MEXICAN LEMON EXPRESSING ANTICROBIAL PROTEINS BY A NOVEL GENETIC TRANSFORMATION METHOD OF CITRUS TREES

Rebeca Zecua-Nájera, Roberto Ruíz-Medrano, Jesús Hinojosa-Moya, Beatriz Xoconostle-Cázares
CINVESTAV del I.P.N. Departamento de Biotecnología y Bioingeniería
México, D.F. CP. 07360. bxoconos@.cinvestav.mx

Key words: genetic transformation, Agrobacterium tumefaciens, antimicrobial peptides

Introduction *Citrus* genetic transformation has been carried out in many species and hybrid plants. However, most studies on citrus transformation emphasize the use of tissues in juvenile stage as material to perform the transformation since it has been observed a high frequency of transformation and regeneration. However, these plants take many years (6-20 years) until flowering and fruit yield, which makes this method expensive and difficult to carry out (1).
In order to shorten the regeneration time, in this study, it was used seedlings of mexican lime (*Citrus aurantifolia*) for transformation, then grafted onto adult stock plants. Antimicrobials were introduced into the genome of mexican lime by *Agrobacterium tumefaciens*-mediated transformation.

Methods The antimicrobial gene was placed under the strong constitutive cauliflower mosaic virus (CaMV) 35S promoter. For inoculum preparation, an *Agrobacterium tumefaciens* colony containing the plasmid was transferred to LB medium supplemented with carbenicilin (100 µg L^{-1}) and incubated for 48h at 30°C. Acetosyringone (140µm) was added to the medium and incubated for two more hours. The bacterial suspension was centrifugated at 12 000g and re-suspended in Murashige and Skoog salts (MS). Genetic transformation was performed as described by Cervera et al. (2), using in this work seedlings of mexican lime, which were dissected with sterile knives submerged in the bacterial suspension in (MS). The obtained explants were placed in co-cultivation media (CM) (MS plus 2 mg/L of 2,4-D, 2 mg/L of IAA, 1 mg/L of 2,i-P, 8 g/L of agar, pH 5.7) for 48h in darkness at 25°C. Then the explants were transferred to Shoot Regeneration Media (SRM) (MS plus 3 mg/L of BAP, 10 g/L of agar at pH 5.7, supplemented, 500 mg/L of cefotaxime, and 250 mg/L of vancomycin), for the first 30 days were placed in darkness at 25°C. Then the explants were transferred in a new SRM in 16h photoperiod at 25°C. Regenerated explants were grafted onto volkamerian, citrange troyer or Schaub citrus stocks.

Results After 60 days implemented the grafted, the plants showed a completely regeneration and recovery (Fig. 1).

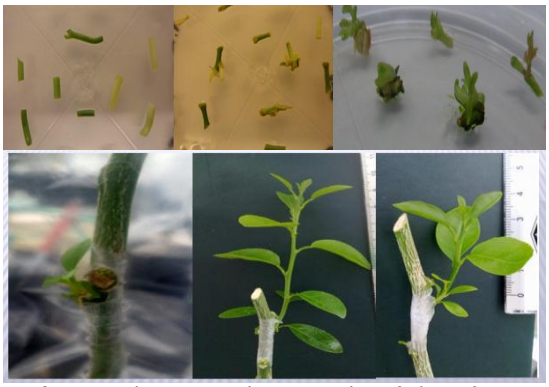

Fig.1 Development and regeneration of plants from genetically transformed explants.

To verify that these plants were successfully transformed, genomic DNA was purified and employed as template for transgene amplification.

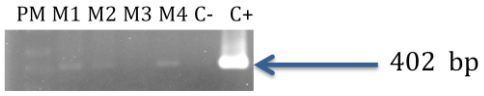

Fig. 2 Electrophoresis gel: PM, ladder; M1-M4, samples; C-, negative control; C+, positive control.

Conclusions Plants of mexican lime expressing antimicrobial proteins were obtained from explants that were successfully transformed with *Agrobacterium tumefaciens*.

Acknowledgements RZN received a fellowship from CONACyT México. Research was supported by a competitive grant from SENASICA.

References

1. He, Y., et al. (2011). *Scientia Horticulturae*. 128, 99-107.
2. Cervera, M., et al. 2004. *Methods Mol. Biol.* 286, 177–187.