Glutamine synthetase (GS) of wheat

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Contributor: Yihao Wei

Glutamine synthetase isoforms of wheat play distinct roles in nitrogen assimilation for their different kinetic properties, tissue locations, and response to nitrogen regimes.

Keywords: wheat ; glutamine synthetase ; nitrogen assimilation ; kinetic properties ; localization

Glutamine synthetase (GS), the key enzyme in plant nitrogen assimilation, is strictly regulated at multiple levels, but the most relevant reports focus on the mRNA level.

1. Introduction

Nitrogen (N) is a key limiting factor in the yield and quality of crops, and large quantities of nitrogen fertilizers are required to attain maximal growth and productivity[1][2]. To increase crop production, nitrogen fertilizers are often applied excessively, leading to severe nitrogen pollution on a global scale[3][4]. Therefore, there is a need to improve nitrogen use efficiency (NUE) to make agriculture more sustainable[4][5].

In order to improve crop NUE, glutamine synthetase (GS; EC 6.3.1.2) has been studied numerous times owing to its essential role in the assimilation of inorganic N[1][6][7][8][9][10]. GS catalyzes the ATP-dependent fixation of ammonium (NH_4^+) to glutamate (Glu) to form glutamine (Gln)[11]. Plant GS is classified into two groups according to its subcellular location: Cytosolic glutamine synthetase (GS1) and chloroplast glutamine synthetase (GS2) [12][13]. GS2 is encoded by a single gene, while GS1 is encoded by a multigene family[5].

2. GS isozymes are Involved in Gln Synthesis

Although all GS isozymes are involved in Gln synthesis, GS isozymes play different roles in nitrogen assimilation or transportation in plants. GS2 is involved in assimilating NH_4^+ derived from photorespiration and nitrate (NO_3^-) reduction[14][15]. GS1 has multiple isoforms with distinct affinities for NH_4^+ and glutamate[16][17], and each GS1 isoform may have a different function in nitrogen assimilation or transportation. Wheat is an important crop for mankind. individual wheat GS (TaGS) isozymes are classified into four subfamilies: TaGS1, TaGSr, TaGSe, and TaGS2[9]. Thomsen et al. clustered GS isozymes of cereals into four categories: GS1;1, GS1;2, GS1;3, and GS2[5]. Based on the cluster of TaGS isoforms, researchers renamed TaGS1, TaGSr, and TaGSe genes as TaGS1;1, TaGS1;2, and TaGS1;3, respectively.

The physiological functions of GS isozymes have been studied according to the cellular localization and expression characteristics. In Arabidopsis, the green fluorescent protein (GFP) signal driven by the AtGln1;1 promoter is recorded in the epidermal cells of the root elongation zone and can affect primary root development in response to exogenous N provision[18]. The promoter of AtGln1;2 can drive reporter gene expression in the mesophyll and vasculature of developed leaves[19][20]; vascular cells, cortex, and epidermis of roots[18][21]; epidermal cells of sepals; and veins of petals and stamens[18]. The mRNA level of AtGln1;2 can be upregulated to relieve NH_4^+ toxicity under ample nitrate (NO_3^-) supply and high NH_4^+ supply conditions[19][20][21]. Promoter::GFP fusion has shown that AtGln1;3 expression is localized in the pericycle, suggesting a role in loading glutamine to the xylem[21]. A more recent study showed that β-glucuronidase (GUS) activity driven by Gln1;1−5 promoters was localized in phloem companion cells but in veins of different order, and AtGln1;1, AtGln1;2, and AtGln1;3 act together for N remobilization and seed filling[22].

In maize, ZmGln1−3 in the mesophyll cells has a role in the synthesis of Gln following NO_3^- reduction until plant maturity[7][23]. ZmGln1−4 in bundle sheath cells has a role in the reassimilation of NH_4^+ released during protein degradation in senescing leaves[7][24]. In rice, OsGS1;1, with its transcript located in vascular tissue of mature leaves, has a role in grain filling[25][26]. OsGS1;2, with its transcript located in surface cells of roots in an NH_4^+-dependent manner, is important in the primary assimilation of NH_4^+ taken up by rice roots[16][27]. OsGS1;3 transcript is mainly expressed in the spikelet, indicating a key role in grain ripening and/or germination[28]. In wheat, TaGS1 transcript is present in the perifascicular sheath cells, and TaGSr transcripts are confined to the vascular cells[9][29]. During leaf senescence, TaGS1

and TaGSr have high mRNA levels, suggesting major roles in assimilating ammonia during the critical phases of remobilization of nitrogen to the grain[9]. However, since GS genes are highly homologous and their gene products are indistinguishable at the protein level by any GS antibody, previous studies about the cellular localization and expression characteristics of individual GS isozymes were mainly focused on the mRNA level[30].

In cells, the inorganic nitrogen assimilation process that GS participates in consumes a substantial amount of energy; therefore, GS must be tightly regulated at the gene, transcript, and protein level[5][11][31][32]. The regulation of each step of this process may affect the localization and activity of GS. In transgenic alfalfa, constitutively overexpressed GS1 genes significantly increased the level of GS1 transcripts in the leaves, but it did not significantly change the level of GS1 polypeptides[33]

3. Conclusion

In plants, each GS isozyme plays a different role in nitrogen metabolism, and the expression of GS is strictly regulated at multiple levels[5][11][31][32]. GS proteins are responsible for the catalytic activity. However, previous studies about GS isoforms mainly focused on the mRNA level. In this study, using antibodies specific to individual TaGS isozymes, the expression differences of TaGS isoforms at the protein level were analyzed. Moreover, some new functions of TaGS isoforms were discovered by analyzing the effects of N supply on their expression and localization at the protein level, and their kinetic properties and nitrogen metabolism.

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