The effect of expression levels of *AtGR1* and *AtGR2* genes on the sulfur assimilation pathway in *Arabidopsis thaliana* plants

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Sulfur is an important element in plant nutrition required for the synthesis of several essential metabolites such as amino acids, *viz.* cysteine and methionine, and glutathione. Glutathione (GSH) plays diverse roles in plants, including preventing oxidation damage. During oxidative stress, two oxidized GSH molecules form a glutathione disulfide complex through a disulfide bond (GSSG). These complexes are degraded in vacuole or are recycled by reduction, which is catalyzed by the enzyme glutathione reductase (GR) in a process that requires NADPH as an electron donor. In plants, two genes encode for GR: GR1, which is located in the cytosol; and GR2, which is located in the chloroplast. It was expected that the overexpression of GR would lead to a higher GSH content and tolerance to oxidative stress. However, the effect was deceptive, since several plants were indeed more tolerant, while others were not, and some were more sensitive.

Transgenic *Nicotiana tabacum* plants over-expressing GR1 and GR2 genes from *Arabidopsis thaliana* (*AtGR1* and *AtGR2*) showed high GR activity and elevated cysteine and GSH levels, together with a high GSH/GSSG ratio, but they did not differ from wild type plants regarding their tolerance to oxidative stress. The high GSH levels in these plants might result from higher GR activity that reduced its degradation, but it cannot explain the higher cysteine levels. Thus, we assume that the elevation in these two metabolites results from GR overexpression that increased cysteine synthesis through the sulfur assimilation pathway. A high level of cysteine enables the accumulation of GSH, as previously shown. The hypothesis of this research proposed (as theoretically previously suggested) that when GSH donates two electrons to the catalytic reaction of adenosine 5' phosphosulfate reductase (APR), which is known to be the rate-limiting enzyme sulfur assimilation pathway, GSH is oxidized, and thus GR is required to reduce GSSG to GSH in order to maintain APR activity and make this pathway efficient.

To examine this possibility further, these two genes were overexpressed in Arabidopsis plants, which, together with *gr1* and *gr2* mutants, were compared to wild type plants and transgenic plants having an empty vector. The results showed that compared to the control plants, plants overexpressing GR have a higher level of GR activity, a high GSH/GSSG ratio, and higher contents of cysteine, glutathione and methionine, while the mutants exhibit lower levels in all of these parameters. The levels of sulfide, the product of APR, increased significantly in transgenic plants but decreased significantly in the mutants; however, the levels of sulfate did not change compared to the control in transgenic plants but increased in the mutants. These findings support the hypothesis that GR plays an important role in the sulfur assimilation pathway in Arabidopsis and apparently in other plants. In addition, this work offers a new way of enhancing a plant's nutritional value by increasing the levels of both sulfur amino acids, keeping the level of glutathione high for its different functions.