

Plant Regeneration from Suspension Culture Protoplasts Established from Hypocotyl-Derived Callus of Two *Coffea arabica* Genotypes

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SUMMARY

Regeneration of plants of *Coffea arabica* from protoplast-derived calli was achieved through the induction of somatic embryogenesis. Protoplasts were enzymatically isolated from 5-day-old cell suspension cultures, which were established from hypocotyl-derived callus. A high yield of protoplasts was released from the suspended cells (1.15×10^6 protoplasts ml⁻¹ packed cell volume) when treated with an enzyme mixture containing 1.5% Cellulase Onozuka R10, 1.0% Macerozyme R10 and 0.5% Driselase. Culture of protoplasts in an agarose-solidified medium resulted in sustained proliferation of microcalli, which were transferred to callus-growing medium and subsequently to embryo-induction medium. Embryoids, which developed, were grown on embryo-germination medium into plantlets. Plants with normal morphological features have been successfully transferred to the greenhouse. The establishment of this protoplast system should facilitate novel breeding approaches in *C. arabica*.