

## Using mannose as a positive selection of transformed *Carica papaya* l. var 'Eksotika'

### ABSTRACT

The main objective of this study is to develop marker-free transgenic papaya plants via positive selection using phosphomannose isomerase (pmi) gene. Phosphomannose isomerase (PMI) is an enzyme that converts mannose-6-phosphate to fructose-6-phosphate, a glycolysis intermediate that supports the growth of plant cells. To establish a marker-free positive selection system using this PMI, the effect of mannose on the growth and development of embryogenic 'Eksotika' papaya callus was evaluated. One-month old embryogenic calli were cultured on Murashige and Skoog (MS) medium in which 60 g/L sucrose in the original recipe was replaced with different concentrations of mannose and sucrose. Mannose was supplied as the sole carbon source or in combination with sucrose at 0, 5, 10, 15, 20, 25 or 30 g/L. Embryogenic calli cultured on medium supplemented with a ratio of 0:60 g/L mannose: sucrose was used as a control. The results after six sub-cultures showed that most of the embryogenic calli transferred on media containing only mannose turned brown. Higher concentrations of mannose resulted in higher percentage of brown calli (dead). Mannose at 30 g/L mannose was found to be effective for screening transformed embryogenic calli. Evaluation of papaya transformation Efficiency using this positive selection system was pursued using 650 one-month-old embryogenic calli *Agrobacterium*-transformed with pNOV2819 harboring the pmigene. Only transformed cells are capable of utilizing mannose as a carbon source to grow. After five months on mannose selection, all 67 putative transformants obtained were PCR-positives for the pmi gene.

**Keyword:** 'Eksotika' papaya; Positive selection; Phosphomannose isomerase (pmi); *Agrobacterium*-mediated transformation