OSMOTIC STRESS INDUCED CALLUS PROLIFERATION AND EMBRYOGENIC CALLUS INITIATION DURING SOMATIC EMBRYOGENESIS FROM LEAF EXPLANTS OF *HEVEA BRASILIENSIS*

R.G. Kala, R. Jayashree and A. Thulaseedharan

Rubber Research Institute of India, Kottayam-686 009, Kerala, India

Received: 18 October 2023 Accepted: 20 November 2023

Kala, R.G., Jayasree, R. and Thulaseedharan, A. (2023). Osmotic stress induced callus proliferation and embryogenic callus initiation during somatic embryogenesis from leaf explants of *Hevea brasiliensis. Rubber Science,* **36**(2): 209-216.

Somatic embryogenesis and plant regeneration was achieved using leaf explants of *Hevea brasiliensis* clone RRII 105. Modified MS medium containing calcium nitrate [$Ca(NO_3)_2.4H_2O$] and phytohormones was used for callus induction. The concentration of calcium nitrate was optimized for enhanced callus induction. Callus proliferation was carried out by sequential subculture (2-3 times) at an interval of 50 days in solidified proliferation medium. The combined effect of different concentrations of $Ca(NO_3)_2.4H_2O$ and sucrose in enhancing callus proliferation and texture improvement was studied. After proliferation, the callus was sub-cultured in embryo induction medium containing phytohormones and devoid of charcoal. Polyethylene glycol (MW 6000) was added to the above medium for providing osmotic stress. Compact friable calli, which developed in the medium containing PEG (5.0 g L^{-1}) solidified with phytagel (3.5 g L^{-1}), were transferred to the medium fortified with activated charcoal (2.0 g L^{-1}) for embryogenic callus initiation. Osmotic stress was induced through addition of different concentrations of sucrose (30-80 g L^{-1}) and PEG (5-10 g L^{-1}). Induction of osmotic stress by addition of sucrose at 60 g L^{-1} and PEG at 8.0 g L^{-1} in the embryo induction medium containing phytohormones led to embryogenic calli initiation. Concentration of the solidifying agent phytagel (0.5%) for enhanced induction of embyogenic callus and somatic embryogenesis was also optimized. The present study reports embryogenic callus initiation induced by osmotic stress provided in the medium aiding somatic embryogenesis from leaf explants of *Hevea brasiliensis.*

Key words: Embryogenic calli, *Hevea brasiliensis*, Somatic embryogenesis.

INTRODUCTION

Somatic embryogenesis is a multistep regeneration process starting with formation of dedifferentiated tissue called callus which is then redifferentiated to form pro-embryogenic masses followed by formation of somatic embryos, maturation and plant regeneration. This can be defined as a process in which a bipolar structure resembling a zygotic embryo develops from a non-zygotic cell without vascular connection with the original tissue (Arnold *et al.*, 2002). Since *Hevea* is cross pollinated the seeds are highly heterozygous. Hence development of amenable plant regeneration systems through somatic embryogenesis from elite

Correspondence: Kala R.G. (Email: rgkala@rubberboard.org.in)