EFFECT OF PICLORAM ON INDUCTION OF FRIABLE EMBRYOGENIC CALLUS IN RUBBER AND CHARACTERIZATION OF EMBRYOGENIC POTENTIAL BY CYTOCHEMICAL ANALYSIS

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Received: 29 July 2024 Accepted: 10 September 2024

Jayasree, K.P., Raheena, K.T. and Raj, R. (2024). Effect of Picloram on induction of friable embryogenic callus in rubber and characterization of embryogenic potential by cytochemical analysis. *Rubber Science*, **37**(2): 250-260.

In many plants, both monocots and dicots, picloram has been successfully used for induction of somatic embryogenesis. However, the effect of picloram on the somatic embryogenesis of rubber has not been studied so far. In the present study, immature anther explants were cultured on a modified MS medium supplemented with varying concentrations of picloram (1.0, 2.0 and 3.0 mg L^{-1}) in combination with BA (1.0 mg L^{-1}). Embryogenic calli were subsequently sub-cultured in embryo differentiation medium which composed of modified MS with BA and picloram (0.5 mg L^{-1} each). Friable embryogenic callus could be effectively induced on medium containing 1.0 and 2.0 mg L^{-1} picloram. This combination gave the highest percentage of callus induction (100%), proliferation and callus fresh weight (1.07 and 0.76 g) without significant difference. Moreover, the differentiation of globular and heart-staged somatic embryos was also observed. A double staining test with acetocarmine and Evan's blue showed positive reaction to both dyes, indicating the existence of both embryogenic and non-embryogenic cells in the friable primary callus. After transfer to the embryo induction phase, a larger percentage of produced calli was heavily stained with acetocarmine, which were identified as embryogenic calli. However, in the friable translucent primary callus, cell mass reacting more strongly to Evan's blue detected the presence of more non-embryogenic cells. Additionally, PCR amplification using genomic DNA revealed presence of *SERK* gene in both embryogenic and non-embryogenic calli.

Keywords: Embryogenic callus, *Hevea brasiliensis*, Non-embryogenic callus, Picloram, Somatic embryogenesis

Hevea brasiliensis (Muell. Arg.), commonly called the Para rubber tree, is the major commercial natural rubber (NR) producing tree, and it accounts for more than 90 per cent of NR production worldwide. Genetic improvement through conventional breeding remains a challenge in rubber due to its long breeding cycle and highly heterozygous nature. Biotechnology through genetic engineering holds great potential for the genetic improvement of this crop and *in vitro* regeneration *via* somatic embryogenesis

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