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Poster Abstract - E.01

TRYPTOPHANE GENE IS USEFUL FOR EARLY DETECTION OF POSITIVE TRANSFORMED FRUIT CROP PLANTS BY EXCLUDING INCIDENTAL *AGROBACTERIUM* CONTAMINATION

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Actinidia deliciosa, Citrus spp, Olea europaea, Pirus communis, Prunus avium

The *Agrobacterium* permanence in plant tissues after co-cultivation often persists during multiple subculture transfers on selective media even using higher concentrations of antibiotics.

Expression of *nptII* in viable agrobacteria that has not been eliminated from transformed tissue after co-cultivation can cause problems with the truly transformation detection in putative plantlets since the presence of the *nptII* gene hosted in the bacterial plasmid not eliminated can provoke apparent resistance of plantlets to kanamycin selection. The neomycin phoshotransferase enzyme must be produced by plant tissue to supply for themselves the resistance to the antibiotic kanamycin giving an healthy appearance in plants to consider them as a putative transformed. However, these putative plantlets subjected to PCR analysis may give a false positive determinations since the inside presence of *Agrobacterium* in plant tissues at the time of sampling, due to ambiguous can provide the *nptII* gene from the bacterial DNA which can be amplified DNA from both plant and bacterial. Putative transformed plantlets can be wrong judged by resistance to kanamycin even if they were found PCR positive to any of the genes intended to transfer. To avoid this misinterpretation, we have searched sequences in gene as unique bacterial sequence to design specific primers exclusively for the *Agrobacterium.* The tryptophan gene (*trpr*) of *Agrobacterium tumefaciens* appears the appropriated candidate for this goal and on the sequence of the corresponding Pseudomonas gene, already present in GenBank, we designed specific primers for early confirmation of successful plant cell transformation. The absence of tryptophan amplified fragment in transgenic plant as in wild type plants indicates that any contamination is acting in the tissues, therefore, it provides an early and efficient transformation diagnostic test. In fact, every time that the amplification reaction have been conducted with the different strain of *A. tumefaciens* and on other bacteria species we have found the predicted PCR product.

In our lab, transformation of plants was carried out by co-cultivation of several woody crop species together with different strain of A. *tumefaciens*, armed with different genes, followed by selection of putative transgenic explants on Kanamycin. The presence of *nptII* gene and the specific alien gene in every putative transformed plants of citrus, pear, cherry, kiwi and olive was also confirmed by PCR analysis.

In this work we suggest an easy, robust and rapid test to verify the insertion of alien gene and to exclude any contamination of bacteria inside the plant tissue.

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