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Micropropagation of *Quercus pubescens* from buds

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Some plants present challenging obstacles when grown by *in vitro* culture, they may be recalcitrant, hampering the development of reliable regeneration techniques. This 'recalcitrant behaviour' is genetically driven, so it is difficult to control by environmental and nutritional manipulation in microculture. Plants which are difficult to work with are usually perennials with complex seasonal life cycles. *Quercus* species show short growth spurts per season, when they stop growing until the next season. Such species may be called episodic and are not always successfully used in microculture and biotechnology. Micropropagation has the advantage of genetic homogeneity and avoids difficulties related to obtaining sterile plant material.

This research work dealt with the micropropagation of *Quercus pubescens* with the aim of enhancing the percentage proliferation and rooting from microcuttings. The microcuttings, containing apical or axillary buds, were taken from two year-old branches using a clone of *Q. pubescens* grown in the Forest Nursery of Sant Angelo in Vado, Pesaro-Urbino, Marche, Italy. After an initial screening to select the best growth hormones, the proliferation tests were performed using increasing concentration of benzylaminopurine (BA) in the culture medium (0.25; 0.50; 0.75; 1.00 mg/l). From the results it was possible to deduce that BA plays a key role in influencing shoots proliferation, leading to the formation of a callus and to the production of new shoots. Moreover, it was observed that, especially at higher concentrations (from 0.5 to 1.0 mg/l), BA promoted the production of a higher number of shoots, although their length was shorter than expected. At lower concentrations of BA, fewer but longer shoots were produced. Furthermore, these trials have shown that, in order to have a large number of seedlings with an optimal shoot length, it is necessary to divide the process of propagation in two stages. The first phase, using high concentrations of BA, with the aim of increasing the number of new micropropagules; the second stage, using lower levels of BA, to increase the length of the shoots. With regards to root induction, the hormone selected was indolacetic-butirric acid (IBA). For this stage the micropropagules were removed from their proliferation substrate and transferred in

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two different substrates containing different concentrations of IBA (10 and 20 mg/l). Microcuttings were left in the hormone for 24h and 48h. Results showed that the lower hormone concentration gave the best results for root induction; similarly, the shorter period of contact with IBA substrate was more efficient. The development of this protocol can allow for improving in vitro studies of *Q. pubescens* in various areas of research, such as *in vitro* mycorrhization.