

## ***Agrobacterium*-mediated transformation of olive (*Olea europaea* L.) with an antifungal protein from *Aspergillus giganteus*.**

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Broad-spectrum resistance to pests and diseases is difficult to obtain through classical breeding programs, hence, this is a targeted trait for accelerating the development of major olive cultivars using plant transformation technologies. Olive Verticillium wilt, caused by *Verticillium dahliae*, is considered to be an important constraint for cultivation of olive trees (López-Escudero and Mercado-Blanco 2010). Different transgenic approaches have been proposed to engineer plants for resistance to fungal diseases, including production of antifungal proteins (Gurr and Rushton 2005). Regarding this approach, among different antifungal compounds, the antifungal protein (AFP) from *Aspergillus giganteus* can be considered a promising candidate for practical applications in crop protection (Meyer 2007). AFP is a defensin-like protein that belongs to a group of small-sized secretory proteins rich in cysteine residues. The protein possesses *in vitro* antifungal activity inhibiting the growth of several fungal pathogens. Previous work has already shown that *afp* gene can be expressed in transgenic rice plants inducing resistance to the fungus *Magnaporthe grisea* and indicating the usefulness of such approach for protection against rice blast. (Coca et al. 2004)

In this work, transgenic olive plants were generated by *Agrobacterium*-mediated transformation as described by Torreblanca et al. (2010). The AGL-1 strain containing the pBIN61-*afp* binary vector was used. This plasmid contains the *nptII* gene for paromomycin selection and a chemically synthesized codon-optimized *afp* gene under the control of the 35S CaMV promoter. Globular somatic embryos derived from a mature seed of cultivar 'Picual' were transformed obtaining an average success rate around 2%. Plants were regenerated from six independent lines and transgenic nature was confirmed by PCR studying *nptII* and *afp* insertion. With the aim of studying whether the *afp* gene can be used to induce resistance against fungal diseases in olive, susceptibility to the fungal pathogens *Rosellinia necatrix* and *Verticillium dahliae* will be evaluated. In addition, the inhibitory effect of proteins extracts from transgenic leaves on the *in vitro* growth of these fungal pathogens will also be examined.

**Key words:** *Olea europaea*, Antifungal protein, *Aspergillus giganteus*, Transgenic olive.

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Research supported by projects: P11-AGR-7992 and AGL2011-30354-C02-01