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Overexpression of a Glutathione S-Transferase gene from *P. vulgaris* L. improves salt stress Tolerance in Transgenic Tobacco Plants

G. Voulgari[1], P Madesis[2], N. Lambrou[3], **I.Nianiou – Obeidat[1]**

[1] *Laboratory of Genetics and Plant Breeding, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 261, Thessaloniki GR-54124, Greece*
[2] *Institute of Applied Biosciences (IN.A.B.), C.E.R.TH., 6th km Charilaou-Thermis Road, Thermi, GR-57001, Greece*
[3] *Laboratory of Enzyme Technology, Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, Gr-118 55, Athens*

Abstract

Glutathione S-transferases (GSTs) are multifunctional proteins and forms major part, of plant cellular detoxification system and antioxidant enzyme network. Previously, a novel GST gene *Pv*GSTU3-3 has been isolated from roots of *Phaseolus vulgaris L.* plants. The isoenzyme shows high antioxidant catalytic function and acts as hydroperoxidase, thioltransferase, and dehydroascorbate reductase. In the present study, with a view to investigate the biological function of *Pv*GSTU3-3 a constitutive plant overexpression vector of *Pv*GST3-3 was constructed and transferred into tobacco (*Nicotiana tabacum.* L. cv Xanthi) plants via *A. tumefaciencs.* The *Pv*GSTU3-3 gene was successfully integrated into the genome of the transgenic tobacco lines as confirmed by Real time PCR and expressed in the transformants, validated through quantitative reverse transcription PCR. Three tobacco transgenic lines overexpressing *Pv*GSTU3-3 tested for their salt tolerance (200mM NaCl) under *in vitro* conditions. All lines were more tolerant compared to wt plants, as demonstrated by the increased root length. These results suggest that *Pv*GSTU3-3 isoenzyme can mediate physiological pathways that enhance salt stress tolerance.

Introduction

Salinity 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 salt tolerance. Plants, in order to limit oxidative damage under stress condition, have developed a detoxification system, 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. Nevertheless, very little information is available on the involvement of GSTs in response to drought and salt stresses, although changes in the GSH pool and glutathione reductase and glutathione peroxidase activities in dehydrated plants were described (Loggini et al.,1999; Galle´ et al., 2009). In this research we isolated a GST gene from *P.vulgaris* (*Pv*GSTU3-3).Transgenic tobacco plants over-expressing *Pv*GSTU3-3 were obtained and salt tolerance were studied.

Results

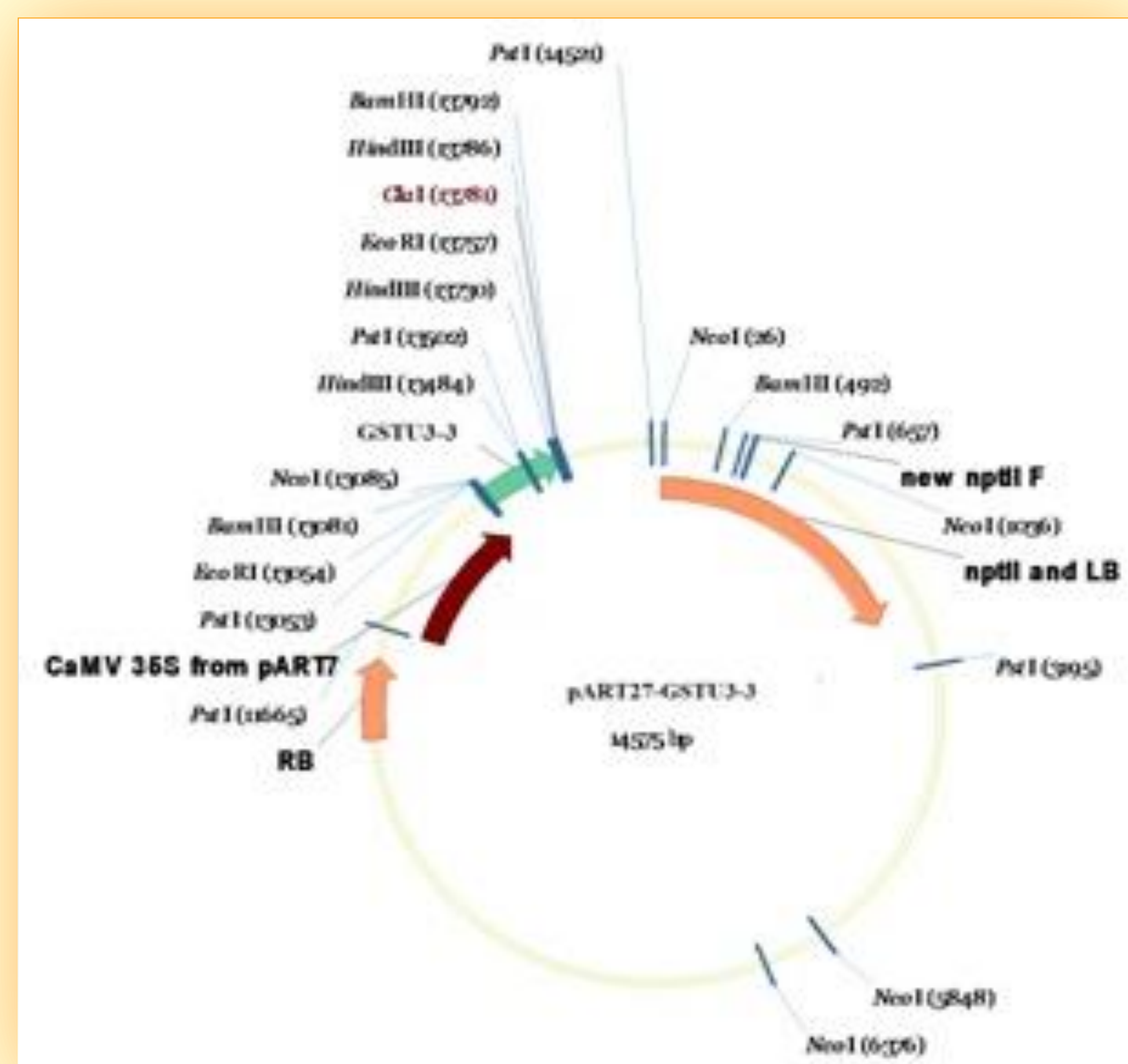
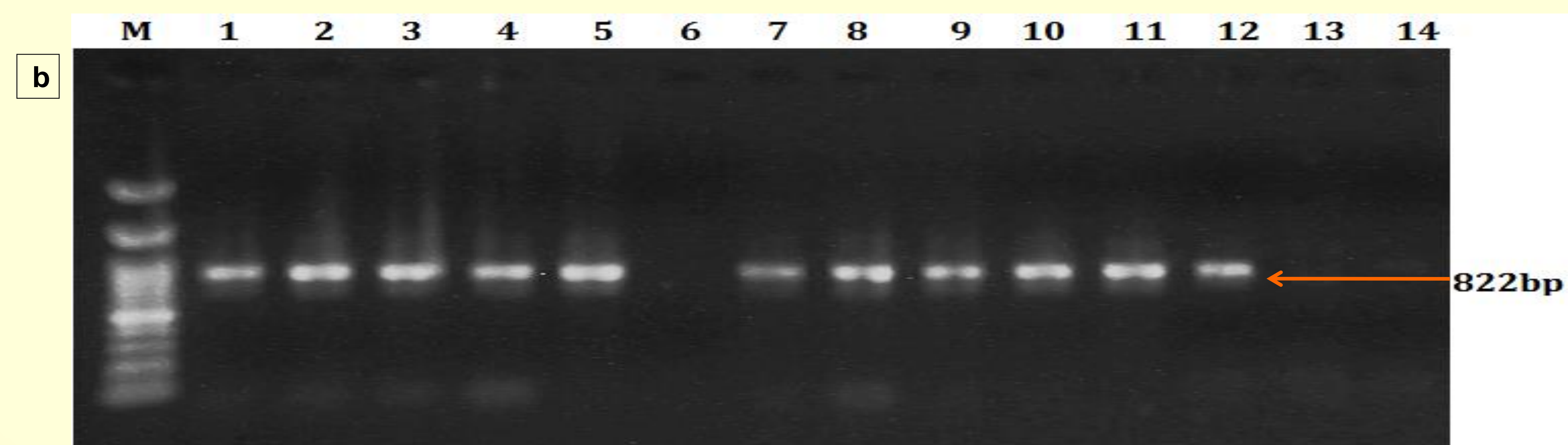


Fig 1. Schematic map of *A. tumefaciens* pART27GSTU3-3 vector constructed for overexpressing *Pv*GSTU3-3 gene in *N.tabacum* plants



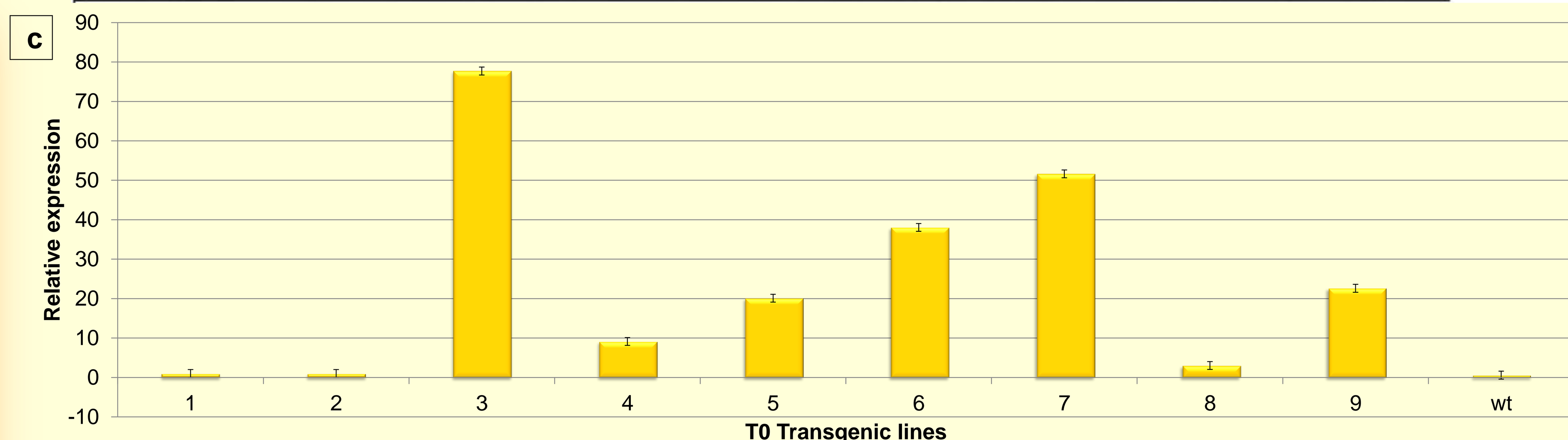


Fig. 2 (a) Regeneration of putative transformed tobacco plants **(b)** PCR analysis of independently transgenic tobacco plants (11 and 12), positive marker (13), negative marker (14) H_2O (M), molecular marker 100bp. Lanes with bands at 822bp indicate individual plants that contain the *Pv*GSTU3-3 gene (c) qRT-PCR expression analysis of *Pv*GSTU3-3 in transgenic tobacco plants, (wt) non-transformed plants; (1-9) independent transgenic lines

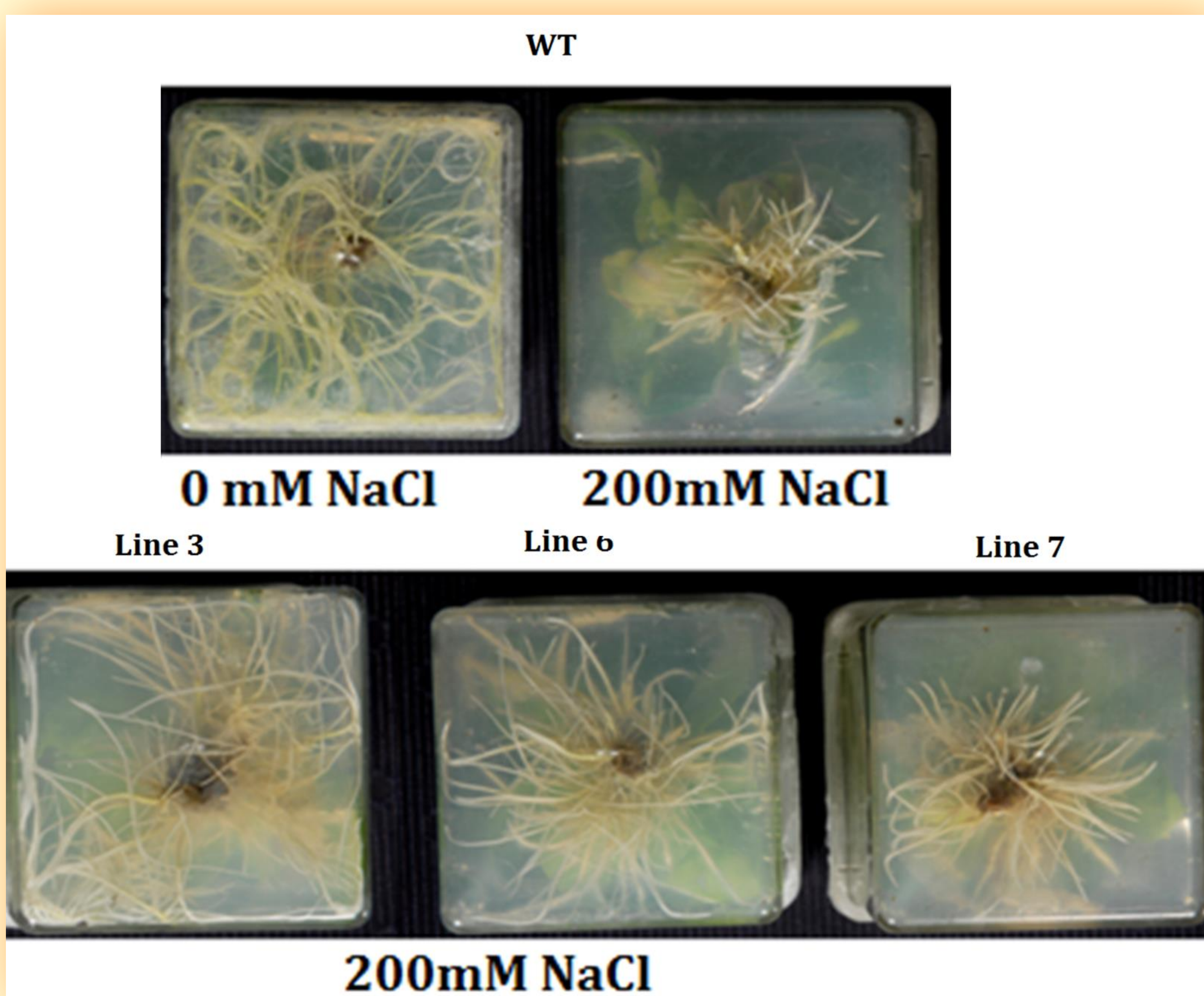


Fig 3 Root growth comparison between transgenic and wt plants after salt stress for 30 days in MS medium with 200mM NaCl. (WT) non-transformed plants (Line 3,6 and 7) independent transgenic lines

Conclusion

- **Nine transgenic lines were verified by RT-PCR analysis. The 822bp fragment were detected in the independent lines whereas were not found in wt plants.**
- **The transgenic lines showed varied transcript levels as compared to no detectable expression in wt plants.**
- **The selected transgenic lines, were more tolerant to salinity compared to wt plants as demostrated by the longer root length.**
- **These results suggest that *Pv*GSTU3-3 can be used as a suitable candidate to improve salinity and oxidative stress tolerance in crop plants**

Material and Methods

*Pv*GSTU3-3 was cloned into the binary plant expression vector pART27. The resulting plasmid, harbored the *Pv*GSTU3-3 gene under the control of the CaMV35S promoter. The *npt II* gene was used as the selective marker and the gene was introduced into leaf discs from tobacco by *A. tumefaciens*-mediated method. Transformed shoots were selected on MS medium supplemented with 50mg/L kanamycin. Ten T0 plants were assayed for transgene insertion by PCR obtain a 822 bp fragment. In order to confirm the expression of *PvGSTU3-3* in transgenic lines qRT-PCR was performed. The results expressed as relative expression based on Livak and Thomas method (2001). To test salt response of T0 transgenic plants, micropropagated plants from the three selected T0 transgenic lines as well as wild type plants were transferred to MS medium supplemented with 200 mM NaCl..The plants were grown with a day/night cycle of 16/ 8 h at 25oC. After 30 days, the root growth were evaluated. The experiment was performed by three replicate plants per treatment.

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