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ORIGINAL ARTICLE

Analysis of glutamate homeostasis by overexpression of *Fd-GOGAT* gene in *Arabidopsis thaliana*

Takashi Ishizaki · Chieko Ohsumi · Kazuhiko Totsuka · Daisuke Igarashi

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Abstract Glutamate plays a central role in nitrogen flow and serves as a nitrogen donor for the production of amino acids. In plants, some amino acids work as buffers: during photorespiration, ammonium derived from the conversion of glycine to serine is promptly reassimilated into glutamate by the glutamine synthetase (GS-2)/ferredoxin-dependent glutamate synthase (Fd-GOGAT) cycle. The glutamate concentration is relatively stable compared with those of other amino acids under environmental changes. The few studies dealing with glutamate homeostasis have but all used knockouts or mutants of these enzymes. Here, we generated *Fd-GOGAT* (*GLU1*)-overexpressing *Arabidopsis* plants to analyze changes in the amino acid pool caused by glutamate overproduction under different ammonium conditions controlled by CO_2 concentration, light intensity and nitrate concentration. Under photorespiratory conditions with sufficient ammonium supply, aspartate increased and glutamine and glycine decreased, but glutamate barely changed. Under non-photorespiratory conditions, however, glutamate and most other amino acids increased. These results suggest that the synthesized glutamate is promptly converted into other amino acids, especially aspartate. In addition, ammonium supply by photorespiration does not limit glutamate biosynthesis, but glutamine and glycine are important. This study will contribute to the understanding of glutamate homeostasis in plants.

Keywords *Arabidopsis* · Fd-GOGAT · Glutamate synthase · Photorespiration · GLU1

Abbreviations

CaMV	Cauliflower mosaic virus
GOGAT	Glutamate synthase (glutamine 2-oxoglutarate aminotransferase)
Fd-GOGAT	Ferredoxin-dependent GOGAT
GS	Glutamine synthetase
FW	Fresh weight
LHCB	Light-harvesting chlorophyll-*a*/*b*-binding protein
MS	Murashige and Skoog
PNS	Plant nutrients and sugar

Introduction

Glutamate is a key molecule in cellular metabolism and is the primary compound for the synthesis of amino acids and other metabolites. In plants, most glutamate is synthesized by two distinct glutamine synthetase/glutamate synthase (GS/GOGAT) cycles, one of which involves NADH-dependent GOGAT, the other of which involves ferredoxin-dependent GOGAT (Fd-GOGAT). Fd-GOGAT utilizes light energy to reassimilate the vast amounts of ammonium derived from photorespiration (Keys et al. 1978; Forde and Lea 2007). As glutamate occupies a major portion of the free amino acid pool, its amount stays relatively constant under various conditions (Geiger et al. 1998).

C. Ohsumi · K. Totsuka · D. Igarashi (✉)
Institute of Life Sciences, Ajinomoto Co., Inc.,
1-1, Suzuki-cho, Kawasaki-ku,
Kawasaki, Kanagawa 210-8681, Japan
e-mail: daisuke_igarashi@ajinomoto.com

Present Address:
T. Ishizaki
Accelrys K.K., Nishishinbashi TS Building 11F,
3-3-1 Nishishinbashi, Minato-ku, Tokyo 105-0003, Japan

Homeostasis of amino acids is well studied in mammals. Glutamate transporters in the brain control the extracellular glutamate concentration to prevent it from rising to neurotoxic levels (Takahashi et al. 1997). Elevated levels of extracellular glutamate are implicated in the neurodegeneration associated with Parkinson's disease (Han et al. 1997). Increased glutamate levels are correlated with inhibition of cysteine transport in HIV-infected patients (Droge et al. 1992; Han et al. 1997). These studies indicate that maintaining the glutamate pool within optimal limits is important in humans.

There are many reports of amino acid profiling in plants, but only a few deal with amino acid homeostasis (Forde and Lea 2007). In mature plants, amino acids are transported in the xylem and phloem, usually in the form of asparagine and arginine, which have a high N:C ratio and are thus appropriate for nitrogen storage (Lea et al. 2007). In developing plants, however, the mobility of amino acids produced in leaves is limited: the amino acids are either transported to developing leaves and roots or are stored as protein, predominantly rubisco (Staswick et al. 1994). Since glutamate is a highly reactive compound (being an amino-group donor in many reactions), and yet its level is maintained, there should be mechanisms that sustain the glutamate pool (Forde and Lea 2007).

In theory, glutamate is synthesized from two pathways: one from direct assimilation of ammonium to 2-oxoglutarate and the other from amino acids via aminotransferases. As soluble amino acids are abundant metabolites in plants, Forde and Lea (2007) hypothesized that the glutamate level is maintained by interdependent modulation of the pool size of the other major amino acids. To test this hypothesis, we generated plants overexpressing *GLU1*, one of two ferredoxin-dependent glutamate synthase (*Fd-GOGAT*) genes found in *Arabidopsis thaliana* (Coschigano et al. 1998), and analyzed their amino acid pool changes in comparison with the wild type. In addition, we measured the amounts of amino acids under various nitrogen and carbon flux conditions to determine which amino acids are primarily responsible as buffers for sustaining the glutamate level. This information could provide important knowledge about glutamate homeostasis in plants.

Materials and methods

Plant materials and growth conditions

All plants were grown under the following moderate conditions for 3 weeks, if not specified explicitly (for convenience, normal conditions). *Arabidopsis thaliana* Col-0 wild-type and transgenic plants were grown on 5 cm rockwool cubes (Nittobo, Tokyo, Japan) under a 14 h light/10 h dark photoperiod at a light intensity of 60 μmol m^{-2} s^{-1} at 23°C. Deionized water and fertilizer solution were each supplied once a week. The fertilizer contained 5 mM potassium phosphate buffer (pH 5.8), 0.1 mM Fe(III)-EDTA, 2 mM $MgSO_4$, 3 mM $Ca(NO_3)_2$, 0.1 mM $MnCl_2$, 0.5 μM $CuSO_4$, 30 μM $ZnSO_4$, 1 μM Na_2MoO_4, 0.1 μM $CoCl_2$, 2 mM KCl, 2.5 mM NH_4NO_3, and 2 mM KNO_3.

Construction of transgenic plants overexpressing *GLU1*

To generate *GLU1*-overexpressing plants, we used the pBIDAVL-GWR1 vector containing the Gateway conversion cassette (Invitrogen) and the kanamycin resistance gene, as described (Nakazawa et al. 2003). The 2,000 bp upstream promoter region of chlorophyll-*a/b*-binding protein/LHCII type II (LHCB2.2; At2g05070) was amplified with primers GSP1 (5′-GCCAAGCTTTGGATGGTTACT TGATATATTTTCG-3′) and GSP2 (5′-GCCCTCGAGAA TCGTAATCTTTGTTTAACTAC-3′). The fragment was digested with *Hin*dIII and *Xho*I and inserted into the *Hin*dIII/*Sal*I site of pBIDAVL-GWR1 to obtain pLHCB-GWR1, harbouring the LHCB2.2 promoter region and the Gateway conversion cassette. To prepare full-length *GLU1* (At5g04140), we cloned the N and C termini separately. The 1893-bp C terminus was amplified with GSP3 (5′-AG AGGATCCTATCCGTTGGA-3′) and GSP4 (5′-CCGAGC TCCTAAGCCGATTGAAATGTGACTTC-3′). The fragment was digested with *Bam*HI and *Sac*I and cloned into the *Bam*HI/*Sac*I site of pLHCB-GWR1 to generate pLHCB-GLU1C, harbouring the *LHCB2.2* promoter region and the C-terminal half of *GLU1*. The N-terminal fragment was amplified with GSP5 (5′-GCCTCTAGAGGATCC ATGGCGATGCAATCTCTTTCCCCTG-3′) and GSP6 (5′-ATAGGATCCTCTCCACCTTC-3′) to obtain the remaining 2973 bp of the *GLU1* N-terminus. It was inserted into the *Bam*HI site of pLHCB-GLU1C to create pLHCB-GLU1, containing the full-length *GLU1*. The p35S-GLU1 vector harbouring the CaMV 35S promoter and the full-length *GLU1* was prepared by digesting pLHCB-GLU1 with *Xba*I and *Sac*I, and the extracted fragment was cloned into pBIDAVL-GWR1. pLHCB-GLU1 and p35S-GLU1 were introduced into Arabidopsis by means of *Agrobacterium tumefaciens*-mediated transformation (Clough and Bent 1998). The transgenic plants were grown on half-strength MS agar medium (Murashige and Skoog 1962) containing 100 μg mL^{-1} kanamycin for screening and on plant nutrients and sugar (PNS) agar medium for analysing amino acids (Haughn and Somerville 1986).

Analysis of *GLU1* expression

Total RNA of leaves was prepared with an RNeasy Plant Mini Kit (Qiagen). First-strand cDNA was synthesized with Oligo-d(T) 12–18 primers (GE Healthcare) and SuperScript II reverse transcriptase (Invitrogen). Real-time PCR was performed on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems) with SYBR Green PCR Master Mix protocols (Applied Biosystems). Specific primers for *GLU1* (5′-TTAATTGAAGCACATGTGGA AAA-3′ and 5′-TTGCCAGAAGAGAGGTAGATACTTT-3′) and *ACTIN2* (5′-GGTAACATTGTGCTCAGTGG TGG-3′ and 5′-GGTGCAACGACCTTAATCTTCAT-3′) were used for quantification. Relative abundances of mRNA were determined by using the delta Ct method and normalized against the expression of endogenous standard *ACTIN2*, as described in the manufacturer's manual (Applied Biosystems).

Preparation of crude extracts for enzyme activities

The above-ground tissues of seedlings grown on agar plates containing fertilizer as nutrients and 1% sucrose were harvested at midday, frozen and ground to a fine powder by MM-300 Mixer Mill (Qiagen). The ground powder was added to extraction buffer (50 mM KH_2PO_4–KOH pH 7.5, 100 mM KCl, 5 mM EDTA, 2 mM 2-oxoglutarate, 1 mM DTT) and mixed to ensure complete suspension. After centrifugation at 10,000 rpm for 10 min, supernatant was applied to an Ultrafree filter (UFV5BGC00, Millipore) for concentration and desalination. The total protein concentration was assayed by a Bio-Rad protein assay kit.

Fd-GOGAT activity was determined using reduced methyl viologen as the electron donor (Lea et al. 1990; Esposito et al. 2005). The reaction mixture consisted of 200 mM KH_2PO_4–KOH pH 7.5, 10 mM glutamine (Gln), 10 mM 2-oxoglutarate, 15 mM methyl viologen, and extract. After 5 min of pre-incubation at 30°C, the reaction was started by the addition of reductant solution (47 mg Na_2S2O_4, 50 mg $NaHCO_3$ in 1 mL of reaction solution). After 30 min of incubation at 30°C, the reaction was terminated by adding five times amount of ethanol and by heating 98°C for 5 min.

GOGAT activity was calculated by determination of the rates of glutamate by amino acid analyzer L-8800 (HITACHI).

Quantification of amino acids

Harvested leaves, flowers and siliques were frozen in liquid nitrogen and homogenized in an MM-300 Mixer Mill (Qiagen). Amino acids in homogenized samples were extracted twice in 80% ethanol and once in water at 80°C, then treated with diethylether for purification, as previously described (Igarashi et al. 2003). After evaporation, the dried samples were dissolved in five times volume of their fresh weight of 20 mM HCl, and quantified by amino acid analyzer (L-8800, Hitachi Hitechnologies), which uses cation-exchange chromatography and ninhydrin reactions (Noguchi et al. 2006). All data were stored as Microsoft Excel files (Microsoft). The statistical calculations and drawing of the heat map were done with R Statistics packages (The R Project for Statistical Computing) and Pipeline Pilot (Accelrys).

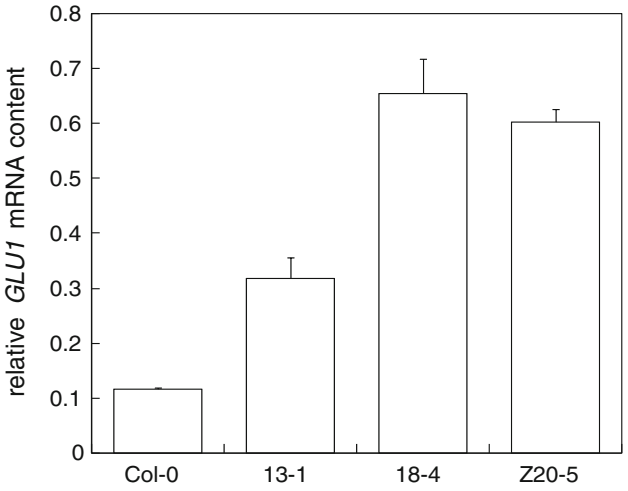

Fig. 1 Expression of *GLU1* mRNA in the wild-type (Col-0) and three *GLU1* overexpression lines. Total RNA was isolated from rosette leaves of three-week-old plants and analyzed by quantitative real-time PCR. The mRNA contents were quantified relative to *ACTIN2* as an internal standard. Each *bar* represents the average of more than three independent experiments with error bars of +SE

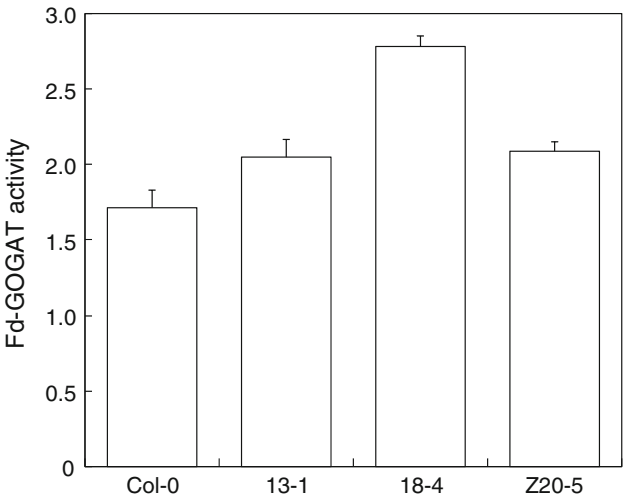

Fig. 2 Fd-GOGAT activity of the wild-type and *GLU1* overexpression lines. Glutamate generation in partially purified protein extracts for 30 min after addition of the electron donor were quantified to estimate Fd-GOGAT activity (n mol (h g)$^{-1}$ of soluble protein). The +SE of four independent experiments is indicated with *error bars*

Table 1 Leaf amino acid pool in three-week-old plants of the wild-type and the overexpression lines under "normal" conditions, 60 μmol m^{-2} s^{-1}

(nmol/mg FW)	Wild-type		Overexpression lines								
	Col-0	SE	13-1			18-4			Z20-5		
				SE	v. WT (%)		SE	v. WT (%)		SE	v. WT (%)
Glu	9.51**	0.55	10.09	0.20	106	11.24	0.42	118	11.17	0.19	117
Asp	5.87**	0.26	6.19	0.05	105	7.25	0.39	123	6.83	0.16	116
Gln	3.09**	0.04	2.22	0.11	72	1.79	0.16	58	1.74	0.09	56
Ser	2.29**	0.11	2.43	0.10	106	3.28	0.32	143	3.13	0.04	136
Ala	2.26*	0.19	1.86	0.04	82	2.12	0.19	93	2.10	0.12	93
Thr	1.49*	0.03	1.37	0.02	92	1.87	0.04	125	1.81	0.05	121
Asn	0.53	0.02	0.52	0.02	97	0.56	0.05	106	0.54	0.02	102
Pro	0.45*	0.01	0.52	0.04	114	0.54	0.06	120	0.50	0.04	112
Gly	0.42	0.01	0.26	0.06	64	0.31	0.01	74	0.96	0.01	230
Val	0.18**	0.01	0.21	0.00	115	0.24	0.01	130	0.24	0.01	131
Lys	0.14	0.04	0.15	0.03	101	0.19	0.08	133	0.22	0.07	150
Phe	0.12	0.02	0.13	0.01	104	0.14	0.00	114	0.15	0.00	119
Arg	0.12	0.02	0.10	0.00	82	0.12	0.02	101	0.15	0.02	132
Leu	0.07	0.01	0.07	0.02	103	0.06	0.01	92	0.07	0.02	108
Ile	0.07	0.01	0.08	0.02	122	0.08	0.01	118	0.09	0.01	134
His	0.07	0.02	0.04	0.00	65	0.05	0.01	74	0.04	0.01	59
Total	26.68*		26.23		98	29.83		112	29.74		111

Values represent the average of three independent experiments, standard error (SE) and percentage compared to the wild-type. The asterisks next to amino acid names indicate the overexpression lines have significant difference on the specified amino acid compared to the wild-type by Mann–Whitney *U* test (* $P < 0.05$ and ** $P < 0.01$)

Results

Construction of transgenic *Arabidopsis* overexpressing *GLU1*

We tested two different promoters to generate *GLU1*-overexpressors: the CaMV 35S promoter and the 2,000 bp upstream region of the Arabidopsis *LHCB2.2* gene. We obtained many transgenic plants in the T_2 generation with the former, but all of the plants had unchanged, or even suppressed, *GLU1* expression (data not shown). In contrast, some of the plants with the latter promoter had higher *GLU1* expression than that of the wild type, Col-0 (Fig. 1). The expression in line 18-4, which is a highest levels of *GLU1* expression, was approximately 6× that of the wild type. We used the T_4 generation of homogeneous lines 13-1, 18-4 and Z20-5 for further analysis.

Enzyme activities of *GLU1* overexpression lines

Fd-GOGAT activity was measured by the glutamate generation rate of partly purified soluble protein extracts of above-ground tissues of 2-week-old seedlings, using reduced methyl viologen as the electron donor, as described in "Materials and Methods". Line 18-4 had a 62% increase in the rate ($P < 0.01$), and 13-1 and Z20-5 showed slight but statistically significant increases ($P < 0.01$ and $P < 0.05$, respectively) compared with the wild type (Fig. 2).

Amino acid pool in the overexpression lines

Sixteen amino acids out of the 20 protein-forming ones in leaves under normal air (0.03% of CO_2) and moderate light conditions (60 μmol m^{-2} s^{-1}) were quantified to determine the effect of the *GLU1* overexpression. Although we did not obtain statistical significant results, the means and standard errors revealed trends tendencies in overexpression. The amount of glutamate increased in all three lines by 6–18% compared with the wild type (Table 1). In addition, the total amino acid pool size and the amounts of aspartate, serine, threonine, proline, valine, lysine, phenylalanine and isoleucine increased by more than 10% in two of the three overexpression lines. In contrast, the amounts of glutamine, alanine, glycine and histidine decreased, and asparagine and leucine showed little change.

Table 2 Leaf amino acid pool in 3-week-old plants of the wild-type and the overexpression lines under stronger conditions, 300 μmol m^{-2} s^{-1}

(nmol/mg FW)	Wild-type			Overexpression lines								
	Col-0	SE	v. Normal conditions (%)	13-1			18-4			Z20-5		
					SE	v. WT		SE	v. WT (%)		SE	v. WT (%)
Glu	10.31	1.25	108	11.27	0.33	109	10.01	0.85	97	9.37	0.41	91
Gln	5.36**	0.90	173	5.21	0.18	97	3.34	0.62	62	3.08	0.69	57
Ser	4.59	0.49	200	5.57	0.37	122	4.19	0.90	91	4.79	0.59	104
Asp	4.26**	0.63	73	6.06	0.28	142	6.61	0.93	155	6.67	0.47	157
Gly	3.14	0.33	754	2.82	0.14	90	2.52	0.31	80	2.70	1.06	86
Ala	2.60	0.37	115	2.45	0.15	94	2.63	0.36	101	2.28	0.17	88
Thr	1.22	0.12	82	1.41	0.15	116	1.34	0.13	110	1.33	0.15	109
Pro	0.73	0.20	161	0.65	0.00	89	0.57	0.08	79	0.49	0.02	67
Asn	0.67	0.07	127	0.74	0.01	110	0.70	0.09	104	0.66	0.09	99
Val	0.26	0.03	140	0.28	0.01	109	0.24	0.03	93	0.23	0.01	90
Phe	0.14	0.03	112	0.12	0.01	84	0.10	0.01	72	0.10	0.02	73
Lys	0.12*	0.01	87	0.15	0.01	119	0.14	20.04	115	0.18	0.01	144
His	0.12	0.00	183	0.13	0.02	106	0.15	0.05	124	0.16	0.02	133
Arg	0.07	0.00	57	0.08	0.02	120	0.06	0.01	92	0.07	0.01	101
Ile	0.06	0.01	93	0.06	0.00	102	0.06	0.00	105	0.06	0.00	91
Leu	0.05	0.00	69	0.06	0.01	129	0.05	0.01	116	0.05	0.01	110
Total	33.68		126	37.04		110	32.73		97	32.23		96

Values represent the average of three independent experiments, standard error (SE) and percentage compared to plants under normal conditions or the wild-type under the same conditions. The asterisks next to amino acid names indicate the overexpression lines have significant difference on the specified amino acid compared to the wild-type by Mann–Whitney *U* test (* $P < 0.05$ and ** $P < 0.01$)

Amino acid pool under photorespiratory conditions

The changes in amino acid pool size in leaves of the plants grown under stronger light intensity (300 μmol m^{-2} s^{-1}) were quantified to examine the effect of overexpression under ammonium-producing photorespiratory conditions. As shown in Table 2, in the specified condition Gly and Ser contents were increased, implying photorespiratory activity was enhanced by the high light intensity. Compared with the wild type, the overexpression plants accumulated aspartate, threonine, lysine, histidine and leucine by more than 10% in two of the three lines (Table 2); most of them were aspartate-family amino acids, and the ratio of increase in aspartate was highest under these conditions (maximum 57%). No morphological differences between the wild-type and overexpressing plants were observed.

Amino acid pool under non-photorespiratory conditions

The change in amino acid pool size in leaves of the plants grown under a high CO_2 level (0.3%) was quantified to examine the effect of overexpression under non-photorespiratory conditions. Compared with the wild type, levels of all amino acids except phenylalanine and undetectable histidine were increased in the overexpression lines (Table 3).

Amino acid pool under various conditions

In addition to the three conditions described above (condition NL, HL and CO_2 in order of description; Fig. 3), the pool changes under six other conditions were examined: in leaves during transition from moderate light (60 μmol m^{-2} s^{-1}) to stronger light (300 μmol m^{-2} s^{-1}) for 24 h under normal CO_2 (condition NL–HL) and high CO_2 (condition CO_2–HL); in seedlings with low nitrogen (5 mM KNO_3) under weak light (30 μmol m^{-2} s^{-1}, condition LLLN), low nitrogen under relatively stronger light for seedlings (100 μmol m^{-2} s^{-1}, condition HLLN), high nitrogen (10 mM NH_4NO_3) under low light (condition LLHN), and high nitrogen under relatively strong light (condition HLHN). The results are summarized as a heat map based on the $\log_2$ ratio of the amino acid pool of the *GLU1* overexpression line 18-4 relative to the wild type (Fig. 3). The amount of glutamate was slightly increased under some conditions but remained stable compared with that in the wild type under most conditions (shown in white or pale red). Instead, the amounts of aspartate and

Table 3 Leaf amino acid pool in three-week-old plants of the wild-type and the overexpression lines elevated CO_2 concentration, 0.3%

(nmol/mg FW)	Wild-type			Overexpression lines								
	Col-0	SE	v. Normal conditions (%)	13-1			18-4			Z20-5		
					SE	v. WT (%)		SE	v. WT (%)		SE	v. WT (%)
Glu	5.43**	0.25	57	6.74	0.69	124	7.50	0.82	138	8.33	1.34	153
Asp	4.26**	0.22	73	5.56	0.52	130	6.12	0.75	144	6.49	0.90	152
Ala	2.03**	0.22	90	2.52	0.10	124	2.59	0.37	128	2.72	0.15	134
Gln	2.32	0.05	75	2.37	0.35	102	1.88	0.37	81	2.29	0.39	99
Asn	0.80	0.05	151	1.07	0.20	133	1.07	0.20	133	1.16	0.23	145
Thr	0.64**	0.02	43	0.85	0.08	134	0.95	0.15	149	1.05	0.12	164
Ser	0.53**	0.09	23	0.69	0.07	130	0.80	0.06	152	0.91	0.12	171
Pro	0.38	0.03	83	0.34	0.02	90	0.37	0.10	100	0.48	0.08	127
Arg	0.16*	0.04	135	0.18	0.01	117	0.26	0.01	165	0.29	0.03	184
Val	0.14**	0.01	78	0.18	0.01	125	0.20	0.02	144	0.21	0.01	150
Lys	0.05**	0.01	35	0.06	0.01	130	0.08	0.00	167	0.08	0.00	153
Ile	0.05*	0.01	69	0.06	0.00	129	0.07	0.01	152	0.07	0.00	158
Leu	0.04**	0.00	67	0.06	0.00	131	0.06	0.00	138	0.07	0.01	151
Gly	0.05	0.01	12	0.06	0.01	107	0.06	0.01	114	0.06	0.01	125
Phe	0.05	0.00	40	0.04	0.00	89	0.05	0.01	93	0.04	0.00	91
His	n/d	n/d		0.04	0.00		0.05	0.01		0.05	0.01	
Total	16.94		63	20.82		123	22.12		131	24.30		143

Values represent the average of three independent experiments, standard error (SE) and percentage compared to plants under normal conditions or the wild-type under the same conditions. The asterisks next to amino acid names indicate the overexpression lines have significant difference on the specified amino acid compared to the wild-type by Mann-Whitney U-test (* for $p < 0.05$ and ** for $p < 0.01$)

threonine increased significantly under most conditions (shown in red). In contrast, the levels of glutamine and glycine decreased under many conditions (shown in blue). Levels of other amino acids tended to increase, but their ratios were decreased or unchanged under strong light, especially at low nitrogen concentrations (conditions NL–HL, HL and HLLN).

Discussion

Recent reports indicate that amino acid metabolism is highly controlled by environmental and developmental conditions such as nitrogen supply, light/dark, and transition from vegetative to reproductive stage (Miyashita and Good 2008; Lemaître et al. 2008; Diaz et al. 2008).

We investigated how the glutamate pool is maintained by modulating glutamate biosynthesis using Fd-GOGAT-overproducing *A. thaliana* plants and profiling of free amino acid contents in leaves of pre-mature plants. Since glutamate biosynthesis depends on the supply of an alpha-amino group and a carbon skeleton, we grew the plants under photorespiratory and non-photorespiratory conditions, and we quantified the amount of amino acids in their leaves to determine how the amino acid profiles were affected by glutamate overproduction.

The increase in glutamate production caused by the overexpression was small under most conditions except elevated CO_2 (Fig. 3). At first sight, the increases in Fd-GOGAT mRNA and enzyme activity were not enough to accelerate glutamate synthesis (Figs. 1, 2). However, since the basal amount of Fd-GOGAT protein is large in leaves (Yamaya et al. 1992), a small increase in percentage would be effective on metabolisms of glutamine and glutamate. In addition, the overexpressors increased glutamate levels under elevated CO_2, indicating that suppression of photorespiration by CO_2 might increase the availability of 2-oxoglutarate as a reactant in glutamate biosynthesis (Table 2). This idea is supported by the decrease in glutamine level under nearly all conditions, as glutamine is used by Fd-GOGAT to generate glutamate, and by a report that the external supply of 2-oxoglutarate caused a significant accumulation of glutamate (Forde and Lea 2007).

In contrast to that of glutamate, the aspartate pool was increased in all conditions in accordance with the overexpression. This increase suggests that the aspartate aminotransferases that utilize glutamate and oxaloacetate are activated by the overexpression, but we could not confirm

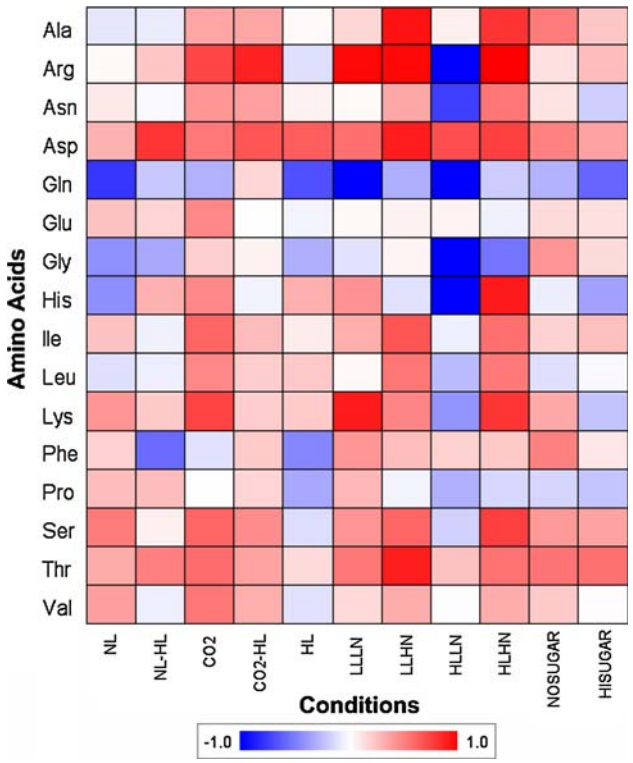

Fig. 3 Heat map representing the $\log_2$ ratio of amino acid pool size in leaves or seedlings of a *GLU1* overexpression line (18-4) relative to the wild-type under 11 different conditions. *Red* indicates increase, and *blue* indicates decrease. The ratio more than 1 or less than −1 were cut off. His in CO_2 represents the data from single experiment as histidine was hardly detectable in that condition

the upregulation of any aspartate aminotransferase genes in GeneChip analysis (data not shown). Since the production of many other amino acids that are derived from aspartate was induced at the same time, the aspartate flux from glutamate might have been increased by the overexpression.

Glycine and serine, produced during photorespiration (Igarashi et al. 2003), showed opposite results to each other under many conditions. The increase in serine and the decrease in glutamine and glycine suggest that ammonium reassimilation, which should be indirectly activated by the overexpression, might cause a shortage of ammonium, accelerating the conversion of glycine to serine at the cost of glutamine, as normally occurs under photorespiratory conditions.

Asparagine and arginine, which both act as nitrogen deposits in plants (Lea et al. 2007), showed similar changes under most conditions. Notably, both were decreased under stronger light and low nitrogen, indicating that they tended to be mobilized under nitrogen shortage even in pre-mature plants.

In conclusion, our results suggest the most overproduced glutamate is converted into aspartate and other relatively stable amino acids—especially those of the aspartate group as a part of amino acid homeostasis in plant. These features could be due to the nature of glutamate; higher reactivity as a donor in many reactions, and that hampered the approach to increase glutamate in plant drastically. As amino acid in plant is vital force for all animals, the attempt to increase glutamate in plant is important and these results may contribute to the creation of crops with increased amino acids.

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