

Comparison between a conventional culture system and Plantform bioreactor in *Quercus robur* micropropagation

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Quercus robur is a recalcitrant species when the *in vitro* propagation is carried out. Indeed, although a protocol for *in vitro* propagation of juvenile Italian genotypes of this species has been previously reported, the proliferation rate of shoots still remains unsatisfactory. Plantform bioreactor is a new system recently developed to improve *in vitro* plant cultures, based on a temporary immersion system (TIS) with a ventilation that ensures the regular air renewal inside the vessel. Hence, in this study the Plantform bioreactor was tested with the aim to evaluate its efficiency in promoting shoot proliferation from axillary buds, comparing the results with those obtained on gelled medium, with a conventional culture vessel. The effect of the two culture systems previously applied was moreover evaluated during the rooting phase, carried out on agarized medium. Finally, the influence of the culture environment on some features of leaf anatomy were considered, to assess the possible establishment of the micropropagated plants. Nodal segments (10-15 mm, on average) of *Q. robur* were taken from previously established *in vitro* shoot cultures and placed horizontally both in Microbox vessels, on Woody Plant Medium (WP) added with sucrose (20 g L⁻¹), agar (6 g L⁻¹) and 6-benzylaminopurine (BA), (0.2 mg L⁻¹), and in Plantform with the same medium devoid of agar. Two different conditions of temporary immersion were tested in Plantform: 12 min/8 h (Plantform 1) and 8 min/16 h (Plantform 2). Air was renewed for 15 min/4 h. Difference in terms of Relative Growth Rate (RGR), based on fresh weight of the shoots, were recorded after 10 weeks of culture. RGR was higher in Plantform 1 and Plantform 2 than in Microbox, while only in Plantform 2 the number of shoots per explant was higher than in Microbox. Shoots previously cultured in Microbox, however, seem to show the best percentage of rooting. No difference in stomata frequency emerged among leaves isolated from shoots cultured in Microbox, Plantform 1 and *in planta* (acclimatized plants) and this value was significantly lower in Plantform 2 than in all the other samples. Leaves taken from Microbox showed a stomatal surface area very similar to acclimatized plants and also with young leaves from adult trees. Plantform seems to be a very effective method to promote a rapid and vigorous growth in *Q. robur* shoot cultures. Conversely, the growth of the shoots in Microbox seems to favour the following rooting. The possibility to modulate the time of immersion and the duration of air renewal in Plantform bioreactor opens many opportunities to obtain the “best condition” for *in vitro* propagation of oak. The “environmental” factors inside the *in vitro* culture vessels, confirmed to affect some anatomical leaf features, involved in acclimatization of micropropagated plants.