# Analysis of the hormonal manipulation during *in vitro* induction of adventitious roots in *Castanea sativa* Mill.

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Adventitious rooting is a complex process still poor understood, and is a crucial step for the clonal propagation of elite genotypes of woody species. Plant development is modulated by genetic and environmental factors, which have effects on auxin biosynthesis, metabolism, transport and signaling pathways. Auxins play an essential role in many developmental processes, particularly during the induction phase of adventitious rooting. The identification of molecular players regulated by auxin that control the rooting process will help to the practical improvement of root formation in difficult-to-root genotypes.

The aim of this study was to evaluate the physiological and molecular responses of chestnut tissues to the hormonal manipulation by the application of plant growth regulators during the *in vitro* induction of adventitious roots. Two types of explants, chestnut microshoots and leaf petioles of chestnut microshoots were used. Explants were treated with indole-3-butyric acid (IBA) alone or in combination N-1-naphthyl-phthalamic acid (NPA), an inhibitor of polar auxin transport. Rooting response was determined based on number and length of roots and kinetics of rooting. Samples were collected at different time points for gene expression analysis.

In both microshoots and petiole systems, auxin promoted the initiation of adventitious roots. In leaf petioles most of roots were developed from a callus originated in the base of the petiole. The application of NPA reduced the rooting ability in terms of rooting rates and also in the delay of the onset of the rooting process compared with the controls treated only with IBA.

The use of leaf petioles as explants for adventitious rooting provided a number of advantages: 1) an increase in the potential number of samples, as an average of 3-4 leaf petioles can be obtained from a single microshoot, 2) a reduction in the complexity of tissues to be analyzed, as it focuses in the zone were roots are initiated, 3) the avoidance of the side-effects potentially caused by the polar auxin transport that basipetally moves endogenous auxin from shoot apex in the microshoots, 4) the exclusion of other developmental processes that occur simultaneously in the shoot as a consequence of auxin treatments, which allows to characterize auxin-responsive genes involved only in root formation.

Key words: adventitious roots, auxin, chestnut, leaf petiole, NPA.

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