# Morphophysiological factors regulating rooting of pedunculate oak (*Quercus robur* L.) *in vitro*

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Keywords: *Quercus robur*, micropropagation, *in vitro* culture, adventitious rooting, plant growth regulators

The aim of this study was to investigate the morphophysiological factors regulating the rooting capacity of pedunculate oak (*Quercus robur* L.) shoots previously propagated under *in vitro* conditions, with particular emphasis on the role of plant growth regulators during root induction. Efficient vegetative propagation of forest tree species remains a major challenge, especially in long-lived woody plants such as oaks. Improving rooting efficiency in micropropagation systems is therefore essential for the large-scale production of high-quality planting material and for the conservation of valuable forest genetic resources.

Rooting was evaluated separately under *in vitro* and *ex vitro* conditions in order to compare the effectiveness of controlled culture environments with acclimatization-based rooting approaches. Initially, shoots were incubated for 48 h in culture medium supplemented with indole-3-butyric acid (IBA; 0 or 100 μM) and 20 g $l^{-1}$ sucrose. Subsequently, the shoots were transferred either to *in vitro* media containing meta-topolin (mT; 0 or 4 μM) and 40 g $l^{-1}$ sucrose and maintained for 40 days, or to a perlite/peat substrate (3:2) placed in plastic boxes under *ex vitro* conditions. Under *ex vitro* conditions, mT (0 or 4 μM) was applied as a foliar spray every 7 days for a total period of 60 days. In both *in vitro* and *ex vitro* experiments, treatments were arranged in a factorial design consisting of two IBA levels and two mT levels (2 × 2).

Shoots previously exposed to IBA for 48 h produced a greater number of roots and exhibited more vigorous root growth compared with untreated controls. Clear differences were also observed between the two rooting systems. Root development under *in vitro* conditions was more efficient and uniform (67–87% rooting) than under *ex vitro* conditions (40–50% rooting), suggesting that the presence of exogenous carbohydrates promotes more stable root induction and development. Moreover, shoots previously treated with 100 μM IBA exhibited a thicker lower epidermal layer and a significantly thicker spongy parenchyma, regardless of mT supplementation, while the ratio of palisade to spongy parenchyma decreased. In leaves collected from *ex vitro* saplings, only the thickness of the spongy parenchyma differed between treatments and was independently influenced by both factors, IBA and mT. Plants previously exposed to IBA exhibited a thicker spongy mesophyll, regardless of the presence of mT. Similarly, plants grown *ex vitro* under conditions with mT also had a thicker spongy mesophyll.

The results indicate that short-term treatment of oak microshoots with IBA can substantially improve rooting efficiency in pedunculate oak micropropagation protocols. The study highlights the importance of optimizing hormonal treatments and culture conditions to enhance the vegetative propagation of this ecologically and economically important forest tree species.