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Explant Type, Culture System, 6-Benzyladenine, Meta-Topolin and Encapsulation Affect Indirect Somatic Embryogenesis and Regeneration in *Carica papaya* L.

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A protocol to propagate papaya hybrid plants through indirect somatic embryogenesis was developed considering the effect of explant type, culture system, particular cytokinins and encapsulation, in different stages of the process. Optimal 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations for non-embryogenic callus formation ranged between 9.0 and 27.1 μM in half-cut seeds, while higher concentrations were harmful. Non-embryogenic callus was also obtained with 22–158 μM 2,4-D from hypocotyl segments. Callus with embryogenic structures was only obtained in half-cut seeds cultured in the darkness on half-strength Murashige and Skoog culture medium supplemented with 2,4-D, while hypocotyl segments and isolated zygotic embryos failed to produce this type of callus regardless of the 2,4-D and sucrose (30 and 70 g l^{-1}) concentrations tested in this study. Both, embryogenic callus development and quantity of somatic embryos formed per embryogenic callus, which ranged between 11 and 31 units after 14 months, required 2,4-D, but without any effect of the concentration. Histological studies confirmed the multicellular origin of the somatic embryos. In further steps, liquid medium induced over four times more somatic embryos than agar-gelled medium and showed significantly higher production of globular somatic embryos (85 vs. 57%). Both, 6-benzyladenine (BA) and meta-topolin (Mtop) stimulated sprouting (40–45%) of the somatic embryos (development of shoots only) in concentrations of up to 2.7 and 10 μM, respectively. Sprouting probability showed a 2nd order polynomial trend despite the range of concentration used for each cytokinin. This is the first report about the positive effect of Mtop on the apical shoot development of *Carica papaya* somatic embryos known to the authors. Radicle growth was observed in 5% or less of the cultivated embryos, regardless of the BA concentration. Finally, all encapsulation conditions tested (2.5, 3.5, and 4.5% sodium alginate, combined with 50 and 100 mM $CaCl_2$) reduced sprouting of somatic embryos