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SOMATIC EMBRYOGENESIS FROM THE *IN VITRO* ROOT EXPLANTS OF TRANSGENIC *HEVEA BRASILIENSIS* AND VALIDATION OF STABLE GENE INTEGRATION

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Plant genetic transformation offers a potential tool for crop improvement in *Hevea brasiliensis* by the integration of agronomically useful genes with stable expression. The objectives of the present study were to develop a method for rapid regeneration of transgenic plants *via* somatic embryogenesis using root explants and validation of stable foreign gene integration. Actively growing root explants were collected from germinating *in vitro*-derived MnSOD transgenic somatic embryos (RRII 105), sliced into thin segments and placed on callus induction medium. Callus induction and proliferation were observed within four weeks of explant inoculation. Half-strength Murashige and Skoog (MS) basal medium containing B5 vitamins and growth regulators (1.4 µM each of 2,4-D and Kin, 1.0 µM each of NAA, BA and GA_3) showed the highest embryo induction frequency of 42 per cent. Maximum conversion of pro-embryos into heart, torpedo and cotyledonary stage was achieved by incorporating 40 g/L sucrose and 4 g/L phytagel in the embryo induction medium. The highest percentage of fully developed plantlets was obtained in half-strength modified MS basal medium containing 1.3 µM BA, 1.5 µM GA_3 and 2.5 µM IBA. Molecular confirmation of the presence of the transgene was performed by polymerase chain reaction with *npt*II and MnSOD gene-specific primers and Southern hybridization with *npt*II gene-specific probe. Regeneration of transgenic plants could be achieved within eight months using this protocol.

Keywords: Genetic transformation, *Hevea brasiliensis*, MnSOD, Somatic embryogenesis, Stable gene integration, Transgenic plant.

INTRODUCTION

Hevea brasiliensis (Para rubber), belonging to the family Euphorbiaceae, is the major source of commercial natural rubber, and accounts for 99 per cent of the world natural rubber (NR) production and 49 per cent of the elastomer sale market. Increasing demand for NR as well as pressure on availability of land and socio-economic constraints necessitate the expansion of rubber cultivation to the non-traditional/ marginal areas, which are exposed to severe environmental stresses such as extreme cold, elevated temperature and high light intensities, resulting in poor performance of the plants. Susceptibility of *H. brasiliensis* to

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