THESIS TITLE : DETECTION OF NPT-II AND CP GENES IN PUTATIVE PAPAYA TRANSFORMANTS

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ABSTRACT

Expression of NPT-II gene in putative papaya transformants was detected using NPT-II ELISA method. Crude extracts from 20 lines of KN of young and old leaves were used for comparison the level of enzyme NPT-II. The result showed clear positive reaction in the transgenic line of KN (222-3 line) and no reaction in the normal line of KN. Among 20 tested lines of putative papaya transformants, expression of NPT-II gene was varied. However, the level of NPT-II from young leave was higher than old leave. To make more precision result of an experiment, at least three replications were recommended.

Extracted DNA from leaves of untransformed, transgenic papaya plants and putative transformants using ultrafast NaOH method were used as template

amplification of CP gene by polymerase chain reaction (PCR) technique and detected by gel electrophoresis. The result showed that the CP gene was amplified from young and old leaves of line 222-3 and not from untransformed papaya plant. Among 20 lines of putative transformants, expressed DNA fragment of CP gene was detected from young leaves of 9 transgenic lines ie. KN 44-7, 56-1, 226-1, 227-1, 319-1, 378-2, 440-8, 469-4 and 634-1, and from old leaves of 6 transgenic lines ie. KN 44-7, 45-2, 226-1, 476-2, 469-4 and 634-1. Using DNA microprep method, amplification of CP gene was detected from DNA of young and old leaves of line 222-3 and not from untransformed papaya plant. The CP gene was also detected from DNA extracted from young leaves of 5 transgenic lines ie. KN 226-1, 227-1, 476-2 and 634-1, and from old leaves of 6 transgenic lines ie. KN 226-1, 314-1, 440-8, 469-4, 476-2 and 634-1. Therefore, DNA extraction from young leaves of papaya using ultrafast NaOH method was the best condition. These achieved data will be used for choosing an effective method for furthur screening of transgenic papaya lines which resistant to PRSV.