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Poster Abstract – D.44

EXPANSIN: EXPRESSION PATTERN, SUBCELLULAR LOCALIZATION AND RECOMBINANT PROTEIN PRODUCTION

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Expansins are the most important cell wall proteins involved in the regulation of cell wall enlargement in growing cells, acting in pH-dependent manner (McQueen-Mason et al., 1992; Cosgrove 2000; Lee et al., 2001). Gene-expression studies have shown that many expansin genes are expressed in a pattern that is consistent with their involvement in growth of specific organs or cell type. To identify the promoter sequence of *PhEXP1A*, a *P. hybrida W138* dTph1 insertion library has been screened and we isolated 1008 bp upstream of *PhEXP1A* coding sequence. Analysis of the *PhEXP1A* promoter sequence revealed the existence of several *cis*-acting transcriptional factors characteristic of expansin genes. To investigate the spatial and temporal expression of *PhEXP1A* during plant development, we constructed transgenic *P. hybrida* plants harboring a recombinant reporter gene (GUS) under *PhEXP1A* promoter sequence control. Furthermore, to examine whether PhEXP1A is capable of translocanting in the cell wall, plants of *Petunia* were transformed with a DNA construct encoding eGFP-EXP1A fusion proteins in C-terminal region (*PhEXP1A:GFP*) under the *CaMV 35S* promoter and examined their subcellular localization.

The production of active recombinant expansins A has proven largely unsuccessful and the crystal structure is still undefined. Their mechanism of action, not defined yet, appears to involve the disruption of hydrogen bonds between cellulose microfibrils and cross-linking glycans predisposing cellulose sling and consequently cell expansion. Another aim of this work is the production and purification of recombinant PhEXP1A, a first expansin A isolated in *Petunia hybrida*. Here, we report the construction and the expression of recombinant expansinA from *Petunia hybrida* in a bacterial expression system. The coding sequence of *PhEXP1A* was introduced into pDEST17 bacterial expression vector, using Gateway® Technology and highly expressed in derivatives C41(DE3) of the *Escherichia coli* strain BL21(DE3).