the complexity of the transcriptional response in glu1-2 mutant leaves (Figures 5 and 6). Notably, among genes upregulated in glu1-2 leaves GO terms related to metabolic processes of nitrogen and carbohydrate compounds (Figure 5), the regulation of transcription and the response to a diversity of stimuli are overrepresented. GO categories related to the latter two are also overrepresented among downregulated genes in glu1-2 leaves, in addition to photosynthesis and pigment/porphyrin biosynthesis (Figure 6). Although the complexity of GO-term networks enriched in the glu1-2 mutant roots versus wild-type roots is reduced (Figure 7), the
Figure 4 Metabolic pathways covered by the transcriptional changes affecting the $glu1$-2 mutant. Overview of expression changes in metabolic pathways in $glu1$-2 mutant leaves (A) and roots (B) using the MapMan software [12]. Represented are only the genes showing a significant ($P = 0.01$) change in expression between the $glu1$-2 mutant and the wild-type and that have been attributed to the respective bins by MapMan. Genes whose expression is increased or decreased in the $glu1$-2 mutant tissue versus the corresponding wild-type tissue are shown by an increasingly intense blue and red colour, respectively. The graduation can be seen on the scale that is represented in the top right corner of each subfigure. A change in expression of $\log_2 = 2.0$ scale was chosen as giving full saturation.
T-DNA insertion in Fd-GOGAT1 has nevertheless a considerable impact on the root transcriptome as evidenced by the number of affected genes (i.e. 687) and the affected GO-terms (Additional files 2 to 4).

The large number of genes with modified expression and the analyses described above indicate that a large number of pathways and processes are seemingly affected in the glu1-2 mutant. Interestingly, glutamate biosynthesis and nitrogen metabolism are not often identified as such, and if so are indicated as induced in leaves. However as these pathways most likely constitute the plant’s primary response to the knock-down of Fd-GOGAT1, the expression data of genes involved in these pathways will be analysed in a first part. In a second part the focus will be put on some of the processes that, despite the differences in algorithms and category definitions and terms, are recurrently identified by the performed analyses. These processes most likely constitute

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**Figure 5** Network representations of enriched GO categories amongst genes induced in glu1-2 mutant leaves. Representations generated by ClueGO [17] of functionally grouped networks of enriched GO categories among genes whose expression is induced in leaves of the glu1-2 mutant compared to leaves of the wild-type. GO terms are represented as nodes based on their kappa score level ($\geq 0.3$), only networks with at least three nodes being represented. The node size represents the term enrichment significance. The label of the most significant term is used as leading group term.
a secondary response of the mutant plant and encompass photosynthesis and related processes as well as aspects of a multiple stress response.

Even within the selected pathways and processes that will be presented below it is out of scope to present and discuss all aspects in the text. The reader is therefore kindly referred to the respective Additional files containing the detailed information about affected genes.

**Analysis of genes involved in glutamate biosynthesis or related pathways whose expression is affected in leaves or roots of the glu1-2 mutant**

**Changes in expression levels of genes involved in glutamate biosynthesis and related pathways**

In order to show how glutamate metabolism and related pathways are affected in the glu1-2 mutant, an overview of the major genes and enzymes involved (Table 2) is
Figure 7 Network representations of enriched GO categories amongst genes affected in glu1-2 mutant roots. Representations generated by ClueGO [17] of functionally grouped networks of enriched GO categories among genes whose expression is induced (A) or repressed (B) in roots of the glu1-2 mutant compared to roots of the wild-type. GO terms are represented as nodes based on their kappa score level (≥ 0.3), only networks with at least three nodes being represented. The node size represents the term enrichment significance. The label of the most significant term is used as leading group term.
Table 2 Genes that are involved in major glutamate-related pathways and that are differentially expressed between the glu1-2 mutant and the wild-type mutant

| EC # | Enzyme/protein | Abbreviation | Gene ID   | log₂ root | log₂ leaf |
|------|----------------|--------------|-----------|-----------|-----------|
| 1.4.1.7 | Fd-dependent glutamate synthase | Fd-GOGAT 1/GLU1 | AT5G04140 | -5.738    |           |
| 1.4.1.7 | Fd-dependent glutamate synthase | Fd-GOGAT 2/GLU2 | AT2G41220 | 1.070     |           |
| 1.4.1.14 | NADH-dependent glutamate synthase | NADH-GOGAT | AT5G53460 | 0.712     |           |
| 1.7.1.1 | nitrate reductase | NIA2/NR2 | AT1G37130 | 0.438     |           |
| 1.7.7.1 | nitrite reductase | NIR1 | AT2G15620 | 0.687     |           |
| 6.3.1.2 | glutamine synthetase | GLN1.1 | AT5G37600 | 1.296     |           |
| 6.3.1.2 | glutamine synthetase | GLN1.3 | AT3G17820 | 0.669     |           |
| 6.3.1.2 | glutamine synthetase | GLN1.4 | AT5G16570 | -0.580    |           |
| 6.3.5.4 | asparagine synthetase | ASN1 | AT3G47340 | -2.913    |           |
| 6.3.5.4 | asparagine synthetase | ASN2 | AT5G65010 | -0.486    |           |
| 3.5.1.1 | asparaginase | AT3G16150 | -0.481    |           |           |
| 2.6.1.1 | aspartate aminotransferase | ASP1 | AT2G30970 | 0.582     |           |
| 2.6.1.1 | aspartate aminotransferase | ASP2 | AT5G19550 | 1.132     |           |
| 2.6.1.1 | aspartate aminotransferase | ASP3 | AT5G11520 | 0.709     |           |
| 2.6.1.1 | aspartate aminotransferase | ASP4 | AT1G62800 | 0.354     |           |
| 2.6.1.2 | alanine-2-oxoglutarate aminotransferase | AlaAT1 | AT1G7290 | 0.438     |           |
| 2.6.1.2 | alanine-2-oxoglutarate aminotransferase | AlaAT2 | AT1G72330 | -0.680    |           |
| 2.1.2.10 | glycine dehydroxymethyltransferase complex – H protein | GmGDH3 | AT3G2470 | 0.593     |           |
| 1.8.1.4 | glycine dehydroxymethyltransferase complex – L protein | GmLDPL1 | AT3G1240 | 0.689     |           |
| 1.4.2 | glycine dehydroxymethyltransferase complex – P protein | GmLDPL1 | AT4G32010 | 0.439     | -1.126    |
| 2.1.2.11 | serine hydroxymethyltransferase | SHM1 | AT4G37930 | -0.513    |           |
| 2.1.2.11 | serine hydroxymethyltransferase | SHM3 | AT4G32520 | 0.532     |           |
| 2.1.2.11 | serine hydroxymethyltransferase | SHM5 | AT4G31930 | 0.535     |           |
| 4.1.1.15 | glutamate decarboxylase | GAD3 | AT2G02000 | 1.441     |           |
| 2.6.1.19 | gamma-aminobutyric acid transaminase | GABA-T1 | AT3G22200 | 0.720     |           |
| 1.2.1.4 | succinic semialdehyde dehydrogenase | SSADH1 | AT1G9440 | 0.516     |           |
| 1.1.186 | ketolacit reductoisomerase | KAR8 | AT3G58410 | 0.380     |           |
| 4.2.1.9 | dehydroxycic acid dehydratase | DHAD | AT3G23940 | 0.857     |           |
| 6.3.2.2 | glutamate-cysteine ligase | GSH1 | AT4G21200 | 0.587     |           |
| 6.3.2.3 | glutathione synthetase | GSH2 | AT5G27380 | 0.855     |           |
| 1.4.1.3 | glutamate dehydrogenase | GDH2 | AT5G0140 | 1.126     |           |
| 2.3.1.1 | N-acetyltransferase | NAGS | AT4G37670 | 0.755     |           |
| 6.3.5.5 | carboxamyl-phosphate synthase | CPS | AT3G27420 | 0.696     |           |
| 6.3.5.5 | carboxamyl-phosphate synthase | CPS | AT1G29900 | 0.701     |           |
| 2.1.3.3 | ornithine carbamoyltransferase | OTC | AT1G73330 | 0.436     |           |
| 6.3.4.5 | arginosuccinate synthase | AS | AT4G24830 | 0.596     |           |
| 2.3.1.1 | N-acetyltransferase | NRT1.1 | AT1G12110 | 0.432     |           |
| 6.3.5.5 | carboxamyl-phosphate synthase | CPS | AT3G21670 | -0.703    |           |
| 2.1.3.3 | ornithine carbamoyltransferase | OTC | AT1G32450 | 0.382     |           |
| 6.3.4.5 | arginosuccinate synthase | AS | AT1G66870 | 1.760     |           |
| 2.3.1.1 | N-acetyltransferase | NRT1.7 | AT1G12450 | 0.501     |           |
given below, starting from the endpoint (i.e. glutamine and glutamate synthesis) and expanding to the steps leading up to glutamate, before focussing on the pathways that utilize glutamate in the production of other compounds. This is complemented by analysis of certain amino acid contents in the glu1-2 mutant.

Changes in expression levels of genes encoding glutamate synthases and glutamate synthetases

Comparison of expression levels in our microarray assays indicates that *Fd-GOGAT1* (GLU1; *At5g04140*) is more highly expressed in leaves than in roots (signal intensity: \( \log_2 = 10.84 \) versus \( \log_2 = 5.06 \); \( \Delta \log_2 = 5.78 \)) of 18 day old *A. thaliana* wild type plants (Col-0 ecotype) (Additional file 1). This is in accordance with earlier published results and gene expression data publicly available [5,6,18,19]. As expected, the expression of *Fd-GOGAT1* (Table 2) was downregulated in the glu1-2 mutant. There was a log2 ratio of -5.74 between the expression levels of *Fd-GOGAT1* in leaves of wild type and the mutant. In roots the difference was -0.54 (only significant at \( P = 0.05 \)). It should however be noted that residual levels of transcripts for *Fd-GOGAT1* are still detectable on the microarrays from the glu1-2 mutant, and that these are similar in roots and leaves.

The gene *GLU2* (*At2g41220*) encoding *Fd-GOGAT2*, the second *A. thaliana* Fd-dependent glutamate synthase, is upregulated (\( \log_2 = 1.07 \)) in leaves but not affected in roots of the glu1-2 mutant. The NADH-GOGAT (EC1.4.1.14) encoding gene *At5g53460* is also slightly upregulated (\( \log_2 = 0.71 \)) in glu1-2 leaves but not affected in roots (Table 2). This could indicate a partial recovery of the loss of the plastid-localized Fd-GOGAT1 by these enzymes in leaves as NADH-GOGAT and Fd-GOGAT2 are localized to plastids. *Fd-GOGAT2* has however higher expression levels in roots than in leaves and is therefore more likely involved in primary nitrogen assimilation in roots [5]. In addition, NADH-dependent glutamate synthase activity only makes up a small percentage of the total glutamate synthase activity in *A. thaliana* leaves and NADH-dependent activity is not affected in Fd-GOGAT deficient mutants [7,9]. It should also be noted that posttranscriptional regulation has been hypothesised for Fd-GOGAT in tobacco and *A. thaliana* [20,21].

Glutamine is the substrate of GOGATs for the synthesis of glutamate (Figure 8) and glutamine synthetases (EC 6.3.1.2) catalyse the synthesis of glutamine from ammonium \( (\text{NH}_4^+) \) using ATP. Of the five putative *A. thaliana* genes encoding the cytoplasmic glutamine synthetase 1 (GS1), two were upregulated (*At5g37600/GLN1.1* and *At3g17820/GLN1.3*) whereas one (*At5g16570/GLN1.4*) was slightly downregulated in *glu1-2* leaves. The gene *At5g35630* encoding glutamine synthetase 2 (GS2), which is dual-targeted to the plastid and mitochondria [22], was not affected. None of the genes encoding glutamine synthetases 1 or 2 were affected in the roots of the *glu1-2* mutant.

| Genes encoding enzymes involved in the primary nitrogen assimilation leading to the formation of glutamine and glutamate |
|--------------------------------------------------------------------------------------------------------------------------|
| Glutamine and glutamate play a central role in the primary assimilation of nitrogen (Figure 1). Hence, genes encoding enzymes catalysing the reactions leading to the formation of glutamine from nitrate are affected in the *glu1-2* mutant (Table 2), mostly in leaves. |
Figure 8 Primary nitrogen assimilation and glutamate metabolism. Simplified schematic representation of the pathways leading to the synthesis and the conversion of glutamate and the way they are affected in the glu1-2 mutant. Only the data related to glu1-2 leaves is depicted. Colour code: blue are upregulated, red are downregulated, violet are both up- and downregulated steps whereas grey are unaffected steps of these pathways. Enzymes: (1) nitrate reductase, (2) nitrite reductase, (3) glutamine synthetase, (4) NADH-GOGAT, (5) Fd-GOGAT, (6) asparagine synthase, (7) asparaginase, (8) aspartate aminotransferase, (9) alanine-2-oxoglutarate aminotransferase, (10) glutamate-glyoxylate aminotransferase, (11) glycine decarboxylase complex, (12) serine hydroxymethyltransferase, (13) glutamate decarboxylase, (14) γ-aminobutyric acid transaminase, (15) succinic semialdehyde dehydrogenase, (16) glutamate-cysteine ligase, (17) glutathione synthetase, (18) glutamate dehydrogenase, (19) acetoxyhydroxidase synthase, (20) ketolacid reductoisomerase, (21) dehydroxyacid dehydratase, (22) branched-chain aminotransferase. Subcellular compartmentalisation is not taken into account in this figure and reductants are only indicated for some of the reactions. The detailed data of the genes depicted in this figure and related data for genes affected in glu1-2 roots is presented in Table 2.
Some of the genes encoding nitrate reductase (NADH-NAR; EC 1.7.1.1) and nitrite reductase (NIR1; EC 1.7.7.1), are upregulated in glu1-2 leaves while unaffected in glu1-2 roots. These enzymes are responsible for the reduction of nitrate and nitrite respectively, leading to the formation of NH4+, which serves as substrate by glutamine synthetase. The upregulation of nitrate reductase expression levels was previously observed in tobacco plants deficient in Fd-GOGAT activity [23].

Glutamine and aspartate are used as substrates for the synthesis of glutamate and asparagine, in an ATP-dependent reaction catalysed by asparagine synthase (ASN; EC 6.3.5.4)(for review: [24]). Of the three dependent reaction catalysed by asparagine synthase synthesis of glutamate and asparagine, in an ATP-tobacco plants deficient in Fd-GOGAT activity [23]. reductase expression levels was previously observed in by glutamine synthetase. The upregulation of nitrate downregulated in glu1-2 genes encoding asparagine synthases, two were downregulated in glu1-2 leaves but none was affected in roots (Table 2). ASN1 (At3g47340) is one of the ten most downregulated genes in the glu1-2 mutant leaves. It has been shown that ASN1 is induced by dark and reduced by light (or sucrose) while ASN2, which is moderately downregulated, is induced by light. These two genes also respond differently to asparagine, glutamine and glutamate with ASN1 expression being induced and ASN2 expression being reduced ([24] and references therein). Overexpressing ASN1 leads to higher asparagine levels in seeds and phloem [25] and Masclaux-Daubresse et al. [21] have recently shown that asparagine synthase can also catalyse the formation of asparagine from aspartate using ammonium directly. None of the four genes encoding asparaginases (EC 3.5.1.1), which are responsible of degrading asparagine into aspartate, were affected in the leaves of the glu1-2 mutant (Figure 8). Hence, the reduced expression of asparagine synthase encoding genes in glu1-2 leaves could indicate lower levels of asparagine. In Fd-GOGAT deficient barley plants, the levels of asparagine are however higher than in wild-type plants [10].

Multiple pathways leading to the transformation and degradation of glutamate are also affected
Glutamate serves directly or indirectly as substrate in the production of a series of compounds, like amino acids, nucleic acids, ureides, and polyamines (Figure 1; [26]). Hence, reducing Fd-GOGAT1 expression could have a knock-on effect on the genes encoding enzymes implicated in these various biosynthetic pathways (Figure 8).

Amino acid biosynthesis
Aspartate aminotransferases (ASPs or AATs; EC 2.6.1.1) catalyse the transfer of the α-amino group of glutamate to oxaloacetate to form aspartate and 2-oxoglutarate. In glu1-2 leaves, four of the five A. thaliana genes (putatively) encoding ASPs [26] are upregulated in glu1-2 leaves. Aspartate is a precursor of asparagine and the aspartate family of amino acids such as lysine, threonine and methionine (Figure 8). From the changes in expression levels of genes involved in these pathways the synthesis of these latter amino acids seems to be induced in glu1-2 leaves.

The transfer of the α-amino group of glutamate to pyruvate to form alanine is catalysed by alanine aminotransferases (AlaAT or AOAT; EC 2.6.1.2) which comprise four members, subdivided into two groups, in A. thaliana [27]. The first group, composed of AlaAT1 and AlaAT2 that possess alanine aminotransferase activity, is slightly induced in leaves but not in roots of the glu1-2 mutant (Table 2). AlaAT1 has recently been suggested to catalyse the reverse reaction (i.e. conversion of alanine to pyruvate) [28], which could lead to a production of glutamate (Figure 8) to compensate for the lack of Fd-GOGAT1. Low levels of glutamate may shift this reaction equilibrium to favour glutamate production. The members of the second group, two peroxisomal enzymes, possess a glycine (or glutamate:glyoxylate) aminotransferase (GGAT) activity in addition to their alanine aminotransferase activity (Figure 8). Glyoxylate is thereby transaminated to glycine, accompanied by the consumption of glutamate and the production of 2-oxoglutarate, during photorespiration. Neither of these two genes is affected in glu1-2 roots (Table 2) but the expression of GGAT2 (At1g70580) is reduced in glu1-2 leaves. The further conversion of glycine to serine involves the glycine decarboxylase complex (GDC) and serine hydroxymethyltransferase (SHMT; EC 2.1.2.1). The glycine decarboxylase complex is composed of four mitochondrial proteins (H, L, P and T) encoded by a total of eight genes in A. thaliana [29]. The genes encoding the two P proteins are both downregulated (Table 2). Knocking out these genes simultaneously provokes a lethal phenotype, also under nonphotorespiratory conditions, which points towards a role of GDC in other metabolic processes than photorespiration [30]. Of the seven A. thaliana genes putatively encoding serine hydroxymethyltransferases, four show a changed expression in glu1-2 leaves (Table 2). SHM1 (At4g37930) encoding the mitochondrial SHMT1 is downregulated. Interestingly, the knock-out mutant shm1-1 also displays a lethal photorespiratory phenotype [31] and a physical interaction between Fd-GOGAT1 and SHMT1 in mitochondria was recently established [8]. Three genes encoding putative cytosolic serine hydroxymethyltransferases were on the other hand almost equally upregulated in leaves. None of the SHMT-encoded genes was affected in glu1-2 roots (Table 2).

Arginine is formed from glutamate and glutamine in a multiple-reaction pathway [32] that shows a slight overall induction in glu1-2 mutant leaves (Figure 8; Table 2).
This may be responsible for increased production of arginine that has been observed in Fd-GOGAT deficient plants and which may prevent excessive accumulation of glutamine [11]. In root tissue of the *glu1-2* mutant none of the transcripts of these genes involved in arginine synthesis were affected.

Valine biosynthesis starts with the condensation of two molecules of pyruvate by acetyl-CoA synthase (AHAS; EC 2.2.1.6), also known as acetyl-CoA synthetase [33]. Neither the gene *At3g48560* encoding the catalytic subunit, nor the genes *At2g31810* and *At5g6290* encoding the regulatory subunit, are significantly affected in the *glu1-2* mutant (Figure 8). The resulting 2-acetolactate is converted to 2,3-dihydroxy-3-isovalerate by ketolactate reductoisomerase (KARI; EC 1.1.1.86) and its gene *At3g58610* is slightly upregulated in *glu1-2* leaves. The gene *At3g23940* encoding dehydroacyclodehydratase (DHAD; EC 4.2.1.9), which catalyses the conversion of 2,3-dihydroxy-3-isovalerate to α-ketoisovalerate, is also induced in *glu1-2* leaves. Branched-chain aminotransferases (BCAT; EC 2.6.1.42) catalyse the subsequent and last step in the synthesis of valine from α-ketoisovalerate, which is accompanied by the conversion of glutamate to 2-oxoglutarate. However none of the BCAT-encoding genes (*At1g10070*, *At3g49680* and *At5g65780*) implicated in this step is affected in the *glu1-2* mutant. Hence, a few of the genes encoding biosynthetic enzymes involved in valine biosynthesis are induced, indicating possibly a slight activation of the valine biosynthetic pathway.

**Glutathione biosynthesis**

Glutamate is used for the synthesis of glutathione in a two-reaction pathway (Figure 8) catalysed by glutamate-cysteine ligase (GSH1; EC 6.3.2.2) and glutathione synthetase (GSH2; EC 6.3.2.3). Both GSH1 (*At4g23100*) and GSH2 (*At5g27380*) are upregulated in *glu1-2* mutant leaves (Table 2). Mutants deficient in GSH1 have been shown to contain lower levels of glutathione and are more sensitive to stresses. Complete knock-out of GSH1 leads to an embryo-lethal phenotype [34]. Uptregulation of glutathione biosynthetic genes in *glu1-2* may be connected to the upregulation of numerous glutathione S-transferases (see later).

**Gamma-aminobutyrate and succinate synthesis**

Under certain conditions glutamate may be converted to gamma-aminobutyrate (GABA) in a cytosolic reaction catalysed by glutamate decarboxylase (GAD; EC 4.1.1.15). The two GAD-encoding genes GAD1 (*At5g17330*) and GAD2 (*At1g65960*) initially identified in *A. thaliana* [35,36] are not affected in the leaves or roots of the *glu1-2* mutant. Of the three additional genes encoding putative GADs that have recently been identified based on homology [37], GAD3 and/or GAD4 (undifferentiating probe) were upregulated in *glu1-2* mutant leaves (Table 2). The gene encoding GABA-T1 (EC 2.6.1.19; *At3g22200*), the γ-aminobutyric acid transaminase that catalyses the conversion of GABA into succinic semialdehyde (SSA), simultaneously producing alanine from pyruvate, is also induced in leaves. The conversion of SSA to succinate by succinic semialdehyde dehydrogenase (SSADH) is also upregulated in *glu1-2* leaves (Table 2).

**Glutamate catabolism**

Glutamate is catabolised into oxoglutarate and ammonium by glutamate dehydrogenase (GDH; EC 1.4.1.2) (Figure 8), a mitochondrial enzyme that exists under the form of homo- or heterohexamers of two subunits in *A. thaliana* [38]. Only GDH2 (*At5g07440*), encoding the β-subunit, is affected in the *glu1-2* mutant, showing surprisingly an increased expression in mutant leaves (Table 2). Although the role of glutamate dehydrogenase in glutamate metabolism has remained controversial for a long time, recent evidence indicates that GDHs are indeed responsible for the deamination of glutamate leading to the formation of ammonium and 2-oxoglutarate [21,39]. Induction of GDH2 expression could hence lead to a further depletion of the pool of glutamate in the *glu1-2* mutant but would simultaneously increase the levels of oxoglutarate to fuel the TCA cycle. Although the physiological role of GDH is currently still unclear, there seems to be a consensus that GDH is not essential for primary nitrogen assimilation. Instead a role of GDH in the breakdown of several amino acids into their corresponding keto-acids under carbon deficiency was proposed [39]. The the α- and β-subunit composition of glutamate dehydrogenase hexamers may also influence its activity and hence its physiological role [40]. Lancien et al. [41] proposed that ammonium and glutamine would favour the amination reaction. In tobacco plants with reduced Fd-GOGAT activity the amminating, but not the deaminating activity, of glutamate dehydrogenase was indeed reported [42].

**Cellular uptake of glutamate**

Amino acids can be exported from their site of synthesis and transported via the vascular tissue to newly developed tissues. In *A. thaliana*, glutamate is one of the predominant amino acids found in the phloem sap and xylem exudates [18]. Members of the *A. thaliana* amino acid permease (AAP) family have been shown to catalyse the low affinity influx of a broad range of amino acids, including glutamate [43]. Of these, AAP1 (*At1g58360*), AAP4 (*At5g63850*) and AAP6 (*At5g49630*) were moderately downregulated, whereas AAP5 (*At1g44100*) was induced in *glu1-2* leaves (Additional
file 1). AAP1 has recently been shown to be involved in glutamate uptake into root cells and may have a role in the efficient use of nitrogen resources in the rhizosphere [44], but its expression was not affected in glu1-2 roots. Only AAP4 (At5g63850) was affected in roots, with a lower expression in the mutant than in the wild-type.

The related LHT1 (At5g40780) gene which appears to encode a high-affinity glutamate influx system [45] was also moderately downregulated in glu1-2 roots but not affected in glu1-2 leaves. A role for LHT1 in root uptake of certain amino acids was also recently proposed, but its role in glutamate uptake may be limited as this was not affected in an lht1 mutant [46].

The gene At2g01170 encoding the recently identified bidirectional amino acid transporter BAT1 capable of import and export of glutamate and potentially involved in amino acid export from phloem to sink tissue [47] is moderately induced in glu1-2 leaves and not affected in roots.

**Changes in expression levels of genes involved in the synthesis of 2-oxoglutarate and the TCA cycle**

The synthesis of glutamate by NADH- and Fd-GOGATs necessitates 2-oxoglutarate, which is thus situated at the interface between C and N metabolism. As it is possible to affect the glutamate pool by feeding 2-oxoglutarate to plants, the supply of 2-oxoglutarate may be a key regulator of glutamate levels [48].

2-oxoglutarate is produced through the TCA cycle (for review: [41]) and several genes involved in the TCA cycle were upregulated in glu1-2 leaves (Figure 8; Additional file 5). These include genes encoding citrate synthase (CSY; EC2.3.3.1), succinate dehydrogenases (SDH; EC 1.3.5.1) and succinyl-CoA ligase (EC 6.2.1.4). Three of the five genes encoding NAD-dependent isocitrate dehydrogenase (IDH; EC 1.1.1.41) subunits are also upregulated in glu1-2 mutant leaves (Figure 8; Additional file 5). This increase in IDH transcript levels in the glu1-2 mutant is in contrast to the situation observed in tobacco plants with reduced Fd-GOGAT activity, where no change in transcript levels was observed. These tobacco plants did however show increased isocitrate dehydrogenase activity [42].

Recently, Lemaitre et al. [49] have shown that mutants lacking one of the three IDH subunits mentioned above do not exhibit changed levels in 2-oxoglutarate, glutamine and glutamate. The glu1-2 mutant leaves also showed an increase in the expression of four genes encoding putative components (EC 2.3.1.61 and EC 1.2.4.2) of the 2-oxoglutarate dehydrogenase system. On the other hand, the genes At2g47510/At5g50950 encoding fumarate hydratases (FUM; EC 4.2.1.2) were downregulated in glu1-2 leaves. Interestingly, none of the TCA cycle-implicated genes mentioned above were significantly affected in glu1-2 roots. These data indicate an overall induction of the TCA cycle in glu1-2 leaves potentially leading to an increase in 2-oxoglutarate production. Higher levels of 2-oxoglutarate have been observed in Fd-GOGAT deficient tobacco plants [11].

2-oxoglutarate can also be synthesised in the cytosol by export of citrate from mitochondria and the subsequent action of aconitases and isocitrate dehydrogenases. Two aconitase-encoding genes (ACO; EC 4.2.1.2) and two genes putatively encoding cytosolic NADP-dependent isocitrate dehydrogenases (IDCH; EC 1.1.1.42) are upregulated in glu1-2 leaves (Additional file 5). Although this could indicate an increase in cytosolic 2-oxoglutarate synthesis, it should however be noted that a role of the latter in cytosolic 2-oxoglutarate production has not yet been established.

**Uptake, transport and distribution of nitrate and ammonium**

Fd-GOGAT1 is a key enzyme in the primary assimilation of nitrogen and knocking it down was expected to change the transcriptional level of genes involved in nitrogen uptake and transport (Table 2).

The NRT1 and NRT2 nitrate transporter families [50] were however only marginally affected. Of the 53 genes that encode putative NRT1 nitrate transporters in *A. thaliana*, five were induced in glu1-2 leaves. Of these, NRT1.1 (At1g12110) has been implicated in stomatal aperture and drought stress [51] and its NO3- sensing role in root architecture was recently described [52]. NRT1.5 (At1g32450) and NRT2.7 (At1g69870) are less well characterised but have been shown to transport nitrate in a heterologous system [50]. The latter of these genes is also slightly induced in glu1-2 roots. On the other hand, eight genes encoding putative NRT1 family members, such as the NRT1.3/NTP3 (At3g21670; [53]), are downregulated in glu1-2 leaves. Out of the seven NRT2-encoding genes, only At5g14570 (NRT2.7) was moderately induced in glu1-2 leaves (Table 2).

An essential role in nitrate uptake has also recently been shown for the NAR2-like protein [54,55]. The gene NRT3.1/NAR2.1 (At5g50200) encoding this protein is induced in glu1-2 leaves (Table 2). None of the genes implicated in nitrate uptake and transport mentioned above was affected in glu1-2 roots.

The ammonium transporter (AMT) proteins, which are encoded by six genes in *A. thaliana*, are likely responsible for high affinity ammonium transport in plant roots [56]. The gene At2g38290 encoding the ammonium
transporter AMT2;1 was upregulated in glu1-2 leaves (Table 2), but its contribution to ammonium uptake in planta has been questioned recently [57].

The nitrate/proton antipporter AtCLCa mediates nitrate accumulation in vacuoles [58,59] and its encoding gene At5g40890 is down-regulated in glu1-2 leaves (Table 2). Several NRT1 gene-encoding proteins are claimed to be involved in nitrate distribution in different cellular compartments and tissues, but detailed evidence has not been provided yet [50].

Two vacuolar tonoplast intrinsic proteins (AtTIP2;1 and AtTIP2;3) have been proposed to be responsible for NH3 transport across the tonoplast membrane in A. thaliana [60]. AtTIP2;1 (At5g16240) is downregulated in glu1-2 leaves, whereas AtTIP2;3 (At5g47450) is downregulated in glu1-2 roots (Table 2).

NMR analysis of glu1-2 mutant roots and leaves reveal differences in amino acid contents

Although the Fd-GOGAT deficient gluS/glS mutants did not seem to be impaired in primary nitrogen assimilation [9], it was later shown that Fd-GOGAT1/GLU1 indeed plays a role in primary nitrogen metabolism [5]. As described above, knocking down Fd-GOGAT1 in the glu1-2 mutant affected several genes involved in primary nitrogen assimilation but the effects at the transcriptional level were more moderate than expected. Metabolite analysis by NMR spectroscopy of glu1-2 mutant tissue was performed to assess metabolic changes in the mutant.

Using this technique, the four amino acids alanine, threonine, valine and glutamine were identified and some changes in their levels in the glu1-2 mutant were revealed (Table 3; Figure 9). Most importantly glutamine contents were increased more than eight-fold in glu1-2 leaves and more than two-fold in glu1-2 roots. This indicates that the loss of Fd-GOGAT1 is not compensated by Fd-GOGAT2 or NADH-GOGAT activity in the glu1-2 mutant. The fact that the latter two enzymes are more active in roots than in leaves [7] is consistent with a smaller increase in glutamine levels in glu1-2 roots than in leaves. Higher glutamine contents have been previously described for Fd-GOGAT deficient plants of A. thaliana, barley and tobacco [9-11,61]. Principal component analysis (PCA) of the NMR spectra indicates that glutamine is to a large extent responsible of the changes detected between the wild-type and the glu1-2 mutant (Figure 9A, left panel). PCI explains 79% of the total explained variance in leaf samples and allows to discriminate between glu1-2 and wild-type leaf samples (Figure 9A, right panel). Conversely to the increase in glutamine, the levels of glutamate are expected to be reduced in the glu1-2 mutant. Reduced levels of glutamate have been described for Fd-GOGAT deficient barley and A. thaliana plants [10,61,62]. The levels of valine were also considerably increased in glu1-2 leaves, consistent with previous reports on Fd-GOGAT deficient tobacco plants ([11]; Figure 9B). This is however difficult to explain by the transcriptional profiling of the glu1-2 mutant. Indeed, only two genes of the valine biosynthetic pathway are slightly upregulated (Figure 8; Table 2) and genes involved in the degradation of valine are not affected. In addition, the diversion of this pathway towards leucine biosynthesis via the use of α-ketoisovalerate seems rather upregulated than downregulated (data not shown).

Reduced foliar levels of alanine have previously been observed in Fd-GOGAT deficient barley and tobacco plants [11,61]. Threonine levels were only affected in glu1-2 leaves, showing a slight reduction (Figure 9B). In Fd-GOGAT deficient tobacco plants the levels of foliar alanine and threonine were respectively reduced and unaffected [11]. The reduced levels of alanine and threonine in glu1-2 leaves are accompanied by an overall upregulation in gene expression in the biosynthetic pathways of these amino acids (Figure 8). Other amino acids were not identified by NMR, but changes in several amino acids have been previously reported in Fd-GOGAT deficient plants [10,11].

Analysis with PathExpress identified starch and sucrose metabolism as being downregulated in leaves and roots of the glu1-2 mutant (see above). This is not surprising considering the close interaction between C and N metabolism through the GS/GOGAT cycle. Differences in the contents of sucrose and glucose were not observed by NMR spectroscopy of glu1-2 leaves and roots (data not shown). This is consistent with results from tobacco plants with reduced Fd-GOGAT activity [42].

Table 3 Contents of some amino acids in wild type and glu1-2 plants

|          | glutamine | valine | alanine | threonine |
|----------|-----------|--------|---------|-----------|
| Leaves   |           |        |         |           |
| wild type| 5.07 ± 0.91| 0.21 ± 0.04| 1.15 ± 0.18| 0.64 ± 0.07|
| glu1-2   | 42.90 ± 0.39| 0.76 ± 0.19| 0.42 ± 0.09|       |
| mutant   | 5.42 ± 0.09|        |         |           |
| roots    |           |        |         |           |
| wild type| 6.48 ± 1.51| 0.61 ± 0.03| 1.16 ± 0.09|       |
| glu1-2   | 15.08 ± 2.71| 0.93 ± 0.16| 1.26 ± 0.17|       |

Glutamine, valine, alanine and threonine contents in glu1-2 and wild type leaves and roots measured by NMR spectroscopy. Values are expressed in μmol g⁻¹ fresh weight ± standard deviation (n = 4). n.i. = not integrated due to overlapping resonances.
Secondary responses of the glu1-2 mutant revealed by transcripational profiling

Photosynthesis and related biochemical processes are affected in glu1-2 mutant leaves

Photosynthesis and related biochemical processes were recurrently identified in our unbiased search of affected pathways (Additional files 2, 3 and 4). Especially the GO term GO:0015979 (photosynthesis), and to a lesser extent GO:0019684, GO:0042548 and GO:0042549, was revealed by GOstat as being affected in several of our datasets (Additional file 2). If only the GO terms related to photosynthesis that were identified by GOstat as being affected are taken into consideration, 37 genes show different expression levels in glu1-2 leaves (Additional file 6). All but one of these are repressed and the level of repression varies from \( \log_2 = -0.32 \) to \(-1.76\). Although the TAIR database to some extent categorises different genes into these GO terms, these are also almost exclusively downregulated (Additional file 6). If all genes included in GO:0015979 and its
subcategories as provided by AmiGO are taken into consideration, the list contains 49 unique genes not identified by GOstat whose expression is affected in the glu1-2 mutant leaves. Of these only five genes are (moderately) upregulated. The photosynthesis related bins of the MapMan software (Figures 4 and 10) identify another 71 genes with changed expression in glu1-2 leaves. All but ten of these are downregulated (Additional file 6). Hence, a total of more than 150 genes attributed to photosynthesis related pathways are affected in glu1-2 leaves, of which approximately 90% are downregulated. In glu1-2 roots, 52 genes attributed to the photosynthesis related GO terms or bins show a differential expression and these are exclusively induced (Additional file 6).

Several genes involved in the Calvin cycle are thus affected in the glu1-2 mutant, with most of them being downregulated in leaves and upregulated in roots (Figure 10). The most downregulated gene in the Calvin cycle in glu1-2 leaves is encoding a putative fructose-biphosphate aldolase (At4g26530).

A process directly linked to photosynthesis and the Calvin cycle is photorespiration, a consequence of the oxygenation of ribulose-1,5-biphosphate (RuBP) by RuBP carboxylase/oxygenase [3]. This oxygenation produces 3-phosphoglycerate and 2-phosphoglycolate, the latter being recycled into 3-phosphoglycerate by the photorespiratory cycle. In this process O2 is consumed in the plastid and CO2 and ammonium are released in the mitochondria. The released ammonium is reassimilated by the GS/GOGAT pathway in the plastid (for review: [63]). The essential role that glutamate synthase plays in this process is evidenced by the fact that mutants deficient in Fd-GOGAT activity exhibit a photorespiratory-dependent lethal phenotype [5,9]. Transcriptional profiling of the glu1-2 mutant indeed shows that genes involved in most of the steps of the photorespiratory cycle are downregulated in leaves (Figure 11; Table 4). These include the genes encoding phosphoglycolate phosphatase (PGLP1; At5g36700) and glyceralate kinase (GLYK; At1g80380) of plastids and mitochondrial serine hydroxymethyltransferase (SHM1;
At4g37930). Knock-out mutants of these genes exhibit a conditional lethal photorespiratory phenotype [31,64,65]. The gene At1g68010 encoding the peroxisomal NADH-dependent hydroxypyruvate reductase HPR1 is downregulated in glu1-2 leaves with no concomitant change of At1g79870 encoding the cytosolic isoform HPR2 that provides a cytosolic bypass to the photorespiratory cycle [66]. The gene encoding the dicarboxylate transporter DiT2.2 is also repressed in glu1-2 leaves. Its role is yet unclear, although the homolog DiT2.1 has been identified as a glutamate/malate translocator with an essential role in photorespiration [67]. A notable exception to the general downregulation of photorespiration-related genes is At2g22500 encoding one of the three recently characterised mitochondrial dicarboxylate carriers (DIC1-3) that are able to transport malate and oxaloacetate among other substrates [68]. In glu1-2 roots the expression of a smaller number of genes involved in photorespiration is affected by the glu1-2 mutation, and all these are upregulated (Table 4). It should also be noted that the changes in expression levels for both leaves and roots are moderate, which may explain the fact that our glu1-2 mutant has a less severe photorespiratory phenotype than the Fd-GOGAT1 mutants.
characterised previously [5,9]. In addition, Takahashi et al. [69] showed that mutants impaired in photore-
spiration have accelerated photoinhibition of the photo-
system II and that this is due to a suppressed repair
process through inhibition of the D1 protein translation.

Due to the dual-targeting of glutamine synthetase 2
(GS2) to the plastid and mitochondria [22], other
schemes of the reassimilation of ammonium than the
photorespiration cycle depicted (Figure 11) and dis-

cussed here, have been proposed [2].

Chlorophyll is synthesised from glutamate. Many of
the reactions of the chlorophyll biosynthetic pathway
are downregulated (Figure 12), although the fold
changes in expression are moderate (Additional file 6).
However, two genes (At1g03630 and At5g54190) encod-
ing protochlorophyllide reductases that catalyse the last
step leading to the synthesis of chlorophyllide a
(Figure 12), show a more pronounced downregulation.
Downregulation of genes implicated in chlorophyll bio-
synthesis and other photosynthesis related pathways,
such as photorespiration (discussed above), in glu1-2
mutant leaves are consistent with its chlorotic pheno-
type (Figure 2). A chlorotic phenotype has been
observed and reduced total chlorophyll contents have
been measured in Fd-GOGAT deficient plants of A.
thaliana, tobacco and barley [5,9,11,61]. Barley plants
with reduced Fd-GOGAT activity have also been shown
to have a reduced chlorophyll a/b ratio [61]. Takahashi
et al. [69] hypothesised that depletion of glutamate in
the Fd-GOGAT mutant may lead to accumulation of
glyoxylate may involve of the
photosynthetic rate.

The glu1-2 mutant displays a multiple stress response
Categorisation of genes affected in leaves and roots of
the glu1-2 mutant presented above showed a high num-
er of genes related to stimulus and stress responses.
More detailed analysis of the affected genes revealed
that these changes in expression can not be attributed to a
specific stress. Indeed, genes responsive to a
multitude of different abiotic stresses, including light,
drought, salt, heat and cold, oxidative stress and osmotic
stress were affected (Additional file 2). This indicates
that knocking down Fd-GOGAT1 leads to a secondary
response that consists in the activation of multiple stress
responses or the activation of mechanisms that are com-
mon to several stresses. Due to the extensive nature of
these transcriptional responses, only a selection will be
presented briefly in the following section and the reader

### Table 4 Genes that are involved in the photorespiratory pathway and that are differentially expressed between the glu1-2 mutant and the wild-type mutant

| EC # | Enzyme/protein | Abbreviation | Gene ID | log₂ root | log₂ leaf |
|------|----------------|--------------|---------|-----------|-----------|
| 3.1.3.18 | phosphoglycolate phosphatase | putative PGLP AtPGLP1 | AT5G36790 AT5G36700 | 0.674 | -0.658 |
| 1.1.3.15 | glycolate oxidase | GOX | AT3G14415 AT3G14420 | 0.655 | -0.464 |
| 2.6.1/2.6.14 | glutamate-glyoxylate aminotransferase | GGAT2 | AT1G0580 | -0.680 |
| 2.6.1/2.6.14 | glycolate oxidase complex – H protein | AtGdh3 | AT1G32470 | 0.593 |
| 2.6.1/2.6.14 | glycolate oxidase complex – H protein | AtGdh3 | AT1G32470 | 0.593 |
| 1.8.1.4 | glycolate oxidase complex – L protein | Atgdp1 | AT3G7240 | 0.689 |
| 1.4.1.2 | glycine decarboxylase complex – P protein | Atgdp1 | AT4G33010 | 0.439 | -1.126 |
| 1.4.1.2 | glycine decarboxylase complex – P protein | Atgdp2 | AT2G26080 | -0.532 |
| 2.1.1.10 | glycine decarboxylase complex – T protein | AtGTD1 | ATG11860 | -0.600 |
| 2.1.1 | serine hydroxymethyltransferase | Srm1 | ATG37930 | -0.513 |
| 2.6.1.45 | serine-glyoxylate aminotransferase | AsGT | ATG8360 | -0.566 |
| 1.1.129 | peroxisomal hydroxypyruvate reductase | Hpr1 | ATIG88010 | 0.705 | -0.689 |
| 2.1.31 | glycerate kinase | Glyk | ATG8380 | -0.554 |
| 1.1.37 | glyoxyosomal malate dehydrogenase | Mdhg1/ pMdH2 | ATG9960 | -0.851 |
| 1.4.1.7 | Fd-dependent glutamate synthase | Fd-GOGAT 1/GLU1 | ATG50440 | -5.738 |
| 1.4.1.7 | Fd-dependent glutamate synthase | Fd-GOGAT 2/GLU2 | ATG4220 | 1.070 |
| mitochondrial dicarboxylate transporter | Mitochondrial dicarboxylate transporter | Dit2 | ATG46280 | -0.439 |
| mitochondrial dicarboxylate transporter | Mitochondrial dicarboxylate transporter | Cdc1 | ATG22500 | 0.702 |

List of genes encoding enzymes involved in the photorespiratory pathway and whose expression is affected by knocking down Fd-GOGAT1 in the glu1-2 mutant. See also Figure 11. The presence of two gene IDs in some boxes indicates a nondiscriminating probe, i.e. the change in expression can not be attributed to a specific gene.
is kindly referred to Additional files 7 to 14 for the details on the affected genes.

**Key stress responsive transcription factors affected in the glu1-2 mutant** Numerous genes (putatively) encoding transcription factors belonging to different families, such as the zinc finger proteins (WRKY, C2H2, CCCH), MYBs and bHLHs (Additional file 7), were affected in the glu1-2 mutant.

WRKY25, whose encoding gene At2g30250 showed the highest induction of WRKY transcription factors in glu1-2 leaves, has been implicated in the defence against *Pseudomonas syringae* [70]. WRKY25 expression has also been shown to be induced in response to heat shock, wounding and oxidative stress. Higher levels of WRKY25 transcripts were detected in cytosolic ascorbate peroxidase Apx1-deficient plants, which maintain a high steady state level of H2O2 and activate ROS defence mechanisms [71].

The C2H2-type zinc finger transcription factor ZAT12 plays an important role in oxidative and abiotic stress response [71-73] and its encoding gene At5g59820, is upregulated in glu1-2 mutant leaves. ZAT12 expression is, like WRKY25, induced by heat shock, wounding and oxidative stress and higher transcripts were also detected in cytosolic ascorbate (Apx1)-deficient plants [71]. ZAT12 expressing plants can tolerate oxidative stress [71] and show an increased freezing tolerance [73].

Five genes (putatively) encoding bHLH transcription factors were among the 5% most repressed genes in glu1-2 leaves. While for BHLH101 (At5g04150), and CCA1 (At2g46830), which are associated with the circadian clock and act as negative regulators of the periodic flowering pathway [74], were strongly downregulated in glu1-2 leaves respectively. A role of LHY and CCA1 in the response to abiotic stresses has also been proposed [75].

**Flavonoid biosynthesis is affected in the glu1-2 mutant** Flavonoids, which were revealed as affected in the gene sets, are a class of compounds that act for...
example as protection against abiotic and biotic stresses and their concentrations increase in response to these [80].

Flavonoids are produced from phenylalanine which is synthesised through the shikimate pathway (Figures 13 and 14). In leaves of the *glu1-2* mutant the phenylalanine metabolism was identified as being negatively affected whereas flavonoid biosynthesis was identified as being positively affected on the transcriptional level (Additional file 2). This could be interpreted as reduced levels of phenylalanine and increased amount of flavonoids and related compounds in *glu1-2* mutant leaves, although tobacco plants with reduced Fd-GOGAT activity accumulate phenylalanine and tyrosine [11].

Although the expression levels of a fair number of genes implicated in the shikimate pathway were affected
in *glu1-2* leaves (Figure 13), a more detailed analysis shows that most genes are only moderately affected. In addition, no clear trend towards up- or downregulation of the pathway is visible (Additional file 8). Indeed, on one hand the genes encoding 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase DAHPS that have been identified as important targets for regulation of the shikimate pathway are not affected in the *glu1-2* mutant. On the other hand, the genes encoding 3-dehydroquinate synthase (DQS) and 3-dehydroquinate dehydratase (DHQD) are slightly induced in *glu1-2* leaves but do not constitute important regulatory steps [80]. One of two putative prephenate aminotransferase-encoding genes (*At2g38400*) is downregulated in *glu1-2* leaves. Prephenate aminotransferase transfers the amino group from glutamate or aspartate for the synthesis of arogenate. Genes putatively encoding arogenate dehydratase that catalyses the last step of the shikimate path-

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**Figure 14 Changes in gene expression in flavonoid biosynthesis of the *glu1-2* mutant.** Simplified schematic representation of the flavonoid biosynthetic pathway and the genes showing different expression levels in *glu1-2* leaves and root. Gene IDs indicated in blue are upregulated whereas those indicated in red are downregulated. Enzymes: (1) phenylalanine ammonia lyase (PAL), (2) cinnamate 4-hydroxylase, (3) 4-coumarate-CoA ligase, (4) chalcone synthase, (5) chalcone isomerase, (6) flavanone 3-hydroxylase, (7) flavonoid 3'-hydroxylase, (8) flavonol synthase, (9) flavonol glycosyltransferase. The figure is based on [80]. The detailed data of the genes depicted in this figure are presented in Additional file 8.
way leading to the formation of phenylalanine, were either induced, repressed or unaffected in *glu1-2* leaves (Figure 13). This complex behaviour of the shikimate pathway in response to various treatments and its regulation by feedback mechanisms has been described [80].

As to the flavonoid pathway as such (Figure 14), it may be worth pointing out that although three (PAL2 to 4) of the four genes encoding phenylalanine ammonia lyases that putatively catalyse the first step of the phenylpropanoid pathway [80] are slightly downregulated in *glu1-2* leaves, PAL1, whose effect on flavonoid accumulation has been shown, was not affected. Among the various affected genes was the gene *At5g13930* encoding chalcone synthase, the first committed enzyme in flavonoid synthesis, which was upregulated in leaves and roots of the *glu1-2* mutant. *CHS* expression has been shown to be induced by sugar, high light, UV and blue light, and phosphorus and nitrogen depletion [80]. Two genes encoding UGTs that catalyse *in vitro* the transfer of glucose from UDP-glucose to the 7-OH position of flavonols were among the twenty most highly upregulated ones in *glu1-2* leaves: UGT73B1 [81] and UGT73C6 [82]. UGTs that are involved in flavonoid biosynthesis are essential for the accumulation of flavonoids [80]. It should however be noted that the UGT73C6 is recognised by a probe that also recognises UGT73C5 and the increased signal could therefore also be due to an increased expression of the latter. UGT73C5 is responsible for glycosylating brassinosteroids [83].

In *glu1-2* roots, PathExpress identifies flavonoid biosynthesis in both down- and upregulated genes, whereas it was revealed by GeneBins only among downregulated genes (Additional files 3 and 4). This downregulation of genes in roots is however hard to sustain by looking at the reduced number of affected genes whose role in this pathway has been confirmed. As to the regulators of the flavonoid biosynthetic pathway, PAP1/MYB75 is the only implicated transcription factor that is affected in *glu1-2* roots, where it is slightly upregulated (Additional file 8). PAP1/MYB75 is a positive regulator of the flavonoid biosynthetic pathway and is strongly induced by nitrogen deficiency [80]. However the genes encoding PAP2 and MYB12, two other transcription factors of this pathway that are also induced by nitrogen deficiency [80], are not affected in the *glu1-2* mutant.

The multidrug resistance-related protein (MRP)-type ABC transporters have been implicated in the vacuolar sequestration of phenolic compounds such as flavonoids and anthocyanins, and flavonoids have been suggested as negative regulators of MDR-type members (for review: [84]). A long distance transport of flavonoids by the MRP-type of ABC transporters was also recently suggested, although the transporter and the mechanism were not identified [85]. In total, 11 of the 15 AtMRP-encoding genes are induced in *glu1-2* leaves, whereas only two are affected in *glu1-2* roots (Additional file 9). The AtMRP2 to AtMRP5 are ATP-dependent pumps for organic ions [86] and, interestingly, AtMRP2 and AtMRP3 are able to transport glutathione S-conjugates and chlorophyll catabolites into vacuoles [87,88]. AtMRP4 [89] and AtMRP5 are supposedly involved in regulating ion channel activities in guard cells but also have transport activity: folate for AtMRP4 and E217G for AtMRP5. Folate mediates large metabolic fluxes mainly during photorespiration as a cofactor of the mitochondrial GDC/SHM (mentioned before) complex [86]. The roles of the other MRP transporters affected in *glu1-2* leaves have not yet been described, although the contribution of AtMRP12 in detoxification seems to be marginal [88] and AtMRP15 may possibly constitute a pseudogene [90]. Based on gene expression, Klein et al. [86] speculate that AtMRP14 could be involved in processes controlling seed integrity and germination efficiency such as the regulation of dormancy.

**Cytochrome P450 monooxygenase-encoding genes are affected in the *glu1-2* mutant** Another group of enzymes related to stress response are cytochrome P450 monooxygenases. They are known to be involved in the synthesis of structural components, hormones, signalling molecules (e.g. SA and JA) and defense compounds (flavonoids, phytoalexins, glucosinolates). A large number of cytochrome P450s are responsive to hormones, signalling molecules and environmental stresses (for review: [91]). The functions of most of the 245 members of this group of enzymes in *A. thaliana* have not yet been identified, and it is therefore impossible to attribute most of these to particular pathways. Cytochrome P450s are categorised into the gene ontology term GO:0006118 (electron transport), which was revealed as being overrepresented (together with its parent GO:0006091) by GOstat analysis (Additional file 2).

In the *glu1-2* mutant cytochrome P450s were especially frequent among the most upregulated and downregulated genes in roots. In total 22 and 39 genes annotated as encoding cytochrome P450 are affected in roots and leaves respectively (Additional file 10), indicating that proportionally a greater number of cytochrome P450s were responsive in roots.

Of the 22 genes affected in roots, 9 are repressed and 13 are induced (Additional file 10). Five cytochrome P450 encoding genes (*CYP712A1*, 76G1, 93D1, 716A2, 96A12 in descending order of induction) are among the 10% most highly induced genes and all but one are
induction in cytochrome P450-encoding genes that show the highest whereas 16 were repressed (Additional file 10). The two tripeptide) with electrophilic compounds, to form non- oxidative damage and metal exposure [104]. AtGSTF2 has also been shown to be repressive to several stimuli and the protein interacts with flavonoids in vitro [105].

UDP-glycosyltransferases (UGTs) use UDP-activated sugars as donor to catalyse the glycosylation of various metabolites and are hence implicated in a series of mechanisms and pathways, including phase II of the detoxification mechanism.

In glu1-2 mutant leaves 45 UGT-encoding genes are affected, which represents a third of the approximately 120 UGT-encoding genes that have been identified in the A. thaliana genome (for review: [106]). Thirty-six are induced in glu1-2 leaves and fourteen of these are among the top 5% induced genes (Additional file 12). UGT73B1 and UGT73C6, already mentioned above, are involved in flavonoid biosynthesis [81, 82]. In vitro essays with UGT73B4 (At2g15490) showed that it was able to glycosylate both the 3-OH and the 4-OH position of the benzoate derivative 3,4-dihydroxybenzoic acid [107]. UGT75B1 (At1g02930) and At1g02920 (At1g02920 dual probe) are responsive to cold and heat stress, oxidative damage and metal exposure [104]. AtGSTF2 has also been shown to be repressive to several stimuli and the protein interacts with flavonoids in vitro [105].

The detoxification of secondary metabolites and xenobiotics is induced in glu1-2 Another indication that glu1-2 mutants deploy a general stress response is the change in expression of genes involved in the detoxification of secondary metabolites and xenobiotics.

Some of the cytochrome P450-encoding genes that are affected in the glu1-2 mutant may play a role in the phase I of this detoxification [98]. Glutathione S-transferases (EC 2.5.1.18; GSTs), also known as glutathione transferases (GTs), are involved in the second phase of detoxification processes. These enzymes catalyse the conjugation of glutathione (GSH-tripeptide) with electrophilic compounds, to form non-toxic derivatives that are ready to be compartmentalised in vacuoles. In addition, GSTs can serve as peroxidases, isomerases and thiol transferases or have non-catalytic functions such as ligand binding and modulation of signalling processes (for review: [99]). Several studies that have analysed the responsiveness of AtGSTs to different stimuli have revealed the complex nature of the regulation of these genes (for review: [100]). In glu1-2 leaves the expression of 26 genes coding for (putative) GSTs belonging to the tau (GSTU), phi (GSTF) and zeta (GSTZ) classes are affected, 20 of these being induced and 6 being repressed. In glu1-2 roots, four glutathione S-transferases are induced and two are repressed (Additional file 11). Eight GSTU-encoding genes are among the 5% most upregulated genes in glu1-2 leaves and AtGSTU24 (At1g17170), the most highly induced of them, was reported to be induced by herbicide treatment, xenobiotic exposure and in the catalase 2 deficient mutant (characterised by intracellular redox perturbation and activation of oxidative signalling). It was hypothesised that AtGSTU24 could be involved in the conjugation of stress-induced catabolites ([101] and references therein). AtGSTU24 has also been shown to be SA inducible [102]. Several of the other highly upregulated GSTU-encoding genes, such as GSTU1, 2, 4, 7, 9, 19, 22, 25 are induced in response to salt stress [103]. As to members of the phi class that are induced in glu1-2 leaves, AtGSTF6/GSTF7 (At1g09290/At1g09290 dual probe) are responsive to cold and heat stress, oxidative damage and metal exposure [104]. AtGSTF2 has also been shown to be repressive to several stimuli and the protein interacts with flavonoids in vitro [105].

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UGT85A1 (At1g22400) and UGT76C1 (At5g05870). Recombinant UGT73C1 is also able to conjugate transformation products of the explosive 2,4,6-trinitrotoluene [111]. UGT84B1 (also known as IAGLU) glucosylates indole-3-acetic acid [112] and the encoding gene At4g15550 is also upregulated in glu1-2 leaves. UGT74F2 (At2g34820) has been described as a salicylic acid glucosyltransferase [107,113] and it has also been hypothesised as playing a role in tryptophan biosynthesis [114].

Several of the UGT-encoding genes that are highly upregulated in glu1-2 leaves have been shown to be stress responsive. Recently, UGT74E2 (At1g05680) was identified as one of eight genes that were rapidly and highly induced upon treatment with the herbicide imidazolinone [95]. This gene was the most highly induced in glu1-2 leaves. UGT73B1, UGT73B2 and UGT73B3 have been implicated in the response to oxidative stress and a role in stress response and resistance to Pseudomonas syringae was shown for UGT73B3 and UGT73B5 [115,116].

Phase III of xenobiotic detoxification in plants consists of storage of the compounds produced by the mechanisms of two first phases. The multidrug and toxic compound extrusion (MATE) efflux carriers may function in this process, although the transport activities and exact roles of most of these have not yet been described [117].

Fourteen of the 58 MATE protein-encoding gene of A. thaliana are induced and five are repressed in glu1-2 leaves (Additional file 13). Three are among the top fourteen induced genes in glu1-2 leaves: AtDTX1 (At2g04040), AtDTX3 (At2g04050) and AtDTX4 (At2g04070). AtDTX1 serves as an efflux carrier for plant derived alkaloids [118]. To our knowledge, the functions of AtDTX3 and AtDTX4 have not yet been reported but AtDTX4 was also among the genes that were rapidly induced by the herbicide imidazolinone [95].

Numerous other responsive genes linked to a number of different stresses/stimuli are induced in glu1-2 leaves Besides the stress responsive genes and those involved in detoxification mechanisms that were discussed in more detail above, numerous other stress-related genes were affected in the glu1-2 mutant. It is beyond the scope of the present article to describe these in detail. This can however be exemplified by the striking overlap between the genes induced in leaves of the glu1-2 mutant and those described by Vanderauwera et al. [119] in the catalase 2 deficient CAT2HP1 plant. Of the 55 genes induced more than 3 fold in the CAT2HP1 plant, 45 were also induced in glu1-2 mutant leaves (Additional file 14), including cytochrome P450-, GST- and UGT-encoding genes already mentioned above. Other similarities in transcriptional responses can be detected between glu1-2 mutant plants and plants submitted to cold treatment [73], heat stress [120] and herbicide treatment [95](data not shown). This also exemplifies that the transcriptional response of the glu1-2 mutant bears the signatures of a multiple stress response.

Conclusions
Knocking down the expression of the gene Fd-GOGAT1 coding for one of the two ferredoxin-dependent glutamate synthases, which catalyse the ultimate step in the biosynthesis of glutamate, and a key enzyme in the assimilation of inorganic nitrogen, has marked effects on the transcriptome of the plant as evidenced by our microarray analysis of the glu1-2 mutant. Even more so as the assayed plants were grown in vitro on 1x MS media supplemented with 3% sucrose, and nitrogen supply should not be limiting either under these conditions. Hence, the transcriptional profiling should be viewed under the angle that the growth conditions that were used in the present study do not constitute the most severe conditions possible for the glu1-2 mutant. This is also indicated by the fact that the glu1-2 mutant described here develops more slowly than wild-type plants when grown on soil, but does eventually complete its life cycle (data not shown). The Fd-GOGAT1 deficient A. thaliana mutants described previously display a lethal phenotype [5,7,9]. It should however be noted that the growth conditions for the glu1-2 mutant used here, did not prevent plants from showing a chlorotic phenotype, although less severe than in previously published studies.

As Fd-GOGAT1 expression is much higher in leaves than in roots of A. thaliana at the steady state ([5]; Additional file 1), the effects were expectedly of a larger scale in the leaves than in the roots, both in regard to the number of affected genes and the levels of regulation. The level of downregulation of Fd-GOGAT1 itself in the glu1-2 mutant is also much more pronounced in leaves than in roots.

Although an effect can be seen on the expression levels of genes involved in primary nitrogen assimilation, glutamate metabolism and related pathways, the number of such genes affected and the scale of changes are more moderate than we expected. This may be explained by the fact that the defect in primary nitrogen assimilation exhibited by Fd-GOGAT1 mutants are specific to conditions when photosynthesis is suppressed [5], which is not the case under the experimental conditions chosen here. Upregulation of Fd-GOGAT 2 and NADH-GOGAT may also compensate to some extent
for the loss of Fd-GOGAT1. Metabolic profiling confirmed the expected increase in glutamine levels in the glu1-2 mutant but revealed also changes in the levels of other amino acids.

Photosynthesis and related pathways are overall down-regulated, which is consistent with the chlorotic phenotype of the glu1-2 mutant and the reduced amount of total chlorophyll measured in gls mutants [5]. The flavonoid biosynthesis was also revealed as being affected, although a more detailed analysis of the affected genes did not reveal major changes in expression levels, except for genes involved in the production of flavonoid glycosides.

The most pronounced effect at the transcriptomic level could however be seen on genes that are responsive to abiotic stresses and stimuli. Genes that had been described before as being responsive to cold, heat and drought could be identified as being upregulated in the mutant, mostly in leaves. Especially striking is the way that oxidative stress response genes and genes involved in detoxification of secondary metabolites are affected in the glu1-2 mutant.

Fd-GOGAT plays an important role in the photosynthetic cycle by participating in the reassimilation of released ammonia. Deregulation of photorespiration in the glu1-2 mutant may lead to a reduced elimination of excess excitation energy, and hence an imbalance in the redox status. In addition NH4+ that accumulates due to the lack of reassimilation through the GS/GOGAT cycle may be perceived by the plant as a toxic compound, triggering a global stress response.

Methods
Plant material
The A. thaliana T-DNA insertion line SALK_019917 for Fd-GOGAT1 (At5g04140) was identified in the SALK T-DNA insertion mutant collection [121], seeds were obtained from the European Arabidopsis Stock Centre NASC [122] and homozygous mutant plants (called glu1-2) were obtained. The T-DNA insertion was checked by PCR amplification using a T-DNA primer and a Fd-GOGAT1 specific primer, and subsequent sequencing of the amplicon. A. thaliana ecotype Col-0 was used as wild-type control in all described assays.

Plant growth conditions
Wild-type Col-0 and mutant glu1-2 seeds were surface sterilised and sown on solid in vitro cultivation medium consisting of 1x Murashige and Skoog basal salt mixture, 3% sucrose, 0.75% phytoagar (w/v), pH5.7. This medium contains 1650 mg ammonium nitrate/L. Seeds were stratified for 3 days at 4°C before being transferred to a controlled growth chamber under a 16 hour light period (light intensity: 75 μmol.m–2.sec–1) at 21-23°C.

Microarray analysis
For microarray experiments of wild-type and glu1-2 mutant plants, four biological replicates of each were processed simultaneously through the following procedure. Leaves and roots of 18 day old in vitro grown plantlets were harvested separately and immediately flash-frozen in liquid N2. Harvesting of tissue was performed two hours after onset of the 16 hour light period. The harvested tissue was stored at -80°C until further processing. Total RNA was extracted from plant tissue (300 mg roots, 500 mg leaves) using the RNeasy Plant Midi kit (Qiagen, Hilden, Germany) following the supplier’s instructions. RNAsin (Promega, Madison, USA) was added to the RNA to the final concentration of 1 U/μl. RNA quality was assessed by standard denaturing agarose gel electrophoresis and RNA concentration was measured with a NanoDrop ND-1000 (Nanodrop, Delaware, USA). To prepare the samples for microarray experiments the “GeneChip Expression Analysis” procedure of Affymetrix (Santa Clara, USA) was followed. Briefly, 10 μg total RNA was processed through a one-cycle cDNA synthesis procedure, the resulting double-stranded cDNA was cleaned up and submitted to the synthesis of biotin-labelled cRNA. This biotin-labelled cRNA was then cleaned up, quantified and fragmented before being hybridized to Affymetrix GeneChip Arabidopsis ATH1 genome arrays. After 16 hours of hybridization the arrays were processed through the washing and staining procedures, before being scanned and analysed. Microarray data files have been deposited in the Gene Expression Omnibus (GEO accession number: GSE20493).

Statistical analysis of microarray data
The microarray data were preprocessed using the Robust Multichip Average (RMA) package [123] as implemented in R [124]. The data were normalized using quantile normalization, and expression measures were produced by fitting the RMA robust linear model. Differentially regulated genes were identified using moderated t-tests discussed in [125] and implemented in the Limma package for R. To adjust for the large number of hypothesis tests made, the q-value [126] associated with each p-value was also calculated. The q-value for a gene is the expected proportion of false positives one will get when calling that gene significant. For a gene to be considered significantly differentially expressed in this experiment its q-value was required to be lower than 0.01. In effect, this means controlling the false discovery rate (FDR) [127] in the experiment at a 0.01 level.

Genome annotation
Genome annotation as given in the tables originates from Affymetrix (Santa Clara, USA). This was supplemented/updated by information provided by The
Arabidopsis Information Service (TAIR; [128]) and the Salk Institute Genomic Analysis Laboratory (SIGnAL; [121]).

Biochemical pathways were assessed according to published data (see referred articles at the respective places in the text) and information provided by the AraCyc database [129], the Kyoto Encyclopedia of Genes and Genomes (KEGG; [130]) and the Expert Protein Analysis System (ExPASy; [131]).

Classification into GO terms were taken over from the software in question (e.g. GOstat, MapMan) but AmiGO [132] and the GO annotation at TAIR [128] were used for more extensive searches.

NMR spectroscopy of total metabolites and principal component analysis (PCA)

Total metabolites were extracted from freeze-dried plant material according to Liang et al. [133] with some modifications. Fresh plant material (0.5 g) was freeze-dried and extracted with 1.5 ml mixture of KH2PO4 buffer (90 mM, pH 6.0) in D2O containing 0.05% TSP (trimethylsilyl propionic acid sodium salt, w/v) and methanol-d4 (1:1). The extract was vortexed for 30 s, centrifuged at 4500 rpm for 10 min and filtered through glass wool. Five hundred microliters of the supernatant were taken for NMR spectroscopy analysis.

NMR spectroscopy was performed at 25°C on a Bruker DRX 600 spectrometer (Bruker, Rheinstetten, Germany) resonating at 600.13 MHz fitted with a 5 mm BBO probe. The frequency lock was done on MeOD. 1D 1H-NMR spectra were recorded with presaturation of the residual water resonance in the interscan delay using a standard Bruker pulse sequence (zgpr). A 90° excitation pulse was used to record 256 FID’s with a spectral width of 8389 Hz averaged into 32 k data points with an acquisition time of 3.91 s. The interscan delay was 3 s. 1H, 1H-COSY NMR spectra with the same spectral width as the 1D spectra were recorded with 2048x512 data points in the F2xF1 directions. Presaturation of the residual water resonance was done in the interscan delay using a standard Bruker pulse sequence (cosyqfpr). 16 FID’s were recorded for each increment in the F1 direction. A square sine bell window function was applied in both directions and zero filling in the F1 direction was applied to give the processed spectrum a resolution of 2048x2048 data points.

The NMR spectra were exported from TOPSPIN and imported into R [124] where the datasets were compiled. SpecAlign [134] was used for peak alignment of the spectra. Principal Component Analysis (PCA) was performed in R using the pls library of Mevik et al.[135]. The loading plots from the analyses were used to identify the resonances that were different between the groups.

Acknowledgements

This work was funded by grants from the Norwegian Research Council (NFR). We would like to thank Torfinn Stasand and Marianne Nymark for excellent technical assistance.

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Authors’ contributions

RK, PW, TSJ, DHII, TRS and AMB drafted the manuscript. RK and PW analysed the transcriptional data. TSJ performed statistical analysis of the transcriptional data. TRS performed NMR spectroscopy and analysed NMR results. TC performed the verification of the T-DNA insertion mutant and a preliminary microarray characterisation on a home-made 2K chip. PW, DHII and AMB designed the study and AMB coordinated the study. All authors read and approved the final manuscript.

Additional file 1: Transcriptional data of glu1-2 Detailed lists of genes whose expression is affected in leaves and roots of 18 day old glu1-2 mutant plants.

Additional file 2: GOstat analysis of glu1-2 transcriptional data

GO biological process terms identified by GOstat [13] as being overrepresented (P = 0.01 with FDR/Benjamini correction) among the genes affected in glu1-2 mutant leaves and roots.

Additional file 3: PathExpress analysis of glu1-2 transcriptional data

Identification by PathExpress [14] of affected pathways among the sets of genes whose expression is affected in the glu1-2 mutant.

Additional file 4: GeneBins analysis of glu1-2 transcriptional data

BINs identified by GeneBins [15] as being affected in the datasets of genes that show a different expression in glu1-2 organs compared to wild type.

Additional file 5: 2-oxoglutarate synthesis and TCA cycle

Genes whose expression is affected in glu1-2 mutant leaves and that encode enzymes involved in 2-oxoglutarate synthesis and the TCA cycle.

Additional file 6: Photosynthesis and related pathways

Genes whose expression is affected in glu1-2 mutant leaves and roots and that encode enzymes involved in photosynthesis related GO categories and bins as identified by GOstat [13], AmiGO [132] and MapMan [12] respectively.

Additional file 7: Transcription factors

Genes encoding WRKY, C2H2, CCH, MYB, bHLH or AP2/ERF transcription factors and that show different expression in the glu1-2 mutant.

Additional file 8: Shikimate pathway and flavonoid biosynthesis

Genes implicated in the shikimate pathway and flavonoid biosynthesis, and that show different expression in the glu1-2 mutant.

Additional file 9: ABC transporters

Genes whose expression is affected in the glu1-2 mutant and that encode ABC transporters.

Additional file 10: Cytochrome P450 monooxygenases

Cytochrome P450-encoding genes whose expression is affected in the glu1-2 mutant.

Additional file 11: Glutathione S-transferases

Genes encoding glutathione S-transferases (GSTs) and that show different expression in the glu1-2 mutant.

Additional file 12: UDP-glycosyltransferases

Genes encoding UDP-glycosyltransferases (UGTs) and that show different expression in the glu1-2 mutant.

Additional file 13: MATE efflux family proteins

Genes coding for multidrug and toxic compound extrusion (MATE) efflux carriers and that show different expression in the glu1-2 mutant.

Additional file 14: Comparison with catalase 2 deficient plants

Comparison of induced transcriptional responses in catalase 2 deficient plants CAT2HP1 and glu1-2 mutant leaves.
dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. Plant Physiol 2006, 140:444-456.

22. Taira M, Valtersson U, Burkhardt B, Ludwig RA. Arabidopsis thaliana GLN2-encoded glutamine synthetase is dual targeted to leaf mitochondria and chloroplasts. Plant Cell 2004, 16:2048-2058.

23. Ferrario-Méry S, Masclaux C, Suzuki A, Valadier MH, Harel B, Fowler CH. Glutamine and alpha-ketoglutarate are metabolite signals involved in nitrate reductase gene transcription in untransformed and transformed tobacco plants deficient in ferredoxin-glutamine-alpha-ketoglutarate aminotransferase. Plants 2001, 213:265-271.

24. Lea PJ, Sodek L, Parry MAJ, Shewry PR, Halford NG. Asparagine in plants. Ann Appl Biol 2007, 150:1-26.

25. Lam HM, Wong P, Chan HK, Yam KM, Chen L, Chow CM, Coruzzi GM. Overexpression of the ASN1 gene enhances nitrogen status in seeds of Arabidopsis. Plant Physiol 2003, 132:926-935.

26. Lea PJ, Azededo RA. Nitrogen use efficiency. 2. Amino acid metabolism. Ann Appl Biol 2007, 151:269-275.

27. Igarashi D, Miwa T, Seki M, Kobayashi M, Kato T, Tabara S, Shinozaki K, Ohsumi C. Identification of photosynthetic glutamate/glutamyl aminotransferase (GGAT) gene in Arabidopsis. Plant J 2003, 33:975-987.

28. Miyashita Y, Dolfurus R, Ismord KP, Good AG. Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. Plant J 2003, 39:1108-1121.

29. Bauwe H, Kolukisaoglou U. Genetic manipulation of glycine decarboxylase. J Exp Bot 2003, 54:1523-1535.

30. Engel N, Daele van den K, Kolukisaoglou U, Margenthaler W, Weckwerth W, Parlk T, Keerberg O, Bauwe H. Deletion of glycine decarboxylase in Arabidopsis is lethal under nonphotosynthetic conditions. Plant Physiol 2003, 134:1328-1335.

31. Voll LM, Jamai A, Renne P, Voll H, McClung CR, Weber AP. The photosynthetic Arabidopsis shm1 mutant is deficient in SHM1. Plant Physiol 2006, 140:59-66.

32. Stocum RD. Genes, enzymes and regulation of arginine biosynthesis in plants. Physiol Plant Biochem 2005, 43:729-745.

33. Binder S, Knill T, Schuster J. ClueGO: a Cytoscape plug-in targeting of the two GDH genes products in leaves and stems of Arabidopsis thaliana and chloroplasts. Molec Res 2004, 29:667-680.

34. Ferrario-Méry S, Ho dgers M, Hirel B, Foyer CH. Prunus domestica photosynthetic serine hydroxymethyltransferase activity requires the mitochondrial accumulation of ferredoxin-dependent glutamate synthase. Plant Physiol Cell 2005, 21:595-606.

35. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 2004, 13:493-505.

36. Beissbarth T. Primary N-assimilation into amino acids in Arabidopsis. The Arabidopsis Book Rockville: American Society of Plant BiologistsSomerville C, Meyerowitz EM 2003.

37. Linka M, Weber AP. Shuffling ammonia between mitochondria and plastids during photorespiration. Trends Plant Sci 2005, 10:461-465.

38. Wang A, Lea PJ, Quick WP, Leegood RC. Photorespiration: metabolic pathways and their role in stress protection. Philos Trans R Soc Lond B Biol Sci 2000, 355:1517-1525.

39. Suzuki A, Knapp DE. Glutamate synthase: structural, mechanistic and regulatory properties, and role in the amino acid metabolism. Photosynth Res 2005, 83:191-217.

40. Coschigno KT, Melo-Oliveira R, Lim J, Coruzzi GM. Arabidopsis gls mutants and distinct Fd-GOGAT genes: implications for photorespiration and primary nitrogen assimilation. Plant Cell 1998, 10:741-752.

41. Ferrario-Méry S, Ho dgers M, Hirel B, Foyer CH. Prunus domestica photosynthetic serine hydroxymethyltransferase activity requires the mitochondrial accumulation of ferredoxin-dependent glutamate synthase. Plant Cell Physiol 2005, 46:1328-1335.

42. Ferrario-Méry S, Ho dgers M, Hirel B, Foyer CH. Prunus domestica photosynthetic serine hydroxymethyltransferase activity requires the mitochondrial accumulation of ferredoxin-dependent glutamate synthase. Plant Physiol Cell 2005, 21:595-606.

43. Udall B, Blasing OE, Gibson Y, Pope F, Hohnen M, Gunther M, Trethewey R, Kamlage B, Poorter H, Stitt M. Multilevel genomic analysis of the response of transcripts, enzyme activities and metabolites in Arabidopsis rosettes to a progressive decrease of temperature in the non-freezing range. Plant Cell Environ 2006, 31:518-547.

44. Masclaux-Daubresse C, Redisd-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A. Glutamine synthetase-glutamate synthase pathway and glutamate
55. De Angeli A, Monachello D, Ephritikhine G, Frachisse JM, Thomine S, Kissen P, Geelen D, Lurin C, Bouchez D, Frachisse JM, Lelievre F, Courtial B, Barbier-Byba H, Barbier-Brygoo H, Frommer WB, Koch W, Arabidopsis thaliana: Nitrate transporters and peptide transporters. FEBS Lett 2007, 581:2290-2300.

56. Yuan YZ, Ou JQ, Wang ZQ, Zhang CF, Zhou ZP, Lin QH: Characterization of a two-component high-affinity nitrate uptake system in Arabidopsis. Physiology and protein-protein interaction. Plant Physiol 2007, 143:305-319.

57. De Angeli A, Monachello D, Ephritikhine G, Frachisse JM, Thomine S, Kissen P, Geelen D, Lurin C, Bouchez D, Frachisse JM, Lelievre F, Courtial B, Barbier-Byba H, Barbier-Brygoo H, Frommer WB, Koch W, Arabidopsis thaliana: Nitrate transporters and peptide transporters. FEBS Lett 2007, 581:2290-2300.

58. Yuan YZ, Ou JQ, Wang ZQ, Zhang CF, Zhou ZP, Lin QH: Characterization of a two-component high-affinity nitrate uptake system in Arabidopsis. Physiology and protein-protein interaction. Plant Physiol 2007, 143:305-319.

59. De Angeli A, Monachello D, Ephritikhine G, Frachisse JM, Thomine S, Kissen P, Geelen D, Lurin C, Bouchez D, Frachisse JM, Lelievre F, Courtial B, Barbier-Byba H, Barbier-Brygoo H, Frommer WB, Koch W, Arabidopsis thaliana: Nitrate transporters and peptide transporters. FEBS Lett 2007, 581:2290-2300.

60. Yuan YZ, Ou JQ, Wang ZQ, Zhang CF, Zhou ZP, Lin QH: Characterization of a two-component high-affinity nitrate uptake system in Arabidopsis. Physiology and protein-protein interaction. Plant Physiol 2007, 143:305-319.
126. Storey JD: A direct approach to false discovery rates. *J R Stat Soc Ser B* 2002, 64:479-498.
127. Benjamini Y, Hochberg Y: Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 1995, 57:289-300.
128. The Arabidopsis Information Service (TAIR). [http://www.arabidopsis.org](http://www.arabidopsis.org).
129. AraCyc. [http://www.arabidopsis.org/biocyc/](http://www.arabidopsis.org/biocyc/).
130. Kyoto Encyclopedia of Genes and Genomes (KEGG). [http://www.genome.ad.jp/kegg/](http://www.genome.ad.jp/kegg/).
131. Expert Protein Analysis System (ExPASy). [http://www.expasy.org](http://www.expasy.org).
132. The Gene Ontology Database. [http://amigo.geneontology.org/cgi-bin/amigo/go.cgi](http://amigo.geneontology.org/cgi-bin/amigo/go.cgi).
133. Liang YS, Choi YH, Kim HK, Linthorst HJM, Verpoorte R: Metabolomic analysis of methyl jasmonate treated *Brassica rapa* leaves by 2-dimensional NMR spectroscopy. *Phytochemistry* 2006, 67:2503-2511.
134. Wong JWH, Cagney G, Cartwright HM: SpecAlign - processing and alignment of mass spectra datasets. *Bioinformatics* 2005, 21:2088-2090.
135. Mevik BH, Wehrens R: The pls package: Principal component and partial least squares regression in R. *J Stat Softw* 2007, 18:2.

doi:10.1186/1471-2164-11-190
Cite this article as: Kissen et al.: Transcriptional profiling of an *Fd-GOGAT1/GLU1* mutant in *Arabidopsis thaliana* reveals a multiple stress response and extensive reprogramming of the transcriptome. *BMC Genomics* 2010 11:190.

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