DIFFERENTIAL REGULATION OF GS-GOGAT GENE EXPRESSION BY PLANT GROWTH REGULATORS IN ARABIDOPSIS SEEDLINGS

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Abstract: Primary and secondary ammonium assimilation is catalyzed by the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway in plants. The Arabidopsis genome contains five cytosolic GS1 genes (GLN1;1 – GLN1;5), one nuclear gene for chloroplastic GS2 isoform (GLN2), two Fd-GOGAT genes (GLU1 and GLU2) and a GLT1 gene coding for NADH-GOGAT. Even though the regulation of GS and GOGAT isoforms has been extensively studied in response to various environmental and metabolic cues in many plant species, little is known about the effects of phytohormones on their regulation. The objective of this study was to investigate the impact of representative plant growth regulators, kinetin (KIN), abscisic acid (ABA), gibberellic acid (GA3) and 2,4-dichlorophenoxyacetic acid (2,4-D), on the expression of Arabidopsis GS and GOGAT genes. The obtained results indicate that GS and GOGAT genes are differentially regulated by growth regulators in shoots and roots. KIN and 2,4-D repressed GS and GOGAT expression in roots, with little effect on transcript levels in shoots. KIN affected all tested genes; 2,4-D was apparently more selective and less potent. ABA induced the expression of GLN1;1 and GLU2 in whole seedlings, while GA3 enhanced the expression of all tested genes in shoots, except GLU2. The observed expression patterns are discussed in relation to physiological roles of investigated plant growth regulators and N-assimilating enzymes.

Key words: Glutamine synthetase; glutamate synthase; abscisic acid; kinetin; gibberellic acid

INTRODUCTION

The glutamine synthetase-glutamate synthase (GS-GOGAT) cycle provides an entry route for reduced inorganic nitrogen into all organic nitrogenous compounds in plants. GS (EC 6.3.1.2) catalyzes ATP-dependent ligation of ammonia and glutamate to yield glutamine, while GOGAT catalyzes the redox transfer of the glutamine amide group to α-ketoglutarate, forming two glutamate molecules. Apart from assimilation of ammonia from primary sources such as nitrate reduction, nitrogen fixation and ammonia uptake, the GS-GOGAT cycle is active during secondary assimilation of ammonia derived from different metabolic processes such as photorespiration, amino acid catabolism and the phenylpropanoid pathway [1]. Consistent with the diversity of metabolic roles, GS and GOGAT are present in most plants as several isoforms. Higher plants contain one nuclear gene for a plastidic GS isoform, GS2, and a small gene family encoding different cytosolic GS1 isoforms [2]. The Arabidopsis genome contains one GS2 gene (GLN2) and five GS1 genes (GLN1;1 – GLN1;5) with specific spatio-temporal regulation [3], different kinetic properties and differential regulation of expression by external N supply [4], suggesting non-redundant functions [4-6]. Two types of GOGAT isoforms, Fd-GOGAT (EC 1.4.7.1) and NADH-GOGAT (EC 1.4.1.14), are localized in the plastids [7]. Arabidopsis contains two Fd-GOGAT genes, GLU1 and GLU2, and one NADH-GOGAT – GLT1, which were also shown to have non-overlapping roles in distinct processes [8].
Given the importance of the GS-GOGAT cycle in providing and recycling organic nitrogen, and the fact that nitrogen availability and its utilization efficiency are limiting factors in plant productivity [1], the effort to understand the regulation and roles of various GS and GOGAT isoforms is not surprising. The key regulators of GS and GOGAT gene expression are light, nitrate, ammonium, amino acids and sugars [4,8-11]. Recently, several classes of phytohormones, namely cytokinins, auxins and abscisic acid (ABA) have been implicated in the regulation of nitrogen transport, assimilation and distribution [12], while evidence of the effects of gibberellins is mostly indirect [13-14]. Here we present a study of the effects of four representative growth regulators on the expression of GS and GOGAT isoforms in Arabidopsis seedlings grown in liquid culture.

MATERIALS AND METHODS

Plant growth conditions and growth regulator treatment

Arabidopsis thaliana ecotype Columbia (NASC ID: N60000) seedling liquid culture was established in vitro, as previously described [15]. The seedlings, cultivated for one month submerged in growth regulator-free liquid MS medium [16] supplemented with 3% sucrose, were transferred to fresh medium containing varying concentrations (10^-8, 10^-7, 10^-6 and 10^-5 M) of 2,4-dichlorophenoxyacetic acid (2,4-D), ABA, kinetin (KIN) or gibberellic acid (GA3). Plant harvesting was carried out 24 h after hormone application; roots and shoots were separated and stored at -70°C.

RNA Extraction and RT-qPCR

Total RNA was isolated from roots and shoots using Trizol (Invitorgen), quantified spectrophotometrically and its integrity was checked by gel electrophoresis. For reverse transcription, 1 µg of total RNA was pretreated with DNase I (Fermentas) followed by reverse transcription using a RevertAid First Strand cDNA Synthesis Kit (Fermentas), with oligo-dT primers, at 42°C, according to the manufacturer’s instructions. Real-time PCR reactions were set with Maxima SYBR Green mix (Fermentas) using cDNA corresponding to 50 ng RNA and 0.3 mM primers (Table 1), in 25 µl total volume. The amplification was carried out on an ABI PRISM 7000 SDS thermal cycler (Applied Biosystems). The qPCR program included initial denaturation (95°C/10 min), 40 cycles of denaturation (95°C/15 s), annealing (58.5°C/20 s) and extension (72°C/30 s), followed by melting curve analyses. Standards for absolute quantification were prepared from PCR products purified by gel electrophoresis and extracted with a GeneJET Gel Extraction Kit (Fermentas) according to the manufacturer’s protocol. Constitutive expression of 18S rRNA was confirmed in parallel using the same poly(dT)16 primed RT mixture [17].

Table 1. Sequences of primers used for qPCR.

<table>
<thead>
<tr>
<th>gene</th>
<th>GenBank</th>
<th>forward (5’→3’)</th>
<th>reverse (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN1:1</td>
<td>NM_123119</td>
<td>AGAACTGTCATGGCGGCTACGT</td>
<td>GTCAGATGCAGTCTCAGTGTC</td>
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<tr>
<td>GLN1:2</td>
<td>NM_105291</td>
<td>AGCGGACACCATGAAATGCTC</td>
<td>GCCAGCTCAACCAGGTCAAA</td>
</tr>
<tr>
<td>GLN1:3</td>
<td>NM_112663</td>
<td>TCGGCCCTGTTGAGGTTATT</td>
<td>CACGTCCACTCTCACGAGAC</td>
</tr>
<tr>
<td>GLN1:4</td>
<td>NM_121663</td>
<td>GGAGTTCCAAGTCCAGTCCC</td>
<td>GGTTTGCCACACCCCATATA</td>
</tr>
<tr>
<td>GLN1:5</td>
<td>NM_103743</td>
<td>CATTGCCCTGGAACAGTGGGAT</td>
<td>CACCGATGTCACGCCATCC</td>
</tr>
<tr>
<td>GLN2</td>
<td>NM_122954</td>
<td>ATGCGCTGGACAGTTGAGGATT</td>
<td>GCTCAGCTGTTCTCAGTGCA</td>
</tr>
<tr>
<td>GLU1</td>
<td>NM_180432</td>
<td>CTTGTGTCCTGTTGTCTGGT</td>
<td>CTTGACCACCTTCCATAGCC</td>
</tr>
<tr>
<td>GLU2</td>
<td>NM_129687</td>
<td>CCTGTGTCCTGGAAGTTGGT</td>
<td>TGACACACCAAAAGCCGCTAGG</td>
</tr>
<tr>
<td>GLT1</td>
<td>NM_124725</td>
<td>TCGAGCTGCCTGGAACCTTCT</td>
<td>CACTTGAGACGACCCCTACG</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>X16077</td>
<td>TGACGGAGAATTAGGTTCG</td>
<td>CAATGGATCCCTCGTTAACG</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The expression data in control plants (Table 2) showed that the GS1 genes generally have slightly higher expression in roots compared to shoots, and that GLN1;2 transcripts are the most abundant in both tissue samples. The expression of GLN1;4 was below the detection threshold in shoots, while GLN1;5 transcripts were not detected in seedlings. These results are consistent with previously published data for plants grown under ample nitrogen supply [4-5,15].

As expected, based on the proposed roles in photorespiration [8-11,18], the expression of the chloroplastic GLN2 was higher in shoots, while Fd-GOGAT GLU1 transcripts were found in shoots only. The GOGAT genes GLU2 and GLT1 were dominantly expressed in roots (Table 2). Previously it was shown that GLT1 is mainly involved in primary assimilation and remobilization of nitrogen [8, 19], while it was proposed that GLU2 encoded enzyme serves to supply a constitutive level of glutamate for the maintenance of a basal level of protein synthesis [8].

The tested plant growth regulators differentially modulated the expression of GS (Fig. 1) and GOGAT (Fig. 2) genes in roots and shoots. KIN had a higher impact on gene expression in roots, where significant repression was found for all GS and GOGAT genes, while the effect on expression in shoots was less pronounced, usually at higher KIN concentrations. This result is consistent with the postulated role of cytokinins, which act in signaling routes that communicate the internal and external nitrogen status of the plant. Cytokinin synthesis is upregulated during N abundance, while high cytokinin levels cause suppression of N-uptake and primary assimilation [12,20].

Treatment with 2,4-D had a more pronounced effect on gene expression in roots, where it downregulated the most abundantly expressed GS1 gene – GLN1;2, plastidic GLN2 (Fig. 1), as well as the two GOGAT genes – GLU2 and GLT1 (Fig. 2). The only gene whose expression was enhanced in both roots and shoots in the presence of high 2,4-D concentrations was GLN1;3. Based on this it can be concluded that 2,4-D and KIN have somewhat overlapping effects on primary N-assimilation; however, 2,4-D shows higher specificity and less potency in the repression of GS1 gene expression. Similar results were obtained when the effect of cytokinins and auxins on the expression of Arabidopsis root-type nitrate transporter genes (AtNRT) was evaluated [12]. Namely, trans-Zeatin showed non-selective repression of all root AtNRT genes, while the effect of indole-3-acetic acid was less pronounced and apparently selective.

Treatment with ABA significantly induced GLN1;1, GLN1;3 and GLU2 genes in entire seedlings, as well as GLN1;4 and GLN2 expression in roots (Figs. 1 and 2). The most affected genes were GLN1;1, whose expression in roots was increased almost 4-fold, and GLU2, which was induced 5.5-fold in shoots. Since ABA is considered one of the main hormonal regulators during abiotic stress responses [21], the ABA-responsive GLN1;1 and GLU2 genes can be considered as stress-responsive genes. Indeed, GLN1;1 is upregulated during cold [22] and salinity stress [23]. Furthermore, among all A. thaliana GS1 single knockout mutants, only GLN1;1ko had an increased stress sensitivity phenotype [22]. The role of GLU2 is not well defined, but it seems that GLU2 is involved neither in primary nitrogen assimilation nor in photorespiratory ammonium removal [8]. Interestingly, Arabidopsis abiotic stress expression maps [24] indicate that GLU2 transcripts accumulate in shoots of plants under osmotic and salt stress.

### Table 2. Absolute expression of GS and GOGAT genes in roots and shoots of control (hormone untreated) plants. The values represent the average copy number per µg of total RNA for three biological repetitions (n = 3) of 10 plants each ± standard error (SE), nd – not detected.

<table>
<thead>
<tr>
<th>gene</th>
<th>root</th>
<th>shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN1;1</td>
<td>2.65 · 10⁶ ± 4.27 · 10⁵</td>
<td>1.38 · 10⁴ ± 2.46 · 10⁵</td>
</tr>
<tr>
<td>GLN1;2</td>
<td>5.31 · 10⁷ ± 6.57 · 10⁶</td>
<td>2.27 · 10⁷ ± 3.29 · 10⁶</td>
</tr>
<tr>
<td>GLN1;3</td>
<td>6.49 · 10⁴ ± 5.28 · 10⁴</td>
<td>5.85 · 10⁴ ± 1.02 · 10⁵</td>
</tr>
<tr>
<td>GLN1;4</td>
<td>3.07 · 10⁴ ± 4.30 · 10⁴</td>
<td>nd</td>
</tr>
<tr>
<td>GLN1;5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>GLN2</td>
<td>7.42 · 10⁴ ± 5.21 · 10⁴</td>
<td>2.29 · 10⁴ ± 3.10 · 10⁵</td>
</tr>
<tr>
<td>GLU1</td>
<td>nd</td>
<td>9.43 · 10⁴ ± 4.23 · 10⁴</td>
</tr>
<tr>
<td>GLU2</td>
<td>1.87 · 10⁵ ± 4.64 · 10⁵</td>
<td>2.85 · 10⁴ ± 2.98 · 10⁵</td>
</tr>
<tr>
<td>GLT1</td>
<td>6.49 · 10⁴ ± 4.37 · 10⁴</td>
<td>7.41 · 10⁴ ± 3.88 · 10³</td>
</tr>
</tbody>
</table>
Previously it was shown that during abiotic stress responses a large portion of Glu synthesized by the GS-GOGAT pathway is used for proline production [25], which is a common osmoprotectant.

GA3 treatment significantly induced the expression of all studied genes in the shoots except GLU2, while its effect on expression in roots was minor or absent. This result indicates that GA3 does not regulate primary assimilation of ammonium in roots, but is involved in ammonium (re)assimilation in shoots. Little is known about the effects of gibberellins on nitrogen metabolism. This class of hormones has been shown to induce the expression of cytosolic GS in pine [26] and to reduce glutamate content in Arabidopsis shoots [14], probably by stimulating its utilization for growth. In view of this, the observed stimulation of GS and GOGAT expression in shoots by GA3 might serve to provide enhanced Glu production, to support gibberellin-stimulated shoot growth.

We have shown that the expression of GS and GOGAT genes in Arabidopsis seedlings grown submerged in liquid culture is comparable to literature data on the expression profiles of these isoforms in seedlings grown under standard conditions. We found liquid culture convenient for investigation of the effects of plant growth regulators on gene expression in different organs of Arabidopsis seedlings, since this system allows for the uniform application of constant concentrations of the investigated substances, without consideration of internal transport issues or concentration fluctuations imposed by foliar or other types of applications. All of the GS-GOGAT genes were found responsive to at least one of the four tested growth regulators, indicating that N assimilation is modulated not only by N supply, availability of carbon skeletons, light and other factors broadly investigated in literature, but also by phytohormones. The results obtained concur well with the available literature data on the specific functions of different GS and GOGAT isoforms. The established system and the obtained results provide a good basis for further investigation into the role of phytohormones in the regulation of N assimilation.
Authors’ contributions: M. Dragićević and S. Todorović contributed equally to this work. A. Simonović, A. Subotić, S. Todorović and I. Dragićević conceived and designed the experiments. M. Dragićević, M. Bogradnović, N. Ghalawenji and S. Todorović performed the experiments. M. Dragićević, A. Simonović and S. Todorović analyzed the data. A. Simonović and S. Todorović contributed reagents/materials/analysis tools. M. Dragićević and A. Simonović wrote the paper.

Conflict of interest disclosure: The authors declare that they have no competing interests.

REFERENCES


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