

HIGHT STABILITY TRANSFORMATION PROCEDURE MEDIATED BY *AGROBACTERIUM TUMEFACIENS* IN CULTIVARS OF PEAR

M. NESTA, M. CACCAVELLO, E. MENDOZA DE GYVES, D. MIANO, E. RUGINI, R. MULEO

Department of Crop Production, Laboratory of Molecular Ecophysiology of Woody Plant, Tuscia University, Via S.C. DeLellis snc, 01100 Viterbo, Italy - muleo@unitus.it

Pirus communis, transformation, *phyA* gene, *GOX* gene, *myb4* gene

In this work we report results related with one of main important process during the delivering of alien gene from one specie to another. Regeneration procedures are important for the success of genetic transformation especially in the recalcitrant species likewise the woody crop plants. Therefore the purpose of the research was to constitute a regeneration system and transformation procedure, using the *A. tumefaciens* (ipervirulent strain EHA105) as a delivering vector, that are reliable, robust and efficient for five cultivars of pear. This work is part of one general strategy, which tries to induce resistance against biotic stress and keep under control the regulation of plant development. To minimize the events of somaclonal variation a fast and good level of transformation should be associated with an elevated level of regeneration events that occur in the surface of leaf explants after the co-culture with the *A. tumefaciens*.

On the analyzed cultivars, Abate Fetel, Kaiser, Williams, Dar Gazi and Conference, we have evaluated the possibility to evocate and induce regeneration events, since the proliferation stage of plantlet donor explants. With this aim we have manipulated the composition of medium of every cultivar, setting up a specific culture medium composition able to modify the physiological conditions of the subsequent leaf explants in co-culture making them more receptive to the regeneration and transformation. Amount of nitrogen, under different chemical form, and different cytokinins were the more suitable compound that influenced the plantlets.

A similar work has been also done for the regeneration medium. Cytokinins, auxins, nitrogen and vitamins were analyzed in multiple combinations to individuate the proper medium composition for each cultivar. We started from two basal salt formulation media, MS and QL, changing hormones (NAA, IAA, TDZ, BAP) and vitamins (MS and QL) and nitrogen. Among the all combination we individuate the optimum medium for improving up to high value regeneration after the co-culture. Comparing our data with that of literature we were able to increase for all cultivar this event with percentage like 40% for Abate Fetel, 70% for Williams, 50% for Kaiser, 88% for Dar Gazi and 90% for Conference.

Some of these media have been used during the procedures of transformation to transfer into the cv Dar Gazi and Conference aliens genes. From the trials we have got more than 26 putative transgenic clone for gene *GOX*, *phyB* and *Myb4*. The insertion of the aliens gene have been evaluate by PCR procedures.

In this work it is reported for the first time the transformation of the cv Dar Gazi, which is a pear cultivar from Persian. The percentage of the transformation events ranged from 2% for Dar Gazi and 1% for Conference. The higher efficiency of Dar Gazi comparing to Conference could be due to the most greater frequency of the regenerated shoots per single explant.