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BRIEF COMMUNICATION

Expression of tea cytosolic glutamine synthetase is tissue specific and induced by cadmium and salt stress

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Abstract

Glutamine synthetase (GS) showed highest expression and activity in bud (youngest topmost leaf) of *Camellia sinensis*, lower in older leaves, while lowest activity in stem and roots. GS expression and activity was increased by ammonium and nitrate and also by cadmium and salt stress but decreased by copper, aluminum, drought, cold and heat stress.

Additional key words: abiotic stress, *Camellia sinensis*, metal stress, N-source.

The importance of GS/GOGAT cycle has been well documented in plant and microbial systems for its role in nitrogen assimilation. The enzyme glutamine synthetase (GS; EC 6.3.1.2) catalyses the amidation of glutamate to generate glutamine at the expense of ATP hydrolysis, and the enzyme glutamate synthase (GOGAT; EC 1.4.7.1; 1.4.1.14) catalyses the reductive transfer of amide N to 2-oxoglutarate for the generation of two molecules of glutamate, one of which is recycled for glutamine biosynthesis (Miflin and Lea 1980). The GS exists in plants as two major isoforms, a chloroplastic (GS2) and a cytosolic (GS1) enzyme, present in different proportion in photosynthetic and non-photosynthetic tissues (Miflin and Habash 2002). Furthermore, GS1 gene family consists of several isoenzymes, and their physiological roles and regulations are rather complex compared with GS2. These isoforms have been reported to be differentially regulated at the level of gene expression by light, inorganic nitrogen, amino acids and soluble sugars (Sakakibara *et al*. 1996, Ishiyama *et al*. 2004).

Recent studies in pine have indicated that cytosolic GS isoform is under the regulation of gibberellins (Gómez-Maldonado *et al*. 2004a), and its promoter was found to be capable of interacting with a MYB transcription factor (Gómez-Maldonado *et al*. 2004b). In addition to transcriptional control, regulation of GS at the post-transcription level has also been reported (Ortega *et al*. 2001). This enzymes activity is under tight control by phosphorylation and interplays with 14-3-3 proteins (Moorhead *et al*. 1999, Huber *et al*. 2002).

In addition to its role in nitrogen assimilation and transport in the form of glutamine, it may play a role in growth and development of plants. In a study Vincent *et al* (1997) observed prematurely flowering in transgenic *Lotus corniculatus* over-expressing cytosolic GS gene from soybean. Early flower and seed development was also observed in transgenic wheat lines containing *Phaselous vulgaris* GS1 gene (Habash *et al*. 2001) and in transgenic oil seed rape over expressing GS1 (Schjoerring *et al*. 2002). The chloroplastic GS provide tolerance to salt stress in rice (Hoshida *et al*. 2000) and in the phloem this enzyme plays a major role in controlling proline production (Brugière *et al*. 1999). In view of its importance, here we report on differential expression of a cytosolic GS isoform in leaf and root tissues of C*amellia sinensis*. Further we studied the effect of metal ions and abiotic stresses on its transcript and activity levels.

Tea (*Camellia sinensis* (L.) O. Kuntze) growing in the

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Abbreviations: CsGS - *Camellia sinensis* glutamine synthetase; DTT - dithiothreitol; GOGAT - glutamate synthase; GS1 - glutamine synthetase 1; GS2 - glutamine synthetase 2; Gln - glutamine; PVP - polyvinyl pyrolidone.

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Institute tea garden was used. For GS activity, apical bud (youngest leaf), the leaves at positions 1st, 2nd and 4th (position with reference to the apical bud), stem and root were used in this study.

For GS activity assay, tissue was ground in liquid nitrogen containing the extraction buffer: 100 mM Tris-HCl (pH 7.8), 10 mM $MgSO_4$, 5 mM glutamate, 0.75 % *Nonidet P-40* (v/v), 5 % glycerol (v/v), 5 mM dithiothreitol (DTT) and 0.5 % polyvinylpyrollidone (PVP) (v/v) and supernatant was used for activity determination following the modified method of Man *et al*. (2005). To study the effect of various treatments, we selected bud tissue only as GS showed maximum transcript and activity in it. For N-source, buds were treated with 100 μM $(NH_4)_2SO_4$ and KNO_3. Metal stresses were given by incubating tea buds in 100 μM $CdCl_2$, $CuCl_2$ and $AlCl_2$. Abiotic stresses were given by incubating tea buds in 100 mM NaCl, keeping buds at room temperature on filter paper (for drought stress), keeping buds in water at 42 °C (for heat stress) and in water at 4 °C (for cold stress). These treatments were given for various duration (2, 4, 6, 12 and 24 h) and then bud were used for activity determination. Control did not receive any treatment and kept in water at room temperature. Only 12 h treated samples were used for total RNA isolation and GS transcript analysis.

To study the GS expression, total RNA was extracted from tissues using *TriZol* reagent (*Invitrogen*, Carlsbad, CA, USA) following the manufacturers instructions. cDNA was synthesized using 1 μg of RNA in the presence of 200 U reverse transcriptase *Superscript*™ III (*Invitrogen*), 0.001 cm^3 of 10 mM dNTPs and 250 ng oligo $(dT)_{12-18}$. Resulting cDNA was used to carryout the PCR reactions with a GS gene (full length GS cDNA was isolated from same tea clone and through blast search it was found to be cytosolic isoform) specific primers; 5'-ATGTCTCTGCTCTCAGATCTC-3' and 5'-TCAT GGCTTCCACAGAATGG-3'. After standardizing the optimal amplification at exponential phase, PCR was carried out under the following conditions: 94 °C, 4 min for 1 cycle; 94 °C, 30 s; 55 °C, 40 s; 72°C, 1 min for 25 cycles. 26S rRNA based gene primers were used as internal control for expression studies (Singh *et al*. 2004).

To study the tissue specific expression of a cytosolic GS isoform from *Camellia sinensis*, its activity was determined in leaves, stem and root tissues. GS showed maximum activity in bud (youngest leaf) and minimum in stem and root tissues (Fig. 1*A*). Further the transcript level also showed the similar pattern being highest in bud and lowest in root tissues (Fig. 1*B*). Differential expression of GS1 in specific tissues or cell types has been reported previously in other plants (Sakakibara *et al*. 1996, Oliveira and Coruzzi 1999, Ishiyama *et al*. 2004).

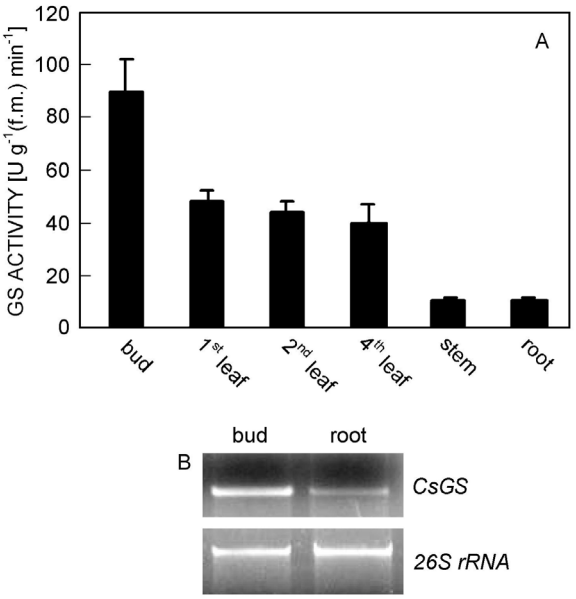

Fig. 1. Glutamine synthetase (GS) activity in various tissues of *Camellia sinensis* (*A*) and GS transcript analysis through RT-PCR in bud and root tissues (*B*). Lower panel indicate the expression of *26S rRNA* used as internal control in RT-PCR. *CsGS* - *Camellia sinensis* GS.

Since GS is involved in nitrogen assimilation, effect of N-sources by incubating bud tissue with ammonium and nitrate source was studied on its activity and transcript levels. Ammonium and especially nitrate enhanced GS activity (Table 1) as well as transcript levels (Fig. 2*A*). Low nitrogen availability is an important growth limiting factor. Therefore, this study suggests

Table 1. Influence of N-sources, various metals [100 μM] and other abiotic stresses on GS activity [U g^{-1}(f.m.) min^{-1}] measured in buds of *Camellia sinensis*. Values are the mean of three independent determinations ± SE.

Time [h]	NH_4^+	NO_3^-	$CdCl_2$	$CuCl_2$	$AlCl_3$	NaCl	Cold	Drought	Heat
0	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2	89.0 ± 6.2
2	89.9 ± 7.6	118.4 ± 2.8	121.0 ± 9.2	65.9 ± 3.6	82.8 ± 6.4	118.4 ± 5.2	82.8 ± 3.2	81.9 ± 4.1	57.9 ± 3.2
4	95.2 ± 4.2	133.5 ± 11.3	133.5 ± 10.4	59.6 ± 2.4	78.3 ± 2.1	110.4 ± 6.4	61.4 ± 2.1	62.3 ± 3.2	45.4 ± 2.1
6	103.2 ± 8.1	138.8 ± 12.4	141.5 ± 6.1	57.0 ± 3.6	78.3 ± 4.1	103.2 ± 8.4	61.4 ± 1.2	51.6 ± 4.6	39.1 ± 1.4
12	134.4 ± 4.2	141.5 ± 7.2	179.8 ± 12.4	57.9 ± 5.4	75.7 ± 3.4	97.9 ± 4.1	59.6 ± 1.4	44.5 ± 1.2	24.9 ± 2.4
24	105.9 ± 9.4	234.1 ± 14.6	95.2 ± 4.8	56.1 ± 2.2	62.3 ± 2.2	97.9 ± 6.2	51.6 ± 2.2	41.8 ± 1.6	16.0 ± 2.8

increasing nitrogen availability by enhancing the activity of GS (Albassam 2001). Both GS transcript and activity levels increased upon N-source treatment documenting its regulation could be at the transcription level.

While studying the effect of various metals on GS, cadmium increased its activity upto 12 h of exposure (Table 1) and also enhanced its transcript levels (Fig. 2*B*). The effect of Cd on activities of the glutamine synthetase-glutamate synthase (GS/GOGAT) cycle, has been reported in earlier studies (Ouariti *et al*. 1997, Gouia *et al*. 2000). Copper and aluminum decreased GS activity from 89.0 to 56.1 and 62.3 U g^{-1}(f.m.) min^{-1}, respectively after 24 h of exposure (Table 1). Similar decreasing effect was reflected through transcript analysis (Fig. 2*B*). The inhibitory effect of Cu on nitrogen metabolism was also observed in tissue cultured *Vitis vinifera* (Llorens *et al*. 2000). However, no report has been available showing the effect of aluminum on GS levels in plants.

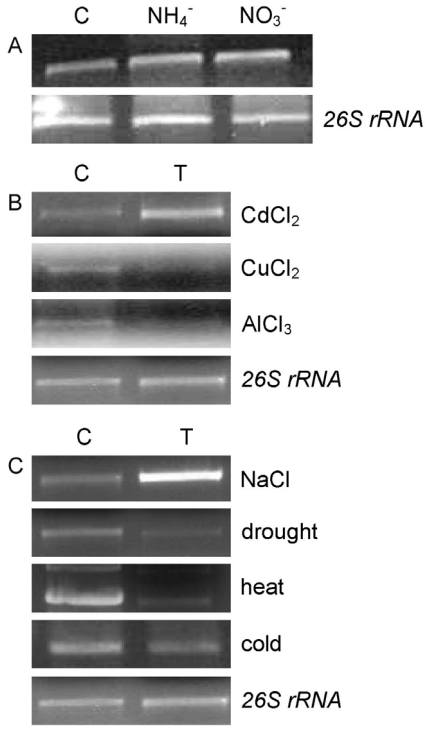

Fig. 2. GS expression in buds of *Camellia sinensis*. *A* - buds were treated with 100 μM $(NH_4)_2SO_4$ or KNO_3 for 12 h, *B* - buds were incubated in 100 μM $CdCl_2$, $CuCl_2$ and $AlCl_2$ for 12 h, *C* - buds were incubated in 100 mM NaCl for 2 h, kept at room temperature on filter paper for 12 h (drought stress), kept in water at 42 °C (heat stress) and in water at 4 °C (cold stress) for 12 h. Lowermost panel in *A*, *B* and *C* indicate the expression of *26S rRNA* used as internal control in RT-PCR. C - control sample where no treatment was given, T - treated sample.

Salt stress increased GS activity upto maximum just after 2 h of exposure and thereafter decreased it, but GS activity remained higher compared to control (Table 1). Hence the effect of salt stress on its transcript level was checked after 2 h of treatment and showed induction (Fig. 2*C*). Earlier the role of chloroplastic GS (GS2) in salt tolerance has been documented in transgenic rice (Hoshida *et al*. 2000). High level of salinity in soil decreased the availability of nitrogen and may therefore be responsible for retardation in the plant growth (Debouba *et al*. 2006). In this study, up-regulation in GS transcript level suggests that by over-expressing GS in plants under constitutive promoter N-availability can be improved even in saline soils. Other abiotic stresses like cold, drought and heat decreased GS activity continuously for 24 h to 51.6, 41.8 and 16.0 U g^{-1}(f.m.) min^{-1}, respectively. Similarly decrease in its transcript level was observed under these abiotic stresses (Fig. 2*C*). However, drought stress had no influence on GS activity in maize (Medici *et al*. 2003). However, earlier reports on cytosolic GS have shown that it is differentially regulated at the level of gene expression by light, inorganic nitrogen, amino acids and soluble sugars (Sakakibara *et al*. 1996, Oliveira and Coruzzi 1999, Ishiyama *et al*. 2004). In peanut seedlings increase in activity of GS was reported upon jasmonic acid treatment (Kumari *et al*. 2006).

The induction of CsGS transcript by both salinity and Cd indicates that different stresses can induce the expression of this gene. This multi-stress response of GS suggests the presence of stress responsive elements in the promoter region of the GS gene. Although differential expression of GS has been shown in different organs like root, leaf, and flower in tomato (Scarpeci *et al*. 2007), this upregulation of the GS reported in response to salinity and cadmium stress is a novel observation.

In this study, we have shown for first time that the expression of a tea cytosolic GS is regulated by different factors and its maximum expression is in bud tissues. Induction in transcript of GS in response to salinity and Cd documents that tolerance to these stresses can be improved in any crop plants by over-expressing GS. Secondly, GS promoter can be used as a inducible promoter to derive the expression of desired gene under such situations. However, the feasibility for its further exploitation in producing abiotic stress tolerance in plants needs further investigation.

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