

A quick protocol to facilitate the selection of putative delayed ripening transgenic papaya lines for field evaluation

ABSTRACT

A quick protocol is needed to determine transgene, ACC oxidase 2 expression in putative transgenic Eksotika papaya lines targeting at selecting potential lines for field evaluation. In this study, three housekeeping genes, 18S ribosomal RNA, 40S ribosomal protein and actin, were used for normalisation of the ACC oxidase 2 gene expression. Comparison with a non-transformed seedling-derived plants revealed that 42 putative transgenic lines exhibited between 2- and 5-fold reduction of ACC oxidase 2 expression level. Based on the gene expression data of the in vitro putative transgenic papaya plants obtained, 24 independent potential lines were selected for field evaluation. Gene expression analysis on field grown papaya plants showed similar profile of ACC oxidase 2 expression levels as compared to in vitro papaya plants. Increase in storage shelf life was also examined in the transgenic lines grown in the field with the most potential transgenic line was 27-3, which required 14 days to achieve full yellow colour index. Overall, the findings in this study revealed that reduction of ethylene was successful in the Eksotika papaya by manipulating the ACC oxidase 2 using the antisense technique. This reflects that future production of longer shelf life Eksotika papaya fruits is possible through antisense technique and it will help boost the papaya industry further by opening up new export markets in distant destinations.

Keyword: Antisense; Transgenic; Real-time PCR; Housekeeping gene; Gene expression