

Transgenic Plant Journal

Abbreviation: Transgenic Plant J. (TPJ)

Print: ISSN 1749-0413

Scope and target readership: *Transgenic Plant Journal* publishes original, peer-reviewed articles dealing with new advances concerning all aspects of fundamental research and technology in the field of plant genetic transformation, transgene transfer and expression.

Transgenic Plant Journal will consider manuscripts that explore the following topics related to transgenic plants:

- 1) Commercialization;
- 2) Field trials;
- 3) Gene silencing mechanisms;
- 4) Marker-assisted breeding;
- 5) Molecular farming and production system enhancement and exploitation through transgenic systems;
- 6) Novel plasmids, enhancers, constructs, or insertion methods;
- 7) Post-translational analysis;
- 8) Public perception, intellectual property, education, (bio)ethical issues;
- 9) RNA silencing mechanisms;
- 10) Safety regulations, environmental impact and containment;
- 11) Use of transgenic plants as experimental tools and in applied studies.

In general manuscripts should provide several molecular (PCR, Southern, Northern, Western) evidence to prove transgenic events/integration. Other histochemical, fluorescent, immunological (e.g. ELISA) or other techniques to support the molecular component are strongly encouraged. Contemporary methods of molecular genetics, genomic analysis, structural and functional genomics, proteomics and metabolic profiling, abiotic stress and field evaluation of transgenic crops containing particular traits are also a vital complement. Manuscripts that link marker-assisted breeding to transgenic plants are also of interest.

Results of transgenic studies that might bring potential benefits (or show potential/actual risks) to health/society are encouraged.

Editor-in-Chief

Jaime A. Teixeira da Silva, Japan

Technical Editor

Kasumi Shima, Japan

Editorial Board and Advisory Panel

Chhandak Basu, USA

Pankaj Kumar Bhowmik, Canada

Eva Casanova, Spain

Hany A. El-Shemy, USA

C. Gopi, India

Wenwu Guo, China

Shuangxia Jin, China

Hiroaki Kodama, Japan

Sean Mayes, UK

Gopi K. Podila, USA

Salehi Jouzani Gholam Reza, Iran

Nedeljka Rosic, Australia

Shigeru Satoh, Japan

Alan Smith, USA

Kin-Ying To, Taiwan

Leena Tripathi, Uganda

Hao Yu, Singapore

Global Science Books, Ltd.
Editorial Office
Miki cho Post Office, Kagawa ken, Kita gun
Miki cho, Ikenobe 3011-2, P.O. Box 7
761-0799, Japan



Head Office: Isleworth, United Kingdom
Accounting: Lagos, Portugal

GSB homepage: www.globalsciencebooks.info
Journals web-page: <http://www.globalsciencebooks.info/Journals/GSBJournals.html>
TPJ web-page: <http://www.globalsciencebooks.info/Journals/TPJ.html>
GSB Japan web-page: <http://www17.plala.or.jp/gsbjapan>
GSB™ is a trademark of Global Science Books, Ltd.

Transgenic Plant Journal ©2008 Global Science Books, Ltd.

All rights reserved. No parts of this journal may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from Global Science Books, Ltd.

For additional copies, photocopies, bulk orders, or copyright permissions, please refer requests in writing to the above address, or apply online.

Cover photos: Top left plate (4 photos): Aseptic carrot root discs four weeks after the inoculation with *A. rhizogenes*: the non-inoculated control (top, left) and the inoculated discs developing hairy roots from meristematic cells of the secondary cambium (bottom, left); Fluorescence of carrot expressing green fluorescent protein (GFP) under the control of CaMV 35S promoter; umbel under white light (top, right) and under blue light (bottom, right) (Baranski, pp 18-38). Top right: Viral suppressor activity can be directly assessed within tobacco leaf infiltrations (Velten *et al.*, pp 1-13). Bottom row: Agronomic field trial of *Helichrysum stoechas* hairy root-regenerated plant line E, N, B, M and control plants (on the right) at flowering (Giovannini *et al.*, pp 54-61).

Disclaimers: All comments, conclusions, opinions, and recommendations are those of the author(s), and do not necessarily reflect the views of the publisher, or the Editor(s). GSB does not specifically endorse any product mentioned in any manuscript, and accepts product descriptions and details to be an integral part of the scientific content.

Printed in Japan on acid-free paper.
Published: June, 2008.

CONTENTS

Jeff Velten (USA), Barry James Pogson, Christopher Ian Cazzonelli (Australia) Luciferase as a Reporter of Gene Activity in Plants	1
Wataru Takahashi (Japan), Hongwei Cai (Japan/China) Transgenic <i>Lolium</i> and <i>Festuca</i>	14
Rafal Baranski (Poland) Genetic Transformation of Carrot (<i>Daucus carota</i>) and Other <i>Apiaceae</i> Species	18
Zhengquan He, Lei Chen, Wei Yao, Jianwu Dai (China) Recent Progress in Cucumber (<i>Cucumis sativus</i>) Transformation	39
Irina E. Dodueva, Elena L. Ilyina, Tatiana N. Arkhipova, Nadezhda V. Frolova, Veronika A. Monakhova, Guzel R. Kudoyarova, Ludmila A. Lutova (Russia) Influence of <i>Agrobacterium tumefaciens ipt</i> and <i>Agrobacterium rhizogenes rolC</i> Genes on Spontaneous Tumor Formation and Endogenous Cytokinins Content in Radish (<i>Raphanus sativus</i>) Inbred Lines	45
Annalisa Giovannini, Carlo Mascarello, Luca Pipino, Antonia Nostro (Italy) <i>Agrobacterium rhizogenes</i> -Mediated Transformation in Mediterranean <i>Helichrysum</i>	54
Hyeon-Suk Lee, Jae-Keun Sohn, Kyung-Min Kim (South Korea) Ectopic Expression of <i>Arabidopsis Rotundifolia</i> 3 Gene in Perilla (<i>Perilla frutescens</i> L.)	62
Mukaddes Kayim (Turkey), Kenneth S. Derrick, Gary A. Barthe, Julia M. Beretta (USA) Expression of Three Different Mutant Green Fluorescent Protein Genes in Transgenic Carrizo Citrange	66
Wei Chee Wong, Rofina Yasmin Othman, Norzulaani Khalid (Malaysia) Improvements in the Efficiency of <i>Agrobacterium</i> -Mediated Transformation of Embryogenic Cell Suspensions of Banana cv. 'Mas' using a Low-Antibiotic Liquid Washing-Assisted Approach	75

Jeff Velten (USA), Barry James Pogson, Christopher Ian Cazzonelli (Australia) Luciferase as a Reporter of Gene Activity in Plants (pp 1-13)

ABSTRACT

Invited Review: Reporter gene systems based upon modified luciferase genes isolated from organisms, ranging from bacteria to insects, have proven to be important tools for plant molecular studies. The biochemical characteristics of these genes combine very high sensitivity with the ability to determine reporter activity non-destructively *in vivo*, allowing many applications in plants that cannot be accomplished using any other single reporter system. The relative ease of *in situ* detection of the firefly luciferase has made it an especially successful reporter for screening mutants in the model plant genetic system, *Arabidopsis thaliana*. The rapid turnover rate of luciferase has aided the characterization of promoters and elements that are influenced by time-sensitive factors such as circadian rhythm (diurnal cycles), gene silencing, and environmental stresses. The high sensitivity of luciferase as a reporter has facilitated the analysis and development of synthetic promoters for plant gene expression. Additionally, the biochemical characteristics of different luciferases have allowed their use as *in situ* indicators of metabolic activity and oxygen levels, as well as direct indicators of *in vivo* protein-protein interaction. The various applications of luciferase-based reporter systems in plants will be the subject of this review.

Wataru Takahashi (Japan), Hongwei Cai (Japan/China) Transgenic *Lolium* and *Festuca* (pp 14-17)

ABSTRACT

Invited Mini-Review: Genetic engineering enables us to exchange genes among species beyond the taxonomic barrier for controlling and expanding the genetic variation of organisms. Although genetic modification of organisms can be accomplished by other asexual techniques, such as artificial mutation and somatic hybridization, so far only genetic engineering can accurately control target phenotypes by controlling foreign gene expression under the control of the optional promoter in the host genome. This indicates that, for plant breeding programs, genetic engineering can produce epoch-making breeding materials with valuable traits that are difficult to generate by conventional breeding. We present here a comprehensive summary of information on the genetic engineering of *Lolium* and *Festuca* species.

Rafal Baranski (Poland) Genetic Transformation of Carrot (*Daucus carota*) and Other *Apiaceae* Species (pp 18-38)

ABSTRACT

Invited Review: Carrot (*Daucus carota* L.) is well known as a model species for plant tissue culture systems. It is also amenable to genetic modifications using both vector and non-vector methods. Several works have been commenced to study factors affecting transgenesis in this species. As a result, optimized transformation protocols have been established. Genetically modified *Ammi*, *Anethum*, *Apium*, *Bupleurum*, *Carum*, *Centella*, *Coriandrum*, *Foeniculum*, *Levisticum*, *Petroselinum*, *Peucedanum* and *Pimpinella* belonging to the *Apiaceae* family have also been obtained. However, unlike carrot, most of them were mainly used for hairy root development after *Agrobacterium rhizogenes*-mediated transformation. Extensive research in carrot has focused on studying gene function and regulation, and plant metabolism. Applied research includes the development of plants resistant to both abiotic and biotic stresses, and modifications of biosynthetic pathways. As the *Apiaceae* family is an important reservoir of condiments and medicinal plants, some species were studied for the ability of their hairy roots to produce pharmaceutically principal secondary metabolites as well as for phytoremediation. This review provides an overview on the essential genetic transformation studies conducted in the *Apiaceae* family with insight into the application of these genetically modified species for basic and applied research.

Zhengquan He, Lei Chen, Wei Yao, Jianwu Dai (China) Recent Progress in Cucumber (*Cucumis sativus*) Transformation (pp 39-44)

ABSTRACT

Invited Mini-Review: Genetic transformation is vital to the transfer of novel genes into vegetable plants as well as the emerging area of functional genomics. However, the successful genetic transformation of cucumber still remains time-consuming and genotype dependent. This paper updates the progress made in recent years toward developing a genetic transformation system for cucumbers. *Agrobacterium*-mediated cucumber transformation offers advantages, such as single copy gene

insertion, minimal rearrangement of DNA, low cost and comparatively high efficiency. The recent developments in cucumber transformation could lead to an increased efficiency of a cucumber breeding program. The most exciting recent progress has been in the production of elevated levels of an anti-aging superoxide dismutase in transgenic cucumber fruits, since cucumber fruits were considered to be one of the most promising economical plant bioreactors which can produce edible pharmaceutical proteins.

Irina E. Dodueva, Elena L. Ilyina, Tatiana N. Arkhipova, Nadezhda V. Frolova, Veronika A. Monakhova, Guzel R. Kudoyarova, Ludmila A. Lutova (Russia) Influence of *Agrobacterium tumefaciens ipt* and *Agrobacterium rhizogenes rolC* Genes on Spontaneous Tumor Formation and Endogenous Cytokinins Content in Radish (*Raphanus sativus*) Inbred Lines (pp 45-53)

ABSTRACT

Original Research Paper: Several inbred lines from a radish (*Raphanus sativus* var. *Radicula* Pers.) genetic collection spontaneously form tumors on the crop roots during flowering. Here we studied the influence of the *Agrobacterium tumefaciens ipt* gene, which controls cytokinin biosynthesis, and the *Agrobacterium rhizogenes rolC* gene, which influences cytokinin metabolism, on tumor formation in the radish lines. Two *in planta* transformation methods – transformation of ovaries via pollen-tube pathway and transformation of seedling apices – were firstly used to obtain transgenic radish plants. Both methods showed high efficiency for radish. Transformation of radish lines by both *ipt* and *rolC* genes induced tumor formation in non-tumorous lines. We noted a several-fold increase of free zeatin concentration in *ipt*-transgenic radish plants while *rolC*-transgenic radish plants showed an increase in zeatin riboside concentration comparing with the untransformed line. We suppose that the tumorigenic effect of *ipt* and *rolC* genes on radish lines is likely to be due to change of phytohormonal balance in the tissues of transgenic plants.

Annalisa Giovannini, Carlo Mascarello, Luca Pipino, Antonia Nostro (Italy) *Agrobacterium rhizogenes*-Mediated Transformation in Mediterranean *Helichrysum* (pp 54-61)

ABSTRACT

Original Research Paper: *Helichrysum italicum* (Roth) G. Don and *H. stoechas* (L.) Moench are aromatic wild species of the Mediterranean region. The plants grow in arid soils, from the cliffs above the sea to the hills, where they flower from May to August. The bright yellow flower heads contain essential oils, flavonoids (helichrysin A and B), tannins and caffeic acid. *Helichrysum* has been used in folk medicine because of its antibacterial, antitoxic, diuretic and antiallergic properties. The flowering stems are also used dried as “everlasting flowers”. *A. rhizogenes* ATCC 15834 wild type strain was effective to induce hairy roots in leaf and root tissue of micropropagated *H. italicum* and *H. stoechas* Italian accessions. Hairy root-lines, originated from independent transformation events, were recovered. *H. stoechas* hairy root-regenerated plant lines, and their self pollinated progeny, have been studied for three years to assess their ornamental value and to test the antibacterial activity of the inflorescences extracts. The transgenic hairy root-derived plant lines showed distinct morphological and physiological growth patterns. Moreover, peculiar characters, induced by *A. rhizogenes* genes, were transmitted to the progeny.

Hyeon-Suk Lee, Jae-Keun Sohn, Kyung-Min Kim (South Korea) Ectopic Expression of *Arabidopsis Rotundifolia 3* Gene in Perilla (*Perilla frutescens* L.) (pp 62-65)

ABSTRACT

Original Research Paper: The *Rotundifolia 3* (*Rot 3*) gene regulates narrow leaf shape in *Arabidopsis thaliana*. We introduced *Rot 3* into perilla (*Perilla frutescens* L.) by *Agrobacterium*-mediated transformation, and analyzed the leaf shape of transgenic plants. Cv. ‘Manbaek’ showed the highest performance in shoot induction (27.3%) compared with that of six tested cultivars. Thirteen transgenic plants were obtained from cotyledon tissues of ‘Manbaek’ with a 9.2% of transformation efficiency. Southern blot analysis revealed that the average copy of the integrated *Rot 3* was 1 or 2 in T₁ transgenic plants. The high level of *Rot 3* mRNA resulted in elongated leaves. On the other hand, *Rot 3* transcript levels were lower in the lines of round leaves than in those from the elongated leaves. These results suggest that leaf shape can be artificially modified and that the heterologous expression of the leaf shape-regulating gene can be used as a new breeding strategy for improving leaf design in perilla.

Mukaddes Kayim (Turkey), Kenneth S. Derrick, Gary A. Barthe, Julia M. Beretta (USA) Expression of Three Different Mutant Green Fluorescent Protein Genes in Transgenic Carrizo Citrange (pp 66-74)

ABSTRACT

Original Research Paper: The expression of three different GFP mutants was studied in Carrizo citrange (*C. sinensis* [L.] Osb. X *Poncirus trifoliata* [L.] Raf.) using strain AGL1 in *Agrobacterium tumefaciens*-mediated transformation. The localization of green fluorescent protein (GFP) expression in citrus tissue was compared with three different GFP mutants: *EGFP.1*, *GFPC3*, and *mGFP5-ER*. All three were driven by the CaMV35S promoter, but *EGFP.1* and *C3* gene cassettes have the double 35S promoter with an AMV enhancer sequence from the Alfalfa mosaic virus (AMV). Strong GFP expression was provided with the double 35S CaMV promoter and AMV enhancer in front of *EGFP* and *GFPC3* in transgenic citrus shoots. However, the brightest expression of GFP was observed in transgenic callus and shoots transformed by *GFPC3*. Most of the stable transgenic shoots survived when transformation was performed with *mGFP5-ER* for GFP expression. GFP expression in transgenic tissue was detected by stereo and confocal microscopy for three different GFP variants. The intensity of GFP fluorescence varied in transgenic plants regardless of the GFP variant. Sixty stable transgenic citrus shoots developed whole plants by *in vivo* shoot tip grafting. In the greenhouse, however, 50% of these transgenic plants were silenced for GFP expression. The presence of transgenes in both silenced and transgenic plants was verified by gene amplification and Southern analysis. GFP synthesis was also confirmed by Western blotting using GFP-PCA only in GFP-expressing shoots and plants.

Wei Chee Wong, Rofina Yasmin Othman, Norzulaani Khalid (Malaysia) Improvements in the Efficiency of *Agrobacterium*-Mediated Transformation of Embryogenic Cell Suspensions of Banana cv. 'Mas' using a Low-Antibiotic Liquid Washing-Assisted Approach (pp 75-85)

ABSTRACT

Original Research Paper: *Agrobacterium*-mediated transformation is a convenient gene transfer process for many plants, including banana. However, the use of various antibiotics using this method, either to eliminate *Agrobacterium* or for screening of putative transformed plants significantly affects plant growth as well as transformation efficiency. In this study, we have improved currently reported methods for transformation of banana embryogenic cell suspension by using a hormone-free Murashige and Skoog (MS) liquid washing-assisted medium supplemented with an optimum concentration of cefotaxime at 50 mg l⁻¹ for bacterial elimination followed by 200 µg l⁻¹ hygromycin for screening of putative transformed plants. This strategy was designed to reduce the antibiotic stress on the transformed plants by accelerating plant regeneration and by shortening the incubation period of the transformed plants on the antibiotic selection medium. The highest number of transformation events was achieved after 30 minutes incubation with *Agrobacterium tumefaciens* strain LBA4404 carrying pCAMBIA/SOC1 encoding a MADS-box transcription factor followed by 4 days of co-cultivation in darkness at 30 ± 1°C. The integration of the *Elaeis guineensis* Jacq. (oil palm) *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) transgene was confirmed by both Southern Blot and real-time RT-PCR analysis.