O-60 (52)
EFFICIENCY OF USAGE INDUCIBLE SITE-SPECIFIC RECOMBINASE AND A BIFUNCTIONAL SELECTABLE GENE FOR PRODUCTION OF MARKER-FREE CISGENIC APPLE PLANTS

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The presence of marker genes, especially antibiotic resistance, in genetically modified plants is of concern in society due to fears associated with risks for the environment and human health. Creation of transgenic plants that do not contain the foreign genetic material, especially bacterial and viral origin largely alleviates the tension. Here we used the pMF system containing of the *Z. rouxii* recombinase R and a CodA-nptII bifunctional selectable gene for produce marker-free transgenic apple plants carrying the super sweet thaumatinII gene from tropical plant *Thaumatococcus daniellii* under control of E8 gene fruit specific promoter and rbsS3A terminator. We have obtained three independent transgenic lines that have been thoroughly analyzed by PCR for the presence of T-DNA sequences. We then used the delayed strategy for the selection of marker-free plants with one checked line contained all parts of expression cassette. After induction of recombinase activity in leaf explants we have obtained on selective media with 5-fluorocytosinemore more than 30 sublines, most of them lost their resistance to kanamycin. PCR and Southern blot analysis revealed that all undesirable genes and sequences between RS sites were removed by recombination process while gene of interest with regulatory elements is present in all obtained plants. However by semi-quantity PCR analysis we can detect expression of thaumatin gene in most, but not all resulted sublines. So, using vector based on pMF system we developed acceptable protocol for production marker-free cisgenic apple plants**.**

Keywords: marker-free plants, apple, Malusxdomestica, thaumatin II, CodA, R/RS recombination system