

## HAIRY ROOT CULTURES FOR THE PRODUCTION OF SECONDARY METABOLITES

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Hairy roots of medicinal and aromatic plants, induced by the soil Gram-negative bacterium *Agrobacterium rhizogenes*, represent a fast growing active tissue with a biosynthetic potential that can be exploited for the production of valuable secondary metabolites (SAITO *et al.*, 1992, *J. of natural products*, **55** (2): 149-162.). Hairy root cultures are hormone independent and can grow as fast as unorganized plant cell suspensions while maintaining a stable differentiated phenotype; furthermore secondary metabolites synthesis is not strictly limited to those that are normally produced in roots of differentiated plants (GIRI, A. and NARASU, M.L., 2000, *Biotechnology Advances*, 18: 1-22.).

*A. rhizogenes* wild type strains 15834 (ATCC) and 1855 (NCP) have been used as natural gene vectors to induce hairy roots in *Astragalus verrucosus*, *Helichrysum italicum*, *Helichrysum stoechas*, *Hypericum perforatum*, *Salvia cinnabarrina*, *Salvia wagneriana* and *Solidago virgaurea*. Leaf, root and stem tissues of micropropagated plants were co-cultivated with each bacterial suspension for 20'. The explants were cultured on basal MS (Murashige and Skoog, *Physiol. Plant.*, 1962, 15:473-497) vitamins and salts mixture, 30g/L sucrose, pH 5.7, agar 8 g/L, without growth regulators for three days and then transferred to the same medium added with cefotaxime (100 mg/L). Explants developed new roots from the wounded tissues after 30 days of culture in dark (*A. verrucosus*, *S. cinnabarrina* and *S. virgaurea*) and light conditions (*H. italicum*, *H. stoechas*, *H. perforatum* and *S. wagneriana*). *H. perforatum* co-cultivated leaf tissues needed to be placed on the basal medium added with 1 µM IAA and 10 µM Zeatin to obtain hairy roots (Di Guardo *et al.*, *J. of Genetic and Breeding*, 2004 in press). The screening of the putative hairy root lines was performed by PCR analysis. The *A. rhizogenes* T-DNA *rolC* gene amplification band of 514 bp (Scorza *et al.*, *J. Amer. Soc. Hort. Sci.* 119(5): 1091-1098, 1994) was detected in the hairy root lines and not in the respective plant controls. Contamination of plant tissue by bacterial cells was excluded by testing the amplification of a 326 bp fragment of the *virC1* gene (Vaira *et al.*, *MPMI* Vol.8, N° 1: 66-73, 1995), which is located outside the bacterial T-DNA and is not transferred to the plant genome. The amplification band was detected only in the *A. rhizogenes* samples. Each hairy root line was cultured and maintained on hormone-free medium for more than three years. Some *H. perforatum* and *S. cinnabarrina* hairy root lines were selected for the scaling up in bioreactor (RITA®, CIRAD (F)) to increase biomass production. When grown in light conditions *H. perforatum* and *H. stoechas* hairy roots spontaneously regenerated plants. The hairy root-regenerated plants showed morphological characteristics (short internodes, increased branching, small

leaves and reduced apical dominance) with a phenotype unique for each independently originated line. *H. italicum* hairy root liquid cultures were extracted and analysed by TLC to detect flavonoids (elichrysin); some *A. verrucosus* hairy root lines produced saponins and in *H. perforatum* hairy root-regenerating plant cultures hypericin and hyperforin were detected by HPLC analysis (Giovannini et al., Convegno SOI, 2004). In this work we report the establishment of hairy root cultures in several aromatic and medicinal species. Each hairy root line has a peculiar phytochemical profile that can be exploited for the production of active substances.