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ISOLATION OF PROTOPLASTS FROM LEAF MESOPHYLL CELLS OF *HEVEA BRASILIENSIS*

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A method has been standardised for the isolation of large number of protoplasts from young leaves of *Hevea brasiliensis*, which could be another source for culturing protoplasts for somatic hybridisation. It was observed that selection of suitable enzymes, their combinations and concentrations, pH of the incubation medium, duration and temperature of incubation are very important in the protoplast isolation procedure. Standardisation of these parameters in the preparation of leaf tissues for enzyme digestion, purification and protoplast viability is discussed. In this study, 95 per cent healthy and viable protoplasts were obtained from young leaves of one-year-old plants digested with 1.0 per cent Macerozyme R10 and 1.5 per cent Cellulase Onozuka R10 along with 0.7 M mannitol as osmoticum at pH 5.5 at 35 °C. Isolated protoplasts were finally purified using sucrose gradient (0.7 M and 0.5 M sucrose solutions) method. Purified protoplasts were found as light green layer between the 0.7 M and 0.5 M sucrose layers. Viability of protoplasts was ascertained using Fluorescein-di-acetate (FDA) staining. The study also indicated the potential of utilising leaf mesophyll cells of young *H. brasiliensis* plants for protoplast isolation and its use for somatic hybridisation studies in crop improvement programmes.

Keywords: Enzymatic digestion, *Hevea brasiliensis*, Mesophyll cells, Protoplasts isolation, Viability.

INTRODUCTION

Protoplasts isolated from higher plants provide a fairly uniform population of genetically similar single units. Plant protoplasts open up a new avenue to utilise them in many molecular biology techniques including the genetic modification of plants. The successful isolation of protoplasts from plant tissues is a prerequisite for their use in physiological, biochemical and virological studies. In addition, protoplast technology can offer a better tool for achieving crop improvement, if plant regeneration from protoplasts can be achieved. *In vitro* fusion of plant protoplast with subsequent regeneration of hybrid plants has been suggested as a technique for introducing greater genetic diversity in plants for breeding purposes. In recent years, a number of methods have been described for accomplishing protoplast fusion (Power *et al.*, 1970; Compton *et al.*, 1999). Somatic hybridisation bypasses biological barriers and creates new evolutionary opportunities that would be difficult to accomplish through natural or conventional breeding techniques.

Isolation of plant protoplasts using enzymatic degradation of cell walls, developed by Cocking (1960) opens up a new

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