

# Efficient Micropropagation Protocol in *Jatropha curcas* L., an Important Biofuel Plant and Establishment of ISSR Based Genetic Fidelity among the Regenerants

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## ABSTRACT

*In vitro* techniques have been perfected for direct multiple shoot induction through cotyledonary node explants for the first time in *Jatropha curcas* L., an important biodiesel plant. Seedling derived cotyledonary nodes when reared on MS medium augmented with 2 mg/l benzyladenine, 20 mg/l glutathione, 20 mg/l adenine sulphate and 10 mg/l silver nitrate induced an average of 6.02 axillary buds in 76% explants. Addition of 0.8 mg/l putrescine to the above medium enhanced the number of shoot buds up to 15. Glutamine at 500 mg/l supplemented to the shoot induction medium proved beneficial for elongation of shoot buds, with several shoots reaching mean length of 3.55 cm. Protein profiling of the aforesaid explants revealed three bands of size 22.83, 28.28 and 31.71 kDa that were poorly visible in control and putrescine lacking cultures and got over expressed in the putrescine treated explants relating their possible role in multiple shoot induction. Rhizogenesis of excised shoots was achieved on half strength MS medium containing 0.25 mg/l indole-3-butyric acid. The micropropagated plants were hardened and transferred to field wherein 90% plants survived and were phenotypically similar to mother plants. Inter-simple sequence repeat (ISSR) marker was employed to evaluate the genetic stability amongst the regenerants with the elite mother plant of *J. curcas*. A total of 286 ISSR fragments ranging from 100 to 1200 bp were generated with 7 ISSR primers in the aforesaid plants. All banding profiles from micropropagated plants were monomorphic and similar to those of mother plant. Dendrogram generated through UPGMA revealed 95% similarity amongst the regenerants confirming the genetic fidelity of the regenerants.

**Key words:** Cotyledonary node, genetic fidelity, ISSR, *Jatropha curcas*, micropropagation, putrescine

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