Improving Corsican pine somatic embryo maturation: comparison of somatic and zygotic embryo morphology and germination

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Abstract:

Clonal propagation of high-value forest trees through somatic embryogenesis (SE) has the potential to rapidly capture the benefits of breeding or genetic engineering programs and to improve raw material uniformity and quality. A major barrier to the commercialization of this technology is the low quality of the resulting embryos. Several factors limit commercialization of SE for Corsican pine, including low initiation rates, low culture survival, culture decline causing low or no embryo production, and inability of somatic embryos to fully mature, resulting in low germination and reduced vigour of somatic seedlings. The objective was to develop a Corsican pine maturation medium that would produce cotyledonary embryos capable of germination. Treatments were arranged in a completely randomized design. Data were analyzed by analysis of variance, and significant differences between treatments determined by multiple range test at P=0.05.

Corsican pine (*Pinus nigra* var. *maritima*) cultures were initiated on modified ½P6 medium. Modifications of the same media were used for culture multiplication and maintenance. Embryogenic cultures were maintained on the same medium semi solidified with 2.5 g/l Gelrite. A maturation medium, capable of promoting the

development of Corsican pine somatic embryos that can germinate, is a combination of ½P6 modified salts, 2% maltose, 13% polyethylene glycol (PEG), 5 mg/l abscisic acid (ABA), and 2.5 g/l Gelrite. After initiation and once enough tissue developed they were grown in liquid medium. Embryogenic cell suspensions were established by adding 0.951.05 g of 10- to 14-day-old semisolid-grown embryogenic tissue to 9 ml of liquid maintenance media in a 250ml Erlenmeyer flask. Cultures were then incubated in the dark at 2022°C and rotated at 120 rpm. After 2.53 months on maturation medium, somatic embryos were selected that exhibited normal embryo shape. Ten embryos were placed horizontally on 20 ml of either germination medium (½strength Murashige and Skoog (1962) salts with 2.5 g/l activated charcoal) or same medium with copper sulphate adjusted to 0.25 mg/l to compensate for copper adsorption by activated carbon. 2% and 4% maltose was substituted by 7.5% and 13% PEG respectively to improve the yield of the embryos. Substitution of maltose with PEG was clearly beneficial to embryo development. When 2% of the maltose was replaced with 7.5% PEG, many embryos developed to large bullet-shaped embryos. At latter stages of development most embryos callused and stopped development. A few short, barrel-shaped cotyledonary embryos formed that were covered by callus on the sides and base. When 4% of the maltose was removed and substituted with 13% PEG, the embryos developed further, emerging from the callus and increasing yield slightly. Microscopic examination of the cultures showed differing morphologies, varying from mostly single cells or clumps to well-formed somatic embryos that resembled early zygotic embryos only liquid cultures with organized early-stag

A procedure for converting and acclimating germinants to growth in soil and greenhouse conditions is also tested. Seedling conversion and growth were highly related to the quality of the germinant at the time of planting. Germinants with larger shoots, longer, straighter hypocotyls and longer roots performed best. When mature zygotic embryos germinate the root emerges, before or coincident with the shoot. In contrast, somatic embryos germinate in reverse sequence, with the cotyledons greening first, then shoot emergence and then, much later, if at all, the appearance of the root. Somatic seedlings, produced from the maturation medium, showed 100% survival when planted in a field setting. Somatic seedlings showed normal yearly growth relative to standard seedlings from natural seed.