Biotic and Abiotic Stress Tolerance in Plants: the Challenge for the 21st Century

BOOK OF ABSTRACTS

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transformation, in order to obtain transgenic plants tolerant to abiotic stresses. In this work we developed a genetic transformation protocol for 3336 clone of *Eucalyptus urograndis*, aiming further transformation with genes related to abiotic stresses. Leaf explants were collected from *in vitro* grown plants maintained on semi solid MS medium containing BAP and ANA. The leaves were cut and placed into a Petri dish containing MS liquid medium with the *Agrobacterium tumefaciens* EHA105 harboring the pCAMBIA2301 vector with the *nptII* and *uidA* genes. After 20 minutes in this liquid co-culture, explants were placed in regeneration semi solid medium containing WPM salts, TDZ and ANA and maintained for 3, 4 or 5 days at 23 ºC and 16 h photoperiod. Afterwards, the explants were washed three times to eliminate the *Agrobacterium* and transferred to fresh regeneration medium supplemented with 12.5 mg L⁻¹ kanamycin and 250 mg L⁻¹ Augmentin. To induce shoots, the explants were transferred to a WPM medium supplemented with BAP and ANA after one month. Kanamycin dose was enhanced every 15 days, when the explants were transferred to fresh medium. In a second experiment, we tested the effect of 100 µM acetoseringone in the liquid co-culture (for four days) and compared to a control, without acetoseringone. The experiments were repeated twice. Forty five and 90 days after the beginning, we carried out a gus histochemical assay to observe the blue staining at the calluses and shoots formed, respectively. Blue spots were observed on calluses in all treatments in both experiments, suggesting that transient expression did not depend on time of co-culture or acetoseringone. However, 90 days later, the shoots tested proved to be escapes, and did not stained. We conclude that the transformation is effective, although the selection step still needs to be improved, and new kanamycin concentrations must be tested in order to regenerate transgenic shoots. Work supported by Embrapa Florestas.

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**Genetic transformation of tobacco plants with a cacao pathogenesis-related protein 4 for tolerance to water stress study**

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Drought is an important environmental factor limiting the productivity of various crops worldwide. The development of crop cultivars with improved adaptation to drought is a major goal in many crop breeding programs. In addition to classical breeding approaches, genetic transformation to introduce candidate genes into plants for better tolerance to water deficit has been successfully developed. Pathogenesis-related proteins (PR proteins) are defined as plant proteins induced in response to pathogen attack. However, it is known that these proteins may also be involved in response to abiotic stresses. The objective of this study was to transform tobacco plants (as plant model for subsequent analysis of cultivated plant such as citrus) with a PR-4b protein from *Theobroma cacao* (TcPR-4b) and to select and test the tolerance of such transformed plants to water/osmotic stress. First, an *in silico* analysis of the TcPR-4b using the BLAST, Pfam, InterProScan, ORF-Finder programs, as well as a search on Cocoa GenDB databank were performed. The TcPR-4b belongs to a small family of PR-4 proteins whose members were mainly located on the chromosomes 5 (five genes) and 10 (one gene). The complete TcPR-4b sequence is 802 bp in length and contains two exons (171 and 258 bp), and one intron (82 bp); the corresponding protein is 142 amino acids in length. For plant transformation experiment, the TcPR-4b cDNA (from cacao-M. *perniciosa* interaction library) was cloned into the pGEM-T Easy vector then subcloned on the pCambia binary vector 1390. Then, *Agrobacterium tumefaciens* strain EHA 105 was transformed with the35S::TcPR-4b::pCambia construction, and the transformed *A. tumefaciens* used for *Nicotiana tabacum* cv. Havana transformation by co-cultivation of leaf segments in selection medium. Transformed shoots from three transformation events are under selection for subsequent *in vitro* water/osmotic stress, using, among others, mannitol and NaCl.

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