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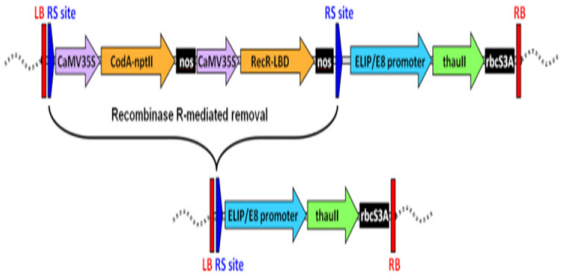
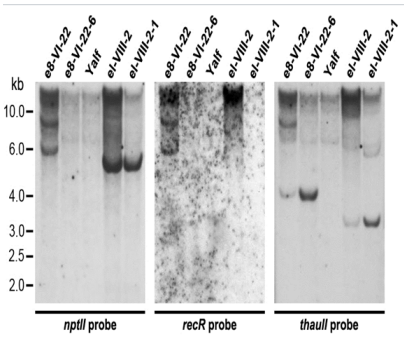
Production of marker-free transgenic tomato and apple plants using inducible site-specific recombinase and a bifunctional selectable gene

Sergey Dolgov[2], Vadim Timerbaev[1] and **Tatyana Mitiouchkina[2]**
[1]Branch of Shemyakin Institute, Russia
[2]Nikita Botanical Gardens, Russia

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 tomato and 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 used two different recombinase induction procedures: early selection of primary calluses and delayed decsametasone treatment of leafs or petioles of transgenic plants with further selection on 5-FC media. As alternative we transformed plants by vector carried only thaumatin expression cassette without any selective markers. Transgenes in this case were selected by PCR analysis of all obtained regenerants. We have obtained 170 transgenic lines of tomato that have been thoroughly analyzed by PCR. After induction of recombinase activity only one fully marker-free transgenic tomato line was obtained. The similar results were obtained by early selection. Direct transformation by thaumatinII cassette resulted two marker free plants too. We suggest that an incomplete excision and chromosomal rearrangements due to the presence of multiple and aberrant or partial T-DNA insertions occur in other cases of easy transform tomato plants. We also have obtained three independent transgenic lines of apple 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 we have obtained 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. Early selection procedure as direct transformation was unsuccessful for production marker free apple plants. pMF system is suitable for production marker-free apple or other recalcitrant crops, as cisgenic tomato plants may be obtained by direct transformation by gene of interest. The research was funded in part by a research grant N° 14-50-00079 of the Russian Science Foundation.





Biography

Dolgov S V is the Head of Laboratory of Expression Systems and Plant Genome Modification "Biotron". During last 25 years, the technologies of *in vitro* cultivation of isolated cells, tissues and organs on artificial media have been developed for more than plant 30 species. Highly effective methods of genetic transformation have been developed for a large number of plants (carrots, tomatoes, pears, apples, strawberries, wheat, duckweed, chrysanthemum), which allow to study the activity of foreign proteins in transgenic plants and obtain varieties with economically valuable traits. Currently, the station of artificial climate "Biotron" researches on the plant physiology and molecular biology (studying of genes that affect the flowering morphology of Compositae), biopharming, protection of plants against biotic and abiotic stresses, field trials of transgenic fruit trees, etc.

dolgov@bibch.ru