

# A NOVEL ANTIOXIDANT $\alpha$ -LIPOIC ACID SIGNIFICANTLY ENHANCING AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION OF *HEVEA BRASILIENSIS* WITH ScHSP31 GENE

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Received: 05 April 2022

Accepted: 10 June 2022

Kala, R.G., Jayasree, R., Melvin, P. and Shivaprasad, P.V. (2022). A novel antioxidant  $\alpha$ -lipoic acid significantly enhancing *Agrobacterium* - mediated genetic transformation of *Hevea brasiliensis* with ScHsp31 gene. *Rubber Science*, 35(1): 1-11.

*Agrobacterium* mediated genetic transformation is an effective tool for crop improvement in *Hevea* since stable integration of agronomically important genes can be brought about in substantially lesser time compared to the conventional breeding. Transgenic *Hevea* plants have been regenerated successfully using standardized protocols developed earlier for genetic transformation. Heat shock protein 31 gene from *Saccharomyces cerevisiae* (ScHsp31) was selected to engineer *Hevea* plants against the biotic and abiotic stresses. The heat shock protein Hsp31 routinely detoxifies Methylglyoxal (MG) which accumulates during abiotic and biotic stresses. Overexpression of ScHsp31 gene has resulted in enhanced stress tolerance of plants. This has been proved in tobacco plants which showed that transgenic expression of ScHsp31 gene confers protection against various cellular stresses. Experiments were carried out for integration of ScHsp31 gene into *Hevea* callus for regenerating transgenics with enhanced stress tolerance. Genetic transformation was carried out in *Hevea* with pBIN-ScHsp31 gene construct in *Agrobacterium* strain LBA4404. The construct also harboured *nptII* gene for antibiotic selection. Friable and actively proliferating embryogenic calli obtained from the leaf cultures of clone RR11 105 were used for *Agrobacterium* infection. Transformation frequency of around 15 per cent was obtained when genetic transformation with pBIN-ScHsp31 gene was carried out in the earlier standardized medium. Kanamycin resistant calli were further proliferated and used for somatic embryogenesis. Effect of antioxidants such as  $\alpha$ -lipoic acid and glutathione on transformation efficiency was studied. Transformation efficiency could be enhanced to around 50 per cent by the addition of 50 mg L<sup>-1</sup>  $\alpha$ -lipoic acid. Gene integration and expression in the transgenic calli lines was studied using semi-quantitative RT-PCR analysis where a 713 bp band was amplified with gene specific primers with no amplification in the untransformed calli. Somatic embryogenesis was also obtained from the transgenic and control calli.

**Keywords:** Antioxidant, Genetic transformation, Heat shock protein, Methylglyoxal

## INTRODUCTION

Development of clones with improved agronomic traits such as stress tolerance and enhanced yield is the need of the hour so as

to extent cultivation to non-traditional areas and to achieve the goal of increasing productivity of the natural rubber producing tree, *Hevea brasiliensis*. Biotechnological