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BRIEF COMMUNICATION

Effect of alginate matrix composition on regrowth of *in vitro*-derived encapsulated apical microcuttings of hybrid aspen

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Abstract

Effect of alginate matrix composition on regrowth performance of encapsulated microcuttings of hybrid aspen (*Populus tremula* L. × *P. tremuloides* Mincx.) was studied. Both high regrowth frequency and viability of explants were registered in all encapsulation mixtures tested. Some ingredients of the matrix (nutrient medium salts, sugars, growth regulators) significantly affected the initial development of the microcuttings. Sucrose appeared to play an important role in the starting stage of the regrowth event.

Additional key words: encapsulation, propagules, *Populus* sp.

Despite of being basic alternative for production of 'synthetic seeds' (Redenbaugh *et al.* 1986, Attree and Fowke 1993, Uozumi and Kobayashi 1995), the encapsulation technique offers attractive opportunities for supplementary applications. The encapsulation of non-embryogenic *in vitro*-derived explants in Na-Ca alginate beads could be useful tool both for transfer of aseptic material between laboratories and for storage of valuable germplasm (Standardi and Piccioni 1998). In realizing the idea of providing an 'artificial endosperm', the nutritive ingredients of the alginate beads are of key importance for both the storage and conversion efficiencies of the propagules encapsulated. Effects of various compositions of sodium alginate matrix on regrowth performance of encapsulated apical cuttings of hybrid aspen were examined in a pilot study.

Shoot apical microcuttings 0.5 - 0.7 cm in length isolated from *in vitro* proliferating clone of *Populus tremula* L. × *P. tremuloides* Mincx. were used in the experiment. Microcuttings were encapsulated in accordance with the following procedure. For coating with sodium alginate, explants were immersed in sterilized encapsulation mixtures with different composition: *a*) distilled water + 4 % sodium alginate (W); *b*) distilled water + 3 % sucrose + 4 % sodium alginate (W+S); *c*) Murashige and Skoog (1962, MS) nutrient medium + 4 % sodium alginate (M); *d*) MS medium + 1.3 μM 6-benzylaminopurine (BAP) + 4 % sodium alginate (M+B); *e*) MS medium + 3 % sucrose + 4 % sodium alginate (M+S); *f*) MS medium + 1.3 μM BAP + 3 % sucrose + 4 % sodium alginate (M+B+S). The alginate-covered microcuttings were dropped into stirred 1.4 % $CaCl_2$ solution and kept 5 min for complexation and formation of capsules. The capsules were rinsed thrice 1 min each time with liquid MS medium supplemented with 1.3 μM BAP and 3 % sucrose.

Encapsulated microcuttings were transferred for regeneration on MS medium supplemented with 1.3 μM BAP, 3 % sucrose and solidified with 0.8 % agar. Glass jars (300 cm^3) with 50 cm^3 nutrient medium were used for all experimental treatments. Each variant consisted of five jars, with five capsules being placed into each of them. Non-encapsulated microcuttings were directly placed on the same medium as a control. The jars were kept in a cultivation room at 24 ± 1 ºC, 16-h photoperiod and irradiance of 40 μmol m^{-2} s^{-1} provided by cool-white fluorescent lamps (*GroLux*™, *Sylvania*, Raunheim, Germany).

After period of four weeks the values of the following parameters were recorded: frequency of regrowth (regrowth is defined as any visual growth activity, *i.e.* breakage of capsule, protrusion of either leaf or shoot

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Abbreviations: BAP - 6-benzylaminopurine; MS medium - Murashige and Skoog medium; PGRs - plant growth regulators.
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outside the bead or presence of roots), frequency of viability (green appearance, lack of necrosis), average fresh mass of explants (per vessel), length of shoots, and number of axillary shoots formed (> 0.5 cm). For estimation of putative effect of the alginate matrix composition on the regrowth parameters data were subjected to analysis of variance (*ANOVA*). The treatment means were compared by using Duncan's multiple range test (Sokal and Rohlf 1996).

One week after the start of the experiment at all variants the encapsulated microcuttings demonstrated high regrowth frequency (96.7 - 100.0 %) (Fig. 1). Similar high values of viability were registered at the end of the experiment (Table 1). The regrowth parameters were significantly influenced ($P < 0.001$) by the composition of the encapsulation mixture. The highest fresh mass was recorded at both M+B+S and M+S formulations, with values exceeding twenty fold increase of the initial one (data not shown). Gardi *et al.* (1999) reported similar results concerning growth performance of encapsulated microcuttings from several woody species after one-month cold storage. Surprisingly, the growth parameters of shoots regenerated from beads with sodium alginate supplemented with sucrose (W+S) did not deviate considerably from both M+S and M+B+S (Table 1), whereas the variant lacking sucrose (M+B) demonstrated significantly ($P < 0.001$) worse regrowth.

Fig. 1. Regrowth of encapsulated apical microcutting of hybrid aspen (*P. tremula* × *P. tremuloides*) one week after placing the bead on MS medium supplemented with 1.3 μM BAP (*bar* = 0.5 cm).

Table 1. Effect of various compositions of Na-alginate matrix on the regrowth performance of encapsulated microcuttings of hybrid aspen (*P. tremula* × *P. tremuloides*). The values presented are means ± SD. Values followed by the same letters are not significantly different at $P < 0.05$.

Variants	Shoot length [cm]	Number of axillary shoots [explant^{-1}]	Fresh mass [g vessel^{-1}]	Viability [%]
Control	2.4 ± 0.4^{b}	2.4 ± 1.1^{b}	0.7 ± 0.2^{d}	100.0
W	2.5 ± 0.6^{b}	2.8 ± 2.2^{b}	1.2 ± 0.3^{c}	100.0
W+S	3.5 ± 0.7^{a}	5.3 ± 1.8^{a}	2.0 ± 0.3^{a}	100.0
M	3.2 ± 0.5^{a}	5.1 ± 1.7^{a}	1.6 ± 0.2^{b}	96.0
M+B	2.5 ± 0.4^{b}	2.9 ± 1.6^{b}	1.1 ± 0.2^{c}	100.0
M+S	3.4 ± 0.8^{a}	4.7 ± 1.5^{a}	2.1 ± 0.2^{a}	100.0
M+B+S	3.3 ± 0.7^{a}	5.2 ± 1.8^{a}	2.1 ± 0.3^{a}	100.0

These findings suggest that the sucrose is an essential ingredient of the sodium alginate matrix, being likely not less important than the PGRs supplements. At the same time, the sodium alginate combined with sucrose *(*W+S*)* demonstrated significant superiority over the pure alginate solution (W). The latter performed in a similar way like the control, with an exception of providing shoots with significantly ($P < 0.001$) higher average fresh mass.

The alginate matrix composition appeared to be an important factor significantly affecting the regrowth performance of the encapsulated hybrid aspen apical segments. The addition of adjuvant components to the alginate solution resulted in general improvement of the regrowth parameters. In particular, sucrose was found to have more pronounced effect than BAP. The requirements for availability of definite ingredients of the hydrogel matrix (inorganics, organics, PGRs, carbohydrates, *etc*.) are likely species-specific, thus suggesting a special attention to be paid to the features of the species of interest.

The results obtained would be useful in further attempts at developing a model system of synseed production based on *in vitro*-derived apical microcuttings. A proper optimisation of the alginate matrix composition may both enhance the regrowth performance of encapsulated explants and directly impact the efficiency and practical applicability of the technique.

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