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Poster Abstract – E.06

ESTABLISHMENT OF TRANSIENT TRANSFORMATION SYSTEMS IN GRAPE LEAVES FOR FUNCTIONAL GENOMICS STUDIES THROUGH GENE SILENCING

URSO S.*, ZOTTINI M.**, RUBERTI C.**, LO SCHIAVO F.**, BISELLI C.*, STANCA A.M.*,VALÈ G.*

*) C.R.A. Genomic and Post-Genomic Research Centre, Via San Protaso 302, 29017 Fiorenzuola d'Arda (PC)
**) Department of Biology, University of Padova, Via Ugo Bassi 58/B, Padova

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Transient transformation of plant leaves represent a useful tool allowing to study gene function in different plant tissues and it is a time-saving procedure especially for species with long generation times avoiding stable transformation. In this work two methods for grape leaves transient transformation have been tested: the use of *Agrobacterium tumefaciens,* which can penetrate into outer leaf tissues, and particle bombardment, useful for: *Agrobacterium* recalcitrant genotypes, different kind of tissues or substrates and when leaves epidermal cells transformation only is required. To establish a method with *A. tumefaciens,* PDS (phytoene desaturase, gene coding for an enzime involved in carotenoid biosynthesis) sequence, integrated in a Gateway® binary vector for gene silencing called pK7GWIWG2D, has been used. Underside leaves have been agroinfiltrated by a syringe without needle and analized 6 and 12 days after infection. Presence of chlorotic spots due to photobleaching, a PDS silencing symptom, has been found into agroinfiltrated sites. PDS gene silencing has been confirmed by semiquantitative RT-PCR of the corresponding gene transcripts into transformed tissues.

As a second possibility, we are setting particle bombardment method for transient transformation using GUS (β-glucoronidase) as gene marker. Different kinds of shooting parameters and pre and post-bombardment treatments of plant tissues have been tested. Leaf cells expressing GUS have been obtained by these experiments. Further implementation to the method are in progress in order to obtain a good level of epidermal cells transient transformation.

In the near future, establishment of these two transformation methods will allow use them, in association with gene silencing system, as tools for functional genomic in order to study gene function in different leaf tissues and, in particolar, to study genes involved in plant response to biotic stress.