

# Generation of phenotypically normal marker-free transgenic plants of *Kalanchoe blossfeldiana* through hairy root induction

Thirukkumaran, G.<sup>ab</sup>, Ntui, V.O.<sup>ab</sup>, Khan, R.S.<sup>ab</sup>, Nakamura, I.<sup>ab</sup> and Mii, M.<sup>ab</sup>

<sup>a</sup> Laboratory of Plant Cell Technology, Graduate School of Horticulture, Chiba University, Matsudo, Japan

<sup>b</sup> Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Sri Lanka

## Abstract

Multi-Auto-Transformation (MAT) vector system consists of positive selection, using the *ipt* or *rol* gene, 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 *Kalanchoe blossfeldiana* Poelln. employing *rol* gene as the selectable marker gene and *gus* gene as a reporter gene. Leaf explants inoculated with pMAT101 produced hairy roots with GUS expression on agar-solidified, half-strength MS medium without both plant growth regulators and selective agent under dark condition. These hairy roots produced 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. This study indicates that the *rol*-type MAT vector could be used for the production of morphologically normal marker-free transgenic *K. blossfeldiana* plants without using selective chemical agents.

## Author keywords

*Kalanchoe blossfeldiana*; MAT vector; Removable cassette; *Rol* gene; Site-specific recombination

## Indexed keywords

**Species Index:** *Kalanchoe*; *Kalanchoe blossfeldiana*