TRANSGENE REPEAT FORMATION AND PROMOTER METHYLATION IN
TRANSGENIC PLANTS

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ABSTRACT

Stable integration of a single copy of delivered DNA into plant cells is most desirable for
production of transgenic plants. However, the majority of transgenic lines have various patterns
of transgene loci. The inactivation of transgenes is often accompanied by transgene
arrangement, multiple copies of the gene and promoter methylation events. This research aimed
to study transgene integration patterns and promoter methylation in transgenic tobacco
(Nicotiana tabacum cv. Samson NN), japonica rice (Oryza sativa ssp. japonica cv. Taichung)
and indica rice (Oryza sativa ssp. indica cv. KDML105).

Leaf disks of tobacco were transformed via Agrobacterium-mediated transformation and
particle bombardment. Using the former technique, the bacterial strain EHA105 harboring
pCAMBIA1301, which contains β-glucuronidase (gus) gene and hygromycin resistant (hpt)
gene driven by CaMV 35S promoter, was employed. All of the transgenic plants maintained
GUS activities with complete T-DNA integration cassette. By contrast, 70% of transgenic lines
produced by particle bombardment showed silencing of gus gene expression. Deletion of gus
was detected in all silent lines.

Using Agrobacterium-mediated transformation, calli of japonica rice were transformed
with EHA105 harboring pCAMBIA1301. GUS activity was detected in 43% of transgenic rice.
The absence of promoter region and gus gene were observed in some silent lines. In indica rice,
shoot apical meristem transformation incorporated with multiple shoot regeneration system was
established in both Agrobacterium-mediated transformation and particle bombardment methods.
The Agrobacterium strain EHA105 was more effective than AGL1 for gene transfer.

Acetosyringone had no effect while sonication at 10 seconds significantly enhanced the
transient expression. In particle bombardment, the effect of acceleration pressures, target
distances and the number of bombardments were studied. None of the parameters affected
multiple shoot regeneration. Southern blot analysis revealed the presence of multiple copies of
gus gene; however, the number of transgenes had no effect on the level of gus expression. RT-
PCR analysis showed that the levels of gus transcripts decreased from the intensive, moderate
and silent GUS-expressing plants, respectively. Using bisulfite genomic sequencing PCR, a
high level of DNA methylation in the CaMV 35S promoter of transgenic plants was observed in
all silent lines. Indeed, the methylations in both symmetrical and asymmetrical sequences were
crucial for transgene inactivation at the transcription level. The investigation of regulation of
transgene expression in relation to transgene integration and DNA methylation may provide
more information for potential manipulation of transgenic plants.

KEY WORDS: GENE SILENCING / TRANSGENE INTEGRATION / DNA
METHYLATION / GENE TRANSFORMATION

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