# Morphological, Physiological and Metabolomic Response of transgenic tobacco plants (*N. tabacum* L.) overexpressing GmGSTU4 under Drought Stress

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## Abstract

**GSTs appear to have a significant role in plants' adaptation under abiotic stress as many isoenzymes are found to be differentially expressed under these conditions yet, little is known about the regulatory functions of GSTs. Wild type and transgenic tobacco plants over-expressing the soybean *Gm*GSTU4 of cultivars Basmas, Burley and Virginia were grown *in vitro* under 100 and 200mM mannitol or in soil (plant pots) by withholding watering for 15 days. However, *Gm*GSTU4 plants did not exhibit significant differences in drought tolerance compared to wild-type plants. Morphological (shoot length, total and root fresh weight) and physiological (chlorophyll content, relative water content and photosynthetic capacity) parameters of transgenic plants did not differ from the wild-type in the presence of 100 or 200mM mannitol or in the soil when watering was halted. Metabolite profiling was used to understand the dynamics between the wild-type and transgenic tobacco response to drought stress. Different metabolic pathways are involved in production of osmoprotectants. These molecules accumulate in plants under stress conditions as adaptive mechanism, which can provide stress tolerance. GmGSTU4 plants did not exhibit difference in drought tolerance compared to wild-type plants, however metabolomics analysis indicated alterations in metabolite profile and increased concentration of sorbitol, glycerol and pyruvic acid. In conclusion, overexpression of GmGSTU4 in transgenic plants did not affect their drought stress tolerance although it has altered their metabolite profile possible because of diverse effects on plant stress tolerance mechanism.**

## Introduction

Drought is one of the most important environmental stress factors that adversely influences plant productivity. Therefore, it is of paramount importance to develop plant crop varieties with enhanced drought tolerance. Plants, in order to limit oxidative damage under stress condition, have developed a detoxification systems, that orchestrates plants cell protection from the cytotoxic effects of ROS, using antioxidant enzymes. GSTs (glutathione transferase) appear to have a significant role in plants' adaptation under abiotic stress as many isoenzymes are found to be differentially expressed under these conditions (Chi et al. 2011; Sappl et al. 2009). A compact connection between GSTs and oxidative damage prevention from abiotic stress conditions was provided by genetic transformation assays of plant GST genes Little is known about the metabolic changes of the plants with altered expression of GSTs. Given the complexicity of plant stress responses metabolomic analysis will help to understand the regulatory role of GSTs and manipulate complex quantitative traits with pleiotropic effect as the drought tolerance. GSTs has been long reported to involved in abiotic stress resistance. Despite that, limited knowledge is available about the involvement of GST in drought stress. For that reason the morphophysiological and metabolic responses of tobacco transgenic lines and WT plants under water deficit conditions were examined .

## Material and Methods

### *Plant material and drought stress treatment*

Wild type and $T_1$ transgenic lines from tobacco plants var. Basmas (BAGST-3), Virginia (VGST-2) and Burley (BUGST-2) overexpressing *Gm*GSTU4 were tested for their drought tolerance under *in vitro* and *in vivo* conditions. For *in vitro* drought testing, seeds were surface sterilized and were sown on MS medium with Mannitol (100 mM and 200 mM). The plants were grown in a controlled environment 16-h-light/8-h dark photoperiod at 25ºC. After 30 days we evaluate the shoot length, total and root fresh weight and chlorophyll content, photosynthetic capacity and relative water content Each experiment was carried out under a completely randomized design The software SPSS 17 was used to handle the results. The data were analysed by LSD and Dunkan test, and mean values under each treatment were compared at p≤ 0.05.

### *Metabolomic analysis*

Metabolomic analysis was carried out using the line BAGST-3 (Benekos et al., 2010 ).Two week old plants were transplanted in MS medium with 70mM mannitol. Leaves were harvested 20 days after imposition of stress. Gas-chromatography coupled to Mass-spectrometry (GC-MS) measurements were performed in a HP6890 GC coupled to a HP 5973 MS. Results were expressed as a response that corresponds to the ratio between the areas of the target metabolite divided by the area of the reference metabolite (ribitol, m/z 319) and reported relative to the dry weight.

## Results

### *Morphophysiological Response of transgenic lines and wt plants under Drought Stress conditions*

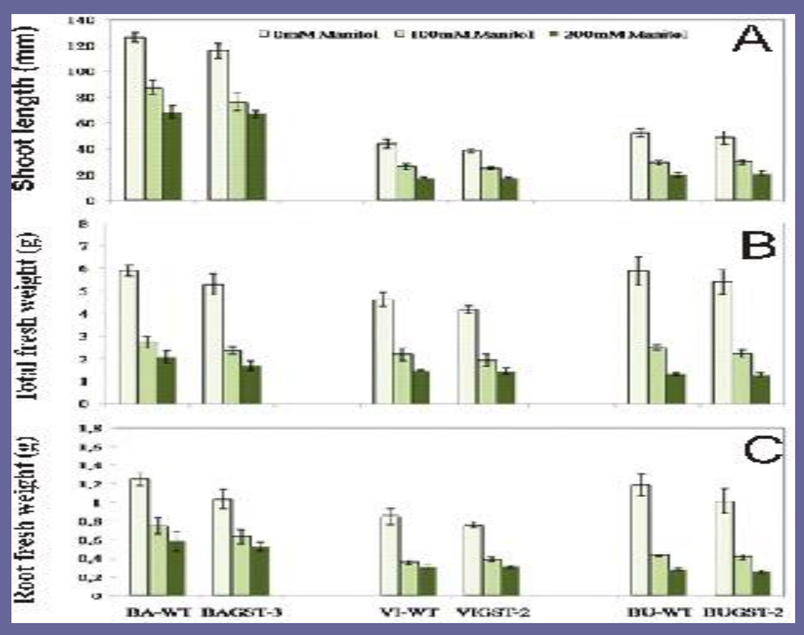


Fig 1. Morphological measurements of transgenic lines and WT plants of the three tobacco cultivars under osmotic stress after 30d in MS medium supplemented with 100 and 200mM mannitol. Shoot length (A) Total fresh weight (B) Fresh root weight (C). Data are the means (± standard deviation, n=4). Lines indicated with * differ significantly from the wt plants P≤ 0,05

GSTs has been long reported to involved in abiotic stress resistance. Despite that, we did not observe any significant differences between wt and transgenic lines in all morphological (Fig.1 A and B) and physiological (Fig.2 ) parameters examined under *in vitro* (mannitol) and *in vivo* drought stress conditions, except from the photosynthetic capacity which was significant reduced in line BUGST2.

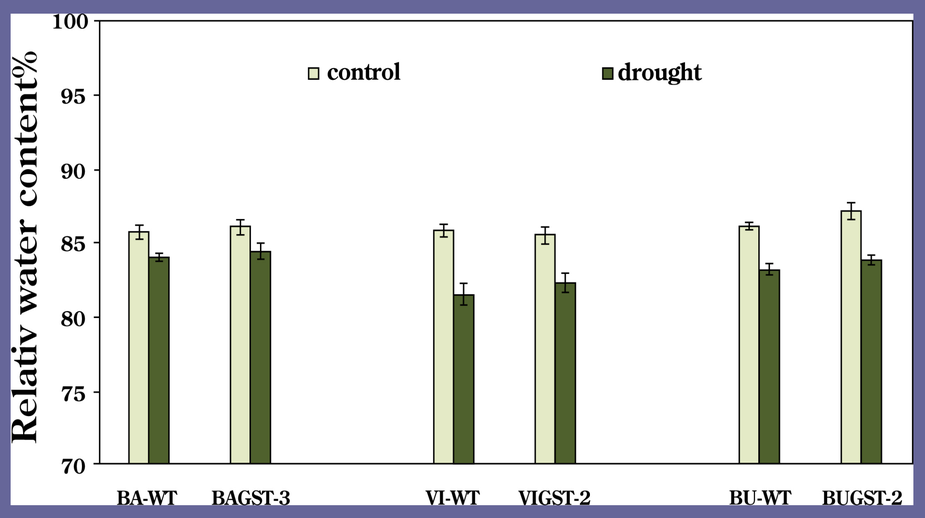


Fig 2. Relative water content of transgenic lines and wt plants of stress conditions 15 days after water restriction, as well as control plants normal watered. data are the means (± standard deviation, n=4). The lines indicated with * differ significantly from the wt plants P≤ 0,05

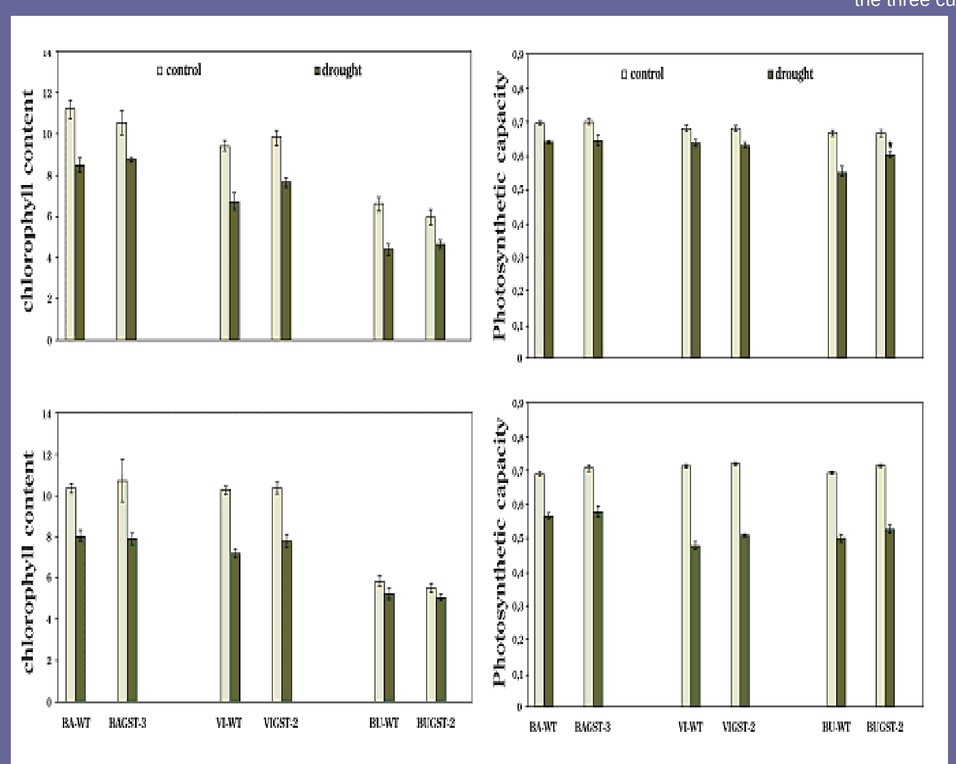


Fig 3.Chlorophyll content (A) and photosynthetic capacity (B) of T1 transgenic lines and wt plants of the three cultivars under in vivo drought stress 7 (A1,B1) and 15 (A2, B2) days after water restriction, as well as control plants normal watered. Data are the means (± standard deviation, n=4).lines indicated with * differ significantly from the wt plants P≤ 0,05


Fig.4 Transgenic lines and wt plants after 30d on MS medium with 100 or 200 mM mannitol

### *Metabolite profiling of transgenic line BAGST-3 and wt plants*

Drought stress in WT plants resulted in increased concentration of manittol (1081-fold), the amino acids glutamine, glycine, proline and hydroxyl-proline (3-, 3.8-, 4.7-, 6.2-fold respectively) as also the disaccharide trehalose. Of the metabolites whose concentration was decreased, the highest decrease was observed for glycerol (13-fold) while citric acid and succinic acid, intermediates of the Krebs cycle, levels dropped off 2.5- and 3.1- fold respectively, In drought stressed *Gm*GST4 plants metabolites that were uniquely increased were the photorespiration intermediate glyceric acid, as well as pyruvic acid and succinc acid, glycolysis and Krebs cycle intermediates respectively.
When directly comparing WT and *Gm*GST4 plants under drought stress, the latter exhibited higher concentration of the osmoprotectants glycerol and sorbitol, as well as intermediates of the Krebs cycle and pentose phosphate pathway. Proline, a major stress metabolite with both antioxidant and osmoprotective function, was decreased in *Gm*GST4 under drought stress.

**Table 1**. Metabolites significantly (P< 0.05) increased or decreased in the different genotypes and conditions comparisons

**Metabolites significantly altered**

| GST4/WT CTR | Fold change | GST4mannitol/GST4 CTR | Fold change |
|---|---|---|---|
| acetyl-L-serine | 8.14 | mannitol | 1692.26 |
| fumaric acid | 6.33 | glycerol | 9.75 |
| hydroquinone | 5.29 | trehalose | 8.44 |
| glycerol 1-phosphate | 2.91 | pyrrole-2-carboxylic acid | 5.83 |
| glycine | 2.22 | glutamine | 5.46 |
| threonine | 1.82 | proline | 5.03 |
| | | quinic acid | 4.11 |
| fructose | 2.81 | glyceric acid | 3.87 |
| glucose | 2.92 | cellobiose | 3.16 |
| isopropyl beta-D-1-thiogalactopyranoside | 4.35 | sorbitol | str. sp. |
| mannose | 8.15 | | |
| **WT mannitol /WT ctr** Fold change | | Sucrose | -1.52 |
| mannitol | 1081.10 | putrescine | -1.79 |
| pyrrole -2-carboxylic acid | 6.95 | acetol | -2.43 |
| trans-4-hydroxy-L-proline | 6.24 | acetyl-L-serine | -6.77 |
| N-acetyl-D-mannosamine | 5.64 | | |
| proline | 4.73 | **GST4/WT mannitol** Fold change | |
| glycine | 3.76 | glycerol | 58.89 |
| quinic acid | 3.34 | maleic acid | 10.83 |
| | | sedoheptulose anhydride monohydrate | 8.93 |
| glutamine | 2.98 | pyruvic acid | 5.18 |
| trehalose | 2.75 | sorbitol | 3.33 |
| sorbitol | str. sp. | glyceric acid | 2.52 |
| | | succinic acid | 1.76 |
| Sucrose | -1.39 | | |
| putrescine | -1.62 | proline | -1.50 |
| citric acid | -2.54 | N-acetyl-D-mannosamine | -4.15 |
| aspartic acid | -2.61 | | |
| succinic acid | -3.07 | | |
| melibiose | -3.32 | | |
| 5-aminovaleric acid | -4.71 | | |
| maleic acid | -6.67 | | |
| glycerol | -13.04 | | |

## Conclusion

- **Transgenic tobacco plants overexpressing GmGST4 fail to respond positively to drought stress treatments possibly due to: Highly specification of GMGST4 to particular stress stimuli**
- **Pleiotropic effects of mannitol accumulation inside the plant, used to induce osmotic stress. However GmGST4 did not perform better in vivo . The observed greater accumulation of the osmopotectants glycerol and sorbitol may have adverse effects in plant growth as these metabolites when they over-accumulate become toxic (Deguchi et al. 2006). Furthermore the reduced proline concentration may restrict plant adaptive potential under drought (Szabados and Savouré 2010).**
- **GmGSTU4 plays a regulatory role which results in significant metabolic alterations under drought stress thus further research need to be undertaken to understand the function of GSTs under abiotic stress for their efficient utilization in I improving plant stress tolerance.**

## References

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