Plant Growth Regul (2006) 50:231–238
DOI 10.1007/s10725-006-9140-4

ORIGINAL PAPER

Water potential is maintained during water deficit in *Nicotiana tabacum* expressing the *Escherichia coli* glutamate dehydrogenase gene

R. Mungur · A. J. Wood · D. A. Lightfoot

Received: 22 March 2006 / Accepted: 13 June 2006 / Published online: 20 October 2006

Abstract Expression of bacterial *gdhA* (glutamate dehydrogenase; GDH; E.C. 1.4.1.1) genes in transgenic plants fundamentally alters plant growth, herbicide tolerance and metabolite profiles. The aim was to correlate *gdhA* expression with water potential during deficit using transgenic *Nicotiana tabacum* cv. 'SR1' (tobacco). Expression of GDH activity from the transgene was significantly correlated with high water potentials during deficit, both after 5 days of water deprivation ($R = 0.91$) and after 6 h after re-watering on day 6 ($R = 0.72$). GDH expression may provide a tool to alter the response of plants to periodic water deficit.

R. Mungur · D. A. Lightfoot
Department of Molecular and Medical Biochemistry, Southern Illinois University, Carbondale, IL 62901, USA

A. J. Wood · D. A. Lightfoot
Department of Plant Biology, Southern Illinois University, Carbondale, 62901 IL, USA

D. A. Lightfoot (✉)
Department of Plant Soil and Agricultural Systems, Southern Illinois University, Carbondale, 62901 IL, USA
e-mail: ga4082@siu.edu

Present Address:
R. Mungur
Max Planck Institute, Pottsdam, Germany

Keywords Amino acids · Glutamate · *Nicotiana tabacum SR1* · Roots-Shoots · Tobacco · Water potential

Introduction

Glutamate and pyruvate are the central metabolites in cellular biochemistry (Fell and Wagner 2001; Coruzzi and Zhou 2001). Glutamate pool fluctuations play a role in regulating the activity of many pathways, particularly in carbon metabolism (Lam et al. 1998), amino-acid metabolism (Noctor et al. 2002) and nitrate assimilation (Stitt et al. 2002). High intracellular glutamate concentrations appear to be associated with phloem loading cells, remobilization, transport and source strength (Terce-Leforgue et al. 2004a; 2004b).

Glutamate dehydrogenases from bacteria (GDH; E.C. 1.4.1.1) and plants (E.C. 1.4.1.2) catalyze a reversible reaction. For assimilation, GDH is one of the few enzymes capable of the reductive amination of an organic acid to produce an amino acid; α-ketoglutarate is used to produce glutamate in the presence of the cofactor NAD(P)H (Wooton 1983). For catabolism, GDH is one of the few enzymes capable of releasing amino nitrogen from amino acids to give a keto-acid and NH_3 that can be separately recycled to be used in carbon metabolism and

amide formation, especially during senescence (Aubert et al. 2001; Loulakakis et al. 2002).

Physiological, molecular and genetic studies have all shown that the enzymes glutamine synthetase (GS; E.C. 6.4.2.1) and glutamate oxoglutarate amido-transferase (GOGAT; E.C. 1.4.7.1; E.C. 1.4.1.13) function as the primary routes of ammonia assimilation in plants (Keys et al. 1978; Lea et al. 1990; Melo-Oliveira et al. 1996). Plant GDH is a NADH-dependent, primarily mitochondrial enzyme activity that has a lower affinity for ammonium than the enzyme GS (Lea et al. 1990). The GDH dependent glutamate catabolism provides both carbon skeletons for the tri-carboxylic acid cycle and energy production during carbon or energy deficit. Mutant and sequence analyses suggest that GDH activity plays a role in abiotic stress resistance (Melo-Oliveira et al. 1996; Syntichaki et al. 1996). Physiological analyses in tobacco showed amination by GDH predominated during ammonium nutrition, in phloem loading cells and during remobilization and senescence (Terce-Laforgue et al. 2004a; 2004b). Therefore, manipulation of GDH activity in plants may alter tolerance of abiotic stress, metabolite remobilization and senescence.

Bacterial GDH (E.C. 1.4.1.1) is NADP dependent and has a low K_m for ammonium (Wooton 1983). Plants were genetically modified using the bacterial GDH to measure the effect of increasing ammonium assimilation to form glutamate in the plant cytoplasm (Lightfoot et al. 1999; 2001; Ameziane et al. 2000a; 2000b). In independent experiments the changes observed included increased herbicide resistance (corn and tobacco; Nolte et al. 2004), improved seed quality (corn; Guthrie et al. 2004) and altered yield potential (corn and tobacco; Lightfoot et al. 2001, 2006). The underlying metabolic changes included an overall increase in the concentration of certain sugars, amino acids, special nitrogen metabolism compounds and ammonium ions within the cell (Ameziane et al. 2000a; Mungur et al. 2005).

Transgenic plants with increased tolerance to water deficit have resulted from increased metabolite and protein accumulation in cells (Sawahel 2003; Park et al. 2005). Since the increased metabolites in the GDH transgenic tobacco included several compatible solutes it was hypothesized that GDH over-expressing plants would maintain normal water potentials for longer than normal plants during water deficit. Here changes in water potential during water deficit are reported among a series of tobacco plants transgenic for GDH compared to non-transgenic plants.

Materials and method

Plant material and growth conditions

Tobacco seeds were obtained from the seed stocks at the Agriculture Research Center, Southern Illinois University at Carbondale (Carbondale, IL). All seed were from *N. tabacum* cv. SR1 transformed with *gdhA* encoding a glutamate dehydrogenase (GDH); with *bar* encoding an acetyl transferase (E.C. 2.3.1.-) that leads to bialaphos and phosphinothricin resistance (BAR); and with *gusA* encoding a beta-glucuronidase (GUS; E.C. 3.2.1.31; Ameziane et al. 2000a). Seeds were sown in 4-inch pots containing a mixture (1:1) of sand and soil (Mungur 2002). Seedlings were thinned to one plant per pot, watered daily and grown on un-shaded benches at the Horticulture Research Center, Southern Illinois University (Carbondale, IL) from 9/99 to 9/03. Seed of each line used are available on request.

Preparation of cell free extracts and GDH assays

GDH assays were performed exactly as described (Ameziane et al. 2000a) with 2 experimental and 3 technical replicates (six measurements). The youngest expanded leaf (3–4 node from the apex) was used for the assays to avoid senescence gradient effects. All preparative steps were carried out at 4°C. The specific activity of aminating NADPH-GDH was quantified by measuring the rate of oxidation of NADPH dependent on reductive amination of α-ketoglutarate. Assays were performed at 25°C. The amount of protein in the extracts was determined by the Bradford assay as described by Ameziane et al. (2000).

DNA and RNA hybridizations

Genomic DNA was isolated using a modified CTAB method as described by Ameziane et al. (2000a). The DNA pellets were dissolved in distilled water overnight at 4°C. Genomic DNA (20 μg) was separated by electrophoresis on 0.8% (w/v) agarose gels and subject to Southern hybridization as described previously (Mungur 2002).

Total RNA was isolated using a method modified by Ameziane et al. (2000a) from 5 to 10 g of leaf tissue. The RNA pellets were dissolved in 100 μl of DEPC-treated water. Northern hybridizations used 10 μg of total RNA separated by electrophoresis at 40mA for 2 h through formaldehyde gels as described previously (McDaniel and Lightfoot 1997).

Water potential measurements

Experiments were repeated twice over three months. Seeds were germinated in 4-inch pots containing a 1:1 (v/v) mixture of sand and topsoil and grown in a controlled environment (16 h light, 300 μmol photons m^{-2} s^{-1}, 23 ± 2°C; 8 h dark, 18 ± 2°C and 85% relative humidity). Seedlings were thinned to one plant per pot two weeks after sowing. Plants were watered daily with 100 ml of a complete nutrient solution containing 10 mM-NO_3^- and 2mM-NH_4^+ during watering. The 90 individual plants represented 15 replicates of each of the six lines planted in a randomized complete block array. At exactly 4 weeks (28 days) water deficit was applied by withholding all water for a period of 5 days, while the control group was watered daily. Samples were harvested from random individuals on days 1–12 on the dates indicated, 3 h before the photoperiod on the days mentioned. In order to study the recovery from drought stress, plants from the stress group were supplied with 200 ml of water, 6 days after the initiation of stress, for a period of 5 days. In order to study wilting some plants from the stress group were not re-watered. Relative water content (RWC) was measured by placing leaf tissue into pre-weighed flasks containing distilled water and calculated according to the formula RWC = [(Fresh Weight – Dry Weight)/(Turgid Weight – Dry Weight) × 100] (Wood et al. 1996). Leaf water potential (ψ_w) was measured on the lowermost fully expanded leaf using a pressure bomb apparatus (PMS Instruments) and a thermocouple psychrometer (Wescor, Inc., Logan, Utah). Apoplastic fractions (AWC) were calculated by extrapolating a line to the approximated curve to read a value off the *x*-axis (Fig. 4).

Results

Nicotiana tabacum cv. 'SR1', T2 seed was generated from a series of independently transformed plants that showed a range of GDH activity from 2 to 25 μmol min^{-1} mg^{-1} protein (Ameziane et al. 2000b). Figure 1 shows evidence that each line was an independent transformant, with genetic architecture consistent with one or two copies of the *gdhA* transgene. Possible exceptions were lines GDH8 and GDH9 that showed very similar genomic architectures around *gdhA*.

The mRNA abundance measured by Northern hybridization (Fig. 2A) and the GDH activity measured by spectrophotometry (see legend above Fig. 2A) were correlated. The mRNA was of high abundance for the lines GDH8, GDH9

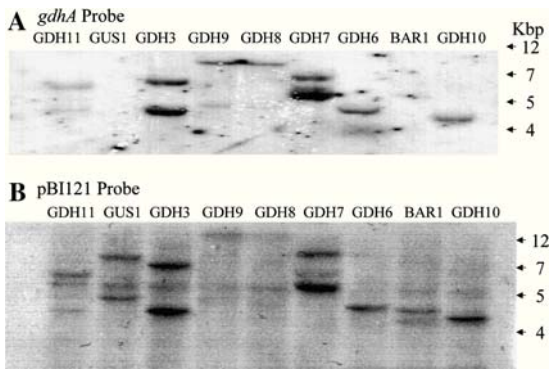

Fig. 1 Southern hybridization of the *E. coli gdhA* gene to genomic DNA from transgenic and non-transgenic *Nicotiana tabacum* cv SR1 lines. About 10 μg of total DNA was digested with *Eco*RI. Panel **A** shows hybridization to the *gdhA* gene probe (1.6 kbp *Xba*I to *Eco*RI fragment). Panel **B** shows hybridization to the pBI 121 probe (whole plasmid). Shown are GDH11 (lane 1), GUS1 (lane 2), GDH3 (lane 3), GDH9 (lane 4), GDH8 (lane 5), GDH7 (lane 6) GDH6 (lane 7), BAR1 (lane 8) and GDH10 (lane 9). Molecular weights of size markers are given in kbp

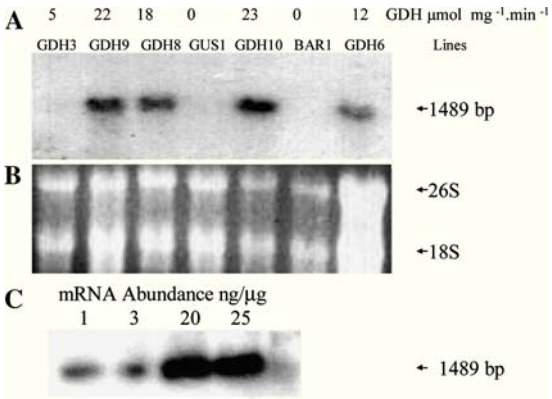

Fig. 2 Northern hybridization of the *E. coli gdhA* gene to transgenic and non-transgenic *Nicotiana tabacum* cv SR1 line leaves. About 10 μg of total RNA was separated by electrophoresis. The molecular weight of the *gdhA* transcript is given in kb. Panel **A** shows results from the *gdhA* gene probe (1.6 kbp *Xba*I to *Eco*RI fragment) that detected the 1489 bp transcript (arrowed). Lanes contained GDH3 (lane 1), GDH9 (lane 2), GDH8 (lane 3), GUS1 (lane 4), GDH10 (lane 5), BAR1 (lane 6) GDH6 (lane 7). The GDH specific activities measured in aliquots of the leaf samples used to make the RNA were 5 ± 0.4, 22 ± 1.8, 18 ± 1.1, 0, 23 ± 2.4, 0 ± 0.0, 12 ± 1.2 μM of NADPH oxidized per mg of soluble protein per minute for the lines in the order shown. Panel **B** shows a fluorograph of the 10 μg of RNA loaded, the 18 and 26 S rRNA bands are arrowed. Panel **C** shows hybridization of the probe from Panel A to 1 ng (lane 1), 3 ng (lane 2), 20 ng (lane 3) and 25 ng (lane 4) of the *gdhA* gene (1.6 kbp *Xba*I to *Eco*RI fragment) after separation by electrophoresis

and GDH10 that produced between 20 μmol min^{-1} mg^{-1} and 23 μmol min^{-1} mg^{-1} protein of GDH activity and moderate abundance for GDH6 that produces about 10 μmol min^{-1} mg^{-1} protein of GDH activity. Transcript abundance was low for the line GDH3 that produces 5 μmol min^{-1} mg^{-1} protein GDH activity. Similar enzyme activities and mRNA abundances were obtained for roots (data not shown). The detection of groups of genotypes with similar gene expression led us to concentrate on lines 3, 6 and 10 that represented low, medium and high GDH lines for further analyses and comparisons to vector and non-transgenic controls.

Analysis of the tobacco seedlings in the growth chamber (Table 1; Fig. 3B) showed that in *N. tabacum* cv. SR1 water potential of the GDH transgenic plants decreased to a far lesser degree (–0.6 to –0.8 MPa) than glucuronidase (GUS1) expressing transgenic plants (–1.2 MPa), and non-transgenic plants. Since GUS1 and non-transgenic plant were nearly identical in response only GUS1 plants are shown. The ability to maintain a water potential normal for that genotype was sustained in GDH plants for a period of 5 days, after which watering was recommenced (Table 2). At day five (maximum water deficit) the difference between the water potentials of stressed and unstressed GDH10 was lowest at 0.12 MPa, GDH6 was at 0.30 MPa, GDH3 was at 0.33 MPa and the GUS1 control was highest at 0.65 MPa. The specific activity of GDH significantly affected this phenotype ($P < 0.05$) over the range of 3–23 μmol min^{-1} mg^{-1} protein measured in GDH3, GDH6 and GDH10 plants. The correlation made using individual plant data ($R = 0.91$) suggested a dependent relationship between GDH activity and water potential. At day six (6 h after re-watering) the difference between the water potentials of stressed and unstressed GDH10 plants was lowest at 0.13 MPa, GDH6 was at 0.17 MPa, GDH3 was at 0.17 MPa and the control was highest at 0.52 MPa. Water potential was significantly correlated with GDH activity after re-watering ($P < 0.05$, $R = 0.72$) and during recovery of the plants. A difference in apoplastic water content was noted between GDH and non-GDH plants after 5 days of water deprivation (Fig. 4). The *x*-intercept of a tangent to both GDH and non-GDH curves gave values of 5% and 15% respectively that were significantly different.

Fresh weights among the stressed GDH plants at day 12 were higher than the GUS1 and untransformed plants with the exception of GDH10 that was similar (Table 1). The same pattern was observed in dry weights and among the unstressed plants. The data suggest a quadratic relationship between GDH expression and plant growth. Either growth is stimulated at lower GDH activities or the high activity (GDH10) plants are inhibited in their growth. Free amino acid concentrations were higher in all the GDH plants compared to the GUS1 and non-transgenic plants suggesting the amino acid concentration were not directly related to the water potential of the GDH plants.

Table 1 Mean differences in water potential (MPa) between water deprived stressed and un-stressed plant among transgenic lines expressing GDH (lines 3, 6 and 10) or GUS (line 1) for day 1–12 related to GDH activity at days 1, 5 and 12 and fresh weights at day 12

	GUS1	GDH3	GDH6	GDH10
A. Water potential differences (MPa)				
Day 1	0.05 ± 0.03	0.07 ± 0.02	0.08 ± 0.06	– 0.13 ± 0.05
Day 3	0.52 ± 0.07	0.13 ± 0.14	0.33 ± 0.07	0.47 ± 0.05
Day 5	0.65 ± 0.15	0.33 ± 0.05	0.30 ± 0.04	0.12 ± 0.02
Day 6	0.52 ± 0.05	0.16 ± 0.06	0.52 ± 0.06	0.13 ± 0.01
Day 7	0.22 ± 0.02	0.05 ± 0.02	0.52 ± 0.12	0.02 ± 0.03
Day 8	0.25 ± 0.05	0.08 ± 0.09	0.17 ± 0.03	– 0.02 ± 0.02
Day 10	0.00 ± 0.25	– 0.05 ± 0.02	0.13 ± 0.05	– 0.05 ± 0.02
Day 12	0.07 ± 0.15	– 0.05 ± 0.02	– 0.08 ± 0.01	0.05 ± 0.02
B. NADP-GDH activity (μmol $min^{-1}mg^{-1}$protein)				
Day 1	0.0 ± 0.0	4.6 ± 0.5	10.3 ± 0.8	23.1 ± 2.4
Day 5	0.0 ± 0.0	4.5 ± 0.3	10.2 ± 0.7	23.0 ± 2.4
Day 12	0.0 ± 0.0	4.7 ± 0.4	10.4 ± 0.9	23.1 ± 2.4
C. Fresh weight (g)				
Day 2 roots	1.79 ± 0.09	3.38 ± 0.21	3.14 ± 0.15	2.54 ± 0.26
Day 12 shoots	6.11 ± 0.35	7.15 ± 0.25	8.50 ± 0.21	5.94 ± 0.35
D. Total free amino acids (nmol mg^{-1}dry weight)				
Day 12	152 ± 16	229 ± 38	234 ± 36	231 ± 32

(A) The mean differences between water potentials and standard errors (SE) among the stressed (S) and unstressed (US) plant at each day among GDH transgenic and GUS transgenic *N. tabacum* cv SR1 lines during a 5-day period of water deprivation followed by a 5-day period of recovery from three replicates and two experiments. Plants were watered 18 h after the day 5 measurements and 6 h before the day 6 measurement. Plants were watered each day from day 6 to day 12. (B) Mean GDH activities measured in the stressed and unstressed plants. (C) Mean Fresh weights of the plants

Discussion

Correlations between the activity of GDH and differences between the water potentials of control and water deprived plants were approximately linear implying phenotypes were dependent on GDH activity (Table 1, Fig. 3). However, after re-watering the relationship between the activity of GDH and differences between the water potentials was not linear. Thus, other factors may interact with GDH induced changes to alter recovery rates. The fresh weight of plants with the highest GDH activity was lower, relative to the other genotypes regardless of treatment. Therefore, the changes induced by NADP-GDH expression in plants may either increase or decrease seedling growth depending on the genotype and environment. Further, the smaller GDH10 plants may resist water deficit to a greater degree due to their lower evaporative leaf area.

The accumulation of about twice the normal concentration of most free amino acids and other solutes has been reported in the leaves and roots of GDH transgenic tobacco (Ameziane et al. 2000b; Mungur et al. 2005). The function of amino acids as osmolytes may explain part of the maintenance of water potential by GDH plants in this experiment. However, since all the GDH plant genotypes tested had similar free amino acid amounts the total concentration does not explain the relationship between the amount of GDH activity and water potential.

Amino acids represent major sinks for nitrogen (glutamate), carbon and reduction equivalents in plants, particularly during stress (Blum and Ebercon 1976; Crowe et al. 1992). The accumulation in plant tissues during water stress may be an adaptive response since amino acids function as osmolytes, as osmoprotectants of macromolecules and scavengers of hydroxyl radicals (Delauney and Verma 1993; Cushman and Bohnert 2000; Chen and Murata 2002). The increased amino acids in the GDH transgenic plants (1.5 fold) probably involved increased glutamate, proline and arginine (Mungur et al. 2005) that are effective osmolytes. In total the metabolites altered by GDH may be sufficient to prolong the period of water uptake from a drying soil.

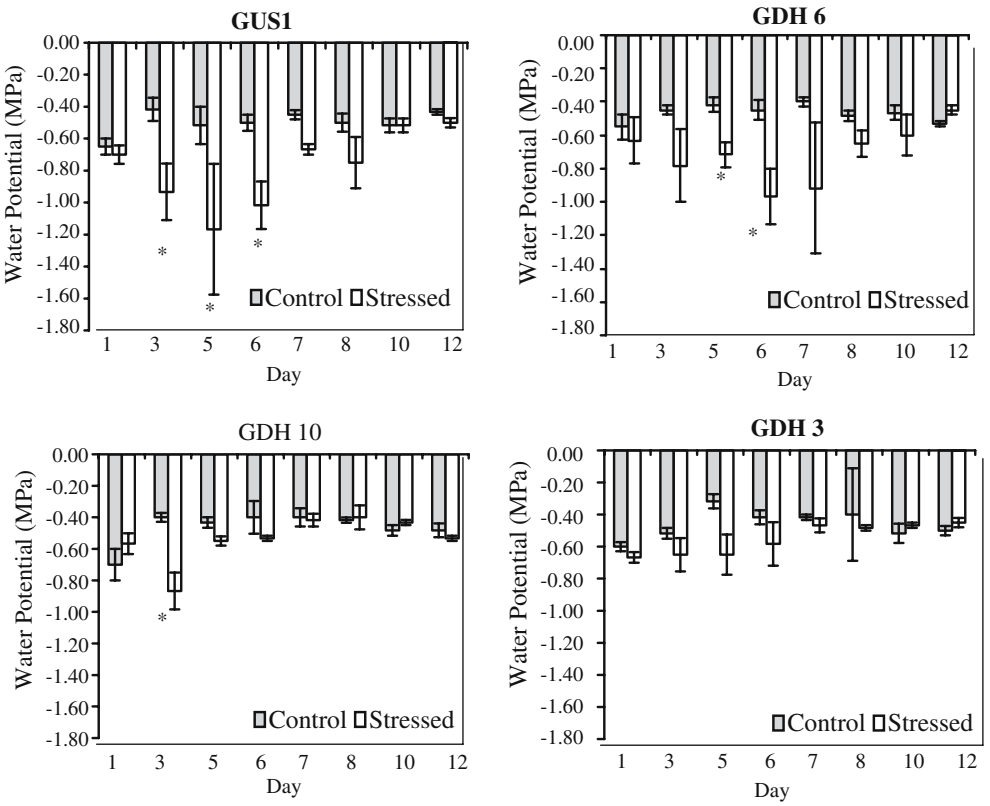

Fig. 3 Responses of *N. tabacum* GDH10, GDH6, GDH3 and GUS1 during water deficit and re-watering. The GDH specific activities measured in aliquots of the leaf samples used to make the RNA were 23 ± 2.4, 12 ± 1.2, 5 ± 0.4, 0 ± 0 μM of NADPH oxidized per mg of soluble protein per minute respectively. Water potentials and standard errors (SE) between control and stressed plant means among transgenic and non-transgenic *N. tabacum* cv SR1 lines during a 5-day period of water deprivation followed by seven days of watering. Each column represents the mean of three replicates and two experiments (six plants). Statistically significant differences ($P < 0.05$) were indicated by an asterisk

The increased amino acid synthesis may be a consequence of greater nitrogen availability. Equally, the degradation of amino acids could be repressed at one of several enzyme-catalyzed steps. Measurements of each amino acid concentration and determination of osmotic potentials would be needed to determine the extent of amino acid accumulation relative to osmotic potentials.

The data presented show the GDH activity was somewhat correlated with maintenance of the difference in water potential during water deficit but not with absolute water potential, seedling biomass or growth. Changes in growth measured in the growth chamber (this work) and in the field (Ameziane et al. 2000) infer that the glutamate made by transgenic GDH in the cytoplasm and the consequent effects on metabolism (Mungur et al. 2005) are complex. GDH can reduce seedling growth in the growth chamber at high GDH activities. Broad metabolic changes appear to underlie the maintenance of water potential during water deficit and the effects on growth. However, biomass yield is the sum of responses to the environment and therefore the sum of several effects of GDH on plants may underlie the yield effects observed in the field (Ameziane et al. 2000a, Lightfoot et al. 2001). A combination of transgenes with cumulative effects on water deficit tolerance (Sawahel 2003; Park et al. 2005) may allow further advances in crop improvement.

Table 2 Water potentials of water deprived stressed and un-stressed plant among transgenic lines expressing GDH (lines 3, 6 and 10) or GUS1

	Control ± SE	Stressed ± SE	Control ± SE	Stressed ± SE
A. GUS1			*B. GDH6*	
Day 1	– 0.65 ± 0.09	– 0.70 ± 0.10	– 0.55 ± 0.130	– 0.63 ± 0.24
Day 3	– 0.42 ± 0.13	– 0.93 ± 0.31	– 0.45 ± 0.050	– 0.78 ± 0.38
Day 5	– 0.52 ± 0.20	– 1.17 ± 0.71	– 0.42 ± 0.076	– 0.72 ± 0.13
Day 6	– 0.50 ± 0.09	– 1.02 ± 0.26	– 0.45 ± 0.100	– 0.97 ± 0.28
Day 7	– 0.45 ± 0.05	– 0.67 ± 0.06	– 0.40 ± 0.050	– 0.92 ± 0.68
Day 8	– 0.50 ± 0.10	– 0.75 ± 0.28	– 0.48 ± 0.058	– 0.65 ± 0.13
Day 1	– 0.52 ± 0.08	– 0.52 ± 0.08	– 0.47 ± 0.076	– 0.60 ± 0.22
Day 1	– 0.43 ± 0.03	– 0.50 ± 0.05	– 0.53 ± 0.029	– 0.45 ± 0.05
C. GDH3			*D. GDH10*	
Day 1	– 0.60 ± 0.05	– 0.67 ± 0.06	– 0.70 ± 0.17	– 0.57 ± 0.12
Day 3	– 0.52 ± 0.06	– 0.65 ± 0.18	– 0.40 ± 0.05	– 0.87 ± 0.20
Day 5	– 0.32 ± 0.08	– 0.65 ± 0.22	– 0.43 ± 0.06	– 0.55 ± 0.05
Day 6	– 0.42 ± 0.08	– 0.58 ± 0.24	– 0.40 ± 0.18	– 0.53 ± 0.03
Day 7	– 0.42 ± 0.03	– 0.47 ± 0.08	– 0.40 ± 0.10	– 0.42 ± 0.07
Day 8	– 0.40 ± 0.50	– 0.48 ± 0.03	– 0.42 ± 0.03	– 0.40 ± 0.13
Day 1	– 0.52 ± 0.10	– 0.47 ± 0.03	– 0.48 ± 0.06	– 0.43 ± 0.03
Day 1	– 0.50 ± 0.05	– 0.45 ± 0.05	– 0.48 ± 0.08	– 0.53 ± 0.03

Water potentials, standard errors (SE) for control and stressed plant means among transgenic and non-transgenic *N. tabacum* cv SR1 lines during a 5-day period of water deprivation followed by a 5-day period of recovery from three replicates and two experiments (see Table1)

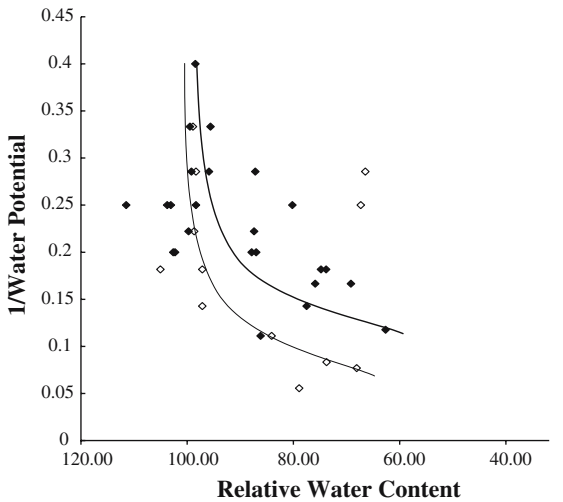

Fig. 4 Apoplastic water content determined in 4-week-old transgenic and non-transgenic *Nicotiana tabacum* cv SR1 lines after 5 days of water deprivation. Solid diamonds and the thicker trend curve represent GDH lines (GDH3, GDH6 and GDH10), open diamonds and the thinner trend curve are non-GDH lines (BAR1 and GUS1 and non-transgenic)

Acknowledgements We thank Scott Nolte for valuable discussions and help with experiments. Plant materials were developed with a grant from the Herman Frasch foundation. Analyses were supported by grants from the Illinois Missouri Biotechnology Alliance and the Illinois Council for Food and Agricultural Research.

References

Ameziane R, Bernhardt K, Lightfoot DA (2000a) Expression of the *Escherichia coli* glutamate dehydrogenase gene in tobacco affects plant growth and development. Plant Soil 221:45–57

Ameziane R, Bernhardt K, Lightfoot DA (2000b) Expression of the bacterial *gdhA* gene encoding a glutamate dehydrogenase in tobacco and corn increased tolerance to the phosphinothricin herbicide. In: Martins-Loucao MA, Lips SH (eds) Nitrogen in a sustainable ecosystem, from the cell to the plant. Backhuys Publishers, Leiden, The Netherlands, pp 339–343

Aubert S, Bligny R, Douce R, Gout E, Ratcliffe RG, Roberts JKM (2001) Contribution of glutamate dehydrogenase to mitochondrial glutamate metabolism studied by C-13 and P-31 nuclear magnetic resonance. J Exp Bot 52:37–45

Blum A, Ebercon A (1976) Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. Crop Sci 16:428–431

Chen TH, Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr Opin Plant Biol 5:250–257

Coruzzi GM, Zhou L (2001) Carbon and nitrogen sensing and signaling in plants, emerging 'matrix effects'. Curr Opin Plant Biol 4:247–253

Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Ann Rev Physiol 54:579–599

Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. Curr Opin Plant Biol 3:117–124

Delauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. Plant J 4:215–223

Fell DA, Wagner A (2001) The small world of metabolism. Nature Biotech 18:1121–1122

Guthrie TA, Apgar GA, Griswold KE, Lindemann MD, Radcliffe JS, Jacobson BN (2004) Nutritional value of a corn containing a glutamate dehydrogenase gene for growing pigs. J Anim Sci 82:1693–1698

Keys AJ, Bird IF, Cornelius MJ, Lea PJ, Wallsgrove RM, Miflin BJ (1978) Photorespiratory nitrogen cycle. Nature 275:741–743

Lam HM, Chiu J, Hsieh MH, Meisel L, Oliveira IC, Shin M, Coruzzi G (1998) Glutamate-receptor genes in plants. Nature 396:125–126

Lea PJ, Robinson SA, Stewart GR (1990) The enzymology and metabolism of glutamine glutamate and asparagine. In: Miflin BJ, Lea PJ (eds) The Biochemistry of Plants. Academic Press, New York USA, pp 121–159

Lightfoot DA, Long LM, Vidal ME (1999) Plants containing the *gdhA* gene and methods of use thereof. US Patent # 5,998,700

Lightfoot DA, Long LM, Vidal ME (2001) Plants containing the *gdhA* gene and methods of use thereof. US Patent # 6,329,573

Lightfoot DA, Mungur R, Ameziane R, Nolte S, Long L, Bernhardt K, Colter A, Jones K, Iqbal MJ, Varsa EC, Young BG (2006) Expression of the *Escherichia coli* glutamate dehydrogenase gene *gdhA* in *Zea mays* alters growth and increases tolerance to water deficit and phosphinothricin. *Mol Breeding* (in review)

Loulakakis KA, Primikirios NI, Nikolantonakis MA, Roubelakis-Angelakis KA (2002) Immunocharacterization of *Vitis vinifera* L. ferredoxin-dependent glutamate synthase, and its spatial and temporal changes during leaf development. Planta 215:630–638

McDaniel K, Lightfoot DA (1997) A gene specific cytokinin responsive mRNA in *Phaseolus vulgaris* leaves. Plant Physiol and Biochem 35:373–380

Melo-Oliveira R, Oliveira IC, Coruzzi G (1996) *Arabidopsis* mutant analysis and gene regulation define a non-redundant role for glutamate dehydrogenase in nitrogen assimilation. Proc Natl Acad Sci USA 93:4718–4723

Mungur R (2002) Metabolic Profiles of GDH transgenic crops. MS thesis, SIUC Carbondale, p 132

Mungur R, Glass AD, Goodenow D, Lightfoot DA (2005) Metabolite fingerprint changes in transgenic *Nicotiana tabacum* altered by the *Escherichia coli* glutamate dehydrogenase gene. J Biomed Biotech 2005:198–214

Noctor G, Novitskaya L, Lea PJ, Foyer CH (2002) Co-ordination of leaf minor amino acid contents in crop species, significance and interpretation. J Exp Bot 53:939–945

Nolte SA, Young BG, Mungur R, Lightfoot DA (2004) The glutamate dehydrogenase gene *gdhA* increased the resistance of tobacco to glufosinate. Weed Res 44:335–339

Park BJ, Liu Z, Kanno A, Kameya T (2005) Increased tolerance to salt- and water-deficit stress in transgenic lettuce (*Lactucta sativa*) by constitutive expression of LEA. Plant Growth Regul 45:165–171

Sawahel W (2003) Improved performance of transgenic glycine-betaine-accumulating rice plants under drought stress. Biol Plant 47:39–44

Syntichaki KM, Loulakakis KA, Roubelakis-Angelakis KA (1996) The amino-acid sequence similarity of plant glutamate dehydrogenase to the extremophilic archaeal enzyme conforms to its stress-related function. Gene 168:87–92

Stitt M, Muller C, Matt P, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A (2002) Steps towards an integrated view of nitrogen metabolism. J Exp Bot 53: 959–970

Terce-Laforgue T, Mack G, Hirel B (2004a) New insights towards the function of glutamate dehydrogenase revealed during source-sink transition of tobacco (*N, tabacum*) plants grown under different nitrogen regimes Physiol. Plant 120:220–228

Terce-Laforgue T, Dubois F, Ferrario-Mery S, de Crecenzo MA, Sangwan R, Hirel B (2004b) Glutamate dehydrogenase of tobacco is mainly induced in the cytosol of phloem companion cells when ammonia is provided either externally or released during photorespiration. Plant Physiol 136:4308–4317

Wood AJ, Sanoeka H, Joly RJ, Rhodes D, Goldsberg PB (1996) Betaine aldehyde dehydrogenase in *Sorghum bicolor*: molecular cloning and expression of two related genes. Plant Physiol 110:1301–1308

Wooton JC (1983) Reassessment of ammonium ion affinities of NADP-specific glutamate dehydrogenases, activation of the *Neurospora crassa* enzyme by ammonium and rubidium ions. Biochem J 209:527–531