ABSTRACT

The booming demand for natural rubber along with the inconsistency related to the production and consumption necessitates the development of superior clones with high yield potential in Hevea. Crop improvement through traditional methods is a long run process due to the lengthy breeding cycle and heterozygous nature of the crop. Hence the release of a new clone through conventional method is time consuming. In Hevea, yield improvement through transgenic technology attempts the transfer of key regulatory genes involved in the rubber biosynthesis pathway. In vitro plant regeneration pathway through somatic embryogenesis is an essential prerequisite for achieving this task. The biosynthetic pathway genes were well characterized by many researchers. It has been documented that among the enzymes catalyzing rubber biosynthesis, the one responsible for the irreversible conversion of 3-Hydroxy 3-Methylglutaryl Coenzyme A (HMG CoA) to mevalonate was said to be rate limiting, since its activity was lower compared to others up to isopentenyl pyrophosphate (IPP). Clonal variations in the 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) activity were established and a significant correlation has been worked out between the enzyme activity and yield of Hevea. Based on these observations an attempt was made to regenerate transgenic plants that overexpress laticifer specific hmgr1 gene in Hevea brasiliensis through Agrobacterium mediated genetic transformation. Different strains and callus types were experimented to ascertain the suitable strain and the tissue type giving excellent transformation frequency in Hevea. Experimental results proved EHA 105 as the best strain giving highest frequency of transformation. Amidst the callus types experimented, the embryogenic calli derived from the zygotic embryos gave maximum cell lines at an efficiency of 67%. Embryogenic suspensions from the anther tissue proved to the best among the clonal explants used, producing cell lines at a frequency of 27%. Influence of compounds and culture conditions beneficial in improving the efficiency of transformation were also assessed among which the incubation temperature during
co-cultivation had a definite role on accelerating the frequency of transformation. Optimum culture condition for *Agrobacterium* mediated transformation was a three day co-culture at 20°C in presence of acetosyringone where an incremental variation in the efficiency of transformation was noticed (32%). The PCR positive cell lines were proliferated and cultured for somatic embryogenesis. Components influencing somatic embryogenesis and plant regeneration from the transgenic cell lines were analyzed. Transgenic embryos were produced from the cell lines at an efficiency of 72%, where the basal salts, organic nitrogen sources, organic supplements, growth regulators, polyamines and carbohydrates had a well defined role. Half strength MS medium with an increase in KNO₃ concentration (3.0 g l⁻¹), omitting NH₄NO₃, resulted in embryo maturation. The impact of stress inducing compounds on somatic embryo maturation was studied. Polyethylene glycol (PEG), abscisic acid (ABA) combinations mediated maturation of *hmgr1* transgenic embryos. Partial desiccation of the embryos improved the germination capacity and a growth regulator combination of GA₃, BA, IAA triggered plant regeneration. The regenerated plantlets were successfully hardened and transferred to net house conditions. Transgene integration was confirmed using PCR analysis and the pattern of integration of the transgene was assessed using Southern hybridization. Gene expression analysis was performed by ELISA technique and northern blotting. This is the first report on the development of transgenic plants in *Hevea* integrated with the laticifer specific *hmgr1* gene. Forty five plantlets were successfully hardened and maintained in the containment facility. Selected transgenic plants were multiplied by bud grafting to study the yield pattern of the plants. Once proved to be positive, *hmgr1* gene can be used to increase latex production of low yielding disease tolerant clones of *Hevea brasiliensis*.

*Keywords: Agrobacterium mediated genetic transformation, Enzyme-linked immunosorbant assay, 3-hydroxy-3-methylglutaryl CoA reductase, Southern hybridization, Northern blotting Polymerase Chain Reaction, Polyethylene glycol*