Molecular cloning and characterization of nitrogen source responsive *GS1* gene from melon

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Abstract

A full-length cDNA clone encoding cytosolic glutamine synthetase (GS1; EC 6.3.1.2) was isolated from melon (*Cucumis melo* L.) for the first time by RT-PCR and RACE approach. The clone, designated as *M-GS1* (accession No. DQ851867), contains 1494 nucleotides with an open reading frame (ORF) of 1068 nucleotides. The deduced 356 amino acid sequence showed high similarity with previously reported GS1s from various plant species. Sequence analysis revealed that the predicted protein contains a GS β-Grasp domain, a GS catalytic domain, and the main conserved motifs characteristic of a plant GS1. The phylogenetic analysis displayed that M-GS1 is related most closely to the GS1 from *Datisca glomerata*. Southern blot analysis indicated that *M-GS1* belongs to a small gene family of 2 or 3 members. *M-GS1* was expressed in all plant tissues without evident tissue specificity, but with different patterns when the melon plants were fed in hydroponic culture with different forms and concentration of nitrogen. Ammonium dramatically enhanced the contents of *M-GS1* transcripts in all tested tissues, while nitrate stimulated *M-GS1* transcription only in the roots and leaves, but not in the stems; glutamate, however, depressed *M-GS1* transcripts in the roots, but resulted in no significant change to the levels of *M-GS1* transcripts in the stems and leaves. Moreover, the same effects were observed at the GS enzyme activity level. These results indicated that melons respond to changes of N nutrition by regulating *M-GS1* expression.

Additional key words: *Cucumis melo*, cytosol, glutamine synthetase, nitrogen forms, relative mRNA level, total GS enzyme activity.

Introduction

The assimilation of inorganic nitrogen into its organic form is critical for plant growth. Glutamine synthetase (GS; EC 6.3.1.2) is a key enzyme in this process, catalyzing the first step in the incorporation of ammonium into glutamate to form glutamine (Gln). In the GS-glutamate synthase (Fd-GOGAT, EC1.4.1.7; NADH-GOGAT, EC 1.4.1.14) cycle, the Gln synthesized in the first step is then used to form glutamate (Glu), the primary N donor in the main biosynthetic pathways of nitrogenous compounds (Marigo *et al.* 1993) and the essential precursor for various secondary metabolites in plants (Gallardo *et al.* 2003). GS in plants can exist as distinct isoforms (McNally *et al.* 1983), one localizing in the cytosol and the other in the chloroplasts (Chen and Silflow 1996). The cytosolic isoform (GS1) generates Gln for N transport or remobilization between different organs (Kamachi *et al.* 1991), and this role is particularly important for *Cucumis* after anthesis and during fruit enlargement when N is remobilized to the reproductive sinks. The other isoform (GS2) functions in chloroplasts to assimilate ammonium generated by photorespiration and nitrate reduction, ameliorating the potential toxic effect of excessive ammonium on the leaves (Ishiyama *et al.* 2004).

There is a family of *GS* genes in higher plants: mostly a single nuclear gene for GS2 but multiple nuclear genes for GS1 (Oliveira and Coruzzi 1999). Since 1956, when the purification and characterization of the first plant glutamine was reported (Miflin and Habash 2002), numerous GS isoenzymes have been isolated from various plant species, and different characterization studies have

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Abbreviations: GOGAT - glutamate synthase; GS - glutamine synthetase; ORF - open reading frame; RACE - rapid amplification of cDNA ends; RT-PCR - real time polymerase chain reaction.
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shown that these *GS* genes can display organ-specific, cell-specific, developmental and temporal patterns of mRNA expression (Oliveira and Coruzzi 1999). They are regulated by a variety of environmental factors such as radiation, temperature, salt stress, heavy metal ion toxicity, carbon sources, and especially by supplementation of N in various forms (ammonium, nitrate, and amino acids) (Rana *et al.* 2008, Li *et al.* 2009, Matas *et al.* 2009).

In the cultivation of horticultural crop melon (*Cucumis melo* L.), N is needed in large quantity but must be strictly administered in different growth phases to ensure yield and fruit quality. Also the composition of different forms of N fertilizers has strong impact on the growth and development of melons (Lee *et al.* 2006, Silva *et al.* 2007, Cabello *et al.* 2009). However, our knowledge of N assimilation in melon is still very limited, and isolation and characterization of GS genes, the key player in plant N assimilation, from the whole family of *Cucurbitaceae* has scarcely been reported to date (Deng *et al.* 2009). Here we report the first isolation and characterization of a cytosolic *GS* gene, *M-GS1*, in melon. To gain some insight toward elucidating the effects described above, the expression of *M-GS1* in different tissues in response to different N nutrition was also investigated.

Materials and methods

Plant culture: Melon (*Cucumis melo* L. var. *reticulatus* Naud.) cv. Chunli (Shanghai Academy of Agriculture, China) seedlings were grown in half-strength Hoagland solution (pH 6.5 ~ 6.8) under continuous irradiance of 1000 μmol(photons) m^{-2} s^{-1} and temperature of 28 ± 2 °C in an climate chamber. When the first pair of true leaves was fully expanded, the melon plants were transferred to pure water for 6 h prior feeding with various N forms. The plants were randomly divided into groups and then grown in half-strength Hoagland solution with either ammonium, nitrate or glutamate as the only N source. Different N concentrations were used: 0.75, 3.75 or 7.50 mM. Tissue samples from the roots, stems and true leaves of the melon plants after adaptation in pure water for 6 h (as control) and after 24-h treatment with different N nutrition were separately collected, immediately frozen in liquid nitrogen, and stored at -80 °C before used for total mRNA extraction and GS enzyme activity measurement.

Cloning of *M-GS1* complete cDNA: Total RNA (5 μg), isolated with *Trizol* reagent (*Invitrogen*, Carlsbad, USA) from melon seedlings, was converted into single-stranded cDNA with *M-MLV* reverse transcriptase kits (*Promega*, Madison, USA). This cDNA was then used as the template in PCR for *M-GS1* cDNA amplification with degenerate primers [GF1: 5'-TGTGATGC(TCA)TACAC(ACT)CC (AT)GCTGG-3' and GR1: 5'-C(AT)CCAGCAATCTC (AT)GT(ACT)ATCCTCTC(CT)-3'], designed based on the conserved regions among known GS1 amino acid sequences. PCR amplification was performed for 35 cycles (94 °C for 60 s, 55 °C for 30 s, and 72 °C for 60 s) followed by a final extension step of 8 min at 72 °C. PCR products were gel purified, cloned into *pGEM-T Easy* plasmid vector (*Promega*), and sequenced by *ABI 377* sequencer (*Perkin Elmer*, Norwalk, USA).

On the basis of this cloned fragment of *M-GS1* cDNA, the rapid amplification of cDNA ends (RACE) approach was then employed to amplify the 3' and 5'ends of *M-GS1* cDNA with the *SMART*™ RACE cDNA amplification kit (*Clontech*, Palo Alto, USA) as previously described by Deng *et al.* (2009). By assembling the sequences of the 3' RACE and 5' RACE products, the full-length cDNA of *M-GS1* was obtained and amplified with the 5'-ready cDNA as template, GF3 (5'-ACGCGGGGCTGCAAA ACTTCAACGTCG-3') and GR3 (5'-CCAAAGGATA ATAGAAACCCATCCT-3') as gene specific primers, using a touch-down protocol strategy: 10 cycles of amplification (94 °C for 45 s, 65 °C→55 °C for 45 s, 72 °C for 120 s), 30 cycles (94 °C for 30 s, 55 °C for 30 s, 72 °C for 120 s), and an extension step of 10 min at 72 °C. The PCR products were gel purified, cloned into *pGEM-T Easy* plasmid vector, and sequenced by *ABI 377* sequencer.

Sequence analysis: The DNA and protein sequence homology search was carried out using the *BLAST* program (http://blast.ncbi.nlm.nih.gov/blast.cgi), and multiple sequences alignment was performed with *Clustal W* and *Genedoc* software. Functional domains and motifs were predicted by Hits database (http://hits.isb-sib.ch) (Dieryck *et al.* 1997). Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version *4* (Tamura *et al.* 2007) by the Neighbor-joining method and the bootstrap value was generated by 1000 repeats.

Southern blot analysis: Total genomic DNA was extracted from the young leaves of melon seedling (17-d-old) using the method of Diallinas *et al.* (1997). Genomic DNA (10 μg) was digested with *Eco*RI, *Sac*I and *Xho*I (*Takara*, Kyoto, Japan), respectively, fractionated in 0.8 % (m/v) agarose gel, transferred on to *Hybond-N*$^+$ nylon membrane (*Amersham Pharmacia Biotech*, Piscataway, USA) and hybridized with a 353-bp *M-GS1* fragment that can be amplified from both the 3' *UTR* (untranslated region) of *M-GS1* in the cDNA and the genomic DNA as a single band of identical sequence with a pair of gene specific primers through PCR. Probe labeling was carried out with *RadPrime* DNA labeling system (*Invitrogen*) according to the manufacturer's instructions. Blot hybridization and membrane washing were performed according to Church and Gilbert (1984).

Real-time quantitative PCR: Total RNA was separately extracted with *Trizol* reagent from the tissue samples treated with DNase I (*Tiangen*, Beijing, China) to remove potential genomic DNA contamination, and reverse-transcribed into the first strand cDNA with *SYBR*®

PrimeScript™ RT-PCR kit II (*Takara*) according to the manufacturer's instructions. Real-time quantitative PCR was then performed using *ABI StepOne*™ cycler (*Applied Biosystem*, Foster City, USA). The primer pair specific for *M-GS1* was rtG-F (5'-CGGAGACCTGCGTCAAACA-3') and rtG-R (5'-CCTCTTCCCTTCCCTTCCTC-3'), targeting a 101-bp *M-GS1* fragment. The primer pair (rtA-F 5'-GAAGCACCACTCAACCC-3' and rtA-R 5'-TCCGACCACTGGCATAG-3') was used to amplify a 123-bp fragment of *M-actin*, the melon house-keeping gene used as the endogenous control (Bouquin *et al.* 1997, Deng *et al.* 2009). These primer pairs were so designed that they generated only a single target band from the cDNA but no band from the genomic DNA in PCR amplifications. PCR was performed according to the specifications of the *SYBR*® *PrimeScript*™ RT-PCR kit II with a protocol as follows: 40 cycles of amplification (95 °C 4 s, 61 °C 15 s, and 72 °C 15 s). Fluorescence signals were collected at each polymerization step. The relative expression level of the *M-GS1* was calculated according to the relative standard curve method as described by Larionov *et al.* (2005). The Ct (threshold of amplification cycles) values, upon which the calculations for *M-GS1* relative expression were based, were the means of triplicate independent PCRs, for both the target and the endogenous control.

Total GS enzyme activity measurement: The same batch of tissue samples collected in feed treatments were separately ground in liquid nitrogen to fine powder, and then homogenized in extraction buffer (2 cm^3 g^{-1} fresh mass) containing 100 mM Tris-HCl (pH 7.6), 1 mM $MgCl_2$, 1 mM EDTA, and 10 mM 2-mercaptoethanol. The suspensions were centrifuged at 4 °C for 20 min (20 000 *g*). The synthetase activity of GS in extracts was determined in a reaction mixture containing imidazole buffer (Rhodes *et al.* 1975). After the mixture was incubated at 37 °C for 15 min, the reaction was terminated by adding acidic $FeCl_3$ [2% (m/v) TCA and 3.5 % (m/v) $FeCl_3$ in 2 % HCl]. Production of L-Glu-γ-monohydroxamate (MHA) was measured with a spectrophotometer (*Shimadzu UV-2201*, Tokyo, Japan) at 540 nm. The results were compared with a standard curve using L-Glu-γ-MHA as the standard. One unit of GS enzyme activity was the enzyme catalyzing the formation of 1 μmol L-Glu-γ-MHA per min at 37 °C.

Statistical analysis: *JMP 6.0* (SAS Institute, Cary, NC, USA) was used for statistical analysis of the real-time quantitative PCR data and total GS enzyme activity data. Values that are means of three replicates were compared using Student's *t*-test with α set at 0.05.

Results

Using the RT-PCR method, a 415-bp cDNA fragment was initially isolated. Nucleotide *BLAST* search showed that this fragment shares high similarity with other plant GS1 genes registered in Genebank database. Based on this cDNA sequence, specific primers were designed to amplify the 5' and 3' flanking regions using the RACE techniques. By 3' RACE cDNA cloning, a 991-bp cDNA fragment was generated, which contained a 298-bp overlapping sequence with the initially isolated 415-bp cDNA fragment. By 5' RACE cDNA cloning, a 441-bp cDNA fragment was obtained, which contained a 55-bp overlapping sequence with the initially isolated 415-bp cDNA fragment. By assembling the sequences of 3' RACE, 5' RACE and RT-PCR products, the full-length cDNA of *M-GS1* was deduced. End sequences of the 5' and 3' fragments were used to design primers to amplify the full-length cDNA (1409-bp; accession No. DQ851867), which contains a 113-bp 5' *UTR* followed by a 1071-bp ORF, coding for a 356 amino acids polypeptide, and a 309-bp 3' *UTR* including a 28-bp polyA tail. Nucleotide sequence analysis of this cDNA revealed that it corresponded to a *GS1* full-length cDNA, thus designated as *M-GS1*.

In silico analysis of the M-GS1 amino acid sequence predicted a molecular mass of 39.2 kDa with the theoretical pI of 5.89. Protein *BLAST* (http://www.ncbi.nlm.nih.gov) revealed that the deduced amino acid of M-GS1 showed 93 % sequence identity with *Datisca glomerata*, 90 % with *Securigera parviflora*, *Avicennia marina*, and *Canavalia lineate*, 89 % with *Brassica napus*, 88 % with *Pisum sativum* and 86 % with *Arabidopsis thaliana* and *Ricinus communis*. Hits analysis indicated that M-GS1 contains a GS β-Grasp domain, a GS catalytic domain, a GS signature, and a putative GS ATP-binding region signature (Fig. 1), as are typical features of a plant GS1 protein. To investigate the evolutionary relationships among different plant GS1 proteins, the amino acid sequences of M-GS1 and other GS1 proteins from different plant species were used in phylogenetic tree construction and analysis. The result showed that M-GS1 forms a clade with the GS1 from *Datisca glomerata* with the shortest distance (Fig. 2), indicating that these two sequences could have evolved from a common ancestor.

Melon total genomic DNA was digested with different restriction enzymes (*Eco*RI, *Sca*I, and *Xho*I, respectively) and blotted on to nylon membrane. Two to three positive bands were detected in each lane, suggesting that M-GS1 is coded by a small gene family of two or 3 members (Fig. 3).

To investigate whether the expression of *M-GS1* was tissue-specific or inducible by exogenously supplied N in different forms, real-time quantitative PCR was performed to measure the relative mRNA levels of *M-GS1* in different tissues of melon plants that were cleaned in pure water for 6 h (control plants) and that were further fed separately with N in different forms and different concentrations for 24 h. Because irradiance has been shown to indirectly

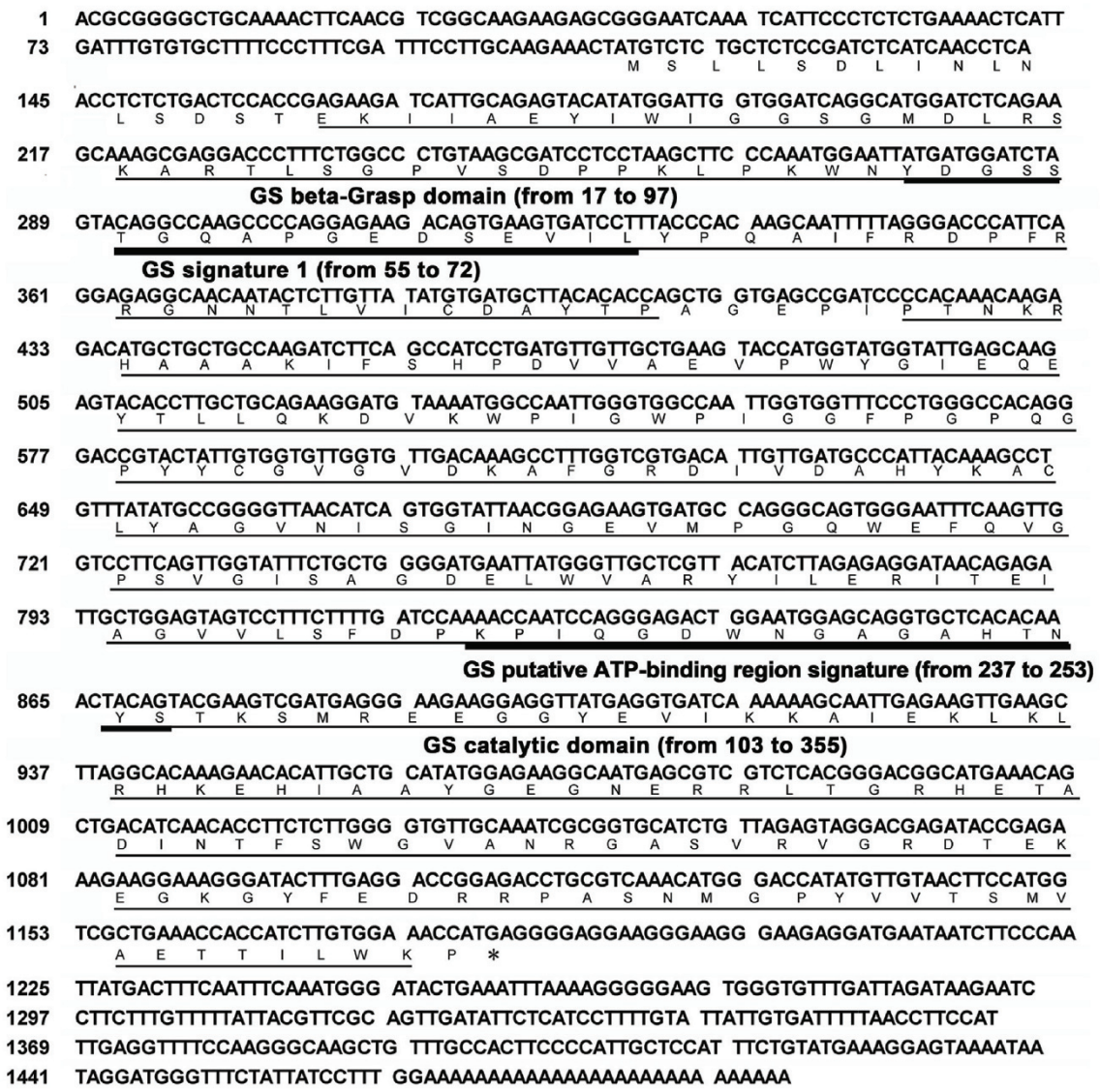

Fig. 1. The full-length cDNA sequence and deduced amino acid sequence of M-GS1. The putative functional domains and signature sequences were underlined.

affect the expression of genes (Oliveira and Coruzzi 1999), the plant growth and treatments were performed under continuous irradiance. The results showed that the expression of *M-GS1* displayed no evident tissue specificity, but was affected by different N forms in a dose dependent manner (Table 1).

In the roots, the expression of *M-GS1* was significantly induced by ammonium or nitrate feeding but was strongly depressed by glutamate feeding, all in a dose dependant manner. By increasing the N concentration from 0.75 to 3.75 or 7.50 mM, the relative mRNA level was enhanced for about 300 % in ammonium feeding, 200 or 300 %, respectively, in nitrate feeding; but was decreased for about 30 or 60 %, respectively, in glutamate feeding (Table 1). In the stems, however, significant regulation on *M-GS1* expression was observed only in ammonium feeding but not in nitrate and glutamate feeding, where the increase of ammonium concentration from 0.75 to 7.50 mM strongly stimulated *M-GS1* transcription. In the leaves, the expression of *M-GS1* was again weakly regulated by glutamate feeding, but significantly induced by ammonium and nitrate feeding: increasing the N concentration from 0.75 to 3.75 or 7.50 mM enhanced the relative mRNA level for about 200 % in ammonium feeding; for about 200 or 600 %, respectively, in nitrate feeding. The same overall patterns of regulation were also observed at GS enzyme activity (Table 1), except that the similar levels of *M-GS1* mRNA did not correspond strictly to homologous levels of GS enzyme activity.

Discussion

In this work, we reported the cloning of the first cytosolic GS gene, *M-GS1*, from melon by the RACE approach. Characterizations including sequence and functional structure analysis, molecular evolution analysis, and

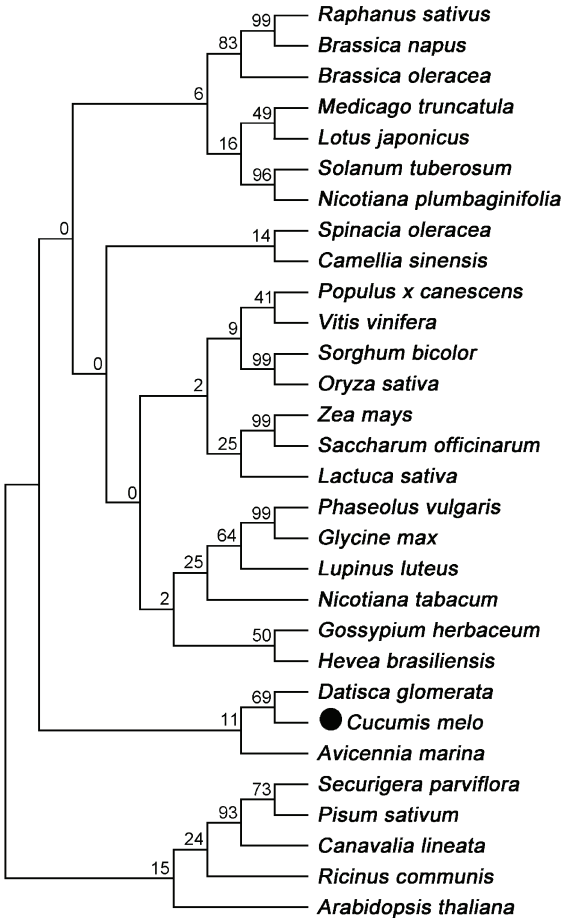

Fig. 2. Phylogenetic relationship of M-GS1 and other GS1 proteins. M-GS1 sequence was placed among the GS1 sequences from families such as *Cruciferae*, *Datiscaceae, Acanthaceae, Brassicaceae, Fabaceae*, *Euphorbiaceae*, *Salicaceae*, *Pinaceae*, *Euphorbiaceae*, *Solanaceae*, *Amaranthaceae*, *Theaceae*, *Malvaceae*, *Vitaceae*, *Poaceae* and *Asteracea*. Protein accession numbers are *Raphanus sativus*, BAA04996; *Brassica napus*, CAA58118; *Brassica oleracea*, ACF42116; *Medicago truncatula*, CAA71317; *Lotus japonicus*, CAA73366; *Solanum tuberosum*, AAG40237; *Nicotiana plumbaginifolia*, P12424; *Spinacia oleracea*, ABU45501; *Camellia sinensis*, BAD99526; *Populus* × *canescens*, AAK49029; *Vitis vinifera*, CAC39216; *Sorghum bicolor*; CAQ86599; *Oryza sativa*, NP_001049424; *Zea mays*, P38561; *Saccharum officinarum*, AAW21273; *Lactuca sativa*, P23712; *Phaseolus vulgaris*, P00965, *Glycine max*, O82560; *Lupinus luteus,* P52782; *Nicotiana tabacum*, CAA65173; *Gossypium herbaceum*, ABW89460; *Hevea brasiliensis*, AAB61597; *Datisca glomerata*, AAR29057; *Cucumis melo*, ABI30732; *Avicennia marina*, AAK08103; *Securigera parviflora*, AAP33167; *Pisum sativum*, CAJ87510; *Canavalia lineata*, AAD52008; *Ricinus communis*, EEF35241; *Arabidopsis thaliana*, AAO42253.

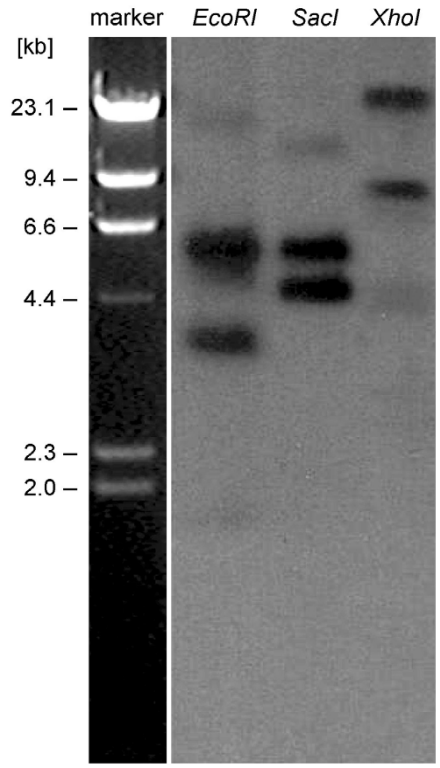

Fig. 3. Southern blot analysis of *M-GS1* gene. Genomic DNA (around 10 μg) isolated from young leaves of melon plants (10-d-old) was digested with *Eco*RI, *Sac*I and *Xho*I, fractionated in 0.8 % agarose-TAE gel, transferred to *Hybond N*-membrane and hybridized with specific ^{32}P-radiolabelled probes for *M-GS1*.

Southern blot analysis were also performed. Sequence analysis revealed that M-GS1 exhibited high similarity with the GS1s previously reported in various plant species and shared structural features characteristic of a plant GS1. The molecular evolution analysis showed that the GS1 isoforms reported so far in different plant species displayed relatively distant evolutionary relationships, and M-GS1 was related most closely to the GS1 from *Datisca glomerata*, implying that they may be diversified from a common ancestor during evolution. Southern blot analysis indicated that *M-GS1* may belong to a small gene family of two or 3 members in melons, similar to the southern blot results in *A. thaliana* and maize, in which GS1 was suggested as encoded for by a small subfamily of genes that varies in number from three to five (Peterman and Goodman 1991, Li *et al.* 1993). All these data strongly suggested that *M-GS1* is a functional *GS1* gene in melons.

It is known that *GS* genes could be differentially regulated in different tissues of plants or by different nutrition. Feeding nitrate to the roots of maize markedly increased the mRNA and corresponding polypeptide levels of GS2 and a root-specific GS (GSr) localized in the extra plastidic compartment, but not those of GS1, while external ammonium was effective only in inducing the

Table 1. Relative mRNA levels of *M-GS1* and GS activity [μmol(L-Glu-γ-MHA) g^{-1}(f.m.) min^{-1}] in different tissues (root, stem and leaves) of melon plants that were cleaned in pure water for 6 h (controls) or that were fed further with various forms of N (ammonium, nitrate or glutamate) at different concentrations (0.75, 3.75 or 7.50 mM) for 24 h. Means ± SE, $n = 6$. Values with the same letter were not significantly different at $P < 0.05$ according to Student's *t*-test.

N form	Conc. [mM]	mRNA root	stem	leaf	GS activity root	stem	leaf
Control	0	0.28 ± 0.04 ^{c}	0.22 ± 0.02 ^{c}	0.26 ± 0.09 ^{c}	1.13±0.12 ^{c}	0.55±0.18 ^{c}	0.95±0.20 ^{c}
NH_4	0.75	0.30 ± 0.01 ^{c}	0.17 ± 0.01 ^{c}	0.23 ± 0.01 ^{c}	1.27±0.25 ^{c}	0.60±0.23 ^{c}	0.87±0.10 ^{c}
	3.75	0.81 ± 0.05 ^{a}	0.78 ± 0.10 ^{ab}	0.63 ± 0.02 ^{b}	3.17±0.28 ^{b}	1.83±0.30 ^{ab}	1.92±0.58 ^{b}
	7.50	0.87 ± 0.03 ^{a}	0.87 ± 0.01 ^{a}	0.65 ± 0.05 ^{b}	4.23±0.58 ^{a}	2.12±0.25 ^{a}	2.03±0.62 ^{a}
NO_3	0.75	0.27 ± 0.01 ^{c}	0.23 ± 0.06 ^{c}	0.28 ± 0.08 ^{c}	0.92±0.15 ^{c}	0.53±0.10 ^{c}	1.10±0.23 ^{c}
	3.75	0.54 ± 0.02 ^{b}	0.18 ± 0.01 ^{c}	0.66 ± 0.11 ^{b}	2.78±0.47 ^{b}	0.58±0.08 ^{c}	2.00±0.35 ^{b}
	7.50	0.85 ± 0.06 ^{a}	0.24 ± 0.01 ^{c}	1.66 ± 0.10 ^{a}	4.08±0.43 ^{a}	0.42±0.20 ^{cd}	3.18±0.30 ^{a}
Glu	0.75	0.26 ± 0.05 ^{c}	0.15 ± 0.01 ^{c}	0.23 ± 0.04 ^{c}	1.15±0.22 ^{c}	0.48±0.18 ^{c}	0.82±0.32 ^{c}
	3.75	0.20 ± 0.02 ^{d}	0.14 ± 0.03 ^{c}	0.22 ± 0.05 ^{c}	0.42±0.13 ^{d}	0.43±0.08 ^{c}	0.87±0.08 ^{c}
	7.50	0.12 ± 0.01 ^{e}	0.19 ± 0.01 ^{c}	0.27 ± 0.04 ^{c}	0.33±0.07 ^{d}	0.45±0.15 ^{c}	0.90±0.17 ^{c}

expression of GSr (Sakakibara *et al.* 1992). In the leaves of maize, however, only GS2 was enhanced by nitrate feeding (Sakakibara *et al.* 1992). Plant *GS1* gene family consists of several isoenzymes, and their physiological roles and regulations are rather complex compared with *GS2* (Rana *et al.* 2008). The expression patterns of *M-GS1*, as found in this study, were similar to those of maize *GS*r, being also positively responsive to both nitrate and ammonium supply, except that *M-GS1* expression displayed no evident tissue specificity. These characteristics of M-GS1 suggest, that in melons it could be a GS1 isoform important for the assimilation of ammonia from both the primary and secondary sources. Significant regulations on *M-GS1* expression in the roots and leaves by both ammonium and nitrate suggest that these two organs are the major locations where M-GS1 functions and inorganic N is assimilated. Oliveira and Coruzzi (1999) reported that addition of sucrose to the cultures induced strong expressions of both *GS2* and *GS1* in *Arabidopsis* and the dramatic induction of *GS2* expression by irradiance was actually mediated in part by the radiation-induced changes in sucrose content. Interestingly, they further showed that these inductions were antagonized by further addition of amino acids to the cultures. In the present study, the expression of *M-GS1* was also depressed strongly in the roots but not affected in the stems and leaves by glutamate, agreeing with the scenario that the metabolic regulation of GS expression in plants is controlled partially by the relative abundance of carbon skeletons *versus* amino acids (Oliveira and Coruzzi 1999). This would allow N assimilation to proceed according to the metabolic status and biosynthetic needs of the plant. However, by treating the leaf discs with glutamate, Masclaux-Daubresse *et al.* (2005) showed that the expression of an early senescence-associated GS1 isoform in tobacco was significantly triggered, and furthermore, similar results were documented for a radish GS1 isoform in a previous study using cell culture systems (Watanabe *et al.* 1997), suggesting diversified roles for different GS1 isoforms.

Generally, a GS activity in different tissues was also regulated by different forms of N (Table 1), however, the changes in *M-GS1* mRNA did not result in equivalent changes in enzyme activity. Ortega *et al.* (2001) also showed in alfalfa plants transformed with GS1 under the control of the 35S (CaMV) promoter accumulation of GS1 transcripts without corresponding increase in the enzyme activity. Although *M-GS1* expressions displayed no evident tissue specificity at mRNA levels, GS activity was higher in the roots and leaves than in the stems in most treatments of the present study. These results suggest that the expression of *M-GS1* might also be post-transcriptionally regulated or there could be other unidentified GS isoenzymes functioning in melons.

In conclusion, the expression of *M-GS1*, the first cytosolic GS gene isolated in melon, was regulated differentially in different tissues of melon by N fertilization. The cloning and characterization of *M-GS1* gene will enable us to investigate further the N assimilation processes in melons at molecular level.

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