Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Review Article **DOI: 10.26479/2026.1204.02**

ADVANCES IN GENETIC TRANSFORMATION OF *ONCIDIUM* ORCHIDS USING PROTOCORM-LIKE BODIES (PLBs)

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ABSTRACT: Oncidium orchids are among the most commercially valued ornamental plants in the global floriculture industry. Genetic transformation has emerged as a critical biotechnological tool for improving trait characteristics including flower color modification, disease resistance, and altered flowering time. Protocorm-like bodies (PLBs) are the preferred target tissue for transformation due to their high totipotency, embryogenic nature, and regeneration capacity. This review examines the current state of genetic transformation in Oncidium orchids using PLBs, covering Agrobacterium-mediated and biolistic transformation methods, selectable markers, reporter genes, and transformation optimization strategies. Applications in developmental regulation, disease resistance, and carotenoid pathway modification are discussed alongside challenges and prospects for CRISPR-Cas9 integration.

Keywords: Oncidium; protocorm-like bodies; *Agrobacterium*; biolistic transformation; orchid biotechnology; PLB regeneration

Article History: Received: July 22, 2026; Revised: July 26, 2026; Accepted: July 28, 2026.

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1. INTRODUCTION

The family Orchidaceae, comprising approximately 25,000–30,000 species across 880 genera, is one of the largest among flowering plants [7]. Within this family, the genus *Oncidium* commonly referred to as "dancing lady orchids" holds substantial commercial importance due to its attractive, long-lasting flowers, with major production centers in Taiwan, the Netherlands, and Southeast Asia.

Conventional breeding strategies remain constrained by long generation cycles, complex polyploid genomes, and high heterozygosity [39], prompting increasing interest in genetic transformation to accelerate trait improvement without disrupting the overall genetic background [3]. The establishment of regenerable explant systems is foundational to any transformation protocol. Among available explant types, PLBs have emerged as the most reliable target for orchid transformation, owing to their embryogenic competence and high regenerative potential [22]. PLBs are somatic embryo-like structures morphologically analogous to the zygotic protocorm of germinating orchid seeds, providing meristematic cells amenable to foreign DNA integration [4]. Significant progress has been made in Agrobacterium-mediated and biolistic transformation of *Oncidium* PLBs, with key advances in efficiency, selectable marker systems, and molecular confirmation of transgenesis [14]. This review synthesizes current knowledge on these aspects and discusses future directions.

Protocorm-Like Bodies as Transformation Targets

Biology and Induction of PLBs

PLBs are globular, meristematic structures originating through somatic embryogenesis from various orchid explants, including leaf segments, root tips, flower stalks, and existing PLBs [22]. Histological characterization of PLBs reveals densely cytoplasmic meristematic cells with prominent nuclei and thin cell walls features associated with high transformation competence [11]. In *Oncidium* 'Gower Ramsey', PLBs have been successfully induced from leaf explants cultured on modified Murashige and Skoog medium supplemented with cytokinins, particularly thidiazuron (TDZ) and 6-benzylaminopurine (BA) [4]. The inclusion of TDZ at 0.5–2.0 mg/L consistently promotes PLB induction and proliferation. Subcultured PLBs maintained at 2–5 mm diameter and replenished every 4–6 weeks provide the most suitable material for transformation experiments [21]; [34].

Advantages of PLBs for Transformation

PLBs confer several advantages over conventional explants for genetic transformation. Their high totipotency ensures that transformed cells can regenerate into complete plants, a prerequisite for stable transgenic lines [29]. The undifferentiated state of PLBs enhances susceptibility to Agrobacterium infection and DNA uptake [10]. The applicability of PLBs across *Phalaenopsis, Dendrobium*, and *Cymbidium* underscores their broad utility, suggesting that *Oncidium* protocols can guide research in related taxa [12].

Methods of Genetic Transformation

Agrobacterium-mediated Transformation

Agrobacterium tumefaciens-mediated transformation exploits the bacterium's natural capacity to transfer T-DNA into plant cells, enabling relatively precise and low-copy integration of foreign DNA [1]. In *Oncidium,* this approach was pioneered by Liau et al. [17], who demonstrated successful T-DNA transfer into *Oncidium* 'Gower Ramsey' PLBs using strain LBA4404 carrying the sweet

pepper ferredoxin-like protein (*pflp*) gene. Transformation was confirmed by PCR and Southern blot analysis, and the regenerated plants expressed the transgene stably across generations. Subsequently, Lee et al. [14] systematically compared transformation conditions across several *Oncidium* cultivars using strains LBA4404 and EHA105, reporting that co-cultivation duration, bacterial density, and PLB developmental stage critically determine transformation frequency. Raffeiner et al. [23] extended this approach to *Oncidium* and *Odontoglossum* species using the ethylene receptor mutant gene *etr1-1*, demonstrating that Agrobacterium-mediated methods can deliver functionally diverse transgenes. Further optimization by Semiarti et al. [25] showed high-frequency transformation in Indonesian orchid species using similar Agrobacterium-based protocols, broadening the taxonomic scope of the technology [26]. The use of acetosyringone during co-cultivation consistently improves T-DNA transfer efficiency by inducing virulence gene expression in the bacterium, a finding applicable across *Oncidium* transformation studies [28].

Biolistic Transformation

Particle bombardment (biolistic) transformation delivers DNA-coated gold or tungsten microparticles into plant cells using high-velocity gas pressure, bypassing the host range limitations of Agrobacterium and enabling simultaneous co-transformation of multiple gene constructs. Niyomtham [21] reported successful co-bombardment of *Oncidium* 'Sharry Baby' PLBs with separate gene cassettes carrying the synthetic green fluorescent protein (*sgfp*), antisense chalcone synthase (*CHS*), and hygromycin resistance (*hptII*) genes. Optimization of six critical parameters—PLB size, DNA concentration, PLB age, presence of spermidine and $CaCl_2$, and pre-bombardment culture duration—substantially improved transient *gfp* expression and stable transformation frequency. Similarly, Chew et al. [5] developed an efficient particle bombardment system for *Dendrobium* species, identifying helium pressure, target distance, and PLB size as key parameters influencing transformation efficiency. Biolistic transformation of selected orchid hybrids targeting shelf life and ornamental improvement, while Kanchanapoom et al. [12] demonstrated efficient biolistic transformation of Phalaenopsis PLBs using EgTCTP transgene, confirming the transferability of biolistic protocols across orchid genera. Biolistic transformation typically yields higher copy number integrations and more complex rearrangements than Agrobacterium, but its independence from host range restrictions makes it an important complementary tool [15].

Selectable Markers and Reporter Genes

Appropriate selectable marker systems are indispensable for recovering transformants from non-transformed PLBs. The hygromycin phosphotransferase II gene (*hptII*), conferring resistance to hygromycin, is the most widely used selectable marker in *Oncidium* transformation . Liau et al. (17) and Lee et al. (14) both employed *hptII* successfully, with selection pressures typically ranging from 3–10 mg/L. Phosphomannose isomerase (*pmi*) enables positive selection by allowing transformed cells to utilize mannose while non-transformed cells are starved. Thiruvengadam et al. [32]

demonstrated that *pmi*-based selection in *Oncidium* 'Gower Ramsey' PLBs yielded efficiencies comparable to antibiotic selection while avoiding antibiotic pressure—an advantage for regulatory compliance. The *pflp* gene encoding sweet pepper ferredoxin-like protein was validated as a dual-function selectable marker and disease-resistance gene by You et al. [38] and Liau et al. [18]. GFP and beta-glucuronidase (GUS) are the most commonly used reporter genes for monitoring transformation efficiency and screening putative transformants [28]; [15].

Optimization of Transformation Parameters

Achieving consistent and reproducible transformation of *Oncidium* PLBs requires systematic optimization of multiple biological and physical parameters. Li et al. [16] demonstrated that osmotic sucrose pretreatment of PLBs significantly enhanced single-cell embryogenesis and biolistic transformation efficiency in *Oncidium*, with 0.2–0.4 M sucrose pretreatment for 4 hours prior to bombardment yielding the highest transient expression rates. Osmotic pretreatment is thought to reduce cellular turgor, facilitating particle penetration and DNA delivery into competent cells. For Agrobacterium-mediated protocols, key variables include strain selection, optical density at inoculation, co-cultivation duration (typically 2–3 days), acetosyringone treatment, and selection medium composition [21]. Lind [19] reviewed modern genetic engineering approaches in Orchidaceae and emphasized that genotype-dependent responses remain a significant source of variation, with transformation efficiencies differing considerably even among cultivars within the same species. Systematic optimization across multiple *Oncidium* cultivars, as performed by Lee et al. [14], is therefore essential for developing broadly applicable protocols. Hormone composition during post-bombardment or post-co-cultivation recovery culture also influences PLB regeneration and transformation outcome, with zeatin and BA being the most commonly used cytokinins in recovery media [24].

Transgenic Plant Regeneration and Confirmation

Following transformation and selection, putative transgenic PLBs are transferred to regeneration medium to produce shoots and roots. In *Oncidium*, PLBs typically require 8–12 weeks on selection medium before regenerated shoots are identified [32]. Molecular confirmation of stable transformation involves a tiered approach: PCR analysis using transgene-specific primers provides initial evidence of integration, while Southern blot hybridization confirms copy number and rules out transient expression [17]. RT-PCR and Western blotting confirm transgene expression at mRNA and protein levels, respectively [31]. Flow cytometry may confirm ploidy stability in regenerated transgenic plants [8]. Stability of transgene expression across vegetative generations is assessed through successive subculture and progeny analysis before field evaluation or commercial release [35].

Applications of Genetic Transformation in Oncidium

Flower Color Modification

The carotenoid biosynthesis pathway in *Oncidium* orchids has been manipulated through RNA interference (RNAi) targeting phytoene synthase (*psy*), resulting in impaired carotenoid accumulation, growth alterations, and modified plastid morphology [20]. These findings provided proof-of-concept for using transgenic approaches to modulate floral pigmentation. Silencing of chalcone synthase (*CHS*)—a key enzyme in the anthocyanin pathway—was also demonstrated through antisense approaches in *Oncidium* 'Sharry Baby', offering a route to novel flower color phenotypes. The broader toolbox for floral color engineering continues to expand with advances in understanding orchid pigment biochemistry [36].

Disease Resistance Enhancement

The *pflp* gene encoding sweet pepper ferredoxin-like protein was shown by Liau et al. [18] to confer significant resistance against bacterial soft rot caused by *Erwinia carotovora* in transgenic *Oncidium* orchids. This finding demonstrated the translational potential of cross-species gene transfer for improving pathogen resistance in commercially important orchids. You et al. [38] further validated *pflp* as a selectable marker with dual functionality, streamlining the development of disease-resistant transgenic lines.

Developmental and Flowering Regulation

Overexpression of the MADS-box transcription factor gene *OMADS1* from *Oncidium* in transgenic *Oncidium* 'Gower Ramsey' significantly promoted early flowering, demonstrating the utility of endogenous orchid genes for accelerating reproductive development [31]; [33] further showed that ectopic expression of two MADS-box genes from *Oncidium* and lily in *Arabidopsis thaliana* altered floral morphology and induced early flowering, providing insights into cross-species regulatory networks. The functional roles of diverse MADS-box factors in orchid reproduction continue to be clarified through transgenic approaches [30]; [36].

Challenges and Future Perspectives

Despite substantial progress, genetic transformation of *Oncidium* orchids remains challenging. Transformation efficiency is generally low, typically ranging from 1–10% of treated PLBs, and is highly cultivar-dependent [14]; [19]. Susceptibility of PLBs to Agrobacterium varies among genotypes, necessitating optimization for each new cultivar [6]. Somaclonal variation from prolonged tissue culture can confound phenotypic analyses, and transgene silencing over successive generations poses long-term stability concerns (25). CRISPR-Cas9 genome editing represents the most transformative future direction for *Oncidium* improvement. Huang et al. [9] demonstrated highly efficient, transgene-free multiplex genome editing using cymbidium mosaic virus-derived vectors—a promising delivery platform applicable to orchids. Zhou et al. [40] established efficient Agrobacterium-mediated transformation of *Cymbidium goeringii*, providing protocols adaptable

to *Oncidium*. Marker-free transformation systems will be important for commercial deployment and public acceptance [2]. Extending transformation to wild *Oncidium* species beyond model cultivars will require genotype-specific optimization [13]; [27]. Functional genomics through transformation will further deepen understanding of orchid-specific processes including mycorrhizal associations, CAM photosynthesis, and pollinator-directed floral chemistry [7]; [26]; [37].

2. CONCLUSION

Genetic transformation of *Oncidium* orchids using protocorm-like bodies has emerged as a well-established platform for both applied trait improvement and fundamental biological inquiry. PLBs provide a uniquely suitable explant system that combines embryogenic competence with large-scale propagability. Both *Agrobacterium* - mediated and biolistic transformation methods have been successfully applied to multiple *Oncidium* cultivars, supported by diverse selectable marker and reporter gene systems. Demonstrated applications span disease resistance, floral pigmentation modification, and developmental regulation. Remaining challenges, including low efficiency, genotype-dependence, and somaclonal variation, are progressively being addressed by improved protocols, novel delivery systems, and next-generation editing tools such as CRISPR-Cas9. Continued investment in *Oncidium* transformation research will yield novel cultivars and scientific insights of enduring commercial and academic value.

ACKNOWLEDGEMENT

The author acknowledge the Department of Botany, Union Christian College, Aluva, for providing facilities for conducting the study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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