World Avocado Congress V. 2003. Abstracts. A-14. pg. 78-79.

A- 14

AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION OF AVOCADO SOMATIC EMBRYOS: A PROTOCOL

C.L. Encina[1], N. Westendorp[1], P. Gil[1], E. Caro[1] and J.R. Botella[2].

[1]Plant Tissue Culture & Biotechnology Lab. Estacion Experimental La Mayora (C.S.I.C.) s/n. 29750 Algarrobo-Costa. Malaga. Spain. E-mail: clencina@eelm.csic.es
[2]Plant Genetic Engineering Lab. Dpt. of Botany. Univ. of Queensland. Brisbane. Qld 4072. Australia.

Avocado embryogenic calli were transformed by *Agrobacterium tumefaciens*. Strains AGL1, AGL0, EHA105, GV310 carrying the plasmid pBI121 were used as vector system for transformation. This plasmid contained the reporter and selectable marker genes NPTII (neomycinphosphotransferase II) and GUS (b-glucuronidase). After infection, explants were cocultivated for 24 h on embryogenic medium (EM) (Pliego-Alfaro and Murashige, 1988), containing 100 mM acetosyringone and furthermore transferred to EM without acetosyringone and with 500 mg/l cefotaxime. One week later, explants were transferred to selection medium consisting in a basal EM with 500 mg/l cefotaxime and 25 mg/l geneticin. Explants under selection were transferred to fresh medium every two weeks during 4 months. Putative transformants identified by growth on geneticin-containing medium were evaluated for GUS expression using the X-glucuronide histochemical assay (Jefferson, 1987) and the polymerase chain reaction (PCR). Strains AGL0 and AGL1 did not produce transformation. Best results were obtained with the strains GV3101 and EHA105, with a 7% and 6% of transformation respectively. Due to the uncontrollable overgrowth of the hypervirulent strain GV3101, the EHA105 strain we finally selected as the best, producing 11 transformed lines.