GREENUPORTO Sustainable Agrifood Production Research Centre

A. thaliana Devoid Of Cytosolic Glutamine Synthetase 1;3 In Leaves and Meristems Is Compensated By Chloroplastidial Glutamine Synthetase

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Introduction

In *A. thaliana* GS is encoded by a 6 isogenes family, five of which have cytosolic (GS1) location (*Gln1;1* to *Gln1;5*) and a plastidic one (*Gln2;0*), all involved in a wide variety of physiological processes throughout plant life cycle. Chloroplast located Glutamine Synthetase (GS2) plays a major role in the reassimilation of ammonium and has been previously shown that its function can be partially replaced by the cytosolic *Gln1;3* and *Gln1;2*, both under photorespiratory and non permitting photorespiratory conditions (Ferreira *et al.,* 2019). Not as relevant as its action in the photorespiration process, GS2 shows some activity at root level associated to nitrate assimilation with joint function of the nitrite reductase (Ishiyama et al., 2004; Prinsi & Espen, 2015; Wei et al., 2020). These factors led to the formulation that, possibly, the GS2 steps in the nitrate uptake (Woodwall & Forde, 1996).
The results of this work endorse the role of GS2 in the assimilation of cytosolic ammonium, further supporting the existence of a compensation mechanism between cytosolic and chloroplastic GS forms.

Results

1 RT-PCR on single GS1 mutants

To verify GS2 leaf expression in different GS1 knock-out single mutants a RT-PCR was performed. The results showed that a lack of *Gln1;3* expression possibly leeds to GS2 overexpression in leaves (**Fig.1**).

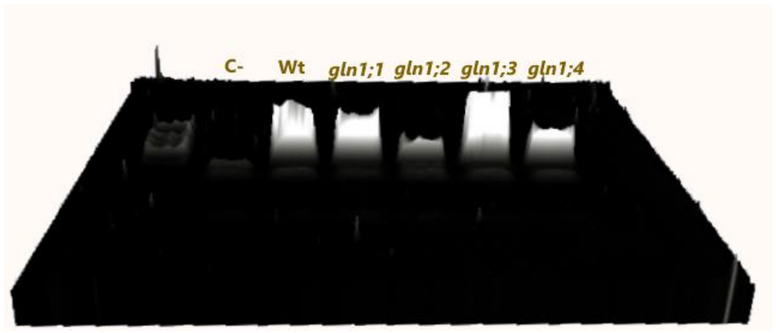

Fig.1 – *Gln2;0* expression in the leaves of distinct GS1 single mutants, by semiquantitative RT-PCR
The synthetized cDNAs were previously quantified to 1,25 µg/µL and made from equally quantified RNA. The *ACT8 was chosen as a reference gene to ensure that all samples were relatively balanced.*

2 RT-qPCR

A Real-Time PCR assay was performed in leaves of the *gln1;3* mutants, to confirm the preliminary results of the semiquantitative RT-PCR. The RT-qPCR confirmed that in the leaves of the *gln1;3* mutant, not only the *Gln2;0*, but also the cytosolic GS genes, *Gln1;1* and *Gln1;2*, were overexpressed (**Fig.2**) .
To go further into this compensation mechanism, the meristems (where the expression of *Gln1;3* is high) of the mutant *gln1;3* were also analysed revealing similar expression patterns as the observed in the leaves.

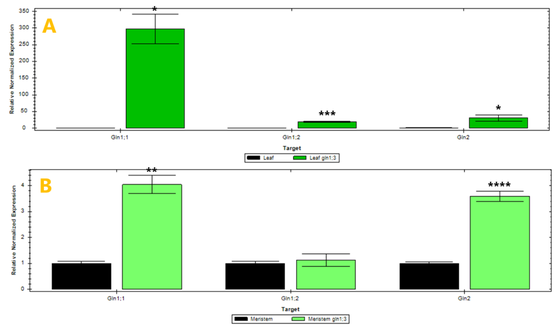

Fig.2 – RT-qPCR *Gln2;0* relative expression in the *gln1;3* single mutant plants
The synthetized cDNAs were previously quantified to 1,25 µg/µL and made from RNA equally quantified. The *AtACT8* and *AtRCE1* were defined as reference genes to ensure the normalization of the expression results. Two month leaves (A) and meristems (B) were determined as sample targets.
*P<0,05, **P<0,01, ***P<0,001, ****P<0,0001

Results

3 GS activity and protein

GS activity quantification and Western blot analysis, using an anti-GS antibody, was performed in leaves of Wild type (Wt) and *gln1;3* plants, in order to confirm the Real-Time PCR results.
The activity assay revealed that in the *gln1;3* mutants the GS activity is significantly higher than in the wild type plants (**Fig.3**) and the increased activity kept up with a higher content of GS1 and GS2 proteins in the mutant plants, as revelled by the western blot (**Fig.4**).
These results further support a compensation mechanism in which the absence of a cytosolic isoenzyme (GLN1;3) leads to an increased expression of the chloroplast, and also other cytosolic isogenes.

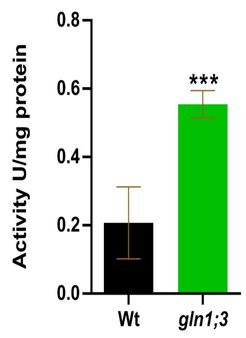

Fig.3 – GS activity in *gln1;3* mutants
Transferase GS activity was performed in wild type and *gln1;3* mutant leaves. Results are expressed as means ±SD of 3 biological replicates assayed in triplicate. ***P<0,001

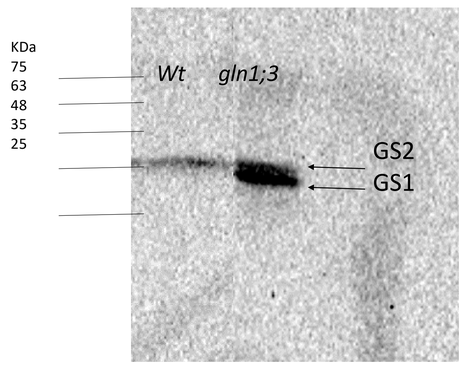

Fig.4 – Western Blot analysis in leaves of Wt and *gln1;3*
The proteins (30 µg) were separated by SDS-PAGE, transferred to nitrocellulose and the membranes proved with an anti-GS antibody. Molecular weight markers and GS1 and GS2 positions are indicated

Conclusions

- Ferreira *et al.*, 2019, described an A. *thaliana* species-specific compensation mechanism, in which the cytosolic GS isoenzymes are overexpressed in GS2 absence. In this research it is shown that this mechanism also occurs in reverse, being possible the compensation of the of GS1 isoenzymes by GS2;
- The knock-out of *Gln1;3* leads to higher GS activity in leaves and greater content of GS1 and GS2 protein;
- The knock-out of *Gln1;3* leads to an overexpression of other cytosolic isogenes in both leaves (*Gln1;1 and Gln1;2*) and meristems (*Gln1;1*);

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