

## FUNCTIONAL CHARACTERIZATION OF A NUCLEOTIDE PYROPHOSPHATASE GENE IN *ARABIDOPSIS THALIANA*

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Antioxidants, such as ascorbate and phenolics, play a crucial role in controlling the level of reactive oxygen species (ROS). Stresses cause an increase in antioxidant demand, leading regulatory mechanisms to be exploited to enhance antioxidant biosynthetic fluxes. Cells perceive increased antioxidant demand throughout signal transduction pathways including H<sub>2</sub>O<sub>2</sub> accumulation and redox changes in photosynthetic electron transport system promoted by stresses (Miyagawa *et al.* Plant Cell Physiol. 41: 311–320 2000; Yabuta *et al.* Plant J. 32: 915–925 2002). In particular, ascorbate is synthesized in plant through alternative pathways, which may differentially contribute to its pool size (Valpuesta and Botella Trends in Plant Science 9(12): 573–577 2004). Previous studies showed ascorbate accumulation well correlates with the nucleotide pyrophosphatase activity (NPP) and overexpression of the *Arabidopsis* NPP gene in transgenic tobacco and potato lines did not result in a modification of the ascorbate biosynthetic flux (Di Matteo *et al.* Proceedings of SIFV-SIGA Joint Congress p: 107 2004). The aim of this work is to functionally characterize the NPP gene in transgenic Columbia 0 *Arabidopsis thaliana* lines overexpressing the homologous gene. The NPP gene under the control of a 35S<sup>2</sup> promoter was transformed into *A. thaliana* genome by the procedure described by Clough e Bent (Plant J 16: 735–43 1998). Seven homozygous transgenic lines harboring single copy of the NPP transgene were functionally characterized. The relative quantification of the NPP gene transcript was assayed in transgenic lines by Real-Time qPCR and showed on average up to a 20-fold increase of the NPP mRNA abundance. Indeed, the evaluated level of leaf ascorbate in transgenic lines nearly doubled the one observed in the untransformed lines. Additionally, some transgenic lines also showed a significant increase of leaf total phenolics concentration as well as monomeric antocyanin concentration increased up to 5 fold. For some transgenic lines, a significant reduction of the NaCl effect on seed germination and on root growth was also observed.

In conclusion, we provided evidences of involvement of the NPP gene in antioxidant accumulation in *A. thaliana* leaves. We also suggested a putative implication of the NPP gene in salt tolerance mechanisms. Further investigation will be addressed to better understand the mechanism of action and the phenotypic expression of the *Arabidopsis* NPP gene.