

EFFECT OF COLD TREATMENT ON CONVERSION OF BLACK LOCUST SOMATIC EMBRYOS

I. Arrillaga¹, J.J. Tobolski², and S.A. Merkle³

¹ Dpto. Biología Vegetal, Facultad de Farmacia, University of Valencia, Avda. Vicent Andres Estelles s/n, Burjasot, Valencia, SPAIN, E-46100, ² Biology Department, Indiana University-Purdue University at Fort Wayne, Fort Wayne, IN, USA 46805 and ³ D.B. Warnell School of Forest Resources, University of Georgia, Athens, GA, USA 30602

The objective of this study was to determine the effect of cold treatment on the conversion of naked or encapsulated black locust (*Robinia pseudoacacia* L.) somatic embryos. The experiment was carried out using one embryogenic line established from an immature seed (Merkle and Wiecko 1989, Merkle 1992) and maintained in F medium (10A4ON; Finer and Nagasawa 1988) supplemented with 3 mg/l 2,4-D. Globular stage somatic embryos were obtained by transferring approximately 0.5 g of proembryogenic masses (PEMs) to hormone-free liquid F medium. Cotyledonary stage embryos were obtained when the globular stage embryos were plated on the same medium solidified with agar. For encapsulation, cotyledonary embryos were placed in a beaker of 2% sodium alginate solution, either dissolved in F medium or distilled water. Then, drops of alginate solution containing individual embryos were pipetted into a beaker of 50 mM calcium chloride solution forming a calcium alginate bead around each embryo. Naked and encapsulated embryos were stored on moist filter paper at 4°C for 0, 15, 45 or 100 days, after which they were tested for germination on half-strength MS medium (Murashige and Skoog 1962). With no cold treatment, 71% of the naked embryos versus 41% of the encapsulated (either in water or F medium) developed into plants. Fifteen days of cold treatment increased germination rates up to 95% for naked embryos, 80% for embryos encapsulated with 2% sodium alginate in water and 75% for embryos encapsulated with 2% sodium alginate in F medium, respectively. Cold treatments longer than 15 days resulted in low germination percentages. Recovered plants were acclimatized and grown in the greenhouse.

References

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