HETEROLOGOUS EXPRESSION OF A PROTEIN WITH AMORPHOGENETIC ACTIVITY ON CELLULOSE FROM *Bjerkandera adusta* IN *Arabidopsis thaliana* PLANTS

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Introduction. The saccharification process during second- generation ethanol production from lignocellulosic material (plant biomass) is a major challenge given the highly recalcitrant structure of cell wall of plants, the main source of fermentable sugar storage in the form of polymers (cellulose and hemicellulose). To make the process more efficient, the use pretreatments that allow disruption of the lignocellulose structure, improving cellulose and hemicellulose accessibility has been proposed (1). The most common pretreatments are the physical and chemical, however biological pretreatments have additional advantages over the first two, such as low cost, safety and environmentally friendly (1). Cell wall remodeling proteins, such as expansins, swollenins and loosenin have recently been considered as a potential biological pretreatment tool. Loosenin is a protein from basidiomycete fungus *Bjerkandera adusta*. It showed to have the ability to modify the structure of cellulose, tested on cotton fibers and make them more susceptible to saccharification (2).
The aim of this work is to generate plants whose cell walls are more susceptible to enzimatic degradation through the heterologous expression of the protein LOOS1 of *B. adusta* in *A. thaliana* plants.

Methods. We cloned the *loos1* gene in a plant expression vector under an inducible promoter, pBINRD29A. For insertion of the construct pBINRD29::*loos1* in the *Arabidopsis* genome *Agrobacterium tumefaciens* 4404 will be used. Plant transformation will be accomplished by the floral dip method. Gene transcription and protein production will be corroborated by RT-PCR and Western blot analysis respectively. To evaluate the effect of the expression of LOOS1 on the susceptibility of the cell wall, plant material of transgenic plants will be treated with cellulolytic cocktails and reducing sugars assays will be performed.

Results. The LOOS1 native signal peptide was replaced by that of calreticulin from *Nicotiana plumbaginifolia*. Then the gene was cloned into the intermediate vector pJET 1.2/Blunt, and thereby transformed cells *E. coli* DH5α. A clone with the correct sequence was selected to carry out subcloning into the expression vector pBINRD29-A. This clone was then transformed into *A. tumefaciens*. The *Agrobacterium* 7 clone with the pBINRD29 :: *loos1* construction was selected for transforming plants of *A. thaliana* strain Columbia by the floral dip method on one month old plants. The first selection of transgenic plants by germination and growth of seeds on plate MS medium supplemented with kanamycin (resistance conferred by the vector) was obtained.

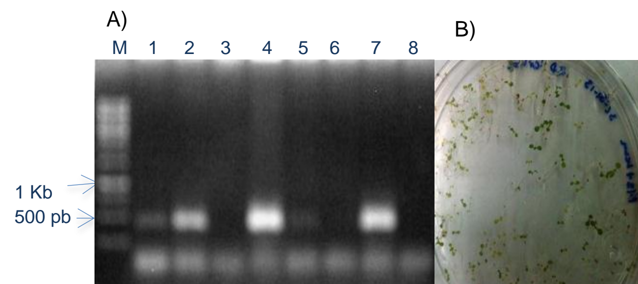

Fig.1 A) PCR in colony of *Agrobacterium tumefaciens* transformed with pBinRD:: *loos1* amplifying the gene *loos1* of 383 bp. M: Molecular weight marker 100 bp Fermentas. 1-8: *loos1* gene amplified by PCR from colonies 1-8. B) The first generation of transgenic plants.

Conclusions. We obtained the first generation of transgenic plants transformed with the construct pBINRD29 :: *loos1*.

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