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Agrobacterium tumefaciens-mediated genetic transformation and regeneration of transgenic plants from leaf explants of *Jatropha curcas*: A candidate biodiesel plant

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Jatropha curcas is an oil bearing species with multiple uses and considerable economic potential as a biofuel crop. A simple and reproducible protocol was developed for *Agrobacterium tumefaciens*-mediated stable genetic transformation of *J. curcas using* leaf explants. *Agrobacterium* strain LBA 4404harbouringthe binary vector pCAMBIA 1304 having sense-dehydration responsive element binding (S-DREB2A), β-glucuronidase (gus), and hygromycin-phosphotransferase (hpt) genes were used for gene transfer. A number of parameters such as preculture of explants, wounding of leaf explants, *Agrobacterium* growth phase (OD), infection duration, co-cultivation period, co-cultivation medium pH, and acetosyringone, were studied to optimized transformation efficiency. The highest transformation efficiency was achieved using 4-day precultured, non-wounded leaf explants infected with *Agrobacterium* culture corresponding to OD_{600}=0.6 for 20 min, followed by co-cultivation for 4 days in a co-cultivation medium containing 100 µM acetosyringone, pH 5.7. Co-cultivated leaf explants were initially cultured on Murashige and Skoog (MS) medium supplemented with 2.27 µM thidiazuron (TDZ) for regeneration of shoot buds, followed by selection on same medium with 5 µg ml^{-1} hygromycin. Selected shoot buds were transferred to MS medium containing 10 µM kinetin (Kn), 4.5 µM6-benzyl aminopurine (BA), and 5.5 µMα-naphthaleneacetic acid (NAA) for proliferation. The proliferated shoots were elongated on MS medium supplemented with 2.25 µM BA and 8.5 µM indole-3-acetic acid (IAA). The elongated shoots were rooted on half strength MS medium supplemented with 15 µMindole-3-butyric acid (IBA), 5.7 µM IAA, 5.5 µM NAA, and 0.25 mg l^{-1} activated charcoal. GUS histochemical analysis of the transgenic tissues further confirmed the transformation event. PCR and DNA gel blot hybridization were performed to confirm the presence of transgene. A transformation efficiency of 29% was achieved for leaf explants using this protocol.

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