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OPTIMISATION OF MEDIA COMPONENTS FOR SOMATIC EMBRYOGENESIS FROM ANTHERS OF *HEVEA BRASILIENSIS*

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Somatic embryogenesis was initiated from immature, anther-derived callus of *Hevea*. Embryogenic callus with prominent proembryos were obtained using modified MS medium along with BAP (1 mg/L), NAA and 2,4-D (1 mg/L) each. Somatic embryos were obtained from these calli when cultured on media containing kinetin (1 mg/L) and IAA (0.1 mg/L). Histological studies of embryogenic callis revealed that the meristematic cells at the periphery of the calli produce somatic embryos. Clonal variation was also observed in callus induction and somatic embryogenesis.

Key words: Anther, Clonal variation, Multiple shoots, Phytohormones, Somatic embryogenesis.

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INTRODUCTION

Tripura is the smallest state of North-East India and the second largest State in area under rubber (*Hevea brasiliensis*) cultivation in the country. The climate of Tripura a non-traditional rubber growing region, is widely different from that prevailing in the traditional region, in terms of low winter temperature, which falls below 10°C (Jacob *et al.*, 1999) with partial soil moisture deficiency (Saseendran *et al.*, 1993) and high summer temperature. In the non-traditional areas of India rubber trees flower during late February to April (Meenattoor *et al.*, 1989; Sudhasowmyalatha *et al.*, 1997) while in the traditional belt, flowering starts in January. Microsporogenesis and anther formation are likely to be affected by variations in climatic pattern. Plant regeneration through somatic embryogenesis and development of synthetic seeds may become advantageous in overcoming such problems in propagation. This method will also facilitate shortening the breeding cycle. Genetic transformation during somatic embryogenesis can also be attempted. Hence induction of haploids from immature anthers of *H. brasiliensis* was attempted at the Regional Research Station of Rubber Research Institute of India at Agartala in Tripura. The optimisation of media components was taken up as this is the most important pre-requisite for successful somatic embryogenesis.

MATERIALS AND METHODS

Young flower buds of RRII 105 and SCATC 93/114 were collected. Their developmental stages were examined under a microscope using aceto-carmine squash method and flower buds of optimum size studied. After thorough washing with Teepol, the buds were surface sterilized with 0.02 per cent mercuric chloride and placed in few drops of Tween 20 for 5 to 7 min, followed by thorough washing with sterilized distilled water. Anthers were dissected out aseptically and cultured on induction medium (20 ml/tube). Half strength of the MS (Murashige and Skoog, 1962) inorganic salts was added to the medium with the normal quantum of organic nutrients. Casein hydrolysate (CH, 100 mg/L), adenine sulfate (AdS, 2 mg/L) and 10 per cent coconut water (CW) were added as organic supplements along with sucrose (3%) and phytagel (2%) for inducing callus proliferation. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 15 min. For callus in-