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Short communication

In vitro regeneration from alginate-encapsulated microcuttings of *Quercus* sp.

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Abstract

Microcuttings with apical and nodal buds from common oak (*Quercus robur* L.) and Turkey oak (*Quercus cerris* L.) in vitro clones were encapsulated into an alginate matrix. Apical segments of common oak showed significantly greater in vitro regeneration potential as compared with the nodal ones. Encapsulated Turkey oak nodal segments demonstrated successful regeneration after different periods (2–6 weeks) of cold storage (4 °C), showing no significant deviation from the regenerative frequency of non-cold-treated segments.

Keywords: Encapsulation; In vitro propagules; *Quercus* sp.; Regeneration; Synthetic seeds

1. Introduction

Since the concept of ''artificial seeds'' was formulated by Murashige (1997), numerous studies were undertaken in this practice-oriented field of plant biotechnology. The most common target for the ''synseed'' technology remains the somatic embryos. However, the encapsulation of microcuttings could be an attractive alternative for producing plantlets in some species recalcitrant to somatic embryogenesis. The technique of encapsulation–

Abbreviations: BAP, N^6-benzylaminopurine; GD, Gresshoff and Doy medium.

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regeneration from different explant types has been demonstrated in a wide variety of plant species, including some woody and forest species—*Olea europea* L., *Morus alba* L., *Morus incana* L., *Betula pendula* Roth., *Betula davurica* Pall., etc. (Standardi and Piccioni, 1998).

Results from a pilot study on the feasibility of the encapsulation technique in two representatives of the *Quercus* genus are presented in this paper.

2. Materials and methods

Apical and nodal segments from common oak (*Quercus robur* L.) and Turkey oak (*Quercus cerris* L.) in vitro clones were used in these experiments. Microcuttings (4–5 mm in length) were used as initial explants. The donor plants were cultivated on modified GD (Gresshoff and Doy, 1972) medium supplemented with 0.88 μM BAP and 7.5 g l^{-1} agar with a 6-week subculturing period at standard culture room conditions (see later).

For encapsulation, microcuttings were immersed for a few seconds with forceps into an encapsulation solution made of the same formulation as the GD modified medium without agar and supplemented with 0.88 μM BAP and 4% (w/v) sodium alginate (Sigma, A 0682). The alginate-covered microcuttings were then transferred in stirring 1.4% (w/v) $CaCl_2$ solution and kept complexing for 10 min. The hardened alginate beads were rinsed thrice for 2 min each time in liquid GD modified medium. Finally, the capsules were placed for regeneration on semisolid modified GD proliferation medium (0.88 μM BAP, 7.5 g l^{-1} agar) with respect to the polarity of the explants. The explants were cultivated in a culture room at 23 ± 1 °C under 16/8 h day/night regime with illumination provided by GroLux white fluorescent lamps (40 μmol m^{-2} s^{-1}). The pH of all the media and solutions were adjusted to 5.7 before being autoclaved at 121 °C and 1.3 kN m^{-2} for 20 min.

In a separate experiment, nodal segments isolated from shoots of a Turkey oak clone were encapsulated according to the procedure mentioned above and kept at low temperature (4 °C) for various periods of time before being transferred for regeneration. Five different low-temperature exposure times (2, 3, 4, 5 and 6 weeks) were evaluated, with a separate non-cold-treated control being directly placed for regeneration. Each variant was tested on 25 explants.

Erlenmayer flasks (100 ml) containing 25 ml medium were used for explant culture, with five capsules being placed into each vessel. The encapsulated Turkey oak segments were kept on filter paper moistened with GD liquid medium, in plastic Petri dishes (90 mm × 15 mm) during the cold storage.

The frequency of regeneration was recorded after 5 and 7 weeks in the first experiment and 4 weeks in the second experiment after beads were placed on the respective regeneration media and conditions. Regeneration was defined as breakage of capsules and/or extrusion of leaf or shoot from the capsule or as sprouting of buds. The multiplication rate (number of sprouted buds) and percentage of viability (green appearance) of the explants were also recorded.

Analysis of variance (ANOVA) and *G*-test (Sokal and Rohlf, 1994) were applied for estimation of putative factorial effects on the multiplication rate and frequency parameters.