

FACTORS PROMOTING *AGROBACTERIUM* MEDIATED GENETIC TRANSFORMATION EFFICIENCY IN *HEVEA BRASILIENSIS*

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In *Hevea brasiliensis*, since conventional methods of crop improvement are elaborate and time consuming, genetic transformation offers a viable approach to breeders for adding desirable traits in a relatively short period. Although, genetic transformation experiments in *Hevea brasiliensis* were initiated two decades ago, reports on the development of transgenic plants incorporated with genes coding for agronomically important traits is limited mainly due to the low efficiency of transformation and plant regeneration protocol in *Hevea brasiliensis*. Therefore the transformation and plant regeneration protocol needs further modifications for developing an efficient and reproducible transgenic plant regeneration system. In the present work, an attempt was made to identify the factors promoting *Agrobacterium* mediated genetic transformation frequency. The texture and friability of the target tissue (immature anther callus) was improved by pre-culturing it on medium containing varying concentrations of calcium nitrate (0-1200 mg/L). The transformation efficiency was significantly enhanced by using this soft friable callus as target tissue. The combined effect of callus pre-culture and acetosyringone concentration on transformation efficiency was also assessed by incorporating different concentrations of acetosyringone (0-200 mg/L) in the co-culture medium. The transformation frequency was increased from 6 to 14% by using the callus pre-cultured for fifteen days on medium containing 800 mg/L calcium nitrate and increasing the acetosyringone concentration to 80 mg/L in the co-culture medium. The transformation event was confirmed by PCR analysis with MnSOD and *nptII* gene specific primers.

Keywords: Acetosyringone, Callus pre-culture, Genetic transformation, Transformation frequency

INTRODUCTION

Increasing demand for natural rubber and the scarcity of land availability for rubber cultivation in the traditional rubber growing regions in India necessitate the expansion of rubber cultivation to the non-traditional areas, which are exposed to extreme climate such as cold, high

temperature and high light intensities *etc.* Even though conventional breeding has achieved significant progress in improving the yield and secondary characters, genetic transformation has immense potential for crop improvement by transferring desirable genes within a short period.