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IN VITRO CULTURE OF IMMATURE EMBRYOS OF *HEVEA BRASILIENSIS*

In vitro culture of plant embryos is a technique to obtain viable offspring following inter specific and inter generic hybridization where routine fertilization fails to produce a full term embryo. The technique has generally been adopted for rescue of the immature hybrid embryos from incompatible crosses and for overcoming short viability of seeds. Although successful fertilization and early embryo development occur in most cases, a number of irregular events take place subsequently, resulting in the eventual death of the embryo and collapse of the seeds (Raghavan and Torry, 1963). A major cause of early embryo abortion is the failure of the endosperm to develop properly. Aseptic culturing of the embryos in a nutrient medium can often overcome this problem. However, the success of the technique depends upon many factors like isolation of embryos from ovules or immature fruits without injury, use of a suitable nutrient medium and induction of continued embryogenic growth and seedling formation.

A very low fruit set (0-3.5 %) is observed in the natural rubber tree, *Hevea brasiliensis* (Warmke, 1951, 1952; Gandhimathi and Yeang, 1984), which could be due to environmental, physiological or genetic reasons. However, normal plants can be raised from mature embryos of *Hevea* using embryo culture technique (Das *et al.*, 1998) thus permitting the incorporation of useful characters into cultivated rubber (Chen, 1984). In spite of several attempts on *in vitro* techniques for multiplication and improvement of *Hevea*, information on embryo culture techniques for rubber is scanty. The present investigation attempts development of a suitable nutrient medium for *in vitro* culture of immature zygotic embryos of *H. brasiliensis*.

Immature fruits (3-16 week old) were collected from the experimental farm of Rubber Research Institute of India at Taranagar, Agartala in Tripura State. After washing thoroughly with detergent, the fruits were surface-sterilized in 0.05 per cent mercuric chloride solution with a drop of Tween 20 for 15 to 20 min and washed well with distilled water. Subsequently, intact seeds were isolated by dissecting the fruits. Two techniques were adopted for excision of embryos avoiding injury. Embryos from 3 to 8 week old fruits were excised by making an incision at the micropylar end and separating out the tiny embryos by applying force at the opposite end while those from 9 to 16 week old fruits were excised by using the blunt end of the scalpel after dissecting the seed longitudinally.

Widely used basal media like White's 'W' (White, 1963), Gamborg's 'B5' (Gamborg *et al.*, 1968) and Murashige and Skoog's 'MS' (Murashige and Skoog, 1972) were tried for culturing embryos and based on the performance, MS medium was selected as the basal medium for embryo germination.

Further growth of the embryos was induced by modifying the MS medium, by incorporating half the concentration of macro and double the concentration of micronutrients and adding 0.05 to 0.2 g/L of Na_2PO_4 and 2-8 per cent sucrose. Coconut water (CW) was supplemented at different doses (5-20%) as the natural plant extract. Casein hydrolysate (CH), the amino acid complex, was tested at different concentrations (50 – 500 mg/L) to observe the growth response of the embryos.

For regeneration, different concentrations (0.1-5 mg/L) of various auxins such as IAA, IBA, NAA and cytokinins like kinetin, BAP and 2iP were tested. GA_3 was added in the media for healthy growth of the leaves. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C under 1.26 kg/