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LIGHT MODULATES ACTIVITY AND EXPRESSION OF GLUTAMINE SYNTHETASE ISOFORMS IN MAIZE SEEDLING ROOTS

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Abstract — In maize roots, continuous illumination inhibits chloroplastic glutamine synthetase (GS2) activity, which decreased in light from 72.8% in 4-day-old to 26% in 10-day-old plants. In dark-adapted plants transferred to light for 6 days, GS2 activity declined from 100% to 41%, but in light-adapted plants transferred to darkness, it increased to the level of the dark control. Changes of cytosolic (GS1) activity were minor, with a similar trend. Quantitative RT-PCR revealed that light/dark treatments moderately affected only transcription of GS1 isoforms, with the exception of GS1-2, which was dramatically induced by darkness and repressed by light.

Key words: Expression, glutamine synthetase, GS1, GS2, GS1-2, maize, light, real-time PCR, *Zea mays*

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INTRODUCTION

Glutamine synthetase (GS, E.C. 6.3.1.2) is a core enzyme of nitrogen metabolism that incorporates ammonia into glutamine. Plant GS enzymes are octamers with ≈ 40 kD subunits. Higher plants usually have one plastidic (GS2) and one or more cytosolic (GS1) isoforms (Hirel and Gadal, 1980; Lam et al., 1996). In maize, GS isoforms are encoded by six nuclear genes, one for GS2 (Snustad et al., 1988) and five for cytosolic isoforms, named GS1-1 through GS1-5 (Sakakibara et al., 1992a; Li et al., 1993). The GS2 is expressed primarily in green leaves, where it reassimilates photorespiratory ammonia, but is also implicated in nitrate assimilation in both leaves and roots (Sakakibara et al., 1992 a, b; Li et al., 1993; Redinbaugh and Campbel, 1993). The major cytosolic isoforms are GS1-3 and GS1-4, which are constitutively expressed throughout the plant, and a pair of root isoforms, GS1-1 and GS1-5 (Sakakibara et al., 1992 a, b; 1996; Li et al., 1993). The GS1-2 isoform is involved in nitrogen remobilization during the grain fill (Muhitch, 2003), but it is also is found in vascular root tissues (Li et al., 1993).

Glutamine synthetase is regulated by light in order to coordinate assimilation of inorganic N with available carbon backbones produced during photosynthesis, and to scavenge toxic ammonia produced during photorespiration. Because of its specific role in reassimilation of photorespiratory ammonia, GS2 is induced by light in leaves of all species examined, including C4 plants (Hirel and Gadal 1982; Sakakibara et al., 1992a, 1992b). Light can modulate gene expression directly, via activation of phytochromes and cryptochromes, and indirectly, by activation of photosynthesis followed by increase in levels of carbon metabolites and other changes in chloroplasts. The effect of light on GS2 expression has been shown to be, at least in part, mediated by phytochrome in pea (Edwards and Coruzzi, 1989; Tjaden et al., 1995), *Arabidopsis* (Oliveira and Coruzzi, 1999), and pine (Elmlinger et al., 1994). Activation of the GS1 gene during imbibition of photoblastic lettuce seeds is also, directly or indirectly, regulated by phytochrome (Sakamoto et al., 1990). However, experiments with phytochrome-deficient *aurea* mutants of tomato revealed that photoreceptors other than phytochrome are also involved in regulation of GS2 expression at the

level of transcription, translation, and post-translational modifications (Migge et al., 1998). The phytochrome-mediated accumulation of GS2 in Scots pine was shown to be on both transcriptional and translational levels, with blue light determining the responsiveness of translational regulation toward phytochrome (Elmlinger et al., 1994). The accumulation of GS2 mRNA in leaves of *Pisum sativum* results from the action of phytochrome, as well as a light-induced increase in photorespiration (Edwards and Coruzzi, 1989). The dramatic induction of GS2 transcript in *Arabidopsis* leaves by light is mediated in part by phytochrome and in part by light-induced changes in sucrose levels, while moderate induction of GS1 transcript by light is primarily mediated by changes in the level of carbon metabolites and not by phytochrome (Oliveira and Coruzzi, 1999). Amino acids were shown to antagonize the sucrose induction of GS1 and GS2, both at the level of transcript accumulation and at the level of enzyme activity in *Arabidopsis* (Oliveira and Coruzzi, 1999; Oliviera et al., 2001; Thum et al., 2003).

Roots of higher plants are involved in the uptake of water and nutrients, anchorage of the plants in the ground, synthesis of plant hormones, and storage functions (Schiefelbein and Benfey, 1991). Light penetration through clay or sand is less than 1% of the incident light at 2.2 mm depth at any wavelength between 350 and 780 nm (Woolley and Stoller, 1978). In addition, light that reaches the soil surface is transmitted through green tissues of the shoot, and thus depleted of the red and enriched in the far-red component. Nevertheless, roots respond to various environmental stimuli, including light. Light promotes phototropism, lateral root formation, and formation and elongation of roots hairs, in addition to which it modulates the gravitropic response (Sato-Nara et al., 2004). Microarray analysis of gene expression in *Arabidopsis* roots in response to light showed that 47 genes were induced more than three-fold in darkness, while five genes were dark-repressed (Sato-Nara et al., 2004).

In experiments testing the effects of low temperatures on GS activity, it was noticed that light-adapted maize seedlings have lower root GS2 activity than in etiolated seedlings (Simonović and Anderson, 2007). The aim of the present work is to further investigate this finding and analyze the effects of light on expression and activity of all GS isoforms in maize roots.

MATERIALS AND METHODS

Light treatments

Maize *(Zea mays)* inbred G50 was grown in pots with Sunshine Germinating Mix #3 for 4 days at 27°C in the dark or under combined fluorescent and incandescent white light (100 $\mu Em^{-2}s^{-1}$), then either kept under the initial light regime or transferred from dark to light and vice versa, as indicated in Fig. 1. Root tissue samples were collected, frozen in liquid nitrogen and stored at -80°C until protein or RNA extraction.

Growing roots in liquid culture

Seeds were rinsed with 70% v/v ethanol, surface-sterilized with 6% sodium hypochlorite with 0.2% v/v Triton X-100 for 7 min, thoroughly rinsed with sterile water, and set out to germinate in water in the dark for 3 days. Approximately 1-cm-long root tips were transferred to 250 ml flasks (≈15 tips / flask) containing 100 ml ½ strength MS minimal organic medium (Gibco BRL) supplemented with 1.5 μM 3-indoleacetic acid and 1 ml/l Gamborg's vitamin solution (Sigma). Roots were cultured in darkness at room temperature on an orbital shaker at 150 rpm. Three-week-old roots were transferred to fresh medium and used to test the effects of light, amino acids, and sucrose concentration. For light treatments, flasks were exposed to white light for 12 h, 1 day, or 4 days. Some flasks were supplemented with amino acids Glu, Gln, Asp, or Asn to 10 mM final concentration and incubated for an additional 12 h in darkness prior to protein extraction. For sucrose treatments, roots were rinsed with sterile water and transferred to flasks with ½ strength MS minimal medium (without sucrose); sucrose was added in concentrations from 0% to 6%, and the flasks were incubated in darkness for 12 h. Root samples were frozen in liquid nitrogen and stored at -80°C until protein extraction.

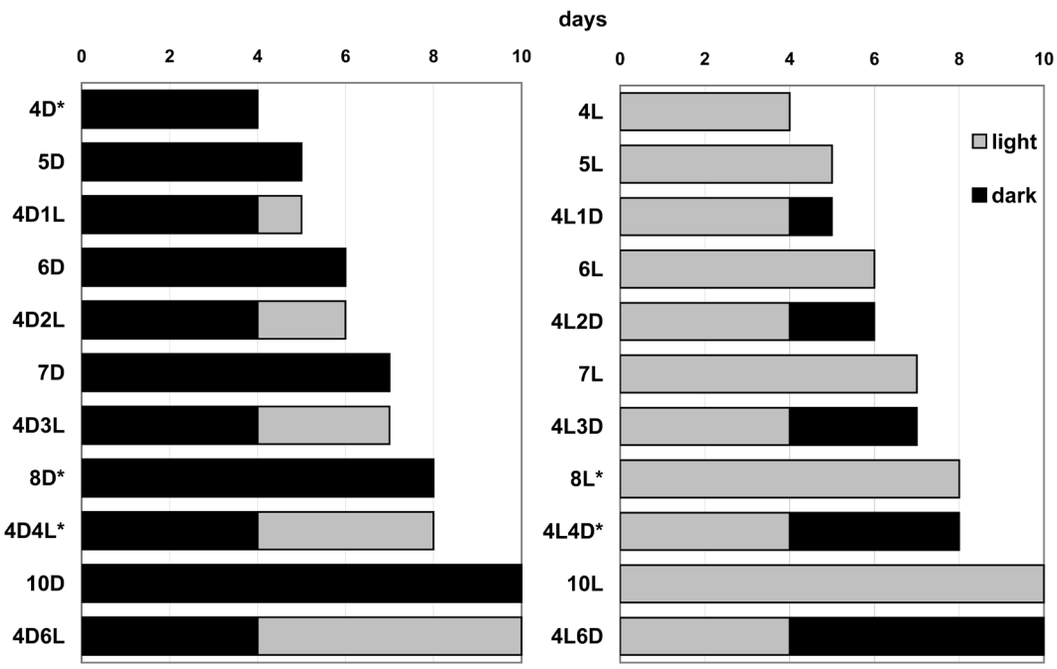

Fig. 1. Light treatments. Left panel: plants were grown in the dark for 4 days (4D) and then transferred to light for 1-6 days (treatments 4D1L, 4D2L, 4D3L, 4D4L, and 4D6L, respectively). For each light treatment, there is a dark-grown control of the same age (5D, 6D, 7D, 8D, and 10D) for comparison. Right panel: plants were grown in continuous white light for 4 days (4L) and then transferred to darkness for 1–6 days (4L1D, 4L2D, 4L3D, 4L4D, and 4L6D, respectively). For each transfer treatment, there is a light-grown control of the same age (5L, 6L, 7L, 8L, and 10L) for comparison. Treatments indicated with an asterisk were used both for enzyme activity studies and for real-time PCR expression studies. Each treatment was repeated three times.

Protein extraction, separation, GS assay, and data analysis

Protein extraction from roots, separation of GS isoforms by NATIVE PAGE, GS activity assay followed by phosphate precipitation, and densitometric analysis of the activity bands were performed as explained earlier (Simonović et al., 2004; Simonović and Anderson, 2007).

RNA isolation and reverse transcription

To study the effect of light on expression of GS isoforms in roots, plants were exposed to five light/dark treatments (indicated by asterisk on Fig. 1). Roots were washed and 0.5 g of tissue was homogenized in liquid nitrogen and used for RNA isolation. Total RNA was extracted using Trizol reagent (Invitrogen Life Technologies) following the manufacturer's protocol with one modification: for the RNA precipitation step, 250 µl of isopropanol and 250 µl of solution containing 1.2 M NaCl and 0.8 M disodium citrate were added, instead of 500 µl isopropanol as suggested. The quality of samples was checked by TBE-agarose gel electrophoresis. To eliminate eventual DNA contamination, a 2-µg quantity of total RNA was treated with RQ1 RNAse-free DNAse (Promega) and then reverse transcribed using an Omniscript reverse transcription kit (Qiagen) following the manufacturer's protocol, with anchored Oligo$(dT)_{23}$ primers (Sigma, 1 µM final concentration) and 10 U/reaction RNAse inhibitor (Promega). Synthesized cDNA corresponding to 200 ng total RNA (2 µl of 20 µl RT reaction volume) was used for real-time PCR reactions.

Quantitative real-time PCR using SYBR Green

The gene-specific primers (Table 1) were designed to amplify 100-140 bp sequences from the 3'UTR of the genes because the coding regions of different

GS1 isoforms share 67-77% identity with GS2 and 80-99% identity among themselves (Sakakibara et al., 1992b; Li et al., 1993). In addition, this approach minimized problems with incomplete reverse transcription, since anchored oligo(dT) primers used for RT bind at the boundary of the 3' polyA tail and 3'UTR, so that the region of interest was reverse-transcribed first. All amplicons, including that for the housekeeping gene actin, had a Tm between 77 and 81°C, and were checked for specificity by "blasting" against the maize database MaizeGDB (http://www.maizegdb.org/). The primers were purchased from Invitrogen. Primer specificity was confirmed by running PCR reactions with control (4D) RT mixture as a template, followed by gel sizing of the amplicons. Real-time SYBR Green PCR assays were set by mixing cDNA templates (2 µl of RT reactions) with 12.5 µl 2x QuantiTect SYBR Green PCR Master Mix (Qiagen), 2 µl combined forward and reverse primers (to give a final concentration of 0.3 µM each), and PCR-grade water to final volume of 25 µl in 96-well optical plates. Each RT reaction was used as a template in seven assays with different primer pairs, and each real-time assay was performed in triplicate corresponding to treatment replicates. Real-time PCR was done using a Stratagene Mx3000P thermal cycler, with the following cycling program: initial activation step 95°C/15 min, denaturation 94°C/15 s, annealing 55°C/30 s, and extension 72°C/30 s for 45 cycles, followed by melting curve analysis.

Data normalization

For normalization of real-time PCR data, we used Liu and Saint's method (2002) for relative quantification, where the efficiencies of individual reactions are calculated from the reaction kinetics using formula (1)

$$(1)\ E = (R_{n,A} / R_{n,B})^{1/(CT,A - CT,B)} - 1$$

where E is efficiency; R_n is SYBR Green fluorescence at cycle n; $R_{n,A}$ and $R_{n,B}$ are R_n at arbitrary thresholds A and B (in this experiment, $R_{n,A}$ = 0.04 and $R_{n,B}$ = 0.07 for all reactions), and $C_{T,A}$ and $C_{T,B}$ are the threshold cycles at these arbitrary thresholds. The efficiency calculated in this way reflects only a fraction of extra yield from a preceding cycle, so the maximum theoretical efficiency is E= 1. The normalization of data was performed using formula (2) (Liu and Saint, 2002):

$$(2)\ R_{0,Tnorm} = R_{0,T} / R_{0,R} = (1 + E_R)^{CT,R} / (1 + E_T)^{CT,T}$$

where $R_{0,Tnorm}$ is normalized target gene expression; $R_{0,T}$ is initial reporter fluorescence for the target gene (GS isoform), which corresponds to the initial number of template molecules; $R_{0,R}$ is initial fluorescence for the reference gene (actin), E_R and

Table 1. Forward and reverse primers for six GS isoforms and actin used for real-time PCR.

Gene	GenBank Accession	primer sequences (5'-3')	amplicon length, bp
GS1-1	X65926	fwd: CTCCAGTGTATTGCTCGGGAAC	104
		rev: CCCAATAAACTGGAAGCACAGC	
GS1-2	X65927	fwd: TGAGCTCTGTGTGTGAGCCG	101
		rev: CCAGAACGAGCACACTGCAG	
GS1-3	X65928	fwd: AAGCGATTGCAAAGCCACTG	101
		rev: CTGTTTTGGCACACCACGAC	
GS1-4	X65929	fwd: TAGCTAGAACACAACAACAGCCAAA	134
		rev: GACACGTCTATTATTGGAGGAGGATTA	
GS1-5	X65930	fwd: GCCCCGTGCTGTCACTTTT	101
		rev: TCGGACTTTCCGAGCAGTACA	
GS2	X65931	fwd: TATAAACCGGTCCGCGACA	112
		rev: CGATGAATCAAAGACAGCCGT	
Actin	ZMU60511	fwd: TCTGCTGAACGCGAAATTGT	101
		rev: ACAGATGAGCTGCTCTTGGCA	

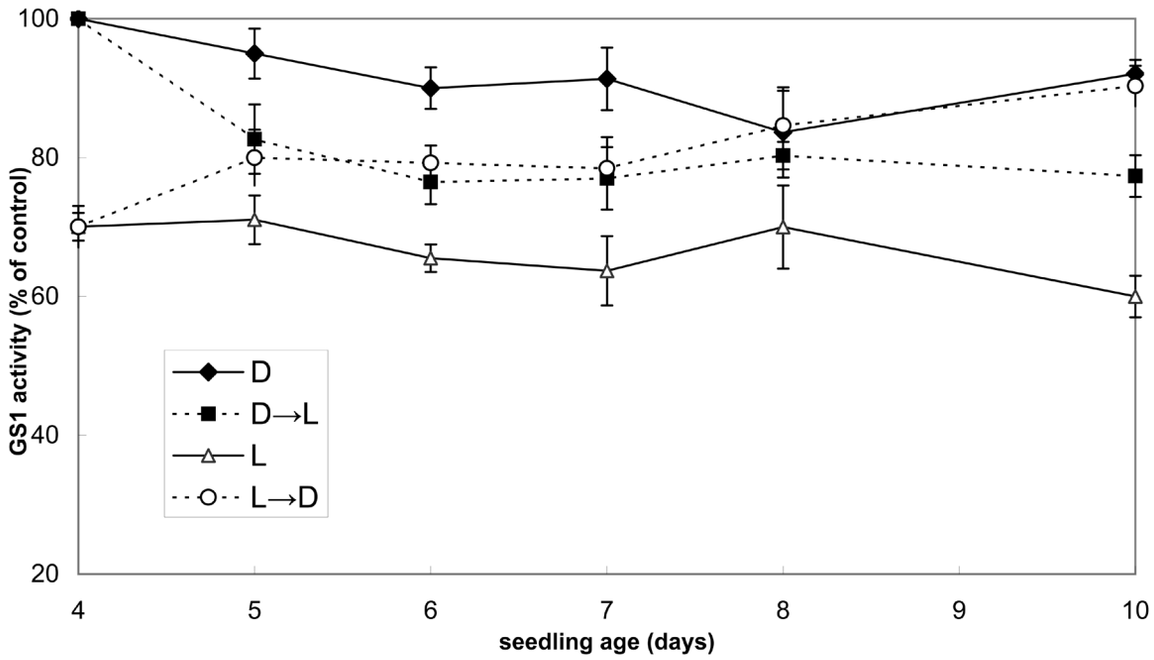

Fig. 2. Time-response curves for the effects of continuous white light, darkness, and darkness/light transfers on GS1 activity in roots. The seedlings were treated as follows: ◆-plants kept in darkness (treatments 4D, 5D,…10D), ■-plants grown in the dark for 4 days and then transferred to light for 1-6 days (4D1L, 4D2L,…4D6L), □-plants grown in continuous white light (4L, 5L,…10L) and ○-plants grown in light for 4 days and then transferred to darkness (4L1D, 4L2D,…4L6D). Soluble proteins extracted from roots were loaded in amounts of 100 μg/lane. The GS1 activity is presented relative to the 4D control (100%). The experiment was repeated three times, and the SE is indicated by vertical bars.

E_T are amplification efficiencies for the reference and target gene, respectively; and $C_{T,R}$ and $C_{T,T}$ are threshold cycles at an arbitrary threshold (0.07) for the reference and target gene. The $R_{0,Tnorm}$ values were then averaged for three replicates, and divided by values for 4D samples, setting the normalized expression for 4D controls to 1.

RESULTS AND DISCUSSION

Effect of light on GS1 and GS2 activities in roots

In experiments with low temperatures (Simonović and Anderson, 2007), it was noticed that light-adapted seedlings have lower root GS2 activity in comparison with dark-grown ones. Preliminary studies with 4-day-old dark-adapted plants exposed to pulses of red and/or far-red light showed that this was not a "classical" R/FR reversible response (Borthwick et al., 1952) and that only prolonged illumination with white light was effective in reducing GS2 activity and, to lesser extent, GS1 activity (data not shown). This could be explained either by an indirect (metabolic) effect of light (Oliviera et al., 2001) or by a PHYA-mediated "high irradiance response" (Casal et al., 1998), a possibility that was not further investigated, due to technical constraints.

The next step was to study the time-course of changes in GS activities over several days following the transfer of dark-adapted plants to light in comparison with plants of the same age grown in the dark. The reverse experiment, involving light-grown plants transferred to darkness, was carried out in parallel (Figs. 2 and 3). The GS1 activity in 4-day-old light-grown plants (4L, 70%) was lower than in dark-grown plants of the same age (4D, control, 100%), and neither changed more than 10% during the aging (Fig. 2). Plants that were transferred from darkness to light showed a decrease in GS1 activity

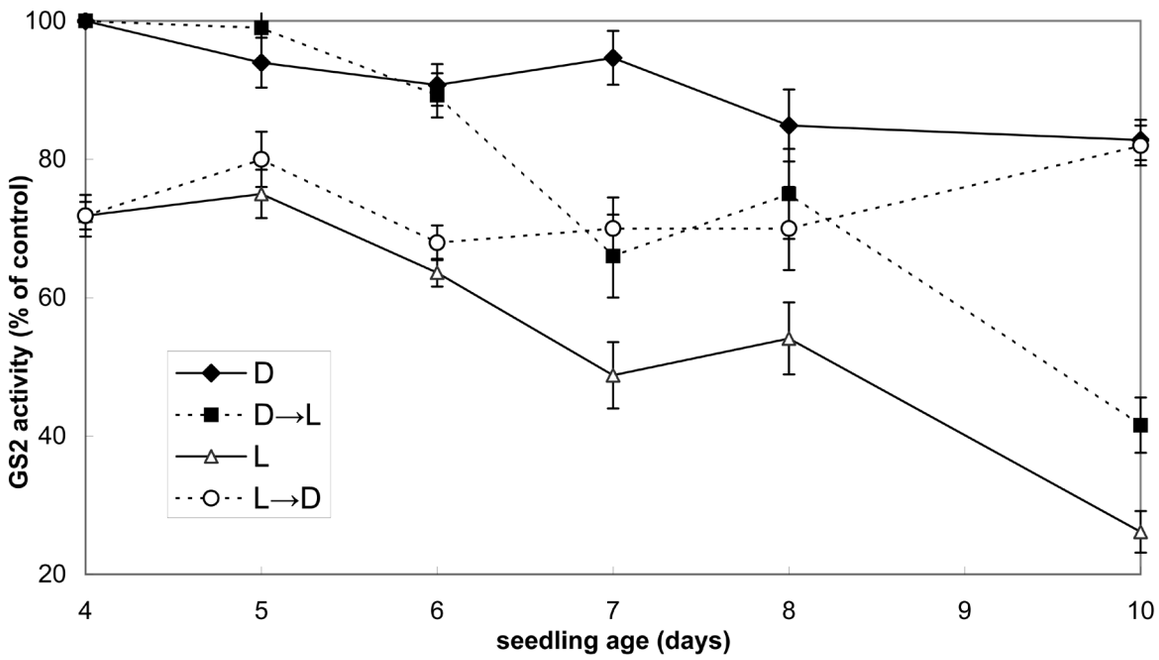

Fig. 3. Time-response curves for the effects of continuous white light, darkness, and darkness/light transfers on GS2 activity in roots. The GS2 activity is presented relative to control (4D) GS2 activity. Legends are as for Fig. 2.

from 100% to 77% after 6 days of illumination, while those transferred from light to darkness exhibited an increase of GS1 activity, from the initial 70% to 90.3% at the end of the experiment.

Changes in GS2 activity upon light/dark transfers had the same trend as GS1 changes, but were much more pronounced (Fig. 3). Plants kept in darkness showed a slight decrease in GS2 during aging (from 100% to 83%), while in dark-adapted seedlings transferred to light GS2 activity decreased to 41.5%. Light-adapted seedlings had initially lower GS2 activity (71.8%), which further decreased in light to 26% over 6 days, but increased upon transfer to darkness to 82% (Fig. 3).

GS activity in cultured roots

A root culture was established to assess whether roots are capable of perceiving the light signal themselves. Optimization of the conditions for culturing untransformed maize roots included testing of liquid and solid media; full-strength and half-strength MS media; and three different auxins, IAA, NAA, and 2,4-D in 1 μM and 1.5 μM concentrations (data not shown). The roots grew best in a liquid medium containing ½ MS with addition of Gamborg vitamins and 1.5 μM IAA. Three-week old primary culture grown in darkness was used. Illumination for 12 h, 24 h, or 4 days had no effect on GS activity in cultured roots (Fig. 4), indicating that roots cannot respond to light directly and that the light signal modulating GS activity is probably coming from shoots. Since sucrose induces GS1 and GS2 transcription and activity in *Arabidopsis,* and the sucrose effect is antagonized by amino acids Glu, Gln, Asp, and Asn (Oliveira and Coruzzi, 1999; Oliviera et al., 2001; Thum et al., 2003), these metabolites were tested as candidates for the "shoot signal", but neither addition of amino acids nor varying sucrose concentrations had any effect (Fig. 4). Several explanations for the lack of an effect of sucrose and amino acids on GS activity in cultured roots are plausible. First, regulation of plant GS enzymes is species-specific, so the importance of metabolic regulation found for *Arabidopsis* may not be general and may not apply to maize, in

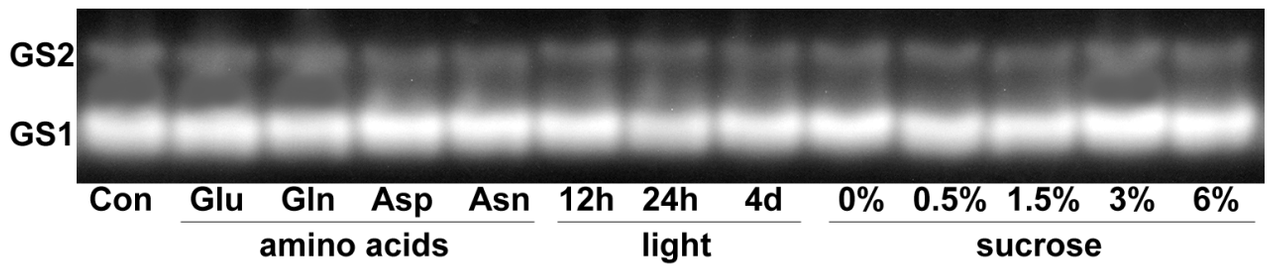

Fig. 4. Effects of light, amino acids, and sucrose on GS activity in cultured roots. Three-week old root cultures grown in the dark were supplemented with 10 mM amino acids for 12 h (left), transferred to sucrose-free MS medium with addition of sucrose in varying concentrations for 12 h (right), or exposed to white light for the indicated time (middle). The experiment was repeated four times, and a typical result is shown. Densitometric analysis of the zymograms revealed that differences among various treatments were less than 9% of activity for either GS1 (lower band) or GS2 (upper band). Con–dark control without any amino acids and with 1.5% sucrose.

which some other metabolites may be of greater significance. Second, it could be that roots in culture respond to signals differently than normal roots. Third, the effect may be transient, in which case the timing of sample collection (12 h after the treatment) could be inappropriate. Finally, it is possible that the abundance of nitrogen sources in the MS medium (9.4 mM KNO_3 and 10.3 mM NH_4NO_3 in ½ MS) overrides all other signals.

Light differentially affects expression of GS isoforms in roots

To assess whether the observed light modulation of GS activity is based on transcriptional regulation, the fluorescence-based kinetic reverse-transcription polymerase chain reaction (real-time PCR) was used, as the most sensitive method for quantification of transcripts (B u s t i n , 2000). Total RNA

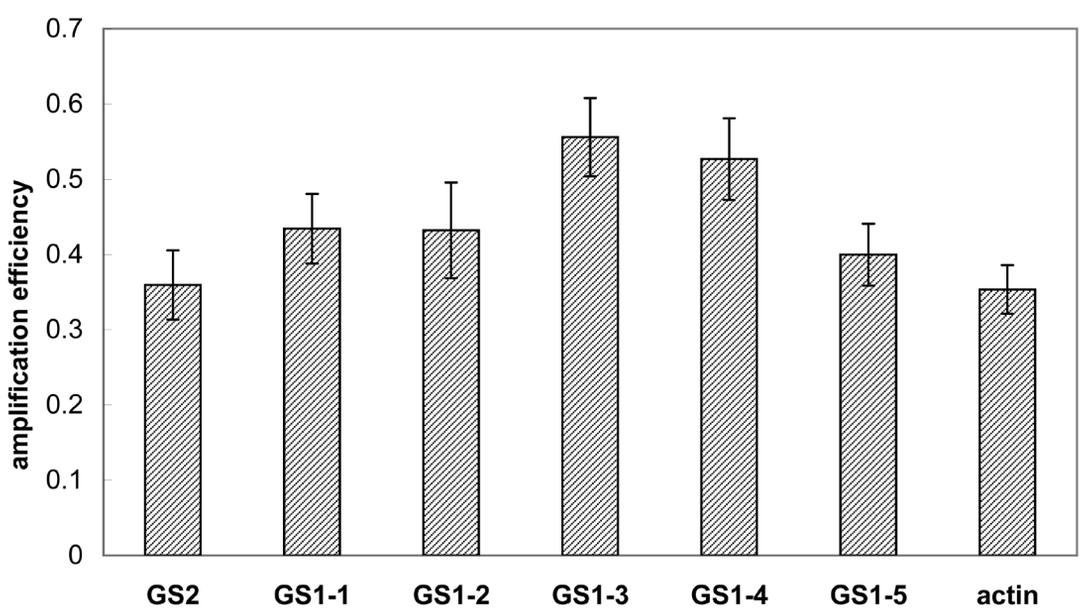

Fig. 5. Amplification efficiencies for GS isoforms and actin in control 4D samples. Efficiencies were calculated using formula (1), and the mean values from three (biological) replicates are shown. Vertical lines represent SE.

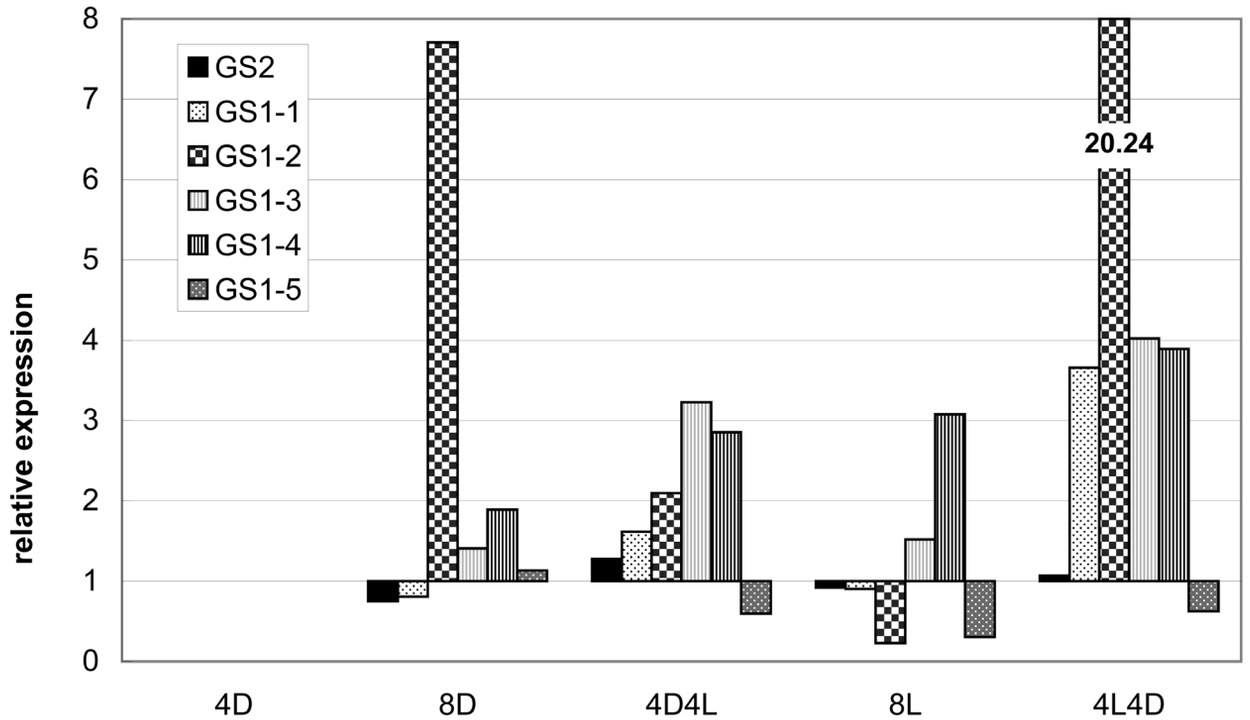

Fig. 6. Relative expression of GS isoforms in roots. The expression of six GS isoforms in response to different light/dark treatments (Fig. 1) was determined by real-time PCR, normalized with actin, and presented relative to 4D controls.

from roots of 4-day-old seedlings grown in the dark (4D, control), 8-day-old seedlings grown in the dark (8D), 8-day-old seedlings grown under light (8L), and seedlings transferred from darkness to light (4D4L) or vice versa (4L4D, Fig. 1) was reverse transcribed and used in real-time assays. The relative amount of GS transcripts from all samples was compared to 4D controls and normalized with actin as a reference (housekeeping) gene. The choice of actin was rather arbitrary, since there are no "perfect" housekeeping genes for which expression does not change at all under varying conditions (Jin et al., 2004). However, actin has been widely used for normalization in different systems (Bustin, 2000), including light experiments with maize (Fuchs et al., 2003).

The efficiencies of amplification for GS isoforms and actin were calculated for each individual reaction, but data are shown only for the 4D sample (Fig. 5). It is clear that different GS isoforms and actin have different amplification efficiencies, but differences were found even for the same target isolated from different tissue samples (data not shown), as well as for biological replicates (as indicated by the standard error bars in Fig. 5).

The expression of different GS isoforms after light/dark treatments shows that light differentially affects different isoforms (Fig. 6). Surprisingly, the GS2 isoform, whose activity decreased in light, was least affected and varied only from 0.75 for 8D to 1.27 for 4D4L treatment, meaning that the described light regulation is likely post-translational. Maize GS2 is probably susceptible to post-translational modification(s), since it was observed that the enzyme isolated from mesophyll cells and BS cells differs kinetically and biochemically (González-Moro et al., 2000). The GS from pea seeds can be regulated allosterically by ADP and ATP, and the activation by ADP may be important when the cellular energy charge is low, as it is in darkness

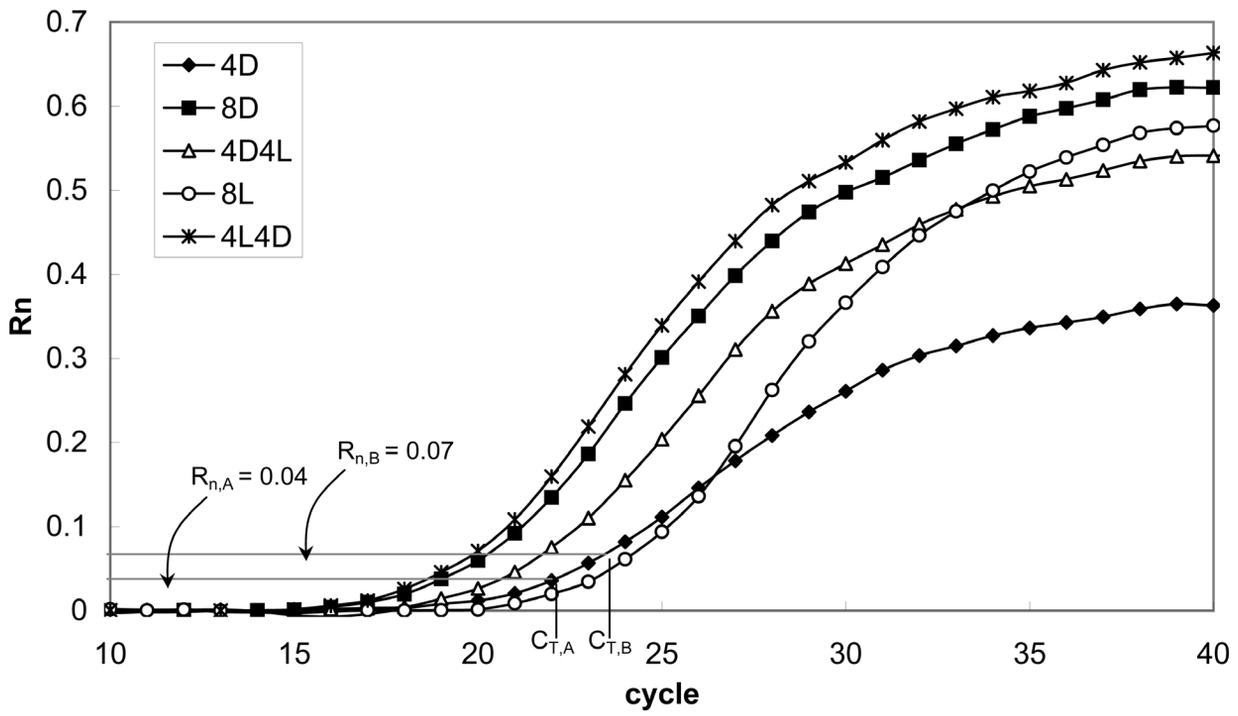

Fig. 7. Real-time PCR kinetic curves for isoform GS1-2. Note that Rn is SYBR Green fluorescence at cycle n; Rn,A and Rn,B are arbitrary thresholds used to calculate efficiencies; and CT,A and CT,B are the corresponding threshold cycles for the 4D kinetic curve.

(Knight and Langston-Unkefer, 1988). If this mode of regulation is applicable to maize, then it may account for the observed activation of GS2 in roots in the dark.

The expression of cytosolic isoforms changed moderately in response to different light/dark treatments, with the notable exception of GS1-2, whose expression changed dramatically (Fig. 6). Transcripts for the major cytosolic isoforms GS1-3 and GS1-4 appeared to be more abundant in 8-day- than in 4-day-old roots and showed a four-fold increase in expression in light-adapted plants that have been transferred to dark (4L4D). This treatment also induced the GS1-1 isoform to a similar extent, while the expression of this isoform in other treatments was practically at the level of the control. The inductive effect of transfer from light to darkness on GS1-1, GS1-3, and GS1-4 may not have physiological significance, since this does not normally happen in the field, but it is in correlation with high total GS1 activity in 4L4D treatment (Fig. 2). The GS1-5 isoform was inhibited by light, nearly two-fold upon transfer from dark to light (4D4L, 0.59) and three-fold in continuous light (8L, 0.3) relative to the control. These results, especially concerning GS1-5, are similar to the described effect of light on the expression of cytosolic isoforms during illumination and greening of leaves (Sakakibara et al., 1992 b). It was found that the total GS1 protein detected by SDS-PAGE did not change during illumination in leaves, but transcripts for individual GS1 isoforms changed slightly: mRNA corresponding to GS1-1, GS1-3 and GS1-4 accumulated to a slight extent during greening, while the GS1-5 transcript decreased and eventually disappeared in leaves after 24 h of illumination (Sakakibara et al., 1992b).

Speculation on the possible role of the GS1-2 isoform in roots

The only isoform that dramatically responded to light/dark treatments in roots is GS1-2, whose

expression increased nearly eight-fold during prolonged growth in the dark (8D, 7.7) and more than 20-fold upon transfer from light to darkness (4L4D, 20.24), but in light-adapted plants was ≈5 times lower than in the control (8L, 0.22, Fig. 6). During work on characterization of cytosolic isoforms in roots of seedlings grown in continuous white light of relatively high intensity (300 μE $m^{-2}s^{-1}$), Sakakibara et al. (1996) found that the GS1-2 transcript was hardly detectible. Additional illustration of light-dependent changes of GS1-2 expression is given as real-time PCR kinetic curves (Fig. 7).

It is well established that GS1-2 has a specific role in nitrogen remobilization, e.g., in conversion of transported nitrogen compounds in phloem of the mother plant to glutamine for delivery to the developing kernel during the grain fill (Muhitch, 1989, 2003; Muhitch et al., 1995, 2002). It is not known whether GS1-2 has any specific role in roots, but several lines of evidence, supported by the current results, indicate that it may have a specific role in mobilization of nitrogen reserves from the seed during seedling establishment and early growth. In maize, the seed remains attached to the young seedling, and amino acids and amides derived from the hydrolysis of endosperm storage proteins support seedling growth (Sivasankar and Oaks, 1995 and references therein). It was shown that if hydrolysis of endosperm reserves in 3-day-old seedlings is prevented, the induction of NR activity by nitrate is much higher in comparison with seedlings where endosperm hydrolysis was permitted (Sivasankar and Oaks, 1995). The authors also showed that the endosperm hydrolysis products Asn and Gln inhibit NR induction by nitrate. Thus, the seedling's ability to use seed nitrogen reserves appears to affect its ability to acquire nitrogen by reductive assimilation. It is reasonable to assume that the opposite way of regulation also exists, in which case the putative GS isoform responsible for nitrogen remobilization from seeds would be (1) inhibited under conditions favorable for nitrate assimilation and (2) induced when reductive nitrate assimilation is limited. It has been shown that NR-inducing treatment with nitrate, as well as treatment with ammonia, reduces the amount of the GS1-2 transcript in roots (Sukanya et al., 1994). On the other hand, in seedlings transferred to darkness, both NR and NiR transcripts steadily decline (Bowsher et al., 1991), while GS1-2 is strongly induced. It can be speculated that dark-grown seedlings, with limited energy and reducing power, enforce nitrogen acquisition from the endosperm in already reduced organic form, where GS1-2 is required for its conversion to Gln for intercellular transport, while light-grown seedlings have sufficient resources to invest in active nitrate uptake and reduction, so that GS1-2 activity is not needed and is hence suppressed. Localization of the GS1-2 transcript in vascular tissue of roots (Li et al., 1993) and GS1-2/GUS activity in the vascular cylinder of stems (Muhitch, personal communication) only supports this idea. A GS isoform with analogous function, named GS3A, has been described in pea (Edwards et al., 1990). The GS3A promoter directed GS3A/GUS expression in phloem of transgenic tobacco, while in germinating seedlings the intense expression of GS3A-GUS transgene in the vasculature of cotyledons revealed its role in the mobilization of seed storage reserves (Edwards et al., 1990). Work is in progress to test the hypothesis about the role of GS1-2 in roots.

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СВЕТЛОСТ УТИЧЕ НА АКТИВНОСТ И ЕКСПРЕСИЈУ ИЗОФОРМИ ГЛУТАМИН СИНТЕТАЗЕ У КОРЕНОВИМА КЛИЈАНАЦА КУКУРУЗА

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Код свих до сада испитиваних биљака светлост стимулише експресију и активност хлоропластне изоформе глутамин синтетазе (ГС2) у листовима, због тога што овај ензим користи амонијак који се ослобађа током фотореспирације. Ми смо показали да у кореновима кукуруза континуално осветљавање инхибира ГС2 активност, која током гајења на светлу опада са 72.8% код 4 дана старих клијанаца на 26% код 10 дана старих биљака. Код биљака гајених у мраку, код којих је ГС2 активност 100%, иста опадне на 41% после 6 дана осветљавања, али ако се клијанци адаптирани на светлост пренесу у мрак, ГС2 активност порасте до нивоа контроле у мраку. Светлост утиче и на активност цитосолне ГС1 на сличан начин, с тим што су промене мање. Анализа количине транскрипата свих ГС изоформи после различитих светлосних третмана коришћењем “real-time PCR” је показала да на транскрипционом нивоу светлост утиче само на ГС1 изоформе, те да је овај утицај релативно мали, са изузетком ГС1-2, која је драматично инхибирана на светлости и индукована у мраку.