Poster Abstract - B.04

ANALYZING Ppi1 GENE EXPRESSION USING PROMOTER-GUS FUSIONS

C. ANZI, L. CORTELLINO, C. SOAVE, P. MORANDINI

Dept. of Biology, University of Milan, Italy

gene expression, proton pump, H+ATPase, Ppi1, GUS reporter

PPI1 (PROTON PUMP INTERACTOR 1) is a protein identified in a two hybrid screen whose N-terminus binds to the *Arabidopsis thaliana* plasma membrane proton pump (PM H⁺-ATPase). The entire PPI1 protein, or fragments thereof, expressed as fusion protein in *E. coli*, are able to stimulate the activity of the proton pump *in vitro* (Morandini et al., 2002 Plant J. 31:487-97).

To study the pattern of *Ppi1* expression we produced transgenic reporter lines with the GUS gene under the control of *Ppi1* promoter. Since the gene presents a large intron before the beginning of the coding region (a so-called 'leader intron'), we decided to test three different constructs. The first contains both the promoter and the leader intron; the second one is lacking the leader intron but still contains the *Ppi1* 5'UTR; the last one is missing the intron and the 5'UTR derives from the TMV.

In an alternative approach to study the function of the PPI1 protein we make use of *Arabidopsis* KO lines bearing a T-DNA insertion. Two lines were characterized in detail for *Ppi1*: an insertion in intron I (line N93) and one in exon VII (line F09) at aa 525. These KO lines were confirmed with RT-PCR and western analysis. The *Ppi1* KO lines do not show evident phenotype when grown in pots. In contrast, KO culture cells seems to grow faster, to release a brown compound and to form tracheary elements at higher frequencies than the WT.