A new system to study auxin responses and gene expression during the
*in vitro* induction of adventitious roots in chestnut.

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European chestnut is difficult to propagate vegetatively from mature tissues. Cuttings
from mature chestnut trees, as well as mature-shoot derived cultures, are difficult to
root. The identification of molecular players that control the rooting process will help to
improve root formation in difficult-to-root genotypes. In our laboratory we engaged in a
search for molecular regulators of adventitious root initiation.

Based on the different rooting response of two lines of chestnut microshoots (juvenile-
like and mature) to auxin treatment, we also developed a simple and highly reproducible
system to induce adventitious roots on leaves excised from these shoots.

The aim of this study was to determine the suitability of the leaf rooting system in
response to auxin and NPA (N-1-naphthyl-phthalamic acid) treatments, compared to the
system of microshoots (Vielba et al. 2011). Besides, we analyzed the expression of two
different genes, the *CsGH3*-2 (*auxin-inducible GH3-like*) and *CsLRR-RLK* (*leucin-rich
repeat receptor-like kinase*) in different organs of chestnut shoots as well as during the
induction of roots in leaves excised from juvenile-like and mature microshoots. The
expression pattern of both genes was also evaluated in rooting-competent leaves
(juvenile-like) treated with auxin and NPA. It has been described that *GH3* genes can
play a crucial role in auxin homeostasis in the rooting area, as they participate in the
control of free auxin levels. The *LRR-RLK* genes are involved in signaling pathways
and meristem maintenance.

Our results indicate that the proposed system of leaves can be used at physiological and
molecular levels to study the rooting process, as the physiological response to auxin is
similar to that shown by microshoots, and it also exhibits a stronger inhibition of root
formation by NPA. *CsGH3*-2 expression revealed that the gene is upregulated by auxin.
Its expression significantly decreased after NPA treatment in auxin-treated juvenile
leaves, suggesting its involvement in the induction of roots. On the other hand, *CsLRR-
RLK* transcripts were localized in the root tip, although the gene was not regulated by
auxin or NPA during the first 24 hours of root induction.

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**Keywords:** Adventitious root, chestnut, *CsGH3*-2, *CsLRR-RLK*, NPA.
