Rubber Science, **28**(1): 31-39, 2015 *RESEARCH ARTICLE*

AGROBACTERIUM-MEDIATED TRANSFORMATION OF *HEVEA BRASILIENSIS* WITH APPLE cDNA ENCODING SORBITOL-6-PHOSPHATE DEHYDROGENASE

P. Kumari Jayasree, S. Divya, R. Supriya and A. Thulaseedharan

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

Received: 18 September 2014 Accepted: 25 February 2015

Jayasree, P.K., Divya, S., Supriya, R. and Thulaseedharan, A. (2015). *Agrobacterium*-mediated transformation of *Hevea brasiliensis* with apple cDNA encoding sorbitol-6-phosphate dehydrogenase. *Rubber Science*, **28**(1): 31-39.

The increasing demand of natural rubber and land unavailability in traditional regions has been resulted the cultivation of rubber in non-traditional regions. However, the severe agro-climatic conditions in non-traditional regions adversely affected growth and yield of rubber. In this context, scope for the introduction of genes conferring tolerance to abiotic stresses is of paramount importance and hence the study was undertaken. Embryogenic callus derived from immature anther of *Hevea brasiliensis*, clone RRII 105 was used as the target tissue for genetic transformation. Kanamycin sensitivity to embryogenic callus was examined by kill curve test and medium with 100 mgL^{-1} kanamycin completely suppressed the growth of untransformed callus and was selected as the suitable concentration for selection of transformants. Addition of ABA was found to be beneficial for embryogenesis and maximum embryo induction was obtained with 2.0 mgL^{-1}. Among various levels of sucrose (2-10%), 4 per cent sucrose enhanced embryo induction efficiency and gradually decreased by higher levels. Mature embryos get germinated and converted into plantlets. GUS histochemical assay revealed expression of *uid*A gene in transformed callus and embryos which was evidenced by the intense blue colour. Polymerase chain reaction confirmed the presence of S6PDH, *uid*A and *npt*II genes in transformed callus and embryos. The transgenes were also detected in plasmid DNA (positive control), but absent in untransformed callus (negative control).

Keywords: *Agrobacterium tumefaciens*, Genetic transformation, GUS histochemical assay, PCR analysis, Sorbitol-6-phosphate dehydrogenase gene

INTRODUCTION

Hevea brasiliensis (Muell. Arg.), commonly known as rubber tree, is a perennial tree crop belonging to *Euphorbiaceae* family. In India, rubber is traditionally grown in Kerala and Kanyakumari district of Tamil Nadu. However, scope for further expansion of rubber cultivation in these areas is very limited and attempts were thus made to cultivate rubber in the non-traditional regions which are exposed to a wide range of abiotic stresses. Considering the severe climatic constraints and crop loss in these areas, there is an urgent need for the introduction of genes conferring tolerance to abiotic stresses. In *Hevea*, genetic manipulation was first explored with the

Correspondence: P. Kumari Jayasree (Email: kumarijaya@rubberboard.org.in)