

Rol Gene Expression Offers the Positive Selection of Marker-free Transgenic *Solanum Melongena*

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Abstract: Safety of genetically modified plants has led to a demand for technologies allowing the production of transgenic plants without selectable markers. Multi-Auto-Transformation (MAT) vector system consists of positive selection, using oncogene *rol* (root loci), with a site-specific recombination and DNA removal system, that generates morphologically normal marker-free transgenic plants without antibiotic selective-agent. This study describes *rol*-type MAT vector (pMAT101) containing *lacZ* gene as a model gene and the removable cassette with *gus* gene in the T-DNA region which was used to produce morphologically normal transgenic *Solanum melongena* employing *rol* gene as the selectable marker gene and *gus* gene as a reporter gene. Leaf explants inoculated with pMAT101 produced twenty one adventitious roots which had fourteen hairy roots with GUS expression on agar-solidified, MS medium without both plant growth regulators and selective-agent under dark condition. These hairy roots produced more than hundred shoots with Ri syndrome such as dwarfism, wrinkled leaves, and an over abundance of roots as a consequence of the morphogenic action of *rol* gene. They eventually produced morphologically normal shoots without GUS expression on the same fresh MS medium under 16h photoperiod. Molecular analysis of DNA from the hairy roots, shoots with Ri syndrome and morphologically normal shoots revealed that the normal shoots had only *lacZ* gene, and the removable cassette consisting of *rol*, R (recombinase) and *gus* genes was excised. In our studies, *rol* gene has been used as positive selection marker with MAT vector system which was designed to remove the oncogene or rhizogene from transgenic plants after transformation by inserting these genes between two directly oriented recombination sites and recover the normal phenotype to overcome the effect of oncogenes. This study proved that the chimeric *rol* genes can be used as a visible selection marker for *Agrobacterium*-mediated transformation of *Solanum melongena*. Moreover the production of phenotypically normal marker-free transgenic plants is possible without using selective chemical agents when employing *rol*-type MAT vector and it is a promising method to save time and work for the generation of marker-free normal transgenic *Solanum melongena* and related species with repeated transformation with various useful genes.

Keywords: MAT vector, Removable cassette, *rol* (root loci) gene, Site-specific recombination