*Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress*
*Potenza, Italy – 12/15 September, 2005*
ISBN **88-900622-6-6**

**Poster Abstract - E.02**

# ANTIOXIDANT-ASSISTED TRANSFORMATION OF *VITIS VINIFERA*

P. CROCE**,** C. VANNINI, M. BRACALE

D.A.S.S., Università degli Studi dell'Insubria, Via Dunant 3, 21100 Varese, Italy - p.croce@infinito.it

*grape transformation, Agrobacterium tumefaciens, Vitis vinifera, antioxidants, DTT*

We have developed an easy and convenient method for the maintenance of embryogenic potential of some Italian grape cultivars of economical relevance. We first tested the susceptibility of different Italian grape cultivars (Trebbiano d'Abruzzo, Trebbiano Toscano, Sangiovese, Regina, Bombino Bianco, and Montepulciano) to produce somatic embryos from immature anthers and ovaries. We then tested the morphogenic capacity of the somatic embryos from the different cultivars in order to find a high efficiency method for the maintenance of embryonic cell culture. With the method we found is possible to generate an indefinite number of somatic embryos suitable for propagation or to set the conditionsfor transformation purposes. This method is based on recurrent cycles of induction of somatic embryogenesis using, as source of cells single somatic embryos. Somatic embryos were able to produce embryogenic callus on a 2,4-D containing medium. The embryogenic callus produced on the induction medium was able to generate somatic embryos on a suitable medium. The embryos formed works as starting material for the following cycle. In any cycle the number of embryos can be increased up to a 20 factor. There's no need of any selection of the material on subcultures. We have been growing our cultures with this method for more than two years with no decrease of embryogenic potential.

We've developed a Agrobacterium mediated transformation protocol for embryogenic calli of Chardonnay and Brachetto. The Green Fluorescent Protein (GFP) reporter gene was utilized to facilitate optimisation of experimental procedures.

Grape tissues experience a severe necrosis following agrobacterium infection that completely inhibit any subsequent regeneration. In order to limit the browning related with the hypersensitive reaction to the pathogen, we introduced a post co-culture step, a liquid culture with 1g/l DTT a strong antioxidant. The aim of the procedure is to limit the hydrogen peroxide in the cell that stimulate the response to the pathogen. The antioxidant effect was evident in both the cultivars tested yielding white calli, similar to prior of the transformation. Transformation efficiency varied according the physiological state of the calli used for transformation.

Transgenic plants have been recovered both from Chardonnay and Brachetto.

Evaluations are underway for the transformation and regeneration of various rootstocks and Vitis vinifera cvs Regina and Sangiovese.