Effect of NPA on adventitious root induction and root development in leaves of chestnut microshoots

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The successful formation of adventitious roots (highly dependent of both the age and the physiological stage of the plant material) is a crucial step for the clonal propagation of elite genotypes of woody species. Adventitious rooting is a complex process, and basic mechanisms controlling the development of new roots are still poorly understood. It is well known that auxins are the compounds most frequently used to modulate adventitious root formation. Auxin biosynthesis, metabolism, transport and signaling pathways play an essential role during the induction phase of adventitious rooting. We are interested in understanding the molecular mechanisms involved in the reprogramming of differentiated cells towards a new cell fate to initiate a root meristem.

The first objective of this work was to evaluate the effect of the auxin efflux inhibitor NPA (N-1-naphthyl-phthalamic acid) during the induction and development of adventitious roots in leaf explants excised from rooting-competent microshoots. Four different treatments were evaluated in this study. Explants were treated with: 1) IBA (indole-3-butiric acid) for 5 days (induction phase); 2) NPA and IBA simultaneously during the induction phase period (5 days); 3) IBA for 5 days, and subsequently transferred to NPA-containing medium for another 5 days and 4) IBA for 5 days and then transferred to NPA-containing medium for another 25 days.

A second objective was to compare the dynamics of *CsSCL1*expression in leaves from rooting-competent and –incompetent shoots in response to auxin. The effect of NPA on the *CsSCL1* expression was also tested in IBA-treated leaves excised from rooting-competent shoots. *CsSCL1* was previously found to be induced by auxin in a phase-dependent manner during the induction of adventitious roots in microshoots.

Our results showed that NPA negatively affected the induction and development of adventitious roots as well as the expression of *CsSCL1*. When NPA was included in the medium for 25 d, the rooting percentage was reduced from 90 to 14%. *CsSCL1* expression was highly induced by auxin only on rooting-competent leaves. NPA treatment reduced the 24h peak of *CsSCL1* expression of IBA-treated leaves and also changed the spatial distribution of mRNA transcripts.

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