LONG TERM MAINTENANCE OF EMBRYOGENIC CALLUS CULTURES AND INDUCTION OF SOMATIC EMBRYOS IN *HEVEA BRASILIENSIS*

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A protocol was developed for long term maintenance of embryogenic callus aggregates induced from immature anther of *Hevea brasiliensis,* clone RRII 105. In *H. brasiliensis*, establishment of embryogenic callus is a long term process coupled with low frequency. Maintenance of embryogenic callus cultures for prolonged duration in regenerable state is one of the important requirements for adopting the technique in genetic improvement of rubber. In this study, factors influencing long term somatic embryogenesis including subculture interval and effects of proline and activated charcoal were examined. Results showed that somatic embryo induction from embryogenic calli subcultured at an interval of 50 days was better than that at intervals of 25 and 75 days indicating that 50 day subculture cycle was more suitable for long term establishment. Addition of proline and activated charcoal at 100 mg L^{-1} and 0.1 per cent respectively improved embryo induction. Genetic stability analysis did not reveal any variation in the RAPD banding pattern in short and long term cultures (eight and 36-months-old). Embryogenic callus aggregates maintained in the above medium retained their embryogenic potential even after 36 months.

Keywords: Embryogenic callus aggregates**,** *Hevea brasiliensis,* Long term somatic embryogenesis, Primary somatic embryos, Secondary somatic embryos

INTRODUCTION

Hevea brasiliensis is a tree crop of great economic importance since it is a commercial source of natural rubber. Compared to bud-grafted rubber plants, somatic embryo derived self-rooted plants showed better field performance in terms of greater girth, higher laticifer numbers and rubber yield (Yang and Mo, 1994; Yuan *et al*., 1998; Chen *et al*., 2002). Thus, somatic plants are promising planting materials for natural rubber production (Chen *et al*., 2002). In *Hevea*, somatic embryogenesis has been developed *via* two procedures *viz*. primary somatic embryogenesis, a short term process and maintained somatic embryogenesis, a long term process (Lardet *et al*., 2009). Despite the report with leaf explants (Kala *et al*., 2005), somatic embryos have been mostly induced from floral explants or maternal tissues (Jayasree, 2017). However, the availability of floral explants or maternal tissues is limited by season, climate and diseases (Hua *et al*., 2010). Moreover, the frequent browning of calli from these explants lead to tissue degeneration and loss

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