Development of synthetic seed technology of silver birch (*Betula pendula* Roth.)

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INTRODUCTION

Synthetic seeds are artificially encapsulated plant tissues. They represent a promising biotechnology-based solution for the conservation of genetic resources. This method can aid in the preservation and propagation of genetically improved reproductive material with high forestry value. Synthetic seeds are small in size making them easier to store, transport, and plant. Encapsulation is crucial for reducing plantlet costs and extending storage times. The aim of this study was to develop a shoot tip encapsulation method for long-term storage of *in vitro* silver birch genetic material.

MATERIALS AND METHODS

Shoot tips (3–5 mm) were used for encapsulation. The tips were immersed in 2% or 3% sodium alginate solutions and pipetted into a 100 mM $CaCl_2$ solution for 30 minutes under gentle stirring, then removed and rinsed three times in sterile distilled water for 5 minutes each. After rinsing, the capsules were placed on filter paper to dry. Once dry, they were transferred into 5 mL tubes, with 15–20 capsules per tube. The tubes were stored in a refrigerator at +4 °C, where the synthetic seeds were kept for seven months.
To assess the influence of the growth medium prior to encapsulation, three different media variants were tested (Fig. 1). To evaluate the effect of capsule composition on shoot regeneration and shoot length, variations in MS and sodium alginate concentrations, as well as storage conditions, were compared (Fig. 2).

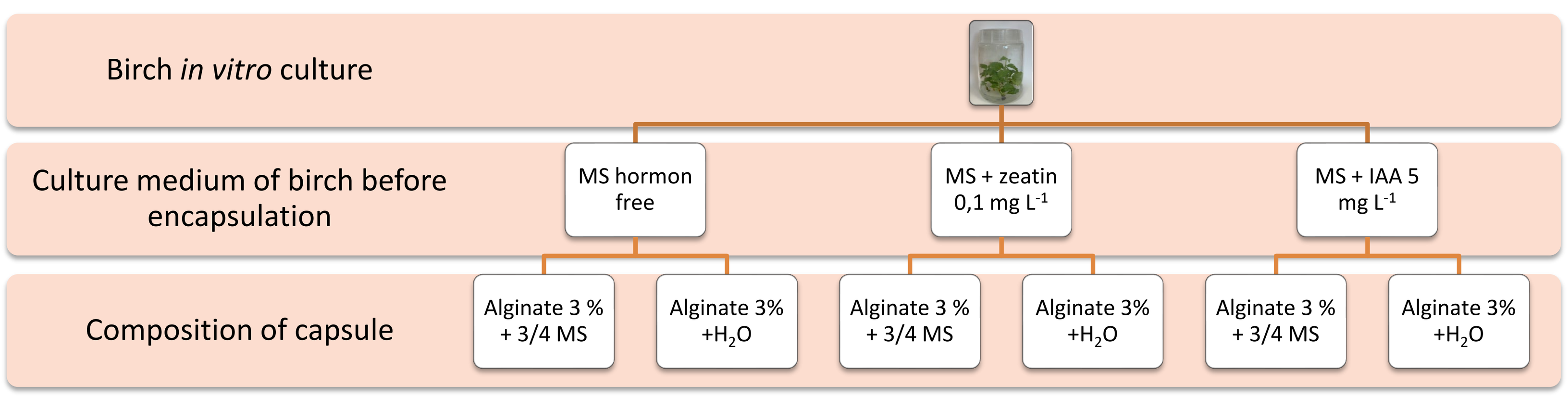

Fig. 1. *Schematic Representation of the Experimental Design: Effects of Pre-Encapsulation Culture Medium and Capsule Composition on the Storage of encapsulated Betula pendula in vitro shoots*

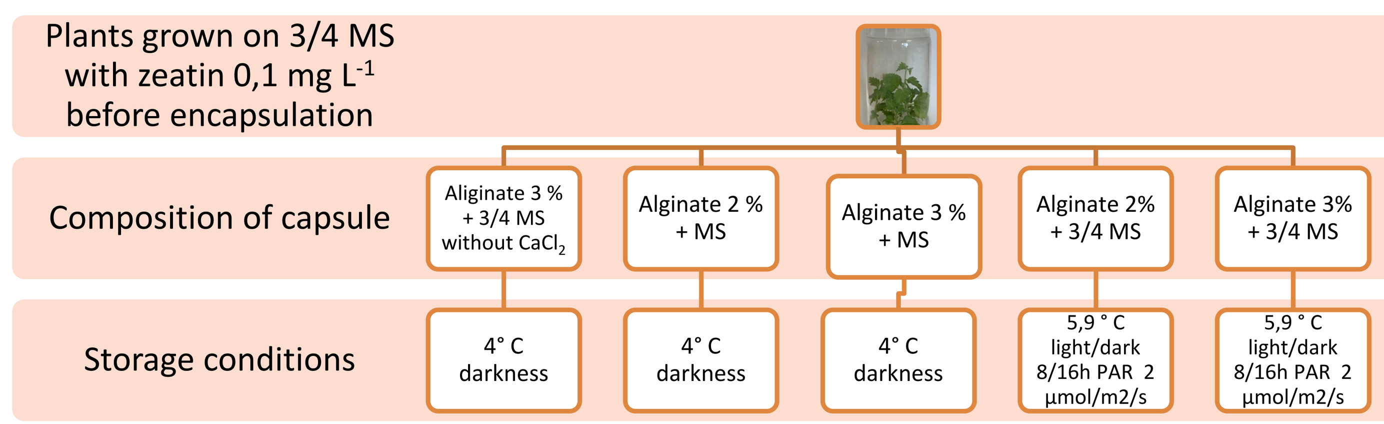

Fig. 2. *Schematic Representation of the Experimental Design: Effect of capsule composition and storage conditions on the storage efficiency of encapsulated Betula pendula in vitro* shoots.

Following storage, the encapsulated shoots were removed from the tubes and transferred to jars. Throughout the experiment, a consistent regeneration medium was used: ¾-strength MS supplemented with 0.2 mg L^{-1} zeatin. Each jar contained six to seven capsules. The jars were placed in a growth chamber maintained at +25 °C, with adequate ventilation and a photoperiod of 16 hours light and 8 hours darkness.

RESULTS

After seven months of storage, it was evident that the regeneration capacity of *Betula pendula* was strongly influenced by the composition of the capsules (Fig. 3). Shoots stored in capsules containing sodium alginate and MS medium showed regeneration rates above 80% across all tested variants. In contrast, capsules composed of sodium alginate dissolved in water showed no viable shoot regeneration.
The length of regenerated shoots (Fig. 4) after six months of storage did not differ significantly among variants where MS medium was included in the capsule composition. The shortest shoots were observed in plants regenerated from capsules containing only water and sodium alginate, with a regeneration rate of just 2.2%. The average shoot length in this group was 1.55 ± 0.64 cm. In comparison, the longest shoots (2.84 ± 1.05 cm) were obtained from capsules containing MS medium, where the explants had been precultured on a medium without phytohormones .
After seven months of storage, significant differences were observed between plant groups precultured on media supplemented with either 0.1 mg L^{-1} zeatin or 5 mg L^{-1} indole-3-acetic acid (IAA). Shoots from explants precultured with zeatin developed significantly longer average shoot lengths.
Comparisons of storage conditions and alginate concentrations showed that 2% alginate with full-strength MS salts yielded the highest shoot lengths and regeneration rates. The absence of calcium chloride in capsules significantly reduced regeneration by nearly 30%. Shoots stored in darkness at +4 °C exhibited the best performance, highlighting the importance of both biochemical and physical storage conditions for synthetic seed technology in *Betula pendula*.

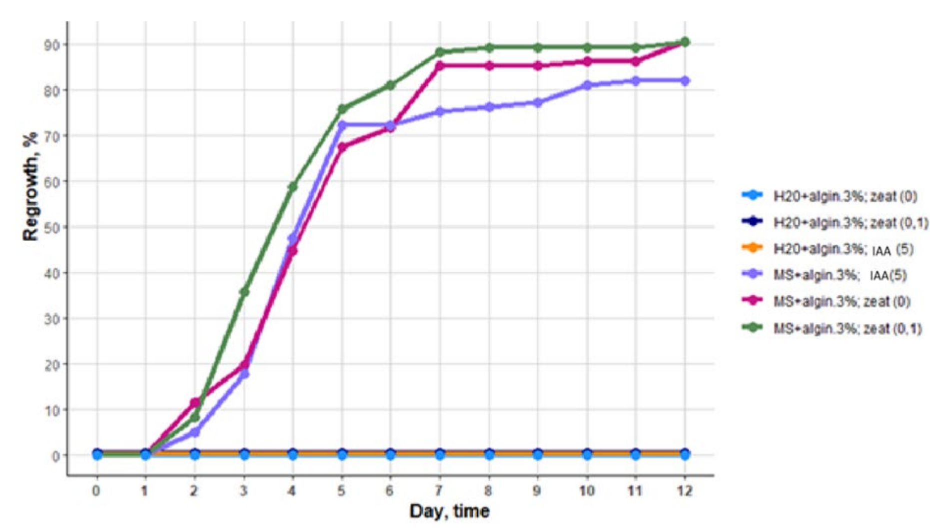

Fig. 3. *Regrowth of encapsulated* Betula pendula *shoot tips after seven months of storage at +4 °C. Capsules were formed using sodium alginate with either hormone-free MS medium or distilled water*

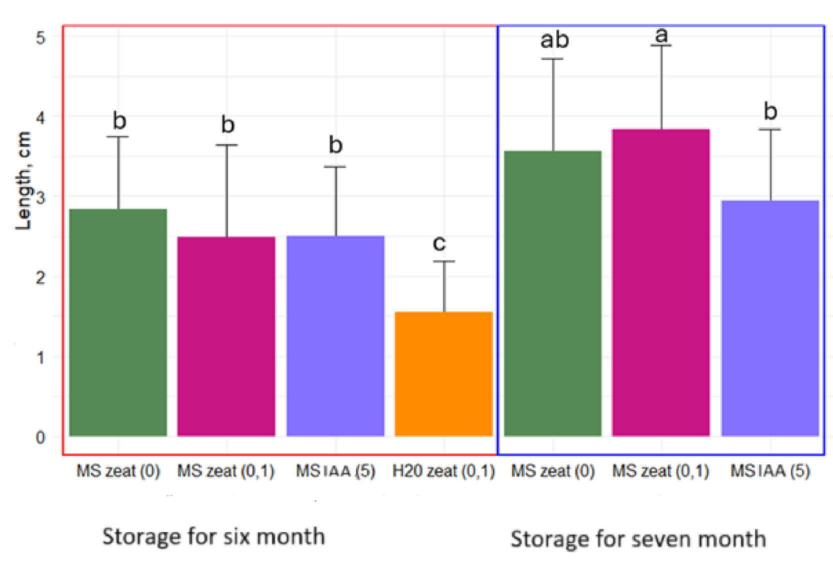

Fig. 4. *Average shoot length of* Betula pendula *after six and seven months of storage at +4 °C. Capsules were prepared using sodium alginate combined with either hormone-free MS medium or distilled water. All shoots were regenerated on MS medium supplemented with 0.2 mg L^{-1} zeatin.*

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