IMPLEMENTATION OF A REGENERATION AND TRANSFORMATION PROTOCOL FOR *VITIS VINIFERA* VARIETIES AND ROOTSTOCKS TO INDUCE GENE SILENCING AGAINST GFLV-GLRaV VIRUS

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Introduction

Grapevine cultivation is penalized by pathological problems with significant impact on production, quality and related costs. This species is affected by numerous viral diseases, such as “fanleaf (GFLV)” and “leaf roll (GLRaV)” diseases, which are the most diffused in Europe. The application of rigorous certification criteria is the only strategy available to control the diffusion of viruses. Traditional breeding techniques are limited for the length of the process and for the difficulties in preserving the characteristics of the grapevine clones. Post Transcriptional Gene Silencing (PTGS) has emerged as alternative tool to induce resistance to virus in several plant species, even by using rootstocks as a source of RNAi controlling plant virus infection. For the application of this technology in grapevine it is really important to have efficient regeneration and transformation protocols for the most important cultivars and rootstocks.

In vitro regeneration and transformation of grapevine

An innovative and efficient regeneration (meristematic bulk) and genetic transformation protocol was developed by Mezzetti *et al.*, 2002 in table grape and then applied to different grapevine cultivars (Vermentino, Albana, Pignoletto, Sangiovese, Aspirant Bouschet) and rootstocks (110 Richter and Kober 5BB). *In vitro* proliferating shoots are cultured in the presence of increasing concentrations of N6-benzyl adenine (BA). After three months the production of meristematic bulk (MB) tissues characterized by a high capacity to regenerate shoots is observed; finally, they are used as starting tissues for *Agrobacterium*-mediated genetic transformation (Fig.1).

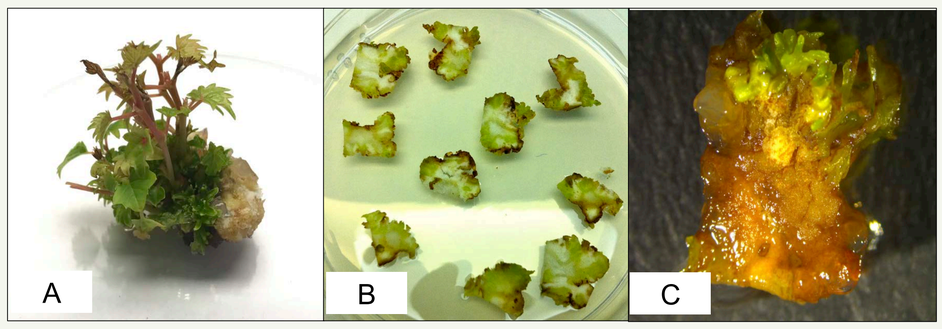


Fig 1. Organogenesis-based transformation method applied to grape (Mezzetti *et al.*, 2002; Xie et al., 2016): a) Meristematic bulk; b) Slices obtained from meristematic bulk prepared for *Agrobacterium*-mediated transformation; c) Regeneration of putative transgenic lines on selective medium.

The MBs created for each clone were used as explants for *Agrobacterium*-mediated genetic transformation protocols with a gene construct that express *e-GFP* as marker gene (Fig.2), in comparison with the efficient table grape cultivar Thompson Seedless.

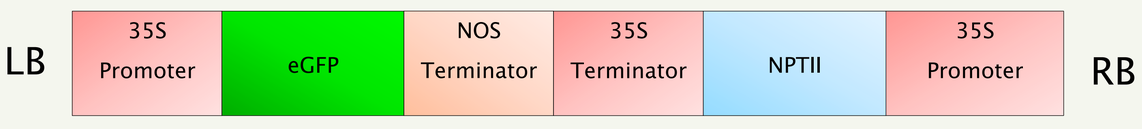
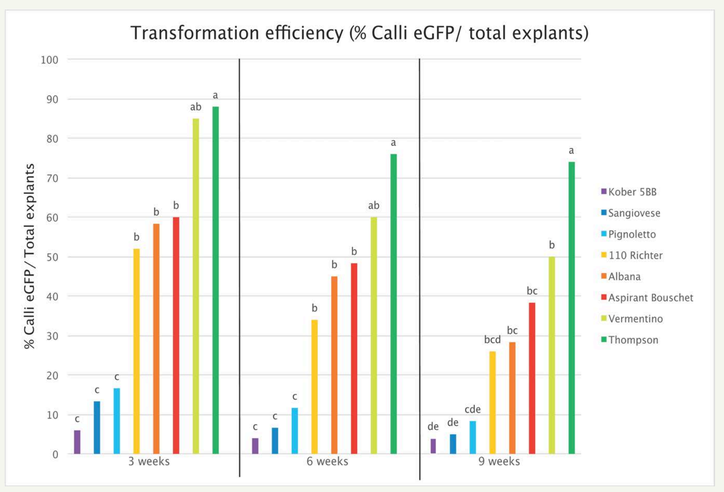


Fig 2. Schematic drawing of the gene construct expressing *e-GFP* and *NPTII* as marker genes.

Results

The genotypes used for the transformation trials showed a different transformation efficiency in terms of meristematic calli expressing eGFP and number of transformed shoots obtained (only the cv. Thompson seedless produced transgenic shoots during the first 9 weeks of selection) (Fig.3 and 4).



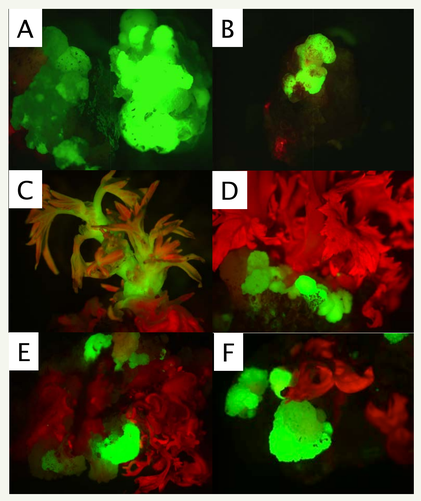


Fig.3 Data showing the percentage of calli expressing eGFP/total explants for each genotype acquired after 3, 6, 9 weeks on media containing kanamycin 70 mg L^{-1}. Different letters compare the means of different genotypes at the same data acquisition time. Means with different letters are significantly different according to Duncan test ($p \leq 0,05$).

Fig. 4 Expression of e-GFP in different transformed grapevine genotypes after 6 weeks on selective medium containing kanamycin 70 mg L^{-1}. Uniform fluorescence with bright green colour was observed in transformed tissues under UV light: a) Albana; b) Pignoletto; c) Thompson seedless; d) Vermentino; e) Aspirant Bouschet f) 110 Richter.

RNAi for the obtainment of virus resistance in plant

Genotypes having the highest regeneration and transformation efficiency will be used for transformation experiments using a hairpin gene construct designed to silence the RNA-dependent RNA polymerase (RpRd) of the GFLV and GLRaV3, which should induce multiple virus resistances (Fig.5).

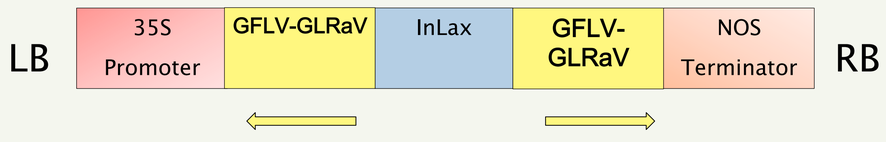


Fig.5 Schematic drawing of hp construct virus resistance

- Mezzetti, B., Pandolfini, T., Navacchi, O., & Landi, L. (2002). Genetic transformation of Vitis vinifera via organogenesis. *BMC biotechnology*, *2*(1), 18.
- Xie, X., Agüero, C. B., Wang, Y., & Walker, M. A. (2016). Genetic transformation of grape varieties and rootstocks via organogenesis. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *126*(3), 541-552.