

Section 3 Abstracts: Chestnut Tree Breeding, Propagation and Physiology

Regeneration of Chestnut Via Somatic Embryogenesis.

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During successive years, different culture media, organic adendas, hormonal treatments and culture conditions were assayed to induce somatic embryogenesis in explants of both juvenile (mature and immature zygotic embryos) and mature tissue (anthers and filaments) from two specimens of different *Castanea sativa* × *C. crenata* clones. Up to now, somatic embryogenesis has been achieved only on explants obtained from immature zygotic embryos, cultured on solid MS medium supplemented with (0.1 mg/1) 2,4-D plus (1 mg/1) Z, or (0.5-1.0 mg/1) 2,4-D with or without (1-2 mg/1) Z, and subsequently transferred to 1/2 MS supplemented with low levels (0.2 mg/1) of auxine and cytokinin. When subcultured on 1/2 MS medium supplemented with 3mM glutamine, (0.2 mg/1) Z and (0.05 mg/1) IBA (maintenance medium), embryogenic callus proliferated forming clusters of shiny, yellowish globular structures, from which somatic embryos developed. After 4 wk of culture, embryos at all stages of development, as well as abnormal structures were observed in same culture. On average, 400 embryos beyond the globular stage were formed per gram (f.w.) of callus. The embryogenic competence of cultures or their ability to produce mature somatic embryos has remained undiminished after 40 mo of repeated subculture on this medium. Although, plantlet regeneration occasionally was achieved, reculturing isolated cotyledonary embryos on maintenance medium resulted in the appearance of secondary embryos or in the development of soft, friable callus with mucilaginous consistency. These calli were used to initiate embryogenic cell suspension cultures, from which somatic embryos developed. Culturing isolated cotyledonary embryos on a medium with no growth regulators generally resulted in the growth of a radicle but not a shoot. Different experiments were carried out to enhance the conversion of somatic embryo into plantlets. Best results were achieved when somatic

embryos were stored at 4 C for up to 9 mo and subsequently cultured on solid MS medium supplemented with 150 mM Fe-Na-EDTA and (0.1-0.2 mg/1) Z. Regenerated plants were successfully acclimated and transferred to soil.