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DEVELOPMENT OF A METHOD FOR CONFERRING RESISTANCE TO GFL AND GLR ASSOCIATED VIRUSES THROUGH POST TRANSCRIPTIONAL GENE SILENCING

PALMA D.*, MOLESINI B.*, SABBADINI S.**, POLVERARI A.*, NAVACCHI O.***, MEZZETTI B.**, PANDOLFINI T.*

*) Dipartimento Biotecnologie, Università di Verona, Strada le Grazie 15, Verona
**) Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche, Ancona
***) Vitroplant Italia, Cesena

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Grapevine (*Vitis vinifera L.)* is one of the most important crop species in the world. Main purposes of grapevine genetic improvement programs are the obtainment of plants able to stand biotic stresses and the improvement of features related to production and fruit quality. The production of resistant cultivars through classic genetic techniques such as crossing, requires very long implementation times. In contrast, the use of genetic engineering represents an effective method to increase resistance to pests and to improve fruit quality and production without altering the agronomic traits of the cultivars. The aim of this project is to develop a genetic engineering method based on post transcriptional gene silencing (PTGS) for conferring resistance against viruses responsible for grapevine fanleaf and grapevine leafroll diseases. Grapevine fanleaf disease is mainly caused by the *Nepovirus* viruses such as *Grapevine fanleaf virus* (GFLV) and *Arabis Mosaic Virus* (ArMV). The grapevine leafroll disease is ascribed to the presence of one or more infectious agents belonging to *Closterovirus* and *Ampelovirus,* "associated" to the disease (Grapevine leafroll-associated viruses, GLRaV). To curtail virus replication by gene silencing, we built a construct for the expression of hairpin transcripts homologous to selected portions of the viral genomes. The PTGS-eliciting construct (*hpViruses GFLV-GLRaV*) containes two 400 bp-long arms placed in inverted orientation, each arm consisting of two fused DNA fragments homologous to the GFLV and GLRaV-3 RNA-dependent RNA polymerase genes, respectively. The hp construct was introduced in *Nicotiana benthamiana* to test its efficacy against GFLV and ArMV infection. Several transgenic lines were identified by PCR and Southern blot analysis. The same construct has been used for *A. tumefaciens*-mediated genetic transformation of grapevine using a protocol based on organogenesis (Mezzetti *et al.*, 2002). Up to now, the material is still in the selection and regeneration phase.